ANALYTICAL CHEMISTRY

Increased Tempo

THE Magic Number," our December editorial, referred to the possibility that the Division of Analytical Chemistry would obtain 2000 members by the end of 1955. This goal has already been passed and the sights are raised still further.

One reason for the increase was illustrated at the Conference on Analytical Chemistry and Applied Microscopy, held a few weeks ago at Pittsburgh. At this meeting, G. Frederick Smith, supersalesman and immediate past chairman of the division, sold more than 100 divisional memberships.

Activities of Smith, K. G. Stone, chairman of the Membership Committee, and other active members have made possible the division's recent rapid growth. This increased membership places responsibilities on the division to provide programs and services of interest to members and thus retain their active membership.

The title "Magic Number" could also be applied to the Sixth Pittsburgh Conference, sponsored jointly by the Analytical Group of the Pittsburgh Section and the Spectroscopy Society of Pittsburgh, because registration at this meeting passed the 2000 mark.

The outstanding success of the Pittsburgh conference has brought with it some problems and headaches. It is literally bursting at the seams. The Hotel William Penn was unable to accommodate all those who wished to attend and could not make sufficient space available for manufacturers and suppliers who wished to demonstrate their wares.

The close proximity of the meeting rooms and exhibition hall has been an advantage, but now with the need for more space the solution may lie in the proposed convention and exhibition hall in Pittsburgh.

Other events of interest to analysts which indicate the increased tempo include the national ACS meeting in Cincinnati. The divisional program is scheduled for three days (March 30 to April 2).

After Cincinnati comes the annual Summer Symposium cosponsored by the Division of Analytical Chemistry and ANALYTICAL CHEMISTRY. The 1955 symposium, to be held at Syracuse University, June 17 and 18, will deal with "The Role of Reaction Rates in Analytical Chemistry."

Analysts on the West Coast will be interested to learn that the 1956 meeting will be staged on the Los Angeles campus of the University of California. Purdue will be host in 1957.

We applaud the decision to take the "show" to California next year. It will afford analysts residing on the West Coast an opportunity to share intimately in the program of the division and will provide a golden opportunity for analysts from other sections of the country to see at first hand a wide variety of industrial and agricultural activity, much of which is peculiar to western states. There are many problems in the industrial field, such as smog control, and in the field of agricultural and food chemistry, the solution of which may well come from analytical chemists.

Another major event this year is the Nuclear Engineering and Science Congress, to be held in Cleveland in December.

The analytical profession is on the march and slated to reach bigger and better heights in the foreseeable future. And that is the way it should be.

Nuclear Congress—Opportunity for Analysts

A RECENT announcement by the Atomic Energy Commission, that it will be selling commercial power from its land-based prototype of a submarine reactor (West Milton, N. Y.) by late summer, indicates the speed with which atomic power is becoming a reality. In this mushrooming field, and in particular its peacetime applications, analysts are playing an increasingly important role.

Analysts will have an opportunity to participate in a scientific meeting which will evaluate the past, present, and future role of atomic energy: the first Nuclear Engineering and Science Congress, at Cleveland, December 12 to 17, 1955.

The congress is being sponsored by the Engineers Joint Council with the active assistance and cooperation of some 20 professional, engineering, and technical societies. The AMERICAN CHEMICAL SOCIETY is a major participating group.

To date the Analytical, Polymer, Physical and Inorganic, Chemical Literature, Water, Sewage, and Sanitation, and Industrial and Engineering Chemistry Divisions have indicated their intention to present papers.

Each participating organization is being given the responsibility for obtaining authors and papers. The individual papers will be assigned to appropriate symposia or general sessions. The authors, however, will be identified with the organization they represent.

To assure a well rounded program, the sponsors are not requiring original presentations. This will allow presentation of papers which may be declassified for presentation at the United Nations "atoms-for-peace" conference at Geneva this year.

Some groups have declined to participate, partially because of an early deadline of April 1 for titles and abstracts of papers.

The deadline for titles and abstracts has been moved up to May 1. Deadline for papers is set for mid-August.

ACS divisions which cannot participate as a unit should encourage individual members who have papers of interest to take part in the congress. Details may be obtained from R. M. Warren, assistant secretary, AMERICAN CHEMICAL SOCIETY, 1155 Sixteenth St., N.W., Washington 6, D. C.

This congress, which may develop into an annual affair, should represent a significant step forward as the era of peacetime applications of atomic energy rapidly comes closer. The ACS through both its divisions and individual members can make a major contribution.

Characterization of Organic Substances by Differential Thermal Analysis

General Experimental Technique

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The methods of differential thermal analysis in studying the thermal characteristics of liquid and solid organic substances have been critically examined. Preliminary results show that the differential thermographic features exhibited by many substances are unique properties. Experimental details and a selection of thermograms are given to illustrate the reproducibility and the distinctive nature of the thermal curves. The thermograms of some synthetic and natural high polymers are presented to indicate the potential importance and usefulness of this analytical tool for the characterization of complex organic substances.

DIFFERENTIAL thermal analysis of inorganic systems has received considerable attention by the mineralogists. The method consists in heating the substance to be studied at a constant rate with calcined alumina. The ensuing exothermic and endothermic pyrolysis reactions are electronically measured against a heat-stable reference material, so that the temperature differences between the sample and the reference material are recorded as a function of temperature.

Despite isolated efforts which have been reported in the past (2, 3, 5, 9, 10), there appears to have been no critical or concerted attempt to appraise the method as a general tool for the organic chemist. With the recent advent of improved instrumental design and the consequent refinement in operational procedure, a systematic investigation was undertaken in order to determine whether this thermal technique can be applied for general organic analysis. This paper is the first in a series outlining the results of studies involving some natural and synthetic polymers.

The aim of the first communication is threefold: to give the experimental details of a reliable technique; to illustrate the order of precision or accuracy that may be expected; and to demonstrate the versatility of the method by its application to some diverse substances.

EXPERIMENTAL

A wide variety of apparatus is available for differential thermal analysis (9). The essential arrangement has been described by Kulp and Kerr (7) and by Whitehead and Breger (11).

The sample holder (Figure 1) consisted of a rectangular metallic block (palladium–stainless steel or Inconel) provided with two vertical sample wells, each of 0.4-ml. capacity. In one well the heat-stable reference substance, calcined alumina, was placed, and in the other, an admixture of the organic substance in alumina. The thermal variations between the reference and the mixture were measured and recorded by means of Chromel-Alumel thermocouples embedded in the wells. The essential arrangement of the thermocouple circuit has been described by Whitehead and Breger (11). The notable modifications in the
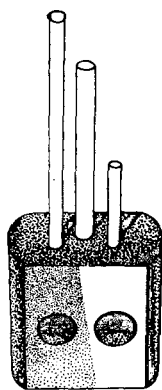
Figure 1. Sample holder

present study included the use of a Leeds & Northrup H2195 Speedomax temperature controller, a Leeds & Northrup direct current Model 9835-B preamplifier, and a Speedomax Type G Model S 600 Series indicating millivolt recorder having a sensitivity of ±0.1 mv. which was expandable to ±10 mv. for microanalysis of 15- to 25-mg. samples.

For consistent results, care was taken to place the sample block symmetrically in the ceramic core of the furnace. Purified nitrogen was then slowly passed through via an inlet provided at one end of the furnace.

After numerous attempts, it was found that, for consistent and uniform reproducibility, a compressed sandwich packing (1) of the sample proved to be the most convenient and reproducible. This was accomplished by packing 150 mg. of the powdered organic material between two layers of calcined alumina and compressing the resulting mixture under a pressure of 200 pounds per square inch.

For most determinations, the samples were heated at the rate of 10° C. per minute up to 1000° C. The 150-mg. samples required the use of only 50 to 70% of the maximum sensitivity of the apparatus. For the more difficultly obtainable organic compounds, the use of 15- to 35-mg. quantities was feasible simply by reducing the volume of the sample wells and using the higher sensitivities of the instrument.
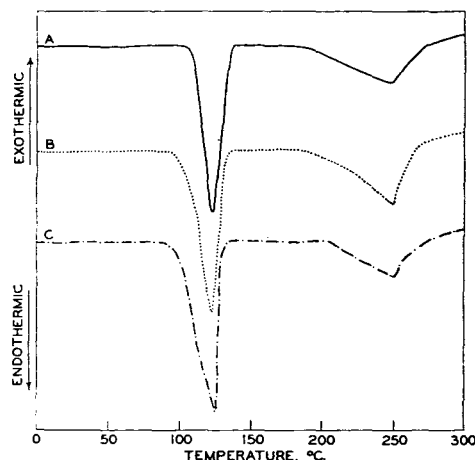

Figure 2. Thermogram of benzoic acid

A, B, C. Benzoic acid

For the analysis of liquids and solutions, the samples were prepared by placing, first, enough alumina in the well barely to cover the thermocouple. Then a 0.15-ml. portion of the liquid was placed on the alumina, followed by an additional amount of alumina to give a sandwich packing as before.

For each substance studied, four to eight determinations were made. The temperatures of the exothermal and endothermal peaks were read graphically to within 5° C. and estimated to within 2.5° C. Duplicate runs showed that the temperatures can be confidently reproduced to within ±5° C. for temperatures below 400° C. and to within ±10° C. in the range of 400° to 1000° C.

RESULTS AND DISCUSSION

The thermograms of benzoic acid shown in Figure 2 demonstrate the reproducibility of this thermoelectric method using the sandwich packing of the samples. The endothermic peaks at 130° and 250° C. corresponded approximately to the reported
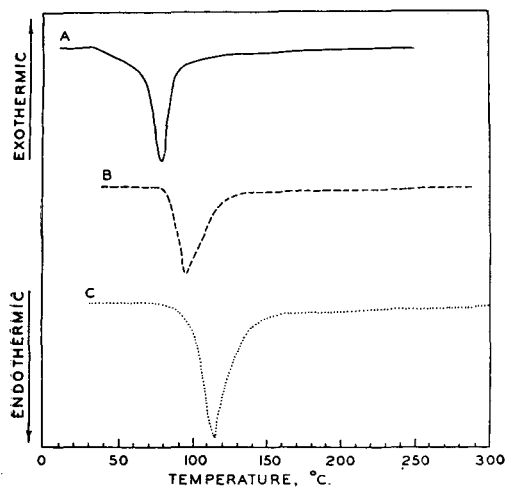
Figure 3. Thermogram of liquids

A. Benzene
B. 50% benzene in toluene
C. Toluene

melting and boiling points of pure benzoic acid. Table I lists these physical constants which have been determined by the differential thermal analysis technique and are compared with the literature values. The observed melting points were about 10° C. higher than the literature values, whereas the boiling points lay more nearly to those reported. The differential thermal temperatures are, of course, dependent upon the rate of heating (1).

The quantitative reproducibility of the thermal curves is given in Table II. Two National Bureau of Standards calorimetric standards were selected. The numerical values listed give the maximum height (in millivolts) of the melting point curves which were graphically readable to within ±0.1 mv. The temperatures are those at which the values were measured.

Table I. Melting and Boiling Points Observed by Differential Thermal Analysis

	Observed, ° C.		Literature, ° C.	
Compound	Melting pt.	Boiling pt.	Melting pt.	Boiling pt.
Ethyl p-aminobenzoate	92	...	92	...
Ethyl propionate	...	100	...	99
Amyl acetate	...	148	...	148
Isophthalic acid	352	...	346	...
α-d-glucose	155	...	146	...
Anthracene	228	330	217	340
Benzyl alcohol	...	210	...	205

Table II. Maximum Height of Endothermic Peaks in Millivolts

Compound	Trial 1	Trial 2	Trial 3	Trial 4
Benzoic acid				
Mv.	6.0	6.4	6.2	5.9
° C.	130	130	130	135
Sucrose				
Mv.	4.8	5.1	5.2	5.2
° C.	195	200	195	195

The order of reproducibility is such that semiquantitative calculations of the heat effects involved in these simple and well-defined endotherms are fairly reliable. For example, by measuring the area of the endotherms and several other quantities it is possible to calculate the heat of fusion (10). As subsequent data will show, despite certain disabilities (10), differential thermal analysis provides a versatile and a reliable technique for qualitative organic analysis, particularly for the identification or characterization of complex organic compounds.

The thermal analysis of liquids and solutions are exemplified by the thermograms in Figures 3 and 4. The boiling points of benzene, toluene, and 50% by weight toluene in benzene were observed at 80°, 112°, and 95° C., respectively, the corresponding literature values being 80°, 110°, and 92.4° C. In Figure 4, the thermogram of a benzene solution containing 50 mg. of acenaphthene clearly exhibits the physical constants of the two components, namely, the boiling point of benzene at 80° C. followed by the melting point of acenaphthene at 95° C. and the boiling point of acenaphthene at about 275° C.
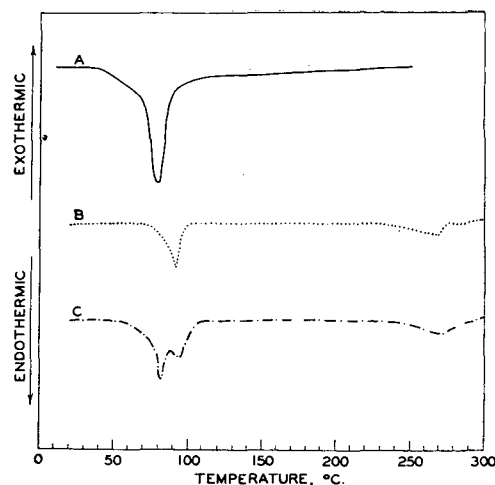

Figure 4. Thermogram of solution

A. Benzene
B. Acenaphthene, 50 mg.
C. Acenaphthene in benzene, 0.15 ml.

The differential thermal analysis of some natural polymers are illustrated in Figures 5, 6, and 7. Although these substances may be considered to be essentially polymers of glucose, the effect of further substitutions as well as the type of molecular configuration resulting from variations in chain linkages have a decided influence upon the shape of the thermograms. In Figure 5, cellulose (curve A) is characterized by an endotherm at 340° C. and an exothermic peak at 655° C., while both glucose and cellobiose, which are considered to be the building units of cellulose,
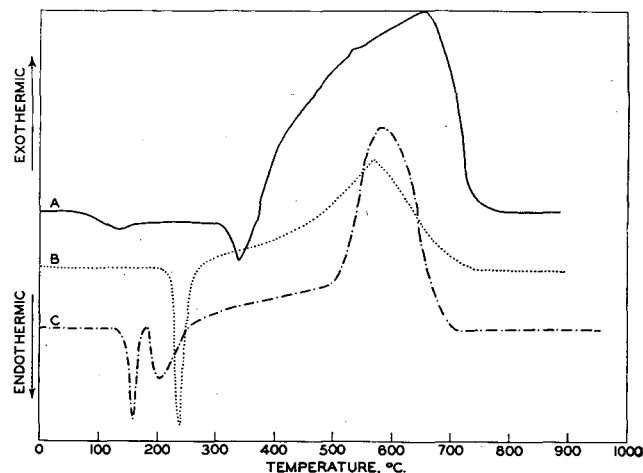

Figure 5. Thermogram of glucose polymers

A. Whatman cellulose powder
B. Cellobiose
C. α-d-Glucose

exhibit a pronounced exothermic peak in the 570° to 580° C. region.

Figure 6 shows the thermal behavior of some polymers containing the carboxy group. Curve C represents a thermogram of Hercules Powder Co. carboxymethylcellulose substituted on the average with 0.5 carboxy groups per glucose unit. The hemicellulose (curve A) was obtained from cotton seed hulls. The chemical structure of this hemicellulose is generally accepted as being composed of glucuronic acid and xylose units.

The predominant influence of the steric factor upon the differential thermal property of certain polymers is exemplified in the thermograms of the starch fractions (Figure 7). Starch is generally considered to be a chain of glucopuranose units joined through 1,4-linkages, in which amylose (curve C) contains linear chains and amylopectin (curve B) the branched polymer. These starch fractions all manifest a series of endothermic reactions between 250° and 350° C. and a distinct exothermic reaction in the 500° to 525° C. region. The differential thermal characteristics of the starch preparations will be the subject of a future communication.
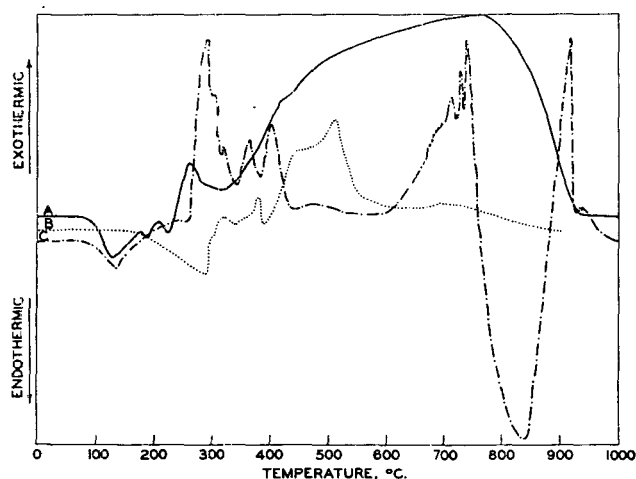

Figure 6.   Thermogram of carboxy polymers

A.   Hemicellulose
B.   Cellulose triacetate
C.   Carboxymethylcellulose

Maltose (Figure 7, curve D) showed an endotherm soon after its fusion, although it was admittedly a very weak one at 185° C. The second endothermic reaction appeared to be typical of most sugars, for example, glucose (Figure 5, curve C) where it occurred at 205° C., immediately following the melting endotherm at 155° C. Cellobiose (Figure 5, curve B), on the other hand, was completely devoid of this second endotherm.

The polypeptides (Figure 8) have been selected to emphasize another aspect of this technique, namely, the fact that marked thermographic changes are brought about by variations in molecular composition, particularly those in a homologous series. The reproducibility of the thermograms is to be noted. Similarly, the consequences of altering monomer composition are illustrated by some commercial synthetic resins (Figure 9). The VYNW (curve B) and VYSL (curve C) resins are vinyl chloride–vinyl acetate straight-chain copolymers of the Canadian Resins and Chemicals, Limited, the former having 97.5% and the latter containing 87.9% vinyl chloride.

Many high polymers when heated at the rate of 10° C. per minute exhibit extensive thermal reactions even up to 1000° C. Complete analysis of such materials, therefore, can become rather time-consuming. Consequently, the feasibility of utilizing a higher heating rate was investigated. The result is shown in

Figure 10.   The thermal analysis undertaken at 18° C. per minute (curve A) gave data which were comparable to those obtained at slower rates of heating. Obviously, because of the increased rate, the exothermic temperatures have been displaced toward higher temperatures; for example, the exothermic peak at 890° C. (curve B) has been shifted to 1025° C. (curve A). This and similar studies indicate that for substances which are characterized by large thermal reactions at relatively high temperatures differential thermal analysis may be carried out more expediently and without serious detriment to pertinent data, by increasing the ráte of heating.
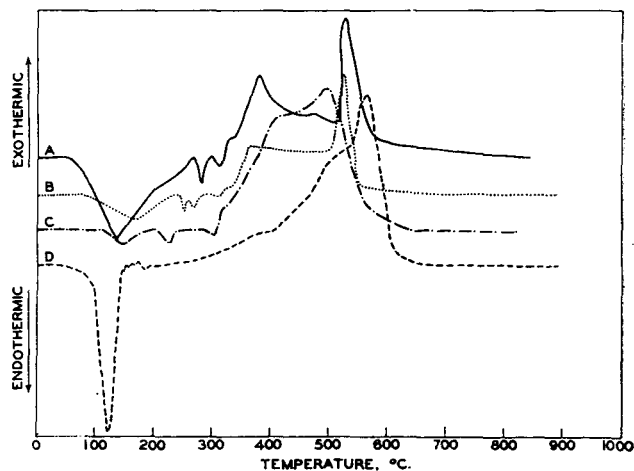
Figure 7.   Thermogram of starch fractions

A.   Cornstarch
B.   Corn amylopectin
C.   Corn amylose
D.   Maltose

Although it is interesting to speculate upon the mechanisms involved in the thermal reactions, such conjectures cannot be entertained confidently until further data become available or until the reaction products have been identified. Certain studies (1) have shown that the alumina can be considered to be essentially inert. Benzoic acid, however, has been found to be an exception. Its thermograms (Figure 11) appeared to indicate that, upon complete fusion, benzoic acid partially formed a

Figure 8.   Thermogram of polypeptides

A.   Glycylglycylglycine
B.   Glycylglycylglycine, duplicate run
C.   DL-Alanylglycylglycine

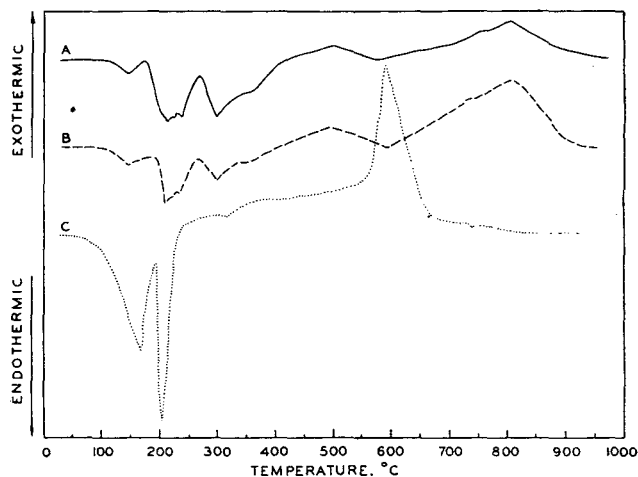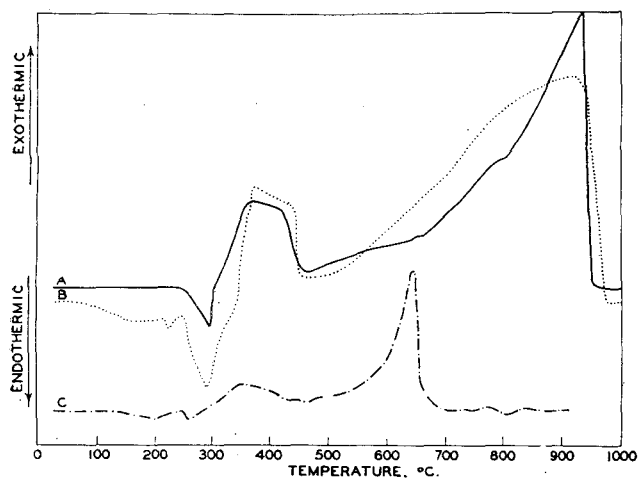Figure 9. Thermogram of synthetic polymers

A. Polyvinyl chloride
B. Vycanac VYNW
C. Vycanac VYSL 400

complex with the alumina which later decomposed to give a large exothermic reaction in the 400° to 550° C. region. In the absence of alumina, this exotherm was completely absent (curve B). Detailed studies relating to the effect of inorganic catalysts other than alumina are now in progress.

The results described herein indicate that further exploratory investigations in the use of this method for general organic analysis would be warranted. Indeed, it can be adapted to suit a variety of experimental conditions. Instead of an atmosphere of nitrogen, it may be desirable in some instances to use carbon dioxide, steam, oxygen, hydrogen, or even vacuum, as for example, in systems having liquids of high boiling points. Furnace atmosphere control has been used successfully in certain inorganic systems (8). In fact, with a hydrogen atmosphere, the technique may be modified for the study of hydrogenation or other similar kinetic measurements as suggested by Kagan (6). Furthermore, electrical circuits are now commercially available whereby multiple recorders may be arranged so that as many as twelve samples can be analyzed simultaneously.

In subsequent papers it will be shown that differential thermal analysis is applicable for the characterization of even the simpler
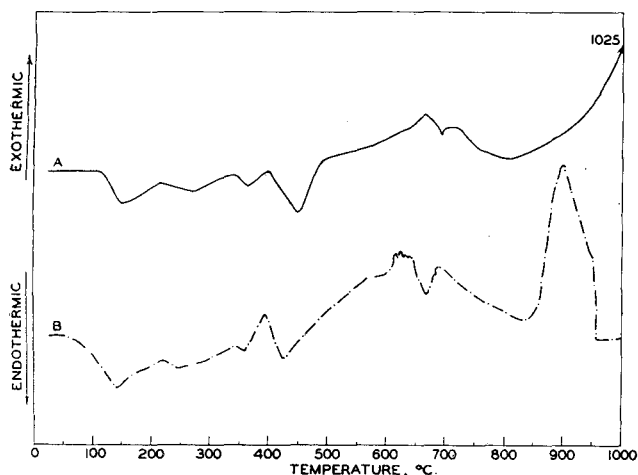
organic substances and mixtures, particularly the isomeric monosaccharides and the oligosaccharides where steric considerations play a decisive role in determining the configuration of the thermograms.

CONCLUSION

The preliminary studies described herein have shown that the temperatures at which a substance exhibits endothermic and exothermic reactions in the presence of calcined alumina are unique characteristic properties of that substance, and the re-
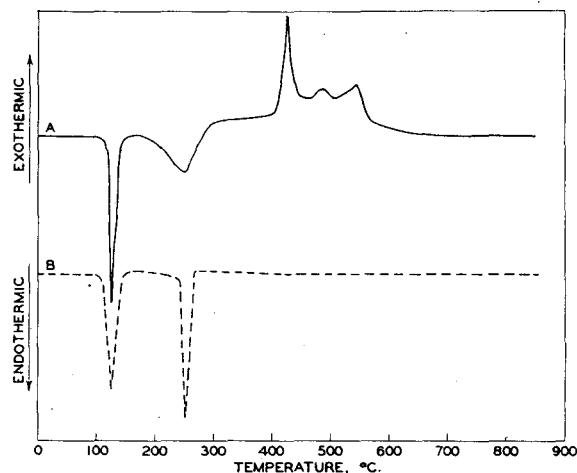
Figure 11. Effect of alumina

A. Benzoic acid in alumina
B. Benzoic acid without alumina

sulting thermograms may be used confidently as a fingerprinting device. Moreover, differential thermal analysis offers unique advantages over other instrumental analyses, especially those involving insoluble colloidal and amorphous aggregates which exhibit diffused x-ray patterns and which, because of their inherent intractable physical state, do not give reproducible infrared spectra (4).

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Figure 10. Effect of rate of heating

A. Sodium polyacrylate, dT/dt = 18° C. per minute
B. Sodium polyacrylate, dT/dt = 10° C. per minute

Differential Measurements of Reflectance

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Reflectance has been explored as a possible means of making direct analyses of difficultly soluble solid mixtures and of substances on chromatograms. For dyed samples of cloth and yarn, differential spectrophotometric measurements of reflectance make possible the more precise determination of small differences in regions of low reflectance. The results can also be interpreted readily in terms familiar to persons interested in defining color differences. A similar study on synthetic mixtures of pigments has shown that analytically useful data can usually be obtained with samples in powdered form.

THE present study was designed to evaluate the applicability of reflectance measurements to problems encountered by analytical chemists. Because measurements can be made directly upon solids, the method should be particularly useful for analyzing samples of difficultly soluble substances, samples containing substances which will, upon being dissolved, react with one another or with the solvent, and chromatograms of colorless substances.

Reflectance measurements of more or less analytical interest have long been applied in the dye, paper, and ceramic industries, where the colors of the products have had to be determined without destruction of the sample (5, 8). Fundamental studies have been carried out almost entirely by physicists interested in describing color. Thus, reflectance data have been obtained by persons primarily interested in converting the results to tristimulus values and thence to units of color difference.

Although differential measurements of reflectance have been gaining increased recognition, the instruments have been tristimulus colorimeters rather than spectrophotometers (1, 3). While the purpose of the present paper is to demonstrate to the analytical chemist the possibility of using reflectance to make direct analyses upon solid samples, it is hoped that persons in industries involving color matching will find the increased information and increased precision resulting from differential spectrophotometric measurements to be of value (2).

APPARATUS AND MATERIALS

All of the measurements of diffuse reflectance were obtained by using a Beckman DU spectrophotometer, a standard Beckman reflectance attachment, and a Beckman photomultiplier attachment.

All of the chemicals were of analytical grade with the exception of two commercial "toners" which were used as adulterants. Weighed amounts of the samples were thoroughly mixed by hand and shaken six times through a U. S. standard No. 100 mesh copper-cloth screen (Tyler). Prints on nylon, rayon, or cotton were available for 16 different dyes, each in a series of concentrations. A variety of shades, yellow, blue, orange, green, brown, scarlet, and violet were represented. The cloths, printed with known concentrations of colored material, were supplied by E. I. du Pont de Nemours & Co., Inc., as were samples of dyed yarns. The samples had previously been judged for shade, strength, and brilliance, by a person experienced in color matching.

PROCEDURES

Cloths, printed with 1-inch stripes of dye separated by plain material, were cut, and a sample of five thicknesses of the stripe alone was mounted on a white card. Care was taken to avoid the use of creased material, as marked deviations were known to re-

sult from an irregular surface. Yarn samples were wrapped around glass plates, $1/8 \times 1^1/4 \times 1^1/4$ inches in such a way that the threads lay against one another rather than bunched on top of one another. Three layers of yarn were used, each successive layer at right angles to its predecessor. The dyed sample of either cloth or yarn was then measured against magnesium carbonate or against another dyed sample which had arbitrarily been selected as a standard.

In dealing with pigments, two methods of mounting were employed. In one case, the dry powdered sample was packed into an inverted crucible cover and held in place with a thin glass slide. In the other case, samples were pressed by means of a flat metal plunger into brass rings backed by polished glass plates in order to produce uniformly flat surfaces for measurement. Although the latter method is preferred, a sample which is so free-flowing that it fails to pack rigidly must be handled by the first method.

In making differential measurements, it was often convenient to set the standard at a value less than full scale. This enabled one to measure samples having greater reflectance than the standard.

RESULTS

Dyed Cloths. As an indication of the advantage to be gained by differential measurements one can compare the results on Diagen Blue MSG solutions which are plotted directly on a scale with magnesium carbonate at 100 in Figure 1 and on a differential basis in Figure 2. The lines on Figure 1, which are crowded together in the region around 600 mμ, are expanded in Figure 2. Small absolute differences in reflectance actually represented large relative differences which are more clearly presented by the differential curve. Similar data have been obtained on other samples where the absolute reflectance of the least concentrated sample was of the order of 2%.
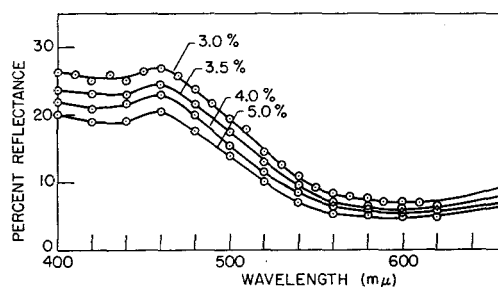

Figure 1. Reflectance, relative to magnesium carbonate, of cotton samples printed with Diagen Blue MSG solution
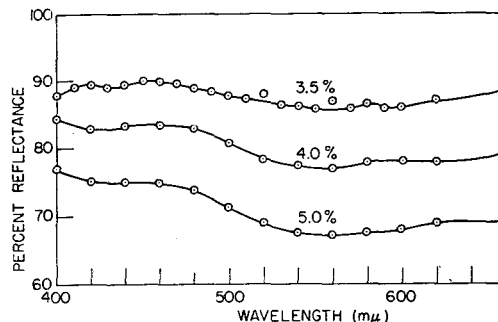

Figure 2. Differential reflectance of Diagen Blue MSG samples relative to the 3% sample as standard at 100% reflectance

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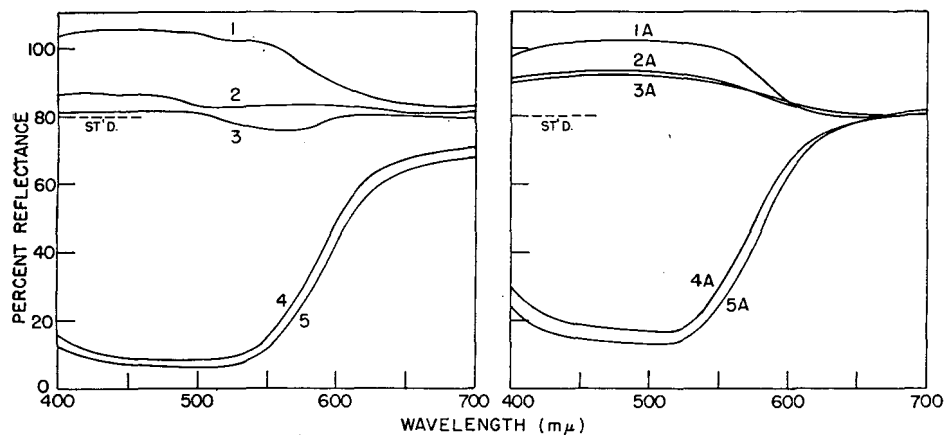
Figure 3.   Normal and differential measurements on cotton prints of Ponsol Golden Orange 4R Double Paste each at two levels of concentration

1.   Preparation 8515A (10.5%) run differentially against preparation 57 (15%) at a scale reading of 80.0
2.   Preparation 8514A run differentially against preparation 57 at 80
3.   Same as curve 2 but with new sample and standard
4.   Preparation 8515A (10.5%) relative to magnesium carbonate at 100
5.   Preparation 57 (15.0%) relative to magnesium carbonate at 100
A curves represent 25% reductions

The precision with which reflectance values could be measured was independent of the standard against which the sample was compared, thereby permitting the concentration of dye in a sample of low reflectance to be determined much more precisely by differential measurement. Reflectance values of a sample were usually reproducible within ±0.2%. Removing the sample (or the standard) from the holder and then replacing it introduced some error, and occasional errors as large as ±0.4% were encountered, though most of the values still fell within ±0.2%.

Covering the sample with another section of dyed cloth from the same stripe produced very interesting results. On "good" prints, the variations from one part of a sample to the next were such that concentration could be determined within ±3% on a relative basis. Thus, against a standard set at 100% reflectance, different samples from a given stripe which should give readings at 50%, gave a spread between 48.5 and 51.5%, while at the same time the value on any given sample could be reproduced to ±0.2%. Because similar results were obtained on a wide variety of samples having different shades and widely different concentrations of dye, it appears that a variation of ±2 to 3% in reflectance must arise from the printing process itself. However, on a number of occasions, variations within a stripe of ±10% or more were encountered together with large relative shifts in shade and dullness. These differences in reflectance did not appear to be due to a faulty roller or poor mixing of a sample, as they usually occurred simultaneously with several stripes on the print. The difficulty appeared to be associated with the printing of large concentrations of dye such that "caking" could occur. One or two of the samples, on close visual inspection, had a somewhat irregular sheen. However, such irregularities could not always be detected visually.

In making color judgments, it is customary to use prints of the dye at two concentrations: one at a high concentration of pure dye—i.e., "full strength"—and one at one fourth that concentration. The latter, because of its greater reflectance, permits any difference in concentration (from a preselected standard) to be detected more readily. Figure 3 shows the spectrophotometric results on duplicate analyses for an orange dye. Sample 8515A is consistent within ±2% reflectance whether compared to the standard at "full strength" or "25% reduction." On the other hand, data for 8514A differ both in magnitude and in quality in going from one strength to another. However, while attempts to reproduce to ±2% the data on the diluted 8514A were successful, those on "full-strength" prints differed almost as widely in

character as those cited for the two concentrations. Similarly, discordant results within single prints were also obtained on other orange samples and on a large number of jade greens.

These studies cast considerable doubt upon the value of the full-strength print for precise qualitative and quantitative comparisons. However, as the eye can usually only distinguish differences in strength of 10% or more, one can understand why such variations have not been reported frequently in the past. The use of a filter photometer undoubtedly can detect large changes, though integration of the response over a broad band of wave lengths probably results in smaller differences than those found spectrophotometrically at the wave length of maximum reflectance. Hence, if a spectrophotometer is available, there appears to be little justification for using full-strength prints.

The interpretation of data obtained from differential comparison of two samples for closeness of match can readily be made. Figure 4 shows that differences in strength appear in shape comparable to the spectrum of the pure dye or to its mirror image so that quantitative judgment of differences in concentration alone ("strength") are straightforward. Any deviations from the expected curve will therefore be due to the presence of a foreign substance, to the effect of the cloth, or to a change in the conditions of dyeing or printing.
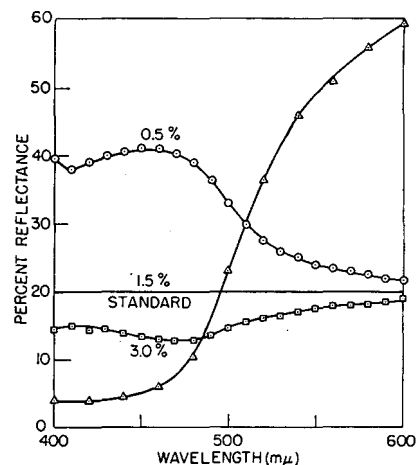
Figure 4.   Effect of concentration on differential curves using acetamine yellow CG on nylon

Fourth curve is reflectance of 1.5% which was used as the differential standard relative to magnesium carbonate

Powders. Of more general interest to the analytical chemist are the studies on mixtures of colored solids which, at the same time, represent problems encountered in measurements on commercial pigments. Figure 5, showing the reflectance of samples containing mixtures of ferric oxide and barium sulfate, illustrates the sensitivity of the method in so far as 0.05% by weight of ferric

Table I. Test of Reproducibility of Packing a Powder

(Using a sample of 0.10% ferric oxide in barium sulfate and magnesium carbonate as standard)

Wave Length, Mμ	Trial		
	1	2	3
400	79.8	79.5	79.3
420	78.8	78.7	78.7
440	77.9	77.9	77.9
460	77.8	77.8	77.8
480	77.5	77.1	77.3
500	77.1	77.2	77.2
520	77.0	77.1	77.0
540	77.0	77.1	77.2
560	80.2	80.3	80.2
580	86.2	86.2	86.2
600	90.8	91.0	90.8
650	93.8	93.7	93.9
700	94.8	95.2	94.8

Figure 6. Effect of barium chromate on reflectance of a mixture of 5% barium chromate and 1% ferric oxide as determined differentially using 5% barium chromate as 100

1. 5% barium chromate in barium sulfate relative to magnesium carbonate
2. 5% barium chromate and 1% ferric oxide in barium sulfate relative to 5% barium chromate
3. 1% ferric oxide in barium sulfate relative to magnesium carbonate

Figure 5. Reflectance of various mixtures of ferric oxide and barium sulfate relative to magnesium carbonate

set at 100 on the reflectance scale, a maximum variation of ±2 units was obtained. Visual examination showed each of the surfaces to be "spotty"—i.e., irregular pressing caused large numbers of small, randomly located areas to be glossy. In spite of this effect, the precision of ±2% was apparently easy to attain. Even more unusual was the fact that samples containing 5% barium sulfate could not be distinguished from pure ferric oxide by reflectance at 400 mμ.

From the analytical standpoint of qualitative detection of substances present in a mixture one must realize that differential

oxide can readily be detected. More striking is the fact that a small but measurable difference in reflectance resulted on successive screenings of barium sulfate. The reflectance at 400 mμ for eight different portions of a sample of barium sulfate was found to be 99.5 ± 0.25% against magnesium carbonate before screening. In one case, after five passes through a 100-mesh copper screen, using a stainless steel spatula to promote 100% passage, the reflectance had dropped to 96.8%; in another case, after three passes through a 150-mesh screen, 95.3%. Therefore, in measuring samples having high reflectances, screening procedures should be carefully standardized.

The reproducibility that one obtains for repeated measurements of a single sample is exactly the same as for a dyed cloth—usually ±0.1 on the 100-unit reflectance scale, or occasionally ±0.2. However, providing that one is working with a homogeneous sample, the reproducibility in going from one portion of a sample to another appears to be better than for the prints. Table I shows the reproducibility obtained at different levels of reflectance for mixtures of ferric oxide and barium sulfate. When five samples of pure ferric oxide were measured against another

Figure 7. Reflectance relative to barium sulfate, of mixtures of barium sulfate and cupric oxide
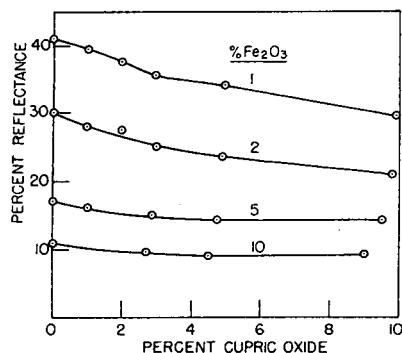
Figure 8. Influence of magnitude of reflectance at 520 mμ ferric oxide–barium sulfate samples on change in reflectance produced by addition of given percentage of cupric oxide
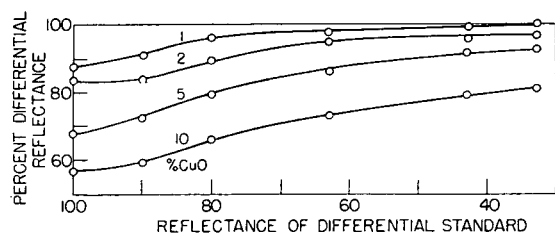
Figure 9. Influence of magnitude of reflectance of 5% barium chromate in barium sulfate on change in reflectance produced by addition of indicated percentages of cupric oxide

measurements of the type described in the present study produce distorted spectra. Thus, the effect of the base material on the spectrum of the component determined differentially is illustrated by Figure 6, which shows 5% barium chromate in barium sulfate alone and in barium sulfate containing 1% ferric oxide. The curve for barium chromate shows a sharp increase in reflectance below 460 mμ where the sample containing ferric oxide absorbs appreciably. Therefore, in interpreting the differential spectrum for a substance, one must consider, also, the spectrum of the base material. Spectra suitable, to a first approximation, for qualitative and semiquantitative analysis of a second compound can only be obtained in absolute terms, so that differential data must be transformed to that basis by calculation before applying the method of Stearns (16). Such information would be desired by the color chemist who wanted to identify an adulterant producing off-shade or dullness in his product.

As an extension of the study of the effect of adulterants, it was of interest to examine the effect of the presence in the sample of a neutral gray substance. Cupric oxide was selected for a series of determinations because each mixture of cupric oxide and barium sulfate gave a characteristic and essentially constant reflectance value (within 1.5 on a reflectance scale of 100 for pure barium sulfate) from 400 to 700 μ. As one might expect, the effect on the reflectance of successive 1% increments of cupric oxide decreased regularly (Figure 7). Such a decrease was also a function of the reflectance of the mixture to which the adulterant was added. This is shown in Figure 8, where the absolute reflectance values at 520 mμ have been plotted for increasing amounts of cupric oxide in barium sulfate containing different percentages of ferric oxide.

Although the change in reflectance produced by adding 1% adulterant was ordinarily greater in going from 0 to 1% than from 1 to 2%, etc., Figure 9 shows that this is not always the case, particularly in regions of low absolute reflectance. The data have been checked with new preparations and found to be

reproducible. In fact, for several systems, such as cupric oxide in ferric oxide and barium sulfate in ferric oxide, the change in reflectance in going from 0 to 1% was less than that from 1 to 2%. In another case, the reflectance increased on going from 2 to 5% cupric oxide in ferric oxide and then decreased at higher percentages. The most striking cases were those in which small (1%) additions of ferric oxide to cupric oxide and vice versa produced mixtures with higher reflectances than the pure compounds alone. These anomalous effects must have been due to inherent optical properties of the systems (refractive index, particle size and shape, or smoothness of the surface), though, in some of the other cases, it may have been due to poor mixing of the samples, such as selective coating of particles of the additive with particles of the base material. It is evident that quantitative statements about the amount of neutral gray component in a material can be made only from standards containing essentially the same concentrations of all the other substances. However, color judgments, which are semiquantitative or, at worst, qualitative, can be made in the large majority of cases, since the values of absolute reflectance are usually not so low as those where the anomalies were encountered.
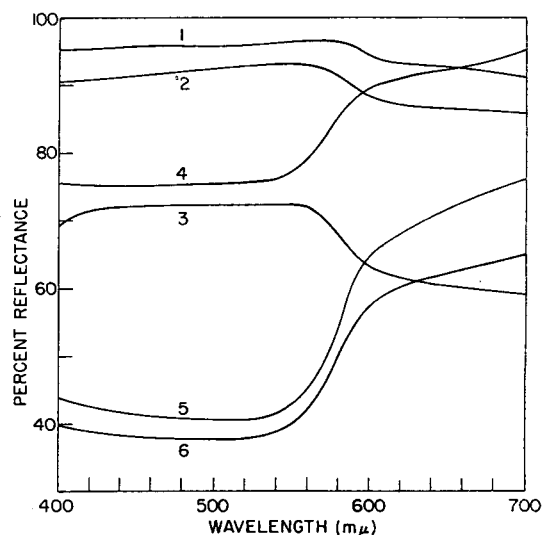
Figure 10. Difference between dullness caused by presence of neutral gray substance and apparent dullness resulting from increase in concentration of colorant

1–3. 1,2, and 10% of cupric oxide added to 1% ferric oxide in barium sulfate relative to latter at a scale reading of 100
4. 2% ferric oxide in barium sulfate relative to 1% ferric oxide at 100
5. 1% ferric oxide in barium sulfate relative to magnesium carbonate
6. 2% cupric oxide added to 1% ferric oxide and measured relative to magnesium carbonate

In terms of detecting the effect of the presence of grayness in a colored base material it is obvious that the addition of grayness to a colored base material will not bring about uniform lowering of reflectance throughout the spectrum. Figure 10 clearly indicates that a proportionately greater lowering of reflectance by the neutral gray component took place in the spectral regions of maximum reflectance of the colored base material, thereby resulting in a differential curve whose shape approximated an inverted spectrum of the original colored medium. Thus, one can readily differentiate between lowerings of reflectance resulting from the presence of neutral grayness and those from an increase in concentration of the colorant. In both cases the reflectance of the sample is less than the standard. However, in the first case, an inverted spectrum of the colored base material is obtained; in

Table II. Effect on Differential Reflectance Values for Ponsol Brown and of Type of Glass on Which Threads Were Wrapped

	Reflectance, %		
Wave Length. Mμ	Black glass (std.)	Clear glass	White glass (opal)
400	100.0	99.3	100.5
440	100.0	98.8	101.5
480	100.0	98.8	101.1
520	100.0	99.1	100.0
540	100.0	98.8	99.1
580	100.0	100.0	98.5
620	100.0	98.7	97.2
660	100.0	99.3	97.7
700	100.0	101.1	98.8

the latter, a spectrum is produced which is essentially that of the colorant.

Dyed Yarns. Brief studies of reproducibility were made of six different pairs of yarn skeins, each consisting of a sample dyed with a "standard" and another dyed with a similar preparation. For each pair, a spectrum of the standard was obtained against magnesium carbonate, and then another of the test sample against the standard. As it has long been recognized by workers in the field of color specification that orientation of fibers is an important variable, one pair was analyzed in detail by the differential method for the following variables: the relative orientation of the yarn in the sample and in the standard, pressing of the yarn against a flat surface just before measurement, and the variations due to background on which the yarn was wrapped.

Tests of the reproducibility of wrapping two samples from a single skein showed that a maximum difference of about 2% (relative) was obtained and that any difference was consistent throughout the spectrum. This difference may have been due to heterogeneity of the skein, but was probably due to variations in winding of the thread on the plate. In order to test the effect of changing the type of glass on which the sample was wrapped, experiments were run using yarn from a single skein of Ponsol Brown ARD on black, white, and clear glasses. Differences between the three sets of samples were usually less than 2% (Table II) but the differences were no longer uniform at all wave lengths for a given sample. This inconsistency is an indication that the background was making a small contribution, which, with white glass backing, became more significant below 520 mμ where the reflectance of the dye was lowest (Table III). For results independent of the glass, the need for one or more layers of yarn, in addition to the three employed, is indicated. Even so, the reproducibility of the data is sufficient for quantitative comparisons, providing the same type of glass and the same number of layers of thread are employed for both the standard and the sample.

Table III also shows that a small but detectable difference may result from removing the sample and replacing it in the holder. The same table confirms the long recognized sources of variation from pressing the mounted yarn onto a flat surface and from changing the direction of yarn in the sample in

relation to that in the standard. However, if the directions of both are changed to the same extent, no effect is observed.

Table III. Studies on Yarns Dyed with Ponsol Brown

(Showing reproducibility of measurements and effects on reflectance of pressing surface of mounted sample and of changing direction of yarn. Samples measured against magnesium carbonate)

Wave Length, Mμ	Removal and Replacement of Given Sample, % R ± Std. Dev. (4 Meas.)	Pressed Surface, (Av. % R of 2 Meas.)	Direction of Threads in Sample Changed 90°, % R ± Std. Dev. (4 Meas.)
400	7.67 ± 0.06	8.25	7.20 ± 0.10
420	5.93 ± 0.10	6.27	5.63 ± 0.07
440	4.79 ± 0.03	5.13	4.63 ± 0.04
460	4.68 ± 0.01	5.10	4.50 ± 0.06
480	5.62 ± 0.03	6.19	5.39 ± 0.15
500	7.76 ± 0.07	8.44	7.55 ± 0.10
540	11.50 ± 0.10	12.20	10.90 ± 0.10
580	14.90 ± 0.10	15.80	13.90 ± 0.10
620	32.50 ± 0.20	33.80	31.40 ± 0.40
660	54.70 ± 0.10	55.80	53.00 ± 0.20
700	62.10 ± 0.20	62.60	51.00 ± 0.30

Plotting of Data. One of the refinements which have often been used in relative measurements, where absolute reflectance values against a primary standard—i.e., magnesium oxide—are not required, is to use as standard a white portion of the cloth or ceramic to which no colorant has been applied. Thus, a recent paper on the determination of the soil content of cloth has suggested that measurements be made against a clean portion of the same type of cloth (12). This automatically adjusts the upper limit of reflectance to 100% for a sample with 0% soil content.

A further refinement that has proved to be very useful in the present study is the use of a high concentration of the colorant, or the pure colorant itself, as the lower limit. In some cases, the difference between the optical properties of the pure colorant and, for example, a cloth dyed with a saturation amount of the colorant may make the latter a preferable limit to use. In one sense, this is an empirical method of cor-
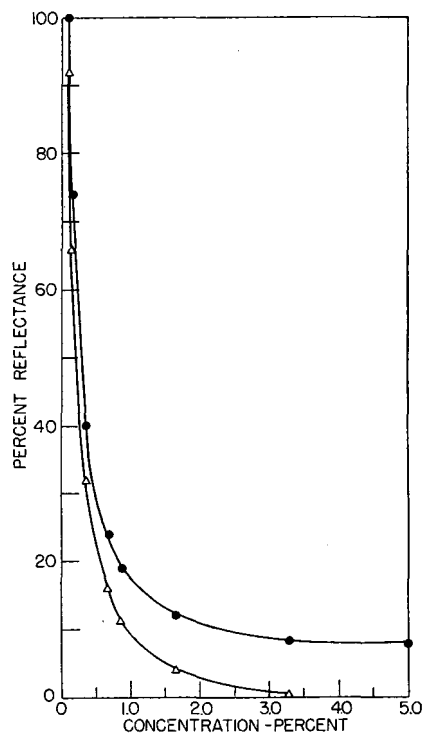

Figure 11. Reflectance at 600 mμ of Blue 871 on cotton as a function of concentration

● R △ $R-R_m$
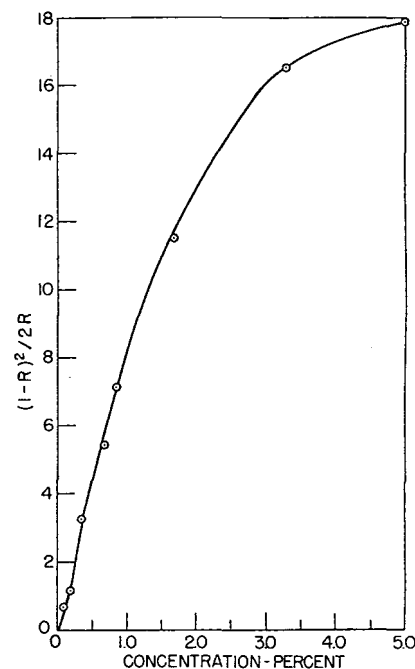
Figure 12. Kubelka-Munk values for Blue 871 on cotton plotted against concentration
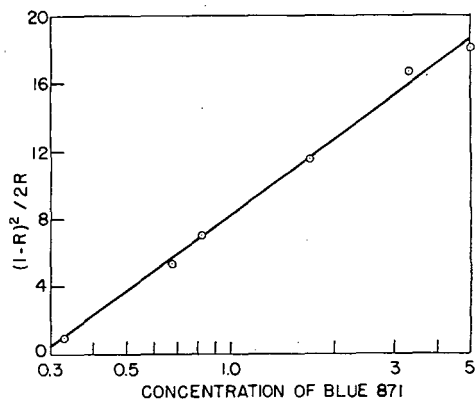
Figure 13. Kubelka-Munk values for Blue 871 on cotton against logarithm of concentration
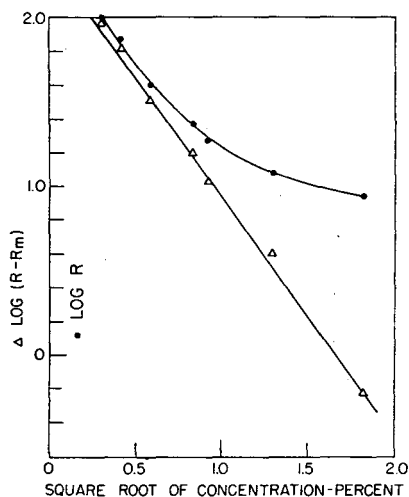
Figure 14. Logarithm of $R - R_m$ for Blue 871 against square root of concentration

recting for the scattering factor which becomes more important at low values of reflectance.

During the course of the present study numerous methods of plotting were examined for prints of Blue 871 on cotton. In Figures 11 to 15, C is the concentration of the colorant expressed in weight per cent, R is the reflectance of the sample, R_B is the reflectance of the pure base material, and R_m is the reflectance of the sample having the highest concentration of dye in the series, preferably close to the limiting reflectance of the pure dye. As a special case, one can set up $(R - R_m)/(R_B - R_m)$, in which the denominator expresses the limits of the system under investigation and the numerator, the position of the sample between those limits. In differential measurements against a standard containing the colorant under investigation, the colored standard may be used as R_B but the concentrations of the other samples must be recalculated to this new base. On the other hand, if the reflectance of the pure base material is used for R_B, the value for R_B must be recalculated so that it is expressed on the same scale as the colored standard—e.g., with a value greater than 100%.

In looking over these figures, it is at once evident that the data for a system such as Blue 871 on cotton always fall on smooth curves, so that any method of plotting could be used for quantitative purposes. However, because a straight line is the easiest form in which to use the data, such plots are usually preferred. A plot of the concentration against $(I - R)^2/2R$, the form of the

Kubelka-Munk expression most often employed (8), produced a linear relationship only for the lower concentrations. However, changing to logarithm of the concentration did result in a straight line over the entire range. It is obvious that in this series of samples the log C vs. log Kubelka-Munk recently proposed for measuring the soil content of fabrics, will fail (12). Another relationship that was linear, for concentrations smaller than 5%, was log $(R - R_m)$ vs. the square root of concentration. Even this plot shows curvature at higher concentrations in which case its linear range could be extended by using $\sqrt{C/(1 - C)}$. Plots of log $(R - R_m)$ vs. C, log C and $\sqrt[3]{C}$ (Figure 15) produced curved lines as did R vs. $\sqrt{C}$.

Similar conclusions have been reached after plotting the data from two series of samples containing barium sulfate plus cupric oxide (1.0 to 50.0%) or ferric oxide (0.05 to 14.3%). Both showed the general validity of using $\sqrt{C}$ over moderate ranges of concentration and $\sqrt{C/(1 - C)}$ over much arger ranges. Figure 16 shows a typical curve. For quantitative purposes, the use of log C vs. $(1 - R)^2/2R$ or $(R - R_m)$ vs. $\sqrt{C}$, or $\sqrt{C/(1 - C)}$, will probably result in linear relationships.

In connection with the use of R_m for defining the lower limit of
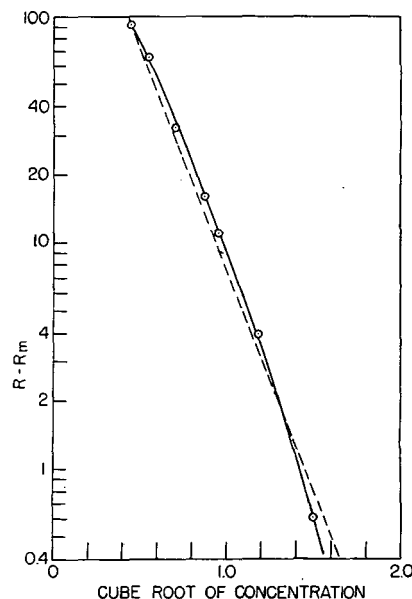
Figure 15. Logarithm of $R - R_m$ for Blue 871 on cotton against cube root of concentration
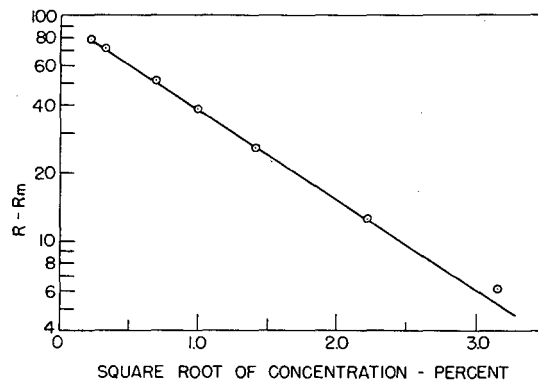
Figure 16. Logarithm of $R - R_m$ for ferric oxide in barium sulfate against concentration of ferric oxide

reflectance, one should realize that curvature, or actual leveling off, of a reflectance plot is bound to occur under conditions where reflectance of the pure colorant may be equal to or greater than that of a mixture to which it is added. In the present study, this would be encountered where it was found that equivalent reflectance values were obtained for pure ferric oxide and for ferric oxide containing 5% by weight of barium sulfate. Even more extreme were certain ferric oxide–barium sulfate mixtures, where, after the addition of small amounts of cupric oxide, greater reflectance was observed than before.

Figure 17. Two runs of Ponsol Golden Orange G compared on both normal and differential basis with standard set at scale reading of 60
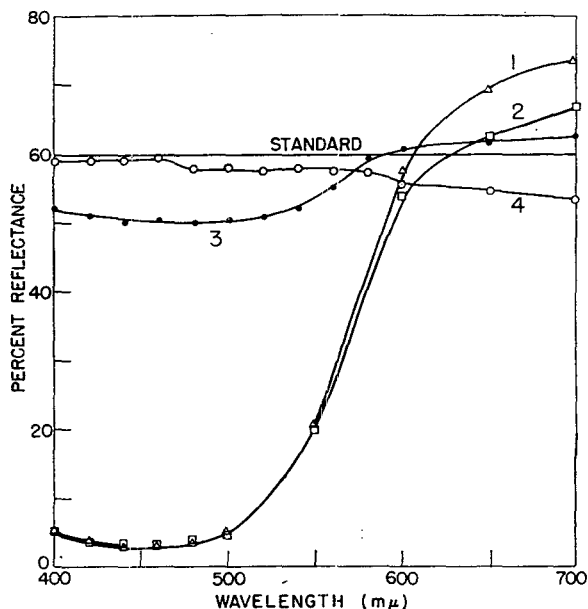
1. Standard preparation relative to magnesium carbonate
2. Sample 3-463 preparation relative to magnesium carbonate
3. Sample 3-183 relative to the standard preparation. Visually judged to be "yellower and brighter" than standard
4. Sample 3-463 relative to the standard preparation. Visually judged to be both "yellower and duller" than standard

Color Matching. Typical spectra of dyed yarns that have been compared differentially are shown in Figure 17 together with the color comparisons of an experienced person. When the curve for a sample crosses that of the standard, there is a high probability that the sample is both "weaker and duller" (or "stronger and brighter") than the standard. On the other hand, metameric pairs may produce this result. In color matching, one must weigh the danger of encountering a metamer with the increased information and somewhat greater sensitivity resulting from the use of relatively narrow spectral slits instead of broad-band filters. Fortunately, the analytical chemist will not be concerned with this choice.

DISCUSSION

Although the present study has been restricted to reflectance in the visible, other regions of the spectrum can be employed. Promising studies have already been reported in the infrared (13-15) where, however, specular rather than diffuse reflectance was employed, probably because it resulted in larger amounts of energy for measurement.

The amount of reflectance, like light transmittance through turbid media, has been shown to be dependent upon particle size (6, 7, 10). The literature showing the effect of particle size on reflectance indicates that deviations greater than about 10% may not be too common. Even so, in dealing with amounts of the or-

der of 10^{-5} mole %, which have already been detected in reflectance studies (6, 9), such accuracy and precision could probably be tolerated.

One would expect the most reliable measurements to result with samples having the same particle size distribution. A recent application of reflectance for the determination of the carbon content of an industrial catalyst falls into this category (11). Similarly, the identification, in situ, of substances in spot tests (17) and on chromatograms, either on paper sheets or on packed columns of powders, would conform to this requirement.

There is good evidence that reflectance is a function of both absorbancy index and refractive index (6, 13), and hence the differences in the latter could also introduce variations. The fact that the refractive index changes with wave length probably means that inconsistencies will result in scanning spectra of preparations having different histories.

The virtual necessity of using a photomultiplier has been observed in several instances where the standard had a low absolute reflectance (less than 3%). An attempt to use a recording spectrophotometer was unsuccessful because the sample reflected insufficient light to permit balance to be obtained. Even when balance could be obtained with a phototube, a photomultiplier usually permitted sufficiently smaller slits to be used to retain much of the fine structure of a spectrum such as that for didymium glass whereas only the major peaks and troughs were found with the standard phototube supplied with the Beckman DU spectrophotometer.

Finally, because of the low light levels being measured, the possibility of errors from stray light is greater (4). In the present study stray light originating outside the monochromator was decreased by draping a large black cloth over the reflectance attachment and phototube assembly.

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A Spectrochemical Procedure of General Applicability

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Spectroscopists have been endeavoring for years to devise a reliable semiquantitative or quantitative spectrochemical procedure which will have universal applicability for the determination of the metallic constituents—particularly the major components—of almost any material, based on the use of a single set of standards and capable of an accuracy of better than ±50% for all determinations. A procedure is described which fulfills most of these requirements. By the judicious use of germanium dioxide as a filler and cupric oxide and graphite as buffers, in both samples and standards, it is possible to establish a common base so that the emission lines used in the analyses are comparable in all kinds of samples and in the standards. Powdered samples are used, but metals, liquids, and metal-organic materials can be readily converted to a powdered salt or oxide. Once standard curves of concentration vs. some function of the intensity of the line of the element to be determined have been established, individual samples can be analyzed with moderate speed.

SPECTROSCOPISTS have been striving for many years to devise a spectrochemical procedure that would have general applicability—one that would be capable of analyzing any sample with moderate accuracy for one or all of its metallic ion constituents, without the necessity of employing separate sets of standards for each basic matrix material involved. Ideally, one would like to be able to do this using only a single spectrogram. For any isolated spectrogram this has not been generally possible, beyond an estimate of the order of magnitude of each element present, even if the absorbance or per cent transmittance of the analytical lines involved is measured accurately. Further information is required, such as knowledge of the behavior of the various element lines in the particular matrix of the spectrogram in question and the energy response characteristics of the emulsion or photoreceiver.

Table I. Schedule of Estimated Ranges

	Per Cent
Principal component	>10
Major components	> 1
Minor components	0.1–3
Impurities	0.01–0.3
Traces	< 0.03
Slight traces	< 0.005
Very slight traces	< 0.001

DISCUSSION

Qualitative analyses are extremely useful and yield valuable information as to what elements are present in a sample, and to some degree, how much. Table I indicates the manner in which qualitative spectrochemical analyses are reported in this laboratory. Rather wide concentration limits are placed on each category, with some overlap between ranges. In the lower concentration ranges, from impurities down, surprisingly good estimates can be made by experienced workers. However, such estimates are highly subjective and in no sense can be considered as quantitative determinations. Going into the higher concentration areas it becomes increasingly difficult to make reliable estimates of concentration.

There are, of course, many good spectrochemical procedures available for the quantitative analysis of almost any material. Hundreds of such methods can be found in the literature, and recently Committee E-2 of the American Society for Testing Materials (ASTM) has published over 50 suggested spectrochemical methods (1). The majority of these methods, however, are specific for the analysis of specific materials for certain elements. All require reliable standards for each matrix involved. Many of these standards are not readily available, and some cannot be made easily in most laboratories, especially for those techniques utilizing metal rods or disks. In any case, where it is required to analyze a wide variety of materials, many sets of standard samples must be kept on hand or made or purchased as required.

Somewhere between the qualitative and the accurate quantitative procedures there is a need for a reliable, objective, semiquantitative procedure with precision within better than ±25%, one which would be applicable to the analysis of a wide variety of samples for most of the common elements. Such a method would be very useful for the analysis and classification of steels, brasses, bronzes, lead, tin, aluminum, magnesium- and zinc-base alloys, ceramics, paint pigments, and other materials.

That a need does exist for a general method of spectrochemical analysis is evidenced by the fact that Group VI, Subcommittee II of ASTM E-2, is engaged in a project to evaluate the various general procedures that have thus far been developed (3–5, 7, 10–13). Many of these procedures are not truly general in scope, but are usually confined to some area of analysis such as that of geological materials, or ceramics, or certain classes of alloys. Nevertheless, they do contain some of the essential factors necessary for wider application.

One of the chief obstacles to devising a universal method is the marked effect of different matrix materials on the behavior of the spectral lines of each individual element to be determined. For example, if iron is determined in a sodium chloride matrix, the transmittance of the iron lines will be very different than if the matrix had been zirconium dioxide, lead, copper, or some other base material.

The logical approach to the elimination of this strong matrix effect is to dilute heavily or buffer samples with some common base material. In this manner the behavior of the elements to be determined will approach that of their behavior in the pure buffer base. What buffer material should be chosen? For widest scope, it should be an element whose oxides and salts behave in an average manner. Its excitation potential and its melting and boiling points should be somewhere in the middle range of all the elements. The extremes of the alkali metals and the highly refractory compounds such as those of zirconium or thorium would obviously be poor choices. Elements and their compounds, such as those of lead, bismuth, tin, germanium, copper, iron, and nickel, should be satisfactory from the standpoint of desirable behavior. It has been found in this laboratory that graphite powder, cupric oxide, lead sulfate, and germanium dioxide are excellent buffer materials, with cupric oxide best for use as an internal standard because of its good distribution of spectral lines in the region from 2400 to 5000 A. Graphite powder is always used with the other buffers. All materials used for buffering must be extremely pure.

A number of papers have been published by workers in this laboratory in which buffers play an important role as an aid to diminishing the matrix effect. The first of these concerned the analysis of copper-base alloys in which brasses and bronzes

were diluted with pure copper (*9*). Here the fact was definitely established that a buffer could be used to reduce the matrix effect appreciably, at least in a particular class of alloys. This was followed by the analysis of triple carbonates (barium, strontium, and calcium) by Hartmann and Prescott (*6*), in which copper was again used for buffering. Methods were also developed for the analysis of aluminum-base alloys and for ceramics and other nonmetallic materials (*2, 8, 10*).

The method for the analysis of ceramics and other nonmetallic materials approached the closest to having general applicability. In this case there was much greater variability in the composition of the samples than for the cases of the copper- and aluminum-base alloys. In the case of ceramics the buffer, cupric oxide, or lead sulfate completely foreign to the matrix, was employed, whereas for the copper- and aluminum-base analyses, samples were buffered with copper and aluminum, respectively. The introduction and the successful use of the foreign buffer opened the way to a method of more general applicability.

All of the above mentioned procedures made use of only one buffer material where solutions were used, and one primary buffer material plus graphite where powders were employed. The ratio of sample to primary buffer varied from 1 to 9 to as much as 1 to 100, depending upon the concentration of the element to be determined. Because of the variability of this ratio, two adverse factors influenced the analysis.

1. Because the relative amount of primary buffer and internal standard varied in different samples and standards, there was some variability in the intensity of the internal standard lines. This led, in some cases, to appreciable errors in the determination of constituent elements.

2. The variable relative amount of sample caused different degrees of matrix influence. Depending upon which elements were present in the sample, this could have a large effect upon the intensity of the spectrum lines of the element to be determined and also upon the intensity of the internal control line, and hence upon their intensity ratios.

In the technique described, in order to reduce further the matrix effect, use is made of an additional buffer, called the diluent. This diluent is incorporated with the samples and standards and is used as a stabilizer as well as a diluent. Germanium dioxide was chosen for use as the diluent because it is readily available in a state of very high purity and satisfied all the requirements of a good buffer. The proportions, by weight, used of sample or standard, diluent, primary buffer and internal standard cupric oxide, and secondary buffer (graphite powder) are: 1 part of (sample or standard plus germanium dioxide), 19 parts of copper(II) oxide, and 20 parts of graphite powder.

The 19 parts of cupric oxide and the 20 parts of graphite powder are invariable. Thus the effect of variable copper content of the system is eliminated except for the case of copper-base alloys. All the manipulation and diluting is done in the 1 part of (sample or standard plus germanium dioxide). The standards are made up on the basis of per cent of element x in (standard plus germanium dioxide), and curves for the per cent concentration *vs.* the $\log I_x/I_{Cu}$ are set up and all calculations made on this basis.

Samples are diluted with germanium dioxide to whatever extent is deemed reasonable to determine the constituent elements of both high and low concentration. This dilution may range from none to 20 times, depending upon the concentration and the sensitivity of the element to be determined. As far as practical, the dilution with germanium dioxide should be such that the concentration of the element to be determined will be between 0.10 and 5.0% with respect to the weight of the diluted sample. Two or more dilutions are usually used when a complete analysis of all constituents is required. If the composition of the sample is completely unknown, a preliminary qualitative analysis will serve as a guide to what the dilution factor should be.

If the ratio of sample to germanium dioxide is 1 to 0, all re-

Table II. Wave Lengths of Analytical Line Pairs

Element	Analytical Line, A.	Internal Std. Line, A.
Aluminum	Al 3082.16 (4) (20)[a]	Cu 2768.88 (20)[a]
	Al 3169.53 (4)	Cu 4248.96 (20)
Antimony	Sb 2877.92 (100)	Cu 2768.88 (20)
	2311.47 (100)	Cu 2768.88 (20)
Arsenic	As 2389.84 (100)	
Barium	Ba(II) 4934.09 (4)	Cu 4704.60 (4)
	Ba 4899.97 (100)	Cu 4704.60 (4)
	Ba(II) 4554.04 (4)	Cu 4480.36 (4)
Bismuth	Bi 2897.98 (4) (20)	Cu 2768.88 (20)
	Bi 3067.72 (4) (20)	Cu 2768.88 (20)
Boron	B 2496.78 (20) (100)	Cu 2768.88 (20)
Cadmium	Cd 3261.06 (20) (100)	Cu 2768.88 (20)
Calcium	Ca(II) 3968.47 (4)	Cu 4248.96 (20)
	Ca 3179.33 (100)	Cu 2768.88 (10)
Chromium	Cr 2780.70 (100)	Cu 2768.88 (20)
	Cr 4254.35 (4)	Cu 4248.96 (20)
Iron	Fe(II) 2719.30 (100) (20)	Cu 2768.88 (20)
	Fe(II) 2599.40 (10) (20)	Cu 2768.88 (20)
Magnesium	Mg 2776.69 (100)	Cu 2768.88 (20)
	Mg 2852.13 (4)	Cu 2768.88 (20)
Manganese	Mn(II) 2605.69 (20)	Cu 2768.88 (20)
	Mn 2798.27 (20)	Cu 2768.88 (20)
Molybdenum	Mo 3132.59 (20)	Cu 2768.88 (20)
Nickel	Ni 3524.54 (20) (4)	Cu 2768.88 (20)
	Ni 3134.11 (20) (4)	Cu 2768.88 (20)
Lead	Pb 2833.07 (100) (20) (4)	Cu 2768.88 (20)
	Pb 2873.32 (10) (20)	Cu 2768.88 (20)
Sodium	Na 3302.32 (100)	Cu 2768.88 (20)
Silicon	Si 2506.90 (100) (20)	Cu 2768.88 (20)
Tin	Sn 2863.33 (100) (20)	Cu 2768.88 (20)
Titanium	Ti(II) 3349.41 (100) (20)	Cu 2768.88 (20)
Zinc	Zn 4810.53 (100)	Cu 4704.60 (4)
	Zn 3345.02 (100)	Cu 2768.88 (20)
Zirconium	Zr(II) 3496.21 (100)	Cu 2768.88 (20)

[a] Figures in parentheses indicate per cent transmittance band in which spectrum lines are measured.

sults can be obtained directly from the standard curves of per cent concentration of the element *vs.* log I_x/I_{Cu}. If the ratio is 1 to 1, a dilution factor of 2.0 is used to multiply the results obtained from the standard curve. If 1 to 5, the factor is 6.0, and so on.

By the above technique the matrix effect is kept at a minimum and the system as far as possible always approximates that of a cupric oxide–germanium dioxide–graphite matrix.

PROCEDURE

Preparation of Samples. Weighed amounts of representative sample, 10 to 100 mg., are diluted and mixed with germanium dioxide in the desired proportion and further mixed as follows with cupric oxide and graphite powder: 1 part of (sample plus germanium dioxide), 19 parts of copper(II) oxide, and 20 parts of graphite powder.

If the sample is already in the form of a powder, this becomes a simple matter of mixing all ingredients in the correct proportions. If the sample is a metal or alloy, a weighed amount of sample is taken into solution or digested in an appropriate reagent, to which cupric oxide, germanium dioxide, and graphite are added in the proper proportions, and dried at 400° C.; or the basic mixture of sample and germanium dioxide is made and dried, then later incorporated with cupric oxide and graphite. The preparation of miscellaneous samples has been discussed fully (*8*). It is always important to know the proportion of sample to germanium dioxide and thus the dilution factor to be employed in the final calculations. Unless maximum sensitivity is required, it is desirable to use germanium dioxide in at least the proportion of 1 to 1 of sample. If the elements to be determined cover the complete range of concentration, two dilutions of sample are required. Samples prepared with dilution factors of 2 and 10 usually suffice to cover the determination of most elements contained in the sample.

Preparation of Standards. These are prepared in exactly the same manner as the samples. National Bureau of Standards samples or other analyzed samples may be used, or synthetic standards may be made by mixing weighed amounts of pure

oxides or salts. In all cases, germanium dioxide should be incorporated in as high a proportion as practical. A concentration range coverage of 0.05 to 10% with a factor of 3 to 5 between successive standards is employed for each element to be determined.

Standards and samples should always be thoroughly mixed and ground in an agate mortar until a smooth uniform mixture of all ingredients is obtained. They are stored in screw cap vials.

Electrode System. The lower sample carrying electrode(anode) is a high purity graphite rod 0.25 inch in diameter and 1 inch long, with a cup cut into the end with a 0.180-inch diameter drill to a depth of 0.125 inch. Each cup is packed level full with its designated sample or standard. A water-cooled electrode holder is used for this electrode.

The upper counter electrode (cathode) is a purified graphite rod $^3/_{16}$ inch in diameter and 1.125 inches long with a flat end. The gap width is maintained at 0.25 inch.

Apparatus. SPECTROGRAPH. A spectrograph is employed which has sufficient resolving power and linear dispersion to separate clearly the analytical lines in the spectrum of the sample in the spectral region 2300 to 5000 A. These conditions are satisfied by commercial grating or quartz prism instruments having a linear dispersion of 0.20 mm. per A. or more at 2800 A. for the determination of all elements covered by this procedure.

RECORDING EQUIPMENT. The spectra are recorded on Eastman Process, SA No. 2, or equivalent emulsions. A neutral three-step filter transmitting approximately 100, 20, and 4% of the incident light is used to aid in bringing all the lines employed in the photometry within a usable absorbance or transmittance range.

DENSITOMETER. The transmittance of the analytical lines is measured on a densitometer having a precision within ±1.5% for transmittances between 5 and 90%.

Excitation. The samples are excited in triplicate, with a series of standards, in the 250-volt, direct current arc at an initial current of 10 amperes, limited by resistance in series. After initial setting, no further adjustments of current are made.

Exposure Conditions.

Spectral region	2400 to 5000 A.
Slit width	Optimum for spectrograph used
Slit length	1.0 mm.
Filters	Step: 100, 20, and 4% (approx.)
Preburn period	None
Exposure period	60 seconds

The spectrograph is illuminated in such a manner that the resulting spectral lines represent radiant energy from the entire arc column except for about $^1/_{64}$ inch from either electrode, which is screened out.

Photography. Emulsion calibration, photographic processing, and photometry are done in accordance with practices suggested by ASTM SM2-1 and SM2-2 (1).

The analytical line pairs used are listed in Table II.

EXPERIMENTAL

Early in this investigation data were obtained to indicate, in some degree, the effect of the matrix upon results. Portions of a sample of cupric oxide, containing 0.24% each of iron, manganese, magnesium, boron, aluminum, chromium, calcium, and zinc were mixed separately with germanium dioxide, lead sulfate, aluminum sulfate, ferric oxide, nickel oxide, and zinc oxide. The proportions used were: 1 part of matrix, 9 parts of copper(II) oxide + additives, and 10 parts of graphite powder.

On a single plate, 3 aliquots of each mixture were arced in the

direct current arc at 10 amperes for 60 seconds, and their spectra recorded. Log I_x/I_{Cu} for each element was calculated, and the three values for each matrix averaged. These data are tabulated in Table III. The matrix material is listed in the left-hand column. Log I_x/I_{Cu} is tabulated for each element opposite the matrix listing.

From these results, it appeared that the influence of the matrix upon the intensity ratios of the various elements involved was moderate. The deviations from average seem to be random, and actually were no greater than the deviations of three exposures with a single matrix. However, the matrix materials involved in this experiment were all, with the possible exception of aluminum, elements in what might be called the "middle" range of behavior, where a minimum matrix influence might be expected.

Table IV. Effect of Matrix on Results

Compound	Actual %	Found, %					
		A.	NaCl, 60%	B.	ZrO₂, 60%	C.	GeO₂, 60%
Al₂O₃	10.0		12.3		8.0		10.1
MgCO₃	10.0		13.6		8.4		10.7
SiO₂	10.0		11.4		9.1		9.9
PbSO₄	10.0		11.9		7.8		10.1
Average deviation			+2.3		−1.7		±0.25
Average % deviation			+23		−17		±2.5

Table V. Suppression of Matrix Effect[a]

Compound	Actual %	Found, %	
		A/3—NaCl	B/3—ZrO₂
Al₂O₃	10.0	10.8	8.3
MgCO₃	10.0	12.3	9.0
SiO₂	10.0	9.6	9.2
PbSO₄	10.0	9.7	9.2
Average deviation		±0.95	−1.08
Average % deviation		±9.5	−10.8

A/3 = 20% NaCl 40% GeO₂
B/3 = 20% ZrO₂ 40% GeO₂

[a] By dilution with germanium dioxide.

What would be the matrix effect of elements of extreme behavior, like volatile sodium compounds on the one hand and refractory zirconium oxide on the other? To test this, three synthetic samples were made. Sample A contained 60% sodium chloride mixed with 10% each of aluminum oxide, magnesium carbonate, silicon dioxide, and lead sulfate. In sample B, zirconium dioxide was substituted for the sodium chloride, and in sample C, germanium dioxide was substituted for sodium chloride. These samples were run as unknowns according to the procedure outlined above. The results obtained are shown in Table IV. As might be expected, the average percentage deviation of the determinations from the true values were small for the germanium dioxide matrix, ±2.5%. With the sodium chloride it was +23% and with the zirconium dioxide −17%. The sodium chloride enhances the log I_x/I_{Cu} for all four elements tested, whereas log I_x/I_{Cu}, for each, was depressed by the zirconium dioxide. The average maximum per cent deviation that might be expected under the most adverse conditions of matrix influence is probably less than ±25%.

In order to test the effect of adding germanium dioxide to an adverse matrix, samples A and B were diluted with germanium dioxide by a factor of 3. The results are shown in Table V. The average per cent deviation was thereby reduced to about ±10%. It is for this reason that germanium dioxide is added to most samples, particularly if sensitivity is not a problem, and an adverse matrix is suspected.

Tables VI to XIII show the quality of the results obtained in the determination of aluminum, chromium, iron, manganese, nickel, lead, silicon, and tin in a variety of materials including re-

Table III. Stability of Log I_x/I_{Cu}, in Different Matrices

Sample	Matrix	Fe[a]	Mn	Mg	B	Al	Cr	Ca	Zn
A	GeO₂	0.61	0.70	0.98	0.53	0.86	0.76	0.53	0.56
B	PbSO₄	0.61	0.72	0.99	0.53	0.85	0.77	0.57	0.62
C	Al₂(SO₄)₃	0.62	0.72	0.97	0.59	...	0.79	0.59	0.57
D	Fe₂O₃	...	0.75	1.01	[b]	0.83	0.72	[b]	0.62
E	NiO	0.58	0.73	0.99	0.49	0.81	0.85	0.51	0.55
F	ZnO	0.66	0.70	0.95	0.51	0.85	0.73	0.48	...
Average		0.62	0.72	0.98	0.53	0.84	0.77	0.54	0.58
Average deviation ±		0.020	0.013	0.020	0.024	0.016	0.033	0.036	0.028
Average % deviation ±		3.2	1.8	2.0	4.5	1.9	4.4	6.7	4.8

[a] Concentration of each element = 0.24% with respect to matrix. 1 part of matrix to 9 parts of copper(II) oxide to 10 parts graphite.
[b] Line interference.

fractories, steels, brasses, bronzes, glass, aluminum, lead, and zinc-base alloys. These results are typical of those that have been obtained on the determination of other elements in the above matrices and in other materials. The average per cent deviation from the National Bureau of Standards values for all the determinations listed is $< \pm 10\%$, with some individual values falling outside of our goal of $\pm 25\%$ accuracy.

Table VI. Determination of Aluminum in NBS Samples

Sample	Al, %	Found, %	Deviation	Deviation, %
NBS 103 (refr.)	11.05	8.4	−2.65	−24.0
	11.05	13.0	+1.95	+17.6
	11.05	11.4	+0.35	+3.2
	11.05	11.0	−0.05	−0.5
	11.05	10.5	−0.55	−5.0
	11.05	12.0	+0.95	+8.6
	11.05	11.2	+0.15	+1.4
NBS 76 (refr.)	19.94	19.60	−0.34	−1.7
NBS 94 (zinc-base)	3.92	3.60	−0.32	−8.2
Average per cent deviation				±7.8

Table VII. Determination of Chromium in NBS Samples

Sample	Cr, %	Found, %	Deviation	Deviation, %
NBS 101b (steel)	18.19	18.0	−0.19	−1.05
	18.19	17.4	−0.79	−4.3
	18.19	19.2	+1.0	+5.5
NBS 87 (Al base)	0.17	0.20	+0.03	+17.7
	0.17	0.165	−0.005	−0.3
NBS 32c (steel)	0.654	0.70	+0.046	+7.0
	0.654	0.65	−0.004	−0.6
NBS 85a (Al-base)	0.23	0.30	+0.07	+30.0
Average per cent deviation				±8.3

Table VIII. Determination of Iron in NBS Samples

Sample	Fe, %	Found, %	Deviation	Deviation, %
NBS 103 (refr.)	11.18	11.4	+0.22	+2.0
	11.18	11.2	+0.02	+1.8
	11.18	12.0	+0.82	+7.3
	11.18	13.5	+2.32	+20.8
	11.18	10.8	−0.38	−3.4
NBS 87 (Al-base)	0.46	0.48	+0.02	+4.3
	0.46	0.47	+0.01	+2.2
	0.46	0.44	−0.02	−4.3
NBS 56a (refr.)	1.52	1.60	+0.08	+5.3
	1.52	1.55	+0.03	+1.9
NBS 76 (refr.)	1.67	1.60	−0.07	−4.2
	1.67	1.55	−0.12	−7.2
Average per cent deviation				±5.4

Table IX. Determination of Manganese in NBS Samples

Sample	Mn, %	Found, %	Deviation	Deviation, %
NBS 87 (Al-base)	0.30	0.30	+0.00	0.0
	0.30	0.31	+0.01	+3.3
NBS 32c (steel)	0.752	0.69	−0.062	−8.0
	0.752	0.61	−0.142	−18.9
NBS 101b (steel)	0.597	0.38	−0.214	−36.0
Average % deviation				±13.2

Table X. Determination of Nickel in NBS Samples

Sample	Ni, %	Found, %	Deviation	Deviation, %
NBS 101b (steel)	8.99	9.2	+0.20	+2.2
	8.99	10.8	+1.8	+20.0
	8.99	9.0	+0.01	+0.1
	8.99	10.2	+1.2	+11.8
	8.99	8.7	−0.29	−3.2
	8.99	7.7	−1.29	−14.3
NBS 87 (Al-base)	0.59	0.74	+0.15	+25.4
	0.59	0.68	+0.09	+15.3
	0.59	0.58	−0.01	−1.7
NBS 32c (steel)	1.2	1.4	+0.20	+16.7
	1.2	1.22	+0.02	1.6
Average per cent deviation				±10.2

Table XI. Determination of Lead in NBS Samples

Sample	Pb, %	Found, %	Deviation	Deviation, %
NBS 37C (brass)	0.97	1.00	+0.03	+3.0
	0.97	0.90	−0.07	−7.2
	0.97	1.0	+0.03	+3.0
NBS 89 (Ba-Pb glass)	16.2	17.7	+1.50	+9.3
	16.2	18.0	+1.80	+11.1
	16.2	15.5	−0.70	−4.3
NBS 124b (ounce met.)	4.78	5.6	+0.82	+17.2
	4.78	5.3	+0.52	+10.9
	4.78	4.3	−0.48	−10.0
NBS 62a (Mn bronze)	0.50	0.53	+0.03	+6.0
	0.50	0.46	−0.04	−8.0
	0.50	0.56	+0.06	+12.0
Average per cent deviation				±8.5

Table XII. Determination of Silicon in NBS Samples

Sample	Si, %	Found, %	Deviation	Deviation, %
NBS 103 (chr. ref.)	3.85	4.6	+0.75	+19.5
	3.85	4.3	+0.45	+11.6
	3.85	4.2	+0.35	+9.1
	3.85	3.6	−0.25	−6.5
	3.85	4.1	+0.25	+6.5
	3.85	4.1	+0.25	+6.5
NBS 87 (Al-base)	6.22	7.0	+0.78	+12.5
	6.22	6.0	−0.22	−3.5
	6.22	6.5	+0.28	+4.5
NBS 56a (phos. rock)	5.15	5.4	+0.25	+4.8
	5.15	5.2	+0.05	+1.0
NBS 76 (refract.)	25.5	25.5	0.00	0.0
NBS 32C (Ni-Cr steel)	0.28	0.24	−0.04	−14.1
	0.28	0.20	−0.08	−28.6
NBS 101b (Ni-Cr steel)	0.48	0.50	+0.02	+4.2
	0.48	0.56	+0.08	+8.4
Average % deviation				±8.8

Table XIII. Determination of Tin in NBS Samples

Sample	Sn, %	Found, %	Deviation	Deviation, %
NBS 37c (brass)	0.96	0.64	−0.32	−33.4
NBS 1246 (ounce met.)	4.69	5.4	+0.71	+15.1
	4.69	5.1	+0.41	+8.8
NBS 53a (Pb-base)	10.23	11.5	+1.27	+12.4
	10.23	12.0	+1.77	+17.3
	10.23	10.3	+0.07	+0.7
	10.23	9.0	−1.23	−12.0
Average per cent deviation				±14.0

CONCLUSIONS

This procedure has general applicability and can be used for analyzing any sample that can be converted into a powdered form. The addition of germanium dioxide as a diluent and stabilizer is the principal factor responsible for the improvement of this procedure over work previously reported (8, 10). The germanium dioxide effectively minimizes the matrix influence of widely different matrices such as sodium chloride and zirconium dioxide. The precision is moderate but adequate for many applications. The method has moderate speed, as once the analytical curves have been constructed for a particular element, determinations can be made without further use of standards, provided that adequate control is exercised over the excitation, exposure, emulsion calibration, and emulsion processing.

The method is objective to a high degree and is comparatively free from the subjectivity of the analyst. Its usefulness has been demonstrated in this laboratory in the following areas: to replace some chemical procedures in the field of ceramics and other nonmetallic materials; to classify a particular alloy within its kind—i.e., the type of aluminum-base, zinc-base, copper-base, steel; to replace many qualitative analyses in the element concentration range above 0.10%, as with this procedure much more accurate information about a sample can be obtained with little more work entailed.

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Infrared Functional Group Analysis of Arylsilanes

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Melting points and chemical analyses of some of the aryl silanes are so similar that other methods of characterizing their structures are required. An infrared spectrometric method is described for the determination of the concentration ratio of phenyl and p-tolyl groups in tetraarylsilanes and hexaaryldisilanes. Accurate results are possible, even though the molar absorptivities of the functional groups are not constant. Determination of group concentration ratios, rather than individual concentrations, permits use of unweighed samples.

INFRARED functional group analyses described to date (1-3, 5-7, 10) have depended upon the constancy of either the peak or integrated molar absorptivity of an absorption band corresponding to a vibration of the functional group being determined. The criterion of constant molar absorptivity is not met by some absorption bands that are otherwise desirable for analytical use because their intensity falls within a desirable range and because they are free from interference from other absorption bands. Such absorption bands can, however, give valuable information about compounds of unknown structure if the molar absorptivity is found to vary in a regular manner with some change in the structure of the compounds under study. If, for example, the molar absorptivity of a group is dependent upon the position of the group along a carbon chain, determination of the molar absorptivity will provide information about the position of the group. Alternatively, if the changes in molar absorptivity cannot be related to the structure of the compounds under study, it may be possible to find an absorption band from another group common to all of the compounds that varies in intensity in a similar manner, so that the ratio of the two molar absorptivities is constant. If such a band can be found, the ratio of the two group concentrations can be related to the ratio of band intensities. The advantages of this type of analysis, which is similar to the internal standard technique used so successfully in emission spectroscopy, are discussed briefly later in this paper.

EXPERIMENTAL

The spectra were recorded on a Baird Associates Model B infrared spectrophotometer and the densitometer attachment of a Perkin-Elmer Model 13 infrared spectrophotometer was used for the determination of peak molar absorptivities. Sodium chloride prisms were used in both instruments. Carbon disulfide was used as the solvent and the cell thicknesses were 0.4 mm. on the Baird instrument and 0.5 mm. on the Perkin-Elmer instrument. The concentration of the sample in the solution varied between 5 and 10 mg. per ml. In all cases readings were made at the absorption maxima rather than at definite wave lengths. The base-line technique was used for the estimation of T_0 values on the Baird instrument, while the cell-in, cell-out technique was used on the Perkin-Elmer instrument.

RESULTS

The compounds studied were a series of silanes and disilanes (4) with only phenyl and p-tolyl groups as substituents on the silicon atoms. Infrared spectroscopic tests indicated that the compounds were 99% pure. The compounds are listed in Table I, and they can be represented by the general formulas $(C_6H_5)_n$-$Si(C_7H_7)_{4-n}$ and $(C_6H_5)_nSi_2(C_7H_7)_{6-n}$, with n having the values 0 to 4 for the monosilanes and 0 to 5 for the disilanes. (Hexa-

phenyldisilane was not studied because of its low solubility in the solvent used.)

Table I lists the peak molar absorptivities of the bands at 12.5 microns for the p-tolyl group and at 13.5 and 14.3 microns for the phenyl group. All three bands have been assigned to out-of-plane carbon-hydrogen bending vibrations of the benzene ring by analogy to the similar bands in the methyl-substituted benzenes, which have been assigned to particular vibrations by Pitzer and Scott (8). These bands were chosen for the analysis because of their high intensity and because they are, in these compounds, free from interference. It can be seen that there are considerable variations in the peak molar absorptivities of these bands, even if the monosilanes and disilanes are considered separately. This is particularly true for the 13.5- and 14.3-micron bands.

If the peak molar absorptivity of the 13.5-micron band of
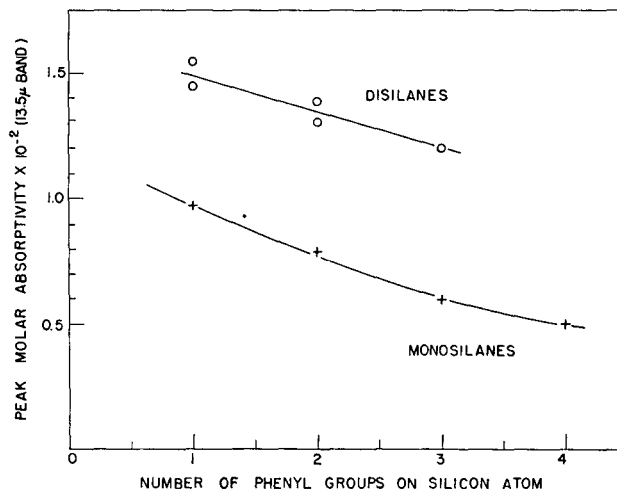
Figure 1. Molar absorptivity of phenyl groups in arylsilanes

13.5-micron band

Table I. Molar Absorptivities of Arylsilanes[a]

Compd. No.	Name of Compd.	Molar Absorptivity, Liters/Mole-Cm.		
		12.5μ	13.5μ	14.3μ
1	Tetraphenylsilane	. . .	50.1	167
2	Triphenyl-p-tolylsilane	145	59.9	193
3	Diphenyldi-p-tolylsilane	140	75.9	226
4	Phenyltri-p-tolylsilane	144	98.0	300
5	Tetra-p-tolylsilane	146	. . .	. . .
6	Pentaphenyl-p-tolyldisilane	171	124	201
7	1,1,2,2-Tetraphenyl-1,2-di-p-tolyl-disilane	169	130	197
8	1,1,1,2-Tetraphenyl-2,2-di-p-tolyl-disilane	158	125	199
9	1,1,1-Triphenyl-2,2,2-tri-p-tolyl-disilane	165	120	200
10	1,1,2-Triphenyl-1,2,2-tri-p-tolyl-disilane	178	154	204
11	1,2-Diphenyl-1,1,2,2-tetra-p-tolyl-disilane	157	144	193
12	1,1-Diphenyl-1,2,2,2-tetra-p-tolyl-disilane	162	137	213
13	Phenylpenta-p-tolyldisilane	161	155	207
14	Hexa-p-tolyldisilane	155	. . .	. . .

[a] Molar absorptivities refer in all cases to the molar concentration of the functional group rather than of the compound. Thus, 1 mole of tetraphenylsilane is equivalent to 4 moles of phenyl groups.

the phenyl group is plotted against the number of phenyl groups on the silicon atom, two smooth curves may be drawn, one for the monosilanes and one for the disilanes as shown in Figure 1. Not all of the disilanes can be included in this plot (compounds 5, 8, 10, Table I), as in some there are different numbers of phenyl groups on the two silicon atoms. It is possible to calculate an expected peak molar absorptivity for these compounds, weighting the peak molar absorptivities obtained from Figure 1 according to the relative numbers of phenyl groups of each type in the molecule. The values thus calculated are found to agree closely with the observed values except for 1,1,2-triphenyl-1,2,2-tri-p-tolyldisilane, where the calculated value is 140 liters per mole-cm, and the observed value is 154 liters per mole-cm.

Figure 3.   Phenyl-p-tolyl absorbance ratio as a function of group concentration ratios

13.5-micron phenyl band
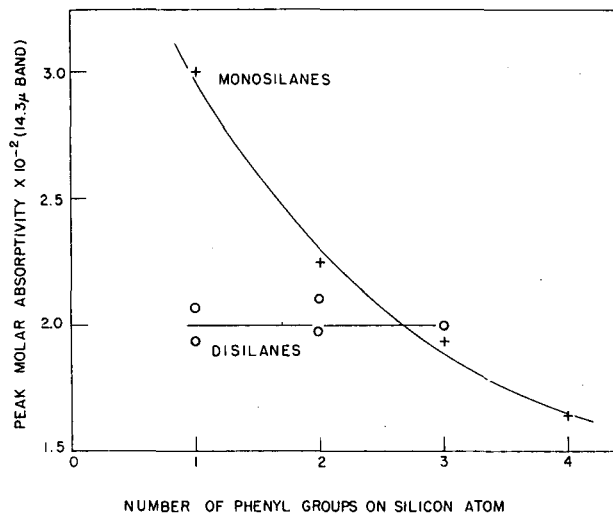
Figure 2.   Molar absorptivity of phenyl groups in arylsilanes

14.3-micron band

Figure 4.   Phenyl-p-tolyl absorbance ratio as a function of group concentration ratios

14.3-micron phenyl band

A similar graph can be made for the 14.3-micron band of the phenyl group, as shown in Figure 2. The peak molar absorptivity of this band is relatively constant for the disilanes but, as for the 13.5-micron band, varies by a factor of about 2 in the monosilanes. The disilanes that could not be included in Figure 2 (compounds 6, 8, and 10, Table I) all have peak molar absorptivities of about 200 liters per mole-cm.

The peak molar absorptivity of the 12.5-micron band of the p-tolyl group does not vary as much as that of the two phenyl group bands. However, there is again a distinct difference in the intensity of this band for the monosilanes and for the disilanes, as well as smaller variations within each group. In 1,1,2-triphenyl-1,2,2-tri-p-tolyldisilane, which had an abnormally high value for the peak molar absorptivity of the 13.5-micron band, the peak molar absorptivity of the 12.5-micron band is also somewhat higher than would be expected from comparison with the other compounds.

Since the molar absorptivity of each band attributable to the phenyl group varies regularly with changes in the structure of the compounds and since the molar absorptivity of the 12.5-micron band of the p-tolyl group is relatively constant, it should be possible to determine the ratio of the two group concentrations in a sample of an unknown compound. The ratio of group concentrations is independent of the weight of sample taken, so an unweighed sample may be used, with the determination of group concentrations proceeding directly from the peak absorbances of the bands rather than from their molar absorptivities. Figures 3 and 4 show the two possible peak absorbance ratios

plotted against the ratio of phenyl to p-tolyl groups in the compounds studied. Two straight lines are obtained in each graph, one for the monosilanes and one for the disilanes. In one case the line representing the disilanes has the greater slope, in the other case the line representing the monosilanes has the greater slope. Advantage can be taken of this fact by plotting the sum of the two absorbance ratios against the ratio of phenyl to p-tolyl groups. This graph is shown in Figure 5. A single straight line passing through the origin is obtained for all of the compounds in the group, with all of the points falling on the line within the precision of measurement.

The fact that a single straight line is obtained in Figure 5 is due to the difference in peak molar absorptivity of the 12.5-micron band in monosilanes compared to disilanes. This difference corrects for a similar difference between the sums of the peak molar absorptivities of the 13.5- and 14.3-micron bands in mono- and disilanes. If it were not for this internal correction, two lines instead of one would have been obtained. Figure 5

can therefore be employed for group ratio determinations regardless of whether the compound is a mono- or disilane, as well as for mixtures of such compounds. In the analysis of mixtures no considerable error should be introduced by variations in the wave length of the absorption maxima since such variations are small. The p-tolyl group bands all fall within the range of 12.47 to 12.50 microns, and the phenyl group bands within the range from 13.40 to 13.45 microns and 14.30 to 14.33 microns. If the sample is known to be a pure compound rather than a mixture, the question of whether it is a mono- or a disilane can be resolved by using the data of Figures 3 and 4.

DISCUSSION

This analytical method has several advantages in addition to yielding quantitative data on functional group concentrations even when the molar absorptivities of the absorption bands used for the analyses are not constant. Because the sample need not be weighed and dissolved in a known amount of solvent, less time is required to obtain the necessary data. The use of peak absorbances, rather than molar absorptivities, saves time in calculating the results. In addition, it was found that virtually the same analytical curves were obtained on the two instruments even though different spectral slit widths were used. The data in Figures 3, 4, and 5 were obtained on a Baird Associates Model B spectrophotometer, operated at normal slit widths and using a base-line technique for the estimation of T_0. The data of Table I and Figures 1 and 2 were obtained on a Perkin-Elmer Model 13 spectrophotometer, using the densitometer attachment. These readings were made at rather narrow slit widths: 0.440 mm. at 12.5 microns, 0.590 mm. at 13.5 microns, and 0.815 mm. at 14.3 microns. In spite of these differences, very nearly the same analytical curves were obtained for the two instruments. For the Baird instrument the slope of the analytical curve is 1.89 and with the Perkin-Elmer instru-

ment the slope is 2.10. The average deviations of the poin from the respective curves were 0.11 on the absorbance rat scale for the Baird instrument and 0.27 for the Perkin-Elm instrument, indicating that the base-line technique was, in th case, self-correcting for some factor affecting the absorptivitie The greater part of the standard deviation value for the Bair instrument came from one point, the absorbance ratio f(pentaphenyl-p-tolyldisilane, and the comparatively large err(here was probably caused by the low absorbance of the 12., micron p-tolyl group band. If this point was not considered i the calculations, the average deviation in Figure 5 was 0.0 Since the closest values of the absorbance ratio are 0.95 for phenyl-p-tolyl group ratio of 1 to 2 and 0.63 for a group ratio (1 to 3, it is unlikely that this method of analysis will fail to di tinguish between different possible structures for a compound.

The variations of peak molar absorptivity of the various banc with the number of phenyl groups on a silicon atom, shown i Figures 1 and 2, probably reflect the electron-donating characte of the p-tolyl group, affecting the electronic structures of th rings. Apparently, this effect is not propagated through mor than one silicon atom. It is not clear why the 14.3-micron ban of the disilanes is not affected in this manner. Other group attached to the same silicon atom can also affect the band inter sities. Two chlorosilanes, diphenyl-p-tolylchlorosilane an phenyl-di-p-tolylchlorosilane, containing both phenyl and p-tol) groups have been studied. Neither of these compounds woul fit on the analytical curve of Figure 5, but an extension of straight line connecting the two points on a similar plot passe through the origin. This general approach is probably als applicable to compounds in which the silicon atom is replace by another metal, though such compounds have not yet bee made available for study.

Certain substituent groups on the silicon atom can give ris to interfering absorption bands. Aliphatic groups on the silico atom absorb strongly in the 12- to 15-micron region (11) an silanols have a strong absorption band, presumably caused by a deformation vibration of the hydroxyl group, at about 11.! microns in the solid and 12.4 microns in solution, the shift rep resenting the effect of hydrogen bonds in the crystal. [Thi band has previously been observed by Richards and Thompsor (9).] The silicon-hydrogen group absorbs strongly at abou 12.5 microns, presumably corresponding to a deformation vibra tion.

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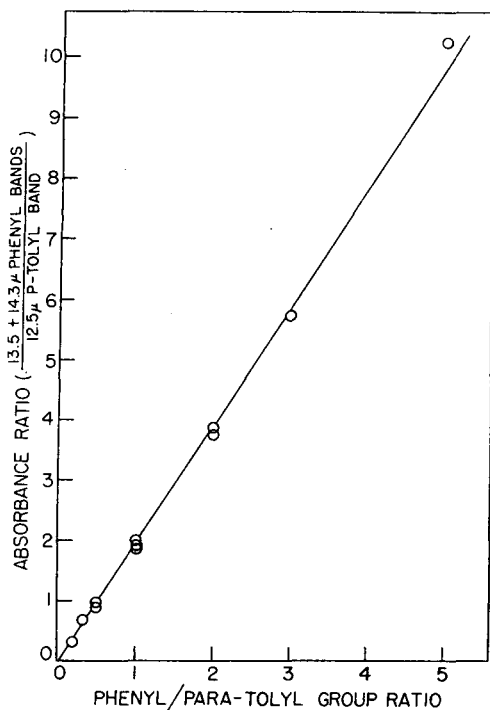**Figure 5. Analytical curve for functional group analysis of tetraarylsilanes and hexaaryldisilanes containing phenyl and p-tolyl groups**

Ultraviolet Spectrophotometric Determination of Styrene and Phthalate and Fumarate Esters in Polyester Resins

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Unsaturated polyester formulations, consisting of polymeric esters of dihydric alcohols and dibasic acids dissolved in a vinyl monomer such as styrene, may be analyzed spectrophotometrically without prior chemical treatment or separation. Phthalate ester and styrene concentrations are determined spectrophotometrically, and the fumarate and/or maleate ester concentration is calculated from relations existing among the components and is used as a correction for the phthalate and styrene concentrations. The method can be of value in the rapid checking of formulations against established specifications.

UNSATURATED polyester formulations, consisting of polymeric esters of dihydric alcohols and dibasic acids dissolved in a vinyl monomer such as styrene, may be analyzed rapidly and easily by an ultraviolet spectrophotometric method, without prior chemical treatment or separation. Such a method can be of value in the checking against established specifications by such consumers as the various government agencies. The successful application of ultraviolet spectrophotometric methods to related problems, such as the determination of phthalic acid in alkyd resins (9), monomeric styrene in polystyrene (6, 7), and polymerized styrene in styrenated fatty acids (4), suggested the applicability of this technique to the polyester resin formulations. Here the two major absorbers are usually the phthalate component of the polyester and monomeric styrene, with a minor contribution from the fumarate or maleate component.

BASIS OF METHOD

A two-component analysis for styrene and phthalate, with a correction for the absorption of the fumarate and/or maleate component of the polyester, is indicated because of the very large differences in the absorptivities of the styrene and phthalate as compared to those of the fumarate and maleate. The analytical wave lengths selected, and the absorptivities determined from the "standards," are summarized in Table I.

Table I. Analytical Wave Lengths and Absorptivities of Chloroform Solutions

	282 Mμ	291 Mμ
Styrene	8.18	5.58
Phthalate	6.85	1.39
Fumarate	1.26	0.663
Maleate	0.404	0.163

The simultaneous equations for the two-component determination of styrene and phthalate, derived from Beer's law, are:

$$A_{291}^{obsd.} = b\, c_s\, (5.58\, C_n + 1.39\, C_p) \qquad (1)$$

$$A_{282}^{obsd.} = b\, c_s\, (8.18\, C_m + 6.85\, C_p) \qquad (2)$$

where A is the observed absorbance at the subscript wave length, b is the cell light path length in millimeters, c_s is the sample concentration in grams per 100 ml., and C_m and C_p are the fractions or per cent concentrations) of the monomeric styrene and phthal-

ate component of the polyester, respectively. Alternatively, the two-component graphical "absorbance-ratio" method (3) may be used. Since no "isoabsorptive point" (3) occurs between the spectra of monomeric styrene and phthalate ester, it is necessary to use a nonlinear plot of absorbance ratio vs. composition which may be prepared from the equation

$$\frac{A_{282}}{A_{291}} = \frac{8.18\, C_m + 6.85\, C_p}{5.58\, C_m + 1.39\, C_p} \qquad (3)$$

STANDARDS AND TEST SAMPLES

Standards. Styrene, 99.75% by freezing point depression (12), containing 50 p.p.m. of tert-butylcresol.
Phthalate, purified (11), as diethyl ester.
Fumarate, purified (11), as diethyl ester.
Maleate, purified (11), as diethyl ester.
Chloroform, Mallinckrodt, reagent grade, for solvent.

Test Samples. Typical polyester resin formulations of known composition were prepared by the polyester resins group of these laboratories. The exact styrene content was checked by distilling out the styrene from a butoxyethyldiglycol carbonate solution of the polyester formulation; the styrene content of this distillate was determined by the one-component ultraviolet spectrophotometric method of McGovern and coworkers (6).

CORRECTION FOR FUMARATE OR MALEATE

The large differences in the contribution to the observed absorbance (absorptivity times concentration) of two of the components (styrene and phthalate) as compared to the third (fumarate or maleate) would impair the accuracy of a three-component spectrophotometric analysis, whether of the three simultaneous equation type or the graphical absorbance-ratio type (3). Consequently, it appeared better to perform a two-component determination of styrene and phthalate and to apply a correction for the contribution of the fumarate or maleate.

As it is known that the polyester is composed of alternating acid and alcohol components, it follows that the sum of the phthalate and fumarate (and/or maleate) units must be equal to the number of alcohol units. If the identity of the dihydric alcohol is known, it is possible to obtain an estimate of the fumarate (or maleate) content using the uncorrected data obtained for the styrene and phthalate concentrations. Thus,

$$\frac{C_p}{FW_p} + \frac{C_f}{FW_f} = \frac{C_g}{FW_g} \qquad (4)$$

where C is the fraction or per cent concentration of the subscript component, p is phthalate, f is fumarate (or maleate), g is glycol (here taken as propylene glycol), and FW is formula weight. For convenience, the dibasic acid was assumed to have lost two atoms of hydrogen during esterification, hence $FW_p = 164.11$ and $FW_f = 114.05$; the propylene glycol is assumed to have lost two hydroxyl groups, hence $FW_g = 42.05$. With no other components present, we also have the relation that

$$C_m + C_p + C_f + C_g = 100\% \qquad (5)$$

Combining these equations,

$$C_m + C_p(1 + FW_g/FW_p) + C_f(1 + FW_g/FW_f) = 100\% \qquad (6)$$

Rearranging,

$$C_f = (1/\beta)(100 - C_m - \alpha C_p). \qquad (7)$$

where $\alpha = 1 + FW_g/FW_p$ and $\beta = 1 + FW_g/FW_f$. Values for α and β for propylene glycol (used in this example) and for some other commonly encountered glycols are given in Table II.

The absorbance contributed by the fumarate (or maleate) at the analytical wave lengths is calculated from the Beer's law equation: $A_f = a_f \times b \times c_s \times C_f$, noting that the c_s term has the dimensions of grams per 100 ml. and the C_f term is a fraction, or per cent. These calculated absorbances are subtracted from the observed absorbances, and the new absorbances used again in the two-component analysis; these values may then be applied again for a better estimation of the fumarate concentration. The alcohol residue concentration is then obtained by difference from 100%.

Table II. Values of α and β for Commonly Encountered Glycols

Glycol	α	β
Propylene	1.256	1.369
Dipropylene	1.610	1.877
Ethylene	1.171	1.246
Diethylene	1.439	1.631

FUMARATE VS. MALEATE CORRECTION

The absorptivities of fumarate and maleate, though low in comparison to styrene or phthalate, differ appreciably from each other, with the fumarate being a stronger absorber, as expected from its trans configuration; this raises a question as to whether the correction should be made for maleate or for fumarate. Although the formulations are generally made up with maleic anhydride or acid, Feuer and coworkers (2) have recently stated that the maleate is changed to fumarate in the course of the reaction. Calculations were made on the test samples using both fumarate and maleate absorptivities; the results are shown in Table III. The fumarate correction is more effective than the maleate in giving better agreement with the preparational data. The differences in the corrections are small, however, and any attempt to proportion the correction in some manner between fumarate and maleate does not seem worth while.

COMPARISON OF PREPARATIONAL AND SPECTROPHOTOMETRIC DATA

The composition of the test samples from preparational data and from the ultraviolet spectrophotometric method described are compared in Table III. The spectrometric data have been obtained by the following steps:

1. Calculation of styrene and phthalate concentrations by two-component absorbance-ratio method (3)
2. Estimation of fumarate from data of step 1
3. Calculation of corrected absorbances, and recalculation of styrene and phthalate concentrations, as in step 1
4. Re-estimation of fumarate from data of step 3
5. Estimation of glycol residue by difference from 100%

APPLICABILITY TO UNKNOWN OR COMPLEX SAMPLES

In application as a control method in manufacturing, the composition of the polyester is known and the appropriate values of α and β are used for the glycol present. In the case of unknown formulations, however, it is necessary to ascertain the qualitative composition prior to application of this method in either the form presented or in a suitably modified form.

The identity of the glycol may be established by the infrared spectrometric method described by Shay, Skilling, and Stafford

(8), and the proper value for α and β may be used from Tab. II, or calculated. In case a mixture of glycols is encountere an estimate may be made using an average value of the tw glycols.

The identity of the dibasic acids may be established by th dibenzylamide infrared spectrometric method described b Stafford, Francel, and Shay (10). In case a third acid is presen it is not possible to apply the correction for fumarate/maleat because the relation among the concentrations of the acids an glycol used in Equations 4 and 5 no longer is true. Howeve the fumarate/maleate may be determined by the direct polarc graphic method described by Hobart (5). This value is the used to correct the styrene and phthalic concentrations obtaine spectrophotometrically. Then since

$$C_m + C_p + C_f + C_{other} + C_g = 100\% \qquad (8$$

and

$$\frac{C_f}{FW_f} + \frac{C_p}{FW_p} + \frac{C_{other}}{FW_{other}} = \frac{C_g}{FW_g} \qquad (9$$

the concentration of the other acid, C_{other}, and of the glycol ma be calculated.

Table III. Comparison of Preparational and Spectrophotometric Data

Sample No.	Maleate corr.	Fumarate corr.	Preparation
	Ultraviolet		
	Styrene		
I	51.6	50.2	50.0
II	63.3	62.6	63.4
III	43.3	41.5	41.3
IV	34.7	32.3	31.2
V	54.4	53.2	51.6
VI	53.5	53.0	53.0
VII	43.6	43.1	40.8
VIII	71.2	70.0	68.8
IX	82.0	80.5	79.6
X	57.8	56.5	55.2
	Phthalate		
I	16.3	15.7	15.5
II	16.6	16.2	16.4
III	19.1	18.6	18.5
IV	20.7	20.0	20.9
V	19.8	19.7	21.7
VI	28.7	28.5	27.6
VII	34.3	33.9	33.9
VIII	18.3	18.0	17.9
IX	9.2	9.0	9.1
X	14.2	13.9	14.1
	Fumarate (Estimated)		
I	20.1	21.8	21.6
II	11.5	12.4	11.4
III	23.6	25.6	25.6
IV	28.4	31.0	31.0
V	15.0	15.9	15.1
VI	7.6	8.9	9.2
VII	9.6	10.4	11.8
VIII	4.9	5.4	6.2
IX	5.4	6.0	6.4
X	18.7	19.3	19.5
	Glycol (by Difference)		
I	12.0	12.3	12.9
II	8.6	8.8	8.8
III	14.0	14.3	14.6
IV	16.2	16.7	16.9
V	10.8	11.2	11.6
VI	10.2	9.6	10.2
VII	12.5	12.6	13.5
VIII	5.6	6.6	7.1
IX	3.4	4.5	4.9
X	9.3	10.3	11.2

Among other possibilities, not commonly encountered at present, would be the presence of monomers other than styrene as solvents, such as diallyl phthalate or triallyl cyanurate. The former would interfere directly, being counted as a phthalate ester along with the phthalate of the polyester. The latter does not absorb appreciably in the ultraviolet above 260 mμ (1

nd would act as a transparent solvent. For either of these pos-
ible monomer solvents present, recourse to a physical separation
f the polyester from the solvent would be necessary.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of W. G.
)eichert with the styrene distillations and of J. G. Koren with
he spectrophotometric data.

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Simultaneous Spectrophotometric Determination of Calcium and Magnesium

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**A reasonably rapid and accurate method for the deter-
mination of small quantities of calcium and magnesium
in water has been developed. This represents the first
simultaneous spectrophotometric determination of cal-
cium and magnesium and depends on obtaining ab-
sorption measurements at a wave length of 630 mμ and
at a pH of both 9.5 and 11.7. On the basis of 43 known
aqueous mixtures of calcium and magnesium con-
taining from 0.3 to 6.0 p.p.m. (expressed as parts per
million of calcium carbonate) of each of these metal
ions and not more than a total of 7.8 p.p.m., it was
found that the average absolute error of the calcium
was 0.12 p.p.m. and the average absolute error of the
magnesium was 0.09 p.p.m.**

MANY polyvalent cations are able to change the color of
the organic dye Eriochrome Black T in basic solutions.
This color change has been used frequently for indicating the
end points in complexometric titrations.

Schwarzenbach and Biedermann (*2*) have studied the indicator
action of the dye with pH changes in the absence of polyvalent
cations. The red color of the dye in basic solutions containing
polyvalent cations has been shown to be the result of complexes
formed between the dye and the cation. Schwarzenbach and
Biedermann (*2*) have investigated the 1 to 1 complexes and
have given stability constants for them. Harvey, Komarmy,
and Wyatt (*1*) pointed out that magnesium forms a complex
containing two dye molecules per magnesium atom. Young
and Sweet (*3*) have shown that, in addition to these complexes,
a 3 to 1 complex is formed with magnesium and 2 to 1 and 3 to 1
complexes exist for calcium.

Harvey, Komarmy, and Wyatt (*1*) used the red color of the
magnesium complex as the basis of a colorimetric determination
of magnesium at a wave length of 520 mμ and a pH 10.1. They
state that calcium interferes and suggest that it be removed from
the solution by precipitation as calcium sulfate.

The purpose of the present work was to develop a convenient
method whereby small concentrations of both magnesium and

calcium could be determined spectrophotometrically without the
necessity of making a separation.

Since the absorption curves for dye solutions containing either
calcium or magnesium have the same general shape, with absorp-
tion maxima in the range 520 to 560 mμ, it was not practical to
base a simultaneous determination on absorption measurements
taken at the same pH and at two different wave lengths. These
absorption curves are shown in a separate publication (*3*).

However, there is a difference in the stability of the complexes
of calcium and magnesium. Although the stability of the calcium
complexes is lower, increasing the pH sufficiently causes the degree
of complex formation of both calcium and magnesium to be es-
sentially complete. Measurement of a dye blank against a
solution containing calcium and magnesium at a sufficiently high
pH gives a reading which is proportional to the total calcium
and magnesium present. As the pH is decreased, the degree of
reaction of both calcium and magnesium decreases, but it de-
creases much more rapidly for calcium than for magnesium.
Therefore, it is possible to choose a lower pH value at which the
degree of reaction of calcium, as compared to that of magnesium,
is quite small. Measurement of a dye blank against a solution
containing calcium and magnesium at this lower pH gives a
reading which is essentially a measure of the amount of mag-
nesium present. Measurement at two pH values provides the
basis for the simultaneous determination presented in the
present paper.

EXPERIMENTAL

Reagents. WATER. The water was triple distilled and was
stored in borosilicate glass. Its specific conductance was less
than 1 micromho.

BUFFER, pH 11.70. Piperidine (78 ml.) was added to about 300
ml. of water. After mixing, 8.5 ml. of concentrated hydrochloric
acid were added. This was diluted to about 500 ml. It was
next adjusted with hydrochloric acid or piperidine so that it gave
pH 11.70 under the same conditions that are used in making the
analytical measurements—that is, 5 ml. of buffer must produce
pH 11.70 in a solution having a total volume of 100 ml., 25 ml.
of which is the alcoholic dye solution that is described below.
All pH measurements were made using a Beckman Model G pH
meter equipped with a micro saturated calomel cell and a Beckman
Type E micro glass electrode. The meter was standardized with
0.05M borax at pH 9.18. Polyethylene bottles were used for the
storage of all buffer solutions.

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mingham. Ala.

BUFFER, pH 9.52. A 8.5-ml. volume of concentrated hydrochloric acid was added to about 300 ml. of water. Concentrated (24 ml.) was added and the total volume was brought up to 500 ml. This buffer also requires adjustment to produce pH 9.52 under the conditions of the determination and is adjusted with ammonium hydroxide or hydrochloric acid in a manner similar to that described for the pH 11.70 buffer. However, 10 ml. of the pH 9.52 buffer are used per 100-ml. total.

CALCIUM SOLUTION, 100 P.P.M. Calcium carbonate (0.1000 gram) was dissolved in 0.3 ml. of concentrated hydrochloric acid. This was quantitatively transferred to a 1000-ml. volumetric flask and diluted to the mark. The concentration of this solution is 100 p.p.m., expressed as calcium carbonate, 40 p.p.m. expressed as calcium, or $1 \times 10^{-3}M$. This solution and all other metal ion solutions were stored in borosilicate glass containers.
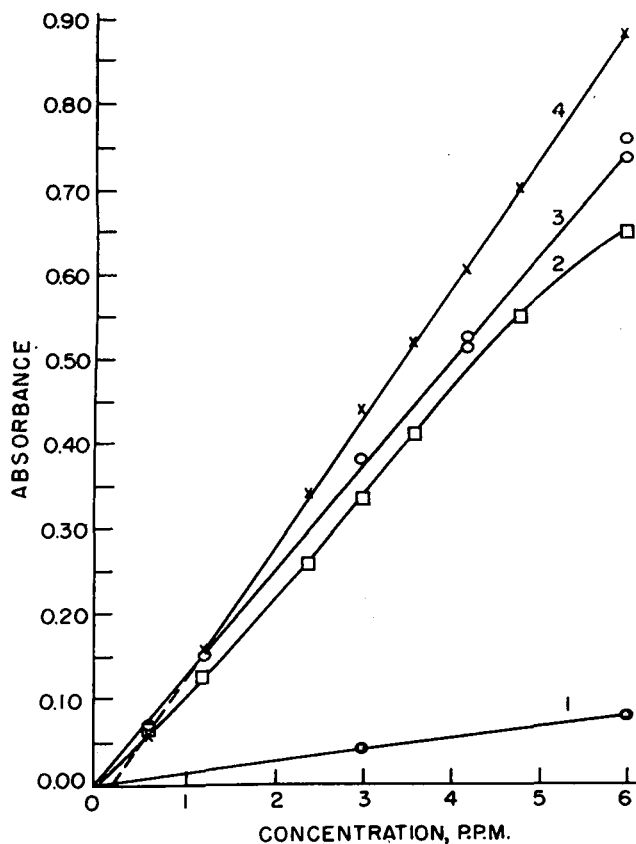
Figure 1. Concentration curves

Concentration, 0 to 6 p.p.m., expressed as calcium carbonate
1. Calcium at pH 9.5 and 630 mμ
2. Magnesium at pH 9.5 and 630 mμ
3. Calcium at pH 11.7 and 630 mμ
4. Magnesium at pH 11.7 and 630 mμ

CALCIUM SOLUTION, 6 P.P.M. Sixty milliliters of the 100-p.p.m. calcium solution were diluted to 1000 ml. with water.

MAGNESIUM SOLUTION, 100 P.P.M. The solution was prepared by treating 0.0913 gram of 3 MgCO$_3$·Mg(OH)$_2$·3H$_2$O in the same way as the calcium carbonate in the preparation of the 100-p.p.m. calcium solution. The resulting solution has a concentration of 100 p.p.m. expressed as calcium carbonate, 24.32 p.p.m. expressed as magnesium, or $1 \times 10^{-3}M$.

MAGNESIUM SOLUTION, 6 P.P.M. Sixty milliliters of the 100-p.p.m. magnesium solution were diluted to 1000 ml. with water.

KNOWN SAMPLES OF CALCIUM AND MAGNESIUM. These were prepared by appropriately diluting the calcium and magnesium solutions.

DYE SOLUTION. One hundred milligrams of Eriochrome Black T (W. H. and L. D. Betz) were transferred to a 250-ml. volumetric flask, using small portions of water, all totaling about 15 ml. One milliliter of pH 11.70 buffer was included in the last portion. The solution was brought up to the 250-ml. mark with 95% grain alcohol. After brief manual shaking, the solution was transferred to a 500-ml. Florence flask. This flask, which was stoppered with a rubber stopper containing a constricted glass tube that

served as a vent, was placed on a reciprocal motor-driven shake for 0.5 hour. The stock dye solution was stored in the dark unt it was used.

Procedure. Add 50 ml. of the solution to be analyzed to a 10(ml. volumetric flask. Add 25 ml. of the dye solution, followed b 5 ml. of the pH 11.70 buffer. Dilute to the mark with watei After mixing, store in the dark for 60 minutes. Measure th absorbance at 630 mμ with a Beckman Model DU spectrophoton eter equipped with 1-cm. Corex cells. Adjust the slit width s that the sensitivity knob is kept at or near the counterclockwis limit. As the blank absorbs more light at this wave length tha the sample, balance the instrument with the sample in the ligh path; then place the blank in the light path and determine it absorbance. The blank must be prepared in exactly the sam way and at the same time as the sample.

Repeat the procedure using 10 ml. of the pH 9.52 buffer in plac of the pH 11.70 buffer.

Calculations. Using the described procedure prepare standar concentration curves, such as those shown in Figure 1, for cal cium at pH 11.70, calcium at pH 9.52, magnesium at pH 11.7(and magnesium at pH 9.52.

From the four concentration curves shown in Figure 1, Equa tions 1 and 2 were obtained. These equations are based on th best straight lines drawn through each of the curves.

$$A_{11.7} = 0.152x + 0.127y - 0.026 \tag{1}$$

$$A_{9.5} = 0.120x + 0.0133y - 0.024 \tag{2}$$
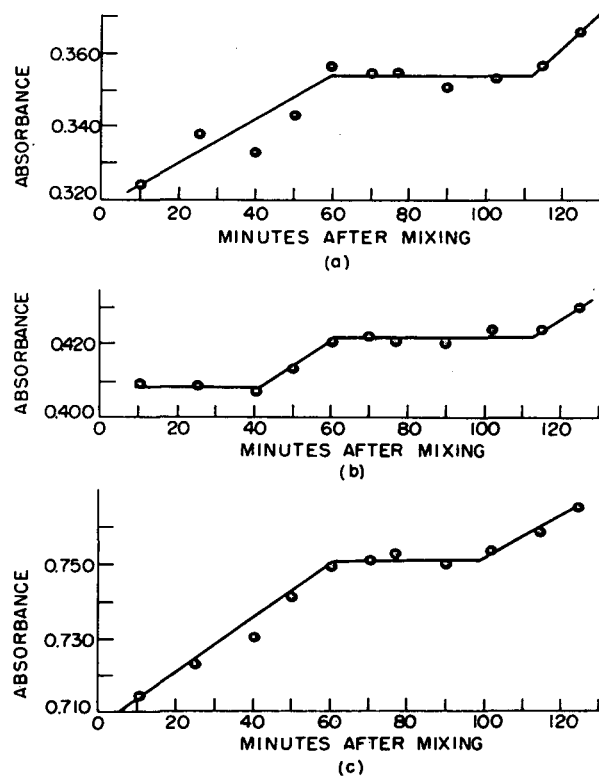
Figure 2. Time study

Concentration, 3.0 p.p.m.; 630 mμ
a. Calcium at pH 11.7
b. Magnesium at pH 11.7
c. Calcium and magnesium at pH 11.7

$A_{11.7}$ is the measured absorbance at pH 11.70 and at 630 mμ; $A_{9.5}$ is the measured absorbance at pH 9.5 and at 630 mμ; x is the concentration of magnesium in the 50-ml. sample in p.p.m. (expressed as calcium carbonate); and y is the concentration of calcium in the 50-ml. sample in p.p.m. (expressed as calcium carbonate). The experimental values of $A_{11.7}$ and $A_{9.5}$ were substituted in these two simultaneous equations and solved for x and y. This has been done for 43 known mixtures of calcium

and magnesium and the results are shown in columns 4 and 6 of Table I.

Equations 3 and 4 were developed in order to make allowance for the slight deviations from linearity in several of the standard curves near the upper or lower ends of the concentration range.

$$A_{11.7} = 0.152x + 0.1267y - 0.026 + 0.0216(1.2 - x) - 0.010(y - 4) \quad (3)$$

The fourth term is used when $x < 1.2$ and the fifth term is used when $y > 4.0$.

$$A_{9.5} = (0.0968 + 0.00473x)x - 0.0133y - 0.025(x - 4)^2 \quad (4)$$

The third term in Equation 4 is used when $x > 4.0$. After solving for x and y by Equations 1 and 2, the indicated terms of Equations 3 and 4 may be used in order to improve the results. This has been done for all of the mixtures of Table I and the results are shown in columns 5 and 7 of Table I.

Table I. Analytical Results

| | Known Mixture | | Experimentally Determined Results | | | | Errors | | | |
| | | | Calcium | | Magnesium | | Calcium | | Magnesium | |
Run No.	Ca, p.p.m. (as CaCO₃)	Mg, p.p.m. (as CaCO₃)	Linear eqs., p.p.m.	Nonlinear eqs., p.p.m.	Linear eqs., p.p.m.	Nonlinear eqs., p.p.m.	Linear eqs., p.p.m.	Nonlinear eqs., p.p.m.	Linear eqs., p.p.m.	Nonlinear eqs., p.p.m.
1	0.6	0.6	0.6	0:6	0.7	0.6	0.0	0.0	0.1	0.0
2	0.6	0.6	0.3	0.3	1.0	0.9	0.3	0.3	0.4	0.3
3	0.6	0.6	0.4	0.4	1.0	0.9	0.2	0.2	0.4	0.3
4	0.6	1.2	0.5	0.5	1.4	1.3	0.1	0.1	0.2	0.1
5	0.6	1.2	0.6	0.6	1.3	1.3	0.0	0.0	0.1	0.1
6	0.6	1.8	0.5	0.5	2.0	2.0	0.1	0.1	0.2	0.2
7	0.3	2.4	0.4	0.3	2.3	2.4	0.1	0.0	0.1	0.0
8	0.6	3.0	0.8	0.8	3.0	3.0	0.2	0.2	0.0	0.0
9	0.6	3.0	0.6	0.6	3.0	3.0	0.0	0.0	0.0	0.0
10	0.6	3.0	0.5	0.4	3.1	3.1	0.1	0.2	0.1	0.1
11	0.6	3.0	0.8	0.8	2.8	2.8	0.2	0.2	0.2	0.2
12	0.6	4.8	0.5	0.6	4.8	4.8	0.1	0.0	0.0	0.0
13	0.6	6.0	1.0	0.8	5.7	5.8	0.4	0.2	0.3	0.2
14	0.6	6.0	1.2	1.0	5.3	5.5	0.6	0.4	0.7	0.5
15	0.6	6.0	1.3	0.8	5.5	5.8	0.7	0.2	0.5	0.2
16	1.2	0.6	1.2	1.2	0.7	0.6	0.0	0.0	0.1	0.0
17	1.2	1.2	1.2	1.2	1.3	1.2	0.0	0.0	0.1	0.0
18	1.2	2.4	1.4	1.4	2.3	2.3	0.2	0.2	0.1	0.1
19	1.2	2.4	1.4	.1.4	2.4	2.4	0.2	0.2	0.0	0.0
20	1.2	3.0	1.2	1.2	3.0	3.0	0.0	0.0	0.0	0.0
21	1.2	6.0	1.5	1.0	5.6	6.1	0.5	0.2	0.4	0.1
22	1.2	6.0	1.8	1.6	5.4	5.6	0.6	0.4	0.6	0.4
23	1.2	6.0	1.6	1.1	5.0	6.0	0.5	0.1	0.5	0.0
24	1.8	0.6	1.9	1.9	0.6	0.5	0.1	0.1	0.0	0.1
25	1.8	0.6	1.8	1.8	0.7	0.6	0.0	0.0	0.1	0.0
26	1.8	6.0	2.3	1.8	5.5	5.9	0.5	0.0	0.5	0.1
27	2.4	0.6	2.5	2.6	0.6	0.5	0.1	0.2	0.0	0.1
28	2.4	0.6	2.4	2.4	0.7	0.6	0.0	0.0	0.1	0.0
29	2.4	2.4	2.4	2.4	2.4	2.4	0.0	0.0	0.0	0.0
30	3.0	0.3	3.1	3.1	0.4	0.3	0.1	0.1	0.1	0.0
31	3.0	0.6	2.9	2.9	0.8	0.6	0.1	0.1	0.2	0.0
32	3.0	0.6	3.0	3.1	0.7	0.6	0.0	0.1	0.1	0.0
33	3.0	0.9	3.1	3.2	1.0	0.9	0.1	0.2	0.1	0.0
34	3.0	1.2	3.0	3.1	1.2	1.2	0.0	0.1	0.0	0.0
35	3.0	2.4	3.2	3.2	2.2	2.2	0.2	0.2	0.2	0.2
36	3.0	2.4	3.1	3.1	2.2	2.2	0.1	0.1	0.2	0.2
37	3.0	2.4	3.1	3.1	2.3	2.3	0.1	0.1	0.1	0.1
38	3.0	3.0	3.0	3.0	3.0	3.0	0.0	0.0	0.0	0.0
39	3.0	3.0	2.9	2.9	3.1	3.1	0.1	0.1	0.1	0.1
40	4.8	0.6	4.9	4.9	0.6	0.5	0.1	0.1	0.0	0.1
41	6.0	0.6	5.9	6.0	0.7	0.6	0.1	0.0	0.1	0.0
42	6.0	1.8	5.8	5.9	1.7	1.7	0.2	0.1	0.1	0.1
43	6.0	1.8	5.6	5.8	1.9	1.9	0.4	0.2	0.1	0.1

DISCUSSION

Alcohol is used in the preparation of the dye solutions, because it yields a solution that is more homogeneous and is easier to handle. Pure water solutions of the dye foam during the process of shaking and during pipetting operations, whereas solutions containing sufficient alcohol do not. In addition, the use of alcohol results in final solutions that change less rapidly with time.

The rather high absorption coefficient of the dye limits the total concentration of the dye and hence of both calcium and magnesium. The total final concentration of dye that was used was 0.01 gram per 100 ml. or $21.65 \times 10^{-5}M$. If the formation of 3 to 1 complexes were complete, this would limit the total metal ion concentration to $7.22 \times 10^{-5}M$, which corresponds to a 50-ml. sample which is 14.44 p.p.m. (expressed as parts per million of calcium carbonate). For a simultaneous determination, y parts per million of calcium in a mixture of y parts per million of calcium and x parts per million of magnesium must produce the same change in absorbance as y parts per million of calcium does in the absence of magnesium. This requires an excess of dye above 3 to 1 and hence reduces the maximum total metal concentration to much less than 14.44 p.p.m. In addition, the high absorption of the dye necessitates the use of a dye blank.

There were two possible choices with regard to the wave length of the measurements. The first was to measure the absorbance in the region of 520 to 560 mμ, where the complexes have their maxima. This region was used in some of the preliminary experiments. However, the results were somewhat erratic. This can be explained in the following way. Although 520 to 560 mμ is the region of the maxima for the complexes, it is also a region in which the absorption curve of the dye is steep, thus making the wave-length setting very critical. The second region that was considered was between 630 and 650 mμ, the region of the dye's maximum. Here the absorption of the complexes is low

and fairly level. Thus the wave-length setting is not so critical. In this region, measurement of a dye blank against the sample solution gives a reading which is primarily due to the disappearance of the dye as a result of complex formation. The exact wave length used was 630 mμ. A wave length of 640 mμ might have been a somewhat better choice.

One of the pH values used in the analysis should be high, so that both the calcium and the magnesium will react as completely as possible with the dye. However, several difficulties arise which place an upper limit on the pH that can be used. Solutions having high pH values are hard on glassware, especially absorption cells. Second, a satisfactory buffer system must be used. Sodium hydroxide and potassium hydroxide are not pure enough. Neither these substances nor any amine hydrochloride were found satisfactory. In so far as could be determined, piperidine reacts with neither calcium nor magnesium and could be obtained free from heavy metal impurities. The pK_b for piperidine is about 11.2. In order to remain safely within the limit of this buffer system's usefulness, a pH of 11.70 was selected. This exact value is arbitrary, although once selected, it is important to maintain it consistently.

Because it was felt desirable to use the same buffer system at the lower pH measurement, a pH of 10.25 was first selected. At this pH the concentration curve for magnesium was lowered only slightly as compared with pH 11.70. The calcium curve, on the other hand, was considerably lower. However, when the absorbances of mixtures were determined at pH 10.25, they were found to be less than the sums predicted from the individual calcium and magnesium concentration curves.

The final choice was pH 9.52. Here the calcium concentration curve is much lower and the magnesium curve is still not less than 75% of its value at pH 11.70. At this pH the absorbance of a mixture of calcium and magnesium is equal to the sum of the individual components. The exact value of 9.52 is not critical, providing that once decided upon it is maintained constant.

Figure 2 illustrates the effect of time on the absorbance measurements. As indicated in these curves, the best time for taking readings is 60 to 80 minutes after the solutions are mixed. Another time factor is the age of the stock dye solution. All analytical measurements were made using stock dye solutions 3 to 5 hours old. Use of stock dye solutions on days subsequent to that on which they were prepared led to lower absorbance values.

For the 43 known mixtures that were tested by the present method, the average absolute error of calcium was 0.17 p.p.m. using the linear equations and 0.12 p.p.m. using the nonlinear equations. The average absolute error of magnesium was 0.17 p.p.m. with the linear equations and 0.09 p.p.m. with the nonlinear equations.

Several determinations were made in order to investigate possible interference of iron and copper. A sample known to contain 3.0 p.p.m. of calcium, 3.0 p.p.m. of magnesium, and 1.0 p.p.m. of copper was found to contain 2.7 p.p.m. of calcium and 3.9 p.p.m. of magnesium. A sample containing 3.0 p.p.m. of calcium, 3.0 p.p.m. of magnesium, and 1 p.p.m. of iron was found to contain 2.9 p.p.m. of calcium and 3.9 p.p.m. of magnesium. This interference is to be expected, not only with iron and copper, but also with other polyvalent cations.

Possible interference of sodium and potassium was investigated by analyzing solutions containing varying known concentrations of sodium and potassium in addition to 3.0 p.p.m. of calcium and 3.0 p.p.m. of magnesium. The results are summarized in Table II.

If the concentration curves that were used in developing the

Table II. Effect of Sodium and Potassium[a]

Concn., P.P.M.	Ca Found, P.P.M.	Mg. Found, P.P.M.
10 Na+	2.8	3.1
100 Na+	3.1	3.2
1 K+	2.8	3.2
10 K+	3.0	3.3
50 K+	3.3	3.3
100 K+	4.1	3.1

[a] Sodium and potassium added as chloride salts.

equations for the simultaneous determination (Figure 1) are extended, they show a pronounced tendency to level off with increasing metal ion concentration. Although it would not be advisable to extend the simultaneous determinations much above 6 p.p.m., standard concentration curves may be used, preferably at pH 11.70, for the determination of solutions known to contain only calcium or magnesium in the range 0.3 to 15 p.p.m.

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Polarographic Behavior of Some Alkyl Phthalate Esters

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The general behavior of the methyl, ethyl, butyl, and octyl esters of o-phthalic acid were investigated at the dropping mercury electrode. In ethanolic solutions, containing quaternary ammonium salts as supporting electrolytes, two well-defined diffusion currents were produced. The reduction process was found to be diffusion-controlled and irreversible for each wave. The effect of pH on the diffusion currents and half-wave potentials of dimethyl phthalate was studied in 75% ethyl alcohol solution buffered over the range of 2 to 12 with Britton and Robinson buffers. Diffusion coefficients were experimentally determined and used to calculate the value of n for the first and second waves. Four electrons are involved in the first reduction and two in the second. Phthalide was established as the intermediate reduction product. Optimum analytical results are secured by measurement of the first wave ($n = 4$). Neutral and alkaline solutions offer the best conditions for measurement of this wave, and the diffusion currents in these media are a linear function of the concentration of phthalate ester.

SEVERAL esters of o-phthalic acid have been investigated at the dropping mercury electrode (10, 11). Two well-defined current-voltage curves have been reported in nonbuffered media. The first wave was chosen for a quantitative study because of the lower reduction potential and the ease of measurement. The authors used this wave for the determination of several phthalate esters in plastics (11). As no data have been reported on such fundamental knowledge as diffusion coefficients,

mechanisms of reduction, and the effect of pH on diffusion currents (i_d) and half-wave potentials ($E_{1/2}$) of alkyl phthalate esters, this paper presents and discusses such data.

APPARATUS AND MATERIALS

Apparatus. A Sargent Model XXI recording polarograph and a Fisher Elecdropode were used in all studies. Half-wave potentials vs. the saturated calomel electrode (S.C.E.) were determined with the Elecdropode (4).

The solutions used in these studies had cell resistances of less than 500 ohms, as determined with a Wheatstone bridge, so the iR drop correction was negligible in computing $E_{1/2}$ values (5). Apparent pH values of the solutions were obtained with a Beckman Model G pH meter.

The capillary used had the following characteristics: At a pressure of 92.7 cm. of mercury, the drop time, t, on open circuit in 0.5M tetramethylammonium chloride solution (75% ethyl alcohol), was 4.90 seconds per drop, the weight, m, of mercury falling per second was 1.36 mg. and $m^{2/3}t^{1/6}$ (3) was therefore 1.60.

Small borosilicate glass beakers (30-ml.) were used as polarographic cells. A rubber stopper to which were attached the dropping mercury electrode, contact electrode, and a glass tube with a fritted disk, was placed over the top of the small beakers. The current-voltage curves were obtained in a constant temperature bath at 30° ± 0.2° C.

Dissolved oxygen was removed from all solutions with pure nitrogen just prior to the polarographic examination. The nitrogen was passed through a portion of the solution being examined polarographically and finally through the solution in the polarographic cell.

Materials. Tetramethylammonium chloride (Matheson Co., practical grade) was used as the supporting electrolyte for polarographic and diffusion coefficient studies, and for the large scale electrolyses.

Britton and Robinson buffers (1) were used in studies on the

effect of pH on $E_{1/2}$ and i_d values. The buffer was added to 95% ethyl alcohol containing varying amounts of the phthalate esters, so that the final solution was 0.1M in buffer. The 95% ethyl alcohol was obtained from General Chemical Co., New York, N. Y., and was not further purified.

Both gelatin and methyl red (0.001%) were used as maxima suppressors in these studies, although gelatin appeared to be the better.

The alkyl phthalate esters used in this work were as follows:

Dimethyl phthalate, Eastman Kodak Co., n_D^{20} = 1.5148 and boiling point = 282° C.

Diethyl phthalate, Eastman Kodak Co., n_D^{20} = 1.5006 and boiling point = 295° C.

Dibutyl phthalate, Fisher Scientific Co., n_D^{20} = 1.4933.

Dioctyl phthalate, Ohio Apex Co., n_D^{20} = 1.4871.

The esters were purified further by distillation before using.

The phthalide used in these studies was prepared according to directions given in "Organic Syntheses" (2). The melting point of the final product was 73–74° C. Phthalaldehyde was prepared by the method of Shirley (8). Potassium chloride, c.p. grade, was used in standardizing the cells for diffusion coefficient measurements.

Redistilled mercury, c.p. grade, was used as the anode in all work.

EXPERIMENTAL

Diffusion Coefficient Measurements. The diffusion coefficients were determined by a procedure adapted from that employed by Stokes (9). In this method, porous diaphragm cells were used, which embodied a magnetic stirring mechanism. The cells were calibrated by the usual method of allowing 0.1M potassium chloride to diffuse into water at 25° C. until 25% had passed through the diaphragm. The diffusion constant of potassium chloride for these conditions is known to be 1.867 × 10^{-5} sq. cm. per second (9).

Large Scale Electrolysis Studies. These studies consisted of preparation of the desired intermediate under carefully controlled conditions at the cathode and separation and identification of the products of electrolysis.

PREPARATION OF DESIRED INTERMEDIATE. Optimum conditions were previously determined polarographically and the following were used in these studies:

Cathode potential vs. S.C.E. = −1.90 volts (this voltage was on the plateau between the two waves obtained polarographically). The potential was controlled within ± 20 mv. during the experiment with a Potentiostat, similar in circuit to that of Lingane and Jones (6).

Supporting electrolyte, 75% ethyl alcohol, 0.5M in tetramethylammonium chloride. This solution had an "apparent" pH of 6.5 at the start of the experiment but was around 11 at the end.

Concentration of phthalate ester, about 5 grams in 500 ml. of solution. The experiments were conducted at room temperature (about 25° C.) in a divided cell at a mercury cathode.

ISOLATION OF ELECTROLYTIC REDUCTION PRODUCTS. After most of the alcohol had been removed by distillation, the catholyte was acidified with hydrochloric acid and extracted with ether. Evaporation of the ether extract yielded about 1.5 grams of solid material, which was treated with Darco and recrystallized from water (melting point, 73–74° C.). This product was shown to be phthalide from an x-ray powder pattern and a mixed melting point with an authentic sample.

Evaporation of the mother liquor from the phthalide crystallization yielded another product which was treated with Darco and recrystallized from water (melting point, 197–201° C.). This product was shown to be phthalic acid from an x-ray powder pattern and a mixed melting point with an authentic sample.

RESULTS AND DISCUSSION

Polarographic Studies. Dimethyl, diethyl, dibutyl, and dioctyl phthalate were investigated in an alcohol-water system at the dropping mercury electrode. Two well-defined diffusion currents were obtained with each ester in a nonbuffered system that was 75% ethyl alcohol and 0.5M in tetramethylammonium chloride. The "apparent pH" of these solutions was 6.5. Half-wave potentials and diffusion-current constants for each wave are given in Table I. The half-wave potentials do not appear to vary a great deal between esters; however, the diffusion-current constants vary significantly. The diffusion currents are directly proportional to the carboxyl content of the molecule. The first wave is better defined and is recommended for analytical

Table I. Half-Wave Potentials and Diffusion-Current Constants for Some Alkyl Phthalate Esters and Phthalide[a]

Phthalate Ester	Concentration, mM	$E_{1/2}$ vs. S.C.E., Volts		$i_d/Cm^{2/3}t^{1/6}$	
		1st wave	2nd wave	1st wave	2nd wave
Dimethyl phthalate	3.37	−1.77	−2.10	4.75	2.80
Diethyl phthalate	2.48	−1.80	−2.10	4.46	2.47
Dibutyl phthalate	2.01	−1.82	−2.09	4.01	2.15
Dioctyl phthalate	1.34	−1.84	−2.10	3.26	1.82
Phthalide	1.67	−2.05	...	3.65	...

[a] 75% ethyl alcohol, 0.5M tetramethylammonium chloride, 0.001% gelatin.

Table II. Alkyl Phthalate Esters in Buffered Solution[a]

Phthalate Ester	Concentration, mM	$E_{1/2}$ vs. S.C.E., Volts		$i_d/Cm^{2/3}t^{1/6}$	
		1st wave	2nd wave	1st wave	2nd wave
Dimethyl phthalate	6.70	−1.73	−2.12	4.33	2.83
Diethyl phthalate	5.30	−1.75	−2.12	4.03	2.80
Dibutyl phthalate	4.13	−1.78	−2.09	3.60	2.40
Dioctyl phthalate	2.70	−1.82	−2.09	2.98	2.41

[a] Apparent pH = 10.7, 60% ethyl alcohol, 0.5M tetramethylammonium chloride, 0.1M buffer, 0.001% gelatin.

work. The lower reduction potential for this wave will produce less interference in the drop time of the capillary and give more reproducible results.

Two well-defined diffusion currents were also obtained with each ester in a buffered system that was 0.1M buffer, 0.5M tetramethylammonium chloride, and 60% ethyl alcohol. The apparent pH of this solution was 10.7. Half-wave potentials and diffusion-current constants for each wave are given in Table II. The half-wave potentials are slightly lower in the buffered system. The $E_{1/2}$ values for the first wave for dimethyl phthalate, as shown in Tables II and III, agree very well, although the concentrations of the ester and the ethyl alcohol content of the solutions were very different. The diffusion-current constants for the first waves of the esters are slightly lower in the buffered solutions (Tables I and II). Since the pH at the electrode surface changes in an unbuffered solution, the waves are generally drawn out in these media and may give higher diffusion-current constants. In this work, the $E_{1/2}$ values did not seem to be dependent upon pH (Table III) and interpretation of the data in unbuffered media seems justified.

Table III. 0.86mM Solution of Dimethyl Phthalate at Different pH Values[a]

Apparent pH	$E_{1/2}$ vs. S.C.E., Volts		$i_d/Cm^{2/3}t^{1/6}$		Remarks
	1st wave	2nd wave	1st wave	2nd wave	
6.0	...	...	...	...	Waves poorly defined
6.5	−1.72	...	2.15	...	Fairly well defined wave
7.2	−1.72	−2.04	2.59	4.74	First wave well defined Second wave ill defined
9.0	−1.71	−2.03	4.53	3.84	First wave ill defined at end Second wave well defined
10.3	−1.72	−2.04	4.59	3.37	Both waves well defined
11.6	−1.72	−2.04	4.65	3.43	Both waves well defined

[a] 75% ethyl alcohol, 0.5M tetramethylammonium chloride, 0.1M buffer, 0.001% methyl red.

The effect of a buffered solution on $E_{1/2}$ and i_d values of dimethyl phthalate is shown in Table III. The half-wave potentials remain the same as the pH increases, while the diffusion current constant for the first wave increases steadily with an increase in pH. Data obtained in the more acidic solutions (apparent pH values of 3.8 and 4.9) indicated no diffusion current arising from a phthalate ester; only hydrogen ion waves appeared in this pH range. Neutral and alkaline solutions (pH values above 6.0) gave well-defined diffusion currents for di-

methyl phthalate. Since alkaline solutions may tend to saponify the esters, an unbuffered salt solution may be just as effective for recording waves in alkaline solution without this danger. At the start of the reduction the solution around the drop becomes strongly alkaline because of the removal of hydrogen ions from the solution immediately surrounding the drop.

In order to test the electrode process for diffusion control, the height of the mercury column (h) was changed and the effect on i_d with this change was studied (Table IV). From the data it appears that the diffusion current (i_d) is dependent upon the height of the mercury column (i_d varies as $h^{-1/2}$) and thus the reduction is diffusion controlled. Since $E_{1/2}$ appears to depend upon concentration (Table V) and plots of log $i/(i_d - i)$ do not give a linear relationship, the process at the cathode apparently is "irreversible." This is usual for most organic reductions at the dropping mercury electrode.

Table IV. 2.48mM Solution of Diethyl Phthalate in 75% Ethyl Alcohol[a]

Mercury Column (h), Cm.	i_d, μa.		$i_d h^{-1/2}$	
	1st wave	2nd wave	1st wave	2nd wave
92.7	18.0	10.1	1.87	1.05
69.2	16.2	9.0	1.95	1.08
58.4	14.4	8.2	1.88	1.07
45.7	12.7	7.3	1.88	1.08

[a] 0.5M tetramethylammonium chloride with 0.001% gelatin.

Table V. Effect of Concentration on $E_{1/2}$ Values of Dimethyl Phthalate[a]

Concentration, mM	i_d, μa.		$E_{1/2}$ vs. S.C.E., Volts	
	1st wave	2nd wave	1st wave	2nd wave
0.674	5.16	3.54	−1.75	−2.07
1.685	12.65	7.88	−1.76	−2.08
3.370	25.60	15.10	−1.77	−2.10
6.600	51.52	30.60	−1.80	−2.15

[a] 75% ethyl alcohol, 0.5M tetramethylammonium chloride, 0.001% gelatin.

Diffusion Coefficient Measurements. The porous diaphragm cell method with magnetic stirring was selected for this work because of simplicity and accuracy. The use of magnetic stirring simultaneously limits the diffusion path to the interior of the diaphragm and stirs the compartment of the cell. Identical conditions of diffusion path length and homogeneity of the bulk solutions are assured even if the solutions differ from the calibrating solution in solvent and viscosity, as was the case in these measurements. The concentration of the solutions in the compartments at the conclusion of a diffusion experiment were determined polarographically and the diffusion coefficient, D, was calculated using the equation

$$D = \frac{1}{K_t} \log \frac{C_1 + \frac{V_2}{V_1} C_2}{C_1 - C_2}$$

where K is the cell constant determined by potassium chloride calibration, t is the diffusion time, V_1 and V_2 are the compartment volumes, and C_1 and C_2 are the final concentrations in the two compartments. Table VI presents experimentally determined diffusion-coefficient data and n values as calculated from the Ilkovič equation ($n = i_d/607D^{1/2}Cm^{2/3}t^{1/6}$) for diethyl phthalate, dioctyl phthalate, phthalide, and phthalaldehyde in 75% ethyl alcohol solution that was 0.1M in tetramethylammonium chloride. In calculating C_1 and C_2, the compounds were allowed to diffuse for 200 to 300 hours so that greater precision could be obtained by measuring large diffusion currents polarographically. Well-defined waves were obtained in all experiments. The

Table VI. Diffusion Coefficients and n Values for Some Phthalate Esters, Phthalide, and Phthalaldehyde[a]

Compound	$D \times 10^5$, Sq. Cm. Sec.$^{-1}$	n[b], 1st Wave	n[b], 2nd Wave
Diethyl phthalate	0.394	3.90	1.97
Dioctyl phthalate	0.238	3.76	2.04
Phthalide	0.526	2.37	. . .
Phthalaldehyde	0.169	1.83	. . .

[a] 75% ethyl alcohol, 0.1M tetramethylammonium chloride, 0.001% gelatin.
[b] Calculated from measured D values and Ilkovič equation.

values of n were calculated from the average of three experimental values of D.

Diffusion coefficients may be calculated from the Stokes-Einstein equation:

$$D_{SE} = \frac{2.96 \times 10^{-7}}{\eta(V_m)^{1/3}} \text{ sq. cm. per second at } 25° \text{ C.}$$

where η = solution viscosity in dyne seconds per sq. cm. and
V_m = molar volume, molecular weight/density

However, viscosity and density data must be accurately determined for the solution and compound, respectively, and the molecule in question must be large (7). The authors compared values of n for some nitrate esters as determined from D values calculated with the Stokes-Einstein equation and D values determined experimentally by the magnetic stirring method reported herein (12). The data indicated that considerably more reliable results could be obtained for the number of electrons, n, involved in the reduction of organic molecules at the dropping mercury electrode with experimentally determined values for the diffusion coefficient (D). The values of n for the alkyl phthalates reported in Table VI are definitely 4 for the first wave and 2 for the second wave, while n values for phthalide and phthalaldehyde are 2.

Mechanism of Reduction. From the polarographic data and the number of electrons involved in the reduction of one molecule (as calculated from Ilkovič equation with experimentally determined values of D), the reduction of diethyl phthalate at the dropping mercury electrode appears to take place in two steps. The first step requires four electrons and the second step two electrons. The most likely intermediate products involving a four-electron change were thought to be phthalaldehyde (Equation 1) or phthalide (Equation 2).
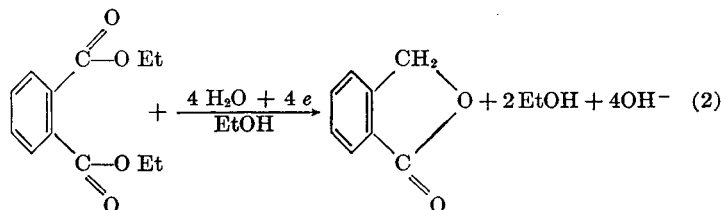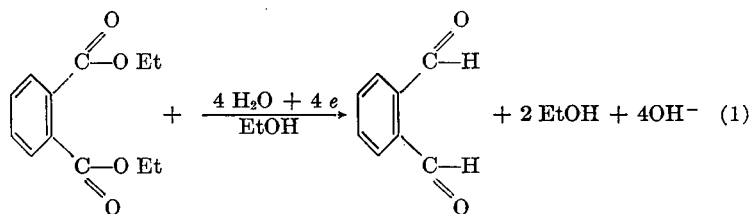
As the reduction appeared to take place in two well-separated steps involving four and two electrons, respectively, the nature of the reduction products might be determined and these data would help in verifying the electrode reaction. A considerable

increment in voltage (Table I) occurred between the first and second waves of diethyl phthalate. This increment was enough to allow a large scale controlled electrolysis of a solution of diethyl phthalate to be made.

In addition, samples of phthalide and phthalaldehyde were examined polarographically. Phthalaldehyde produced two polarographic waves at more positive potentials than diethyl phthalate. The half-wave potential of the first wave vs. a mercury pool was -1.15 volts, while the second wave vs. a mercury pool was -1.57 volts. The first wave disappeared on standing. The second wave appeared to overlap slightly with the first wave for diethyl phthalate (Table I). Phthalaldehyde was therefore not considered to be the intermediate product in the reduction process. Phthalide produced one well-defined wave with a half-wave potential nearly the same as that for the second wave of diethyl phthalate (Table I). Thus, if phthalide were the intermediate product in the electrode reaction, the addition of phthalide to a solution of diethyl phthalate should increase only the second wave. Additions of phthalide to a $1mM$ solution of diethyl phthalate did increase only the second wave.

Three large scale electrolysis experiments failed to produce phthalaldehyde as a reduction product. In each case phthalide was isolated in good yields.

o-Toluic acid was at first thought to be the end product in the reaction. However, no o-toluic acid was isolated from the reaction mixture upon reducing phthalide at a mercury cathode. In this case the cathode potential was raised to -2.20 volts vs. S.C.E. This potential was well on the limiting value of the diffusion current for phthalide as determined polarographically. The product isolated in the reduction of phthalide appeared to have the properties of an aldehyde as shown by infrared spectrum and chemical tests. It was noncrystalline as determined from an x-ray diffraction pattern of a powdered sample.

On the basis of these experimental data the intermediate product in the polarographic reduction of diethyl phthalate is phthalide (Equation 2).

The end product in the electrode process is so far unknown. From these experiments it seems likely that the second step in the polarographic reduction is the following:
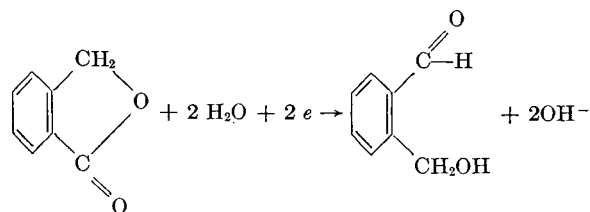
However, under the conditions of the large scale electrolysis experiments, the product is not stable and only a resinous material was recovered from the catholyte.

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Polarography of Humic Acid—Like Oxidation Products of Bituminous Coal

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A study of the polarographic behavior of humic acids was undertaken because of the sensitivity of polarography to changes in conditions imposed during electrolysis. It was assumed that if the humic acids have abnormal properties, indications of such abnormalities might be observed at the dropping mercury electrode. Under suitable conditions three well-defined reduction waves were observed. The waves were long and drawn out but otherwise appeared to be normal and were affected by drop time, temperature, buffer, carrier ions, pH, and concentration in a typical manner. The first wave was most persistent of the three and was believed to be due to the reduction of nitro groups. The second wave was best developed in alcoholic solutions of 30 to 40% alcohol. The third wave was best developed in aqueous solutions containing a salt such as potassium chloride. The structures responsible for the second and third waves have not been identified. The polarographic behavior of the humic acids indicates that these substances can be studied successfully by this means. Diffusion currents observed for the first wave suggest that the molecular weight of these acids is less than 1000.

CERTAIN properties of the humic acid–like oxidation products of bituminous coal appear to be anomalous (1) and for this reason the behavior of these acids at the dropping mercury electrode was studied. Polarography was not selected to prove structural features of the humic acids but rather because of its sensitivity to changes in conditions imposed during electrolysis. If the humic acids have abnormal properties, it was assumed that indications of such abnormalities might be observed at the dropping mercury electrode.

The humic acids selected for study were of a sample prepared by the treatment of a high-volatile A bituminous coal with hot concentrated nitric acid (2). The humic acids constituted about

65% of the carbon of the original coal and therefore are believed to contain an important part of the structure of the coal unit molecules. The nitric acid treatment introduces 4.6% nitrogen into the humic acids, and it has been estimated that 68.4% of the nitrogen (total nitrogen including that part of the original nitrogen in the coal remaining after treatment) was in an oxidized state, probably nitro groups. These groups were expected to undergo polarographic reduction, as well as possible ketonic or quinoidal structures that might have been produced by the oxidizing action of the hot nitric acid or olefinic structures present in the original coal molecules, although natural humic acids have been reported as not being reducible (10).

EQUIPMENT

A Leeds and Northrup Electrochemograph Type E polarograph was used. To minimize large oscillations during the growth of individual mercury drops and to facilitate thereby the measurement of the current-voltage curves, a high degree of damping was imposed on the recording galvanometer. As a result, the half-wave potentials recorded were shifted to more negative values. The amount of the shift was -0.04 volt. Consequently, the half-wave potentials reported on the humic acids presumably should be increased by this amount.

The capillary used was a 10-cm. length of marine barometer tubing. A constant mercury level apparatus described by Lingane and Laitinen (11) was employed to maintain a constant drop rate. The capillary characteristics were: drop time, 4.0 seconds per drop; m for the capillary, 1.59 mg. per second; and for $m^{2/3}t^{1/6}$, 1.72 mg.$^{2/3}$ per second (open circuit).

The cells used were standard H-type, permanent external anode cells described by Lingane and Laitinen (11), using the saturated calomel electrode (S.C.E.). The cell in use was immersed in a constant temperature water bath at $25° \pm 0.5°$ C. Cells were used until the porous plugs were harmed by the strongly alkaline solutions and then they were replaced. The resistance of the cells was about 600 ohms.

A Beckman Model H-2 pH meter was used to obtain the pH of all solutions studied.

MATERIALS

In addition to the nitric acid-oxidized coal humic acids mentioned above, two other samples were examined. The source of the coal, the method used in preparing the humic acids, and the analyses appear in Table I.

Sample 1, used in most of the present work, was a portion of a large sample prepared previously from Upper Freeport seam coal and stored under nitrogen (2). Sample 2 was prepared from Pittsburgh-seam, high-volatile A bituminous coal (3), using the same method as for sample 1. Sample 3 was prepared from the same coal (12) using air oxidation (4) at 200° C. for 234 hours. Humic acids from sample 1 were also reduced with an equal quantity of Devarda's alloy at room temperature in dilute sodium hydroxide solution. Following reduction, the acids were precipitated with hydrochloric acid and washed with water until free from chloride ion. This resulted in some loss by peptization of the humic acids and consequent recovery of 88%.

All other reagents used met AMERICAN CHEMICAL SOCIETY standards of purity and were tested with the polarograph to ensure that no interference occurred in the potential range under study. Clark and Lubs borate buffer was used from pH 7.5 to 9.0 and Kolthoff and Vleeschouwer sodium carbonate–Borax and disodium phosphate buffers from 9.4 to 10.6 and 10.9 to 11.7, respectively. At pH 12.5 no buffer was added to the 0.1N sodium hydroxide solution.

PROCEDURE

Humic acid solutions were prepared by dissolving weighed amounts of the acids in predetermined volumes of 0.1N sodium hydroxide, the volume depending upon the final pH desired. After solution of the acids was complete and the buffer added, any additional solvent, salts, or other material was introduced, and the solution was diluted to volume in a volumetric flask. When the sodium carbonate–Borax buffer was used with no sodium hydroxide, it was found difficult to get the acids dispersed satisfactorily in sodium carbonate, but if the acids were first dissolved in 0.1N sodium hydroxide followed by the addition of an equivalent amount of sodium bicarbonate, the acids were well dispersed and the net result so far as the buffer and the pH were concerned was the same as though the acids had been dis-

Table I. Source of Coal and Analyses of Humic Acids on Moisture-Free Basis

| | Amount of Elements Present, % | | |
	Sample 1, Upper Freeport[a]	Sample 2, Pittsburgh[a]	Sample 3, Pittsburgh[b]
Carbon	61.4	61.4	62.9
Hydrogen	3.2	3.2	2.2
Nitrogen	4.6	4.6	1.4
Sulfur	0.5	0.4	1.0
Oxygen (by difference)	28.9	28.4	28.3
Ash	1.5	2.0	4.2

[a] Oxidized with concentrated nitric acid.
[b] Oxidized with air at 200° C.

Table II. Effect of Mercury Drop Time[a]

Drop Time, Seconds	First Wave		Second Wave	
	$E_{1/2}$	i_d	$E_{1/2}$	i_d
3.5	-0.64	0.85	-1.68	0.45
4.0	-0.63	0.75	-1.66	0.13
5.0	-0.64	0.58	b	b
6.0	-0.65	0.55	b	b

[a] Humic acids. 0.100 gram per liter; Clark and Lubs borate buffer: 0.1M, pH: 9.1.
[b] These waves were too small to be measured.
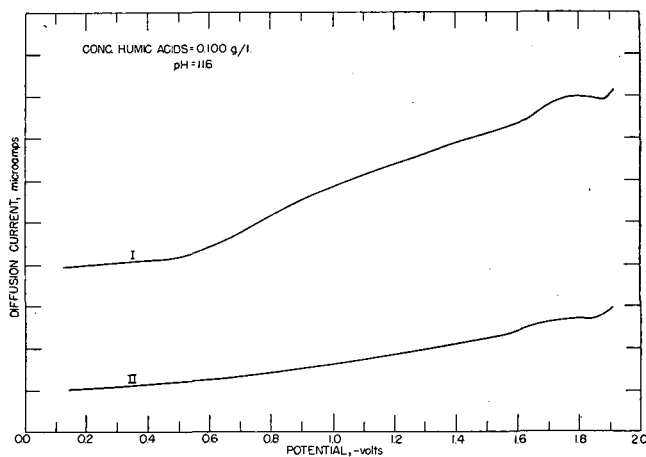
Figure 1. Polarograms of humic acids

I. Nitric acid-oxidized coal humic acids
II. Humic acids reduced by Devarda's alloy

solved in sodium carbonate solution. The type of buffer seemed to have little effect on either the half-wave potentials or the diffusion currents.

To prepare solutions of different concentrations of humic acids, aliquots were removed from the stock solutions as prepared above and diluted with an identically prepared solution containing no humic acids. The pH of each solution was determined before polarographing. After the humic acid solutions had been placed in the cell, nitrogen freed from oxygen by passing through alkaline pyrogallol was bubbled through the solution for 15 minutes before beginning the run. Ordinarily the nitrogen was bubbled through water before passing through the test solution, but when alcoholic solutions were studied, an alcohol-water solution of the same concentration was used. When check runs were made on the same solution, nitrogen was bubbled through the solution for 5 minutes before beginning the check run.

The effect of temperature on the first wave of sample 1, over the range of 3° to 38° C., was to increase the diffusion current an average of 1.65% per degree. Since this temperature coefficient is within the normal range, it appears that the humic acids behave normally with respect to temperature. All subsequent data were obtained at 25° ± 0.5° C.

The effect of mercury drop time on diffusion currents is shown in Table II for the first and third waves. A normal decrease in diffusion current with increasing drop time is observed. Further studies on the effect of drop time indicated that more reproducible waves were obtained at 4 seconds and, although greater diffusion currents were observed at smaller drop times, 4 seconds was chosen as standard.

Table III.　Effect of Humic Acids on Reduction of Thallium(I)ᵃ Ion

TlCl Molality	Humic Acids, Gram per Liter	i_d, μa.	Calcd. i_d, μa.
0.001	0.000	4.55	...
0.001	0.025	4.77	4.82
0.001	0.100	5.40	5.25
0.001	0.200	5.69	5.79
...	0.025	0.27	...
...	0.100	0.70	...
...	0.200	1.24	...

ᵃ Clark and Lubs borate buffer 0.1M; pH 9.0; potassium chloride: 0.25M.

DISCUSSION OF RESULTS

The nitric acid–oxidized coal humic acids (sample 1) dissolved in buffered alkaline solutions gave polarograms of the type shown by Curve I in Figure 1. Previous reduction of the acids with Devarda's alloy resulted in polarogram II. Under more favorable conditions, the unreduced acids exhibited at least three reduction waves. Polarograms of this type are shown in Figure 2, which also shows the effect of adding varying amounts of potassium chloride as a supporting salt.

All of the waves obtained under varying conditions were of the sloping character shown in Figures 1 and 2, but considering the heterogeneous character of the organic matter from which coals were made, it is significant that the waves are as well defined as they are. Possibly the sloping character of the waves reflects the heterogeneity of the general structure of the humic acid molecules, although the appearance of discernible waves must be due to the reduction of characteristic structures present in the majority of the molecules.

The mercury droplets falling to the bottom of the electrode chamber showed a remarkable resistance toward coalescence, suggesting that humic acids or ions were adsorbed on the surface of the mercury droplets (8). As this might have an adverse effect on the polarography of the humic acids, an investigation of the behavior of the acids in the presence of a "pilot" ion, which gives a well-defined wave in the same region, was made. For this purpose the thallous ion was selected, not only because it meets the requirement above but also because it is one of the few ions that does not precipitate the humic acids.

In buffered alkaline solutions the first wave of the humic acids

and the thallium wave were superimposed and could not be distinguished. When the concentration of the thallium ion was such that its wave was more than three times the humic acid wave, the slope of the thallium wave was practically unchanged and the wave height due to the presence of both reducing substances was additive within the limitations imposed by the inaccuracies of reading the humic acid waves (see Table III). As no decided variation in the thallium wave resulted from the addition of humic acids, it was concluded that the reduction waves for the humic acids are normal for these substances and that their slope and height are not affected by the possible presence of an adsorbed layer on the mercury droplets.
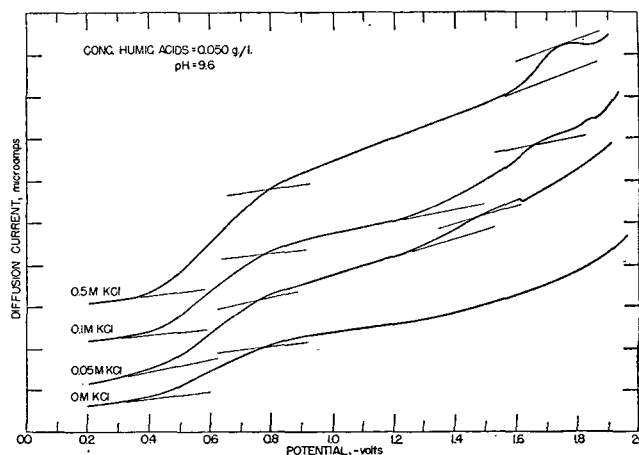

Figure 2.　Effect of adding potassium chloride

The addition of ethyl alcohol to the solvent medium also had a pronounced effect upon the reduction waves of the humic acids. The polarographic constants obtained from solutions containing 0.500 gram of humic acids and up to 50% alcohol are shown in Table IV. Since the addition of alcohol to aliquots of the original solution, made with varying amounts of 0.1N sodium hydroxide and 0.1M boric acid, resulted in progressively more alkaline solutions, the pH of each solution is recorded also.

The effect on the first wave of adding alcohol was to increase the negative half-wave potential, but as the addition of alcohol increased the pH of the solutions, which also has the effect of increasing the potential, it may be presumed that the primary cause of the shift was the change in pH. On the other hand, 40 to 50% concentration of alcohol induced this wave to split into two waves at the lower pH values. The second of these waves appeared in the range of −0.9 to −1.1 volts. No attempt was made to establish the chemistry underlying this behavior. The effect of alcohol on the wave height on the first wave seemed to show no definite trend. Very likely the variations shown in Table IV are due essentially to the difficulties of measuring the sloping waves.

Table IV.　Polarography of Alcoholic Solutions of Humic Acidsᵃ

Alcohol Concn., Vol. %	pH of Soln.	$-E_{1/2}$	i_d	pH of Soln.	$-E_{1/2}$	i_d	pH of Soln.	$-E_{1/2}$	i_d	pH Soln.	$-E_{1/2}$	i_d
					First Wave							
0	8.2	0.62	1.15	8.4	0.63	1.22	9.0	0.67	1.44	9.6	0.75	2.00
10	8.4	0.63	1.07	8.8	0.63	1.04	9.3	0.68	1.46	9.9	0.77	1.91
20	8.6	0.62	0.81	9.0	0.66	1.08	9.5	0.70	1.41	10.1	0.77	1.79
30	9.0	b	...	9.6	0.67	1.01	9.8	0.73	1.45	10.4	0.76	1.90
40	9.3	c	...	9.8	d	...	10.1	0.74	1.56	10.7	0.78	1.75
50	9.7	e	...	9.9	0.72f	1.14	10.3	0.76	1.59	11.0	g	...
					Second Wave							
0	8.2	h	...	8.4	1.45	g	9.0	1.42	g	9.6	1.57	1.10
10	8.4	1.45	0.17	8.8	1.54	0.51	9.3	1.47	0.45	9.9	1.56	1.24
20	8.6	i	0.18	9.0	1.59	0.69	9.5	1.53	1.19	10.1	1.62	2.75
30	9.0	1.62	0.41	9.6	1.65	1.88	9.8	1.65	3.00	10.4	1.62	3.42
40	9.3	b	...	9.8	1.66	2.98	10.1	1.64	3.32	10.7	1.60	2.85
50	9.7	1.61	1.79	9.9	1.68	2.77	10.3	1.52	1.48	11.0	1.58	2.03
					Third Wave							
0	8.2	g	...	8.4	1.75	0.18	9.0	1.81	0.35	9.6	g	...
10	8.4	g	...	8.8	g	...	9.3	1.72	0.30	9.9	g	...
20	8.6	1.67j	0.18	9.0	1.75	0.40	9.5	1.74	0.53	10.1	g	...
30	9.0	c	...	9.6	g	...	9.8	g	...	10.4	g	...
40	9.3	b	...	9.8	g	...	10.1	g	...	10.7	g	...
50	9.7	g	...	9.9	g	...	10.3	1.76	0.90	11.0	g	...

ᵃ Concentration, 0.500 gram per liter; no buffer.
ᵇ Wave distorted.
ᶜ Two waves (1) $E_{1/2}$ = −0.69, i_d = 1.61; (2) $E_{1/2}$ = −1.11, i_d = 0.82.
ᵈ Two waves (1) $E_{1/2}$ = −0.66, i_d = 0.79; (2) $E_{1/2}$ = −0.91, i_d = 0.65.
ᵉ Two waves, difficult to read.
ᶠ Extended, but could be read.
ᵍ Wave too small to read.
ʰ Two small waves at −1.39 and −1.57.
ⁱ Wave drawn out from −1.40 to −1.55.
ʲ Also a fourth wave $E_{1/2}$ = −1.81, i = 0.22.

The second wave was best developed in alcoholic solutions of 30 to 40% and at pH values above 8.8 to 9.0. The half-wave potentials tended to increase markedly with alcohol content at the lower pH values, but at higher values the effect was less important. Diffusion currents rose rapidly with increasing concentration of alcohol to 30 to 40% and then declined. No direct proof is available, but it is suspected that in the higher alcohol concentrations the humate ions tend to associate (13) in a way that interferes with the second wave reduction to a greater extent than the first wave, because if still more alcohol is added, sodium humates precipitate.

The third wave was erratic and poorly defined in alcoholic solutions. Usually, only a slight inflection indicated its presence and often the appearance of a maximum or dip in the trace, as shown in Figure 2, interfered with measuring the wave.

Attempts were made to improve the character of the reduction waves in alcoholic solutions by the addition of salts, but were abandoned because of the salting out effect. The addition of salts to aqueous solutions, however, was marked and was studied in greater detail. Potassium chloride gave the best results of those tried, which include potassium bromide, potassium nitrate, and sodium sulfate. The effect of increasing concentration on the first wave was to increase the wave height, as shown in Figure 2. Small concentrations tended to develop the second wave, while larger concentrations, $0.5M$, produced a striking improvement in the third wave at the expense of the second. On the basis of these results a detailed study of aqueous solutions containing $0.5M$ potassium chloride was made. Polarographic constants for the first and third waves are given in Table V for varying concentrations of humic acids in buffered solutions of varying pH values.

The data in Table V show that the half-wave potentials of the first wave were not constant when either the concentration of the humic acids or the pH was varied and tended to shift to more negative values with increasing concentration and pH. The shift of half-wave potentials to more negative values with increasing pH has been discussed by Kolthoff and Lingane (9), who note that this effect appears to be connected with the mechanism of reduction. The diffusion currents, also, are not proportional to the concentration of the humic acids. Instances of t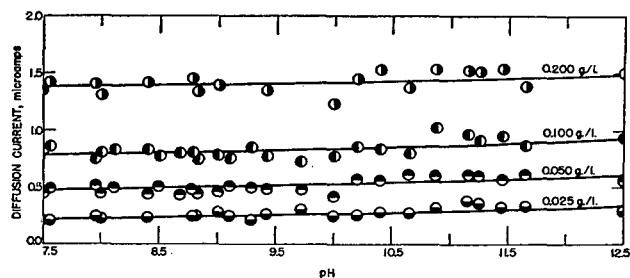his kind are known and appear to be governed by the actual rate of the electrode reaction in which the limiting current is not a linear function of concentration (7). Considering the strong tendency of humic acids to associate, it seems probable that the effect of concentration and perhaps pH on both half-wave potentials and diffusion currents can be explained on this basis. The tendency for the diffusion currents to rise with increasing pH is shown in Figure 3.

Table V. Polarographic Constants of Humic Acids Dissolved in Aqueous Buffered Solutions of Varying pH Containing 0.5M Potassium Chloride

Concn. Humic Acids, Grams/Liter	pH Obsvd.	0.025 $-E_{1/2}$	i_d	0.050 $-E_{1/2}$	i_d	0.100 $-E_{1/2}$	i_d	0.200 $-E_{1/2}$	i_d
First Wave									
Borate buffer	7.5	0.49	0.19	0.51	0.44	0.55	0.80	0.59	1.36
	8.0	0.52	0.22	0.53	0.44	0.58	0.80	0.61	1.30
	8.4	0.55	0.23	0.57	0.44	0.60	0.83	0.64	1.42
	8.8	0.56	0.24	0.59	0.48	0.61	0.80	0.67	1.45
	9.0	0.58	0.28	0.60	0.46	0.63	0.78	0.67	1.39
Carbonate-borax buffer	9.4	0.60	0.26	0.59	0.48	0.63	0.77	0.67	1.35
	10.0	0.62	0.25	0.63	0.42	0.66	0.77	0.68	1.23
	10.4	0.65	0.28	0.66	0.56	0.67	0.83	0.71	1.53
	10.7	0.66	0.27	0.67	0.61	0.68	0.79	0.72	1.37
Disodium phosphate buffer	10.9	0.67	0.32	0.68	0.60	0.71	1.02	0.73	1.53
	11.3	0.68	0.36	0.70	0.60	0.72	0.91	0.75	1.51
	11.7	0.72	0.33	0.72	0.61	0.74	0.86	0.74	1.38
No buffer	12.5	0.76	0.29	0.78	0.55	0.80	0.95	0.80	1.50
Third Wave									
Borate buffer	7.5	1.63	0.53	1.65	1.07	1.68	1.93	1.76	5.40
	8.0	1.62	0.26	1.63	0.47	1.67	1.11	1.70	3.06
	8.4	1.61	0.18	1.68	0.30	1.66	0.71	1.69	2.18
	8.8	1.62	0.15	1.65	0.24	1.67	0.33	1.68	1.18[a]
	9.0	1.61	0.14	1.66	0.10	1.72	0.33	1.60[b]	1.08[a]
Carbonate-borax buffer	9.4	1.63	0.11	1.65	0.09	1.69	0.25	1.73	0.37
	10.0	1.62	0.10	1.66	0.08	1.69	0.25	1.72	0.44
	10.4	1.63	0.07	1.65	0.18	1.67	0.27	1.72	0.40
	10.7	1.64	0.10	1.66	0.12	1.69	0.23	1.71	0.50
Disodium phosphate buffer	10.9	1.62	0.15	1.69	0.18	1.68	0.30	1.69	0.51
	11.3	1.63	0.14	1.63	0.16	1.67	0.26	1.69	0.55
	11.7	1.61	0.11	1.64	0.20	1.67	0.20	1.69	0.48
No buffer	12.5	1.59	0.18	1.62	0.23	1.64	0.32	1.67	0.45

[a] Total i_d for coalesced second and third waves.
[b] Rerun at a lower current range (2) and two waves observed. (1) $E_{1/2} = -1.46$, $i_d = 0.47$; (2) $E_{1/2} = -1.74$, $i_d = 0.74$.

Figure 4. Comparison of polarograms of air-oxidized and nitric acid-oxidized bituminous coal humic acids

The structural grouping responsible for the first wave appears to be the nitro group introduced into the humic acid molecules by the concentrated nitric acid used in oxidizing the coal. The observed half-wave potential not only agrees with reported values of known nitro compounds and reduction with Devarda's alloy eliminates this wave, but when nitric acid is replaced by another method of oxidation, such as air oxidation, this wave does not appear. This is shown in Figure 4, in which polarograms obtained from both nitric acid-oxidized and air-oxidized, Pittsburgh-seam coal humic acids, samples 2 and 3 of Table I, are compared under identical conditions. The polarogram of the

Figure 3. Diffusion current of first wave vs. pH

air-oxidized coal humic acids shows no wave in the -0.6 to -0.7-volt region characteristic of the nitric acid–oxidized coal humic acids. Two other waves do appear at about -1.0 and -1.65 volts. The first wave seems to be characteristic of air-oxidized coal humic acids and no doubt is caused by the reduction of a group introduced by air oxidation. The second wave appears in the same region as the third wave of the nitric acid–oxidized coal humic acids, particularly
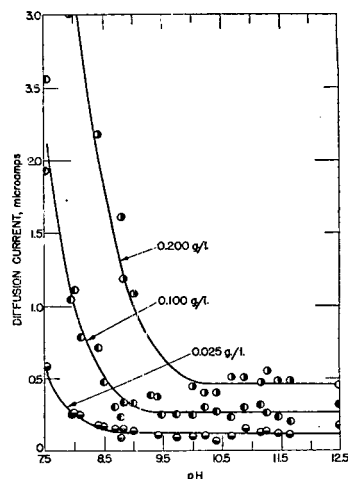
Figure 5 Diffusion current of third wave vs. pH

under conditions which bring about the greatest separation of the third wave from the second. The polarogram of the nitric acid–treated Pittsburgh seam coal humic acids is very similar to those obtained from the Upper Freeport seam coal humic acids prepared by the same method. As the composition of these two preparations is also very similar, the basic constitution of humic acids derived from different coals, but of the same degree of metamorphism or rank, may be essentially the same.

The half-wave potentials of the third wave, given in Table V, were fairly constant, considering the difficulty of measuring these waves, and usually appeared between -1.6 and -1.7 volts. On the other hand, the diffusion currents, at all concentrations of humic acids, fell rapidly as the pH was increased from 7.5 to about 8.8. In this pH range the second wave, at about -1.4 volts, did not appear and therefore may have been combined with the third wave. Above pH 8.8 the second wave did appear at -1.4 volts but was too small to be measured. The change in diffusion current of the third wave with pH is plotted in Figure 5. The structures responsible for the second and third waves have not been identified.

One of the uncertainties of the oxidized coal humic acids is their true molecular weight, and because this is of fundamental importance to coal chemistry, estimations of the molecular weight of the humate ion have made using the Ilkovič equation (5):

$$i_d = 607\ nD^{1/2}Cm^{2/3}t^{1/6}$$

where i_d = diffusion current
n = number of electrons transferred
D = diffusion coefficient

C = concentration
m = rate of flow of mercury
t = drop time of mercury

Among these factors, values of n and D involve the greatest uncertainties. Assuming an alkaline reduction of a nitro group for the first wave and that each humate ion contains one nitro group, a transfer of four electrons seems most probable. An estimation of the diffusion coefficient of the humate ion was made using the Stokes-Einstein equation (6):

$$D = \frac{RT}{N} \times \frac{(4\pi Nd)^{1/3}}{6\pi\eta(3M)^{1/3}}$$

assuming that the humate ions were spherical, that they were not solvated, and that the viscosity of the solution was that of pure water. Since none of these assumptions is quite correct, calculations based upon these assumptions must be considered to be indicative of the magnitude of the molecular weight only; nevertheless the results appear to be significant.

Upon substituting into and rearranging the Ilkovič equation, the following relationship was obtained:

$$M^{7/6} = 607\ n\left(\frac{RT}{6\eta(\pi N)^{2/3}(3/4)^{1/3}}\right)^{1/2} \times \left(\frac{d^{1/6}m^{2/3}t^{1/6}}{1}\right) \times \left(\frac{S}{i_d}\right)$$

where M = molecular weight
n = 4 electrons
R = 8.315×10^7 ergs per ° K.
T = 298° K.
η = 8.93×10^{-3} dyne seconds per sq. cm.
N = 6.03×10^{23}
d = 1.6 grams per cc.
m = 1.592 mg. per second
t = 4.0 seconds
S = sample, in milligrams per liter
i_d = diffusion current, in microamperes

Substituting experimental values of sample weight and diffusion current taken from Table V for the first wave into the equation above gave the molecular weight values shown in Table VI. From the data in Table VI it appears that the apparent molecular weight increases with concentration of the humic acids. A similar increase was observed when the molecular weight was determined cryoscopically in catechol (13). In this case the increasing molecular weight was ascribed to association of the humic acid molecules which presumably are flat carbonlike molecules. Molecular weights estimated from diffusion currents obtained in the more basic solutions, as shown in Table VI, appear to be consistently smaller than in the less basic solutions possibly because of more effective dispersion of the humate ions.

While the molecular weights given in Table VI are considerably larger than those reported in catechol or acetamide solution (13), they are of the same order of magnitude. No doubt the observed molecular weights are too large because of the probability that not all of the humic acid molecules contain nitro groups, that the molecules are not spherical but are flat, and that the humate ions are very extensively hydrated. In view of these difficulties, the molecular weights presented in Table VI seem to indicate that the true molecular size of these humic acids is well below 1000.

Table VI. Calculated Molecular Weights of Humate Ion

Concn. Humic Acid Mg./Liter	pH 9 i_d	pH 9 M	pH 11 i_d	pH 11 M
25	0.24	875	0.33	664
50	0.50	845	0.60	722
100	0.81	1012	0.90	924
200	1.42	1133	1.50	1077

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Chromatography of Organic Acids with Nonesterifying Solvents

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During the chromatography of plant acids by elution analysis, it became desirable to isolate some of the free acids. The simplest procedure was to use an eluent which could be easily evaporated from effluent fractions. For this purpose several ketones were tried as eluents. Most procedures for organic acid chromatography described eluents containing alcohols, which would esterify the untitrated organic acids upon distillation of the effluent. The ketones in combination with halogenated hydrocarbons gave good separations of many organic acids. Except for the separation of citric from isocitric acid the ketones were useful substitutes for alcohols. Circular paper chromatography was used to check the purity and identity of acid fractions. The latter technique was found to be rapid and capable of detecting 0.5 to 1 γ of most nonvolatile organic acids. The drying time of the developed paper chromatograms was reduced from several hours to 20 minutes by heating the papers at 170° to 180° C.

THE use of ketones has been developed for the chromatography of organic acids, particularly the nonvolatile acids found in plant tissues. Such acids have often been chromatographed on silicic acid columns with alcohols in the eluents. It has been reported that alcohols may esterify small amounts of organic acids during their slow passage through a long column (*3*) and during the preparation of acid samples (*4, 8*). Ketones have good solvent properties without this disadvantage during the separation and subsequent recovery of organic acids. Furthermore, when eluents must be changed during the operation of a column, the concentration of ketone may be increased abruptly without eluting inorganic acid from the internal phase as often happens when alcohols are used (*3, 8*). Lower toxicity and frequently lower cost also may be cited as advantages of ketones over alcohols. However, the ketones did not make some separations as well as alcohols did, particularly the separation of citric acid from isocitric acid.

REAGENTS AND APPARATUS

4-Methyl-2-pentanone (methyl isobutyl ketone, Carbide and Carbon Chemicals Co.) and methylene chloride (Eastman Kodak Co.) were used without redistillation. All solvent mixtures used for column chromatography were equilibrated with 0.5N sulfuric acid (water phase to organic phase, about 1 to 20). A loose plug of fine glass wool in the separated organic phase quickly removed suspensions of water droplets when the container was shaken. Mallinckrodt's analytical reagent 100-mesh silicic acid was used without any pretreatment. It had a water content such that it lost 10.7% of its weight when dried for 24 hours at 100° C. A glass column was prepared by sealing a 5-cm. length of 7-mm. diameter and 1-mm. bore capillary tubing at the end of a glass tube 20 cm. long and 8 mm. in inside diameter. The end of the capillary was drawn to a tip.

Solvents used for paper chromatography were not specially purified except that U.S.P. ether and chloroform were washed with distilled water. Whatman No. 1 filter paper of 24-cm. diameter was used as such for chromatograms or was sometimes cut down to fit in a 20-cm. desiccator.

PROCEDURE FOR CHROMATOGRAPHY

Preparation of Column. One gram of silicic acid was ground in a glass mortar with 0.5 ml. of 0.5N sulfuric acid and suspended in a small volume of the organic solvent to be used initially. A filter paper disk punched with a cork borer was placed above

the capillary tube sealed into the chromatographic tube. The column was filled with solvent, and air pressure was applied to sweep air bubbles out of the filter paper disk. The slurry of silicic acid was poured into the glass tube before the last of the solvent used to displace air had passed through the paper disk. The column was packed under 3 pounds per square inch air pressure. The samples were introduced into the column in aqueous solution from a paper disk by a slight modification of an earlier method (*9*). The sample of free nonvolatile organic acids was generally taken to dryness or near dryness on a spot plate. Small volumes of acetone and a glass tube with a capillary tip were used to transfer the sample onto a 0.03-inch-thick filter paper disk (Eaton-Dikeman). The disk was dried in an air stream and put on the silicic acid column after the organic solvent had just drained down to the silica. Any residual sample in the spot plate was then picked up in two successive 0.025-ml. portions of 0.5N sulfuric acid with the same capillary tube used before. Each 0.025-ml. portion was picked up in three or four passes of the capillary and allowed to drain into the column by resting the capillary tip on top of the paper disk. Uneven hydration of the column was avoided by moving the capillary tip about the filter paper disk. The addition of the aqueous phase to the undersaturated column facilitated complete transfer of the sample. It also decreased the tailing compared to that observed when the acids were displaced from the paper disk by the eluent.

Elution by Changing Solvents. Twenty fractions, about 1 ml. each, were collected from the initial solvent, 25 volume % of 4-methyl-2-pentanone in methylene chloride. The remaining solvent was poured off by inverting the column. The second solvent, 60% of 4-methyl-2-pentanone in methylene chloride, was used for fractions 21 to 50. The second solvent was similarly replaced by 100% 4-methyl-2-pentanone, and about 50 additional fractions were collected. A time-flow fraction collector was used, and air pressure was maintained (0.75 to 2 pounds per square inch) to collect each 1-ml. fraction in about 2 minutes. The eluted acids were titrated in the test tubes with 0.01N sodium hydroxide; a stream of carbon dioxide–free air was used for mixing during titration.

Table I. Representative R_f^a Values of Organic Acids on Circular Paper Chromatograms

Acid	Solvents[b]		
	A	B	C
Oxalic	<0.10	<0.10	<0.10
cis-Aconitic	0.18	<0.10	<0.10
Tartaric	0.19	<0.10	<0.10
Citric	0.26	<0.10	<0.10
Isocitric	0.27	<0.10	<0.10
Maleic	0.29	0.22	0.26
Malic	0.38	<0.10	<0.10
α-Ketoglutaric	0.37	0.18	0.20
Glycolic	0.49	0.19	0.23
Malonic	0.51	0.20	0.17
Tricarballylic	0.59	0.10	0.13
Lactic	0.65	0.38	0.42
Succinic	0.67	0.31	0.41
trans-Aconitic	0.67	0.14	0.11
Glutaric	0.77	0.48	0.57
Fumaric	0.79	0.43	0.32

a The R_f values are relative to movement of Sudan III as 1.00. These values are averages and show only the relative positions of the acids.

b Solvent A, 25 volumes of washed chloroform with 75 volumes of *n*-butyl alcohol, the mixture equilibrated with 0.1 its volume of 10% formic acid.

Solvent B, 90 volumes of methylene chloride with 8 volumes of *n*-propyl alcohol and 2 volumes of 85% formic acid.

Solvent C, 14 volumes of methylene chloride with 1 volume of 98% formic acid.

Procedure for Paper Chromatography. The circular paper chromatographic procedure followed was similar to that of Saifer and Oreskes (*7*) and of Airan *et al.* (*1*). Desiccators of 20- or 25-cm. inside diameter were used as chambers. The support for the filter paper was a 19-cm. diameter crystallizing dish or a 24-cm. glass pie plate. The rectangular wick (3 × 25 mm.) was cut radially from the center of the paper. The bottom of the Petri dish well was about 2 to 2.5 cm. below the filter paper, and the liquid level was adjusted to give flow rates of about 2 to 2.5 cm. per hour. Covers to hold the filter papers down were

not essential but were often used for the smaller papers. A satisfactory cover was made by placing a 20-cm. watch glass, convex side up, on top of the paper. A handle was attached through a hole cut in the center of the watch glass.

The free acids or their sodium salts were applied to the paper in 6- to 9-mm. diameter spots; these were equally spaced about the center of the paper in nine positions on a circle of 1.5-cm. radius. For further identification of acids from natural materials after column chromatography, a small amount of the titrated unknown acid was spotted adjacent to a mixture of authentic salts anticipated to be similar to the unknown. The authentic salts added were equivalent to about 5 γ of free acid per spot. A heavy spot of Sudan III in acetone was placed at the center of the paper. This dye closely followed the solvent front, and R_f values were measured using the leading edge of the dye front as the 1.0 reference point. The dye front was allowed to move at least 7 cm. before stopping the run. The solvents used are described in Table I.
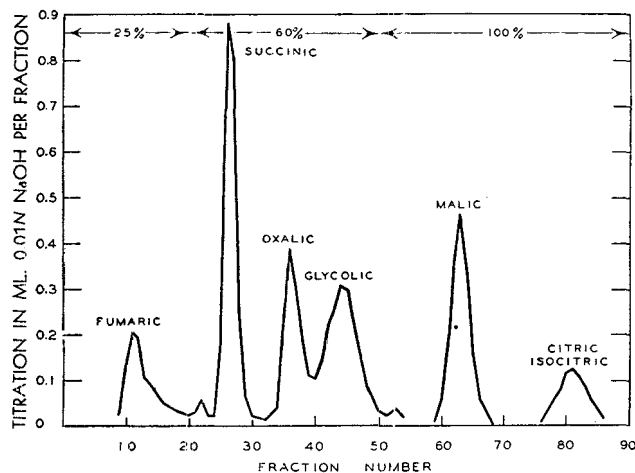

Figure 1. Separation of organic acids on a silicic acid column with increasing concentrations of 4-methyl-2-pentanone in methylene chloride

After the papers were removed from the desiccators, the excess solvent was allowed to evaporate for a few minutes in a hood and the papers were then placed for 20 to 30 minutes in an oven heated to 170° to 180° C. This high temperature removed formic acid by decomposition. The results of oven drying were equivalent to those from drying in an air stream overnight. The short time of heating did not affect the nonvolatile acid spots appreciably. Immediately after being heated, the papers were sprayed with bromocresol green (440 mg. per liter of 80% ethyl alcohol plus sodium hydroxide to a dark green color). The acid spots, which were yellow on a blue background, often were intensified by briefly reheating the papers and spraying them lightly with water or indicator solution. The bright spots lasted for several weeks, even though the papers were unprotected in the laboratory.

RESULTS AND DISCUSSION

Column Chromatography. Ketone solvents have proved useful in separating many organic acids on a single small column of silicic acid with only 3- to 4-hour running time. A typical separation achieved with this method described is shown in Figure 1. The volume percentages of 4-methyl-2-pentanone in the mixture with methylene chloride were: tubes 1 through 20, 25%; tubes 21 through 50, 60%; tubes 51 through 90, 100%. The small peaks at tubes 22 and 52 were shown by paper chromatography to be residual fumaric and glycolic acids which were pushed off abruptly by the change of solvent. A number of other acids overlapped the acids shown. The fractions in which they occurred were: lactyl lactic 7 through 10, glutaric 17 through 22, lactic 23 through 26, α-ketoglutaric 26 through 30, trans-aconitic 28 through 32, malonic 27 through 32, and cis-aconitic 52 through 54.

The column was able to handle at least 0.1 meq. of each of the acids. The coincidence of the citric and isocitric acid peaks is the most serious case in which ketones did not separate acids as well as did alcohols (4, 9). The system described suffered also from tailing of fumaric acid and incomplete separation of oxalic and glycolic acids. The use of a larger amount of silicic acid will improve the separations, but the solvent combinations employed probably will not separate all the overlapping acids appearing with succinic acid, nor will they separate citric from isocitric acid. trans-Aconitic acid was completely separated and lactic acid partially separated from succinic by collecting fractions 20 to 50 using 45 volume % of 4-methyl-2-pentanone in methylene chloride as the eluent. The range of solvent combinations was limited because the position of oxalic acid shifted from overlapping glycolic acid to overlapping succinic acid as the percentage of ketone was increased. However, the difficulties enumerated will not always be encountered because the overlapping acids often will not be present together.

If paper chromatography showed that succinic and α-ketoglutaric acids were both present, they were separated by chromatographing that fraction on a buffered silicic acid column (1 gram of silica plus 0.5 ml. of 7% potassium phosphate buffer pH 3.0; eluted with 20 vol. % of 2-butanone in 4-methyl-2-pentanone). Succinic acid was eluted in the third through sixth 0.5-ml. fraction followed by α-ketoglutaric acid in fractions 9 through 25.

Ketones tested other than 4-methyl-2 pentanone included acetone, 2-butanone, 3-pentanone, 2-octanone, 2,6-dimethyl-4-heptanone (diisobutyl ketone), and 2,4-dimethyl-3-pentanone (diisopropyl ketone). The 2,4-dimethyl-3-pentanone, being less polar than 4-methyl-2-pentanone, was a very good eluent for the more soluble acids but it was not available in large amounts. As ketones of increasing molecular weight were used, their polarity became too low to move citric acid, and their flow rates through the columns became slow. As the molecular weight of the ketone decreased, more nonpolar solvent was required to separate the organic acids. Even acetone-chloroform mixtures gave some satisfactory separations. None of the ketones alone or in combination with other ketones effected a good separation of succinic acid from oxalic acid. 4-Methyl-2-pentanone was a suitable solvent because it was readily available and because by itself it separated malic and citric acids.

Methylene chloride was used in combination with ketones to give the desired separations, and, in general, gave sharper acid peaks than did chloroform; however, the latter solvent was satisfactory if used at a starting concentration of 45% with 4-methyl-2-pentanone. Solvent integration (2), using upright and inverted pear-shaped separatory funnels as reservoirs, gave results similar to those obtained by successively changing solvents.

Paper Chromatography. Paper chromatography was useful for verification of the identity of acid peaks from a column. Because of the horizontal position in circular paper chromatography, the rate of flow of solvent was mainly dependent on the width of the wick and the distance between the paper and solvent surface. The latter dimension was not strictly standardized because it was more reliable to identify an acid by its position next to a standard than by its absolute R_f value under supposedly standard conditions. Identification with standards was achieved readily on the developed papers, because the radial flow and the solvents described produced sharp bands from the original spots. A minimum difference of 0.06 in R_f was required for separation of spots as reported by Rao and Dickey (6). During periods of low relative humidity the filter papers were too dry, and the spots developed with solvent C (Table I) were very diffuse. This was corrected by storing the filter paper in humid air.

Typical R_f values are shown in Table I. All listed acids except citric and isocitric acids were distinguished from the others by column and/or paper chromatography. Citric and isocitric acids may be separated on silica gel columns using 35% n-butyl alcohol in chloroform as eluent (9). This method of paper chro-

matography lent itself to the use of rapidly moving solvents, because their flow rates could easily be controlled by the width of the wick.

Chromatograms were completed within 2 to 4 hours and furnished a rapid check on information obtained by column chromatography. High-temperature drying quickly removed formic acid. Complete removal of formic acid eliminated obscuring background when the papers were sprayed with indicator and brought the sensitivity of a pH indicator spray within that of silver nitrate sprays (5). Five micrograms of any of the acids showed clearly, and 0.5 to 1 γ of most acids was detected.

ACKNOWLEDGMENT

The author is indebted to Eugene Herrling for assistance in preparing the illustrations.

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Titration of Elemental Sulfur with Solutions of Sodium Cyanide

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The purpose of this investigation was to determine whether the reaction between elemental sulfur and cyanide ion could be applied to the volumetric determination of sulfur in acetone extracts. It was found that such a titration is practical and that certain acid-base indicators can be used to detect the end point. The method is simple and rapid and requires only a single, fairly stable standard reagent. The accuracy which can be obtained by the proposed procedure is comparable with the other methods for this analysis.

MANY of the methods for the analysis of elemental sulfur require a preliminary separation of the element by extraction with acetone; such is the case with rubber products, plant spray residues, and sulfur-bearing ores. This is followed by determination of the sulfur in the acetone extract. Among the methods proposed for accomplishing the latter step is the oxidation of the sulfur to sulfate by bromine followed by gravimetric analysis by the usual procedures (7). Castiglioni (3) has proposed a volumetric procedure which involves conversion of the sulfur to thiocyanate in the acetone solution, followed by destruction of the excess cyanide with formaldehyde and titration of the thiocyanate with standard silver nitrate. Hardman and Barbehenn (4) recommend conversion of the elemental sulfur to cuprous sulfide by immersing a copper gauze in the acetone extract. The sulfide formed is then liberated with acid and determined iodometrically. Mark and Hamilton (6) have determined sulfur in acetone extracts by addition of an ammoniacal solution of cuprous sulfate. The cuprous sulfide formed is measured turbidimetrically. The ASTM method (1) for the determination of elemental sulfur in acetone extracts involves conversion of the sulfur to thiosulfate by prolonged heating with a solution of sodium sulfite. The thiosulfate is then determined iodometrically.

In some recent work on the colorimetric determination of small quantities of free sulfur in hydrocarbons (2) it was observed that the reaction between elemental sulfur and cyanide in aqueous acetone solutions proceeds rapidly and quantitatively toward the formation of thiocyanate. It occurred to the authors that this reaction might offer a rapid and simple method for the direct volumetric determination of sulfur in acetone extracts. An investigation has indeed disclosed that such a titration of sulfur

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with a standard solution of cyanide is entirely feasible. End-point detection can be accomplished readily because of the large change in hydrogen ion concentration which occurs when a slight excess of the standard cyanide is added to the solution. This change may be detected by the use of suitable acid-base indicators or by potentiometric measurements.

This method based upon the above appears to offer certain advantages over the other methods for the determination of sulfur in acetone extracts: The method is more rapid than most; only a single, fairly stable standard solution is required; and finally, an accuracy comparable with the other procedures can be obtained.

REAGENTS AND SOLUTIONS

Elemental Sulfur. Flowers of sulfur were recrystallized once from carbon disulfide, dried at 60° C., and stored over magnesium perchlorate.

Solvents. The acetone and isopropyl alcohol used in this work were of technical grade.

Organic Sulfur Compounds. The organic sulfides, disulfides, and mercaptans used were obtained from Eastman Kodak and were not subjected to further purification.

Standard Sulfur Solutions. Solutions containing known concentrations of elemental sulfur were prepared by refluxing weighed quantities of the purified sulfur in acetone until solution was complete. The resulting solutions were then transferred to volumetric flasks and diluted to the mark with acetone. Approximately 35 mg. of elemental sulfur will remain in 100 ml. of acetone at room temperature.

Sodium Cyanide in Isopropyl Alcohol, approximately $0.05F$. About 2.4 grams of sodium cyanide were dissolved in 200 ml. of water and diluted to about 1 liter with isopropyl alcohol.

Silver Nitrate, $0.05F$.

Bromocresol Purple Indicator, 1% solution.

Bromothymol Blue Indicator, 1% solution.

PROCEDURE

Standardization of Cyanide Solutions against Elemental Sulfur. Accurately weigh about 0.16 gram of recrystallized sulfur and dissolve in 400 ml. of acetone by refluxing. Cool and dilute to exactly 500 ml. with acetone. Transfer a 100-ml. aliquot of this solution to an Erlenmeyer flask, add about 20 ml. of water, and bring the solution just to boiling on a hot plate. Remove from the hot plate, add 3 to 4 drops of the bromocresol purple indicator, and titrate with the cyanide solution to a distinct bluish purple color. Reheat the solution. This should cause the indicator to return to the yellow-green color. Continue the additions of reagent and the heating until a permanent bluish

purple is obtained. Near the end point 20 to 30 seconds are required for the reaction to take place. Calculate the formality of the cyanide solution as follows:

$$F_{NaCN} = \frac{mg.\ of\ S\ taken}{ml.\ of\ NaCN \times 32.07 \times 5}$$

Standardization of Cyanide Solutions against Silver Nitrate. Transfer 50 ml. of the standard cyanide solution into a flask and add 150 ml. of water, 8 ml. of 6N ammonium hydroxide, and 0.6 gram of potassium iodide. Titrate to the first permanent turbidity with a standard 0.05F solution of silver nitrate. This end point corresponds to the reaction of two cyanide ions with each silver ion to give the complex $Ag(CN)_2^-$; the formal concentration of the cyanide solution can be calculated as follows:

$$F_{NaCN} = \frac{ml.\ of\ AgNO_3 \times F_{AgNO_3} \times 2}{ml.\ of\ NaCN}$$

Determination of Sulfur in Acetone Extracts. Take an aliquot of the acetone solution of such a size that it contains between 10 and 80 mg. of elemental sulfur. Add a volume of water equal to approximately one fifth the volume of acetone present. Bring the solution to boiling, add 3 to 4 drops of bromocresol purple indicator, and titrate as directed in the section on standardization of cyanide solutions against elemental sulfur. Calculate the milligrams of elemental sulfur in the aliquot as follows:

$$Mg.\ of\ S = ml.\ of\ NaCN \times F_{NaCN} \times 32.07$$

EXPERIMENTAL

End-Point Detection. In the proposed method of analysis, the end point is detected with an acid-base indicator. The sodium cyanide reagent is highly hydrolyzed in the aqueous acetone solvent, whereas the thiocyanate formed is not. As a result, the addition of a slight excess of the standard solution results in a marked decrease in the hydrogen ion concentration which can be readily detected.
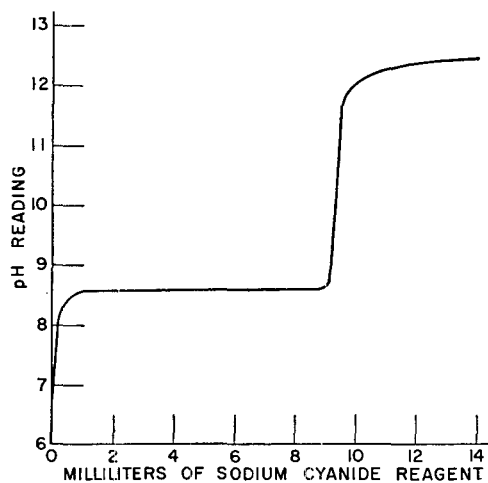

Figure 1. Titration curve for sulfur with sodium cyanide reagent

Concentration of cyanide solution 0.0462F; 13.6 mg. of sulfur

Figure 1 illustrates the magnitude of the changes in hydrogen ion concentration which occur in a typical titration. In this example, 13.5 mg. of elemental sulfur were dissolved in 100 ml. of acetone, about 20 ml. of water were added, and the solution was titrated with a 0.0462F solution of sodium cyanide. Empirical pH readings were obtained with an ordinary glass-calomel electrode system which had been calibrated against an aqueous buffer solution. A well defined end point was obtained.

Several acid-base indicators were investigated for possible

application to the titration. Two of these, bromocresol purple and bromothymol blue, exhibited sharp color changes at a point corresponding exactly with the potentiometric end point. Of the two, the bromocresol purple appeared to be somewhat more satisfactory and was used in most of the work reported herein.

Rate of Reaction. At room temperature, the rate of the reaction between cyanide ion and sulfur was found to be slow enough to make titrations inconvenient. For example, near the equivalence point at least 5 minutes were required for equilibrium to be achieved after addition of 0.1 ml. of reagent. However, by maintaining the temperature just at the boiling point of the solution, the reaction took place rapidly enough to make the titration practical. The higher temperature appeared to have no effect on the behavior of the indicators. At the boiling temperature, the reaction rate is such that 20 to 30 seconds must be allowed between additions of reagent when the titration is within 0.5 ml. of the end point. With a little practice, the titration can be easily completed in 5 minutes.

Table I. Comparison of Sulfur and Silver Nitrate as Primary Standards for Sodium Cyanide Solutions

Primary Standard	S Taken, Mg.	0.0639F AgNO₃ Used, Ml.	NaCN Used, Ml.	Formality of NaCN
S	77.50	...	10.37[a]	0.0465
S	73.50	...	9.86[a]	0.0466
AgNO₃	...	18.18	25.00	0.0465
AgNO₃	...	18.20	25.00	0.0465

[a] Volumes required for titration of aliquots equivalent to one fifth of sulfur taken.

Table II. Stability of Aqueous Isopropyl Alcohol Solutions of Sodium Cyanide

Time of Standing, Days	Formality vs. S
0	0.464
2	0.464
5	0.462
6	0.462
13	0.462
26	0.461

Standardization of Sodium Cyanide Solutions. Both elemental sulfur and silver nitrate were found to be suitable for the standardization of the aqueous isopropyl alcohol solution of sodium cyanide used as the standard reagent in the method. When sulfur was used it was found necessary to recrystallize the ordinary flowers of sulfur once from carbon disulfide before application as a primary standard. In standardizing against silver nitrate a modification of the Liebig-Denigès titration (5) for cyanide was used. The modification involved increasing the amount of potassium iodide and decreasing the quantity of ammonia recommended for the titration in aqueous solution. This was necessary in the presence of isopropyl alcohol as was shown by titration of several aqueous solutions of exactly known cyanide concentration, to which had been added amounts of isopropyl alcohol corresponding to the amount which would be present in the standardization of an alcoholic cyanide solution.

Table I shows a comparison of results of the standardization of a cyanide solution by the two methods. Nearly identical results are obtained and either substance is suitable as a primary standard. The data are of additional interest inasmuch as they indicate that the reaction between sulfur and cyanide proceeds quantitatively and involves a 1 to 1 ratio of the reactants.

Stability of Cyanide Solutions in Isopropyl Alcohol. During the preliminary work on this method, standard solutions of sodium cyanide in acetone were used. However, these were found to be unstable, decreasing in formality by several per cent each day. This lack of stability probably arises from reactions between the cyanide and acetone to give the cyanohydrin which may further decompose by hydrolysis. Solutions of sodium

Table III. Effect of Certain Compounds on Determination of Elemental Sulfur

Compound Added	Wt. of Compound, Mg.	Wt. S, Mg.	
		Present	Found
n-Butyl sulfide	200	17.80	17.87
Ethyl disulfide	40	15.60	15.60
n-Butyl mercaptan	20	23.04	21.05
	20	33.05	26.27
Petroleum ether	8000	15.60	15.60

Table IV. Analysis of Acetone Solutions of Elemental Sulfur

Sulfur, Mg.		Relative Error, %
Taken	Found	
7.80	7.79	0.1
10.10	10.11	0.1
15.60	15.53	0.5
15.60	15.60	0.0
15.60	15.64	0.3
15.60	15.55	0.3
23.40	23.49	0.4
31.20	31.20	0.0
	Average	0.2

cyanide in a solvent consisting of 80% by volume of isopropyl alcohol and 20% by volume of water were found to be remarkably stable. This is illustrated in Table II. These data indicate that the alcoholic cyanide solutions are considerably more stable than simple aqueous solutions of sodium cyanide which are reported to decrease in formality by 0.3% per day (5).

Effect of Water Concentration on Titrations. It was found desirable to have between 15 and 20% by volume of water in the acetone solvent at the beginning of the titration. With smaller amounts of water, there was a tendency for sodium cyanide to precipitate and become unavailable for reaction with the sulfur. Concentrations of water higher than 20% appeared to cause the rate of the reaction to decrease appreciably.

Interferences. The effect of a number of substances on the proposed procedure was investigated. Table III shows that an excess of either a typical aliphatic sulfide or disulfide does not cause any alteration of the results. On the other hand, mercaptans do interfere. This interference undoubtedly results from the reaction between the mercaptans and elemental sulfur which is catalyzed by the basic reagent.

Metallic ions such as silver, mercury, or cadmium interfere with the titration by forming stable complexes with the cyanide reagent. Apparently the complexed cyanide ions do not readily react with the sulfur.

Results. Table IV shows results obtained by the proposed method of aliquots of acetone solutions containing known quantities of elemental sulfur. The average relative error of these determinations was found to be 0.2%. The standard deviation was 0.3%.

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Iron(II) Perchlorate as a Reductant in Glacial Acetic Acid

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A reducing agent was required which could be used for nonaqueous titrations of oxidants. Iron(II) perchlorate in glacial acetic acid was satisfactory for the determination of chromium trioxide and sodium permanganate in glacial acetic acid without the addition of water. An amperometric end point with two active electrodes was most suitable.

STUDIES still in progress in this laboratory led to the investigation of iron(II) perchlorate as an analytical reagent for the determination of oxidants in glacial acetic acid. Aqueous iron(II) perchlorate has received limited attention as a reductant in the establishment of oxidation potentials of various cerium(IV) complexes (1). Iron(II) chloride has been used in glacial acetic acid (3), but its low solubility in this solvent limits its applicability. Iron(II) perchlorate is much more soluble; solutions in excess of 0.1N with respect to iron(II) are easily prepared.

Using acetic acid solutions of iron(II) perchlorate standardized against aqueous potassium dichromate, solutions of sodium permanganate and chromium trioxide in glacial acetic acid can be analyzed without the introduction of aqueous reagents.

REAGENTS AND APPARATUS

Baker's analyzed acetic acid was further purified by distillation away from chromium trioxide followed by a second distillation from potassium permanganate. The iron(II) perchlorate hexa-hydrate and perchloric acid (70%) were obtained from the G. Frederick Smith Chemical Co. Baker's analyzed chromium trioxide and Fisher Scientific Co. C.P. grade sodium permanganate were used as oxidants.

A Fisher Elecdropode, sensitivity 0.025 μa. per scale division, equipped with 2-cm. 18-gage platinum wire electrodes, was used for detection of the equivalence point (2). The solution being titrated was stirred under a nitrogen atmosphere with a magnetic stirrer.

The iron(II) perchlorate solutions were prepared in the following manner: Acetic anhydride in slight excess over that necessary to react with the water present in the iron(II) perchlorate was added to the acetic acid. After the acetic acid had been flushed with nitrogen, the approximate weight of iron(II) perchlorate hexahydrate was added. This solution was left under a nitrogen atmosphere for a minimum of 2 hours but frequently much longer. A measured volume of this solution was added to a solution of 5 ml. of 85% phosphoric acid in 20 ml. of water. The resultant solution was analyzed by titrating to the diphenylamine color change with a solution of primary standard potassium dichromate (4). A potentiometric titration showed the equivalence point to be coincident with the color change when the iron(II) solution was titrated under these conditions.

In the experimental work the iron(II) perchlorate solutions were used for the analysis of acetic acid solutions of sodium permanganate and chromium trioxide.

In the permanganate studies an approximate weight of sodium permanganate was added to the desired amount of acetic acid. Sodium permanganate was used in preference to the corresponding potassium salt because of its much greater solubility in this medium. The actual concentration of the solution prepared in this manner was found by titrating a weighed quantity of

primary standard sodium oxalate in the usual manner. This titration was done in acetic acid as well as in aqueous media; in an aqueous medium acidified with 2 ml. of sulfuric acid in 25 ml. of water, it gave a better end point. Regardless of the medium, the fading of the permanganate color at the equivalence point makes the end point somewhat indeterminate. By observing the persistence of the permanganate color for 45 seconds, the values for the sodium permanganate concentration were obtained.

The solutions of chromium trioxide were prepared by adding the approximate weight of chromium trioxide to the desired quantity of acetic acid. The chromium trioxide was standardized by adding a measured volume of the acetic acid solution to an excess of 10% aqueous potassium iodide in a glass-stoppered flask. The reaction mixture was left in the dark for 30 minutes. The liberated iodine was then titrated to a starch end point with aqueous standard sodium thiosulfate.

STABILITY OF IRON(II) PERCHLORATE

When no precautions are taken, acetic acid solutions of iron(II) perchlorate are slowly oxidized by air. This is to be expected, because oxygen is much more soluble in glacial acetic acid than in water. Evidence for the relative stability of the iron(II) solutions stored under nitrogen and under air is presented in Table I. In order to minimize decomposition, it is desirable to store the solution under an atmosphere of nitrogen. When passed through the solution being titrated, nitrogen minimizes air oxidation of the iron(II) which would lead to high values for the concentration of oxidant. Utilizing nitrogen, the equivalence point is sharp and definite, yielding more accurate and reproducible results.

Table I. Stability of Iron(II) Perchlorate

Under Air		Under Nitrogen	
Days	Normality	Days	Normality
0	0.0265	0	0.0265
1	0.0258	1	0.0264
3	0.0240	3	0.0265

Table II. Sensitivity at Equivalence Point[a]

Potential, Mv.	$\frac{\Delta i}{\Delta Ml.}$, $\frac{\mu A.}{Ml.}$
50	1.2
75	3.6
100	5.5
125	7.9
150	10.2
175	10.4
200	10.5

[a] 50 ml. of $0.0102N$ $Fe(ClO_4)_2$ titrated with 25.20 ml. of $0.0194N$ $NaMnO_4$.

DETECTION OF EQUIVALENCE POINT

Potentiometric measurements with platinum-calomel electrodes were too unstable for practical use. The solution being titrated was too highly colored under the conditions employed to permit the utilization of color indicators because of the deep red-brown color of the iron(III) formed during the titration. However, an amperometric method with two active electrodes was possible for detecting the equivalence point. To increase the sensitivity, 0.5 ml. of 70% perchloric acid was added to the solution and a potential of 150 mv. was applied across the electrodes. This value was obtained experimentally from the data given in Table II, 150 mv. being the minimum potential giving the greatest sensitivity under the conditions employed.

Sulfuric acid and 85% phosphoric acid were tried in lieu of perchloric acid, but the results were unsatisfactory. When sulfuric acid was used, a precipitate was formed, causing erratic results. The solution remained clear when phosphoric acid was used, but the titrations gave values too low compared with the standardization and the results were not reproducible.

DETERMINATIONS WITH IRON(II) PERCHLORATE

Sodium permanganate was determined by titrating a measured volume of the iron(II) perchlorate solution with the permanganate

Table III. Determination of Sodium Permanganate

N of $Fe(ClO_4)_2$	Days	N of $NaMnO_4$ from $Fe(ClO_4)_2$ Titration	N of $NaMnO_4$ from $Na_2C_2O_4$
0.1103	0	0.0901	0.0897
0.1103	0	0.0900	0.0903
0.1103	1.5	0.0637	0.0635
0.1103	1.5	0.0636	0.0637

Table IV. Determination of Chromium Trioxide

N of $Fe(ClO_4)_2$ by $K_2Cr_2O_7$	N of CrO_3 from $Fe(ClO_4)_2$ Titration	N of CrO_3 from Iodometric Determination
Titration of Standard $Fe(ClO_4)_2$ Solution		
0.0679	0.1087	0.1089
0.0679	0.1088	0.1089
0.0679	0.1087	0.1089
0.0710	0.0683	0.0684
0.0710	0.0684	0.0684
Titration of CrO_3 with Standard $Fe(ClO_4)_2$		
0.0710	0.0685	0.0684
0.0710	0.0684	0.0684
0.0710	0.0683	0.0684
0.0710	0.1087	0.1089
0.0510	0.1159	0.1161
0.0510	0.1158	0.1161

solution or by adding excess iron(II) to the permanganate solution and back-titrating the excess iron(II) solution. This procedure was adopted in preference to the direct titration of permanganate with the iron(II) solution, since the decomposition of the sodium permanganate is accelerated by the addition of perchloric acid.

A desired volume of the standardized iron(II) perchlorate solution was pipetted into the titration beaker containing enough acetic acid to cover the electrodes while nitrogen was being passed through the solvent. To this solution 0.5 ml. of 70% perchloric acid was added, and the titration was carried out with the sodium permanganate solution of unknown concentration. The titration is conducted by adding the reagent rapidly at first and then dropwise as the equivalence point is approached. The approach to the end point is indicated by the magnitude of the decrease in the galvanometer reading. In the course of the titration, the current flow reaches a maximum, then decreases, and finally falls off to zero at the end point. With this value a calculation of the permanganate concentration is made.

The results are presented in Table III. The values derived from the nonaqueous titration of permanganate with iron(II) perchlorate solutions are in good agreement with those found from oxalate titrations within the limits of error of the methods.

The two values obtained are indicative of the stability of the permanganate solutions. In spite of the precautions taken for purifying the acetic acid, a significant amount of decomposition occurs in a relatively short time. Restandardization immediately prior to use is therefore necessary.

Table IV presents the results found in the analysis of chromium trioxide solutions. These data show the feasibility of a direct titration of the chromium trioxide solution with one of iron(II) perchlorate or by the reverse procedure—i.e., titrating a known concentration of iron(II) with the chromium trioxide solution. With both titrations the results are in good agreement with those obtained iodometrically.

The titrations were conducted in essentially the same manner as in the permanganate analyses. The solution being titrated is acidified with 0.5 ml. of perchloric acid and the titrant is added with nitrogen flowing throughout the titration. When the iron(II) solution is being titrated, the galvanometer behaves in the same manner as in the permanganate titration, the current flow passing through a maximum and proceeding to zero at the end point. In titrating the chromium trioxide solution, the galvanometer shows the current passing through a small maximum

and falling off to zero as the end point is reached. The next addition of the iron(II) solution causes a large increase in the current flow.

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Measurement of Refractometric Dry Substance of Sucrose Solutions

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Accuracy of refractometric readings is important in controlling purity and concentration of liquid sugar products. An evaluation has been made of the accuracy and precision of readings for two commercial refractometers. The standard deviation of the error of observation is 0.03% solids for the Bausch and Lomb precision sugar refractometer and 0.07% solids for the Zeiss sugar refractometer. The study indicates a small error in the international scale of refractive indices of sucrose solutions in the range from 50 to 75% sucrose, about 0.07% solids at 66% solids. This is in qualitative agreement with findings of others and points to the need for additional work to provide a sound basis for revision of the scale.

REFRACTOMETERS are employed widely in the sugar industry to measure the dissolved solids content of both pure and impure sucrose solutions. Generally, a sugar industry laboratory may be expected to have several of these instruments, sometimes including a variety of models. Refractometers may be located at plant operating stations or in routine control laboratories to provide information for prompt process or product control, and they may find application in research laboratories for process study or other investigations. The result of a refractometric measurement may be used directly as an expression of concentration in per cent solids by weight or it may be used as part of other laboratory procedures—for example, in purity determinations or an adjustment of solutions for color readings.

Although the principal emphasis of the present discussion is in reference to the sugar industry, the data can be recalculated for use in other processing industries. Almost without exception, the industrial instruments in use in these industries in this country fall into the classification of critical angle refractometers.

Refractometers which are now commercially available give very satisfactory results, particularly for routine analytical work. On occasion, however, when more precise results are desired, certain questions of procedure and instrument accuracy arise. Some of these problems have been investigated recently in this laboratory and this paper discusses the results. In particular, two factors have been of primary concern: the determination and correction of errors in zero point adjustment; and the relative accuracy of the Zeiss sugar refractometer (Figure 1) and the Bausch & Lomb precision refractometer (Figure 2). In investigating the latter problem, discrepancies were encountered in the international scale for converting refractive index to per cent sucrose. Inasmuch as this experience parallels that of other observers, this point has also been investigated in some detail.

GENERAL BACKGROUND

The refractive index of a sugar solution is dependent on the concentration of the solution. Consequently, tabulations have been developed relating index of refraction to per cent sucrose in pure sucrose solutions (7) and the sucrose content of such solutions may be determined by reading the refractive index. In fact, some instruments have a per cent sucrose scale mounted directly on the instruments.

While this scale is correct for pure sucrose only, the effect exerted upon refractive index by other sugars and soluble impurities is near enough to that for sucrose to make the reading on this sucrose scale extremely useful even for impure solutions. Refractometers are accordingly utilized to measure the solids content of process liquors and of final liquid products in cane sugar refineries and beet sugar factories. They are also used as
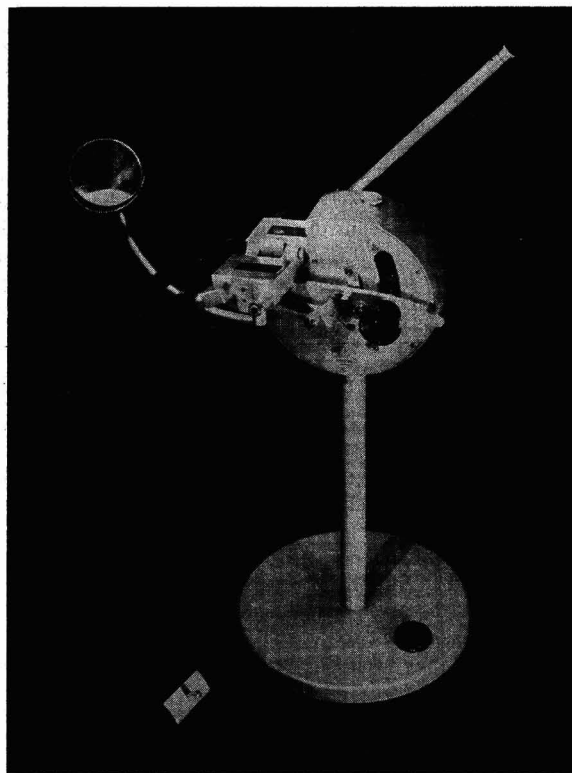

Figure 1. Zeiss sugar refractometer

analytical tools in the determination of apparent purity of liquid and solid sugar products. In this latter procedure, an approximation to per cent sucrose is obtained by a polariscope reading while the solids content is indicated by a refractometer reading. The quotient of these values gives apparent purity.

For many applications an accuracy of 0.1 or 0.2% solids is adequate. However, greater accuracy is sometimes desired, particularly for certain investigative problems and for apparent purity determinations of high purity liquid products. It was particularly the desire to improve the accuracy of refractometer readings for the latter purpose that led to the investigation described herein. Variations of the order of 0.1 or 0.2% in refractometer readings are too great if usefully accurate purity values are to be determined for products whose total impurity is only 0.2 or 0.3%.
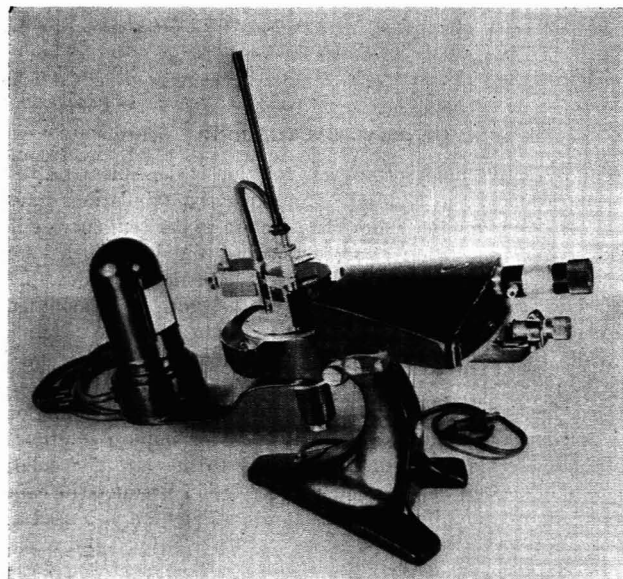

Figure 2. Bausch & Lomb precision refractometer

The approach to this problem has been almost entirely practical and operational. There has been no attempt to determine and analyze prism or optical characteristics of the instruments under consideration. The primary objective was to determine whether the Zeiss sugar refractometer, which had been used for many years for routine control purposes, could be satisfactorily employed to obtain the more accurate determinations necessary for the purity analyses, or whether it would be desirable to use the reportedly more accurate Bausch & Lomb precision refractometer.

GENERAL LITERATURE REVIEW

A review of the literature indicates little material bearing directly on the aspect of refractometry discussed here. Weissberger (12) has reviewed theory, techniques, and instrumentation. Tilton (11) has analyzed the errors in commercial refractometry. Application of refractometers to sugar analysis is discussed in several handbooks: Browne and Zerban (5), Spencer and Meade (10), a National Bureau of Standards circular (1). Forrest (6) has discussed instrumental design for the Bausch and Lomb precision refractometer. Finally, directions for use of the instruments have been supplied by the manufacturers (2–4, 14).

The relationship between per cent sucrose and refractive index has received considerable attention. The international scale for sucrose solutions was adopted by the ninth session (1936) of the International Commission for Uniform Methods of Sugar

Analysis (7). This scale has been placed on most instruments bearing a sugar scale since that time. It received further consideration at the 1953 meeting of the U. S. National Committee of this commission (8).

DISCUSSION OF INVESTIGATION

The investigation involved the zero point (distilled water) calibration of the instruments, the relative accuracy and precision of the instruments in terms of per cent sucrose, and the calibration of the Bausch & Lomb refractometer in terms of refractive index and consideration of apparent inaccuracies in the international scale above 50% sucrose. The results of this investigation are considered in sequence in the following sections.

Determination of Distilled Water Corrections. Refractometers as supplied by the manufacturer have scales which are sufficiently accurate for many purposes without the application of scale corrections. However, for maximum accuracy it may be necessary to make a detailed study of the scale in order to provide information to correct for the minor but possibly significant errors in scale calibration. These errors are of two principal types: those resulting from inaccurate positioning of the scale with reference to some basic point and errors from the unavoidable slight inaccuracies in engraving scale divisions. Refractometer calibrations for the purpose of determining these errors may include readings with one or more of the following standards: glass blocks of accurately known refractive indices; distilled water; organic liquids of accurately known indices; and sugar solutions of accurately known composition.

The manufacturer usually provides a single glass block or plate with each instrument as a standard for positioning of the scale. However, because of its convenience, because it is easier to read, and because it is the normal solvent for sugar, distilled water is often used as a reference standard for this purpose. Any of the other reference standards mentioned could be used to establish the proper scale position.

Scale calibration with sugar solutions is discussed here in connection with the determination of relative accuracy of the two instruments studied, while the use of glass plates and organic liquids is considered as part of the calibration of the precision refractometer in terms of refractive index. At this time, however, consideration is given to the use of distilled water readings in establishing the appropriate corrections for inaccuracy of scale positioning.

Distilled water readings are made on refractometers as a matter of routine control. When significant discrepancies are detected between the observed value and the true value (0.0% solids or the corresponding value on arbitrary scales), an adjustment is made mechanically in the position of the scale. For most purposes, this is adequate. However, such adjustment involves some measure of uncertainty and, furthermore, observers differ in their interpretation of the zero point. This latter is a point which merits elaboration. Since the two sides of the critical shadow line, dark and light, are not symmetrical in appearance, different observers might set the index line in slightly different positions, particularly if the shadow line is not razor sharp. A particular observer might be expected to show the same bias whether reading water or sugar solutions. Therefore a distilled water correction should at least partially compensate for such personal bias.

Thus for readings of the highest accuracy which naturally involve replicated readings, a measurable zero point error is normally observed. However, there was some question regarding the proper procedure for calculation of corrections to observations on sugar solutions on the basis of measured zero point errors, particularly as the scales for refractive index and per cent solids are not linear. The basic problem, therefore, was the determination of suitable corrections for zero point displacements. These determinations took two forms. In one case, the zero point was
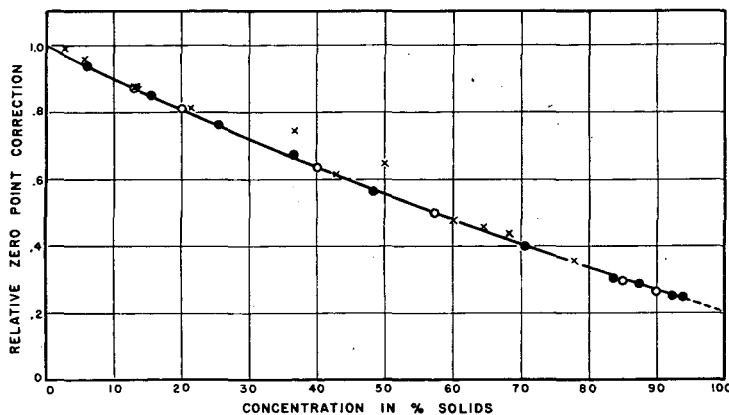
Figure 3. Relative zero point corrections for Zeiss refractometer

X. Measured by observing sugar solutions before and after shifting scale
● From eyepiece field measurements
○ From hair length measurements

A - SCALE READS CORRECTLY
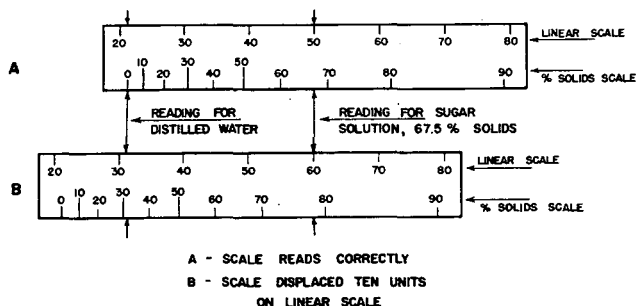B - SCALE DISPLACED TEN UNITS ON LINEAR SCALE

Figure 4. Effect of scale displacement of precision refractometer

deliberately displaced by an exaggerated amount and the effect on readings at other points on the scale was observed. The second approach involved an analysis of the relative length of scale units at various positions on the scale and a subsequent calculation of the relative effect of a shift in the zero point.

ZEISS REFRACTOMETER. The zero point of the Zeiss refractometer was displaced by an amount equivalent to about 6% solids at the zero point. A series of 10 sugar samples was read on the refractometer before and after this zero point displacement. The differences in the readings on the sugar solutions were then expressed as fractions of the zero point displacement and are plotted in Figure 3. Also plotted in Figure 3 are two sets of points obtained by direct observation of the spacing of scale marks, in one case as compared with a short length of hair, in the other case compared with the distance from the index line in the center of the field to the top of the field as observed in the eyepiece. The displacements or corrections (relative to that at the zero point on the scale) as obtained by the three methods agree well.

For the Zeiss refractometer, then, the correction to be applied at any point on the scale for slight displacements in the zero point may be obtained by multiplying the relative correction for that point on the scale, as determined from Figure 3, by the actual zero point displacement. Obviously, if the zero point is displaced in the negative direction the correction is additive. To illustrate the use of Figure 3, suppose that the mean of a number of observations of the distilled water reading is +0.050% solids. Then, if the sugar solution has a mean reading of 66.000% solids, the zero point correction is −0.44 × 0.050% solids. The corrected reading is 65.978% solids.

PRECISION REFRACTOMETER. The Bausch & Lomb refractometer differs from the Zeiss refractometer in that it does

not have a per cent solids scale directly on the instrument. Instead, it is provided with an arbitrary scale, which, by means of a suitable conversion table, may be translated to per cent solids. The arbitrary scale is linear with respect to the arc on which it is mounted, both for convenience in etching and so that a vernier can be used. That an adjustment of the index causes uniform change in scale reading was verified by test.

However, the relationship between scale units and per cent solids is not linear. This relationship is shown in Figure 4, along with the effect of a highly exaggerated displacement of the scale. For example, a displacement of 10 units on the linear scale corresponds with a displacement of 31 units in per cent solids at 0% solids and only about 11 units in per cent solids at 67.5% solids.

The curve of Figure 5 has been calculated directly from the data in the precision refractometer conversion tables (4). Figure 5 is similar to Figure 3 for the Zeiss refractometer and it gives the correction for any point on the per cent solids scale relative to the displacement observed at 0% solids. In practice, in the case of the precision refractometer, the zero point correction could be applied as a uniform correction to the arbitrary scale reading. However, for results already expressed as per cent solids, Figure 5 is useful.

A comparison of Figures 3 and 5 for the two refractometers indicates the same trend but a difference in magnitude. This difference is attributable to the difference in refractive index and measuring angle of the prisms used in the two instruments. Thus, for instruments of different design or appreciably different prism constants, the relative zero point corrections would be expected to be different.
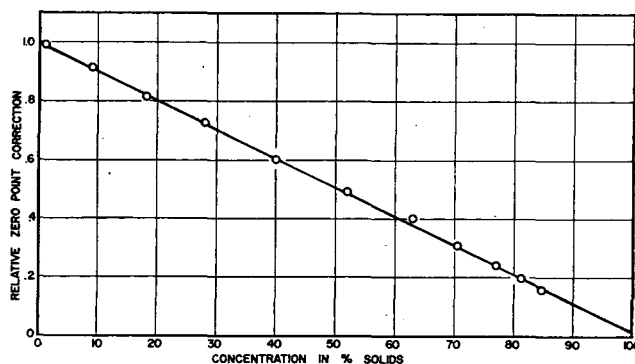
Figure 5. Relative zero point corrections for precision refractometer

The foregoing results could also have been secured from the basic equations for a critical angle refractometer. The following formula, whose derivation was given in an early edition of a Bausch & Lomb Catalog (2), expresses the relationship between refractive index of the unknown and the angle, measured from the normal, of the light emerging from the prism:

$$n_x = \sin b \cos a + \sin a \sqrt{n_g^2 - \sin^2 b} \qquad (1)$$

in which

n_x = refractive index of unknown sample
n_g = refractive index of glass measuring prism (constant)
a = refracting angle of the prism (constant)
b = angle from the normal of light emerging from the prism

Equation 1 can be differentiated to give the following for a and n_g constant

$$\frac{dn_x}{db} = \cos a \cos b - \frac{\sin a \sin b \cos b}{\sqrt{n_g - \sin^2 b}} \qquad (2)$$

The term dn_x represents the increment of refractive index corresponding with an increment in the angle b (measured in radians).

Since a and n_g are constants for any particular measuring prism, it is evident that Equation 2 can be used to compute relative increments in terms of refractive index, providing the constants are known. Such calculations have been made for the precision refractometer using Forrest's (6) values for a and n_g. The results, as would be expected, were essentially identical to those derived from a consideration of the scale relationships.

Comparison of Precision and Accuracy of Zeiss and Precision Refractometers. The particular instruments compared were, first, the Bausch & Lomb precision refractometer, Model 33-45-01, prism series 711, and instrument serial 7722. A varitran Model V-1M autotransformer and a Sola constant voltage transformer were used in the light circuit to provide constant illumination and to provide the stable voltage required to start the sodium light.

The Zeiss refractometer (Model 1, serial 138994) prisms were illuminated by light from a 100-watt incandescent bulb located about 1 foot from the mirror.

The comparison involved a determination of both the relative reproducibility of readings and the absolute accuracy of the refractometers.

TESTS FOR PRECISION. The initial tests were made to determine the reproducibility of measurements on the refractometers. Five observers participated in this phase of the test program.

Test samples were prepared as follows: Confectioner's sugar (a California and Hawaiian Sugar Refining Corp. coarse-grain sugar boiled from high purity liquor, the purest sucrose readily available) was dissolved in water to give approximately the desired density (no attempt was made at this time to obtain an exact concentration of sugar). The solution was shaken in a separatory funnel until the absence of striae indicated homogeneity. Then some 40 or 50 small vials were filled and closed immediately with rubber stoppers. In order to minimize evaporation, the samples were kept until used in a glass desiccator in which, in place of the usual desiccant, a dish of the solution corresponding to that in the vials was exposed to the atmosphere. A number of samples were prepared in this way and read on the refractometers to cover a broad range of densities.

Each observer made three readings on each instrument. A sample from a new vial was used for each reading. Thus, each observer used six vials of each sample. If there appeared to be some distinct irregularity about a particular reading, that reading was discarded and a sample from a new vial was used. A medicine dropper was employed by most observers to introduce the samples to the prism cavity, although some observers preferred to use a hard rubber rod to spot samples on the Zeiss prism. In all cases, readings were estimated as closely as possible and recorded results, after temperature corrections, were kept to the nearest 0.01% solids. Average and standard deviations were carried to 0.001% solids.

From the 15 separate observations for each sample and each refractometer it was possible to calculate the standard deviation of a single observation at each concentration of the sugar solutions. (Statistical analysis showed significant differences between observer means as compared to the within-observer variances. It may be expected, therefore, that for any single observer the standard deviation of observation would be somewhat less.) These standard deviations for the two instruments have been plotted in Figure 6. A quick examination of this graph indicates that the standard deviations for readings with the precision refractometer are somewhat less than half those with the Zeiss refractometer. In fact, the standard deviation with the precision refractometer varied from 0.02% with distilled water up to about 0.04% with solutions of more than 70% solids. The standard deviation of observations with the Zeiss refractometer varied from about 0.04 to 0.08% for the same solutions, although in one case the calculated standard deviation was as

Table I. Comparison of Observed Sensitivity with Reported Sensitivity

Instrument	Reported by Manufacturer		Obsd. % Solids
	Refractive index	% solids	
Zeiss	0.0001 –0.0002	0.1–0.2 (14)	0.04–0.08
Bausch & Lomb precision	0.00003–0.000015 (6)	0.2–0.007	0.02–0.04
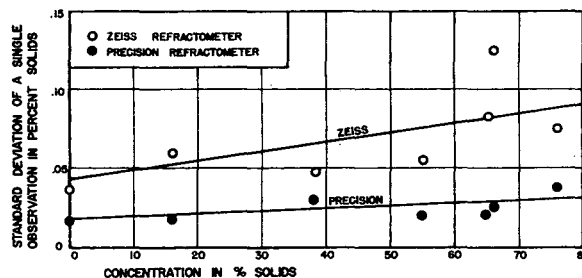
Figure 6. Comparative precision of Bausch & Lomb precision refractometer and Zeiss sugar refractometer

high as 0.12% solids. From these data it may be concluded that the precision refractometer will give results with less than half the error of the Zeiss refractometer when each instrument is read carefully.

It may be of interest to compare these observed sensitivities with those stated in the manufacturers' literature. This comparison is shown in Table I.

In general, the Zeiss instrument was shown to be more precise and the precision instrument somewhat less precise than stated. However, these tests in this laboratory were made on sugar solutions under specific conditions which, in all probability, differ from the manufacturer's conditions. It should not be assumed that the comparison is valid for all conditions.

In particular, per cent solids readings at higher points on the scale should be more reproducible (6). Reference to Figures 3 and 5 shows that the per cent solids scale markings for higher concentrations are spread farther apart. If it is only a matter of reading the angle, results should be more precise at higher per cent solids readings.

On the other hand, Figure 6 shows a reverse trend for both refractometers; random error in terms of per cent solids appears to be greater for high concentrations. Correspondingly, it has been observed that the shadow line for sugar solutions becomes less sharp and distinct for the higher concentrations. Whether this is due to inadvertent evaporation causing nonhomogeneity of sample or whether it is due to decreased resolution for larger angles is not clear.

TESTS FOR ACCURACY. While the foregoing tests indicated the relative precision of the Zeiss and Bausch & Lomb refractometers, they of course did not permit conclusions with respect to the absolute accuracy of the two instruments. Tests in regard to this latter point required the preparation of solutions of accurately known per cent solids composition and the determination of the readings obtained from the instruments for such samples. Accordingly a number of samples were very carefully prepared by the following procedure:

A Florence flask weighing about 85 grams, with a capacity of about 300 ml. and provided with a tapered ground-glass stopper, was carefully washed, dried, cooled, and weighed with the stopper. Confectioner's sugar, previously dried in a desiccator, was funneled into the flask so that no sugar dust adhered to the neck of the flask. The flask was stoppered and weighed. Then distilled water in sufficient quantity to give the desired density was pipetted into the flask without wetting the neck. The flask was again stoppered and weighed. The dry stopper was removed and

replaced immediately with a second ground glass stopper which had been coated with petrolatum to prevent evaporation. The flask was shaken to dissolve the sugar. For more concentrated solutions (above 65% solids), warming was employed to obtain complete solution. The flask was then cooled to near the dew point and unstoppered and the neck was wiped clean of the petrolatum. The sample was poured quickly into a dry separatory funnel and vials were filled as before. In this case, however, it was possible to fill only some 12 to 15 vials, so only two observers were employed in the tests.

From the weight of sugar and water employed, it was possible to calculate exactly the solids contents of the various solutions. These were recorded as concentrations determined in air with brass weights. (This is the basis on which per cent solids–refractive index relationships are normally reported.) A sufficient number of samples was used to give adequate coverage of the entire density range.

Each observer read samples from two or more vials in each sample set until he felt he had obtained accurate readings. His results were averaged for each sample set and for each refractometer. Then an average for the sample was obtained from the individual averages for the two observers. Finally the differences of these composite averages from the calculated per cent solids value for each sample were determined. These have been plotted in Figure 7. It may be noted that these observations indicated that both instruments would generally provide results within 0.5% solids of the correct value over the full density range. Thus, each instrument appeared to be in reasonably satisfactory adjustment.

Each of these instruments has a zero point displacement. Consequently, Figure 8 has been prepared showing refractometer readings corrected for zero point displacement in the manner discussed. The Zeiss refractometer gave values which were slightly less than the correct values and the Bausch & Lomb refractometer gave values which were somewhat greater.

Within the limits for precision, both refractometers appear to be equally accurate below about 50% solids. Above this point, readings on this particular precision refractometer appear to be slightly less accurate. This unexpected result led to a rather careful evaluation of this upper portion of the scale.

Figure 8. Comparative accuracy of Bausch & Lomb precision refractometer and Zeiss sugar refractometer with zero point correction
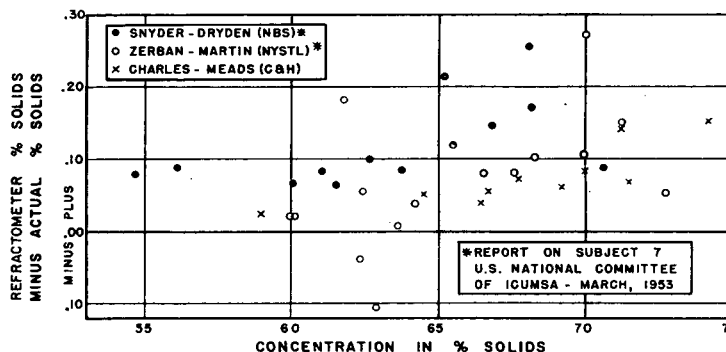
Figure 9. Apparent error of international scale for converting refractive index to per cent solids

Figure 7. Comparative accuracy of Bausch & Lomb precision refractometer and Zeiss sugar refractometer without zero point correction

Accuracy of International Scale of Refractive Indices. The accuracy of per cent solids determinations from refractometric readings depends, in addition to the factors already discussed, upon the soundness of the conversion from index of refraction to per cent solids. The conversion currently in use is based on the international scale adopted by the International Commission for Uniform Methods of Sugar Analysis in 1936 (7). This conversion has been assumed to be the best available up to this time. Recently in a report of the subcommittee, it was pointed out that there appears to be considerable uncertainty as to the accuracy of the international scale at the higher densities (8). Work of

Zerban and Martin at the New York Sugar Trade Laboratory and Snyder and Dryden at the National Bureau of Standards has shown deviations in the range from 50 to 75% solids which are in the same direction as those noted in the present investigation and plotted in Figure 8.

The data of these other observers have been plotted in Figure 9, along with corresponding data from the present investigation. Systematic positive deviations from the international scale are shown for the region from 54 to 75% solids. The wide scattering of these results is disturbing and prevents definite conclusions at this time as to the precise value of errors in the international scale. Results of this investigation appear to be somewhat more consistent than those of other observers; also the apparent deviations from the international scale are smaller than those of others.

In considering these data for sugar solutions, it should be noted that they have been obtained in the region of near saturated and supersaturated solutions. Such solutions involve the greatest technical difficulties in securing precise results. For instance, it is necessary to heat the sugar and water mixture to effect solution. In doing this, it is essential to guard against decomposition and evaporation.

The apparent deviations shown in Figure 9 represent differences between observations of per cent solids readings and per cent solids calculated from the known weights used in preparing the solutions. In the case of the results of the other investigators, the statement is made that a carefully calibrated Bausch & Lomb precision refractometer was used with no mention of how the calibration was performed. In the case of the results from this laboratory, the data shown in Figure 9 were taken from Figure 7 with all points being corrected for zero point displacement.

TEST OF REFRACTIVE INDEX CALIBRATION OF PRECISION REFRACTOMETER

The deviations of Figure 9 represent error in the international scale only if the refractometer reads correctly in terms of refractive index. To check the refractive index calibration of the

Table II. Observations with Glass Test Pieces

(Apparent error[a] in refractive index and equivalent deviation in terms of per cent solids.
Precision refractometer Serial No. 7722)

Plate A 1.43382 (56.4% Solids)		Plate B 1.46210 (68.8% Solids)		Plate C 1.46617 (70.4% Solids)		Plate D 1.46585 (70.3% Solids)	
R.I. × 10⁶	% solids	R.I. × 10⁶	% solids	R.I. × 10⁶	% solids	R.I. × 10⁶	% solids
+39	+0.018	+89	+0.037	+63	+0.026	+83	+0.035
+25	+0.012	+66	+0.027	+39	+0.017	+78	+0.033
+01	+0.001	+69	−0.029	+45	+0.019	+61	+0.026
+29	+0.013	+64	+0.027	+04	+0.002	+63	+0.026
−17	−0.008	+48	+0.020	+42	+0.018	+78	+0.032
−15	−0.007	+76	+0.032	+43	+0.018	...	...
−27	−0.017	+78	+0.032	+41	+0.017	...	...
+02	+0.001	+40	+0.017	+38	+0.016	...	...
Mean +03	+0.002	+66	+0.028	+39	+0.017	+73	+0.030

[a] Observed refractive index (corrected for zero point displacement) minus engraved value of the test piece.
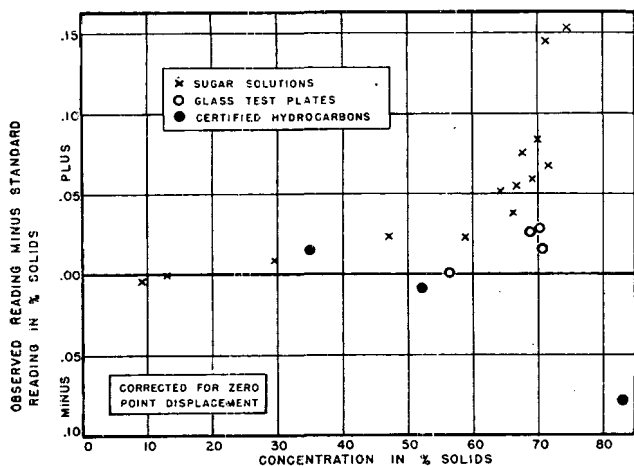
Figure 10. Calibration of precision refractometer

instrument used in this work, a series of readings of several standards was made. These served principally to indicate further complexities in the entire problem of refractometry, while leaving the true refractive index calibration of the precision refractometer somewhat in doubt.

Glass Plates. A series of readings of four glass test pieces was made on the precision refractometer. The results of these tests are shown in Table II. In general, each estimate of the apparent error represents the mean of five observations. The mean result for each plate, corrected for zero point displacement, is plotted in Figure 10. It is difficult to know how much importance to give to the results.

In the first place, considerable care is required in making readings with these plates. It is necessary to observe interference bands to ascertain their number and orientation to ensure that the wedge of liquid between the glass test blocks and the prism is not situated so as to affect the refracting angle (3, 12). (The precision refractometer has a special lens attachment for viewing the back face of the prism and the interference bands. This procedure is less easily done on other refractometers.) Considerable variability in the present results may indicate some shortcoming of technique.

Secondly, there are no certifications of the values of refractive index for the glass blocks. Tilton (11) questioned the engraved refractive index of many such plates, finding errors as large as 0.0001 in refractive index.

Standard Hydrocarbons. Another method of calibration involves the use of standard hydrocarbons of known refractive index (9, 12). These are listed in Table III, along with the values of refractive index at 20° C. and corresponding per cent solids figures. The certificates provided by the National Bureau of Standards with the hydrocarbons list values of n_D for 20°, 25°,

and 30° C. From these data the terms shown in Table III were computed, from which one can obtain the n_D corresponding to any given temperature within that range.

It was originally hoped that an unequivocal calibration of the scale in terms of refractive index could be obtained using these standard hydrocarbons. However, the use of these materials also led to complications.

First observations with the hydrocarbons seemed to deviate from those for the glass plates. It was suspected that ambient temperature, as it varied from 21° to 30° C., was exerting a significant influence upon observed results. The temperature coefficients of refractive index of the hydrocarbons are about five times as great as that of water and about three times as great as those of concentrated sugar solutions. (The change of refractive index with temperature is roughly inversely proportional to the change of volume with temperature.) For the readings on the hydrocarbons, therefore, errors due to temperature effects are larger than when dealing with sugar solutions.

By covering the instrument with a fiberboard box and heating the interior with a light globe, it was ascertained that there were very real effects of variations in ambient temperature. The apparatus was too crude, however, to permit the determination of precise quantitative corrections.

Readings of refractive index for the liquid standards were made over as extreme a range of room temperatures as could be obtained. Rough estimates of the effect of ambient temperature on refractometer readings obtained in this way are indicated in Table IV.

Since the dependability of air temperature correction factors was questionable, the procedure was adopted of maintaining the circulating water temperature close to the air temperature. It was hoped that temperature gradients in the prism block would be minimized, thus avoiding any uncertainty as to the true temperature of the prism. It was necessary, of course, to calculate the refractive index of the certified sample at the particular temperature used. This was done, using the data of Table III. Results obtained in this way are shown in Table V.

The authors have misgivings about this procedure also, inasmuch as when a series of readings was made on distilled water in this way, a highly significant correlation was found between the

Table III. Standard Hydrocarbons

NBS Certified Hydrocarbon	n_D^{20}	Corresponding Approx. % Solids	Refractive Index (× 10⁵) as Function of Temp., ° C.
2,2,4-Trimethylpentane	1.39145	35.0	140123 − 48.5C − 0.02C²
Methylcyclohexane	1.42312	51.1	143348 − 52.6C + 0.04C²
Toluene	1.49693	83.2	150743 − 49.7C − 0.14C²

Table IV. Effect of Air Temperature on Measured Refractive Index of Standards

(Precision refractometer, Serial No. 7722; circulating water at 20° C. Range of air temperature from 12° to 26° C.)

Liquid	Change in Refractive Index per ° Change in Ambient Temp., ° C.
2,2,4-Trimethylpentane	−0.000015
Methylcyclohexane	−0.000017
Toluene	−0.000024
Water	−0.0000035

Table V. Observations with Standard Hydrocarbons

(Apparent error[a] in refractive index and equivalent deviations in terms of % solids. Precision refractometer Serial No. 7722)

2,2,4-Trimethylpentane		Methylcyclohexane		Toluene	
R.I. $\times 10^6$	% solids	R.I. $\times 10^6$	% solids	R.I. $\times 10^6$	% solids
+35	+0.019	−27	−0.013	−142	−0.053
+30	+0.016	−37	−0.018	−189	−0.071
+21	+0.012	−04	−0.002	−204	−0.077
+15	+0.008	+16	+0.007	−209	−0.079
+34	+0.019	−09	−0.005	−204	−0.077
+28	+0.015	−34	−0.017	−265	−0.099
+24	+0.013	−01	−0.001	−228	−0.086
+24	+0.013	−08	−0.004	−206	−0.077
+36	+0.020	−25	−0.012	−232	−0.087
+48	+0.026	−30	−0.015	−192	−0.072
+37	+0.020	−07	−0.004	−194	−0.073
+34	+0.019	−21	−0.010	−226	−0.085
Mean +31	+0.017	−16	−0.008	−208	−0.078

[a] Observed refractive index (corrected for zero point displacement) minus refractive index calculated from certified values.

temperature (common to air and instrument, range 10° to 26° C.) and the distilled water reading, corrected for water jacket temperature using a smoothing of Schönrock's data (1, Table 127). Each degree increase in temperature effected a decrease of 6.1 $\times 10^{-6}$ in refractive index units (equivalent to 0.0043% solids). The explanation for this is not clear.

The effects mentioned are only indicative of the air temperature control problem which was not explored completely. It appears that control of room temperature is essential for precise calibration. Unfortunately the authors did not have a constant temperature room.

It appears important to assess the effect of air temperature upon the readings for concentrated sugar solutions reported. The air temperature was not recorded at the time of these readings.

It is felt that the effect is relatively small. Measurable effects were found for distilled water and solutions of 24% solids, about 3.5 $\times 10^{-6}$ unit of refractive index per degree Centigrade change in air temperature for both. If it is assumed that the effects on concentrated solutions are proportional to the effect of water temperature, then, based upon Schönrock's data (1, Table 127), each degree increase in air temperature corresponds with a decrease of 7 $\times 10^{-6}$ units of n_D, equivalent to 0.003% solids at 70% solids. All readings were made in air temperatures between 22° and 28° C., including distilled water readings.

In terms of scale readings this estimated effect at 70% solids is about three times that for distilled water. The net effect at 70% solids would be about $^2/_3 \times 0.003\%$ solids per degree Centigrade change in air temperature. Thus, the results for high concentrations at an average temperature of 25° C. might be estimated to be too low by about 5 $\times 0.002$ or 0.010% solids, with an uncertainty due to this cause of ±0.006% solids. No correction for this effect has been applied to the sugar readings.

Tables II and V show the apparent scale errors of the instrument in terms of refractive index and per cent solids. The data have been corrected for error in distilled water reading. The mean deviations are plotted in Figure 10. For comparison, there are plotted also the results for sucrose solutions as heretofore shown in Figures 7 and 9 (corrected for zero point).

The interpretation of the over-all picture shown in Figure 10 is not clear-cut. The authors have hesitated to attempt a statistical analysis for two reasons: (1) It was difficult to assign weights to the different standards. Certifications have not been made of the refractive index values of the glass plates. On the other hand, temperature corrections for the hydrocarbons are somewhat doubtful; and (2) the deviations of the mean values for the test points from a smooth curve were greater than should be expected on the basis of distribution of observed results for each individual standard.

There may be reason to ignore the tests on toluene. The

refractive index of toluene, 1.497, approaches closely the index of the prism of the precision refractometer. The index is 1.51711 for a prism of Series 711, as found in the instrument used in this work, Serial No. 7722 (3). Apparently the resolution is diminished as the top of the scale is approached. The results obtained for toluene should probably be ignored in attempting to establish the calibration of the instrument.

The consistency of results with glass plates would seem to indicate that the refractometer reads slightly high in n_D near 70% solids. Thus the apparent error in the international scale near this point would appear reduced to about +0.04% solids instead of +0.06 or +0.07% solids, as Figure 9 would indicate.

The following would seem to be reasonable interpretations: There are small but real errors in the refractive index scale of the particular instrument tested; there are small but real positive deviations in the international scale of refractive indices above about 50% solids; and improved techniques must be employed before reliable results can be obtained. The last would include use of a constant temperature room and use of carefully calibrated glass test pieces as well as liquid standards. The uncertain accuracy of the international scale for concentrations above 50% solids is evident and additional investigative work is indicated.

One more point should be recognized. By making tables of differences it was shown that the Bausch & Lomb precision refractometer conversion table for per cent sugar (4) was not properly smoothed. Weitz has made the same comment (13). In the present studies graphical methods were used to smooth and interpolate the conversion tables. For purposes of further study, using the precision refractometer, it seems desirable to improve the smoothing and to carry results for per cent sucrose to the third decimal place. Probably the first step is to smooth the table of refractive indices of sucrose to the fifth decimal place. This will be especially desirable if the refractive indices in the higher range can be more carefully fixed.

ACKNOWLEDGMENT

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Determination of Carbon Monoxide in Air Pollution Studies

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The NBS colorimetric indicating gel, previously developed for the detection of carbon monoxide in air, can be used to determine very low concentrations if several refinements of the method are employed. A method is described for determining amounts of 1 to 0.1 p.p.m. and 10 to 25 p.p.m. to 1 p.p.m. The method may be applied in studies of air pollution.

IT IS sometimes useful to know the amount of carbon monoxide in polluted air, even though toxic amounts do not occur. If all local sources of this gas are known, its concentration in the air may contribute to the study of pollutant sources. Thus, a correlation of traffic data with the concentration of carbon monoxide and oxidants in air may give information concerning possible pollution from automobiles. The approximately tenfold increase of carbon monoxide observed during smog conditions in Los Angeles (2) served as one measure of pollutant build-up during temperature inversion. If the amounts of acetylene, discovered in the Los Angeles air and found to be characteristic of auto exhaust gases (2), and of nitrogen dioxide are correlated with carbon monoxide, the possible significance of the automotive traffic in pollution might be further studied. Under favorable and controlled conditions, carbon monoxide might also serve as a key in source studies involving industrial plants.

The rapid determination of small amounts of carbon monoxide by the NBS colorimetric indicating gel has been described (1). The indicating gel is a granular yellow solid that turns green (eventually blue) in the presence of carbon monoxide, and is usually sandwiched in a glass tube between sections filled with white guard gel. The guard gel is specially purified and dried silica gel, which removes interfering substances, particularly water vapor, from the gas to be tested. The indicating gel is prepared from purified silica gel by the addition of palladium and molybdenum salts and the subsequent treatment described by Shepherd.

Field and laboratory methods have been outlined. The field method compares commercially prepared tubes with fixed color standards painted or printed on paper. Air to be tested is drawn through the indicating tube by a rubber aspirator bulb equipped with a rate-controlling valve. The presence of carbon monoxide is indicated when the yellow gel turns green. The field method is widely used where physiological considerations are important, but it is not sufficiently accurate for careful studies in air pollution. However, the laboratory method (1) has an accuracy of 5 to 10% of the amount of carbon monoxide involved, and can detect and estimate extremely small amounts such as 1 part in 500,000,000 and much less. The laboratory method involves a colorimetric comparison with freshly prepared indicating tubes that have been exposed to known concentrations of carbon monoxide. These special tubes are easily prepared, and are free from the small variations which occur in the commercially prepared tubes. The laboratory method has now been developed for the determination of carbon monoxide in the very small amounts which may occur in areas of air pollution.

ANALYTICAL METHOD

At a constant rate of sampling, the color developed by the reaction of carbon monoxide with the gel is determined by the product of the concentration of carbon monoxide and the time

[1] Deceased, September 17, 1953.

of reaction. Thus, if the sampling rate through the indicating tube and the concentration of carbon monoxide in the air sample are kept constant, the duration of sampling controls the color response; and by varying this reaction time, an air sample of known composition, passed through especially prepared indicating tubes, can be used to produce color standards corresponding to known concentrations of carbon monoxide. The color produced by reaction with the unknown air can be compared with the standard tubes. Color standards can be prepared in the laboratory, and simultaneously indicating tubes exposed to air at various locations in the field. Comparisons of knowns and unknowns may be made several hours later in the laboratory. If preferred, samples of the unknown air may be brought to the laboratory, or the standard color tubes can be prepared in the field.

Apparatus and Reagents Required. The material requirements are simple—a flow meter registering 100 to 250 cc. per minute, an interval timer or stop watch, a rate-controlling valve, a pump or aspirator for drawing air samples through the indicating tubes, indicating and guard gels, and one or more standard mixtures of carbon monoxide in air, compressed in cylinders. The preparations of the indicating and guard gels, and the standard mixtures of carbon monoxide in air, have been described (1). Two companies licensed by the Secretary of Commerce to manufacture the gels are the Mine Safety Appliances Co., Pittsburgh 8, Pa., and Parmelee Plastics Co., Kansas City 6, Mo.

Preparation of Special Indicating Tubes. Indicating tubes for this type of determination are prepared as they are needed, and usually may be used throughout the same day as prepared.
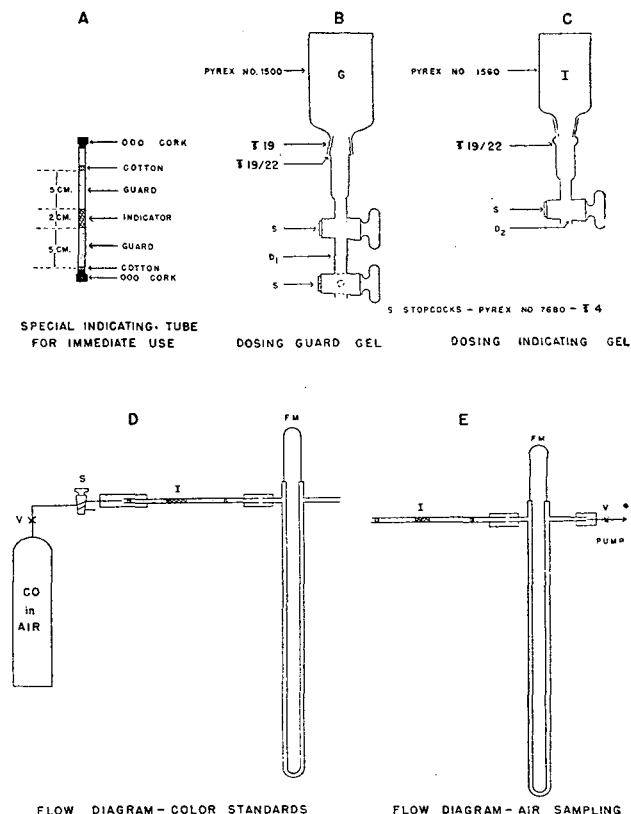

Figure 1. Schematic drawing of apparatus

When greenish rings appear at the ends of the indicating gel, where it is in contact with the guard gel, the tubes should be discarded. The tubes are prepared as follows:

Select 7-mm. borosilicate glass tubing of uniform bore. (Tubes used in this work were 5.1- to 5.2-mm. bore.) Cut in 15-cm. lengths and fire polish. Clean with fuming sulfuric acid and distilled water and dry carefully; or heat at 500° C. for 1 hour in the presence of air; or select freshly drawn tubing which has been protected from dirt. Stopper one end of the tube with an 000 cork, and insert a small wad of adsorbent cotton which will form a loose pad against the cork. Fill the tube with a 5-cm. length of guard gel, followed by a 2-cm. length of indicating gel, and this, in turn, by a second 5-cm. length of guard gel. Insert a second cotton pad, tap the side of the tube twice gently against the edge of a bench, and gently tamp the second cotton pad against the guard gel. Stopper the tube with a second cork. The tube will thus be arranged as shown in Figure 1, A. When air is badly contaminated with strong oxidants or reductants—such as ozone, nitrogen dioxide, halogens, or unsaturated hydrocarbons—an extra tube of guard gel may be necessary. At the prescribed sampling rate, carbon monoxide produces an even color throughout the indicating gel. Other reducing agents are indicated by a dark ring at the inlet, and oxidants by a bleached ring. If such rings occur and interfere with the colorimetric procedure, more guard gel must be used.

The tubes are conveniently filled from simply constructed dosing apparatus such as sketched in Figure 1, B and C. All ground-glass surfaces are used dry, without lubricant; and all glassware must be cleaned with fuming sulfuric or fresh chromic-sulfuric acid, thoroughly rinsed with distilled water, and oven-dried. The volume of D_1 is adjusted to deliver the 5-cm. layer of guard gel to the indicating tube, and D_2, to deliver the 2-cm. layer of indicating gel. Gels may be stored in the stoppered reagent bottles, placed in a desiccator (with dry flange) over guard gel, but the indicating gel should not be stored this way for long periods—i.e., until the top layer turns greenish. Long-term storage of either gel, and particularly the indicating gel, should be in reagent bottles with necks previously drawn out to 9-mm. tubing and sealed off. Unused guard gel may be reactivated by heating 8 hours at 250° C., but, on cooling, the gel must be protected from all vapors (1).

Tubes prepared in this manner are not as convenient to use as the sealed tubes manufactured for field use, but they are capable of giving considerably more accurate determinations. The indicating and guard gels are taken from single, uniform batches, and resistance to gas flow does not vary greatly.

Procedure for Preparing Standard Color Tubes. Connect the cylinder containing the standard mixture of carbon monoxide in air through needle valve V to stopcock S, to indicating tube and the flowmeter (Figure 1, D). The two rubber connections are of pliable nitrometer tubing. Adjust the sampling rate to 100 ml. per minute. Remove the indicating tube and retain it for future adjustments of rate. Do not change the setting of the needle valve. Now prepare the required set of color standards by exposing a series of tubes to the standard mixture according to the predetermined time intervals. Connect each tube to the flowmeter and the stopcock, with the standard mixture escaping to the air. Then turn the stopcock to connect the tube to the standard mixture and simultaneously start the interval timer. At the end of the predetermined period, turn the stopcock to bypass the tube.

In this manner and at the fixed sampling rate of 100 ml. per minute, pass a standard mixture of 10 p.p.m. carbon monoxide in air successively through nine indicating tubes in this order for 130, 130, 120, 110, 100, 90, 80, 70, and 70 seconds. (If the work is to be divided between field and laboratory, the first and last tubes are taken to the field, where they may be used for one day.)

Exposure of Unknowns. The air to be examined usually must be drawn through the indicating tube, using a pump, aspirator, water displacement, or the best means at hand. Take all samples at 100 ml. per minute for direct comparison with the color standards already prepared. At this rate, expose each tube just long enough to be sure that the color developed is slightly darker than the last, 70-second standard tube, but definitely lighter than the first, 130-second standard tube. Record the duration of exposure of the unknown tube for subsequent use in computing the concentration of carbon monoxide. The relationship of concentration of unknown and knowns can be expressed by the equation $\text{p.p.m.}_u = \text{p.p.m.}_k (t_k/t_u)$, where p.p.m._u and p.p.m._k are the concentrations (in parts per million) of carbon monoxide in the unknown and known, respectively, and t_k and t_u are the exposure times of the known and unknown, respectively.

The response of the indicating gel is determined by the product of concentration of carbon monoxide and time of exposure—that is, an exposure for 100 seconds to 0.005% carbon monoxide will produce the same color as an exposure for 50 seconds to 0.010%. This is true over fairly wide limits, but the relationship is not exact, and if the concentration of the unknown is considerably different from that of the known used to prepare the color standards, a known more nearly matching the unknown in concentration should be substituted. Standards of 1, 10, and 50 p.p.m. of carbon monoxide in air are adequate for most work in air pollution. In preparing the color standards, the tubes are exposed in the order dark to light. The colors are compared 3 to 4 hours after the tubes are exposed when operating between field and laboratory.

Procedure for Colorimetric Measurement. Each of the standard color tubes, as it is prepared, is marked with the duration of exposure to the standard mixture. Usually five to seven tubes are prepared, representing the desired increments of carbon monoxide, and designed to bracket the unknown. The tubes are shuffled, and the analyst then arranges them in the order of their color intensity. After the tubes are arranged, the marked exposure times or concentrations are read and recorded. If the order is correct, the colorimetric determination has been success-

Table I. Colorimetric Determination in 0.0001% Range (1 P.P.M.) of Carbon Monoxide in Air

Groups	Experienced 1	2	3	4	Not Experienced 1	2	3	4	And color blind	P.P.M. of CO Actually Found in Unknown[a]
				Arrangement of tubes, seconds[a]						
I	700	700	700	700	700	700	700	700	700	0.75
	850	800	800	850	800	800	800	800	1100	0.85
	800	850	1000	800	850	850	850	850	800	0.95
	900	900	850	1000	900	900	900	1000	900	0.75
	1000	1000	900	900	1000	1000	1000	1100	850	0.85
	1100	1100	1100	1100	1100	1100	1100	900	1000	0.85
	1200	1200	1200	1200	1200	1200	1200	1200	1200	0.85
										0.85
II	700	700	700	700	700	700	700	700	700	0.95
	800	800	800	800	800	800	800	800	800	0.85
	850	850	850	850	850	850	850	850	850	0.85
	900	900	900	900	900	900	900	900	900	0.85
	1000	1000	1000	1000	1000	1000	1000	1000	1000	0.85
	1100	1100	1100	1100	1100	1100	1100	1100	1100	0.85
	1200	1200	1200	1200	1200	1200	1200	1200	1200	0.85
										0.85
III	700	700	700	700	700	700	700	700	700	0.85
	800	800	800	850	800	800	800	800	800	0.85
	850	850	850	800	850	850	900	900	850	0.85
	900	900	900	900	900	900	850	850	900	0.85
	1000	1000	1000	1000	1000	1000	1100	1100	1100	0.85
	1100	1100	1100	1100	1100	1100	1000	1000	1000	0.75
	1200	1200	1200	1200	1200	1200	1200	1200	1200	0.85
										0.85
IV	700	700	700	700	700					0.95
	800	800	800	800	800					0.95
	850	850	850	850	850					0.85
	900	900	900	900	900					0.85
	1000	1000	1000	1000	1000					0.85
	1100	1100	1100	1100	1100					0.85
	1200	1200	1200	1200	1200					0.85
										0.85
V	700	700	700	700	700					0.85
	800	800	800	800	800					0.85
	850	850	850	850	850					0.85
	900	900	900	900	900					0.85
	1000	1000	1000	1000	1000					0.85
	1100	1100	1100	1100	1100					
	1200	1200	1200	1200	1200				Av.	0.85 ± 0.02

[a] Unknown tube is 850 seconds; should contain 0.85 p.p.m.

fully performed. The unknown is assigned a value between those of the two tubes which bracket it.

Daylight fluorescent lighting is desirable for properly viewing the tubes. The tubes should be placed on a dull white surface, and light should be excluded from both ends of this area. The observer looks down upon the tubes at an angle, so that objectionable highlights do not appear.

When the color increments are small, the first viewing usually requires more time and is not so completely successful as the second or third effort. Fatigue may occur during the fourth or fifth effort.

Time Schedule for Complete Determination. The tubes gradually darken after reaction with the carbon monoxide, even though the color change is almost complete within 1 or 2 minutes after exposure. Within the limits of accuracy desired and expected, it is necessary to arrange a simultaneous exposure of the standard color tubes and the tubes used to examine the unknown air. The colorimetric determination should be made 3 to 4 hours after the tubes are exposed, although longer periods are satisfactory if the indicating gel shows no local darkening at the inlet and/or outlet ends. (Tubes have remained relatively unchanged after standing overnight.) If color standards are prepared in the laboratory, field exposures should be made at a temperature within ±5° C. of that of the laboratory.

Data Showing Accuracy, Reproducibility, and Sensitivity of Method. Three standard mixtures of carbon monoxide in air, prepared as described (1), were used to measure the performance of the method. These standard mixtures contained 0.000101 ±0.000001, 0.00100 ± 0.00001, and 0.00257 ± 0.00001%. For this work, these were considered to be 1, 10, and 26 p.p.m. In computing the values given, it was assumed that the air used in the mixtures was carbon monoxide-free. Blanks were run on the compressed air used, but did not extend over a whole order of magnitude beyond the significant value for the most dilute mixture. No carbon monoxide was indicated in any blank.

Nine observers took part in making the color comparisons— four were experienced, four were not experienced, and one was not experienced and color blind. When a series of such tests was conducted on different occasions, not all of the observers were available at all times.

Indicating tubes were exposed to the three standards on various time schedules, so as to determine the least distinguishable differences and the preference in color range of the majority of observers. These exposures also showed the effect of elapsed time after exposure, the effect of temperature, and the over-all accuracy, reproducibility, and sensitivity to be expected.

RESULTS AND DISCUSSION

The data given in Table I are for exposures to 1 p.p.m. of carbon monoxide in air. Good colors were developed with a base time of 1000 seconds, which established the fact that the method is sufficiently sensitive for this kind of work. (Greater sensitivity is possible.) Exposures were made from 700 through 1200 seconds, at intervals of 100 seconds, except for a single tube at 850 seconds which was assigned the role of the unknown. With the base time of 1000 seconds, 100 seconds is equivalent to 0.1 p.p.m., 50 seconds to 0.05 p.p.m. The sampling rate was 250 ml. per minute. The average value for carbon monoxide in the unknown is 0.85 ± 0.02 p.p.m. A good observer can determine carbon monoxide to better than 0.1 p.p.m. in this range of concentration.

The data of Table II give results with the 10 p.p.m. mixture. Groups I and III were exposed at intervals of 10 seconds from 70 to 130 seconds, with a base time of 100, and a 95-second tube included as the unknown. Ten seconds is the equivalent of 1 p.p.m. Each group gave one inversion. Average for the 10 determinations is 9.6 ± 0.2 p.p.m.

Groups II and IV were exposed with a 150-second base in intervals of 10 seconds (equivalent to 0.67 p.p.m.). There were 12 inversions altogether in the two groups, illustrating the relative difficulty of differentiating the darker colors. Before being informed of the results, the observers were asked to express a preference for the lighter or darker tubes. Four of the five emphatically preferred the lighter ones. It is important to calibrate the observer with respect to his optimum color range. The 180-second tube of the darker group (II and IV) was exposed 1 hour after the other tubes of this group. It was inverted with the 170 tube, 5 times out of 10, which indicates a difference of approximately 0.34 p.p.m. carbon monoxide for the 1 hour off-schedule

Table II. Colorimetric Determination in 0.0010% Range (10 P.P.M.) Carbon Monoxide in Air

	Observers					
	Experienced				Not experienced	P.P.M. of CO
Groups	1	2	3	4		
	Arrangement of tubes, seconds[a]					
I	70	70	70	70	70	
	80	80	80	80	80	
	90	90	90	90	90	
	95	95	95	95	95	9.5
	100	100	110	100	100	9.5
	110	110	100	110	110	9.5
	120	120	120	120	120	9.5
	130	130	130	130	130	9.5
II	140	150	140	140	150	9.5
	145	145	145	150	145	9.5
	150	140	150	145	140	10.5
	160	160	170	160	160	9.5
	170	180	160	180	180	9.5
	180	170	180	190	170	
	190	190	190	170	190	
III	70	70	70	70	70	
	80	80	80.	80	80	
	90	90	90	90	90	
	95	95	100	95	95	
	100	100	95	100	100	
	110	110	110	110	110	
	120	120	120	120	120	
	130	130	130	130	130	Av.　9.6 ± 0.2
IV	140	140	145	140	140	
	145	150	140	145	145	
	150	145	150	150	150	
	160	170	160	160	160	
	170	160	170	180	180	
	180	180	180	170	170	
	190	190	190	190	190	
V	85		85	85	85	
	90		90	90	90	
	95		95	95	95	9.5
	100		105	100	100	9.5
	105		100	105	105	9.5
	115		110	110	110	9.5
	110		115	115	115	9.5
VI	90		85	85	85	9.5
	85		90	95	90	8.75
	95		95	90	95	9.5
	100		100	100	100	9.5
	105		105	105	105	9.5
	110		110	110	110	
	115		115	115	115	Av.　9.4 ± 0.1
VII	85		85	85	85	
	90		90	90	90	
	95		95	95	95	
	105		100	100	100	
	100		105	105	105	
	110		110	110	110	
	115		115	115	115	

[a] Unknown tube is 95 seconds.

exposure. Groups V, VI, and VII represent a more ambitious effort. Using the lighter color range with a 100-second base time, tubes were prepared at 5-second (0.5 p.p.m.) intervals from 85 to 115. Out of 84 tubes arranged by four observers there were only 5 inversions, which indicates the possibility of determining carbon monoxide to within 0.5 p.p.m. in this range of concentration. The average for these groups is 9.4 ± 0.1 p.p.m.

Table III gives the data observed with the 26 p.p.m. mixture. Again a light (I and III) and a dark (II and IV) series were examined. The base times were 60 and 100 seconds, and the increments, 5 seconds. At the 60-second base, 5 seconds are equivalent to 2.2 p.p.m.; at the 100-second base, 5 seconds correspond to 1.3 p.p.m. In the entire series of tests (I through IV) there were no inversions on the part of the five observers. The preference was for the lighter series, 45 to 75 seconds. Working with the lighter color range, a series of tubes was prepared with a 60-second base and 2-second (0.9 p.p.m.) steps from 54 to 66 seconds. In two trials (V and VI) the same group of five observers made but two inversions; five additional inversions were made by two additional observers. If the base time is taken as the unknown exposure, average for all observers is 26.1 ± 0.2 p.p.m.

The data of the foregoing three tables established the accuracy of the method as about 10%, the reproducibility about 5 to 10%, and the sensitivity sufficient for this work.

Effect of Increasing Color with Elapsed Time. Most of the color is developed on the indicating gel during the exposure to the air containing carbon monoxide, but the initial color slowly darkens with time. The significance of this change was shown in the following experiment. At 8 A.M., a series of tubes was exposed for 80, 90, 100, 110, and 120 seconds to 10.0 p.p.m. of carbon monoxide in air. These tubes were arranged twice by each of three observers in the order of color intensity without an inversion. This set of tubes now served to measure the apparent intensity of additional tubes, each exposed for 100 seconds, at 9:30, 10, 11 A.M., 12 M., 1, and 2 P.M. Five minutes after each additional tube was exposed, the standard series, plus all additional tubes, were arranged in the order of apparent color intensity. In all cases the freshly exposed tubes were placed below the standard corresponding to 8.0 p.p.m. (80 seconds). With the lapse of time, the apparent intensity of a tube increased roughly 1 color step (1.0 p.p.m.) per hour until the reading was between 9.0 and 10.0 p.p.m., which position was then maintained for the following 24 hours. Three hours after exposure, the tubes were within 1.0 p.p.m. of the correct value as measured by the original color standards. Thus, a set of standards prepared early in the morning would serve to measure unknowns exposed throughout the day if the color comparisons were made 3 hours or more after the unknown exposures, and a systematic error of the order of minus 10% were acceptable.

The reason for the instruction previously given to prepare standard color tubes in the order dark to light is now explained. This order takes advantage of the color drift. Reversing the order will cause more inversions in arranging tubes with very small color increments—e.g., a set representing increments of 0.5 p.p.m. of carbon monoxide in the range 10 p.p.m.

Effect of Temperature. The sensitivity of the indicating gel changes in a regular manner over the range of normal atmospheric temperatures. The direction and extent of this dependence are summarized in Table IV.

Table IV. Effect of Temperature on Sensitivity of Indicating Tubes

Exposure Temp., ° C.	Relative Sensitivity[a]
60	2.7
50	1.3
40	1.2
30	1.0
25	1.0
20	1.0
10	0.7
0	0.6
-10	0.3
-20	0.1

[a] Corresponding sensitivities relative to sensitivity at 25° C.

The values given were derived from colorimetric-time measurements made on 26 p.p.m. carbon monoxide in air, with rate of flow constant. If the results obtained are to be accurate to 10% of the concentrations measured, corrections must be applied for field tubes exposed at temperatures more than 5° C. different from the exposure temperature of the color standards. This procedure is feasible for temperatures of 0° and above but is not satisfactory—for example, at −20° C., where the sensitivity is lowered by a factor of ten. At such low temperatures, it may be necessary to keep the sensitivity at a usable, known level by artificially heating the tube and incoming sample to a constant temperature.

Correlation of Field and Laboratory Measurements. If the unknowns are to be exposed in the field and the color standards prepared in the laboratory, a time schedule must be observed, and significant differences in temperature must be taken into account. If there is a wide variation in the concentration of the unknowns, more than one set of color standards must be prepared. The time schedule can be definitely predetermined, and complete sets of color standards can be prepared even though all are not used. When the outside temperatures are abnormally low, more consistent results may be obtained if samples of the unknown air are taken to the laboratory for the exposures and color comparisons.

Table III. Colorimetric Determination in 0.00257% Range (25.7 P.P.M.) Carbon Monoxide in Air

Group	Elapse of Time after Reaction, Hours	1	2	3	4	1	2	4	P.P.M. of CO
				Arrangement of tubes, seconds					
I and II	1	45	45	45	45	45			
		50	50	50	50	50			
		55	55	55	55	55			
		60[a]	60[a]	60[a]	60[a]	60[a]			
		65	65	65	65	65			
		70	70	70	70	70			
		75[b]	75[b]	75[b]	75[b]	75[b]			
		80	80	80	80	80			
		85	85	85	85	85			
		90	90	90	90	90			
		95	95	95	95	95			
		100[c]	100[c]	100[c]	100[c]	100[c]			
		105	105	105	105	105			
		110	110	110	110	110			
III and IV	2	45	45	45	45	45			
		50	50	50	50	50			
		55	55	55	55	55			
		60[a]	60[a]	60[a]	60[a]	60[a]			
		65	65	65	65	65			
		70	70	70	70	70			
		75[b]	75[b]	75[b]	75[b]	75[b]			
		80	80	80	80	80			
		85	85	85	85	85			
		90	90	90	90	90			
		95	95	95	95	95			
		100[c]	100[c]	100[c]	100[c]	100[c]			
		105	105	105	105	105			
		110	110	110	110	110			
V and VI	1	54	54	54	54	54	56	54	26
		56	56	56	56	56	54	56	26
		58	58	58	58	58	58	58	26
		60[d]	60[d]	60[d]	60[d]	60[d]	62	62	26
		62	62	62	64	64	60[d]	60[d]	26
		64	64	64	62	62	64	64	27
		66	66	66	66	66	66	66	27
		54	54	54	54	54	54	54	26
		56	56	56	56	56	56	56	26
		58	58	58	58	58	56	56	26
		60[d]	60[d]	60[d]	60[d]	60[d]	60[d]	60[d]	26
		62	62	62	62	62	64	62	26
		64	64	64	64	64	62	64	26
		66	66	66	66	66	66	66	26
								Av.	26.1 ± 0.2

[a] Base time, 60 seconds, where 5 seconds are equivalent to 2.2 p.p.m.
[b] Base time, 75 seconds, where 5 seconds are equivalent to 1.7 p.p.m.
[c] Base time, 100 seconds, where 5 seconds are equivalent to 1.3 p.p.m.
[d] Base time, 60 seconds, where 2 seconds are equivalent to 0.9 p.p.m.

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Kjeldahl Method with Sealed Tube Digestion

Factors Influencing Ammonia Decomposition

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The sealed tube digestion procedure employs temperatures considerably higher than those of the conventional Kjeldahl method, and loss of ammonia due to thermal decomposition is of primary concern. This investigation was undertaken to determine what factors influence ammonia loss in this procedure. Ammonia bisulfate decomposed to nitrogen gas at temperatures greater than 500° C. The quantity of sulfuric acid used for a digestion may affect ammonia recovery and prolonged digestion may be responsible for oxidation of appreciable quantities of ammonia. Addition of small amounts of water to the digestion mixture markedly increases the stability of ammonia nitrogen in sulfuric acid. Ammonia is oxidized by sulfur trioxide or by oxygen over the same temperature range. Excellent agreement between the milligram and microgram procedures was found.

THE work of White and Long (4) on the Kjeldahl digestion procedure in sealed tubes at 470° C. offers a major improvement in the reliability of this method for nitrogen determination. The subsequent work of Grunbaum, Schaffer, and Kirk (2) on the decomposition of ammonium sulfate at higher temperatures suggested that rigorous conditions were required to avoid ammonia loss in the course of the digestion, and also showed, as did the work of White and Long, that catalyst addition was unnecessary to effect decomposition of the organic material. Baker (1) confirmed the work of Grunbaum and coworkers and stated that ammonia actually was lost above 420° C. when a mercuric sulfate–selenium catalyst was employed for the digestion.

The present investigation had several objectives. It was desired to identify the oxidation product or products of ammonia at elevated temperatures in sealed tubes and to establish a set of conditions for the digestion which would suppress the ammonia decomposition and result in a less critical control of temperature. Finally, it seemed important to compare results between the milligram and microgram scale analyses to determine whether or not surface or other effects play a role in the digestion of microgram quantities of material which are not evident with milligram samples.

EXPERIMENTAL

The apparatus and procedure used for the analyses of microgram samples were identical with those described previously (2); the data of the previous investigation demonstrated the reliability of the method. The borosilicate glass digestion tubes used in the milligram scale were 22 to 25 cm. long, having an inner diameter of 12 mm., a 3-mm. wall thickness, and a volume that approximated 25 ml. This volume is approximately threefold greater than that for the digestion tubes used by White and Long. The distillation apparatus employed was described by Kirk (3). The milligram method deviated from that described by White and Long in that smaller quantities of sulfuric acid were used for the digestion and that the sulfur dioxide and carbon dioxide were driven off by heating the contents of the bomb to gentle boiling before transfer to the distillation apparatus. This eliminated the need for the addition of the base to the distillation apparatus ahead of the digestion mixture. Omission of the catalyst eliminated the need for the addition of sodium thiosulfate to the alkaline distillation mixture. The temperature range investigated for the digestion of both the milligram and microgram samples varied from about 450° to 625° C. The error in reading furnace temperature was less than 5° C.

RESULTS

Grunbaum and coworkers (2) showed that ammonia from organic, nitrogen-containing samples did not decompose as readily as ammonium sulfate during digestion and that the conditions established by White and Long, 0.5-hour digestion at 470° C., were optimum for the digestion of organic samples.
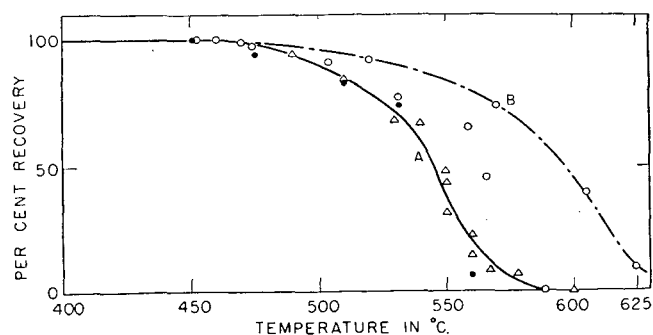

Figure 1. Ammonia recovery from ammonium sulfate with varying temperatures of digestion for 0.5 hour

A. ● 5.2 γ of nitrogen in 0.8-ml. tube (6.5 γ per ml. space) with 10 microliters of concentrated sulfuric acid
○ 26 γ of nitrogen in 0.8-ml. tube (33 γ per ml. space) with 10 microliters of concentrated sulfuric acid
△ 1250 γ of nitrogen in 25-ml. tube (50 γ per ml. space) with 250 microliters concentrated sulfuric acid
B. ○ 1100 γ of nitrogen in 25-ml. tube (44 γ per ml. space) in oxygen gas

In this study similar experiments (2) concerning ammonium sulfate decomposition were repeated on both the milligram and microgram scales. Easily controlled conditions—i.e., sample size, temperature, quantity, and concentration of sulfuric acid— were investigated. Curve A of Figure 1 illustrates the effect on ammonia recovery of varying the digestion temperature. The sample sizes and amounts of sulfuric acid are listed in the figure. Employing the microgram diffusion procedure, the 125-γ ammonium sulfate samples, in general, show a greater stability than the 25-γ ammonium sulfate samples. The ammonia recovered from 6-mg. ammonium sulfate samples, however, does not indicate a greater ammonia stability. While it would seem that the rate of decomposition of ammonia should depend upon the concentration of ammonium sulfate as indicated by the microgram analyses, the uncertainty associated with the temperature of the bomb contents during the digestion discouraged further study of this nature in conventional, sealed tubes. With the exception of the two points at 560° and 566° C., the curve fits the data within the experimental error. The volumes of acid for the microgram and milligram determinations afforded about the same over-all gas pressures. In both cases, a liquid as well as gas phase existed at temperatures somewhat below 530° C. Above this temperature the sulfuric acid was completely vaporized.

Oxidation of Ammonia to Nitrogen Gas. Grunbaum and coworkers have suggested that the ammonia loss could be ascribed to oxidation of the ammonia to nitrogen gas. To confirm that this process was occurring, a 5.28-mg. sample of ammonium sulfate and 250 μl. of concentrated sulfuric acid were sealed in a

25-ml. bomb tube under vacuum (10^{-4} mm. of mercury), and the contents were digested under conditions in which the ammonia was quantitatively lost (624° C. for 0.5 hour). The composition of the gas phase was determined by use of the mass spectrograph. The sulfur trioxide and water were removed from the gas mixture by the proper control of temperature and the remaining gas showed 66.22 mole % of sulfur dioxide, 25.54 mole % of nitrogen, and 8.24 mole % of oxygen. A calculation of the quantity of nitrogen gas from the pressure, volume, and temperature values listed from the mass spectrographic analysis indicated that within experimental error the ammonia was oxidized quantitatively to nitrogen gas (gas recovery = 105%).

The amount of sulfur dioxide formed in the course of the reaction was only in approximate agreement with the ratio from the equation for the net reaction, $SO_2/N_2 = 3$. Some of the sulfur dioxide is lost because it has an appreciable solubility in sulfuric acid at room temperature. Additional loss may be incurred in removing water vapor and traces of sulfur trioxide. The presence of oxygen is not due to contamination but rather to the decomposition of sulfur trioxide at the temperature of the digestion. In the temperature region where the dissociation of sulfur trioxide becomes appreciable, either sulfur trioxide or oxygen may oxidize ammonia to nitrogen gas. For this reason, it is of interest to compare the decomposition of ammonia by oxygen with that by sulfuric acid.

Curve B of Figure 1 shows the results of a series of determinations in which weighed quantities of ammonium chloride were sealed under 1 atmosphere of oxygen gas in 25-ml. tubes. The oxidation of ammonia occurs in approximately the same temperature region but at a slower rate in pure oxygen than in sulfuric acid. This effect may be due to greater concentration of oxidizing agent when sulfuric acid is present or to a different mechanism in the oxidation process. The fact that oxidation of ammonia starts at approximately the same temperature in the presence of oxygen as when the sample is digested with sulfuric acid would seem to indicate that oxygen is responsible for the ammonia loss.

Curve A of Figure 2 shows the oxygen pressure due to the dissociation of sulfur trioxide which would exist in a 25-ml. sealed tube if 250 μl. of concentrated sulfuric acid were completely vaporized at the temperatures listed. These calculations were carried out assuming that gaseous sulfuric acid does not exist as a major species. For these temperatures and pressures the validity of this assumption is uncertain. For the purposes of this comparison no corrections were made for gas imperfections, as the calculations were intended only to demonstrate the effect quantitatively, and the uncertainty as to the species present does not warrant the lengthier and more exact treatment. In the oxidation of ammonium sulfate no large amount of sulfur dioxide
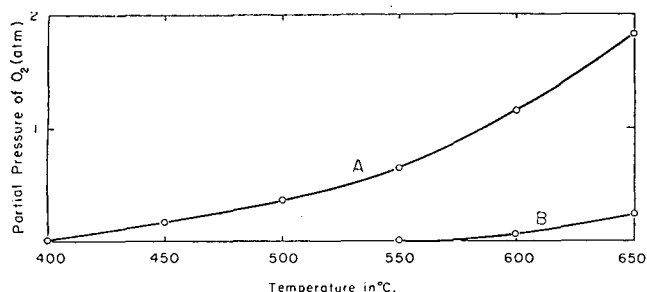
is produced and, as a consequence, the oxygen pressure may approximate the amount calculated for curve A, Figure 2.

Curve B illustrates the reduced partial pressure of oxygen which would occur if a 25-ml. bomb tube containing 10 mg. of acetanilide and 250 μl. of concentrated sulfuric acid were sealed under vacuum and digested at the indicated temperatures. As in the case of curve A, the calculation has been made assuming that the concentrated sulfuric acid is completely dissociated to sulfur trioxide and water vapor. This lowering of the oxygen pressure due to the large amount of sulfur dioxide which is formed in the destruction of the organic matter could account for the increased stability observed when an organic nitrogen-containing sample is digested.

The data discussed so far did not rule out the possibility that in this temperature region ammonia may also decompose to give nitrogen and hydrogen gas. Table I indicates that the reaction does not proceed according to this path. Ammonium sulfate and ammonium bisulfate samples were sealed in a bomb tube in the presence of air and heated for 0.5 hour at 550° C. These samples lost more ammonia than the stoichiometric amount permitted by the reduction of sulfur trioxide to sulfur dioxide, but this is explained by the presence of oxygen from the air contained in the sealed tube. When the same experiments were repeated in evacuated tubes and the samples were heated to 620° C., the extent of oxidation was decreased. The reaction proceeded until the stoichiometrically available amount of sulfur trioxide was reduced to sulfur dioxide as shown by the loss of one third of the ammonia in ammonium sulfate while two thirds was lost with ammonium bisulfate. Within the certainty of the experiment, the reaction did not continue beyond the utilization of sulfur trioxide. It was concluded from these measurements that ammonia was not lost by the reaction

$$2NH_3(g) = N_2(g) + 3H_2(g)$$
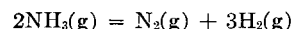
Table I. Oxidation of Ammonia in Absence of Sulfuric Acid

Sample	Furnace Temp., ° C.	NH₃ Recovered, %
In Air		
$(NH_4)_2SO_4$	550	48.4
NH_4HSO_4	550	20.9
In Vacuum		
$(NH_4)_2SO_4$	620	66.4
NH_4HSO_4	620	31.4
NH_4Cl	620	98

Effect of Concentrated Sulfuric Acid. The qualitative observation that ammonia was not decomposed as rapidly when an organic nitrogen-containing sample was digested as when ammonium sulfate was heated under comparable conditions (2) led to an investigation of the effect of reactant and product concentrations in the digestion mixture. The variation in the amount of sulfuric acid added was investigated first. The results of measurements carried out in 25-ml. sealed tubes are shown in Figure 3. The reproducibility of the values plotted is of the order of 10 to 20%. The minimum in this curve is associated with the quantity of sulfuric acid that produces a two-phase system. Amounts of sulfuric acid less than about 500 μl. (approximately 20 μl. of concentrated sulfuric acid per milliliter of tube volume) result in a gaseous system in this temperature range; amounts greater than about 500 μl. produce a two-phase system. The distribution of the ammonia between the two phases diminishes the rate of oxidation of ammonia in the course of the digestion. The species responsible for the oxidation of ammonia (either sulfur trioxide or oxygen) will occur only in the gas phase and at the surface of the liquid.

Figure 3 indicates that the least desirable quantity of sulfuric acid to use is that in which the vapor pressures have reached a

Figure 2. Partial pressure of oxygen gas from thermal dissociation of sulfur trioxide gas using 250 μl. of concentrated sulfuric acid in 25-ml. sealed tubes

A. Calculation based on complete dissociation of sulfuric acid to sulfur trioxide gas and water vapor at temperatures listed
B. 10 mg. of acetanilide digested to form sulfur dioxide gas, carbon dioxide gas, and water vapor assuming that the sulfuric acid is completely dissociated to sulfur trioxide gas and water vapor

maximum but no appreciable excess above this saturation amount is present. The bulk of the work reported in this paper has been performed under conditions which permitted investigation of the gaseous process and avoided the uncertainties introduced by a two-phase system. The starred point of Figure 3 represents the recovery of ammonia when 250 µl. of 30% fuming sulfuric acid were used instead of the regular, concentrated acid. As would be expected the loss of ammonia is greater in the presence of an increased amount of the oxidizing agent.

Figure 3. Effect of varying amounts of sulfuric acid on decomposition of 6 mg. of ammonium sulfate in 25-ml. sealed tubes at 550° C.
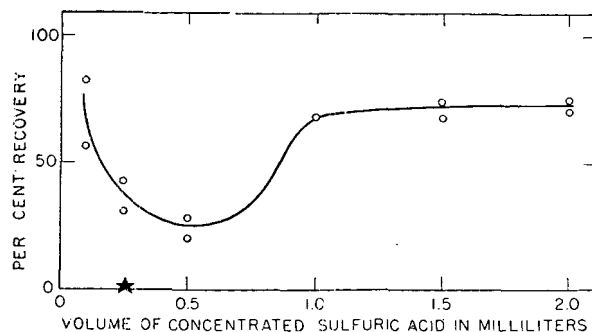
Digestion period 0.5 hour
★ Recovery of ammonia using 250 microliters of 30% fuming sulfuric acid

Effect of Digestion Time. It was suggested (2) that temperature played a more important role than time in the digestion process. It was thought that after the 0.5-hour digestion period equilibrium had been obtained. Work by Baker (1) with organic samples at 420° and 465° C. corroborated this belief. Further work, however, in temperature ranges where the oxidation occurs at a more rapid rate, has shown that equilibrium for the oxidation is not obtained in such a short time interval even at temperatures where the reaction rate is relatively rapid. Table II shows two sets of data: one in which the sample and acid were sealed in the presence of air and the second in which the tube was evacuated before sealing.

Table II. Variation in Ammonia Loss from 6-Mg. Ammonium Sulfate Samples with Time

Composition of Digest, µl.	Temp., ° C.	Time of Digestion, Hour	NH₃ Lost, %
	In Air		
250, H₂SO₄	550	0.5	57, 69
		1.0	85
	In Vacuum		
100, 1M H₂SO₄	620	0.5	63
		1.0	87

Roughly the same fraction of ammonia is lost in the second half-hour interval as in the first. This means that even at these considerably higher temperatures equilibrium conditions are not obtained in an hour's heating time and that at digestion temperatures approximating 470° C., establishment of equilibrium is sufficiently slow that a 0.5-hour heating period causes no serious loss. This argument appears to be confirmed by the work of Baker on aminoacetic acid at 465° C. The theoretical amount of ammonia is found in a 0.5-hour digestion period, but after 3 and 4 hours at the same temperature the analysis shows an ammonia recovery of 98.7%. Further data are given below with respect to loss with time for tryptophan.
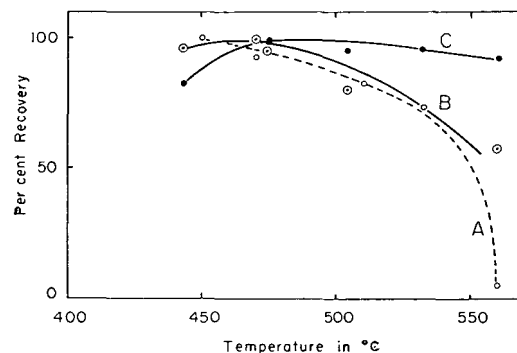

Figure 4. Effect of water on recovery of ammonia from egg albumin using 250-γ samples in 0.8-ml. sealed tubes

A. ○ Ammonium sulfate with 10 µl. of concentrated sulfuric acid
B. ⊙ Egg albumin with 10 µl. of concentrated sulfuric acid
C. ● Egg albumin with 10 µl. of concentrated sulfuric acid plus 10 µl. of water

Table III. Effect of Water above Its Critical Temperature on Ammonium Sulfate Digestions^a

Sample	Digestion Reagent Added, µl.	NH₃ Recovered, %
(NH₄)₂SO₄	None	66^b
	100, H₂O	74
	100, 1M H₂SO₄	37
	100, concd. H₂SO₄	0

^{a} Digestion of ammonium sulfate at 620° C. for 0.5 hour on milligram scale in vacuum.
^{b} Ammonia oxidized represents quantitative reduction of sulfur trioxide to sulfur dioxide.

Effect of Added Water. Initial experiments in which distilled water was added to the sulfuric acid digestion mixture indicated that the presence of water vapor suppressed the rate of ammonia loss (Table III). The determinations show definitely that the presence of water suppresses the decomposition of ammonia.

Figure 4 illustrates the effect of added water on the determination of nitrogen in egg albumin. Curve A is included for comparison of the rate of oxidation of ammonium sulfate in 10 µl. of concentrated sulfuric acid as compared to that of egg albumin in a comparable amount of sulfuric acid. From these two curves it was concluded that the presence of sulfur dioxide does not suppress the decomposition of ammonia, as the major difference in the two sets of experiments lies in the much larger quantity of sulfur dioxide formed in the decomposition of egg albumin than in the oxidation of ammonium sulfate. This observation is in agreement with those listed in Table II, which showed that equilibrium is not attained in 0.5-hour digestion periods. Since time of digestion and pressure of sulfur dioxide both indicate that an equilibrium state for the net reaction is not reached in a 0.5-hour digestion period, it is necessary to seek another explanation for the suppression of oxidation by the addition of water to the mixture as shown by curve C.

A plausible reason for the decreased rate of oxidation in the presence of greater quantities of water vapor is associated with the quantity of oxidizing agent available to react with the ammonia species. A large increase in water pressure in the system will decrease the equilibrium pressure of sulfur trioxide as a result of the formation of sulfuric acid. Consequently, whether the oxidizing species is sulfur trioxide or oxygen, the pressure of either one should be lowered appreciably. This is confirmed by the observation on the milligram scale that a liquid phase is present when 250 µl. of concentrated sulfuric acid and 250 µl. of water are heated to temperatures as great as 560° C. in a 25-ml.

sealed tube. While the liquid phase was not actually observed on the microgram scale, the ratio of the volume of the bomb tube to the volume of concentrated sulfuric acid plus water implies that this also is true on the microgram scale.

Figure 5 shows the effect of digestion time on the decomposition of tryptophan and of ammonium sulfate at 550° C. Curve A indicates the rate of decomposition when 250 µl. of concentrated sulfuric acid were used to digest the sample and curve B illustrates the decomposition rate when 250 µl. of concentrated sulfuric acid plus 250 µl. of distilled water were used. This temperature was selected because ammonia is rapidly oxidized by 250 µl. of concentrated sulfuric acid at 550° C., and the effect of water can be shown using digestion periods of short duration.
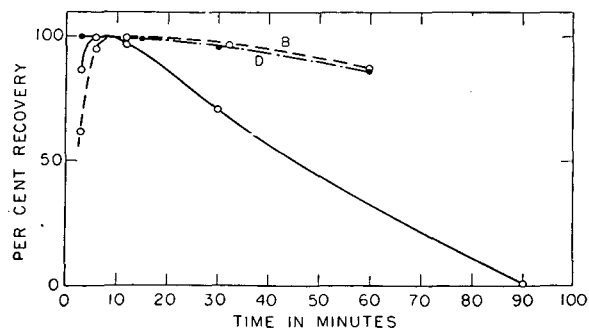

Figure 5. Effect of water on recovery of ammonia from tryptophan and from ammonium sulfate at 550° C. in 25-ml. sealed tubes

A. O 9 mg. of tryptophan plus 250 µl. of concentrated sulfuric acid
B. O 9 mg. of tryptophan plus 250 µl. of concentrated sulfuric acid plus 250 µl. of water
C. ● 6 mg. of ammonium sulfate plus 250 µl. of concentrated sulfuric acid
D. ● 6 mg. of ammonium sulfate plus 250 µl. of concentrated sulfuric acid plus 250 µl. of water

The organic material was destroyed in less than 10 minutes in a furnace heated to 550° C. in both cases, but the rate of oxidation of ammonia was decreased considerably by the increased pressure of water (total pressure approximately 30 atmospheres). The closed circle points, C, represent the ammonia loss when 6 mg. of ammonium sulfate were digested with 250 µl. of concentrated sulfuric acid at this temperature. Curve D illustrates the increased stability of ammonium sulfate when 250 µl. of water and 250 µl. of concentrated sulfuric acid were added to 6 mg. of ammonium sulfate at 550° C. The reason for obtaining 100% ammonia recovery before appreciable loss occurs probably is the length of time required for the contents of the bomb to reach the temperature of the furnace. Because organic matter is decomposed by sulfuric acid at a rapid rate even at considerably lower temperatures than 400° C., it is likely that by the time the sample has reached the temperature region where oxidation of ammonia occurs the organic portion of the sample has been completely oxidized. This is confirmed by curve D, where little difference is observed between the rate of decomposition of ammonia from tryptophan and ammonia from ammonium sulfate.

Figure 6 shows that when tryptophan was digested at 530° C. with 500 µl. of 9M sulfuric acid a digestion period of 10 to 30 minutes yielded a recovery of ammonia equal to or greater than 99%. Comparable experiments on the microgram scale have shown that the digestion period may be shortened to a few minutes. This shorter period is due undoubtedly to the much smaller heat capacity of the smaller system, which permits the maximum temperature to be reached in a considerably shorter time.

As a result of work by Grunbaum and coworkers (2) in addition to the present investigation on the sealed tube Kjeldahl diges-

tion method, it is recommended that for the microgram diffusion procedure the use of catalyst be avoided. The presence of mercuric sulfate and sodium thiosulfate impairs the rate of diffusion of ammonia. Thus the time gained in digestion by the presence of catalyst does not compensate for that lost in the subsequent diffusion step. White and Long have shown that with milligram quantities of material the digestion rate is very appreciably increased by the use of relatively large quantities of mercuric oxide catalyst at 470° C. Baker has shown that when a mercury-selenium catalyst is employed, loss of ammonia occurs at 465° C. and he recommends a digestion temperature of 420° to 450° C. As White and Long have shown that a substance as refractory as nicotinic acid can be digested in 30 minutes without catalyst, and it has been found in this investigation that tryptophan and egg albumin also yield quantitative recovery at 470° C. when digested for 30 minutes, the authors have continued to avoid the use of catalyst in the analysis of milligram amounts of material, since speed of an individual digestion greater than this has not been an important factor. If the temperature of the digestion furnace is difficult to control, an additional margin of security can be achieved by the addition to the digestion mixture of a small quantity of water. The critical temperature of water is exceeded and about 10 µl. of water should be added for each milliliter of gas space, regardless of the quantity of concentrated sulfuric acid added. Temperatures greater than 500° C. were employed to investigate the rate of loss of ammonia and possible means of retarding such loss. It is not recommended that a temperature greater than that proposed by White and Long be used in the sealed tube digestion procedure.
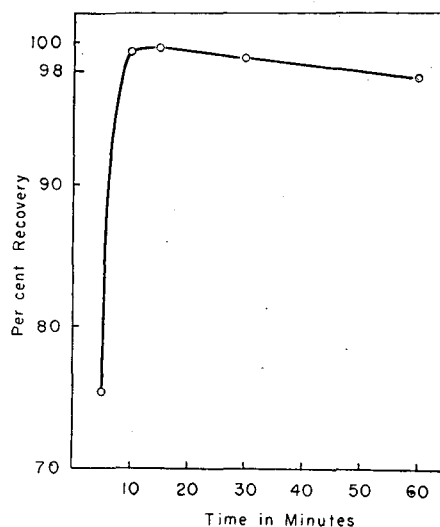
Figure 6. Effect of water on recovery of ammonia from tryptophan at 530° C. using 9-mg. quantities in 25-ml. sealed tubes

Digestion mixture contains 250 µl. of concentrated sulfuric acid and 250 µl. of water

SUMMARY

The investigation of conditions for the sealed tube Kjeldahl digestion demonstrated that ammonia loss in the digestion is due to oxidation of ammonia to nitrogen gas. Excellent agreement existed between the results on the milligram and microgram scales. Oxidation of ammonia by sulfur trioxide or by oxygen (alone) occurs over the same temperature range. The difference in rate of the oxidation probably is due to differences in the partial pressure of the oxidizing species.

The data indicate that 0.5-hour digestions are more than adequate for the destruction of the organic portion of a substance with compounds as refractory as tryptophan and that prolonged digestion can result in the oxidation of appreciable quantities of ammonia. Recently it has been found that several compounds containing quaternary nitrogen groups gave very poor recovery of ammonia nitrogen by the sealed tube method, whereas the conventional Kjeldahl procedure proved satisfactory. A preliminary investigation has shown that the rate of increase of digestion temperature plays an important part in the resulting conversion to ammonia. Further study is being conducted on this problem.

Because the addition of water to the digestion mixture decreases the rate of oxidation of ammonia, temperature control need not be so precise if the digestion mixture contains small amounts of water, and temperatures somewhat greater than 470° C. may be used without loss of ammonia.

A further study of the chemistry of the ammonia oxidation step is being conducted. It is hoped that such a study will lead to a better understanding of the factors which may be controlled in this oxidation.

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Determination of Hydroxy and Amino Compounds by a Chlorine-36—Isotope Dilution Method

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A modification of isotope dilution analysis is proposed by which it is possible to determine hydroxy and amino compounds. The compound to be analyzed is quantitatively converted to a chlorine-containing derivative, and this is determined by an ordinary isotope dilution method. The principle has been applied to determination of phenol, pyrocatechol, methanol, ethylene glycol, aniline, and ethylenediamine.

RADIOACTIVITY measurements can be performed easily and with good precision on chlorine-36 compounds (5). Chlorine-containing compounds may thus be determined with a corresponding precision by chlorine-36 dilution methods (1, 4).

In this paper a method is suggested by which non-chlorine-containing compounds may be analyzed by a chlorine-36 dilution method. The compound to be analyzed is quantitatively converted to a chlorine-containing derivative, and this is determined by an ordinary isotope dilution method (4).

The principle is a modification of the method of Keston and coworkers for the determination of amino acids (3). The amino acids, as radioactive derivatives, are analyzed by a reversed isotope dilution method, which has the advantage that isolation of the desired compound always is possible. However, the impurities present in the mixture are highly radioactive and a very rigorous purification of the compounds is necessary.

By the method here suggested no radioactive impurities will be present. It may sometimes be rather difficult to isolate a given compound, but when it can be done the method may be considered as absolutely specific for the compound in question.

In comparison with ordinary isotope dilution analysis the principle proposed has the following advantages: radioactivity measurements are done with chlorine-36, and therefore good precision is obtainable; with one radioactive compound a whole group of compounds may be analyzed; and the reference compound of the analysis is a derivative of the compound to be analyzed. The derivative is usually much easier to prepare in a pure state than the compound itself.

The disadvantage of the principle is that the compound to be analyzed must be quantitatively converted to a derivative.

For the purpose 3-chloroanisic acid (4-methoxy-3-chlorobenzoic acid) has been shown to be useful. Radioactive 3-chloroanisic acid can be prepared with an acceptably high yield of chlorine-36. Using an excess of 3-chloroanisoyl chloride, hydroxy and amino compounds may be quantitatively chloroanisoylated. The derivatives obtained are crystalline and have sharp melting points.

PREPARATION OF MATERIALS

Anisic Acid (*p*-methoxybenzoic acid). A commercially available product was recrystallized twice from toluene: melting point, 184.0–184.5° C.

3-Chloroanisic Acid. The compound was prepared by chlorination of anisic acid by the method of Hopkins and Chrisholm (2). The crude product was crystallized twice from toluene: melting point, 216–217° C.

3-Chloroanisoyl Chloride. A suspension of 175 grams of 3-chloroanisic acid in 250 ml. of purified thionyl chloride was refluxed until a clear solution appeared, and then further for 15 minutes. After removal of the excess of thionyl chloride under diminished pressure the 3-chloroanisoyl chloride was distilled at 1 mm. at 120° to 125° C. Yield, nearly quantitative; melting point, 64° to 65° C. Analysis: calculated for $C_8H_6O_2Cl_2$, saponifiable chlorine, 17.3%; found, 17.4%.

Chloroanisoylation of Hydroxy Compounds. The hydroxy compound and 50% excess of 3-chloroanisoyl chloride dissolved in pyridine were heated to reflux for 20 minutes. After absolute alcohol was added to decompose excess 3-chloroanisoyl chloride, the mixture was poured into water. The precipitate was removed by filtration, washed with water, and crystallized to constant melting point from an organic solvent.

Chloroanisoylation of Amino Compounds. The preparation was the same as described above, except that the reaction mixture was allowed to stand at room temperature for half an hour. If reaction occurs at refluxing temperature, each amino group may be partly dichloroanisoylated.

For monohydroxy and monoamino compounds it may be advantageous to use an excess of the hydroxy or amino compound. Solvents for crystallizations were dioxane, absolute alcohol, methanol, and ligroine.

Radioactive Compounds. 3-Chloro(36)-anisic Acid. The

method mentioned here (2) was used with some modifications. Radioactive elementary chlorine produced from 11.08 grams of active silver chloride (4) was absorbed in 120 ml. of $1N$ sodium hydroxide, 3.90 grams of anisic acid were added, and the solution was placed in a closed flask provided with a dropping funnel. The flask was evacuated to a pressure of about 200 mm. and from the dropping funnel 130 ml. of $1N$ nitric acid were forced into the evacuated space by the atmosphere. This was followed by 10 ml. of a 0.5% solution of sodium hydrosulfite to reduce unreacted chlorine. During the procedure the flask was shaken by hand. The precipitated 3-chloro(36)-anisic acid was removed by filtration, dried, and crystallized twice from 75 ml. of toluene. Yield, 3.22 grams; melting point, 216–217° C.

The product was prepared with an activity of 1 microcurie per milliequivalent.

Chlorine-36 was regenerated from chlorine-containing fractions as silver chloride. Yield of 3-chloro(36)-anisic acid of chlorine consumed was about 75%.

Chloro(36)-anisoylation of Monohydroxy and Monoamino Compounds. 3-Chloro(36)-anisic acid was refluxed with 4 to 6 times as much thionyl chloride and excess thionyl chloride was removed as described earlier. The residue of crude 3-chloro-(36)-anisoyl chloride and 100% excess monohydroxy or monoamino compound, dissolved in pyridine, were refluxed for half an hour. The reaction mixture was then poured into water and the crude product obtained was purified by two crystallizations from an organic solvent. The mother liquor from the crystallizations was saponified and some 3-chloro(36)-anisic acid could be regenerated. Yield of chloro(36)-anisoylated compounds based on 3-chloro(36)-anisic acid consumed were 60 to 90%. The melting point of a product differed not more than a few tenths of a degree from that of the pure compound.

Chloro(36)-anisoylation of Dihydroxy and Diamino Compounds. The reaction was first carried out with an excess of hydroxy or amino compound as described above. An excess of inactive 3-chloroanisoyl chloride was then added. After the reaction had finished alcohol was added, the mixture was poured into water, and the product was purified as mentioned above.

APPARATUS AND MEASUREMENTS

Determination of Purity. The purity of a derivative was determined from the melting point (4).

The molar melting point depression must be determined for each component to be analyzed.

Radioactivity Measurements. The technique has been described (5).

A ratio of two activities was determined with a statistical error corresponding to a standard deviation of 0.6 to 0.7%.

ANALYTICAL PROCEDURES

Reagents. 3-Chloroanisoyl chloride; distilled, melting point, 64–65° C.

3-Chloroanisoyl derivative of compound to be analyzed; pure.
3-Chloro(36)-anisoyl derivative of compound to be analyzed.
Pyridine; reagent grade, dried over barium oxide.
Dioxane; alcohol (99.5 to 99.9%); methanol; ligroine; all of reagent grade.

Preparation of Active Solution. Dissolve an amount of the 3-chloro(36)-anisoyl derivative corresponding to 0.7 microcurie in 100 ml. of dioxane.

Preparation of Standard Sample. Weigh accurately about 200 mg. of the 3-chloroanisoyl derivative (inactive) and 1.5 ml. of active solution. Add dioxane to the mixture until a clear solution is obtained by boiling. Pour into water and recrystallize the product.

Procedure. Weigh in a test tube an amount which is estimated to contain about 1 meq. of the compound to be analyzed. Add 3 ml. of pyridine and about 0.8 gram of 3-chloroanisoyl chloride and shake until the 3-chloroanisoyl chloride has dissolved. If a hydroxy compound is to be analyzed, reflux for 20 minutes; if an amino compound is to be analyzed, allow to stand at room temperature for half an hour. Add 3 ml. of alcohol and boil for a moment. Add 1.5 ml. of active solution, and determine the amount accurately by weighing before and after addition. Heat the mixture to clear solution and pour into 100 ml. of water. Remove crystals by filtration, and wash with water, and finally with 2 ml. of methanol. Crystallize from an appropriate solvent until the melting point differs by less than 1° from that of the pure 3-chloroanisoyl derivative. Determine the purity from the melting point. Measure the specific activity (activity per unit weight) of this final sample as a ratio of the specific activity of the standard sample (correct for background and self-absorption).

Table I. 3-Chloroanisoyl Derivatives

Compound	M.P., ° C.	Formula	Analysis, % Cl Calcd.	Found
Phenol	141.7–142.0	$C_{14}H_{11}O_2Cl$	13.51	13.46
Pyrocatechol[a]	174.4–174.9	$C_{22}H_{16}O_6Cl_2$	15.86	15.82
Methanol	94.0– 94.3	$C_9H_9O_2Cl$	17.69	17.78
Ethylene glycol[a]	186.5–186.7	$C_{18}H_{16}O_6Cl_2$	17.76	17.80
Aniline	169.2–169.5	$C_{14}H_{12}O_2ClN$	13.56	13.63
Ethylenediamine[a]	243.7–244.1	$C_{18}H_{18}O_4Cl_2N_2$	17.85	17.74

[a] Di-(3-chloroanisoyl) derivative.

CALCULATION

$$B = \left(\frac{1}{r} \times \frac{A}{a} \times b + Ay \times \frac{1-r}{r} \right) \times \frac{P}{100}$$

and

$$\text{Mg. of compound} = \frac{\text{molecular weight of compound} \times B}{\text{molecular weight of chloroanisoyl derivative}}$$

where

$$r = \frac{\text{specific activity of final sample}}{\text{specific activity of standard sample}}$$

A = milligrams of active solution added

B = milligrams of 3-chloroanisoyl derivative present after reaction with 3-chloroanisoyl chloride

a = milligrams of active solution used in preparing standard sample

b = milligrams of 3-chloroanisoyl derivative used in preparing standard sample

y = milligrams of 3-chloroanisoyl derivative per milligram of active solution

P = per cent purity of final sample

EXPERIMENTAL

The method has been applied to the assay of the following compounds:

1. Phenol, analytical grade.
2. Pyrocatechol, c.p., the product was recrystallized twice from benzene, melting point, 104.5–105° C.
3. Methanol, reagent grade, water-free.
4. Ethylene glycol; the product was obtained as a middle fraction by distillation of a technical product. $n_D^{25} = 1.4298$.
5. Aniline, reagent grade.
6. Ethylenediamine; a commercial product was dried with potassium hydroxide and distilled in a vacuum. Titration with $1N$ hydrochloric acid showed a content of 81.9% ethylenediamine (monohydrate 76.9%). The values in Table IX represent the amounts of 100% ethylenediamine calculated from the titration value.

The data concerning the 3-chloroanisoyl derivatives are listed in Table I.

In the experiments the samples for radioactivity measurements were purified until the melting point differed by less than 0.2° from that of the pure substance. The correction due to the purity is then very small and an estimated melting point depression of 0.5° per per cent of impurity was used.

Only in the analysis of methanol was the difference in melting points about 1°; the melting point depression was determined to be 0.5° per per cent content of ethyl-3-chloroanisate.

DETERMINATION OF PHENOL

The derivative was purified by crystallizing from 10 ml. of alcohol, 5 ml. of alcohol, and twice from 10 ml. of methanol.

DETERMINATION OF PYROCATECHOL

The derivative was purified by two crystallizations from 25 ml. of alcohol.

DETERMINATION OF METHANOL

The usual procedure had to be modified. About 3 meq. of methanol were required in the analysis and 1.2 grams of 3-chloro-

anisoyl chloride were added. The derivative was crystallized twice from 5 ml. of methanol and once from 5 ml. of ligroine (Table V).

DETERMINATION OF ETHYLENE GLYCOL

The derivative was crystallized twice from 10 ml. of a dioxane-alcohol mixture (1 to 1).

DETERMINATION OF ANILINE

The derivative was crystallized from 5 ml. of alcohol and twice from 10 ml. of 61% alcohol.

DETERMINATION OF ETHYLENEDIAMINE

The derivative was crystallized from 20 and 10 ml. of alcohol.

DISCUSSION

Experiments with varying excess of 3-chloroanisoyl chloride (Tables III and VIII) show that the chloroanisoylation may be considered to be quantitative. Even moderate amounts of water are permissible (Tables II and IX).

When it is possible to isolate the desired derivative, a content of homologs will not fundamentally disturb the determination. It was usually considered as outside the scope of this investigation to develop methods of purification when homologs were present. However, the methods indicated may often give sufficient purification (Tables II and VII).

Table II. Determination of Phenol

Phenol, Mg.	Recovered Mg.	Recovered %
103.8	103.5	99.7
103.5	103.5	100.0
103.8	103.9	100.1
100.8	101.8	101.0
101.2[a]	101.9	100.7
103.2[a]	103.3	100.1
103.5[a]	104.0	100.5
104.6[b]	105.3	100.7
104.6[c]	104.7	100.1
104.4[c]	103.7	99.3
104.0[d]	98.1	94.3

[a] 5 mg. of o-, 5 mg. of m-, and 5 mg. of p-cresol added to sample.
[b] 10 mg. of water added to sample.
[c] 25 mg. of water added to sample.
[d] 50 mg. of water added to sample.

Table III. Effect of Varying Excess of 3-Chloroanisoyl Chloride on Determination of Phenol

Phenol, Mg.	3-Chloroanisoyl Chloride, Mg.	Recovered Mg.	Recovered %
100.9	400	100.2	99.3
102.4	600	103.1	100.7
104.2	600	104.4	100.2

Table IV. Determination of Pyrocatechol

Pyrocatechol, Mg.	Recovered Mg.	Recovered %
64.9	64.0	98.6
63.5	63.2	99.5
64.3	63.9	99.4
65.3	64.4	98.6

Table V. Determination of Methanol

Methanol, Mg.	Recovered Mg.	Recovered %
97.2	96.5	99.3
95.6	97.3	101.8
95.6	95.6	100.0
96.7	96.5	99.8
96.6	97.2	100.6

Table VI. Determination of Ethylene Glycol

Ethylene Glycol, Mg.	Recovered Mg.	Recovered %
39.8	39.3	98.5
39.0	38.3	98.2
40.6	40.4	99.5
39.2	38.7	98.7

Table VII. Determination of Aniline

Aniline, Mg.	Recovered Mg.	Recovered %
94.8	95.1	100.3
98.5[a]	98.0	99.5
98.0[a]	97.8	99.8
99.1[a]	98.0	98.9
98.4[a]	97.5	99.1

[a] To the sample were added 5 mg. of o- and 5 mg. of p-toluidine.

Table VIII. Effect of Varying Excess of 3-Chloroanisoyl Chloride on Determination of Aniline

Aniline, Mg.	3-Chloroanisoyl Chloride, Mg.	Recovered Mg.	Recovered %
97.2	300	97.2	100.0
94.7	400	95.0	100.3
96.0	600	96.1	100.1

Table IX. Determination of Ethylenediamine

Ethylenediamine, Mg.	Recovered Mg.	Recovered %
24.95	23.5	94.2
25.35	23.7	93.5
24.85	23.7	95.4
25.15	24.0	95.4
25.05[a]	23.9	95.4
26.40[a]	24.6	92.8
25.60[b]	21.0	82.2
26.05[b]	21.4	82.1

[a] 10 mg. of water added to sample.
[b] 25 mg. of water added to sample.

In some of the series (Tables IV, VI, and IX) values a little too low are found. It seems reasonable to suppose that the compounds analyzed were not pure.

The precision of the final result will usually be determined mainly by the precision of the radioactivity measurements.

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Corrections

In the article on "Automatic Titrating and Recording Apparatus for Microbiological Assays" [Eades, C. H., Jr., McKay, B. P., Romans, W. E., and Ruffin, G. P., ANAL. CHEM., 27, 123 (1955)] reference (9) should read: McKay, B. P., and Eades, C. H., Jr., Ibid., 27, 123 (1955). In the article on "Electromagnetic Laboratory Valve" [McKay, B. P., and Eades, C. H., Jr., ANAL. CHEM., 27, 163 (1955)] the reference given in the fourth line of the first paragraph should read: Eades, C. H., Jr., McKay, B. P., Romans, W. E., and Ruffin, G. P., ANAL. CHEM., 27, 123 (1955).

In the article on "Potentiometric Titration of Very Weak Acids [Deal, V. Z., and Wyld, G. E. A., ANAL. CHEM., 27, 47 (1955)] the second sentence under Solvent Effect on page 48 should read: "Carboxylic acids, on the other hand, titrate as moderately weak acids in water, as weak acids in dimethyl formamide, and as strong acids in ethylenediamine."

Reproducibility of Mounting of Solid Samples of Chlorine-36 Compounds for Radioactivity Measurements

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A series of measurements with four different organic chlorine-36 compounds is described. Three different persons have prepared samples.

SEVERAL years' experience has shown that radioactivity of solid samples of chlorine-36 compounds can be determined accurately.

EXPERIMENTAL

Equipment. Geiger-Müller counter. An ordinary end-window counter was used [Madsen-tube (1)]; window thickness, 3 mg. per sq. cm.; window diameter, 30 mm.; background, about 12 counts per minute.

Scaler. Brüel and Kjær electronic counter 6501.

Aluminum dishes. The samples were mounted in aluminum dishes, 14 mm. in diameter, 2 mm. high. A dish was used for only one determination.

Mounting of Samples. An amount of 50 ± 5 mg. of the crystalline compound is placed in an aluminum dish, a few drops of methanol or acetone are added, and the slurry is smoothed with a nickel spatula during evaporation of the suspending agent. Finally the sample is dried under an infrared lamp.

counts and the whole counting procedure was performed twice—i.e., a relative activity was determined with a statistical counting error of 0.61%.

CALCULATION

The relative activity of a sample is calculated from:

$$a_x = \frac{\text{counts per minute of sample}}{\text{counts per minute of standard}}$$

Corrected activity is calculated from:

$$a_{50} = \frac{50}{x}\, a_x + (x - 50) \times 0.0060$$

where

a_x = relative activity of x-dl mg. sample

a_{50} = relative activity of the sample corrected to correspond to a weight of 50 mg.

The last term in the expression is the self-absorption correction and has been determined from the measurements here described.

Table I. Activity Measurements of Different Compounds

Operator	Compound I Weight, mg.	Compound I Measured	Compound I Corrected	Compound II Weight, mg.	Compound II Measured	Compound II Corrected	Compound III Weight, mg.	Compound III Measured	Compound III Corrected	Compound IV Weight, mg.	Compound IV Measured	Compound IV Corrected
Very skilled	45.78	0.942	1.001	46.70	0.957	1.004	47.95	0.972	1.001	48.00	0.975	1.003
	50.16	1.001	0.999	48.15	0.975	1.001	50.20	1.005	1.002	50.15	1.008	1.005
	51.10	1.001	0.986	49.25	0.997	1.007	51.00	1.014	1.000	51.00	1.016	1.002
	53.87	1.044	0.992	53.26	1.054	1.009	53.32	1.046	1.000	52.95	1.036	0.996
	57.55	1.108	1.008	54.65	1.048	0.987	57.22	1.087	0.992	56.70	1.091	1.002
	Av.		0.997			1.001			0.999			1.002
	S		0.008			0.009			0.004			0.003
Medium skilled	44.62	0.922	1.000	45.69	0.958	1.021	46.35	0.941	0.993	47.75	0.991	1.023
	47.25	0.965	1.004	47.78	0.989	1.022	48.62	0.971	0.990	49.10	0.983	0.995
	50.15	1.002	1.000	50.84	1.010	0.999	49.86	0.990	0.992	50.17	1.000	0.997
	51.14	1.020	1.005	53.33	1.051	1.005	51.60	0.997	0.976	51.05	1.014	0.999
	54.25	1.059	1.000	55.93	1.060	0.983	53.80	1.038	0.987	55.64	1.082	1.006
	Av.		1.002			1.006			0.988			1.004
	S		0.002			0.016			0.007			0.011
Unskilled	46.44	0.920	0.970	47.32	0.980	1.018	45.40	0.929	0.994	47.00	1.022	1.069
	49.25	0.993	1.003	49.01	0.972	0.985	48.90	0.972	0.987	48.40	0.986	1.008
	52.75	1.040	1.002	50.82	1.018	1.006	51.86	1.017	0.991	48.84	0.971	0.987
	54.50	1.057	0.997	51.90	1.032	1.006	53.27	1.035	0.992	53.90	1.050	0.997
	55.80	1.076	1.000	54.49	1.042	0.984	54.90	1.043	0.979	56.32	1.084	1.000
	Av.		0.994			1.000			0.989			1.012
	S		0.014			0.015			0.006			0.033

Materials. The compounds were prepared for other purposes (2, 3), and were chosen because they differ appreciably in solubility and crystal structure.

Compound I. 4-Chloro-2-methylphenoxyacetanilide, melting point 130° C.

Compound II. 2,4-Dichlorophenoxyacetanilide, melting point 111° C.

Compound III. Methyl-3-chloroanisate, melting point 94° C.

Compound IV. Pyrocatechol-di-(3-chloroanisate), melting point 175° C.

Compound II has a cottonlike structure and had to be mounted in the dish with acetone. Methanol was used for the other compounds.

The activity of the compounds was 1000 to 2000 counts per minute per 50 mg.

Counting. The samples from one compound were all counted against a standard sample of the same compound having a weight of 50.0 mg. Each sample was counted to about 20,000

DISCUSSION

The results (given in Table I) show that errors introduced by preparing the samples usually are small in comparison with the counting error.

In the author's laboratory a chlorine-36 dilution analysis is

Table II. Duplicate Determinations in Routine Analyses

No. of Duplicate Detns.	Deviation between Duplicate Detns., %
24	0 −0.5
14	0.5–1.0
11	1.0–1.5
8	1.5–2.0
6	2.0–2.5

used as routine analysis (*2*). Counting is carried out with 4-chloro-2-methylphenoxyacetanilide as described in this note. A series of duplicate determinations has been performed, and Table II gives the deviations between these. Calculation of these results shows that a single determination is performed with a standard deviation of 0.79%. The small difference from the statistical counting error (0.61%) shows that no other factors have a serious influence.

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Reduction of Nitroguanidine by Titanium(III) Chloride

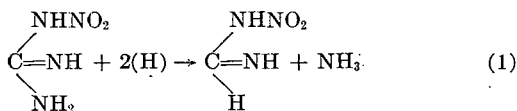WARREN W. BRANDT[1], JOHN E. DeVRIES, and E. ST. CLAIR GANTZ

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The reduction of nitroguanidine by titanium(III) chloride in 1 to 1 hydrochloric acid was studied with the addition of iron(II), in a 20 to 1 ratio to nitroguanidine. The redox reaction is 98% complete for consumption of 8 equivalents of titanium(III). The route and mechanism of the iron "catalyzed" reaction was also studied. The end products of the reaction are guanidine and ammonia. It is proposed that the function of the iron(II) is to stabilize, by complexation, hydroxylaminoguanidine and to make this intermediate susceptible to further reaction with titanium(III). The reduction of nitroguanidine using a 20 to 20 to 1 ratio of titanium(III), iron(II), and nitroguanidine is quantitative and could be used for assay of nitroguanidine.

THE reduction of nitro groups with titanium(III) has become a standard analytical method for their quantitative determination. The usual procedure involves adding an excess of titanium(III), boiling for a short period, and back-titrating with iron(III) (*3*). Nitro groups in compounds such as nitrobenzene require 6 equivalents of titanium per mole. In general, the reduction is carried out in strongly acidic solution.

The extension of this method to the nitramine group revealed that in these cases the nitro group required only 4 equivalents of titanium(III). Kouba and coworkers (*2*) determined nitroguanidine quantitatively by this method. However, when they attempted to determine RDX (hexahydro-1,3,5-trinitro-s-triazine) by the same procedure the 4-equivalent reduction per nitro group was only 60% complete. They found that by introducing iron(II) to the reaction mixture the reduction was within 1% of theory for 4 equivalents.

Zimmerman and Lieber (*7*) extended the investigation of titanium(III) reductions to include several nitroammonocarbonic acids. When iron(II) was introduced into the reduction of nitroguanidine and nitroaminoguanidine, they found that the reaction then approached a total of 8 equivalents per mole instead of the usual 4. A new path of reduction was proposed to explain this phenomenon. Their proposal involved reductive cleavage of the nitroguanidine as the first step, and subsequent reduction of the nitramino group to a hydrazine.

$$\begin{array}{c} \text{NHNO}_2 \\ \diagup \\ \text{C=NH} \\ \diagdown \\ \text{NH}_2 \end{array} + 2(\text{H}) \rightarrow \begin{array}{c} \text{NHNO}_2 \\ \diagup \\ \text{C=NH} \\ \diagdown \\ \text{H} \end{array} + \text{NH}_3 \qquad (1)$$

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$$\begin{array}{c} \text{NHNO}_2 \\ \diagup \\ \text{C=NH} \\ \diagdown \\ \text{H} \end{array} + 6(\text{H}) \rightarrow \begin{array}{c} \text{NHNH}_2 \\ \diagup \\ \text{C=NH} \\ \diagdown \\ \text{H} \end{array} + 2\text{H}_2\text{O} \qquad (2)$$
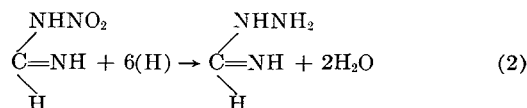
Recently, Sternglantz (*5*) has shown that under weakly acidic conditions, using citrate buffer, nitroguanidine consumes 6 equivalents of titanium(III) per mole.

The current investigation was undertaken in order to study in more detail the iron(II)–catalyzed reduction of nitroguanidine by titanium(III) and to attempt to demonstrate the mechanism of the function of the iron(II).

APPARATUS

Because of the instability of titanium(III) in air, all reagent solutions were kept under an atmosphere of carbon dioxide both in storage and in the burets. The reaction flasks were all connected to reflux condensers by ground-glass joints. The former were also equipped with a small inlet side arm for introducing a stream of carbon dioxide over the surface of the solution. This flow of carbon dioxide over the surface was started following the initial degassing of the acid to be used and continued through the final titration with iron(III) without interruption. All carbon dioxide passing into the reaction flasks was passed through bubblers, so that the rate of flow was readily visible at all times.

REAGENTS

A 0.4N titanium(III) solution was prepared in 1 to 1 hydrochloric acid. It is convenient to use titanium hydride (Metal Hydrides, Inc., Beverly, Mass.) as recommended by Wagner *et al.* (*6*). A 1.0N iron(II) solution was prepared in 1 to 1 hydrochloric acid from reagent grade iron(II) sulfate. Reagent grade iron(III) alum [$\text{Fe(NH}_4)(\text{SO}_4)_2.12\text{H}_2\text{O}$] was used to prepare a 0.3N iron(III) solution in 1 to 1 hydrochloric acid. All hydrochloric acid was Baker and Adamson c.p. reagent, 37 to 38%. Nitroguanidine was prepared by recrystallization of a commercial sample from water. Potassium nitrate was Baker and Adamson reagent grade.

Nitrosoguanidine (melting point 165° C.) was prepared by the procedure described by Davis (*1*). Aminoguanidine hydrochloride was prepared from Eastman's white label aminoguanidine bicarbonate.

The titanium(III) and iron(II) solutions were standardized against National Bureau of Standards potassium dichromate using sodium diphenylbenzidine sulfonate indicator. The iron(III) was standardized against the titanium(III).

REDUCTION PROCEDURE

The weighed sample was dissolved in 50 ml. of 1 to 1 hydrochloric acid and the solution degassed with carbon dioxide for 5 to 7 minutes. The titanium(III) and iron(II) were then added and the solution was refluxed. The mixture was then cooled in a water bath and titrated with iron(III) to a thiocyanate end point. The time of reflux was measured from the start of vigorous bubbling.

In the reactions of potassium nitrate and nitrosoguanidine the

1 to 1 hydrochloric acid was degassed and the titanium(III) and iron(II) were added before addition of the weighed sample.

All results reported have been corrected by means of a blank containing all the reagents except the compound being reduced. In general, the blank encountered was equal to approximately 0.1 equivalent of reduction per mole.

RESULTS AND DISCUSSION

Over-all Reduction of Nitroguanidine. The reduction of nitroguanidine in 1 to 1 hydrochloric acid was found to require 4 equivalents per mole in the presence of a 20 to 1 mole ratio of titanium(III) to nitroguanidine when refluxed for 30 minutes as reported (7). The addition of a 20 to 1 excess of iron(II) caused the predicted increase in reduction (7). Although Zimmerman and Lieber reported a maximum of 7.6 equivalents per mole, the above conditions gave consistent results averaging 7.8 equivalents of reduction per mole. This represents approximately 98% reduction, which was deemed sufficiently close to quantitative for the current investigation. A 25% decrease in the amount of titanium(III) caused a decrease of about 0.2 equivalent per mole. Increasing the iron(II) concentration or the time of reflux caused no appreciable change.

In order to determine the function of the iron(II), a stepwise investigation of the reduction of nitroguanidine was undertaken. In these experiments a calculated amount of titanium(III) was added in order to provide an integral number of equivalents per mole of nitroguanidine. In one series, titanium(III) was refluxed alone with the nitroguanidine for 5 minutes, followed by the addition of 20 to 1 iron(II) and titanium(III) and refluxing for an additional 20 minutes. In the other series, the usual 20 to 1 excess of iron(II) was added with the titanium(III), refluxed for 5 minutes, then excess titanium(III) was added followed by an additional reflux period of 20 minutes. The over-all equivalents of titanium(III) consumed per mole were determined.

The results, shown in Table I, demonstrate that the iron(II) is not accomplishing any reduction itself at any step in the process. In the second series a 20 to 1 excess of iron(II) is always present, but the total amount of reduction corresponds to the concentration of titanium(III) added initially.
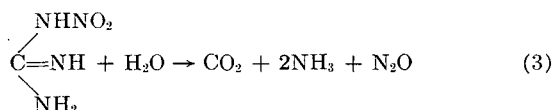
Table I. Stepwise Reduction of Nitroguanidine

Ti(III) Alone for Initial Reflux, Eq./Mole		Ti(III) and Fe(II) for Initial Reflux, Eq./Mole	
Ti added	Consumed	Ti added	Consumed
2.0	2.0	2.0	2.0
3.0	2.8	3.0	3.0
4.0	3.6	4.0	3.9
5.0	3.7	5.0	4.9
6.0	3.7	6.0	5.9
7.0	3.8	7.0	6.7

End Products of the Reduction. The second step in the investigation was the identification of the products of the 8-equivalent reduction of nitroguanidine. A method was developed for the determination of any ammonia which might be formed during the reaction. First, crystalline potassium iodate was added until the disappearance of the last traces of iodine coloration. Sodium sulfite crystals were then added until the solution was free of iodine monochloride. Nitrogen gas was bubbled through the solution for 15 minutes in order to remove the excess sulfur dioxide. The solution was then transferred to a Kjeldahl flask and ammonia distilled as in the usual Kjeldahl procedure. The determination was completed by titration with standard alkali.

By utilizing the above method it was possible to demonstrate the production of appreciable quantities of ammonia during the reaction. The results were too variable to permit a formulation of the exact number of moles of ammonia for each mole of starting material, but the value was approximately unity. Ammonia would be expected as one of the products in the mechanism proposed by Zimmerman and Lieber (see Equation 1). It might

also be formed by hydrolytic degradation (Equation 3); however, the reduction is 90% complete or better, and therefore the latter reaction could not explain the quantity of ammonia formed unless the product of reduction were itself cleaved. This possibility was tested by putting aminoguanidine hydrochloride through the above procedure as a blank. Only traces of ammonia were found.

$$\begin{array}{c} \text{NHNO}_2 \\ | \\ \text{C=NH} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3 + \text{N}_2\text{O} \\ | \\ \text{NH}_2 \end{array} \qquad (3)$$

In order to isolate other products of the reaction, a typical 8-equivalent-per-mole reduction was carried out and titrated. The iron was oxidized with hydrogen peroxide and extracted with isopropyl ether. The solution was then neutralized with sodium hydroxide until the hydrous titanium(IV) oxide precipitated. After filtration, a saturated aqueous solution of picric acid was added to the filtrate. A heavy yellow precipitate settled out on standing. After being twice recrystallized from ethyl alcohol the solid melted at 333° to 334° C. X-ray diffraction patterns agreed with the published results for guanidine picrate (4) and with prepared standards. This demonstrates the presence of guanidine as an end product of the 8-equivalent reduction of nitroguanidine. The amount formed was sufficient to classify it as a major product and not the result of a side reaction. The effect of hydrogen peroxide in 1 to 1 hydrochloric acid and subsequent neutralization was checked with aminoguanidine in a simulated reaction mixture, since aminoguanidine was the end product proposed by Zimmerman and Lieber (7). No appreciable quantity of guanidine was formed.

The presence of guanidine does not fit into the mechanism proposed by Zimmerman and Lieber. A cleavage of the N—N bond at some point during the reduction of nitroguanidine is required. The simple possibilities for reduction of nitroguanidine to guanidine plus ammonia are presented in Figure 1.

Figure 1. Possible reactions in reduction of nitroguanidine to guanidine and ammonia
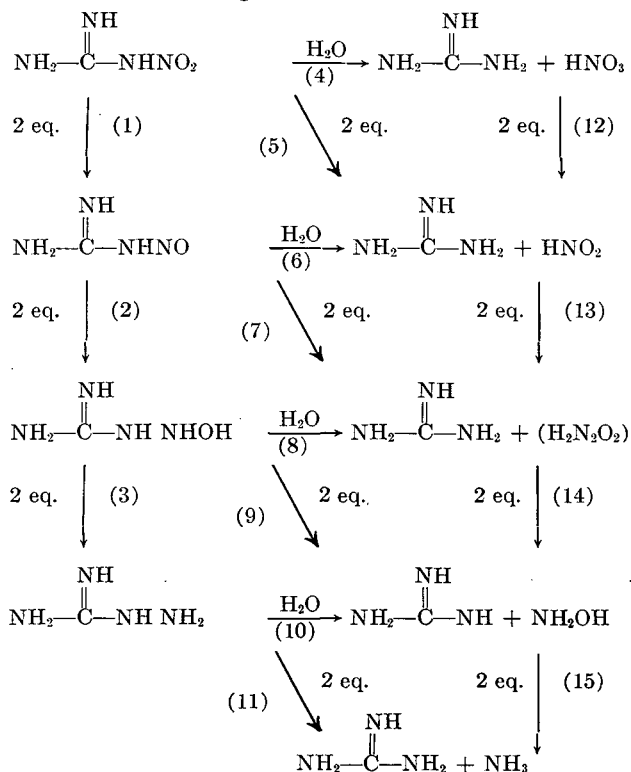
The reduction of nitroguanidine to aminoguanidine followed by reductive cleavage to ammonia and guanidine (Reactions 1,2,3, and 11, Figure 1) would seem to be a plausible path. It has been reported that aminoguanidine is not reduced by titanium(III) (7), but the reaction in the presence of iron(II) had not been checked. A sample of aminoguanidine was introduced into the usual reduction mixture for an 8-equivalent reduction and refluxed 30 minutes. The average of several runs gave a 1% reduction. It was therefore assumed that the path of reduction did not proceed through aminoguanidine. This eliminates Reactions 3, 10, and 11, Figure 1, and requires cleavage of a reduction product of nitroguanidine prior to the aminoguanidine stage; presumably at the 2- or 4-electron step.

In addition to generating guanidine, such a cleavage of a nitroguanidine reduction product would furnish a compound which is an intermediate in the reduction path from nitrate ion to ammonia. Therefore, the reduction of nitrate ion in 1 to 1 hydrochloric acid by titanium(III) was investigated.

Over-all Reduction of Nitrate. In these reactions, the potassium nitrate used as the source of nitrate ion was added after all reagents were present. The results of closely agreeing determinations with titanium(III) alone as the reductant gave 3.8 equivalents per mole, which was interpreted as a 4-equivalent reduction (Reactions 12, 13, Figure 1). In the presence of a 20 to 1 ratio of iron(II) in addition to the titanium(III) the results of four determinations gave an average of 7.3 equivalents per mole, which was interpreted as an 8-equivalent reduction (Reactions 12–15, Figure 1). Considerable effervescence occurs early in the reduction, which might explain the relatively poor percentage of reduction observed. These results indicate that iron(II) is effective in causing the reduction of nitrate by titanium(III) to go beyond the 4-electron point in a fashion similar to its influence on the reduction of nitroguanidine.

Two-Equivalent Reduction of Nitroguanidine and Nitrate. Thus far it appeared that iron(II) was producing some species in the course of the reduction of nitroguanidine which titanium(III) was able to reduce. The determination of where this phenomenon takes place—i.e., at what stage in the reduction—was the next point investigated. In order to enable a decision to be made concerning where the nitroguanidine reduction joins that of nitrate (Reactions 12 to 15, Figure 1), the latter was studied as a comparison.

In these reactions the previously described scheme of adding an integral number of equivalents of titanium(III) per mole was utilized. In some cases the iron(II) was included for the initial reflux; in others it was added afterward with the excess titanium(III).

Upon refluxing a mixture of 2 equivalents of titanium(III) per mole of nitrate ion with excess iron(II) for 10 minutes, a reproducible 3-equivalent reduction takes place. This demonstrates the ability of iron(II) to reduce the 2-equivalent reduction product of nitrate ion—i.e., nitrite ion. The solution is an intense brown, indicating the expected nitrosyl iron(II) complex ion. When these results are compared to those in Table I, it becomes apparent that nitroguanidine and nitrate are completely different after reduction by 2 equivalents of titanium(III) per mole. This comparison eliminates Reactions 4, 5, and 6, Figure 1, as possible steps in the reduction of nitroguanidine.

If nitrate ion is refluxed 5 minutes with 2 equivalents of titanium(III) per mole (Reaction 12, Figure 1) and this is followed with excess titanium(III) and iron(II), the reduction proceeds a total of 2.2 moles where 8 (Reactions 12–15, Figure 1) would be expected if the 2-equivalent product (nitrite) were stable. The instability of the nitrite ion is further demonstrated by repeating the experiment and changing the initial reflux time from 5 minutes to 1 minute, and finally to merely standing 5 minutes without heating. The total equivalents of reduction obtained increase with the order of treatment listed, reaching a maximum of 6.4 equivalents per mole for the initial 5-minute cold standing period followed by reflux with excess titanium(III) and iron(II).

A similar study with nitroguanidine points out further dissimilarities between the 2-equivalent reduction products of nitroguanidine and nitrate. An initial 2 equivalents of titanium(III) per mole refluxed for 5 minutes and followed by excess titanium(III) for 20 minutes gave an average of 3.6 equivalents of reduction per mole, indicating excellent stability of the system with 2 equivalents of titanium(III) per mole. However, when the initial 2 equivalents of titanium(III) per mole are refluxed 5 minutes and followed with titanium(III) and iron(II), the total equivalents per mole observed are only 4.9 instead of close to 8.0. This appears to contradict the immediately preceding conclusion concerning good stability.

The most reasonable 2-equivalent reduction product of nitroguanidine would appear to be nitrosoguanidine (Reaction 1, Figure 1). In order to compare the observed phenomena in the reduction of nitroguanidine, a sample of nitrosoguanidine was prepared. The decomposition of nitrosoguanidine in 1 to 1 hydrochloric acid is so rapid that it was possible to obtain only an average of 1.5 for the expected 2-equivalent reduction with titanium(III) and 5.1 with titanium(III) and iron(II). If nitrosoguanidine is added to iron(II) alone in 1 to 1 hydrochloric acid, an intense brown color is formed immediately, indicating a very rapid cleavage of nitrosoguanidine to nitrite ion and guanidine (Reaction 6, Figure 1). In the light of these results, it is apparent that the addition of 2 equivalents per mole of titanium(III) to nitroguanidine does not produce appreciable quantities of nitrosoguanidine. This requires a postulation that the first reaction of nitroguanidine with titanium(III) is essentially a 4-equivalent reduction (Reactions 1, 2, Figure 1). This postulate finds some support in the previous discussion of the stability of the products of the 2-equivalent reduction with titanium(III). The observed contradictory results would appear reasonable if it is assumed that the first 2 equivalents of titanium(III) reduce half of the nitroguanidine a total of 4 equivalents and this 4-equivalent product is unstable. Further titanium would thus reduce the remaining nitroguanidine, giving a good over-all percentage reduction, whereas the titanium-iron mixture would have only half of the nitroguanidine remaining to work on, and an over-all reaction of 6 equivalents would be the maximum possible.

A confirmation of the initial 4-equivalent reduction of nitroguanidine was obtained polarographically. It was first determined that in 1 to 1 hydrochloric acid nitroguanidine gave a reduction wave which could be used. Then a series of three samples was examined. The first contained just nitroguanidine in 1 to 1 hydrochloric acid and gave a 142 μa. wave at $E_{1/2} = -0.24$ volt vs. the mercury pool. The second solution contained an identical amount of nitroguanidine which had been refluxed 10 minutes with 2 equivalents of titanium(III). This solution gave a wave of 71 μa. at $E_{1/2} = -0.30$ volt. The third was identical except that 4 equivalents of titanium were used. It gave a wave of 6.6 μa. at $E_{1/2} = -0.40$ volt. The titanium(IV) present in the last two samples gave a wave at -0.05 volt which did not interfere with the accuracy of the desired measurements. The mercury pool became contaminated by products of the reaction in the last two samples, which might explain the shifting potential.

These data demonstrate the presence of one half of the nitroguanidine after the initial 2-equivalent reduction since the concentration is proportional to the wave height in microamperes. Thus an investigation of the 4-equivalent reduction product became a necessity.

Four-Equivalent Reduction of Nitroguanidine and Nitrate. Nitroguanidine was refluxed initially with 4 equivalents per mole of titanium(III) and excess iron(II). In some cases this reaction was followed by the addition of excess titanium and a second reflux period of 20 minutes. The initial reflux time was

Table II. Four-Equivalent Reduction of Nitroguanidine[a]

No Subsequent Reduction		Excess Ti(III) Added for Second Reflux Period	
Time of reflux, min.	Average total eq./mole	Time of initial reflux, min.	Average total eq./mole
2	3.6	2.5	7.1
4	3.8	10	6.7
10	3.9	15	6.3
30	4.0	30	6.2

[a] Four equivalents of titanium(III) and excess iron(II) initially present in all runs.

Table III. Four-Equivalent Reduction of Nitrate Ion[a]

No Subsequent Reduction		Excess of Ti(III) Added for a Second Reflux Period	
Time of reflux, min.	Average total eq./mole	Time of initial reflux, min.	Average total eq./mole
2.5	3.9	2.0	5.1
10	4.0	5	4.4
		10	4.0

[a] Four equivalents of titanium(III) and excess iron(II) initially present at all runs.

Table IV. Volume of Gas Evolved in 4-Equivalent Reduction of Nitroguanidine and Nitrate Ion

Nitroguanidine, Ml.[a]		Nitrate Ion, Ml.[a]	
Fe(II) absent	Fe(II) present	Fe(II) absent	Fe(II) present
35.4	12.9	40.4	39.4
35.8	13.6	40.6	41.6

[a] 1.92 millimoles of compound used with 10 minutes' boiling in all cases.

varied in order to determine the relative speed of the reduction reaction and the decomposition of the product. The results are shown in Table II. These results show that in the presence of iron(II) the 4-equivalent reduction product of nitroguanidine is stable in refluxing 1 to 1 hydrochloric acid.

For comparison, the same type of study was conducted with nitrate ion. In this case the product of a 4-equivalent reduction was shown to be rather unstable. The results are summarized in Table III. They demonstrate that although the reduction is completed very quickly, the product is unstable and has completely disappeared after 10 minutes' reflux. This is considerably different from nitroguanidine (Table II).

A further demonstration of the difference between the 4-equivalent reduction of nitroguanidine and nitrate ion is given by the following experiments. These also served to confirm the previously discussed difference in behavior of the 4-equivalent reduction product of nitroguanidine in the presence and absence of iron(II). This series of experiments involved collecting the gas evolved during the course of reduction. The gas was swept through the system with carbon dioxide and collected over 30% potassium hydroxide. The results are given in Table IV. The difference in volume of gas produced when iron(II) is present or absent showed a lack of identity for the 4-equivalent reduction products of nitroguanidine and nitrate. Thus Reactions 4 to 8, Figure 1, are eliminated.

CONCLUSIONS

It would now appear that the following mechanism takes place in the reduction of nitroguanidine. A 4-equivalent reduction occurs initially (Reactions 1 and 2, Figure 1), and this is followed by a reductive cleavage of hydroxylaminoguanidine (Reaction 9, Figure 1). The latter reaction is effectively simultaneous with the subsequent reduction of the hydroxylamine (Reaction 15, Figure 1). The reduction of hydroxylamine hydrochloride was investigated separately and found to consume two equivalents of reducing agent. The reaction with titanium(III) proceeded rapidly. The reaction with iron(II) proceeded slowly. Although a small amount of reduction of hydroxylamine would be

expected at the 6 and 7 equivalent steps in Table I, it apparently is not formed in any appreciable quantity. This could mean that the reaction did not go through hydroxylamine, or that it utilizes the last 4 equivalents in a concurrent fashion, producing no appreciable concentration of hydroxylamine. To eliminate hydroxylamine from the reduction path would require that no cleavage of nitroguanidine or its simple reduction products could occur at an N—N bond, since these would all give precursors of hydroxylamine. This leaves only a complex procedure in which a coupled product is formed which cleaves to give guanidine and ammonia and allows an over-all 8-equivalent reduction to occur. It seems more reasonable to assume that the reduction goes through hydroxylamine rapidly without producing a final measurable concentration.

The 4-equivalent product is the essential point in the function of the iron(II). Without the iron(II), titanium(III) is unable to bring about further reduction and the product is rapidly decomposed, probably by hydrolytic cleavage to hyponitrous acid which also decomposes rapidly. This fact was confirmed by the data in Table IV and by mass spectrographic analysis of the gas from a 4-equivalent reduction of nitroguanidine by titanium(III). With iron(II) present, the compound is stabilized to hydrolytic cleavage, and altered in such fashion as to permit reductive cleavage by additional titanium(III). Although it is a hypothetical compound, hydroxylaminoguanidine is the simplest 4-equivalent reduction product to propose. It is expected to be unstable; thus it would be necessary only to have this compound stabilized by the presence of iron(II) in order to make its postulation logical. It has been shown that large excesses of iron(II) are necessary in order to approach an 8-equivalent reduction. The total equivalents of reduction increases asymptotically toward 8 as the concentration of iron(II) increases. This would indicate also that the function of the iron(II) is not catalytic. It appears rather to be the type of function which would be expected if the excess iron(II) were affecting a displacement of equilibrium. The most reasonable stabilization of the hydroxylaminoguanidine would be through some interaction compound, possibly of the nature of a metal-organic complex ion. It would be expected that such a species would be quite unstable in 1 to 1 hydrochloric acid and that large excesses of iron(II) would be necessary to promote its formation. It is not unreasonable to assume that such interaction could modify the electronic distribution in the hydroxylaminoguanidine sufficiently to facilitate a reductive cleavage of the N—N bond by titanium(III).

Attempted isolation of the postulated hydroxylaminoguanidine was unsuccessful. Reaction mixtures, upon neutralization, effervesced considerably, and guanidine was identified in appreciable quantities. This is the expected product of hydrolytic cleavage of hydroxylaminoguanidine. The compound will probably have to be isolated in combination with iron(II), the only form in which it has been found to be stable.

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Structure of Reducing Disaccharides by Lead Tetraacetate Oxidation

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The use of lead tetraacetate oxidation for determining the structure of reducing disaccharides is described. Measurements included the production of formic acid and formaldehyde and the consumption of lead tetraacetate. A wide variety of hexose and pentose disaccharides was examined. Only a few milligrams of each compound was required and the oxidation periods were usually 5 to 6 hours. Each position of the glycosidic linkage was associated with a characteristic oxidation pattern. The oxidation behavior of monomethyl monosaccharides was similar to that of the corresponding disaccharides, allowances being made for the contribution of the nonreducing end units in the latter compounds. Results agreed generally with the thesis that reducing sugars are oxidized as the cyclic hemiacetal and were explained, in some instances, by the formation of stable formate esters. A crystalline galactobiose isolated from a partial hydrolyzate of *Acacia pycnantha* gum was characterized by oxidation as 3-O-D-galactopyranosyl-D-galactose.

THE characterization of disaccharides and higher oligosaccharides produced by partial degradation of a polysaccharide constitutes an important aspect of the determination of structure of the polymer. For instance, the finding that maltose could be obtained in high yield from starch (11) furnished an early unequivocal proof of the 1,4-α-D-linkage in the polysaccharide. Modern developments in chromatography (8, 14, 22) greatly facilitate the isolation of oligosaccharides and permit small quantities of polysaccharide material to be examined conveniently. However, with these advantages the need has also arisen for methods to characterize isolated products on a scale not always practicable with the well-established methylation and periodate oxidation techniques. For this purpose, the use of infrared spectroscopy shows much promise (3) and periodate oxidation on the microscale may find limited application (20).

The present paper considers the use of the lead tetraacetate oxidation (1, 6) for characterizing oligosaccharides on the micro scale. Results obtained from oxidation of a representative group of reducing disaccharides have shown that the reaction may indeed be useful for determining the structure of such compounds. The oxidations required only a few milligrams of material and were both convenient and rapid. The rate of formic acid production was determined from the evolution of carbon dioxide in the Warburg respirometer (17, 21). In addition, the reaction mixture was analyzed at chosen intervals for consumption of lead tetraacetate and for other products of the oxidation.

HEXOSE DISACCHARIDES

This group comprised compounds of known constitution having either a 1,3-, 1,4-, or 1,6- linkage. No 1,2-disaccharide was included, but the closely related compound 2-O-methyl-D-galactose was examined, and 3-, 4-, and 6-O-methyl-D-galactose were also oxidized for comparison with the corresponding disaccharides. A crystalline galactobiose of unknown constitution, obtained by partial hydrolysis of *Acacia pycnantha* gum (7), was oxidized under the same conditions and a probable structure was assigned by comparing its oxidation behavior with that of the known compounds.

The rates of formic acid production by hexose disaccharides in which the nonreducing end units are glucosides and galactosides are given in Figures 1 and 2.

Disaccharides having a 1,6- linkage rapidly yielded approximately 5 moles of formic acid (Figures 1 and 2, curve 1), four being presumably from the reducing end and one from the nonreducing end. The production of acid from the 1,3-disaccharide, laminaribiose (4) (Figure 1, curve 3), was much less than from the 1,6-disaccharide, isomaltose (curve 1), though noticeably greater than from the 1,4-disaccharide, maltose (curve 4). In maltose the acid appeared to be derived almost exclusively from the nonreducing end, since its rate of production was very close to that of methyl α-D-glucopyranoside (curve 5). Similarly, the 1,4-disaccharide, lactose (Figure 2, curve 4), and its corresponding glycoside, methyl α-D-galactopyranoside (curve 5), yielded acid at very nearly equal rates. The approximate rates of formic acid production from 1,2-disaccharides are predicted (Figures 1 and 2, curve 2) and are based on oxidation data for 2-O-methyl-D-galactose (Figure 3) superimposed on the curves for methyl glucoside and galactoside, respectively (Figures 1 and 2, curve 5). The oxidation behavior of the galactobiose (Figure 2, curve 3), when allowance was made for differences in the contribution of nonreducing ends, corresponded most closely to that of laminaribiose and therefore suggested the presence of a 1,3- linkage.

In addition to indicating the position of a glycosidic linkage, the rate of acid production may sometimes designate the relative position of the sugar units in a mixed disaccharide. Thus the rapid initial rate of acid production from lactose, corresponding as it does to that from methyl galactoside, is in accord with the established structure in which the galactose unit is known to comprise a pyranose nonreducing end.

Each position of the glycosidic linkage was associated with a characteristic consumption of lead tetraacetate. The calculated consumption of oxidant at the reducing end demonstrated this pattern even more clearly (Table I, column 5). Correction for the nonreducing end was made by assuming its consumption of lead tetraacetate to be equivalent to that of the corresponding methyl glycoside. Most of the oxidation appeared to take place within the first 15 minutes, in which time the reducing ends of the 1,3-, 1,4-, and 1,6- disaccharides consumed about 1, 2, and 3 moles of oxidant, respectively. By requiring 1 mole of oxidant, the galactobiose again conformed to the pattern of the known 1,3-linked disaccharide.

The data for consumption of oxidant and acid production suggest that the compounds were oxidized in the cyclic hemiacetal form—i.e., in accordance with the thesis of Criegee (6). In this form the reducing end of a 1,3-disaccharide (I, R =

Table I. Consumption of Lead Tetraacetate by Hexose Disaccharides

Disaccharide	Time, Min.	Total Consumed[a]	Consumed by Corresponding Glycoside[a]	Consumed by Reducing End
Melibiose (1,6-)	15	4.31	1.40	2.91
	50	5.33	1.72	3.43
Isomaltose (1,6-)	15	3.53	0.50	3.03
	50	4.37	1.01	3.36
Lactose (1,4-)	15	3.24	1.40	1.84
	50	3.88	1.72	2.16
Maltose (1,4-)	15	2.30	0.50	1.80
	50	3.13	1.01	2.12
Laminaribiose (1,3-)	15	1.60	0.50	1.07
	50	2.12	1.01	1.11
Galactobiose	15	2.41	1.40	1.01
	50	2.96	1.72	1.24

[a] Corrected for lead tetraacetate consumed in oxidation of formic acid.

glycosyl) can be oxidized only at the 1,2-glycol group and a formyl ester (II) is likely to be produced rather than free formic acid (*19*). Further oxidation of II is then dependent on removal of the ester group. Hence the yield of acid from laminaribiose at the outset was of the same order as from methyl α-D-glucopyranoside and one mole of oxidant was consumed by the reducing end. However, the ester was apparently slowly hydrolyzed and eventually the quantity of acid from the laminaribiose considerably exceeded that obtained from the glycoside.

HCOH HCOH ROCH HCOH HC— H₂COH (I)

HCOH ROCH HCOH HCO—CH H₂C— (II)

HCOH HCOH HOCH HCOR HC— H₂COH (III)
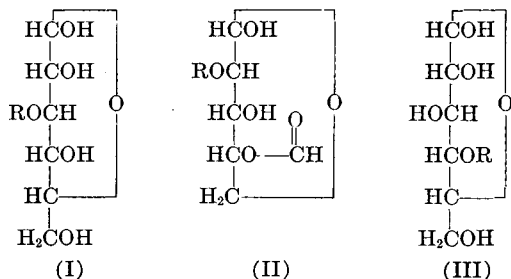
A 1,4-disaccharide (III, R = glycosyl) presents two points of attack at the reducing end (at the 1,2- and 2,3-glycol groups), and two moles of oxidant were, in fact, consumed within the first 15 minutes by the 1,4- compounds examined. It was therefore to be expected that carbon 2 would rapidly be liberated as formic acid, but the results suggest that the acid produced came almost exclusively from the nonreducing end. Possibly the oxidation occurred first at the hemiacetal-glycol group to give a formate ester (IV) in which a new hemiacetal-glycol group was formed, and further oxidation yielded a diformate ester (V). Since additional oxidation would then be dependent on hydrolysis of both ester groups, the rate of acid production would be slow. Support for the postulated formation of the second ester group is provided by the finding that a 1,4-disaccharide in the pentose series (X) yielded one mole of formic acid from the reducing end within 15 minutes reaction time (Figure 4), presumably because no suitably oriented hydroxyl group was available for formation of the second ester as in IV. Further, what appears to be a diformate ester of D-erythrose (possibly V, R = H), rather than a monoester, has been prepared by oxidation of D-glucose with two moles of lead tetraacetate (*18*).

HCOH HOCH HCOR HCO—CH H₂C— IV

CHO HCOR HCO—CH H₂CO—CH V

HCOH HCOH HOCH HCOH HC— H₂COR VI

CHO CO—CH H₂COR VII
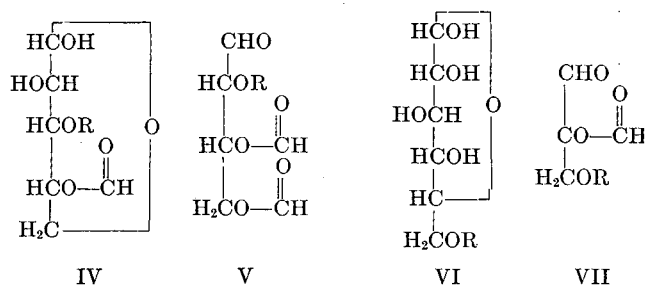
In contrast, there was little evidence of ester formation during oxidation of a 1,6-disaccharide (VI, R = glycosyl). By analogy with the other disaccharides the expected product would have been a formate ester (VII), but its formation would have entailed the release of only 2 moles of acid from the reducing end rather than the 4 moles found. On the other hand, in agreement with the formulation VII, 3 moles of oxidant were consumed by the reducing end within the first 15 minutes and the fourth mole required a much longer period (Table I). Possibly, then, the ester was actually formed but was unstable and easily hydrolyzed. Under the same conditions of oxidation (*18*, *19*), reducing sugars form moderately stable esters, whereas uronic acids, which are structurally akin to 1,6-disaccharides, appear to form no esters or very unstable ones. Little is known of the relative stability, for example, of primary and secondary formate esters and of the influence on stability of neighboring groups. Hence the observed variations in behavior can only be recorded at present rather than explained.

Oxidation of the cyclic hemiacetals of the disaccharides would not be expected to yield formaldehyde since cleavage of carbon 6, the source of the formaldehyde, is prevented (*6*). Thus, no formaldehyde was found with the chromotropic acid reagent (*12*) at the outset of the oxidation of 1,3- and 1,4-disaccharides (Table II). However, on more prolonged oxidation measurable quantities of formaldehyde were liberated and at a much faster rate from laminaribiose than from the 1,4-disaccharides. These differences in rate could be readily reconciled with the postulated reaction products since only one formyl ester group of II, but two ester groups of V, had first to be hydrolyzed before further oxidation could take place with the eventual production of formaldehyde. The results provided an additional index for distinguishing the 1,3- from 1,4- and 1,6-disaccharides, the latter two producing traces of formaldehyde only on prolonged oxidation. Accordingly, the galactobiose again appeared to contain a 1,3- linkage, since it closely resembled laminaribiose in the production of formaldehyde (Table II).

The postulated products from oxidation of a 1,3- and 1,4-disaccharide—II and V, respectively—constitute a pentose and a tetrose derivative, which may be readily differentiated. The disaccharides were therefore oxidized with a small excess of lead tetraacetate and the products were hydrolyzed and examined on the chromatogram. The expected products, arabinose from laminaribiose and erythrose from maltose and lactose, were found. Further, the yield of pentose from laminaribiose, as estimated with the orcinol reagent (*15*), was 1.07 moles, whereas 1,4- and 1,6-disaccharides yielded negligible
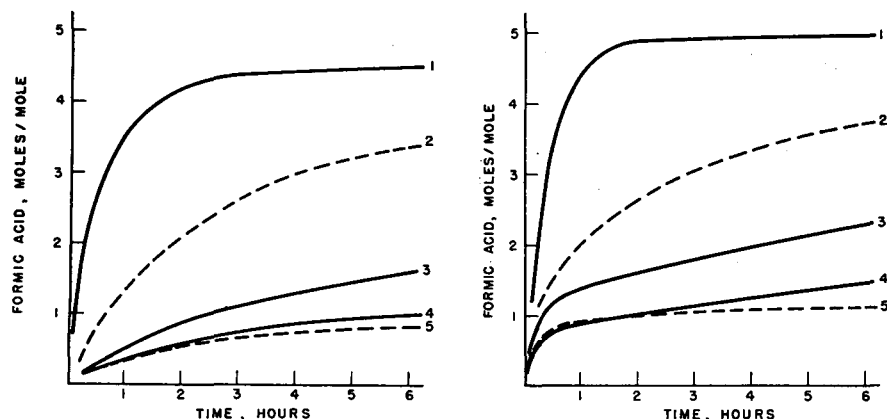
Figures 1 and 2.   Production of formic acid (evolution of carbon dioxide) during oxidation of hexose disaccharides with lead tetraacetate

Contains compounds in which the nonreducing end is a glucoside

1. 1,6-Isomaltose
2. Calculated for a 1,2-disaccharide
3. 1,3-Laminaribiose
4. 1,4-Maltose
5. Methyl α-D-glucopyranoside

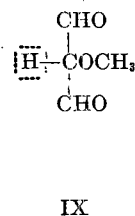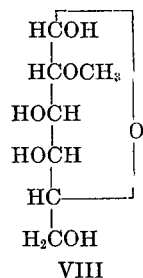Contains compounds in which the nonreducing end is a galactoside

1. 1,6-Melibiose
2. Calculated for a 1,2-disaccharide
3. Galactobiose (unknown linkage)
4. 1,4-Lactose
5. Methyl α-D-galactopyranoside

quantities of pentose. The galactobiose, behaving as a 1,3-disaccharide, on oxidation gave 0.97 mole of a pentose which, on the chromatogram, corresponded to lyxose, the pentose expected by cleavage of carbon 1 from galactose.

MONOMETHYL HEXOSES

The close structural relationship of monomethyl hexoses to the disaccharides suggested an examination of their oxidation behavior; 2-, 3-, 4-, and 6-O-methyl-D-galactose served as representative compounds. Results of the oxidations were in close agreement with those of the corre-

Figure 3. Production of formic acid (evolution of carbon dioxide) during oxidation of monomethyl D-galactoses with lead tetraacetate

1. 6-O-Methyl
2. 2-O-Methyl
3. 3-O-Methyl
4. 4-O-Methyl

sponding disaccharides, allowance being made for the contribution of the nonreducing units in the latter compounds. Thus the rapid production of 4 moles of formic acid from 6-O-methyl-D-galactose was reminiscent of the behavior of the 1,6-disaccharides and the small yields of acid from the 3- and 4-substituted galactoses were characteristic also of the 1,3- and 1,4-disaccharides (Figure 3 and compare Figures 1 and 2).

The acid yields were in general accounted for by the measured consumptions of oxidant (Table III) but, as found for the corresponding disaccharides, the 4-substituted sugar produced very little acid although it consumed two moles of lead tetraacetate. In further agreement with the disaccharide results, little or no formaldehyde was produced during early stages of the oxidation from the 3-, 4-, and 6-O-methyl derivatives (Table III) and only the 3-O-methyl derivative yielded pentose (Table III). An especially interesting member of the group was 2-O-methyl-D-galactose, for which the disaccharide counterpart was not examined and which, therefore, provided an indication of the results to be expected on oxidation of a 1,2-disaccharide. Its yield of two moles of formic acid and one mole of formaldehyde readily distinguished it from the other monomethyl galactoses. In accounting for these products it must be assumed that the cyclic hemiacetal form of the compound (VIII) was easily opened. Also, an extra mole of oxidant was consumed, possibly by the active hydrogen of the dialdehyde (IX) formed in the reaction.

```
HCOH
 |
HCOCH3
 |                          CHO
HOCH                         |
 |        O            |H--COCH3
HOCH                         |
 |                          CHO
HC-------
 |
H2COH

  VIII                       IX
```

From the results presented it appears reasonable to conclude that the oxidation pattern of each hexose disaccharide and monomethyl hexose differs sufficiently from that of the others to recommend the reaction for determining structure of unknown compounds. The use of periodate oxidation for identifying monomethyl glucoses has been described by Lemieux and Bauer (13). Although there are several criteria on which to base a choice of
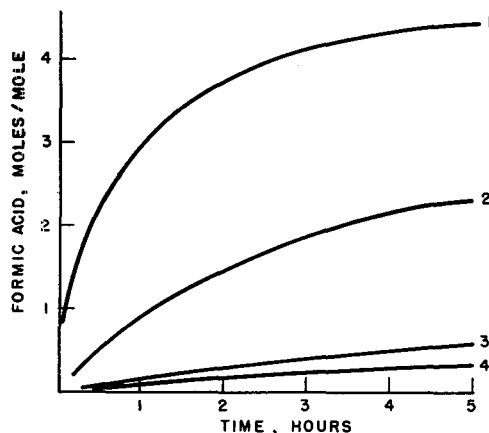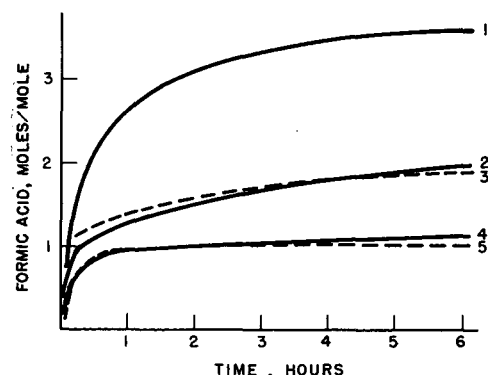
Figure 4. Production of formic acid (evolution of carbon dioxide) during oxidation of pentose disaccharides with lead tetraacetate

1.
2. 5-O-D-Xylopyranosyl-L-arabinose
3. 1,4-Xylobiose
. Methyl β-D-xylopyranoside (1 mole of acid on the graph corresponds to 0 mole, 2 moles corresponds to 1 mole)
4. 3-O-β-L-Arabopyranosyl-L-arabinose
5. Methyl α-L-arabopyranoside

structure, some properties are particularly distinctive: the large yield of acid from the 6-, formaldehyde from the 2-, pentose from the 3-, and tetrose from the 4-substituted compound. Such unique features may sometimes permit the detection of one compound in the presence of the others. Also, the reaction may be at least partly useful for higher oligosaccharides, as the reducing ends are likely to behave similarly to those of disaccharides.

Since the oxidation pattern of the galactobiose conformed exclusively to that of a known 1,3-disaccharide, it was designated 3-O-D-galactopyranosyl-D-galactose.

PENTOSE DISACCHARIDES

This group consisted of compounds with 1,3-, 1,4-, and 1,5-glycosidic linkages, and 2-O-methyl-D-xylose as a model for 1,2-disaccharides. The results generally paralleled those of the corresponding members of the hexose series. Thus 5-O-D-xylopyranosyl-L-arabinose (2) rapidly gave approximately the expected 4 moles of acid (Figure 4, curve 1), by far the highest yield, and consumed 4.7 moles of lead tetraacetate (Table IV). The production of 1 mole of acid (Figure 4, curve 4) and the consumption of 3 moles of lead tetraacetate (Table IV) by the arabinose disaccharide isolated from the ϵ-galactan of larch (10) was in

Table II. Production of Formaldehyde by Hexose Disaccharides

(Moles/mole)

Time, Hr.	Laminaribiose	Galactobiose	Maltose	Lactose
3	0.14	0.06	0[a]	0[a]
5	0.16	0.11	0[a]	0[a]
9	0.21	0.20	Trace	Trace

[a] Colorimeter readings were actually less than blank reading.

Table III. Oxidation of Monomethyl Galactoses with Lead Tetraacetate[a]

(Moles/mole)

Derivative	Formic Acid	Oxidant Consumed[b]	Formaldehyde	Pentose
2-O-methyl	2.1	4.1	1.0	0
3-O-methyl	0.5	1.8	0.1	0.5
4-O-methyl	0.3	2.4	0[c]	0
6-O-methyl	4.4	4.4	0[c]	0

[a] Reaction time, 5 hours.
[b] Corrected for lead tetraacetate consumed in oxidation of formic acid.
[c] Colorimeter readings were actually slightly less than blank reading.

accord with the assigned structure, 3-O-L-arabopyranosyl-L-arabinose., The pyranose configuration of the nonreducing end was further confirmed by the almost identical rates of acid production from the disaccharide and from methyl α-D-arabopyranoside (curve 5). The 1,4-disaccharide, xylobiose(X), differed from the corresponding hexose member by yielding an additional mole of acid (curve 2) although the consumption of oxidant by both disaccharides was the same. The very rapid release of the first mole was probably from the reducing end chiefly, since the rate of appearance of the second mole closely paralleled the rate of acid production from methyl β-D-xylopyranoside (curve 3). Apparently, therefore, carbon 2 was oxidized directly to free formic acid but carbon 1 was retained in the formate ester (XI) which was highly stable during the oxidation period used. This interpretation is compatible with that attached to the oxidation of 1,4-hexose disaccharides since the structure of xylobiose affords no possibility for a second ester group as in VI.
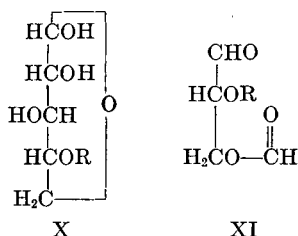

X   XI

Table IV. Oxidation of Pentose Disaccharides with Lead Tetraacetate[a]

Disaccharide	Formic Acid, Moles/Mole	Oxidant Consumed, Moles/Mole
Arabinobiose (1,3-)	1.1	2.9
Xylobiose (1,4-)	1.9	3.9
Xylosidoarabinose (1,5-)	3.6	4.5

[a] Oxidation period 6 hours.

In 5 to 6 hours of reaction time, 2-O-methyl-D-xylose yielded 1 mole of formaldehyde as well as 1 mole of formic acid. This behavior suggests that a 1,2-pentose disaccharide should be readily distinguished from other pentose disaccharides, which yield little or no formaldehyde under the same conditions.

EXPERIMENTAL

Materials. The monomethyl galactoses, 3-O-β-L-arabopyranosyl-L-arabinose (amorphous powder) and 5-O-D-xylopyranosyl-L-arabinose (sirup), were kindly provided by J. K. N. Jones. The crystalline octaacetate of isomaltose was obtained through the courtesy of M. L. Wolfrom. The acetate was deacetylated with 0.05N sodium methylate (24), the base was neutralized with acetic acid, and the sirup remaining after distillation of the methanol was dissolved in 90% acetic acid and used directly in the oxidations. The galactobiose (melting point 159° to 160.5° C., $[\alpha]_D^{25}$ + 62.2) was isolated from a partial hydrolyzate of Acacia pycnantha gum (7). Other carbohydrates were pure crystalline compounds.

Lead tetraacetate was prepared by the procedure recommended by Vogel (23).

All other compounds were of reagent grade quality.

Methods. The oxidations were carried out in a constant-volume type of Warburg respirometer at 27° C., the general procedure having been described previously (17, 21). The vessel chamber contained 1 ml. of a solution of 20 mg. of lead tetraacetate and 10 mg. of potassium acetate in 90% acetic acid and the side arm contained 0.2 ml. of 90% acetic acid in which was dissolved 0.3 to 1.0 mg. of the substrate. The side arm of the blank vessel contained 0.2 ml. of 90% acetic acid. After equilibration and mixing, the production of formic acid was determined from the observed increase in pressure due to evolved carbon dioxide.

At chosen intervals the quantity of lead tetraacetate consumed was determined by transferring the vessel contents to a 125-ml. flask with 8 ml. of "stopping solution" [10 grams of potassium iodide and 50 grams of sodium acetate in 100 ml. of water (5)]

and titrating the liberated iodine with standard 0.005N sodium thiosulfate.

Formaldehyde was determined as follows: The reaction mixture was transferred with acetic acid to a 5-ml. volumetric flask which contained 0.2 ml. of 10% oxalic acid in acetic acid; the oxalic acid rapidly reduced excess oxidant and caused precipitation of the divalent lead. An aliquot (depending on the sample) of the supernatant was diluted to 1 ml. with water and heated for 30 minutes on the boiling-water bath with 10 ml. of chromotropic acid reagent (14). The solution was clarified by centrifuging and the colorimeter reading was made at 570 mμ. The blank was always appreciably colored, even when the acetic acid was previously distilled over chromium trioxide, but standard solutions of erythritol, oxidized under the same conditions, yielded the theoretical quantity of formaldehyde.

Pentose was determined with the orcinol reagent (15): The sample to be analyzed was prepared as described for the determination of formaldehyde. An aliquot (depending on the sample) of the supernatant was diluted to 3 ml. with water, and was heated on the boiling-water bath with 3 ml. of orcinol reagent for 35 minutes. The solution was clarified by centrifuging and the colorimeter reading was made at 660 mμ.

The oxidations in which one mole of pentose was obtained from 1,3-disaccharides were carried out in the following manner: One milligram of the disaccharide was dissolved in 0.01 ml. of water and diluted to 0.1 ml. with acetic acid. Five milligrams of lead tetraacetate dissolved in 0.4 ml. of acetic acid was added. At intervals, 0.05-ml. aliquots were removed and treated with excess oxalic acid in acetic acid; pentose was determined as described. A maximum yield of pentose was obtained in 2 hours. Maltose and lactose were examined under the same conditions. Other aliquots of the oxidation mixtures were also treated with excess oxalic acid, diluted with water, and heated on the boiling-water bath with a small quantity of Amberlite IR-120 resin. The products of hydrolysis were separated on the paper chromatogram using ethyl acetate–acetic acid–water (3 to 1 to 3) (9), and butanol–ethyl alcohol–water (4 to 1 to 5) (16), the rate of travel of the compound compared with that of known sugars.

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Determination of Combined Acetic Acid Content of Cellulose Acetate
Gravimetric Method

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A gravimetric method for the determination of the per cent of combined acetic acid content of acetone-soluble cellulose acetate has been established. The method is based on weighing the cellulose regenerated from the ester by means of complete saponification in an aqueous-alkaline medium. Such a method gives results which compare favorably with those obtainable by the usual volumetric methods. It is anticipated that this new technique will be applicable to cellulose acetates having solubility properties different from those of the products examined here.

D URING the past 50 years numerous papers have been published relating to studies carried out both in research institutes and in industrial laboratories on the determination of the percentage of combined acetic acid in cellulose acetate.

For an extensive and up-to-date documentation on this subject, the reader is referred to Krüger (5), Heuser (4), and Dorée (2), and to the report of a subcommittee of the Division of Cellulose Chemistry (1) of the AMERICAN CHEMICAL SOCIETY.

The Eberstadt method (3) and the Ost method (6) have stimulated most of these studies. Both methods involve the volumetric determination of acetic acid—with previous alcohol-alkaline saponification (Eberstadt method) or acid hydrolysis and distillation (Ost method).

The work presented here describes a new gravimetric method which has given very good results in the determination of the percentage of combined acetic acid of the secondary acetone-soluble acetate having a 54 to 55% acetic acid content.

With this method the percentage of combined acetic acid is obtained from the weight loss of the ester by its transformation to cellulose by complete saponification.

The saponification is carried out in an aqueous-alkaline medium.

The regenerated cellulose is collected on a filter, washed well, dried to a constant weight, and weighed accurately.

If A is the weight of the dried cellulose acetate, and C is the weight of the dried cellulose, the acetic acid content, t (expressed as acetyl value), is given by the following equation:

$$t = \frac{4300 \, (A - C)}{42 \, A}$$

while the acetic acid content, T (expressed as acetic acid), is given by

$$T = \frac{6000 \, (A - C)}{42 \, A}$$

EXPERIMENTAL

Samples. Two samples of cellulose acetate having the following properties have been examined.

Sample A. American cellulose acetate of the type used in the production of plastics. It contained about 54% of combined acetic acid, and was in powder form which could be passed through a sieve of 840-micron mesh.

Sample B. Italian cellulose acetate of the usual type for use in rayon production. It contained about 55.5% of combined acetic acid, and was in flake form. After being milled in a Forplex mill it became pulverized and could be passed through a sieve of 840-micron mesh.

Materials. In the volumetric method (control test) reagents free of carbonates and of carbon dioxide were used, as the influence of such factors on the results is well known. Neutral glassware and burets of precision corresponding to those certified by the National Bureau of Standards were used.

In the gravimetric method normal filter paper, washed by water and dried to constant weight, was employed. The reagents were not specially controlled, as their influence is without importance in this case. The same considerations apply to the glassware.

Drying was carried out in a thermostatically controlled oven; the temperature of 105° C. was kept to within ±2° C.

The saponifications were carried out at room temperature (about 20° C.) in 500-ml. flasks with narrow necks and ground-glass stoppers.

The flasks were rotated on a roller mixer at 60 r.p.m.

Test Methods. VOLUMETRIC METHOD. The cellulose acetate was dried for 5 hours at 105° C. and cooled in a desiccator. A 2.5-gram sample was placed in a 500-ml. flask, and 100 ml. of 0.5N sodium hydroxide solution were added. The stoppered flask was placed on a roller mixer for 48 hours at 20° C. Then the flask was opened, the stopper and the neck were thoroughly washed, and 0.5N sulfuric acid solution was added until there was an excess of about 1 ml. (phenolphthalein as indicator). The well-stoppered flask was allowed to stay on the roller mixer for 5 hours, and it was then titrated until a pink color appeared and remained after vigorous stirring.

A blank test was made under the same conditions.

GRAVIMETRIC METHOD. The same technique was used as for the volumetric method. At the end of the titration the contents of the flask were quantitatively passed through a folded filter of 130-mm. diameter. The cellulose was washed with 500 ml. of water at 20° C. using small quantities at a time, and then with 200 ml. at 70° C. The filter was allowed to stay 12 hours in the oven at a temperature of 50° C., was placed on a weighing glass, and was then replaced in the oven at 105° C. until a constant weight was achieved (about 2 hours).

Table I. Comparison of Volumetric and Gravimetric Methods of Determining Combined Acetic Acid in Two Samples of Cellulose Acetate

Test No.	Acetic Acid Content, %	
	Volumetric method	Gravimetric method
Sample A		
1	53.94	54.00
2	53.94	54.00
3	53.97	53.84
4	53.96	54.00
5	53.94	54.00[a]
6	53.95	54.00[a]
Av.	53.95	53.97
Sample B		
1	55.68	55.77
2	55.68	55.81
3	55.70	55.75
4	55.68	55.85
5	55.66	55.88[a]
6	55.68	...
Av.	55.68	55.81

[a] Infrared dried.

The final drying to constant weight can also be carried out by using an infrared radiating lamp of 0.250 kw., the distance between the lamp surface and the bottom of the weighing glass being 20 cm. This type of drying was found to be more rapid (about 1 hour).

EXPERIMENTAL DATA

The results obtained in tests carried out by the same operator at different times are given in Table I.

CONCLUSIONS

It will be seen from Table I that the new method allows the combined acetic acid content of secondary acetone–soluble cellulose acetate to be determined with great accuracy. Small differences in the results obtained by the new method and by the volumetric method can be justified by experimental errors and by the fact that salts and other water-soluble impurities, always present in the commercial products, give incorrect results in a diametrically opposite way.

The gravimetric method can be used to help establish the accuracy of other methods including those using saponification, and to analyze mixed esters and other esters.

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Precipitation of Pyrophosphate and Triphosphate with Tris(ethylenediamine)cobalt(III) Chloride and Hexamminecobalt(III) Chloride

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Precipitation reactions of the condensed phosphates were studied for the purpose of discovering specific reagents for their determination. Tris(ethylenediamine)cobalt(III) chloride [Co(en)₃Cl₃] precipitates triphosphate but not pyrophosphate at pH 3.5 and pyrophosphate but not triphosphate at pH 6.5. The precipitates dried at 110° C. are $Co(en)_3H_2P_3O_{10}.2H_2O$ and $Co(en)_3HP_2O_7$. Orthophosphate, trimetaphosphate, and tetrametaphosphate are not precipitated. Triphosphate can be precipitated from a mixture which contains pyrophosphate, but some of the latter is coprecipitated and some of the triphosphate is left in solution. The distribution of pyrophosphate and triphosphate between precipitate and solution was determined by phosphorus-32–tagged phosphates. $Co(en)_3Cl_3$ may prove a valuable reagent for triphosphate, notwithstanding the influence of pyrophosphate on the precipitation of triphosphate. In contrast to $Co(en)_3Cl_3$, hexamminecobalt(III) chloride [Co(NH₃)₆Cl₃] precipitates $P_3O_{10}{}^{-----}$ and $P_2O_7{}^{----}$, instead of $H_2P_3O_{10}{}^{---}$ and $HP_2O_7{}^{---}$, and the yield of both is increased by increasing pH. Orthophosphate is also precipitated, so $Co(NH_3)_6Cl_3$ is not a potentially valuable reagent. The triphosphate precipitate dried at 110° C. is $Na[Co(NH_3)_6]_3(P_3O_{10})_2$.

HEXAMMINECOBALT(III) chloride has been suggested as a reagent in qualitative microchemical tests for pyrophosphate (*3*) and triphosphate (*10*). Recently the amperometric titration of pyrophosphate with this reagent has been described (*6*). The possibility of a stepwise variation in composition, size, and charge made Werner cations an attractive class to investigate as specific precipitants for the various phosphate anions.

This background led to a study of hexamminecobalt(III) and the related tris(ethylenediamine) and (propylenediamine)-cobalt(III) ions as precipitants for use in the determination of pyrophosphate and triphosphate in the presence of each other and in the presence of orthophosphate, trimetaphosphate, and other phosphates. Most of this paper deals with the precipitation of phosphates, especially pyrophosphate and triphosphate, by hexammine- and tris(ethylenediamine)cobalt(III) chlorides, the description of resulting precipitates, and the coprecipitation of pyrophosphate with the tris(ethylenediamine)cobalt(III)

triphosphate precipitate. Certain analytical applications are also mentioned.

CHEMISTRY

Qualitative Solubility. A striking difference in the solubilities of the hexammine- and tris(ethylenediamine)cobalt phosphates, silicates, and sulfates is shown in Table I. Sulfate and silicate were included because their separation from the phosphates is often a problem. Tris(propylenediamine)cobalt(III) chloride was also tested, but it did not precipitate any of the phosphates except the polyphosphate with the longest chain length. Tris(ethylenediamine)cobalt(III) chloride showed promise as a specific precipitant, for of the phosphates and other anions tested only pyrophosphate and triphosphate precipitated and these precipitates were obtained at different pH values. With hexamminecobalt(III) chloride, pyrophosphate and triphosphate both precipitated at all pH values, indicating that a method for one in the presence of the other was unlikely.

Materials. Hexamminecobalt(III) chloride (*1*) [Co(NH₃)₆Cl₃] and tris(ethylenediamine)cobalt(III) chloride (*14*) [Co(en)₃Cl₃] were prepared according to published procedures. Tris(propylenediamine)cobalt(III) chloride [Co(pn)₃Cl₃] was prepared by the same method as Co(en)₃Cl₃, making allowance for the different molecular weight of propylenediamine. All were recrystallized and dried at 110° C. before use. Their identities were verified by analysis.

Sodium triphosphate was prepared from a commercial product by salting it out of aqueous solution with alcohol four times and,

Table I. Precipitations with Excess Reagent[a]

Sodium Salt in Solution	Co(NH₃)₆Cl₃[b] pH			Co(en)₃Cl₃[b] pH		
	12.5	7.5	4.5	12.5	7.5	4.5
Polyphosphates						
$\bar{n} = 11$	X	X	X	L	L	L
$\bar{n} = 5.1$	X	X	X	L	L	L
Triphosphate	X	X	X	0	0	X
Pyrophosphate	X	X	X	0	X	0
Orthophosphate	X	X	0	0	0	0
Tetrametaphosphate	X	X	X	0	0	0
Trimetaphosphate	0	0	0	0	0	0
Sulfate	X	X	X	0	0	0
Silicate, SiO₂/Na₂O = 2.6	X	X	X	0	X	0

[a] In 50 ml. of mechanically agitated solution at room temperature there were 3.00 millimoles of cobalt reagent and 0.250 gram of sodium salt except for triphosphate (1.65 millimoles) and sulfate, orthophosphate, pyrophosphate (2.50 millimoles). The pH was maintained constant with NaOH or HCl.
[b] X = precipitate; 0 = no precipitate; L = an oily liquid separated.

Table II. Amperometric Titrations with Hexamminecobalt(III) Chloride

[40 Ml. of solution, titrated with $0.0549M$ $Co(NH_3)_6Cl_3$]

No.	Taken, Millimoles (a) $Na_4P_2O_7$	(b) $Na_5P_3O_{10}$	(c) Na_2HPO_4	Results, Ml. Found	Theor.		Rel. Error, %	pH[a]
1	...	0.150	...	4.09	4.10 b	...	-0.2	12
2	...	0.150	...	4.13	4.10 b	...	0.7	12
3	...	0.150	...	4.13	4.10 b	...	0.7	11
4	0.209	0.150	...	7.87	7.91 a + b	...	-0.5	8.8
5	0.209	0.150	...	7.93	7.91 a + b	...	0.2	8.8
6	0.209	0.300	...	12.01	12.01 a + b	...	0.0	8.5
7	...	...	0.150 b	2.69	0.00 a	2.73 c	...	12
8	...	...	0.150 b	2.58	0.00 a	2.73 c	...	10
9	0.209	...	0.150 b	3.87	3.81 a	6.54 a + c	...	...
10	0.209	...	0.150 b	6.37	3.81 a	6.54 a + c	...	12
11	0.218	...	0.150 b	3.55	3.97 a	6.70 a + c	...	8.7

[a] pH was measured after titration. Before titration NaOH or NH₃ was added except to sample numbers 4, 5, 6, and 8, which were not adjusted, and to 9, to which was added $NH_4^+-NH_3$ buffer.
[b] Only 10% alcohol was present.

after air drying, was weighed as $Na_5P_3O_{10}.6H_2O$. The water loss on heating was 22.8% (theory 22.7%) and the per cent phosphorus pentoxide as determined by titration after hydrolysis was 44.5% (theory, 44.7%). Reagent grade sodium pyrophosphate, $Na_4-P_2O_7$, was recrystallized and dried. Preparation of radioactive pyrophosphate and triphosphate for tracer experiments is described elsewhere (11). Sodium trimetaphosphate, $Na_3P_3O_9.H_2O$, was recrystallized at 50° C. from an aqueous solution of the commercial material (Monsanto Chemical Co., St. Louis, Mo.). Sodium tetrametaphosphate was salted out of a water solution of Cyclophos (Victor Chemical Works, Chicago, Ill.) with ethyl alcohol at 35° C.

The sodium silicate was weighed from an analyzed clear stock solution made by diluting and filtering a commercial product. The silicon dioxide–sodium oxide weight ratio was 2.60. The polyphosphates were commercial samples characterized by the average number of phosphorus atoms per ion, $\bar{n}$, assuming only linear ions present. The chain lengths were calculated from the titration between end points before hydrolysis, W, the titration between end points after hydrolysis, S, and the determined orthophosphate (8), o, from the relation, $\bar{n} = \dfrac{2S}{W + o}$. This is similar to the chain length formula used by Van Wazer (12) but differs in that it gives the average number of phosphorus atoms per ion including orthophosphate. The polyphosphate with $\bar{n} = 10$ was Calgon (Calgon, Inc., Pittsburgh, Pa.) and with $\bar{n} = 5.1$ was Quadrofos (Rumford Chemical Works, Rumford, R. I.).

Hexamminecobalt(III) Phosphates. In the presence of sodium ion Jorgensen (4) and later workers (6) have shown that the precipitate with pyrophosphate, neglecting water of crystallization, is $NaCo(NH_3)_6P_2O_7$. With trisodium orthophosphate the precipitate $Co(NH_3)_6PO_4$ has been reported (2, 9).

Table III. Titration of Triphosphate with Tris(ethylenediamine)cobalt(III) Chloride

[40 Ml. of solution titrated with $0.0487M$ $Co(en)_3Cl_3$ and buffered with $0.15M$ NaAc–$1.8M$ HAc, pH 3.7 to 4.1]

| Sample, Millimoles $Na_4P_2O_7$	$Na_5P_3O_{10}$	Results[a] Used, ml.	Rel. error, %
...	0.2000	4.13	0.5
...	0.2000	4.13	0.5
0.0218	0.2000	4.18	1.7
0.0218	0.2000	4.20	2.2
0.0654	0.2000	4.27	4.0
0.0654	0.2000	4.30	4.3

[a] Theory, 4.11 ml.

Amperometric titrations of triphosphate with the reagent (Table II) showed that the precipitate contained three hexamminecobalt(III) ions per two triphosphate ions. The precipitate contained sodium, so that its formula is presumably $Na[Co(NH_3)_6]_3(P_3O_{10})_2$. Analysis of the precipitate made at pH 9.0 and dried at 110° C. supported this formula, although close checks for the theoretical nitrogen content were not obtained. Analysis, calculated for $Na[Co(NH_3)_6]_3(P_3O_{10})_2$: N, 24.9; P, 18.4; Co, 17.5; found: N, 23.8; P, 18.4; Co, 17.8. Above

about pH 10, although the hexamminecobalt(III) to triphosphate ion ratio was maintained, the gravimetric yield was 1 to 2% high and analysis showed a slight decrease in per cent nitrogen, cobalt, and phosphorus, possibly due to the inclusion of some alkali.

Tris(ethylenediamine)cobalt(III) Phosphates. Amperometric titrations (Table III) indicated that for precipitates of both pyrophosphate and triphosphate the tris(ethylenediamine)-cobalt(III) to phosphate ion ratio was 1 to 1. Analysis of precipitates dried at 110° C. showed their compositions to be $Co(en)_3HP_2O_7$ and $Co(en)_3H_2P_3O_{10}.-2H_2O$. Analysis, calculated for $Co(en)_3-HP_2O_7$: N, 20.6; P, 14.9; titratable H, 0.243; found: N, 20.3; P, 15.1; titratable H, 0.253. Analysis, calculated for $Co(en)_3H_2P_3O_{10}.2H_2O$: N, 15.8; P, 17.5; titratable H, 0.380; H_2O, 6.80; found: N, 16.0; P, 17.5; titratable H, 0.377; H_2O, distillation followed by Karl Fischer 6.64.

The same x-ray powder diffraction pattern was obtained for the air-dried triphosphate precipitate as was obtained after drying at 110° C. Crystals sucked dry on the sintered-glass filter contained 7.9 to 8.4% water. Apparently the precipitate came down as the dihydrate and was not dehydrated by oven drying. Usually ill-formed tabular crystals showing parallel extinction were observed but sometimes bluntly terminated needles with oblique extinction were also present. On the universal stage the needlelike crystals proved to be identical with the tabular crystals. Optical properties and x-ray powder diffraction data useful for identifying these and the pyrophosphate crystals have been published (7).
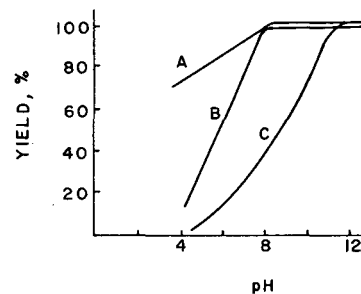
Figure 1. Effect of pH on precipitations with $Co(NH_3)_6Cl_3$

$Co(NH_3)_6Cl_3$ $0.060M$

A. $Na_5P_3O_{10}$ $0.025M$
B. $Na_4P_2O_7$ $0.050M$
C. Na_2HPO_4 $0.050M$

The pyrophosphate precipitate taken from the filter contained 4.3 to 4.5% water and after drying at room temperature and 47% humidity contained 4.02% water, indicating a monohydrate (theory, 4.17%). The x-ray patterns of the air-dried and anhydrous precipitates were different (7).

Effect of pH on Completeness of Precipitation. The curves of Figure 1 show the per cent of theoretical gravimetric yield obtained when orthophosphate, pyrophosphate, and triphosphate were separately precipitated with hexamminecobalt(III) reagent at various definite pH values. The pH as indicated by a glass electrode was maintained constant with sodium hydroxide or hydrochloric acid. Data for similar experiments with tris-(ethylenediamine)cobalt(III) reagent are given in Figure 2. The maximum yields of $Co(en)_3HP_2O_7$ and $Co(en)_3H_2P_3O_{10}.2H_2O$ were obtained at about pH 6.5 and 3.5, respectively, where $HP_2O_7^{---}$ and $H_2P_3O_{10}^{---}$ should be present in nearly the maxi-

mum concentrations. This type of curve, where the yields pass through maxima at certain pH values, contrasts with those obtained with hexamminecobalt(III) chloride where the salts were normal phosphates and the yield remained high above a certain pH. The curves of Figure 2 suggest that tris(ethylenediamine)cobalt(III) ions may be useful in detecting and determining triphosphate in the presence of pyrophosphate.

Precipitations with Tris(ethylenediamine)cobalt(III) Chloride. In solutions of pure salts, triphosphate precipitated almost quantitatively (98 to 99.5%) between pH 3.0 and 4.0 and pyrophosphate precipitated in yields of around 93% at pH 7.0 (Figure 2). However, when both phosphates were in the same solution, precipitation of triphosphate from solutions containing from about 3 to 1 to 1 to 1 molar ratios of triphosphate to pyrophosphate (Table IV, 20 to 40% sodium pyrophosphate) gave yields in excess of 100% [the yield being based on the amount of triphosphate present and the composition $Co(en)_3H_2P_3O_{10}.2H_2O$]. In solutions containing excess pyrophosphate precipitation was far from complete (Table IV, 50 to 70% sodium pyrophosphate).

Radioactive pyrophosphate, $Na_4P^{32}_2O_7$, was used to check for coprecipitation of pyrophosphate with triphosphate and Na_5-$P^{32}_3O_{10}$ was used in other experiments to determine the concentration of triphosphate ions in the solution. Results obtained with tagged triphosphate and tagged pyrophosphate are given in Table IV The values in parentheses were calculated assuming the pyrophosphate to be present as $Co(en)_3HP_2O_7$ (see Discussion). Those for triphosphate in solution were calculated from the triphosphate taken, the weight of the precipitate, and the weight of $Co(en)_3HP_2O_7$ as determined from tagged pyrophosphate; the values for pyrophosphate in the solid which are enclosed in parentheses were calculated from the weight of precipitate and the weight of $Co(en)_3H_2P_3O_{10}$.-$2H_2O$ in the precipitate as determined by tagged triphosphate. The solubility of $Co(en)_3H_2P_3O_{10}.2H_2O$, determined by the use of tagged triphosphate, under conditions of precipitation (room temperature, pH 3.5, 0.0018M excess $Co(en)_3Cl_3$), was 3.01 mg. per 100 ml.

The contamination of the $Co(en)_3H_2P_3O_{10}.2H_2O$ precipitate by pyrophosphate is proportional to the mole fraction of the dissolved phosphate, before precipitation, which is pyrophosphate (Figure 3).

Powder x-ray diffraction data for the oven-dried samples of Table IV did not show a second phase even for the precipitate with the greatest contamination. The absence of lines in the back reflection region and the width of the lines made it impossible to measure accurately the slight changes which appeared as the amount of pyrophosphate in the sample increased. However, for the spacings of 2.63 and 2.40 A. there seemed to be a regular increase in the diffraction angle, 2θ, which amounted to about 0.5° going from a sample with 0% to one with 11.5% pyrophosphate as $Co(en)_3HP_2O_7$. The pattern for a sample with 15.5% contamination was similar but showed some discontinuities such as the spacing formerly of 2.63 A. merging with one formerly of 2.57 A.
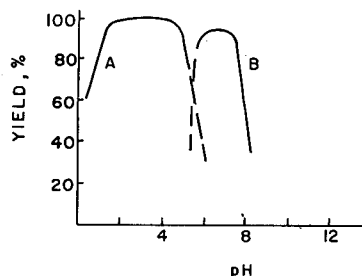
Figure 2. Effect of pH on precipitations with $Co(en)_3Cl_3$

$Na_5P_3O_{10}$ (A) and $Na_4P_2O_7$ (B) were 0.020 to 0.050M and $Co(en)_3Cl_3$ was in excess 0.021 to 0.060 M

PYROPHOSPHATE, MOLE FRACTION OF DISSOLVED PHOSPHATE
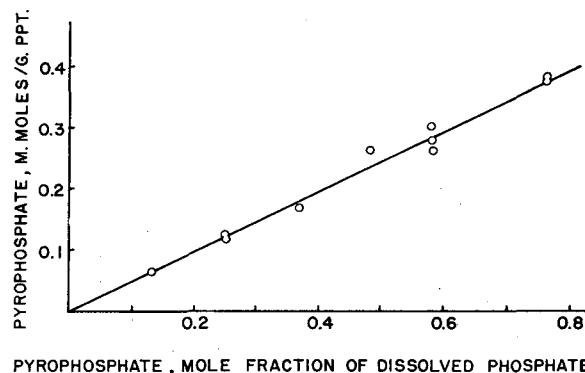
Figure 3. Pyrophosphate contamination of $Co(en)_3$-$H_2P_3O_{10}\cdot 2H_2O$ precipitate

Attempts to precipitate pyrophosphate in the presence of triphosphate at a pH of 6.5 failed in a solution containing a mole ratio of one pyrophosphate to four triphosphate [0.0200M $Na_4P_2O_7$, 0.0800M $Na_5P_3O_{10}$, 0.1200M $Co(en)_3Cl_3$] and the yield was less than for pure pyrophosphate at a mole ratio of two pyrophosphate to three triphosphate (total phosphate concentration 0.1000M, reagent 0.1200M). When more than half of the phosphate was pyrophosphate about the same yield as for pure pyrophosphate was obtained (93% for 0.200M $Na_4P_2O_7$ solution with reagent 0.0240M). The apparent solubility of $Co(en)_3HP^{32}_2O_7$ in a solution 0.0100M in $P_3O_{10}^{-----}$ and 0.0230M in excess $Co(en)_3^{+++}$ at pH 7 was determined by the P^{32} tag to be 0.98 mg. of $Co(en)_3HP_2O_7$ per ml. In addition the precipitate must have contained triphosphate, for the precipitate contained only 94% of the $P_2O_7^{----}$ required by its weight and formula.

The purification by repeated precipitation of a radioactive pyrophosphate–contaminated precipitate, prepared by adding $Co(en)_3Cl_3$ to a solution of pyrophosphate and triphosphate (315 ml., 16.0 millimoles of $Co(en)_3Cl_3$, 14.0 millimoles of $Na_5P_3O_{10}$, and 3.6 millimoles $Na_4P^{32}_2O_7$), at pH 2.5 is shown in Figure 4. The precipitate was filtered off at each step and a sample was taken for counting, the remainder being dissolved in dilute sodium hydroxide solution and reprecipitated by adjusting the pH to 2.5.

Discussion. The purification of the pyrophosphate-contaminated triphosphate precipitate by repeated precipita-

Table IV. Precipitation of Triphosphate in Presence of Pyrophosphate with $Co(en)_3Cl_3$

[Total volume 110 ml.; pH 3.5; $Co(en)_3Cl_3$ 1.20 millimoles]

Taken, Millimoles		Gravimetric Yield, % of Theor.	Found, by Radioactive Count, Millimoles			
			Triphosphate		Pyrophosphate	
$Na_4P_2O_7$	$Na_5P_3O_{10}$		Solid	Solution	Solid	Solution
0.000	1.000	99.1	1.01	0.0062	....	...
0.000	1.000	98.7	1.01	0.0062	.....	....
0.138	0.900	100	...	(0.024)	0.0318	0.107
0.267	0.800	101	...	(0.029)	0.0503	0.221
0.267	0.800	102	...	(0.025)	0.0523	0.225
0.415	0.700	101	...	(0.044)	0.0622	0.366
0.554	0.600	101	...	(0.058)	0.0827	0.479
0.692	0.500	99.3	0.444	0.0512	(0.068)	...
0.692	0.500	95.5	...	(0.077)	0.0703	0.621
0.692	0.500	102	...	(0.054)	0.081?	0.601
0.969	0.300	66.5	0.169	0.131	(0.040)	...
0.969	0.300	72.7	...	(0.116)	0.0432	0.916

tions (Figure 4) is much slower than would be expected for a simple mixture of components, one of which (pyrophosphate) is soluble in the absence of the other under the conditions of the precipitation. A straight line would represent a distribution of pyrophosphate between precipitate and filtrate which did not change with composition of the precipitate, or, in other words, a rate of purification proportional to the amount of impurity present. Although the reprecipitations can be made rapidly, the number of reprecipitations necessary to get a relatively pure product is too great for this to be a convenient method for carrying out an isotope dilution determination of triphosphate.

Figure 4. Purification of Co(en)₃H₂P₃O₁₀⁻-2H₂O
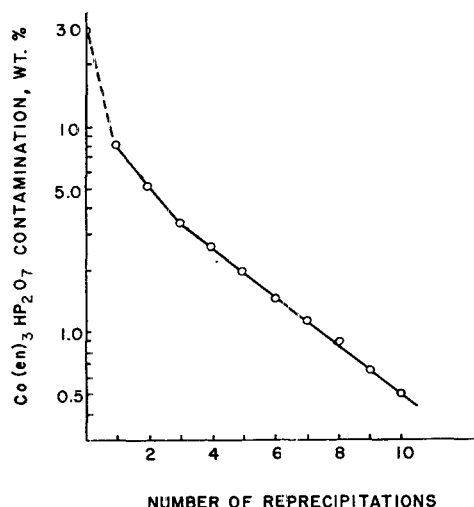
The reasons for use of Co(en)₃HP₂O₇ as the formula for pyrophosphate in the solid should be explained. It is the formula of the solid precipitating from pyrophosphate solutions between pH 6 and 8 although at pH 3.5 no precipitate is formed. More acid was liberated in precipitating a pyrophosphate-triphosphate mixture than was liberated in precipitating pure triphosphate, as would be expected if the precipitated ion was HP₂O₇⁻⁻⁻. It therefore appears reasonable to assume Co(en)₃HP₂O₇ as the formula for pyrophosphate in the solid, although direct proof for its existence at low pH values was not obtained.

In precipitations from solutions containing up to 0.692 millimole of pyrophosphates per 0.500 millimole of triphosphate (Table IV), the ratio of triphosphate in the solution to pyrophosphate in the solid ranged from 0.5 to 1 and averaged 0.7. Difference figures were used in calculating the values of the ratio so that they are not very accurate and involve the foregoing assumption of the formula of the pyrophosphate in the precipitate. However, for the composition containing 0.500 millimole of triphosphate, the ratio can be calculated directly from experimental data and is 0.68. If it can be assumed from this evidence that 1.5 Co(en)₃HP₂O₇ precipitates for each Co(en)₃H₂P₃O₁₀ that does not, the moles of phosphorus in the solid remain constant, the slight observed increase in yield with increased contamination of the precipitate with pyrophosphate is explained, and a little extra Co(en)₃Cl₃ is used in agreement with amperometric titration results for solutions containing pyrophosphate and triphosphate (Table III).

When pyrophosphate was in greatest excess, about three triphosphate ions were in solution for every pyrophosphate ion that was in the precipitate. Most of this change was caused by the low yield (high solubility) of the precipitate—that is, it con-

tained 4 moles of triphosphate per mole of pyrophosphate and dissolved to such an extent that the ratio of triphosphate in solution to pyrophosphate in the solid was raised from 0.5 to 1 to 3.

ANALYTICAL APPLICATIONS

Amperometric Titration Method. Solutions of the phosphates were titrated with solutions of Co(en)₃Cl₃ or Co(NH₃)₆Cl₃ as a convenient means of studying the stoichiometry of precipitation and of evaluating their use as analytical reagents. The titration cell designed by Laitinen and Burdett (5) which provides for continuous stirring by a gas stream was used. The required voltage was supplied and current was measured by a Leeds & Northrup Electrochemograph, Type E. The recommended (6) applied potential of −0.65 volt vs. standard calomel electrode was used for titrations with Co(NH₃)₆Cl₃; an applied potential of −0.80 volt vs. standard calomel electrode was chosen for Co(en)₃Cl₃ titrations after inspection of appropriate polarograms. Gelatin (0.01%), supporting electrolyte (0.10N sodium nitrate), and alcohol (usually 20%) were added to the phosphate solutions. The supporting electrolyte was omitted from certain experiments in which an acetic acid–acetate buffer was used. Volume corrections were made but were never large.

Titrations with Hexamminecobalt(III) Chloride. The amperometric titrations of pyrophosphate with hexamminecobalt(III) chloride solution as described by Laitinen and Burdett (6) were found to be satisfactory, except that other phosphates interfere more than indicated. They state that sodium triphosphate does not precipitate in 0.1M concentration and 20% alcohol. The data of Figure 1 indicate triphosphate is likely to interfere because in their recommended pH range of 9 to 12 it is obtained in as good yields as pyrophosphate. This is confirmed by the data of Table II which show that triphosphate titrates quantitatively in the presence or absence of pyrophosphate.

Orthophosphate is also more likely to interfere than indicated. Laitinen and Burdett (6) have indicated that 4 × 10⁻³M pyrophosphate can be determined in the presence of 4 × 10⁻²M orthophosphate if only 10% alcohol is present. However, great difficulty was experienced in titrating solutions which contained orthophosphate because it precipitated slowly and in amounts depending upon the pH. From pH 10 to 12 most of the orthophosphate, alone or with pyrophosphate, precipitated (Table II). When sodium monohydrogen phosphate–pyrophosphate mixtures were titrated with no pH adjustment or in a solution buffered at pH 8.7, little orthophosphate precipitated (Table II).

If alcohol does not change the shape of the curve of yield vs. pH appreciably, Figure 1 indicates that the best pH for precipitating pyrophosphate and triphosphate but not orthophosphate lies between about 8 and 10. It is certain that above pH 10 orthophosphate interferes. At lower pH values its presence constitutes a potential source of interference.

Titration of Triphosphate with Tris(ethylenediamine)cobalt(III) Chloride. When triphosphate is titrated with this reagent, orthophosphate and other possible interfering substances remain soluble except for pyrophosphate. It coprecipitates, as described previously, in amperometric titrations. As can be seen from Table III the titration is satisfactory for triphosphate alone but is spoiled when pyrophosphate is present in about a tenth of the molar concentration of triphosphate. However, the remarkable freedom from interference by other phosphates, sulfate, and silicate suggests that a study of ways of minimizing or correcting for the coprecipitation would be worth while. A colorimetric method for triphosphate has been developed along these lines (13).

phate and who has made many helpful suggestions. Also they are indebted to H. W. Lampe of the radiochemical laboratory.

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Statistical Comparison of Three Methods for Determining Organic Peroxides

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The polarographic method for determining hydroperoxides was compared with the more commonly used Wheeler iodide and stannous chloride chemical methods. The Latin square experimental design and statistical analyses were used to determine the relative accuracy and precision of the results obtained. The three methods gave results which were not significantly different for high purity Tetralin hydroperoxide. For two hydroperoxide samples of lower purity and for three samples of autoxidized methyl oleate, the chemical methods gave values which were significantly higher than those by the polarographic method. With pure hydroperoxides the three methods apparently yield identical results, but with impure products the polarographic method may give more reliable values because it is more specific than the chemical procedures.

BARNARD and Hargrave (1) recently presented a critical review of chemical methods used for determining organic peroxides and reported that these methods have many sources of error. For example, the methods for ferrous ion oxidation are unreliable unless carried out carefully under controlled conditions; the commonly used iodide oxidation methods are subject to error because of the addition of iodine to olefinic double bonds and the effect of sample size.

Barnard and Hargrave developed a modified stannous chloride procedure, which when tested on peroxides and hydroperoxides of high purity (99 to 100%) gave theoretical values with an average standard deviation of only 0.32%.

The present investigators have used extensively a modification of the Wheeler (8) iodide methods for determining organic peroxides. Recently they developed a polarographic method, which, in contrast with chemical methods, distinguishes between peroxides and hydroperoxides and is specific for determining both. It was hoped that a statistical comparison between the stannous chloride, Wheeler iodide, and polarographic methods might explain some of the anomalous results which the present investigators had observed between the last two methods. They believed that a statistical appraisal might also show the relative precision and accuracy of the three methods.

To make a proper statistical evaluation of the three methods, not only should a variety of peroxidic samples and a sufficient number of replicates be included but consideration should be

given to the stability of the peroxide samples with respect to time and other conditions, such as exposure to air and room temperature during sampling. To make the statistical comparison of the three methods and to include the factors of stability, a series of statistically designed experiments based on a 3 × 3 Latin square arrangement (2, 4, 10) was conducted.

STATISTICAL DESIGN OF EXPERIMENT

The 3 × 3 Latin square was designed to include the three methods, three aliquots of each peroxidic material being analyzed, and the three different times at which the analyses were made. This randomized block arrangement was repeated for each of the six peroxidic materials included in the study. The Latin square arrangement is as follows:

Methods	Aliquots		
	1	2	3
I	M	W	F
II	W	F	M
III	F	M	W

where I, II, and III are the polarographic, iodide, and stannous chloride methods, respectively; 1, 2, and 3 are the three undiluted aliquots of a peroxidic sample; and M, W, and F are the days (Monday, Wednesday, and Friday) on which the analyses were made.

An analysis of variance as described by Snedecor (7) was then applied to the data obtained by the Latin square arrangement so that the effect of methods (polarographic vs. chemical and Wheeler iodide vs. stannous chloride), aliquots, times, interaction, and interaction within the three individual methods could be evaluated. In this experiment the three undiluted aliquots of each original material were transferred to separate containers and each of these aliquots were analyzed by the three methods on the three different days. The Snedecor F ratio, obtained by dividing in turn the mean squares for the methods, aliquots, times, etc., for each sample by the mean square for interaction, was compared to critical F values at the 5% level to determine whether the mean squares were statistically significant or not.

To determine if a difference exists between the values obtained by the three methods, the least significant difference was calculated. This consisted in comparing the mean values of the three sets of duplicates of the three undiluted aliquots for one method and one sample with the corresponding mean values ob-

tained by another method on the same sample. The corresponding duplicates in each set were averaged, and the differences (d), between the two averages obtained. These three differences were squared, giving d_1^2, d_2^2, and d_3^2, which were then substituted in the following equation:

$$s_d^2 = \frac{1}{n-1}(d_1^2 + d_2^2 + d_3^2 - n\bar{d}^2)$$

where s_d^2 is the variance of the differences, n is the number of set of duplicates being compared (in this case, 3), and $\bar{d}^2$ is the square of the average difference. Then $\sqrt{s_d^2} = s_d$, the standard deviation of the differences, and $\frac{s_d}{\sqrt{n}} = s_{\bar{d}}$, the standard error of the differences between means, were obtained. The critical t value for the 2 degrees of freedom at the 5% level, 4.30, and $s_{\bar{d}}$ were used in calculating the limits of differences. A 5% risk was accepted on each comparison so that the combined comparison had a risk somewhere between 5 and 10%. These values were inserted in the following expression:

$$\left|\frac{\bar{d}-D}{s_{\bar{d}}}\right| \leq 4.30$$

where $\bar{d}$ = average of differences between each pair of means
D = observed differences between means of two methods
$s_{\bar{d}}$ = standard error of the differences between the three means

Transposing,

$$|\bar{d} - D| \leq 4.30\, s_{\bar{d}}$$

and eliminating absolute values,

$$-4.30\, s_{\bar{d}} \leq D \leq 4.30\, s_{\bar{d}}$$

then adding $\bar{d}$ to the term of the expression, we obtain

$$\bar{d} - 4.30\, s_{\bar{d}} \leq D \leq \bar{d} + 4.30\, s_{\bar{d}}$$

From this expression the least significant differences were calculated between the Wheeler iodide vs. the polarographic and between the Wheeler iodide vs. the stannous chloride methods.

EXPERIMENTAL

Preparation of Materials. Tetralin (1, 2, 3, 4-tetrahydronaphthalene) hydroperoxide, melting point 54–54.5° C., and cumene hydroperoxide, boiling point 65° C. at 0.1 mm. of mercury and $n_D^{20} = 1.5221$, were prepared as previously described (5). The autoxidized methyl oleate samples were prepared by autoxidation of methyl oleate followed by precipitation of unoxidized methyl oleate as the urea complex (3).

Procedures. POLAROGRAPHIC METHOD. The polarographic procedure has been described (9). A Sargent Model XXI polarograph was used to obtain the current-voltage curves; the electrolytic solution was 0.3M lithium chloride in 50 to 50 methanol-benzene; the electrolytic cell was a modified H-cell (9), and the capillary had m and t values of 3.116 mg. per second and 1.53 seconds, respectively, yielding a capillary constant of 2.29. The diffusion currents, i_d, were calculated from the wave heights of the hydroperoxide waves for the test samples with half-wave potentials of about -0.90 volt. The hydroperoxide content of the samples was calculated as follows:

$$\% \text{ hydroperoxide} = \frac{i_d \times 100}{C \times K}$$

where i_d = diffusion current, microamperes
C = concentration of sample in H-cell, moles per liter

K = diffusion current constant of a standard hydroperoxide, microamperes per mole per liter

This K value (5.85 ± 0.05 for the capillary used) had been established (9) by analyzing highly purified samples of cumene, Tetralin, and cyclohexene hydroperoxides.

STANNOUS CHLORIDE METHOD. The stannous chloride method has been described by Barnard and Hargrave (1). A weighed sample containing 0.75 to 1.0 meq. of peroxide is dissolved in acetic acid (10 ml.) in a 250-ml. Erlenmeyer flask, which is then evacuated to 20 mm. of mercury and filled with nitrogen. Fifteen milliliters of 0.1N stannous chloride solution are added from a pipet, and the flask is immediately re-evacuated and filled with nitrogen, the latter procedure being repeated twice. After standing for 1 hour at room temperature, a boiling solution consisting of 5 ml. of stock ferric solution, 1 gram of ammonium chloride, and 45 ml. water is added. The mixture is kept at 75° C. for 30 seconds and then rapidly cooled, and 10 ml. of phosphoric acid solution are added. The ferrous ion is titrated with 0.05N potassium dichromate solution and 6 drops of indicator solution; (0.25% solution of diphenylamine sulfonic acid in water); the end point is a sharp transition from green to violet. Blank determinations are carried out in a similar manner.

$\%$ peroxide =

$$\frac{(\text{blank} - \text{titer}) \times \text{dichromate normality} \times \text{mol. wt. of peroxide} \times 100}{2000 \times \text{weight of sample}}$$

IODIDE METHOD. The iodide method was a modified Wheeler method (6, 8). Twenty milliliters of 3 to 2 acetic acid–chloroform are introduced into a glass-stoppered 250-ml. iodine flask. A weighed sample adjusted to give the same final volume of reagent as the stannous chloride method is transferred to the flask and flushed with nitrogen. Two milliliters of freshly prepared 50% solution of potassium iodide in water are added, and the flask is again flushed quickly with nitrogen. After 15 minutes, 50 ml. of water are added, and the liberated iodine is immediately titrated with 0.1N sodium thiosulfate. The entire procedure is so arranged that the contents of the reaction flask are not in contact with any appreciable amount of dissolved or atmospheric oxygen at any time until the water is added.

Under these conditions, the liberated iodine rises to a maximum concentration in less than 15 minutes, and thereafter remains constant. Peroxide values obtained with reaction times up to 2 hours are no different from those obtained in 15 minutes.

$\%$ peroxide =

$$\frac{\text{ml. of thiosulfate} \times \text{normality} \times 0.008 \times 100}{\text{wt. of sample} \times \% \text{ peroxide oxygen in pure compound}}$$

Table I.   Statistical Evaluation of Peroxide Methods by Latin Square Design

	I Tetralin Hydroperoxide			II Cumene Hydroperoxide			III Impure Cumene Hydroperoxide		
Aliquot	1	2	3	1	2	3	1	2	3
Methods									
Polarographic	M	W	F	F	M	W	M	W	F
Wheeler iodide	W	F	M	M	W	F	W	F	M
SnCl₂	F	M	W	W	F	M	F	M	W
Polarographic	98.55	98.27	98.29	94.78	94.56	94.27	69.19	70.52	70.76
	98.68	100.30	98.97	94.31	94.57	95.43	68.70	71.72	69.65
Av.	98.62	99.29	98.63	94.55	94.57	94.85	68.95	71.12	70.21
Wheeler iodide	99.28	97.60	99.38	98.19	96.20	98.57	77.18	77.75	78.71
	98.87	99.18	100.00	99.70	96.20	98.87	77.28	75.38	79.47
Av.	99.08	98.39	99.69	98.95	96.20	98.72	77.23	76.57	79.09
SnCl₂	98.43	92.48	97.69	98.56	99.10	96.85	78.79	78.12	78.59
	99.51	91.98	95.80	98.66	99.96	96.03	77.54	76.72	74.37
Av.	98.97	92.23	96.75	98.61	99.53	96.44	78.17	77.42	76.48

	IV High Level Autoxidized Me Oleate (A)			V High Level Autoxidized Me Oleate (B)			VI High Level Autoxidized Me Oleate		
Aliquot	1	2	3	1	2	3	1	2	3
Methods									
Polarographic	W	F	M	W	F	M	F	M	W
Wheeler iodide	F	M	W	F	M	W	M	W	F
SnCl₂	M	W	F	M	W	F	W	F	M
Polarographic	73.37	72.83	73.06	69.54	69.71	69.53	20.84	20.75	20.89
	71.16	74.49	72.61	68.19	67.84	69.45	20.18	20.65	19.67
Av.	72.27	73.66	72.84	68.87	68.78	69.49	20.51	20.70	20.28
Wheeler iodide	77.61	77.00	78.23	78.44	80.00	79.26	21.97	22.38	21.76
	77.61	77.41	77.21	77.82	80.49	79.67	21.76	21.33	21.56
Av.	77.61	77.21	77.72	78.13	80.25	79.47	21.87	21.86	21.66
SnCl₂	76.67	79.54	81.52	80.30	78.42	78.18	22.27	23.38	23.71
	72.96	78.52	82.54	81.41	78.56	78.49	23.57	23.77	23.21
Av.	74.82	79.03	82.03	80.86	78.49	78.34	22.92	23.58	23.46

Table II. Table of Mean Squares[a]

Methods	DF	I	II	III	IV	V	VI
Polarographic vs. chemical	1	0.76	23.31	109.65	53.06	208.28	8.48
Wheeler iodide vs. SnCl₂	1	1.27	0.08	0.11	1.86	0.00	3.47
Aliquots	2	0.21	0.43	0.18	10.73	0.03	0.08
Times	2	0.15	1.02	0.05	11.85	2.55	0.03
Interaction	2	2.97	3.47	3.33	4.69	0.91	0.09
Within polarographic	3	...	0.26	0.48	1.31	0.88	0.32
Within Wheeler iodide	3	...	0.40	1.03	0.20	0.13	0.20
Within SnCl₂	3	...	0.24	3.55	2.47	0.23	0.34

[a] Basis means of duplicates.

RESULTS AND DISCUSSION

Table I shows the six Latin squares for the six peroxide samples, including the duplicate peroxide values and their means expressed as percentage peroxide obtained on the respective days by the three peroxide methods. It is evident that high purity Tetralin hydroperoxide (I) is approximately 99% hydroperoxide, whereas the high purity cumene hydroperoxide (II) has a slightly lower hydroperoxide content. The impure cumene hydroperoxide (III), containing approximately 75% hydroperoxide, had originally been pure but had decomposed during a long storage period at room temperature. The two high-level autoxidized methyl oleates (IV, V) contained approximately 75% hydroperoxide and the low-level autoxidized methyl oleate (VI) had only 21% hydroperoxide. These samples were chosen because they represented a fairly wide range of hydroperoxide contents and provided samples containing different levels of decomposition impurities.

Table III. Limits of Differences between Means of Duplicates

	Compound	Wheeler Iodide– Polarographic, %	Stannous Chloride–Wheeler Iodide, %
I	Pure Tetralin hydroperoxide	$-0.2 \le D \le 1.8$	$-3.0 \le D \le 5.2$
II	Pure cumene hydroperoxide	$-0.4 \le D \le 7.0$	$-2.2 \le D \le 6.2$
III	Impure cumene hydroperoxide	$3.0 \le D \le 12.1$	$-1.0 \le D \le 3.9$
V	High-level autoxidized methyl oleate A	$2.3 \le D \le 6.9$	$-0.2 \le D \le 6.1$
V	High-level autoxidized methyl oleate B	$7.6 \le D \le 12.9$	$-0.2 \le D \le 3.9$
VI	Low-level autoxidized methyl oleate	$-1.0 \le D \le 1.6$	$-0.4 \le D \le 2.6$

The two values, 92.48 and 91.98%, in Table I, obtained for aliquot 2 on Monday for high purity Tetralin hydroperoxide (I) using the stannous chloride method were obviously not in line with the remaining data in this Latin square. Some erratic behavior or gross experimental error had probably occurred. A supplied mean value of 98.15% was calculated for this position in the block by use of the "missing plot procedure" (2). In all subsequent treatment of these data this calculated value was used instead of 92.23%, the mean of the observed pair of values.

Analyses of variance using the means rather than the two individual values were then applied to the above data for each sample. The results of the analyses of variance are shown in Table II, wherein are represented the mean sum of squares (on the basis of the means of two duplicates) for methods, aliquots, times, interaction, and finally the interactions within the individual methods.

The exceedingly large mean squares (critical at the 1% level) obtained in the comparison of the polarographic vs. the chemical methods for all the samples except Tetralin hydroperoxide (I) were anticipated, as previous work had shown that for impure peroxidic samples the polarographic method tended to give lower results than the Wheeler iodide method. Only in the case of high purity Tetralin hydroperoxide (I) did the values obtained

by the polarographic and chemical methods yield a mean square with a noncritical F value at the 5% level. This is consistent with the assumption which is suggested by the means that the chemical methods are measuring substances which are not reducible polarographically and which are presumably not hydroperoxides.

In contrast, the mean squares obtained in the comparison between the Wheeler iodide and the stannous chloride methods show that only in the case of low level autoxidized methyl oleate (VI) was a critical F value at the 5% level obtained. This shows that the two chemical methods generally yield very similar results. The noncritical mean squares obtained for aliquots and for times indicates that the subdivision of the sample into aliquots did not significantly affect the peroxide values obtained by the three methods, and further that performing the analyses on three different days of the week had no significant effect. This indicated that the peroxidic samples were relatively stable during the week's period covered by the experiment even though the samples were exposed to atmospheric oxygen in room temperatures and light for several hours on each of the three days of the experiment. At other times they were stored at $-5°$ C. under nitrogen.

The remaining mean squares which appear in Table II under the items entitled interaction within polarographic, within Wheeler iodide, and within stannous chloride did not yield critical F values at the 5% level. The mean squares for the within methods interactions were not calculated for high purity Tetralin hydroperoxide because of the replaced value. A further comparison of these mean squares on a probability basis showed that no definite decision could be arrived at concerning which one of the three methods was the more precise.

Table III shows the range of differences between means to be expected at the 5% level for the Wheeler iodide vs. the polarographic and the Wheeler iodide vs. the stannous chloride methods. The range in which the observed difference, D, falls shows that the Wheeler iodide method can be expected to give markedly higher values than the polarographic method. Also, the means of duplicates obtained from the stannous chloride method have a tendency to be somewhat higher than those from the Wheeler iodide method.

The table also shows that the means of duplicates obtained using the stannous chloride method had a tendency to be somewhat higher than those obtained by the Wheeler iodide method.

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Conductometric Titration of Sulfuric and Hydrochloric Acids and Their Mixtures in Anhydrous Acetic Acid

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Sulfuric and hydrochloric acids and their mixtures have been titrated conductometrically in anhydrous acetic acid with several alkali acetates. The results represent the first time that the neutralization end points of the two hydrogens of sulfuric acid have been definitely differentiated in acetic acid. The conductometric plots for this acid exhibit two breaks corresponding to the neutralization end points of the first and second hydrogens. Differences in the plots obtained from titrations with different alkali acetates agree with predictions based on conclusions of earlier investigators. Conductometric titration plots of mixtures of sulfuric and hydrochloric acids in acetic acid with lithium acetate were found to exhibit three breaks corresponding to the neutralization of one of the two hydrogens of sulfuric acid, the hydrochloric acid, and the second hydrogen of sulfuric acid, in that order. Quantitative estimations of sulfuric and hydrochloric acids in such mixtures, based on the three breaks, are in agreement with the amounts present.

$\mathbf{I}$N CONNECTION with other studies being carried out at these laboratories, conductometric titrations of sulfuric and hydrochloric acids and their mixtures have been carried out in acetic acid. The results are of particular interest because they represent so far as known the first time that the neutralization end points of the two hydrogens of sulfuric acid have been definitely differentiated in this solvent. These findings, together with a rationalization of the observed facts, are presented at this time not so much because of possible direct analytical application of the conductometric method to the particular acids studied but rather because they cast some light on the complex behavior of acid-base reactions in acetic acid and other solvents of low dielectric constant which are enjoying wide usage in analytical fields.

Apparently very little work has been done on conductometric titrations of acids in acetic acid. Perchloric and hydrobromic acid have been titrated conductometrically in acetic acid with sodium acetate by Kolthoff and Willman (4) and perchloric acid has been titrated conductometrically with a number of organic amines by Hall and Spengeman (3). Although these investigators have shown the general feasibility of the method, there has been very little sustained interest in this field.

SULFURIC ACID

The general type of conductometric titration plot yielded by sulfuric acid solution in acetic acid when titrated by an alkali acetate solution is shown in Figure 1. There are two significant breaks in the curve which correspond very closely to the stoichiometric reactions:

$$MAc + H_2SO_4 \rightarrow MHSO_4 + HAc \qquad (1)$$
$$MAc + MHSO_4 \rightarrow M_2SO_4 + HAc \qquad (2)$$
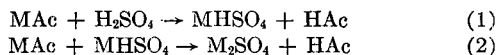
It is evident that the plot is different from the usual acid-base conductometric plots found in aqueous systems. The initial increase in the conductivity in the figure can be attributed to the higher degree of dissociation of the alkali bisulfate into current-carrying alkali ions and bisulfate ions as compared to the original sulfuric acid molecules. The drop in conductivity following neutralization of the first hydrogen can be ascribed to the lower degree of dissociation of the alkali sulfate, which was progressively formed in the system, as compared to the alkali bisulfate. Although straight-line approximations are used in the plot of extrapolation purposes, theoretically the actual curves are probably much more complex involving square root and higher power terms of concentration. In practice, however, a linear plot seems to be a fair approximation in the concentration range studied.

This explanation, based on the assumption that alkali sulfates are very slightly dissociated in acetic acid, is certainly a rea-
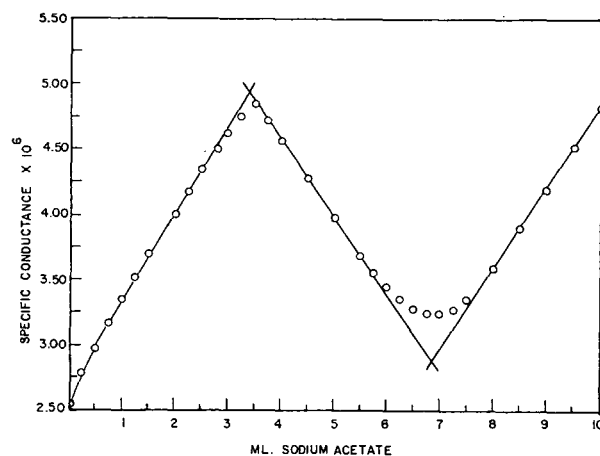

Figure 1. Conductometric titration of 100 ml. of 0.0176M sulfuric acid in acetic acid with 0.500M sodium acetate at 25° C.

First break corresponds to conversion of sulfuric acid to sodium bisulfate; the second break corresponds to conversion of sodium bisulfate to sodium sulfate
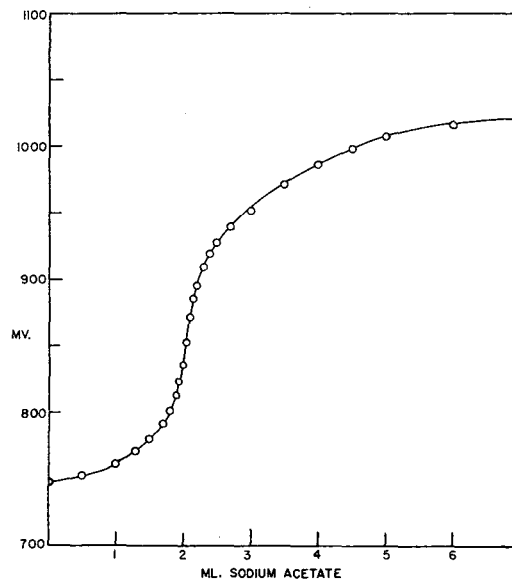

Figure 2. Potentiometric titration of 2 ml. of sulfuric acid (0.049M) with sodium acetate (0.0507M) in glacial acetic acid

sonable one. Because of its high central charge, it seems logical to expect, for example, the triple ion $Na_2{}^+SO_4{}^{--}$ to be less dissociated than the ion pair $Na^+HSO_4{}^-$. The effective negative charge tending to bind sodium ions would appear to be considerably greater in the case of I than in the case of II because of the greater electrophilic nature of the proton as compared to the sodium ion.

$$SO_4Na^- \qquad SO_4H^-$$
$$\text{I} \qquad\qquad \text{II}$$

Potentiometric plots which are presently widely used in non-aqueous titrimetric determinations do not, in contrast to the conductometric plots, bring out this behavior. As shown in Figure 2 the potentiometric titration curve of sulfuric acid in acetic acid with sodium acetate exhibits only a single break corresponding to the titration of the first hydrogen. From potentiometric response alone there is very little to indicate that the second hydrogen can be titrated.

From a purely theoretical standpoint, the glass electrode, moreover, is not necessarily the best indicator of the extent of these acid-base reactions. It can be shown that the extent of such reactions is roughly governed by the constant

$$K = \frac{K_a K_b}{K_{HAc}\, K_{ab}} \qquad (3)$$

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

Since the extent is not purely a function of the strengths of the acid and of the base alone, an acidity indicator is thus not necessarily the best indicator of these reactions.

Further information as to the nature of this system can be obtained by comparing the types of conductometric titration plots exhibited by different bases. The magnitude of the break in going from the bisulfate to the sulfate would be expected to be strongly influenced by the nature and size of the cations involved if the rationalization is valid.

Thus, if potassium acetate were used to titrate the acid, a less pronounced break would be expected, since the larger size of the potassium ion (as compared to the sodium ion) would re-
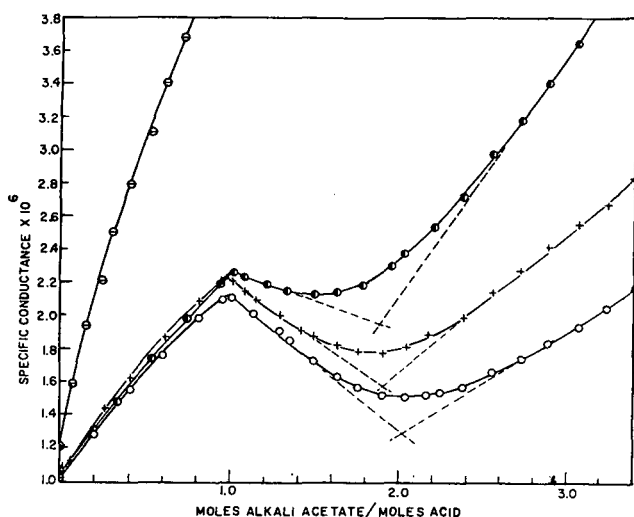
Figure 3. Conductometric titrations of 100 ml. of 0.00586M sulfuric acid in acetic acid with 0.200M alkali acetates at 25° C.

⊖ Triamylammonium acetate
◐ Potassium acetate
+ Sodium acetate
○ Lithium acetate

The first break corresponds to conversion of sulfuric acid to the respective alkali bisulfates; the second break to the conversion of the alkali bisulfates to the sulfates

Table I. Conductometric Determination of Sulfuric Acid in Acetic Acid

Titrant	Millimoles Added	Millimoles 1st Hydrogen Found	Millimoles 2nd Hydrogen Found
NaAc	1.74	1.72	1.73
	0.889	0.904	0.903
LiAc	0.586	0.587	0.588
	0.586	0.580	0.586
KAc	0.586	0.580	0.520
	0.586	0.580	0.510
$(C_5H_{11})_3NHAc$	0.492	0.497	...
	0.586	0.583	...

duce the coulombic forces involved in the ion-pair formation. Similarly, the use of a base containing a very large organic cation might result in a curve in which the break is almost completely suppressed. With a base containing a relatively small cation, such as lithium acetate, one would expect the break to be even more pronounced than that produced by sodium acetate. These predictions are based on the conclusions of Kolthoff and Willman (4).

All of these expectations were borne out experimentally. In Figure 3, conductometric titration plots of sulfuric acid titrated (top to bottom) with triamylammonium acetate, potassium acetate, sodium acetate, and lithium acetate are shown. Because of the limited solubilities of lithium and potassium sulfates in the system, it was found necessary to employ a lower concentration of the acid in obtaining these plots than that used in Figure 1. This resulted in somewhat poorer end-point breaks. It is apparent, nevertheless, that the degree of the first break decreased progressively in the order lithium > sodium > potassium > triamylamine. The point of break for the amine does not appear on the graph but consisted of a very slight positive break.

In Table I, analytical results shown in Figure 3 are given for each end point. Although no serious efforts were made to obtain highly accurate data, because of the type of extrapolation used as discussed previously, these results nevertheless indicate for most cases good stoichiometric relationships.

MIXTURE OF SULFURIC AND HYDROCHLORIC ACIDS

Although it is impossible to distinguish the alkalimetric titration end points of mixtures of hydrochloric and sulfuric acids by potentiometric means either in water or in acetic acid, acceptable end points can be detected by use of conductometric means in the latter solvent. From the results of the present investigation, it appears that one of the two hydrogens of sulfuric acid is first titrated, then hydrochloric acid, and finally the second hydrogen of sulfuric acid.

The conductance curves obtained on titrating 0.119M hydrochloric acid alone in acetic acid with 0.200M lithium acetate, sodium acetate, potassium acetate, and triamylammonium acetate are shown in Figure 4. Significant breaks were obtained in the case of triamylammonium acetate and lithium acetate. A slight break was obtained with sodium acetate. These breaks correspond closely to the stoichiometric reaction

$$HCl + NaAc \rightarrow NaCl + HAc$$
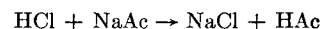
The initial rise in conductance can be ascribed to the formation of the chloride salts which appear to be more highly conducting than hydrochloric acid. The positive break obtained in the case of triamylammonium acetate indicates that this base is more highly dissociated than the chloride salt. The absence of any discontinuity in the plot obtained with potassium acetate indicates that potassium acetate and potassium chloride are effectively dissociated to about the same degree. The negative breaks obtained in the case of sodium and lithium acetate indicate that these bases are probably less dissociated than their respective chloride salts in these systems.

Determinations of hydrochloric acid made with the previously mentioned bases (except potassium acetate) are shown in Table II. The slightly low results obtained were undoubtedly due to the high volatility of hydrochloric acid in acetic acid.

Titration of several different molar ratios of mixtures of sulfuric acid and hydrochloric acid with lithium acetate are shown in Figure 5. The initial rise in conductance was due to the formation of bisulfate. The first break corresponds to the stoichiometric end point of removal of one of the two hydrogens of sulfuric acid. The subsequently greater rate of increase in conductance upon further addition of base indicates that the lithium chloride formed is somewhat more conducting (dissociated?) than lithium bisulfate. The second break in the conductance curve corresponds to the stoichiometric end point of the neutralization of the hydrochloric acid in the system. The decrease in conductance after the second break is explained in the same manner as before, the results of marked tendency for ion-pair formation between the sulfate ion and the lithium ions.

The amounts of hydrochloric acid and sulfuric acid found, calculated on this basis, agree reasonably with the amounts added as shown in Table III.

GENERAL DISCUSSION

These conductometric results are indicative of the vast difference between acid-base behavior in water and in nonaqueous solvents, especially of low dielectric constant. From purely theoretical considerations, titrimetric differentiation in water of sulfuric acid, bisulfate, and hydrochloric acid is impossible either potentiometrically or conductometrically, all three acids being effectively of the same strength. Yet, as is evident from the present study, relatively sharp end points can be detected for mixtures of these acids in acetic acid.
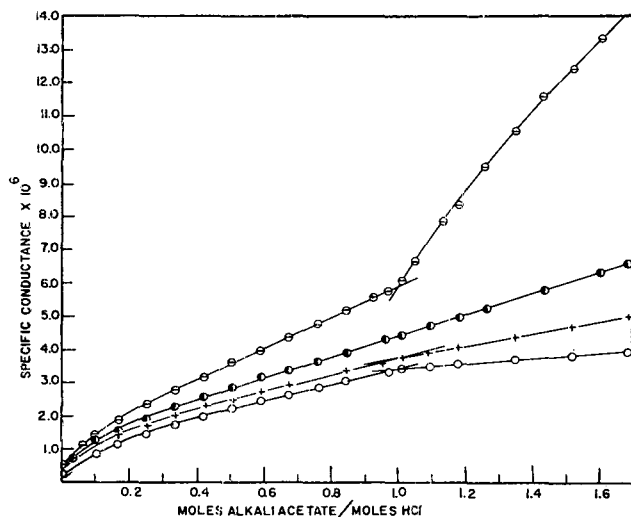
Figure 4. Conductometric titrations of 100 ml. of 0.0119M hydrochloric acid in acetic acid with 0.200M solutions of alkali acetates at 25° C.

⊖ Triamylammonium acetate
◑ Potassium acetate
+ Sodium acetate
○ Lithium acetate

It is of some interest to note that potentiometric titrations of these same acids in acetic acid actually give only little indications of the reactions taking place. No significant breaks are found which can be correlated to the individual end points. This behavior, as discussed before, is probably due to the fact that the extent of the neutralization reaction is not dependent only on the strength of the acid and the base, as in water, but also on the relative dissociative tendency of the salt which is formed.

Although in the somewhat naive explanations of the observed

Table II. Conductometric Determination of Hydrochloric Acid in Acetic Acid

Titrant	Millimoles Added[a]	Millimoles Found
NaAc	1.19	1.11
	1.19	1.12
LiAc	1.19	1.20
	1.19	1.10
(C₅H₁₁)₃NHAc	1.19	1.19
	1.16	1.16

[a] Because of the high volatility of hydrogen chloride in acetic acid, it was difficult to maintain accurate concentration of the gas in solution.

Table III. Conductometric Estimation of Mixtures of Hydrochloric and Sulfuric Acids in Acetic Acid with Lithium Acetate

Sample	Acids Added	Millimoles of Acid Added	Millimoles of Acid Found	
			1st H	2nd H
1	HCl	0.559	0.594	...
	H₂SO₄	0.589	0.580	0.526
2	HCl	1.16	1.16	...
	H₂SO₄	0.589	0.540	0.560
3	HCl	0.559	0.626	...
	H₂SO₄	1.18	1.12	1.20

conductometric plots it was tacitly assumed that the conducting species were essentially simple ions, it must be recognized that, especially at higher concentrations, complex ionic agglomerates play important roles in the over-all process of electrical conductance (2). Because of the complexity of these particular systems, however, their full and complete analysis is unfeasible. The simplified picture presented earlier appears to be sufficiently valid to provide a working hypothesis for analytical studies in these areas.

EXPERIMENTAL

Apparatus. A cylindrical jacketed borosilicate glass vessel, 5 cm. in diameter and 12 cm. deep, was used as the conductance cell. A fitted rubber plug was provided with holes for the electrodes, stirrer, and buret tip. The electrodes were brightly polished platinum plates with a cross-sectional area of 2.25 sq. cm. Distance between the plates was less than 2 mm. The cell constant was determined using 0.01M potassium chloride and was found to be 0.06. It was found necessary to thermostat the conductance cell because of an appreciable temperature
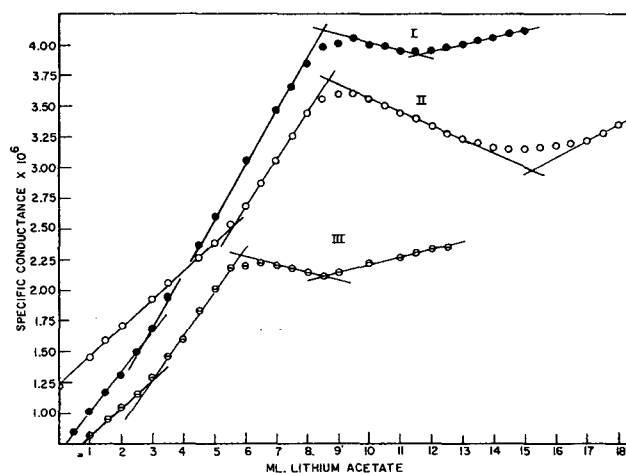

Figure 5. Conductometric titrations of mixtures of sulfuric and hydrochloric acids in acetic acid with lithium acetate at 25° C.

Plots for titrations of mixtures of hydrochloric and sulfuric acids with 0.200M lithium acetate are shown:
I.   1.16 millimoles of hydrochloric acid and 0.589 millimole of sulfuric acid
II.   0.559 millimole of hydrochloric acid and 1.18 millimoles of sulfuric acid
III.   0.559 millimole of hydrochloric acid and 0.589 millimole of sulfuric acid

coefficient of electrical conductance in acetic acid. Water from a constant temperature bath circulating through the cell jacket permitted temperature control to ±0.2° C. of the desired temperature. A Leeds & Northrup conductance bridge (catalog No. 4866) was used to determine the conductance values. In order to increase the sensitivity of the bridge at high resistances a cathode ray oscilloscope (Type 304-H, Allen B. Dumont Laboratories) was connected in parallel with the null point galvanometer of the bridge. The null point of the bridge was determined from the resulting screen pattern of the oscilloscope:

Chemicals and Reagents. ANHYDROUS ACETIC ACID. Reagent grade acetic acid was rendered anhydrous by refluxing over boron acetate (4) for 4 hours and subsequently distilling the anhydrous acid. The water content of acetic acid prepared by this method was less than 0.02% by the Karl Fischer method.

STANDARD SULFURIC ACID. A standard solution of sulfuric acid in anhydrous acetic acid was prepared by diluting absolute sulfuric acid (5) with acetic acid. The molarity of the acid solution was determined by barium sulfate precipitation.

STANDARD LITHIUM, SODIUM, AND POTASSIUM ACETATE. Accurately weighed quantities of these reagent grade salts previously dried overnight in a vacuum desiccator were dissolved in anhydrous acetic acid and diluted to volume.

STANDARD TRIAMYLAMMONIUM ACETATE. Commercial triamylamine was purified by distilling several times under reduced pressure, rejecting the first and last 20% of the distillates. Accurately weighed quantities of the amine were dissolved in anhydrous acetic acid and diluted to volume. The molarity of the resulting solution was checked by titrating with standard perchloric acid in acetic acid, using quinaldine red as the indicator. It was noted that acetic acid solutions of the triamylamine used developed a deep red color upon standing for several days.

STANDARD HYDROCHLORIC ACID. Anhydrous hydrochloric acid was passed into cool anhydrous acetic acid until fairly saturated. The resulting solution was diluted with acetic acid and the molarity was determined by silver chloride precipitation. Frequent restandardization of this solution was found necessary owing to the high volatility of hydrochloric acid in acetic acid.

Procedure. Accurately measured quantities of previously standardized acids were pipetted into the conductance cell and diluted to a volume of 100 ml. with anhydrous acetic acid. The solution was stirred until it had attained the equilibrium temperature of the thermostated vessel. Small increments of standardized base were added and the solution was stirred for about 30 seconds. The conductance reading was taken when it became constant after stirring had stopped.

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Purification, Purity, and Freezing Points of Sixty-four American Petroleum Institute Standard and Research Hydrocarbons

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The purification and determination of freezing point and purity are described for 64 hydrocarbons of the American Petroleum Institute Standard and Research series, including 11 paraffins, 3 alkyl cyclopropanes, 1 alkyl cyclopentane, 4 alkyl cyclohexanes, 24 mono-olefins, 12 alkyl benzenes, 3 dicycloparaffins, 3 dinuclear aromatics, 1 cycloparaffin-aromatic, and 2 olefin-cycloparaffins. Values of freezing points and cryoscopic constants are reported.

THE investigation reported is a continuation of the work of producing highly purified hydrocarbons of the API Standard and Research series (2, 5–9). This paper describes the purification and determination of purity and freezing points of 64 hydrocarbons, which include 11 paraffins, 3 alkylcyclopropanes, 1 alkyl cyclopentane, 4 alkyl cyclohexanes, 24 mono-olefins, 12 alkyl benzenes, 3 dicycloparaffins, 3 dinuclear aromatics, 1 cycloparaffin–aromatic, and 2 olefin–cycloparaffin hydrocarbons. The final lots of material labeled API Standard are sealed in vacuum in glass ampoules and made available as API Standard samples of hydrocarbons, by the Carnegie Institute of Technology. (Twenty-seven of the Standard hydrocarbons are also available from the National Bureau of Standards, Washington 25, D.C.) The material labeled API Research is made available in appropriate small lots through the American Petroleum Institute Research Project 44 for loan to qualified investigators for the measurement of needed physical, thermodynamic, and spectral properties.

Table I gives the names of the 64 compounds, the laboratories providing the starting material, details concerning the first and succeeding distillations or other methods of purification, the character of the plot of the freezing point of the hydrocarbon part of the distillate as a function of its volume, and the volumes of the final lots of API Standard and Research material.

The procedures followed in the process of purification and determination of purity were the same as those described in previous papers (3, 5–9). Details of the distillation apparatus and operations also have been described (4, 10).

Figures 1, 2, and 3 show graphically the results of some typical distillations. Figures 1, 2, and 3 represent the cases where the purest material is, respectively, largely in the forepart of the distillation, in the middle of the distillation, and in the after part of the distillation. In each figure plots are given for refractive index, boiling point, freezing point, and purity, as a function of the volume of the hydrocarbon part of the distillate. As emphasized in the previous reports, the blending of fractions of distillate for the preparation of material of the highest purity can be done safely only on the basis of the freezing points.

Table II gives the following information for the compounds measured: the kind of time-temperature curves, whether freezing or melting, used to determine the freezing point; the freezing point of the actual sample; the calculated value of the freezing point for zero impurity; the value of the cryoscopic constant, determined from the lowering of the freezing point on the addition of a known amount of a suitable impurity (3, 4); and the resulting calculated amount of impurity in the API Standard and Research materials.

Table I. Information on Purification of 64 API Standard and API Research Hydrocarbons

Compound	Laboratory[a] Providing Starting Material	Hydrocarbon Charged for Distillation Volume, liters	Purity, mole %	Kind[c]	Azeotrope-forming substance[d]	Amt. of hydrocarbon in the azeotropic distillate[e], % by vol.	Number of equivalent theoretical plates in the distilling column[b]	Rate of collection of distillate, ml./hr.	Time of distillation, hours	Location of purest material in distillate[f]	Volume of Selected Sample API Standard, ml.	API Research, ml.
2,2-Dimethyl-heptane	APIRP45	1.84	...	Reg.	...	..	200	4.5	480	M	350	100
		4.00[g]	...	Reg.	...	..	200	4.5	1032	M	700	240
3,3,5-Trimethyl-heptane	NBS Auto. Sec.	4.66	...	Reg.	...	..	200	4.5	1104	M	...	...
		3.65	...	Azeo.	Bu Cell.	77	200	4.5	1104	M		
		2.54	...	Reg.	...	..	200	4.0	840	M	1070	375
n-Undecane	APIRP6	5.74	85.4 ± 0.2	Reg.	...	..	130	8.5	744	A	...	...
		3.45	...	Azeo.	Me Carb.	60	200	4.5	1656	F	...	...
		2.56	91.7 ± 0.4	Urea[i]	...	...	...	...	...	..	950	150
		0.20[h]	85.4 ± 0.2									
		1.10[g]	83.7 ± 0.2									
2-Methyldecane	APIRP45	3.00[j]	99.82 ± 0.08	Reg.	...	..	200	6.0	576	A	1240[k]	420[k]
n-Dodecane	APIRP6[l]	6.00[j]	...	Reg.	...	..	125	8.0	792	M	...	...
		5.46[m]	99.78 ± 0.03	Reg.	...	..	130	5.0	1176	A	...	...
		1.67[j]	99.91 ± 0.03	Azeo.	Me Carb.	48	130	5.5	936	M	2190[k]	575[k]
n-Tridecane	APIRP42	1.95	99.87 ± 0.06	Urea[i]	...	..	...	...	...	..	1130	350
n-Tetradecane	APIRP6[l]	10.11	93.4 ± 0.2	Reg.	...	..	125	6.2₅	1800	M	...	...
		3.80	99.58 ± 0.10	Urea[i]	...	..	...	...	...	..	1320	400
n-Pentadecane	APIRP42	2.20	99.89 ± 0.05	Urea[i]	...	..	...	...	...	..	1430	455
n-Hexadecane	APIRP6[l]	9.86	90.2 ± 0.4	Reg.	...	..	125	8.0	1200	M	...	...
		3.33	99.82 ± 0.07	Urea[i]	...	..	...	...	...	..	2350	500
n-Heptadecane	APIRP42	1.54	99.84 ± 0.05	Urea[i]	...	..	...	...	...	..	900	350
n-Octadecane	APIRP6[l]	5.00	98.60 ± 0.08	Reg.	...	..	130	2.0	2472	A	...	...
		2.03	99.90 ± 0.08	Urea[i]	...	..	...	...	...	..	1150	450
1,cis-2-Dimethyl-cyclopropane	APIRP45	2.69	99.5 ± 0.1	Reg.	...	..	200	2.5	840	M	(170[n]	...
		1.00	...	Reg.	...	..	200	4.0	360	F	605	110[i]
		0.17	...	Azeo.	Methanol	91	200	4.0	168	F	45	70
1,trans-2-Dimethyl-cyclopropane	APIRP45	2.70	96.0 ± 0.3	Reg.	...	..	150	2.5	864	M	738	148
1,1,2,2-Tetra-methylcyclo-propane	APIRP45	1.95	99.87 ± 0.05	Azeo.	Ethanol	70	200	4.5	672	M	1065	320
n-Decylcyclo-pentane	APIRP45	4.03	99.75 ± 0.18	Reg.	...	..	200	1.2₅	3288	A	1070	368
1-Methyl-cis-4-isopropylcyclo-hexane	APIRP45	5.34[o]	...	Reg.	...	..	200	4.5	1368	..	...	...
		0.96[o]	...	Azeo.	Bu Cell.	55	200	3.2₅	696	..	...	...
		1.88[p]	...	Azeo.	Bu Cell.	55	200	4.5	1008	A	...	...
		3.37[p]	...	Reg.	...	..	135	4.0	792	A	...	...
		1.17[q]	...	Azeo.	Bu Cell.	55	200	5.0	624	A	...	...
		1.45[r]	99.78 ± 0.15	Azeo.	Bu Cell.	55	200	7.5	528	A	880	205
1-Methyl-trans-4-isopropylcyclo-hexane	APIRP45	5.34[o]	...	Reg.	...	..	200	4.5	1368	..	...	...
		0.96[o]	...	Azeo.	Bu Cell.	59	200	3.2₅	696	..	...	...
		2.67[s]	...	Azeo.	Bu Cell.	59	200	4.5	1224	M	...	...
		2.06	99.60 ± 0.04	Reg.	...	..	200	4.2₅	720	M	(840[t]	310
		0.45	...	Azeo.	Bu Cell.	59	200	2.2₅	528	F	305	...
2-Cyclohexyl-2-methylbutane (tert-pentyl-cyclohexane)	APIRP45	4.46[j]	...	Reg.	...	..	200	4.7₅	1032	M	...	...
		2.43[j]	...	Azeo.	Me Carb.	60	200	5.0	1224	M	1180[k]	300[k]
n-Decylcyclo-hexane	APIRP45	3.99	97.90 ± 0.16	Reg.	...	..	200	1.2₅	2832	M	...	...
		2.16	98.73 ± 0.14	Xtlzn.[u]	...	..	...	...	...	..	1050	350
2,3-Dimethyl-1-butene	General Motors	6.10[j]	...	Reg.	...	..	135	4.5	1512	M	...	...
		6.29[m]	...	Reg.	...	..	200	4.5	1584	M	...	...
		3.30	...	Reg.	...	..	200	4.5	1152	M	1080	640
2,3-Dimethyl-2-butene	General Motors	5.83	...	Reg.	...	..	130	8.5	840	M	...	...
		3.98	...	Reg.	...	..	200	4.5	1152	A	1275	380
trans-2-Heptene	APIRP45	4.40[j]	99.25 ± 0.06	Reg.	...	..	200	7.0	1200	M	1180[v]	360[v]
trans-3-Heptene	APIRP45	3.74[j]	99.1 ± 0.2	Reg.	...	..	200	4.0	768	M	1010[k]	330[k]
2-Methyl-1-hexene	APIRP45	3.66	...	Reg.	...	..	200	7.0	648	M	1085	330
4-Methyl-1-hexene	APIRP45	5.80[j]	...	Reg.	...	..	200	4.5	1344	M	...	...
		3.83[m]	99.66 ± 0.16	Reg.	...	..	200	4.5	1032	M	1050	360
5-Methyl-1-hexene	APIRP45	3.51[j]	...	Reg.	...	..	200	4.2₅	1176	M	1100[k]	350[k]
2-Methyl-2-hexene	APIRP45	4.74	99.5 ± 0.2	Reg.	...	..	200	4.0	1320	M	1105	360
3-Methyl-cis-3-hexene	APIRP45	5.26	...	Azeo.	Ethanol	57	200	7.0	924	M	...	...
		3.47	...	Azeo.	Ethanol	57	200	7.0	1008	M	1085	370
3-Methyl-trans-3-hexene	APIRP45	3.25[j]	...	Reg.	...	..	200	7.0	696	A	...	...
		3.45[m]	...	Azeo.	Ethanol	60	200	7.0	984	M	1040	300
3-Ethyl-1-pentene	APIRP45	3.50[j]	99.61 ± 0.12	Reg.	...	..	200	7.0	528	A	1130[k]	375[k]
2,3-Dimethyl-1-pentene	APIRP45	3.33	...	Reg.	...	..	200	5.7₅	912	M	...	...
		2.26	...	Azeo.	Ethanol	65	200	4.5	888	M	...	...
		1.65	98.9 ± 0.3	Reg.	...	..	200	3.0	528	A	...	...
		3.20[g]	96.3 ± 0.4	Reg.	...	..	200	4.0	960	M	...	...
		2.75[w]	...	Azeo.	Ethanol	65	200	7.5	648	A	...	...
		2.21	99.64 ± 0.20	Reg.	...	..	200	7.0	456	M	1050	340
3-Ethyl-2-pentene	APIRP45	4.40[j]	...	Reg.	...	..	200	7.2₅	768	M	1080[k]	350[k]
2,3-Dimethyl-2-pentene	APIRP45	4.23	98.4 ± 0.5	Reg.	...	..	200	7.0	720	A	...	...
		2.90	99.24 ± 0.40	Azeo.	Ethanol	56	200	7.0	1008	A	...	...
		2.10	99.55 ± 0.30	Azeo.	Methanol	50	200	7.0	672	A	1000	265
2,4-Dimethyl-2-pentene	APIRP45	3.70	...	Reg.	...	..	200	4.0	1008	A	1040	350
4,4-Dimethyl-cis-2-pentene	APIRP45	2.76	98.98 ± 0.15	Reg.	...	..	200	5.0	696	M	1040	240
2,3-Dimethyl-2-hexene	APIRP45	4.37	98.3 ± 0.3	Reg.	...	..	200	7.0	960	M	...	...
		2.67	98.7 ± 0.2	Azeo.	Me Cell.	70	200	6.0	864	M	...	...
		1.88	99.65 ± 0.12	Reg.	...	..	200	4.0	624	A	...	...
		1.65	...	Azeo.	Me Cell.	70	200	7.0	504	M	950	250
2,2-Dimethyl-cis-3-hexene	APIRP45	3.37	...	Reg.	...	..	200	7.0	600	M	...	...
		1.94	99.77 ± 0.16	Reg.	...	..	200	4.5	600	M	1010	280
2,2-Dimethyl-trans-3-hexene	APIRP45	3.68	...	Reg.	...	..	200	6.0	888	M	...	...
		2.00	...	Reg.	...	..	200	3.0	768	M	1030	316
1-Dodecene	APIRP6[l]	11.20	...	Reg.	...	..	125	8.5	1776	M	...	...
		4.00	99.47 ± 0.10	Reg.	...	..	200	3.5	1368	M	...	...
		3.87[m]	...	Urea[i]	...	..	...	...	...	..	1000	325
1-Tridecene	APIRP42	2.06[j]	99.60 ± 0.09	Azeo.	Me Carb.	30	130	8.5	552	F	665[k]	212[k]
1-Tetradecene	APIRP6[l]	5.40[j]	...	Reg.	...	..	130	2.0	2904	M	...	...
		2.97[m]	...	Urea[i]	...	..	...	...	...	..	1000	300
1-Pentadecene	APIRP42	0.81	99.4 ± 0.1	Azeo.	Bu Carb.	13	130	4.0	1872	M	...	...
		0.62	...	Urea[i]	...	..	...	...	...	..	360	127

Table I. Information on Purification of 64 API Standard and API Research Hydrocarbons (*Continued*)

Compound	Laboratory[a] Providing Starting Material	Hydrocarbon Charged for Distillation Volume, liters	Hydrocarbon Charged for Distillation Purity, mole %	Kind[c]	Azeotrope-forming substance[d]	Amt. of hydrocarbon in the azeotropic distillate[e], % by vol.	Number of equivalent theoretical plates in the distilling column[b]	Rate of collection of distillate, ml./hr.	Time of distillation, hours	Location of purest material in distillate[f]	Volume of Selected Sample API Standard, ml.	Volume of Selected Sample API Research, ml.
1-Hexadecene	APIRP6*l*	13.00	...	Reg.	...	..	125	3.5	3432	M	...	...
		4.42	97.1 ± 0.2	Xtlzn.*u*	...	..	...	...	...		1100	1170
1-Methyl-2-iso-propylbenzene	APIRP45	2.95*j*	99.5 ± 0.1	Reg.	...	..	200	4.2₅	984	A	1155*x*	380*k*
1-Methyl-3-iso-propylbenzene	Atlantic	4.40*v*	...	Reg.	...	..	130	8.5	600	F	...	...
		5.50*m*	...	Reg.	...	..	200	4.5	1368	M	1320	330
1-Methyl-4-iso-propylbenzene	NACA	1.66	...	Azeo.	Me Carb.	78	135	5.0	528	M	...	...
	APIRP45	2.07*o*	...	Azeo.	Me Carb.	78	130	8.5	408	F	...	...
		1.77*m*	99.54 ± 0.10	Azeo.	Me Carb.	78	200	4.5	672	A	1000	225
1,3-Dimethyl-5-ethylbenzene	NACA	2.40	99.58 ± 0.10	Azeo.	Me Carb.	70	200	4.5	772	M	1090	280
1,2,3,5-Tetra-methylbenzene	APIRP45	2.48	99.27 ± 0.06	Reg.	...	..	135	4.5	648	A	...	...
		1.36	99.87 ± 0.03	Azeo.	Me Carb.	52	200	4.5	720	A	740	160
1,2,4,5-Tetra-methylbenzene	Humble	5.40*j*	...	Reg.	...	..	125	12.5	672	M	...	...
		4.45*m*	...	Xtlzn.*u*	...	..	...	...	...	..	1000	490
2-Phenyl-2-methyl-butane (*tert*-pentylbenzene)	APIRP45	4.20*j*	...	Reg.	...	..	125	12.5	456	M	...	...
		2.50*j*	...	Azeo.	Me Carb.	60	200	4.2₅	936	M	1130*k*	360*k*
1-Methyl-3-*tert*-butylbenzene	NACA	3.70	99.26 ± 0.08	Reg.	...	..	200	4.5	888	F	1075	266
1-Methyl-4-*tert*-butylbenzene	NACA	3.44	99.80 ± 0.03	Reg.	...	..	200	4.5	888	M	1135	355
1,4-Diisopropyl-benzene	Atlantic	1.00	99.86 ± 0.05	Azeo.	Me Carb.	42	200	4.0	720	M	630	150
1,3,5-Triethyl-benzene	NACA	3.64	99.88 ± 0.06	Reg.	...	..	130	4.5	1032	M	1980	500
n-Decylbenzene	APIRP45	3.36	99.64 ± 0.16	Reg.	...	..	200	1.2₅	2640	M	...	...
		2.50	99.70 ± 0.16	Xtlzn.*u*	...	..	...	...	...	..	1050	300
cis-Hexahydro-indan (*cis*-hydrindan)	APIRP45	5.00	...	Reg.	...	..	125	12.5	502	M	...	...
		3.67*o*	99.58 ± 0.06	Reg.	...	..	200	4.5	888	A	...	...
		2.70*m*	...	Azeo.	Bu Cell.	62	200	4.5	1272	M	1000	295
trans-Hexahydro-indan (*trans*-hydrindan)	APIRP45	0.70	99.2 ± 0.2	Reg.	...	..	200	7.5	936	A	...	...
		4.00*o*	99.2 ± 0.2									
		3.35	99.61 ± 0.14	Azeo.	Bu Cell.	71	200	7.0	796	M	1175	360
Cyclopentyl-cyclopentane	APIRP45	5.50	99.76 ± 0.04	Reg.	...	..	200	4.5	1320	A	1200	340
Naphthalene	Am. Cyanamid	9.00	99.80 ± 0.04	Xtlzn.*u*	...	..	...	...	...	..	...	...
		6.40	99.87 ± 0.04	Xtlzn.*u*	...	..	...	...	...	..	...	...
		5.40	99.92 ± 0.03	Reg.	...	..	125	6.0	1224	M	2000	{ 560*z* { 505*aa*
1-Methylnaph-thalene (A)[ab]	APIRP6*l*	13.00	95.2 ± 0.2	Reg.	...	..	125	8.0	1800	M	...	...
		10.10	96.8 ± 0.1	Xtlzn.*u*	...	..	...	...	...	..	...	...
		2.39*j*	97.8 ± 0.1	Azeo.	Bu Carb.	24	130	4.0	2160	M	...	...
		2.36	98.8 ± 0.1	Reg.	...	..	200	3.0	864	M	...	...
		0.41*ac*	98.7 ± 0.1	Adsorp.*ac*	...	..	...	...	...	..	1200	250
		7.50*ad*	...	Xtlzn.*u*	...	..	...	...	...	..		
1-Methylnaph-thalene (B)[ab]	APIRP6*l*	4.22	98.5 ± 0.1	Reg.	...	..	200	2.5	1800	A	...	...
		2.22	99.60 ± 0.06	Azeo.	DPG	48	150	6.0	1152	A	875	260
2-Methylnaph-thalene	APIRP6*l*	18.00	97.3 ± 0.2	Xtlzn.*u*	...	..	125	6.0	912	M	...	...
		4.70	99.39 ± 0.10	Reg.	...	..	200	3.0	1008	M	1475	440
		3.00	99.73 ± 0.08	Reg.	...	..	130	5.0	1176	M	...	...
1,2,3,4-Tetra-hydronaphthalene	APIRP6*l*	6.07*j*	96.71 ± 0.20	Reg.	...	..	130	4.5	1176	M	1250	400
		5.15*m*	99.38 ± 0.09	Reg.	...	..	200	4.0	960	A	1050	350
Ethenylcyclo-pentane (vinyl-cyclopentane)	APIRP45	3.92	99.87 ± 0.12	Reg.	...	..	200	4.0	960	A	1050	350
Ethenylcyclo-hexane (vinyl-cyclohexane)	APIRP45	4.06*j*	99.1 ± 0.1	Reg.	...	..	200	7.0	648	M	1100	{ 350*z* { 250*aa*

[a] Abbreviations represent the following laboratories: APIRP45, American Petroleum Institute Research Project 45, Ohio State University, Columbus, Ohio; APIRP42, American Petroleum Institute Research Project 42, Pennsylvania State College, State College, Pa.; APIRP6, American Petroleum Institute Research Project 6, Carnegie Institute of Technology, Pittsburgh, Pa.; Am. Cyanamid, American Cyanamid Co., Calco Chemical Division, Bound Brook, N. J.; Atlantic, Atlantic Refining Co., Philadelphia, Pa.; General Motors, General Motors Corp., Detroit, Mich.; Humble, Humble Oil and Refining Co., Houston, Tex.; NACA, National Advisory Committee for Aeronautics, Lewis Flight Propulsion Laboratory, Cleveland, Ohio; NBS Auto. Sec., Automotive Section, National Bureau of Standards, Washington, D. C.

[b] See (4) and (10) for details.
[c] Azeo., azeotropic; reg., regular.
[d] Me Carb, methyl Carbitol, diethylene glycol monomethyl ether; Bu Cell, butyl Cellosolve, ethylene glycol monobutyl ether; Me Cell, methyl Cellosolve, ethylene glycol monomethyl ether; Bu Carb, butyl Carbitol, diethylene glycol monobutyl ether; DPG, dipropylene glycol.
[e] Approximate value obtained from actual volume of hydrocarbon recovered by extracting the azeotrope-forming substance with water in separatory funnels.
[f] Designations refer to general location of purest material in the hydrocarbon part of the distillate as a function of its volume. F, fore or front of the distillate; M, middle part of the distillate; and A, after part of the distillate.
[g] Second lot of this compound.
[h] Similar to original material.
[i] Fractionation by use of solid molecular compounds with urea (4, Chap. 10).
[j] One of two similar distillations.
[k] Half of this sample obtained from each of similar distillations.
[l] Obtained by purchase of commercially available material.
[m] Material having substantially the same composition from each of previous distillations.
[n] Total API Standard was 820 ml. Total API Research was 180 ml.
[o] Both cis and trans isomers were obtained from this material.
[p] Cis concentrate from first distillation.
[q] Cis concentrate from each of two distillations immediately preceding.
[r] Material having substantially same composition from 2nd, 3rd, and 5th distillations above.
[s] Second lot of trans concentrate, 0.67 liter, plus 2 liters from first distillation.
[t] Total API Standard was 1145 ml.
[u] Purification by crystallization with centrifuging. See (4 Chap. 9).
[v] Part of sample obtained from each distillation.
[w] Material having substantially the same composition from preceding three distillations.
[x] One third of this sample obtained from one distillation and two thirds from other distillation.
[y] One of 4 similar distillations.
[z] (A) sample (Table II).
[aa] (B) sample (Table II).
[ab] Footnote [a], Table II.
[ac] Preceding distillation, 2.05 liters, was divided into 5 equal charges for fractionation by adsorption (4, Chap. 8).
[ad] Material having substantially same composition from the preceding 4 steps of purification.

Table II. Freezing Points and Purity of 64 API Standard and API Research Hydrocarbons

Compound[a]	Kind of Time-Temperature Observations Used to Determine Freezing Point[b]	Freezing Point of Actual Material in Air at 1 Atm. API Standard, ° C.	Freezing Point of Actual Material in Air at 1 Atm. API Research, ° C.	Freezing Point for Zero Impurity in Air at 1 Atm., ° C.	Cryoscopic Constant[b] A, Mole Fraction/ Deg.	Calculated Amount of Impurity in Actual Material[c] API Standard, mole %	Calculated Amount of Impurity in Actual Material[c] API Research, mole %
2,2-Dimethylheptane	M	−113.04	−113.03	−113.00 ± 0.05	0.042	0.17 ± 0.15	0.13 ± 0.10
3,3,5-Trimethylheptane	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
n-Undecane	F	− 25.599	− 25.597	− 25.590 ± 0.010	0.0434	0.04 ± 0.03	0.03 ± 0.03
2-Methyldecane	M	− 48.877	− 48.877	− 48.860 + 0.010	0.0524	0.09 ± 0.06	0.09 ± 0.06
n-Dodecane	F	− 9.600	− 9.599	− 9.595 ± 0.010	0.0621	0.031 ± 0.025	0.025 ± 0.025
n-Tridecane	F	− 5.404	− 5.402	− 5.385 ± 0.012	0.0479	0.09 ± 0.06	0.08 ± 0.06
n-Tetradecane	F	+ 5.853	+ 5.853	+ 5.863 ± 0.008	0.0705	0.07 ± 0.06	0.07 ± 0.06
n-Pentadecane	F	+ 9.916	+ 9.916	+ 9.930 ± 0.010	0.0522	0.07 ± 0.05	0.07 ± 0.05
n-Hexadecane	M	+ 18.147	+ 18.149	+ 18.155 ± 0.010	0.0735	0.06 ± 0.04	0.04 ± 0.04
n-Heptadecane	F	+ 21.964	+ 21.964	+ 21.980 ± 0.010	0.0559	0.09 ± 0.06	0.09 ± 0.06
n-Octadecane	M	+ 28.168	+ 28.168	+ 28.180 ± 0.010	0.0821	0.10 ± 0.08	0.10 ± 0.08
1,cis-2-Dimethylcyclopropane	M	−140.910	−140.900	−140.870 ± 0.015	0.032[e]	0.13 ± 0.05	0.09 ± 0.05
1,trans-2-Dimethylcyclopropane	M	−149.626	−149.626	−149.57 ± 0.03	0.041[e]	0.23 ± 0.12	0.23 ± 0.12
1,1,2,2-Tetramethylcyclopropane	M	− 80.733	− 80.732	− 80.720 ± 0.010	0.0304	0.04 ± 0.03	0.04 ± 0.03
n-Decylcyclopentane	M	− 22.164	− 22.164	− 22.13 ± 0.03	0.0587	0.20 ± 0.18	0.20 ± 0.18
1-Methyl-cis-4-isopropylcyclohexane	M	− 89.866	− 89.841	− 89.80 ± 0.03	0.0307	0.20 ± 0.09	0.13 ± 0.09
1-Methyl-trans-4-isopropylcyclohexane	M	− 86.369	− 86.360	− 86.350 ± 0.010	0.0346	0.07 ± 0.03	0.03 ± 0.03
2-Cyclohexyl-2-methylbutane (tert-pentylcyclohexane)	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
n-Decylcyclohexane	F	− 1.745	− 1.741	− 1.720 ± 0.020	0.0571	0.14 ± 0.11	0.12 ± 0.11
2,3-Dimethyl-1-butene	F	−157.30	−157.30	−157.27 ± 0.03	0.046	0.14 ± 0.13	0.14 ± 0.13
2,3-Dimethyl-2-butene	F	− 74.323	− 74.304	−. 74.280 ± 0.020	0.0236	0.10 ± 0.05	0.06 ± 0.05
trans-2-Heptene	M	−109.508	−109.507	−109.480 ± 0.010	0.0522	0.15 ± 0.05	0.14 ± 0.05
trans-3-Heptene	M	−136.66	−136.66	−136.63 ± 0.03	0.068	0.20 ± 0.15	0.20 ± 0.15
2-Methyl-1-hexene	M	−102.863	−102.858	−102.840 ± 0.015	0.0542	0.12 ± 0.08	0.10 ± 0.08
4-Methyl-1-hexene	M	−141.493	−141.485	−141.45 ± 0.03	0.0522	0.22 ± 0.16	0.18 ± 0.16
5-Methyl-1-hexene	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
2-Methyl-2-hexene	M	−130.377	−130.375	−130.350 ± 0.020	0.0534	0.14 ± 0.11	0.13 ± 0.11
3-Methyl-cis-3-hexene	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
3-Methyl-trans-3-hexene	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
3-Ethyl-1-pentene	M	−127.532	−127.530	−127.480 ± 0.020	0.0283	0.15 ± 0.06	0.14 ± 0.06
2,3-Dimethyl-1-pentene	M	−134.34	−134.33	−134.30 ± 0.10	0.044	0.20 ± 0.15	0.15 ± 0.10
3-Ethyl-2-pentene	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
2,3-Dimethyl-2-pentene	M	−118.36	−118.33	−118.27 ± 0.05	0.045	0.40 ± 0.25	0.27 ± 0.20
2,4-Dimethyl-2-pentene	M	−127.736	−127.730	−127.700 ± 0.010	0.0385	0.14 ± 0.04	0.12 ± 0.04
4,4-Dimethyl-cis-2-pentene	M	−135.517	−135.502	−135.46 ± 0.03	0.0362	0.21 ± 0.11	0.15 ± 0.11
2,3-Dimethyl-2-hexene	M	−115.066	−115.058	−115.000 ± 0.020	0.0443	0.29 ± 0.09	0.26 ± 0.09
2,2-Dimethyl-cis-3-hexene	M	−137.383	−137.380	−137.350 ± 0.025	0.0480	0.16 ± 0.12	0.14 ± 0.12
2,2-Dimethyl-trans-3-hexene	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
1-Dodecene	F	− 35.266	− 35.255	− 35.230 ± 0.020	0.0370	0.13 ± 0.07	0.09 ± 0.07
1-Tridecene	F	− 23.118	− 23.105	− 23.070 ± 0.020	0.0439	0.21 ± 0.09	0.15 ± 0.09
1-Tetradecene	F	− 12.913	− 12.913	− 12.85 ± 0.03	0.0452	0.27 ± 0.13	0.27 ± 0.13
1-Pentadecene	F	− 3.762	− 3.753	− 3.730 ± 0.010	0.0490	0.16 ± 0.05	0.11 ± 0.05
1-Hexadecene	F	+ 4.086	+ 4.106	+ 4.120 ± 0.015	0.0477	0.16 ± 0.07	0.07 ± 0.06
1-Methyl-2-isopropylbenzene[f]	M	− 71.561 (I)	− 71.561 (I)	− 71.540 ± 0.015 (I)	0.0296	0.06 ± 0.04	0.06 ± 0.04
				− 75.24 ± 0.03 (II) (u)	...	...	...
				− 81.53 ± 0.03 (III) (u)	...		
1-Methyl-3-isopropylbenzene	M	− 63.762	− 63.760	− 63.745 ± 0.010	0.0375	0.064 ± 0.038	0.056 ± 0.038
1-Methyl-4-isopropylbenzene	M	− 67.952	− 67.944	− 67.935 ± 0.010	0.0275	0.05 ± 0.03	0.02 ± 0.02
1,3-Dimethyl-5-ethylbenzene	M	− 84.360	− 84.353	− 84.325 ± 0.020	0.0302	0.11 ± 0.06	0.08 ± 0.06
1,2,3,5-Tetramethylbenzene	M	− 23.722	− 23.694	− 23.685 ± 0.010	0.0213	0.08 ± 0.02	0.02 ± 0.02
1,2,4,5-Tetramethylbenzene	F	+ 79.174	+ 79.202	+ 79.240 ± 0.020	0.0205	0.14 ± 0.04	0.08 ± 0.04
2-Phenyl-2-methylbutane (tert-pentylbenzene)	..	...	...	...	...	(0.20 ± 0.15)[d]	(0.15 ± 0.10)[d]
1-Methyl-3-tert-butylbenzene	M	− 41.403	− 41.399	− 41.370 ± 0.020	0.0240	0.08 ± 0.05	0.07 ± 0.05
1-Methyl-4-tert-butylbenzene	F	− 52.541	− 52.539	− 52.515 ± 0.015	0.0183	0.05 ± 0.03	0.04 ± 0.03
1,4-Diisopropylbenzene	M	− 17.040	− 17.038	− 17.030 ± 0.010	0.0281	0.03 ± 0.03	0.02 ± 0.02
1,3,5-Triethylbenzene	M	− 66.432	− 66.432	− 66.415 ± 0.015	0.0204	0.03 ± 0.03	0.03 ± 0.03
n-Decylbenzene	M	− 14.418	− 14.403	− 14.38 ± 0.03	0.0538	0.20 ± 0.16	0.12 ± 0.10
cis-Hexahydroindan (cis-hydrindan)	F	− 36.87	− 36.85	− 36.70 ± 0.05	0.0033	0.06 ± 0.02	0.05 ± 0.02
trans-Hexahydroindan (trans-hydrindan)	M	− 59.57	− 59.57	− 59.44 ± 0.05	0.022	0.29 ± 0.11	0.29 ± 0.11
Cyclopentylcyclopentane	F and M	− 35.361	− 35.360	− 35.345 ± 0.010	0.0285	0.05 ± 0.03	0.04 ± 0.03
Naphthalene (A)	F	+ 80.269	+ 80.269	+ 80.290 + 0.015	0.0182[g]	0.04 ± 0.03	0.04 ± 0.03
Naphthalene (B)	F		+ 80.274	+ 80.290 ± 0.015	0.0182[g]	...	0.03 ± 0.03
1-Methylnaphthalene (A)	F	− 30.624	− 30.590	− 30.480 ± 0.020	0.0151	0.22 ± 0.06	0.17 ± 0.06
1-Methylnaphthalene (B)	M	− 30.498	− 30.496	− 30.480 ± 0.020	0.0151	0.03 ± 0.03	0.02 ± 0.02
2-Methylnaphthalene	M	+ 34.522	+ 34.527	+ 34.58 ± 0.04	0.0152[g]	0.09 ± 0.06	0.08 ± 0.06
1,2,3,4-Tetrahydronaphthalene	F	− 35.838	− 35.814	− 35.790 ± 0.020	0.0288	0.14 ± 0.06	0.07 ± 0.06
Ethenylcyclopentane (vinylcyclopentane)	M	−126.500	−126.498	−126.480 ± 0.020	0.0455	0.09 ± 0.09	0.08 ± 0.08
Ethenylcyclohexane (vinylcyclohexane) (A)	M	−126.773	−126.773	−126.760 ± 0.010	0.0352	0.05 ± 0.04	0.05 ± 0.04
Ethenylcyclohexane (vinylcyclohexane) (B)	M	...	−126.770	−126.760 ± 0.010	0.0352	...	0.03 ± 0.03

[a] (B) following the name of compound designates, for the API Research series, a second (and usually slightly purer) sample of given compound, first sample of which is labeled (A).

[b] F indicates freezing and M indicates melting. For experimental details and definition of cryoscopic constant (3, 4).

[c] The values in this column, except as otherwise noted, were calculated (8, 9) using values of cryoscopic constants and freezing points for zero impurity given in preceding columns.

[d] Estimated by analogy with isomers subjected to similar purification.

[e] Cryoscopic constant determined by procedure of (3, p. 371).

[f] This hydrocarbon has more than one crystalline form. Three forms indicated are labeled I, II, and III in order of decreasing temperature of fusion (or freezing point). Forms other than I will be, at their respective freezing points, in metastable equilibrium with the undercooled liquid, but will be unstable with respect to transition to some other solid form at same temperature and pressure (1 atmosphere); indicated by a letter u in parentheses following the Roman numeral.

[g] Not determined in this investigation. From z tables of American Petroleum Institute Research Project 44 (1).
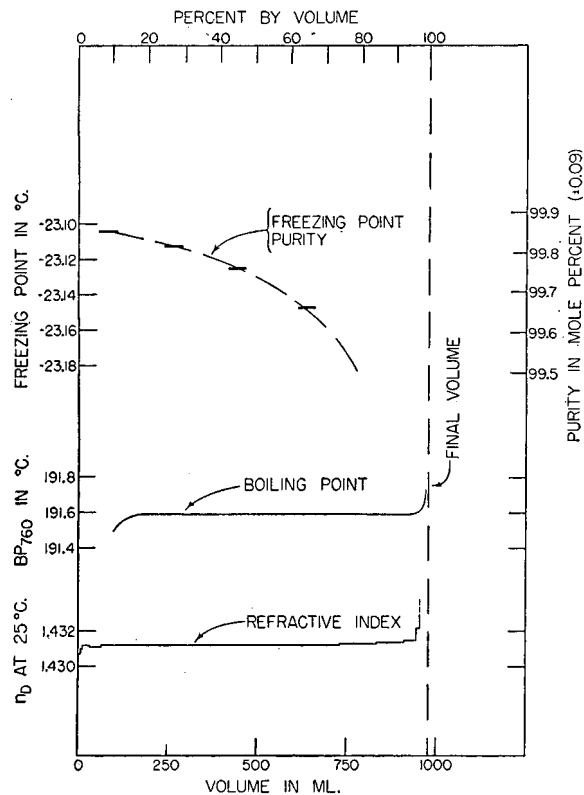
Figure 1. Results of azeotropic distillation of 1-tridecene with diethylene glycol monomethyl ether, methyl Carbitol

Special acknowledgment is made to Charles B. Willingham for his help with the distillations. The authors also wish to express appreciation of the assistance of the following chemists in portions of this investigation: Charlotte M. Kennedy, Laurel F. Soule,
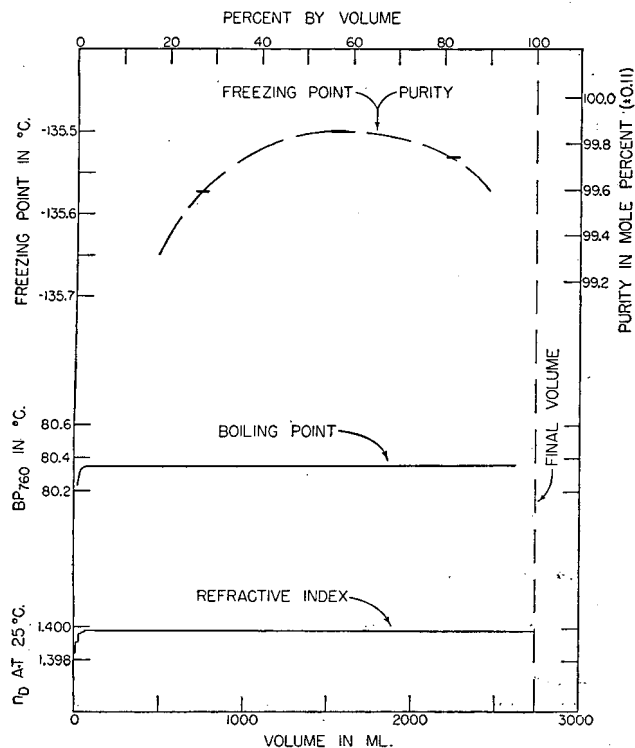
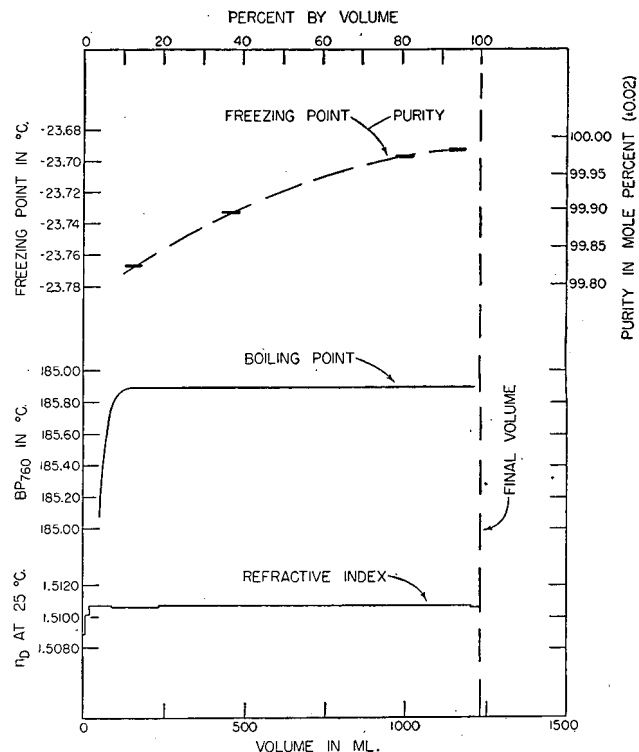**Figure 2. Results of regular distillation of 4,4-dimethyl-cis-2-pentene**

Figure 3. Results of azeotropic distillation of 1,2,3,5-tetramethylbenzene with diethylene glycol mono-methyl ether, methyl Carbitol

Mary G. Beck, Sheila M. O'Toole, Lillian C. Janicik, M. Elizabeth Janes Carlin, Maria C. Lomba, Nilda Zegarra-Paz, David L. Camin, Speros D. Mandamadiotis, Helen F. Flanagan, Janice C. Zimmerman, and Evelyn T. Murphy.

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Purification of Supporting Electrolytes for Polarographic Trace Analysis by Controlled Potential Electrolysis at Mercury Cathode

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A simple and generally applicable method for the removal of heavy metal impurities from supporting electrolytes to be used in polarographic trace analysis consists of electrolyzing the solution with a mercury cathode whose potential is kept constant at a suitable value, and in each of the cases studied reduces the concentration of the impurity to a polarographically undetectable value within 45 minutes of unattended electrolysis.

ONE of the most important problems confronting the trace analyst is that of securing or preparing reagents which are sufficiently free from the substance being determined to give a satisfactorily low blank. Though this problem is by no means peculiar to polarographic analyses, it is perhaps especially severe there because of the relatively large amounts of indifferent salts used. It is not at all uncommon to employ a $1M$ supporting electrolyte in a polarographic procedure in which the substance being determined may be present at a concentration as low as 10^{-5} or $10^{-6}M$. For an analysis to be even feasible—let alone accurate—under these conditions, it is clearly essential that the concentration of the substance being determined in the supporting electrolyte be no greater than $10^{-8}M$. This corresponds to an impurity of about 0.001% or less in the solid salt, which is a standard not satisfied by many commercially available reagents.

There is now reported the development of a method for the purification of supporting electrolytes which is more efficient, simple, and rapid than any other generally applicable method for the removal of traces of heavy metal impurities. It consists of pre-electrolyzing a portion of the supporting electrolyte solution with a mercury cathode, using a potentiostat to maintain the cathode potential constant at a value at which the deleterious impurity is completely deposited. The only evident limitation of the method is the fact that it cannot be used for the removal of elements such as tungsten, vanadium, and uranium, which cannot be reduced to the metallic state by electrolysis of an aqueous solution at a mercury cathode. However, such elements are rarely, if ever, present in significant amounts in reagents used as supporting electrolytes.

EXPERIMENTAL

The potentiostat used in this work was designed in collaboration with Julian M. Sturtevant. It is to be made available commercially by Analytical Instruments, Inc., of Bristol, Conn. A feature especially useful in this work was the fact that the input impedance of the amplifier is high enough to permit the use of a 5-inch calomel reference electrode available from the National Technical Laboratories for use with Beckman pH meters: This is not true of some of its predecessors (*1*).

In principle, all of the purifications described could have been accomplished by the classical constant current electrolytic technique. However, the use of a potentiostat for controlling the cathode potential has a very considerable practical advantage, in that it permits the progress of the purification to be followed by simply observing the magnitude of the electrolysis current, which decreases practically to zero at the completion of the electrolysis. To take full advantage of this indication, oxygen was removed from all solutions by a stream of nitrogen. [Normally oxygen is formed by the electrochemical reaction at a plat-

inum anode in the cell used (*3*) or in any other nondiaphragm cell, but the flow of a rapid stream of nitrogen through the solution keeps the concentration of dissolved oxygen always very small.]

The recording polarograph used has been described (*4*).

APPLICATIONS OF THE METHOD

Removal of Zinc from Sodium Hydroxide. During the development of a polarographic method for the determination of zinc in lead and its compounds, it was found that the percentage of zinc in the best available sodium hydroxide was of the order of 0.001%. Thus the zinc wave in the blank was approximately equal in height to the wave which would have been secured from a sample containing 0.01% of zinc, and would have seriously affected the accuracy and precision of the determination of small percentages of zinc. A typical polarogram of a $2M$ sodium hydroxide solution, secured at one tenth of the highest sensitivity of the polarograph, is shown as curve *a* in Figure 1. The diffusion current of zinc measured from this curve, 0.083 μa., corresponded to 0.012mM zinc.

A portion of this solution was subjected to electrolysis as described above, with the potential of the mercury cathode kept constant at -1.8 volts *vs.* S.C.E. After 30 minutes, a portion of the solution was withdrawn from the cell: Its polarogram is shown as curve *b*. Since no trace of a zinc wave could be detected even at the full sensitivity of the polarograph, it is estimated that the concentration of zinc remaining in solution must have been less than 0.0002mM. The fact that the current on curve *b* is appreciably lower than that on curve *a* even at potentials preceding the zinc wave shows that the original sodium hydroxide also contained traces of other reducible impurities which were removed by the electrolysis.

When a sodium hydroxide solution thus purified was allowed to stand in borosilicate glass, a small zinc wave was observed after some time, doubtless because traces of zinc were leached from the glass. Such solutions should therefore be stored in thoroughly cleaned polyethylene bottles.

Removal of Nickel and Zinc from Ammoniacal Ammonium Chloride. This was of interest in connection with the development of a method for the determination of nickel and zinc in copper and its salts. In the procedure finally evolved, a stock $4M$ ammonia–$4M$ ammonium chloride–$1M$ hydrazine hydrochloride solution was prepared and diluted fourfold in the course of dissolving the sample. Curve *a* in Figure 2 is a polarogram of such a stock solution prepared from the best available chemicals. Curve *b* is a polarogram of the same solution, recorded with slightly higher damping than curve *a*, after electrolysis for 30 minutes at a cathode potential of -1.6 volts *vs.* S.C.E., and it shows no detectable wave for either element. Moreover, it is clear that the solution also contained traces of other reducible substances which were removed by the electrolysis.

Removal of Iron from Citrate Medium. A recently published method (*2*) for the determination of iron in copper-base alloys involves the measurement of the height of the ferric iron wave in a weakly acidic $1M$ citrate buffer after removal of the copper. The amount of iron present in the citric acid used for the preparation of the buffer proved a source of serious annoyance in the determination of traces of iron. A procedure for the preparation

of such a solution free from any polarographically detectable trace of iron has now been devised. The citric acid solution is merely made about $0.1M$ in excess sodium hydroxide and electrolyzed at -1.75 volts vs. S.C.E. for about 30 minutes. This is well beyond the half-wave potential of the reduction wave of the ferrous complex in this medium. When the iron has been completely deposited, the solution is removed from the cell and neutralized to the desired pH with concentrated hydrochloric acid. This modification of the general procedure is necessitated by the fact that the ferrous citrate complex can be reduced to the metallic state at a lower potential than is required to reduce hydrogen ion only in an alkaline solution.

Removal of Alkali and Alkaline Earth Metals from Tetraethylammonium Hydroxide. This separation cannot be accomplished directly by recrystallization because of the high solubility of the hydroxide. Instead tetraethylammonium bromide must be recrystallized several times, then converted to the desired hydroxide by treatment with moist silver oxide. This is a tedious procedure, and the yield obtainable is low enough to make it a rather expensive one as well.
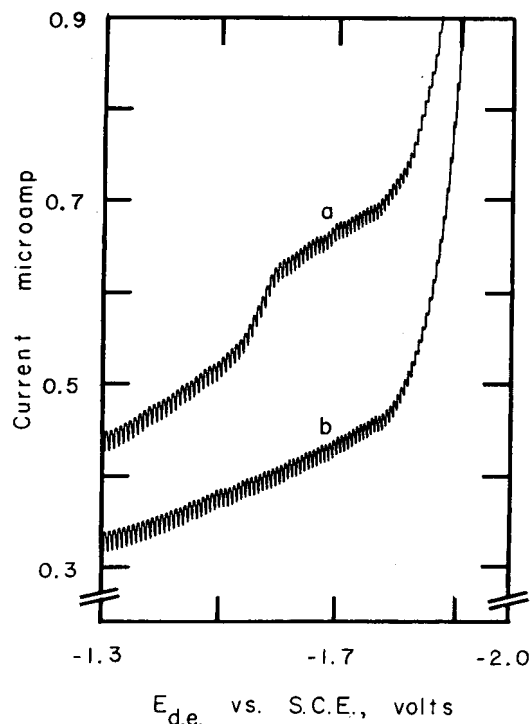
Figure 1. Polarograms of $2M$ sodium hydroxide

(a) Before and (b) after controlled potential electrolysis at -1.80 volts vs. S.C.E.

In 50% ethyl alcohol containing $0.1M$ tetraethylammonium hydroxide the half-wave potentials of the alkali metals range from -1.99 volts for rubidium to -2.31 volts for cesium, and those of the alkaline earth metals range from -1.86 volts for barium to about -2.2 volts for magnesium, while the final current rise does not begin until about -2.4 volts. It has been found that all these metals could be removed by electrolysis at -2.35 volts for about 45 minutes. The current does not decrease quite to zero when the purification is complete, and so the electrolysis should be discontinued when the current has remained constant at a few milliamperes for 10 or 15 minutes.

The fiber-type calomel electrode mentioned is virtually a ne-

cessity in this instance, for no other type so nearly completely prevents transfer of potassium ion from the reference electrode into the solution being electrolyzed. It is convenient to reserve one electrode for such electrolysis and to store it in a tetraethylammonium chloride or hydroxide solution between electrolyses to leach most of the potassium out of the fiber.
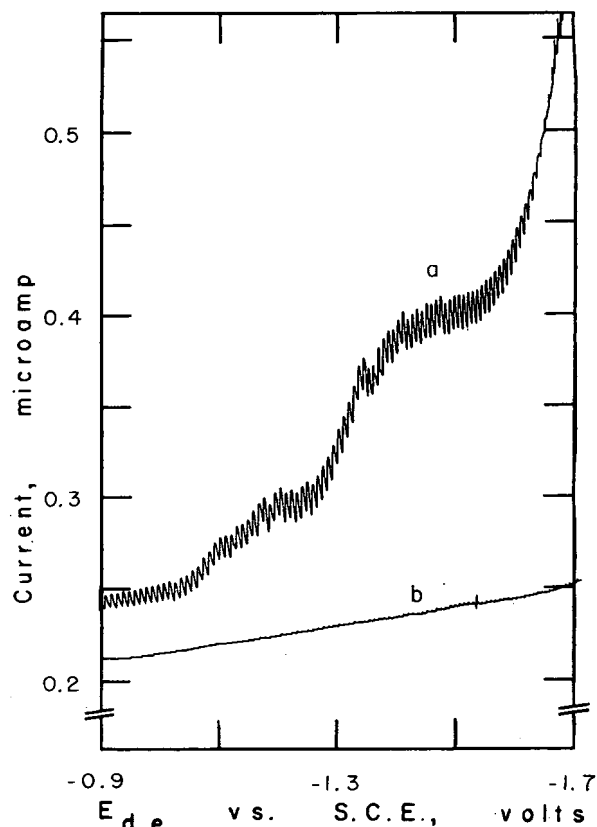
Figure 2. Polarograms of $4M$ ammonia–$4M$ ammonium chloride–$1M$ hydrazine hydrochloride

(a) Before and (b) after controlled potential electrolysis at -1.60 volts vs. S.C.E.

In a typical instance an electrolysis for 30 minutes served to purify a 50% ethyl alcohol–$0.1M$ tetraethylammonium hydroxide so thoroughly that the residual concentration of alkali and alkaline earth metals was too low to be detected polarographically.

Other Applications. Though this technique has as yet been applied only to solutions to be used in polarographic work, it should be useful in various other types of procedures as well. It should, for example, prove convenient in removing such elements as lead and zinc from reagents to be used in colorimetric procedures employing dithizone, which is a ubiquitous problem of colorimetric analysis.

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Complexes of Eriochrome Black T with Calcium and Magnesium

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The complexes of the dye Eriochrome Black T with the alkaline earth metals calcium and magnesium were studied by the method of continuous variations. Evidence is presented which indicates that 1 to 1, 2 to 1, and 3 to 1 complexes exist for both calcium and magnesium. Previous literature on the subject has described only a 1 to 1 complex for calcium and a 1 to 1 and a 2 to 1 complex for magnesium.
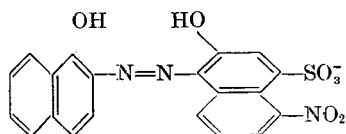
THE dye Eriochrome Black T, has been used to a large extent as a metal ion indicator in analytical chemistry (1-8). Perhaps its most widely known application as a metal ion indicator is in the determination of the total calcium and magnesium content of water, where ethylenediaminetetraacetic acid is used as the titrant.

Schwarzenbach and Biedermann (9) described the indicator action of the dye with variation of pH and have determined the following ionization constants; $K_2 = [H^+][HF^{--}]/[H^2F^-] = 10^{-6.2}$ and $K_3 = [H^+][F^{-3}]/[HF^{-2}] = 10^{-11.55}$. The form H_2F^- is red, the form HF^{-2} is blue, and the form F^{-3} has been described as orange.

Schwarzenbach and Biedermann (9) have shown that both calcium and magnesium form 1 to 1 complexes with the F^{-3} anion and Harvey, Komarmy, and Wyatt (6) reported a 2 to 1 complex for magnesium at pH 10.1.

Some preliminary work on a spectrophotometric determination of calcium and magnesium led the present authors to believe that other complexes in addition to those mentioned existed in solutions containing the dye and calcium or magnesium. In the present paper, the method of continuous variations has been used in order to determine the various calcium and magnesium complexes that are formed with the dye, Eriochrome Black T.

REAGENTS

Water. Triple-distilled water was used in all the experiments. Its conductance was less than 1 micromho.

Buffers. Buffers in the region pH 8.4 to 10 were prepared by mixing 25 ml. of concentrated ammonium hydroxide and the required volume of concentrated hydrochloric acid with sufficient water to make a final volume of approximately 500 ml. Buffers in the region pH 10 to 12 were prepared by mixing 75 ml. of piperidine and the required volume of concentrated hydrochloric acid with sufficient water to make a final volume of approximately 500 ml. All buffer solutions were stored in polyethylene bottles.

Calcium Solution, 0.001M. A 0.1000-gram sample of calcium carbonate (Hach Chemical Co., 99.97% pure) was dissolved in 0.3 ml. of concentrated hydrochloric acid and the solution was diluted with water to 1 liter.

Magnesium Solution, 0.001M. A 0.0913-gram sample of $3MgCO_3.Mg(OH)_2.3H_2O$ (Baker and Adamson, 99.96% pure) was dissolved in 0.3 ml. of concentrated hydrochloric acid and this was diluted with water to 1 liter.

Dye Solution, 8.66 × 10^{-4}M. A 0.100-gram sample of Eriochrome Black T (W. H. and L. D. Betz) was transferred to a 250-ml. volumetric flask by means of small portions of water. The total volume of water used was about 15 ml. One milliliter of pH 11.70 buffer was added and the solution was diluted to 250

ml. with 95% grain alcohol. After mixing, the solution was transferred to a 500-ml. Florence flask, which was fitted with a rubber stopper and a constricted glass tube that served as a vent. This was placed on a reciprocal motor-driven shaker for 0.5 hour. The solution was stored in the dark until it was used.

EXPERIMENTAL

All pH measurements were made with a Beckman Model G pH meter equipped with a Beckman Type E micro glass electrode and a micro saturated calomel electrode. The absorption measurements were made with a Beckman Model DU quartz spectrophotometer equipped with 5-mm. Corex cells. All measurements were made against water as the blank solution. The slit width was adjusted so that the sensitivity knob was maintained at or very near its counterclockwise limit—i.e., a minimum slit width was used for each reading.
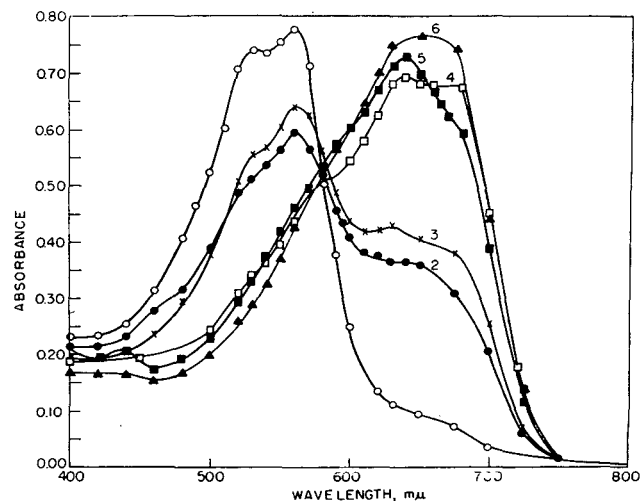
Figure 1. Absorption curves

1. $4.33 \times 10^{-5}M$ Mg, $12.99 \times 10^{-5}M$ dye, pH 11.50
2. $4.33 \times 10^{-5}M$ Ca, $12.99 \times 10^{-5}M$ dye, pH 11.50
3. $4.33 \times 10^{-5}M$ Mg, $12.99 \times 10^{-5}M$ dye, pH 9.32
4. 0 , $12.99 \times 10^{-5}M$ dye, pH 11.50
5. $4.33 \times 10^{-5}M$ Ca, $12.99 \times 10^{-5}M$ dye, pH 9.32
6. 0 , $12.99 \times 10^{-5}M$ dye, pH 9.32

The solutions for Figure 1 were prepared by mixing 5.0 ml. of water or 5.0 ml. of 4.33 × 10^{-4} metal solution (prepared by diluting 43.3 ml. of the 0.001M metal to 100 ml.) with 7.5 ml. of the 8.66 × 10^{-4}M dye and 12.5 ml. of 95% grain alcohol. The appropriate volume of buffer solution (5.0 ml. of pH 9.32 buffer or 2.5 ml. of pH 11.50 buffer) was added and the solution was diluted to 50 ml. with water.

For the continuous variations experiments (see Figures 2 through 4), solution A is 8.66 × 10^{-4}M dye and solution B is 8.66 × 10^{-4}M calcium or magnesium (86.6 ml. of the 0.001M metal solution diluted to 100 ml. with water). Volumes of A and B totaling 10 ml. were pipetted into 50-ml. volumetric flasks. Five milliliters of one of the ammonium hydroxide–ammonium chloride buffers (for pH values between 8.4 and 10), 2.5 ml. of one of the piperidine–piperidine hydrochloride buffers
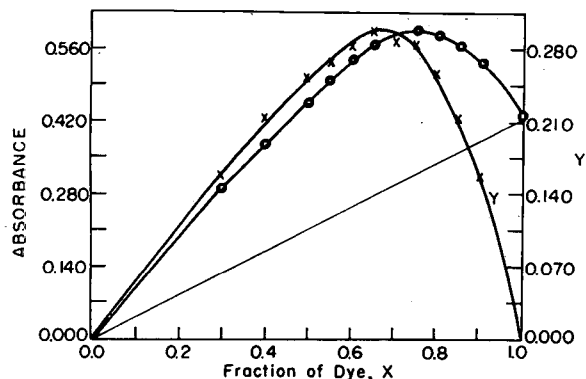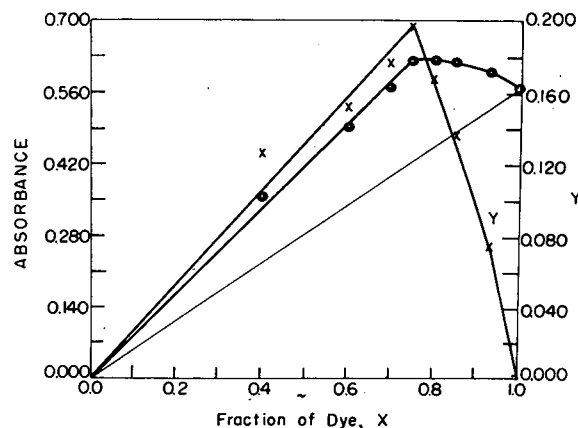
Figure 2. Continuous variation for magnesium at pH 9.52

$\lambda = 540$ mμ, $C_{\text{Mg}} + C_{\text{dye}} = 1.732 \times 10^{-4}M$, $X_{\text{max.}} = 0.675$, $n = 2.08$
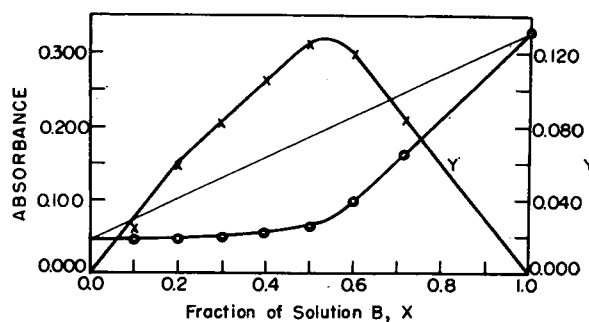
In the continuous variations experiments, the value of n, the number of ligands per cation, is obtained from the relation $n = \frac{X_{\text{max.}}}{1 - X_{\text{max.}}}$, where $X_{\text{max.}}$ represents the fraction of solution B present at the point where the difference curve Y is a maximum. The value obtained for n corresponds to that complex which predominates at the pH of measurement. It was found that n varied with the pH and that at certain pH values no one complex predominates. For magnesium the following values of n were obtained by the method of continuous variations: 1.08 at pH 8.41, 2.08 at pH 9.52, 2.33 at pH 10.11, 2.84 at pH 10.61, and 3.00 at pH 11.70. These values indicate a 1 to 1 complex at pH 8.41, a 2 to 1 complex at pH 9.52, solutions that are composed of mixtures of the 2 to 1 and 3 to 1 complexes at pH 10.11 and 10.61, and a 3 to 1 complex at pH 11.70. The formation of higher complexes increases as the pH increases. This is to be expected if it is assumed that the metal ion coordinates with the F^{---} anion, since the proportion of F^{---} ion in the solution is controlled by the pH and increases as the pH becomes higher.

Figure 3. Continuous variation for magnesium at pH 11.70

$\lambda = 540$ mμ, $C_{\text{Mg}} + C_{\text{dye}} = 1.732 \times 10^{-4}M$, $X_{\text{max.}} = .075$, $n = 3.00$

Figure 4. Continuous variation for calcium at pH 12.4

$\lambda = 540$ mμ, $C_{\text{Ca}} + C_{\text{dye}} = 1.732 \times 10^{-4}M$, $X_{\text{max.}} = 0.75$, $n = 3.00$

(for pH values between 10 and 12), or 1 ml. of pure piperidine (for a pH of 12.4) were then added and the solutions were diluted to 50 ml. with water. These solutions were stored in the dark for 1 hour. Following this period of standing, their absorbance $\left(\text{defined as the function } \log_{10} \frac{I_{\text{blank}}}{I_{\text{soln.}}}\right)$ was measured at 520, 530, 540, 550, and 560 mμ. The curves marked Y represent the differences between the measured absorbance and the absorbance that would be obtained if complex formation had not taken place —i.e., the straight line of no reaction that can be drawn between the ends of the measured values.

In Figure 5, solution A is $4.33 \times 10^{-4}M$ with respect to the dye and $2.165 \times 10^{-4}M$ with respect to magnesium ion—i.e., dye and cation are present in a 2 to 1 ratio in solution A. Solution B is $2.165 \times 10^{-4}M$ dye. Volumes of A and B totaling 20 ml. were pipetted into 50-ml. volumetric flasks. Two and one half milliliters of buffer were added and the solutions were diluted to 50 ml. with water. These were stored in the dark for 1 hour and then measured at 630, 640, and 650 mμ.

DISCUSSION

Figure 1 is a series of absorption curves for the dye Eriochrome Black T with and without calcium and magnesium ions and illustrates the effect of changes of pH and the presence of metal ions.

Figure 5. Modified continuous variation for magnesium at pH 11.70

$\lambda = 640$ mμ, $C_{\text{MgF2}} + C_{\text{dye}} = 2.165 \times 10^{-4}$, $X_{\text{max.}} = 0.54$, $n = 3.18$

For calcium, the following values of n were obtained by the method of continuous variations: 2.1 at pH 10.61, 2.33 at pH 11.70, and 3.00 at pH 12.4. A 2 to 1 complex is indicated for calcium at pH 10.61. At pH 11.70 it appears that the calcium solution is composed mainly of both the 2 to 1 and 3 to 1 complexes. Since the calcium complexes are less stable than those of magnesium, it is necessary to use higher pH values in order to obtain values of n that are comparable to those found for magnesium— for example, for magnesium, $n = 2.33$ at pH 10.11, whereas for

calcium $n = 2.33$ at pH 11.70. By increasing the pH to 12.4 it was possible to obtain a solution that was composed primarily of the 3 to 1 calcium complex.

The values given were all obtained from curves that were drawn at 540 mμ. Curves drawn at 520, 530, 550, and 560 mμ are very similar and indicate the same values of n.

A modified method of continuous variations that was used by Watters and Aaron (10) in their study of copper pyrophosphate complexes was utilized in the present work in order to provide additional evidence for the presence of the 3 to 1 complex for magnesium. This is shown in Figure 5. The value of n, the number of ligands per cation, is obtained from the relation $n = (2 - X_{max.})/(1 - X_{max.})$ where $X_{max.}$ represents the fraction of solution B present at the point where the difference curve Y is a maximum. Since $X_{max.} = 0.54$, $n = 3.18$. This is reasonable since a maximum in the difference curve Y at 0.50 would indicate that a 1 to 1 complex is formed between the 2 to 1 complex and the dye. This is the same as a 3 to 1 complex of dye with metal. A wave length of 640 mμ was used because data at 640 mμ showed greater deviation from the straight line of no reaction than did data that was obtained when using wave lengths between 520 and 560 mμ. Data obtained at 630 and 650 mμ resulted in curves that were very similar to those in Figure 5. This modified method of continuous variations was also used with calcium at pH 11.7 and pH 12.4. The results were not so well defined as those for magnesium. Nevertheless, they indicate reaction between the 2 to 1 complex and the dye.

CONCLUSIONS

The dye Eriochrome Black T forms 1 to 1, 2 to 1, and 3 to 1 complexes with magnesium and also with calcium.

Schwarzenbach and Biedermann (9) suggested that calcium and magnesium have a coordination number of 6 when they form 1 to 1 complexes with the dye. Their proposed formula for these complexes showed one bond with each of the phenolic oxygens, one with each of the azo nitrogens, and one with each of two water molecules. A possible explanation of the 3 to 1 complexes that are indicated in the present work is that the dye anion acts as a bidentate through the two phenolic oxygens or through one azo nitrogen and an adjacent phenolic oxygen, and that the alkaline earth metal ion has a coordination number of 6, thus forming an octahedral complex in which the sp^3d^2 orbitals of the metal are involved in bond formation.

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New Organic Reagent for Silver and Copper

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A red dye prepared from 1-phenyl-3-methyl-5-pyrazol-one, pyridine, sodium cyanide, and chloramine-T was found to give deep blue compounds with silver and cuprous ions. The sensitivity of a test for metals based on the use of this dye is 1 part in 600,000 for silver and 1 part in 250,000 for copper. As a reagent for silver, the dye can be used as an outside indicator for the titration of chlorides or silver.

IN THE course of a search for a satisfactory method of detecting microamounts of cyanides, a blue-green dye was obtained when a mixture of sodium cyanide, pyridine, and 1-phenyl-3-methyl-5-pyrazolone was treated with chloramine-T. While investigating the stability of this coloring matter under various conditions it was found that a red compound was formed when the blue dye was boiled for a short time in aqueous alkaline solution. Investigation of this new red compound, to which the name Zolon Red has been applied, proved that it is a true dyestuff with interesting properties as such but of even more interest because of its properties of forming deep blue insoluble compounds with silver and cuprous ions. This latter property was of sufficient interest to warrant an investigation of its possible uses as an organic analytical reagent.

PREPARATION OF ZOLON RED

Four grams of pyridine and 18 grams of 1-phenyl-3-methyl-5-pyrazolone were stirred to a uniform paste and diluted with 250 ml. of water. Two and one half grams of sodium cyanide were then added, and while stirring, 250 ml. of an aqueous solution containing 14 grams of chloramine-T were added over a period of 10 minutes. A red color formed immediately, rapidly changing through purple to blue. When all of the chloramine-T had been added, the stirring was continued until tests made by placing a drop of the reaction mixture on filter paper gave a pure blue spot with no trace of a red ring. The mixture was allowed to stand until a thick paste of dye separated. This was then filtered with suction and the filtrate, which retained a considerable amount of dissolved dye, was treated with 20 grams of sodium chloride, and the salted out dye was added to the original filter cake. After being pressed down well on the filter, the cake was washed once by displacement with an equal volume of water.

The blue-black dye paste was then transferred to the original reaction vessel and broken up into a thin paste with a small amount of water. Two hundred and fifty milliliters of water and 20 grams of sodium carbonate were added, and the mixture was brought to boiling. This was continued until the conversion to a red dye was complete. The conversion was followed by placing drops of the reaction mixture on filter paper and observing the colors displayed by the spot. When a clear red spot with no blue or purple center was obtained, the heating was terminated and the mixture was allowed to cool to room temperature. The thick deposit of red fibrous crystals which separated was filtered off with suction and washed twice by displacement with a volume of water equal to that of the filter cake. The wet cake, which consisted of the sodium salt of the dye, was broken up in 500 ml. of water and reprecipitated as the free acid of the dyestuff by adding a slight excess of 10% hydrochloric acid. The brick-red precipitate was filtered with suction, washed thoroughly with water, and dried.

The product was very slightly soluble in water, freely soluble in

a variety of organic solvents to an orange color, and soluble in aqueous alkaline solutions to a pure magenta color (absorption maxima, 490 to 530 mμ). The alkaline salts of the dye, in addition to being water-soluble, were also soluble in various organic solvents such as acetone, alcohol, and pyridine.

REACTIONS OF ZOLON RED WITH METALS AND SENSITIVITY

The reactions of Zolon Red with metals were studied by adding a saturated alcoholic solution of the free dye acid to aqueous solutions of the metals at approximately 0.1N dilution buffered with sodium acetate. Only silver and cuprous ions gave deep blue insoluble compounds. Gold gave a red purple precipitate after standing. Mercuric salts reacted after long standing to form a red flocculent precipitate. No reaction under the above conditions was obtained with the following metals: lead, zinc, cadmium, iron, nickel, cobalt, platinum, and palladium.'

The blue compounds of silver and copper when first formed appeared as highly dispersed colloids which, after standing for some time, separated out as a blue-black precipitate. The colloidal suspensions showed a maximum absorption band in the visible spectrum at 590 to 620 mμ. When separated and dried, the solid was almost black, with a strong metallic bronze reflection. The silver compound was found to be stable under ordinary conditions. The cuprous compound was unstable, owing to oxidation to the cupric state. Both the silver and cuprous compounds were destroyed by acids or salts that formed insoluble or nonionized complexes with the metals, the red dye being regenerated in the process. Iodides and thiocyanates will destroy the cuprous–Zolon Red compound.

The sensitivity of Zolon Red to silver and cuprous ions was investigated. Ten milliliters of aqueous solutions of known metal content were buffered with sodium acetate and then tested by adding 6 drops of a saturated solution of Zolon Red in alcohol. Color changes were observed in a 0.75-inch test tube viewed vertically. The limit of sensitivity was found to be: silver, 1 part in 600,000; copper, 1 part in 250,000.

The same sensitivity could be obtained on paper dyed with Zolon Red. In this case the buffered solutions of the metals were applied from a capillary tube to the paper; by this means the insoluble blue compounds were formed and concentrated in a small area.

ANALYTICAL POSSIBILITIES OF ZOLON RED

While the analytical possibilities of Zolon Red have not been thoroughly explored, it has been shown that it can be used as an indicator in the volumetric titration of chloride with silver or vice versa. Although the blue silver complex is destroyed by chlorides with regeneration of the red dye, a sharp end point cannot be obtained when it is used as an internal indicator because of adsorption of the dye and blue complex by silver chloride. However, the end point can be determined sharply with 0.1N

solutions when papers dyed with Zolon Red are used as an outside indicator and in the manner indicated.

STRUCTURE OF ZOLON RED

The structure of Zolon Red has not been determined. However, it appears to be closely related to the blue dye from which it is prepared. This latter substance is a polymethine dye corresponding to the following general formula:

$$CH_3-C-C=CH(-CH=CH)_n-C-C-CH_3$$
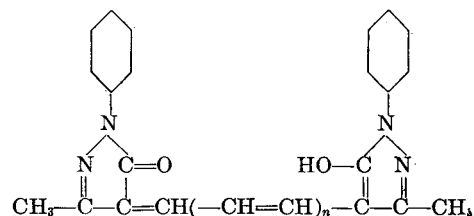
Three dyes of this type are known. The first, $n = 0$, is a yellow substance prepared by Knorr (2). The second, $n = 1$, is magenta, and the third, $n = 2$, the dye under discussion, is blue green. The series is of interest because it illustrates in a striking manner the effect of the increased length of conjugated carbon chain in shifting the maximum absorption band in the spectrum from the short to the longer wave lengths. In this case, the shift is remarkably uniform (1). The same sequence of colors is also noted in the preparation of the blue-green dye.

As prepared in this investigation, three distinct stages in the reaction can be observed. The first, in which the pyridine ring is ruptured by the action of cyanogen chloride, results in the formation of a yellow substance, enolized glutaconic aldehyde, which has the same auxochromophoric system as the yellow dye prepared by Knorr (2). In the second stage of the reaction the aldehyde is condensed with one molecule of the pyrazolone, resulting in an unstable product that has an auxochromophoric system of seven conjugated carbon atoms. This is magenta in color, has weak dyeing properties, and forms blue compounds with silver ions. The resemblance of this compound, both in regard to its color and reactions with silver, to Zolon Red would seem to indicate that the latter also has an auxochromophore of seven conjugated carbons.

A further effect of interest as possibly throwing some light on the composition of Zolon Red is the color that the blue-green dye displays when dissolved in concentrated sulfuric acid. This is a brilliant magenta, which reverts to the original blue-green when the solution is diluted with water and the free acid is neutralized with alkali.

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Improved Apparatus for Zone Electrophoresis

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An apparatus for electrophoresis on paper which features improved cooling, drying, and ease of manipulation is described. An evaluation of the apparatus shows that the mobilities of charged substances in filter paper, as distinguished from those in free solution, may be determined with caffeine as an uncharged reference substance, easily detected by ultraviolet radiation.

THE rediscovery of filter paper electrophoresis by Wieland and Fischer (15) 11 years after the apparently unnoticed description of the technique of Konig (5, 8) has stimulated a rapid development of experimental methods and applications. Investigations in this laboratory in which the resolution of certain proteins, nucleic acids, and nucleotides have been studied have resulted in the development of an improved apparatus.

APPARATUS

In order to study the movement of a charged substance through filter paper for the purpose of separating it from other species or of comparing its rate of movement with that of a known substance for qualitative identification it is necessary to provide a constant voltage distribution in the paper, to control and measure the flow of liquid in the paper, and to localize the zones without distortion or movement during detection procedures. Published methods (3–5, 8–10, 15) for zone electrophoresis show a wide latitude in the efficiency with which these requirements are met.

The apparatus shown in isometric form in Figure 1 resembles that of Kunkel and Tiselius (9) and has certain features which permit control over the foregoing listed requirements. Thus, the apparatus is provided with a top plate which is raised $^1/_{16}$ inch above the paper by a spacer of plastic. This serves the double purpose of permitting an equilibration period under applied voltage before addition of the samples through a sliding panel in the top plate and allowing the removal of the top plate after completion of migration without distortion or movement of the zones.

The evaporation of water from the electrolyte and condensation onto the top plate during the flow of current is prevented by a sprinkler system which sprays cooling water to the bottom of the supporting plate. The efficiency of this means of cooling is such that 0.10 to 0.25 watt per sq. cm. of paper may be applied. The ends of the filter paper are not cooled by the sprinkling system. Polyethylene film is laid in place to prevent evaporation at these positions. Upon completion of the electrophoresis, rapid drying of the paper with a minimum movement of the zones is achieved by application of hot water through the sprinkler.

In cases where ultraviolet absorbing substances are under investigation, observation without interruption of the migration may be carried out in the darkened room by means of ultraviolet radiation applied through the 96% silica glass sliding panel in the top plate.

OPERATION

It is essential for the proper performance of the cooling system that the bottom surface of the supporting glass plate be free from grease. This can be assured by cleaning with alcoholic potassium hydroxide.

The top surface of the supporting plate is coated with a hydrophobic silicone film by rubbing with a Desicote soaked tissue (Beckman Instruments, Inc.). The excess is removed with benzene and any residual hydrochloric acid produced by hydrolysis of the chlorosilanes is rinsed off with water. The edges of the buffer vessels are coated with a bead of silicone stopcock grease and the glass plate placed between them with about $^1/_8$-inch overhang on each end. The grease prevents water leakage into the vessels and also prevents current leakage out of them.

The edges of the buffer vessels are made level, using a spirits level and the adjustable screw feet on the vessels. The sag in the plate is prevented by means of the adjustable center support and the plate leveling completed by means of the adjustable foot on the mounting table.

A siphon is used to equalize the liquid level in the buffer vessels

so that no hydrostatic flow occurs. Leakage of current has been detected when the siphon is left in place and merely clamped off. Therefore the siphon is removed after the initial liquid level adjustment.

The filter paper is cut with a single edge razor blade on a sheet of glass to produce as uniform an edge as possible, since structural variations in the paper affect the flow of liquid due to electroosmosis. Marking of the paper with pencil, as is the usual practice in paper chromatography (2), is not permissible, as the graphite particles act as secondary electrodes and hence cause pH variation as well as oxidation and reduction.

The paper is wet with electrolyte by drawing it rapidly through the liquid in one of the buffer vessels and withdrawing it against the end of the glass plate to aid drainage of excess liquid. Rubber gloves should be used to ensure that no contamination occurs. As rapidly as possible, the fully wet paper is laid on the support plate, starting at the center and taking care to prevent the trapping of small air bubbles between the paper and the glass. Any bubbles may later develop into hot spots and hence should be removed by sliding the sheet around on the plate or by starting over again. It is best to have the paper excessively wet rather than too dry, as drainage is faster than inflow and bubbles are easier to eliminate.

After the paper is centered on the plate, polyethylene cover sheets are placed on the ends of the paper so as to extend about
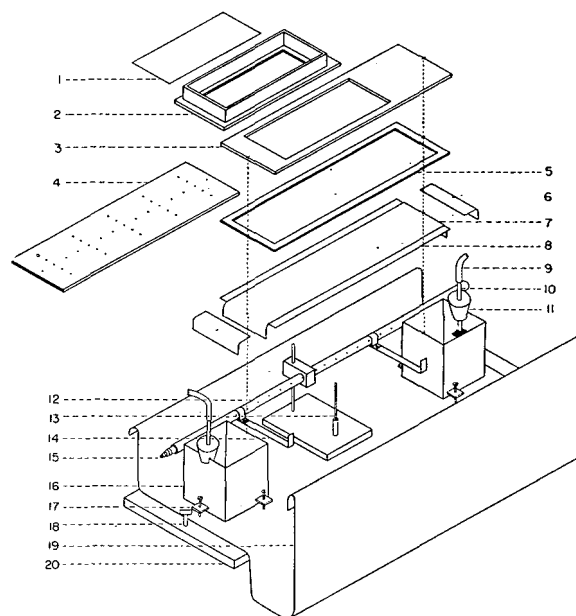
Figure 1. Isometric drawing of apparatus for zone electrophoresis

Scale. 1 cm. of drawing = 15.0 cm. of completed apparatus
1. 96% silica glass viewing panel ground to 1-mm. thickness and polished
2. Lucite mounting box for panel with silicone grease seal
3. Lucite top plate with opening for panel mounting box
4. Alternative top plate with ports covered by greased polyethylene film
5. Spacer strip either glued or held to top plate with grease
6. Polyethylene film to cover paper ends
7. Filter paper sheet
8. Glass plate cut from $^1/_{16}$-inch picture frame glass
9. Power supply lead makes contact through mercury
10. Glass tube with platinum wire sealed in bottom to mount a piece of platinum gauze. Tube is filled with mercury
11. Slotted rubber stopper
12. Sprinkler
13. Adjustable center support for glass plate
14. Sheet metal retainer to prevent force of sprinkler system from shifting glass plate
15. Ring stand
16. Buffer vessel
17. Flange for three-point suspension
18. Adjustable foot for leveling
19. Polyethylene film for collection of cooling water
20. Mounting board

1 inch into the cooled region of the paper, and then the top plate is added and clamped in place with ordinary screw clamps.

In order to allow equilibration of the liquid content of the paper, the sprinkler is turned on and the voltage to be used is applied for from 30 to 60 minutes. A sheet of plastic film serves to collect the sprinkler water and prevent excessive splashing as well as to divert the water down the center well drain. No part of the apparatus is touched while the voltage is applied.

After the equilibration period, the voltage is turned off and the samples are added in from 1- to 5-μl. portions from Kirk-type capillary pipets which may be inserted through ports in the top plate. The starting position is marked by means of an indentation made in the paper with a sharp point.

At any time the progress of ultraviolet absorbing samples may be observed by darkening the room and illuminating the zones with ultraviolet radiation from a mineral light (Ultraviolet Products, Inc.). A yellow filter held in front of the eye eliminates disturbing reflections of the visible output of the lamp. This procedure is used whenever materials of unknown mobility are applied and whenever positions before drying are to be noted.

When the migration is completed, the power is turned off, the sprinkler system turned off, and the cold water drained from it. The ends of the paper are torn off to prevent the inflow of liquid which would occur because of evaporation after the top plate is removed. The top plate is removed and the paper is further cut off 1 inch back from the ends of the supporting plate, since this portion of the paper is not dried as fast as the remainder and any inequality in drying rate causes zone movement. Finally, hot water is turned into the sprinkler. The paper dries in about 10 minutes and is ready for detection of the zones.

Ultraviolet-absorbing substances are detected either by the direct visual method of Holiday and Johnson (7) or by the ultraviolet radiation contact printing method of Markham and Smith (12), modified by affording better contact between papers by means of a nylon net stretched over a hemicylindrical surface and by exposure with an 8-watt germicidal lamp (General Electric Co.). Proteins have been detected by means of the aqueous bromophenol blue method of Kunkel and Tiselius (9).

EVALUATION

In order to evaluate the precision and performance of the apparatus in terms of the constancy of the distribution of voltage in the paper and the control and measurement of the flow of liquid in the paper, studies of each requisite were undertaken.

The first requisite was tested by direct measurement of the voltage distribution. For this purpose a vacuum tube voltmeter was equipped with probes. The probes were inserted through ports at various intervals in the top plate. Measurements that were taken at various points immediately after placing the wet paper in the apparatus and applying the voltage showed a nonuniform field strength throughout the paper. The nonuniformity of field strength gradually disappeared during equilibration until a period of 30 to 45 minutes, wherein the field strength became constant. The period of 30 to 45 minutes applies only to the use of buffers from pH 3.0 to 9.0. A longer period is required for buffers of pH greater than 9.0.

Voltage measurements that are taken within 5 cm. of the anode side of the paper show a reduction under those in the remainder of the paper. This reduction increases with time but can be partially eliminated if polyethylene film is laid in place to prevent evaporation at the ends of the filter paper. Complete elimination of the occurrence has not been found necessary, as no more than 5 cm. of the total 40 cm. of paper develop the nonuniformity. Similar observations have been made by Slater and Kunkel (13).

The precision with which the flow of liquid in the paper could be controlled and measured was determined by studies of the mobility of caffeine and of picrate ion. The pK₁ of caffeine is 0.15 (6); hence, a net charge of zero above pH 3 is expected. The mobility of caffeine, then, should represent the rate of electroosmosis through the paper. The pK of picric acid is 0.8 (6). At any pH value greater than 3, picrate ion has one net charge and the mobility should be independent of the pH. Possible interaction of picrate ions with buffer ions as well as variations in electroosmotic flow which are known to be dependent upon the species of cation (1) were eliminated as variable factors by the use of only sodium ions with a total ionic strength of 0.10.

Table I. Mobilities of Picrate Ions at Different pH Values

pH	Buffer Ion	Picrate Ion[a] To anode	Caffeine[b] To cathode	Picrate Ion[c] To anode
		Sq. cm. per volt per second $\times 10^5$		
3.6	Formate	10.2	1.0	11.2
4.6	Acetate	10.1	1.7	11.8
6.8	Phosphate	8.3	2.6	10.9
9.2	Borate	7.5	4.2	11.7
9.9	Carbonate	8.9	3.4	12.3

[a] Referred to site of application as zero.
[b] Electroosmotic rate.
[c] Referred to caffeine as zero.

A solution which contained both picric acid and caffeine was prepared by the neutralization to pH 7.0 of a solution of picric acid saturated at room temperatures. This solution was finally saturated with caffeine. One-microliter samples of the solution were applied to the paper at various time intervals. After migration the final positions of the zone centers as detected by ultraviolet radiation were marked before drying. This latter precaution was taken because earlier work in this laboratory as well as certain reports in the literature (11) had shown movement of zones upon drying. Comparison of zone positions noted before drying the paper with those after drying showed that the hot water sprinkling system as introduced in this apparatus effectively eliminates the movement of zones during drying.

Mobilities were calculated from the slope of a graph of movement vs. time and the measured voltage distribution. Calculated mobilities were corrected for the difference in viscosity between the buffer and distilled water (14). The paper shrinks 1 to 2% upon drying. No correction for the factor was applied, as it is both small and consistent.

The mobility data are presented in Table I. The mean mobility of picrate ion referred to caffeine as zero mobility in buffers which range from pH 3.6 to 9.9 is 11.6 $\times 10^{-5}$ sq. cm. per volt per second in Whatman No. 1 filter paper with a variation coefficient $\left(\frac{\sigma}{av.} \times 100\right)$ of 4.3%. In 17 studies of the migration of picrate ions in seven different buffers with a range from pH 1 to pH 9.9 the average range from the mean movement at each pH value was ±3.6% for a migration time of 100 minutes at 18 volts per cm.

Caffeine is an easily detected substance which can be used as a reference for the estimation of the rate of electroosmotic flow of liquid through the paper.

The precision obtained is adequate for studies of the effects of the variables of solutions upon the mobility of a given substance. The comparison of substances of similar mobilities, as in qualitative analyses, is best conducted on one sheet of paper.

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Resolution of Ribonucleotides by Zone Electrophoresis

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The study reports the electrophoretic separation of the isomeric ribonucleotides by conditions which emphasize one or more of the following properties of the molecules: the net charge, the mass and geometry, and the formation of complexes. With the exceptions of the 2' and 3' isomers of guanylic acid and the 3' and 5' isomers of adenylic acid, the resolutions described have not been previously obtained by zone electrophoretic techniques. One advantage of the new method is the speed with which the separations may be achieved.

CHROMATOGRAPHIC and spectrophotometric procedures for the analysis of the mononucleotide composition of ribonucleic acids have received widespread attention since the initial work of Vischer and Chargaff (17). Effort has been expended upon conditions for the liberation of the various mononucleotides, their separation, and their quantitative determination. No one of the paper chromatographic solvents described in the literature has been reported to resolve the mononucleotides completely and subsequently permit quantitative estimation by ultraviolet spectrophotometry (3, 4, 10, 15).

At present three isomers of each of the four ribonucleotides are recognized. These isomers have been characterized as the 2', 3', or 5' phosphate esters of adenosine, guanosine, cytidine, or uridine. Their presence in a hydrolyzate of ribonucleic acids is dependent upon the hydrolytic conditions chosen. Separation is accomplished by ion exchange chromatography (6).

The present study reports the electrophoretic separation of the isomeric nucleotides by conditions which emphasize one or more of the following properties of the molecules: the net charge, the mass and geometry, and the formation of complexes.

METHOD

The apparatus and techniques have been described (8). The best conditions for a given resolution by electrophoresis have been selected by consideration of the net charges of the nucleotide molecules as calculated from published dissociation constants, inclusion of mass differences among the nucleotides as frictional differences, calculation of net charge and mass upon formation of complexes with boric acid, experimental verification of the resolutions under the chosen conditions, and incidental observation of unexpected resolutions during experiments which were designed for other purposes.

Before it is concluded that a resolution of two substances is possible, certain precautions must be observed: The mobilities must be compared in one and the same sheet of paper, and a mixture of the two substances must be included in a third channel of the paper.

RESULTS AND DISCUSSION

Separation of 3' Mononucleotides. The titration curves of the four ribonucleotides have been determined by Levene and coworkers (12, 13). These data are for the 3' isomers (6). It is evident from these curves that within the region of pH 3 to 4, the net charges on the nucleotides are sufficiently different to make possible complete separation in an electrical field due to the differences in the dissociation constants of the amino groups and the absence of an amino group in uridylic acid. Figure 1,A, shows the resolution which is obtained in 60 minutes with a field of 30 volts per cm. at pH 3.5 in formate buffer of 0.1 ionic

strength. This resolution has also been found by other workers (9, 16, 18, 19).

Separation of the four 3' mononucleotides occurs also in 90 minutes with a field of 30 volts per cm. at a pH of 9.2 in 0.1M sodium tetraborate buffer, as shown in Figure 1,B. This resolution is expected from the presence of enolic group dissociations in this pH region in uridylic and guanylic acids, the higher pK for the enol group of uridylic acid than for that of guanylic acid, as well as the lower mass of the uridylic acid, and the difference in mass between the cytidylic and adenylic acids.

Mixtures of the mononucleotides may be fractionated best according to the base component by the conditions at pH 3.5, as the percentage difference in mobilities is higher than at pH 9.2.

Separation of Each 5' Isomer from Corresponding 2' and 3' Isomers. The 5' phosphate ester of any ribose nucleoside possesses one characteristic by means of which it may be separated from the 2' and 3' esters of this same nucleoside: the presence of two adjacent hydroxyl groups in the ribose moiety. Complexes of boric acid with adjacent hydroxyl groups have been reviewed recently by Boeseken (2) and Zittle (20). The reaction generally is reported to occur in sodium tetraborate solution at pH 9.2. The complex is a stronger acid than boric acid itself (20), and hence it is strongly negative at pH 9.2 (20). The mobility of the 5' nucleotide in tetraborate buffer is expected to
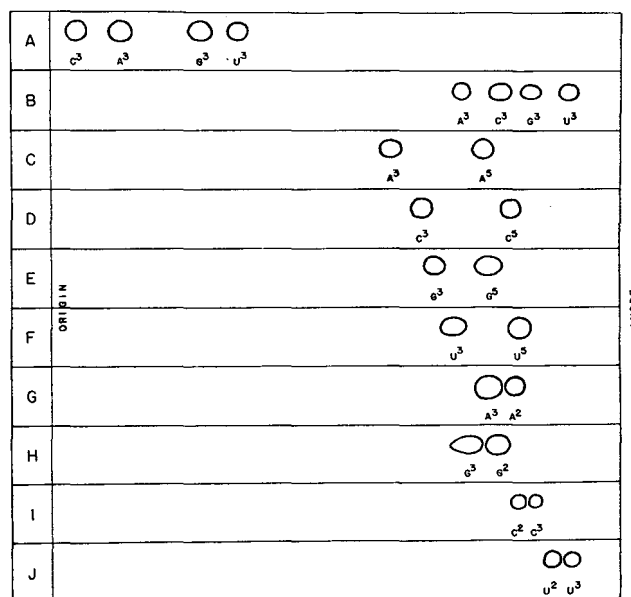
Figure 1. Resolutions of nucleotides

A, G, C, and U refer to adenylic, guanylic, cytidylic, and uridylic acids, respectively. Superscript refers to appropriate isomer. Length of paper shown is 28 cm.

A.   Formate buffer, pH 3.5, ionic strength 0.1, field 30 volts per cm., time 60 minutes

B.   0.1M sodium tetraborate solution, pH 9.2, field 30 volts per cm., time 93 minutes

C-F.   0.1M sodium tetraborate solution, pH 9.2, field 37 volts per cm., time 90 minutes

G.   0.1M sodium bicarbonate, pH 8.0, field 40 volts per cm., time 120 minutes

H.   Formate buffer, pH 3.8, ionic strength 0.1, field 38 volts per cm., time 240 minutes

I.   Phosphate buffer, pH 6.2, ionic strength 0.1, field 32 volts per cm., time 125 minutes

J.   Phosphate buffer, pH 5.8, ionic strength 0.1, field 37 volts per cm., time 148 minutes

be faster than that of the corresponding 2' and 3' nucleotides. Such resolutions were obtained in 90 minutes with a field of 37 volts per cm. at pH 9.2 in 0.1M sodium tetraborate buffer, as shown in Figure 1,C to F. Similar results for the adenylic acid isomers were obtained by Jaenicke and Vollbrechtshausen (11).

Separation of 2' Isomer from Corresponding 3' Isomer. The dissociation constants of the 2' and 3' isomers of adenylic acid and cytidylic acid differ appreciably (1, 5). Resolution of these isomers is expected to occur near the pH values where dissociations are half completed (7). Experiments showed that the 2' and 3' isomers of both pyrimidine nucleotides could be resolved in the region of pH 6 in 0.1 ionic strength phosphate buffer Figure 1,I and J.

It is likely that the separations occur because of net charge differences, as the cytidylic 3' isomer moves faster than the 2' isomer, in accord with the published secondary phosphate dissociation constants (5, 14).

The isomers of adenylic acid are not separable at pH 6. The 3' adenylic acid does not move faster than the 2' isomer at pH 6, as expected from the net charges which are calculated from the reported dissociation constants (1, 4). An explanation for the lack of separation was found accidentally, when it was observed that these isomers are resolved at a pH above 8, as in Figure 1,G. The secondary phosphate groups of both isomers are over 95% dissociated under these conditions and the faster movement by the 2' isomer must be evidence of a steric difference between the isomers which opposes the slight difference in net charge. The net charge difference at a pH below 8, which is expected from the pK values (1, 4), does not cause the 3' isomer to migrate faster than the 2' isomer, owing to the opposing steric effect evident in the difference in mobility above pH 8.

The 2'- and 3'-guanylic acids do not separate at pH 6, but resolution does occur in 240 minutes at 38 volts per cm. in the region of pH 3.8 in formate buffer of 0.1 ionic strength (Figure 1,H). This resolution has also been reported by Davidson and Smellie (9).

SUMMARY

The four 3' ribonucleotides are resolved by electrophoresis in paper in 90 minutes under a field of 30 volts per cm. in both formate buffer of pH 3.5 and ionic strength 0.1 and 0.1M sodium tetraborate solution. Each 5' isomer may be separated from the corresponding 2' and 3' isomers in 90 minutes with a field of 37 volts per cm. in 0.1M sodium tetraborate solution. The 2'- and 3'-cytidylic acids as well as the 2'- and 3'-uridylic acids may be resolved in 150 minutes under a field of 37 volts per cm. in phosphate buffer of pH 5.8 and ionic strength 0.1. The 2'- and 3'-adenylic acids are resolved in 120 minutes at 40 volts per cm. in 0.1 ionic strength buffers of pH greater than 8. The 2'- and 3'-guanylic acids are separated in 240 minutes at 38 volts per cm. in formate buffer of pH 3.8 and ionic strength 0.1.

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A High Rate of Shear Rotational Viscometer

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The assumption that viscosity is independent of shear rate is implicit in conventional viscometry, but is not valid for plastic materials nor for many fluids which are becoming increasingly important in lubrication and other fields. These materials should be studied over a wide range of shear rates. This paper presents information on the design, construction, and typical operation of a rotational viscometer for operation at shear rates up to about 1,000,000 reciprocal seconds. A simple and novel method was devised to deal with the heat generated at high rates of shear in the fluid film. The paper presents calibration data on the viscometer; some indications of the adequacy of the methods for handling the heating within the film; and sample data for several fluids, including two fluids that had been tested by another investigator; and suggests one possible simplification in describing or measuring the viscosity behavior of polymer-thickened oils.

VISCOSITY is one of the important properties that must be dealt with in many liquid flow problems, particularly in bearing lubrication, where viscous forces determine both the load-carrying capacity and the friction of the bearing. Precise methods have been developed for measuring the viscosity of liquids. These methods generally have been developed around Newton's law of viscosity and his assumption that viscosity is independent of the rate of shear. The most widely used method comprises flow through a capillary (1, 2); rotational viscometers (3, 10, 12) are used much less frequently. Ordinarily, viscosity measurements are made at relatively low rates of shear, which vary across the capillary, with respect to time during a determination, and from sample to sample depending upon the viscosity and density of the liquid being tested.

Most pure liquids and mineral lubricating oils are Newtonian in the sense that their viscosity is independent of rate of shear. Many industrially important materials such as blends of mineral oils with polymers, paints, inks, and greases are non-Newtonian

in the sense that they exhibit a viscosity that does depend on rate of shear. For these materials it is desirable to be able to measure viscosity as a function of rate of shear up to shear values that prevail in the machine elements where the materials are used. Rates of shear up to and beyond 1,000,000 reciprocal seconds are not uncommon in lubricated machine elements, and this number was chosen as a reasonable design goal for the instrument to be described.

The measurement of viscosity at high rates of shear has been the subject of a number of investigations (5, 7, 9, 11, 15), and the making of satisfactory measurements is known to present several formidable problems. Regardless of the type of fluid or instrument, the temperature rise due to the shearing results in considerable uncertainty of the actual temperature of the fluid and inadequate handling of this factor will lead to erroneous results. In capillary instruments the pressure on the fluid to produce very high rates of shear becomes very high and may itself affect the viscosity. Also, in capillary instruments the velocity distribution becomes quite uncertain, particularly with plastic materials where "plug flow" may take place.

DESIGN CONSIDERATIONS

A consideration of the problems of high rate of shear viscometry led to a decision to concentrate on the design and development of a rotating cylinder type of viscometer. The test material is contained in the annular space between two concentric cylinders; the inner cylinder is rotated, the resultant shearing forces in the liquid film tend to turn the outer cylinder, and the force required to restrain it from turning is a direct measure of viscosity. The greatest difficulties of this type of instrument, when operated at high rates of shear, are the uncertainties of the temperature of the film and of the film thickness. This is largely due to the normal outward dissipation of the frictional heat which will cause the inner cylinder to be hotter than the outer one. An investigation of these problems led to design details that appeared to minimize these difficulties to such an extent that they should not invalidate viscosity measurements.

It can be shown that, for any given rate of shear, the temperature gradient in the oil film of such a viscometer is proportional to the square of the film thickness:

$$\theta = \frac{\mu R^2 h^2}{2k}$$

where θ = film temperature gradient
  μ = absolute viscosity
  R = rate of shear
  h = film thickness
  k = thermal conductivity

all of which must be of a consistent set of units. Hersey (8) discusses film temperature relationships and expresses them in terms of linear velocity which equals shear rate times film thickness.

Derivation of this equation assumes that the inner member of the viscometer is a heat barrier, that the heat flow is radial, and that the viscosity and thermal conductivity of the film can be expressed by mean values. Calculations of temperature gradients for such an oil film of SAE-10 grade motor oil at a temperature of 100° F. and a rate of shear of 1,000,000 sec.$^{-1}$ are given below:

Film Thickness, In.	Film Temperature Gradient, ° F.
0.0005	32.4
0.0001	1.29
0.00005	0.324

It is clear from the foregoing that the uncertainty due to the temperature gradient within the film can be reduced to minor proportions if high rate of shear is attained by very thin films at low rotative speeds rather than by thick films at high rotative speeds.
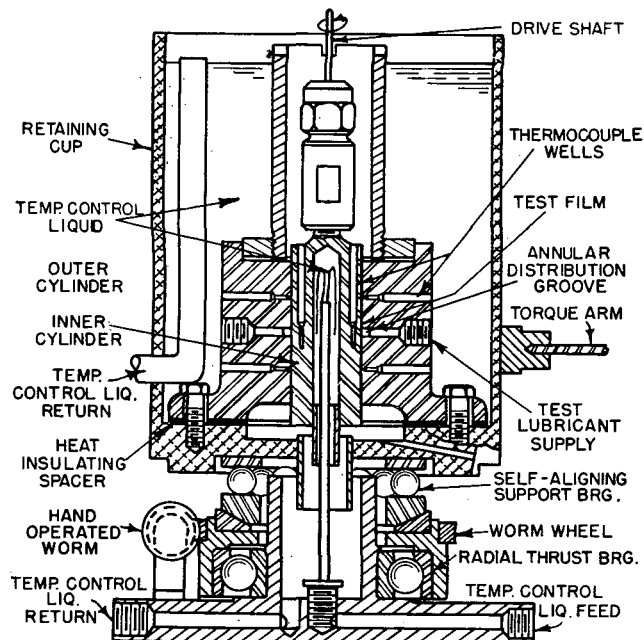
Figure 1.   Section through high rate of shear rotational viscometer

In previous rotational viscometers known to the authors one film boundary has a restricted path for conduction of heat from the liquid film. In the typical case where the inner cylinder loses heat generated in the film only from the ends that protrude into the atmosphere, the film boundary of the inner cylinder runs at a higher temperature than the outer cylinder, and its temperature distribution is unsymmetrical in the axial direction. These conditions contribute to the temperature gradient across the film and, because of differential expansion, make for uncertainty of the clearance between the two cylinders. Blok (4) discusses this problem at some length and cites the mathematical study by Kingsbury (9), Bratt and Duncan (6), Nahme (13), and Hagg (7).

The authors' investigation suggested the following solution to the problem of heat flow distribution. The inner cylinder is bored, and the bore diameter is selected so that the heat path from the film surface to the bore surface of the inner cylinder is equal to the heat path from the film surface to the outer surface of the outer cylinder; the bore of the inner cylinder and the outer surface of the outer cylinder are maintained at the same temperature by being placed in contact with a temperature-control liquid; axial heat flow is minimized by appropriate heat barriers at the ends of the cylinders. Under these conditions the film temperature gradient is reduced to one fourth of the values given previously; differential expansion is nil, provided that both cylinders are of the same material and the axial temperature gradient can be made negligible.

CONSTRUCTION OF APPARATUS

Figure 1 is a section view of the viscometer that has been constructed. The design features are generally evident.

The inner, or rotating, cylinder is bored out and the same temperature control liquid that fills the retaining cup and surrounds the outer cylinder is supplied into the bore.
Axial heat flow is minimized.
The outer cylinder and retaining cup assembly is mounted on self-aligning ball bearings. The shearing force is measured by the torque required to restrain this assembly from rotating. The hand operated worm is used to cancel out the static friction of the support bearings. Torques measured while rotating the worm first in one direction and then in the other direction are shearing torque plus and shearing torque minus support bearing

friction. This cancellation of support bearing friction is significant only for the lowest torques.

The test material is supplied into a groove which extends clear around the circumference of the outer cylinder. It is important to ensure a continuous film. As a preliminary to a test, the test material is supplied into the groove under some pressure, and the inner cylinder is rotated; the pressure is maintained until considerable leakage free of air bubbles occurs from the film. A continuous film is then assumed to exist and the pressure is reduced to a small gravity head for the test work.

The surfaces that form the film are accurately lapped cylinders having a nominal diameter of 1 inch and a nominal axial length of 2 inches. Four inner cylinders are available to produce nominal film thicknesses of 0.00050, 0.00020, 0.00010, and 0.00005 inch. With these clearances and the other design features, it is possible to attain at least 100,000, 250,000, 500,000, and 1,000,000 reciprocal seconds shear rates, respectively, with negligible film temperature gradients. Figure 2 shows a pair of the cylinders. The quality and accuracy of the cylinders are a feature of the apparatus that warrants special mention. They were manufactured by the Acme Scientific Co., Chicago, Ill.

Thermocouple holes are located so that the axial, circumferential, and radial temperature distribution of the cylinders can be determined. The thermocouples are embedded in the bottoms of these holes with copper dental cement. Originally it had been planned to utilize all or most of these couples to deduce a mean film temperature. Experience thus far indicates that it is sufficient to treat the average reading of the couples located approximately $1/16$ inch from the film surface as the film temperature.
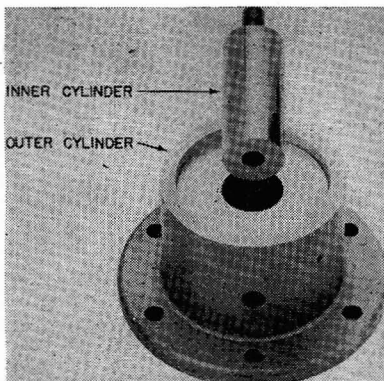
Figure 2. Pair of test cylinders
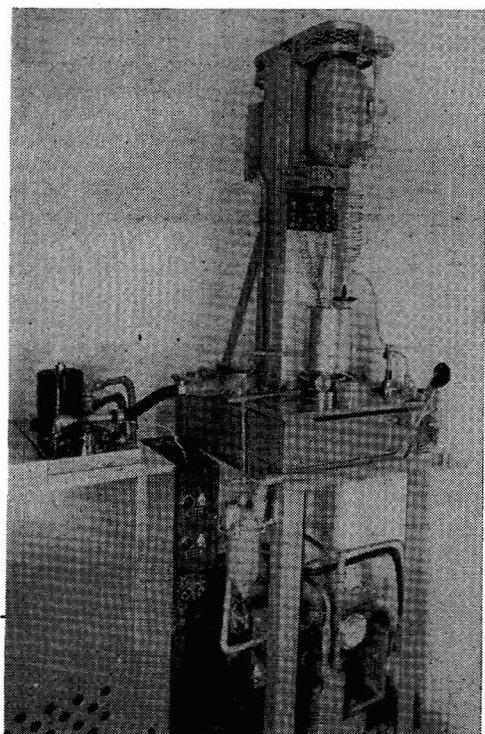
Figure 3. High rate of shear rotational viscometer

Figure 3 is a photograph of the viscometer assembly together with some of the auxiliary apparatus.

The retaining cup is the only part of the viscometer proper that can be seen. A variable-speed hydraulic transmission is mounted directly above the inner cylinder of the viscometer; this unit drives the inner cylinder through a long flexible drive rod which is held at each end in collet chucks. The hydraulic transmission is belt-driven from an 1150 r.p.m. motor, and the hydraulic transmission has an output ranging from 0 to ±100% of its input speed with good speed stability at any setting. Speed of the transmission is measured by an accurate electrical tachometer which does not appear in the photograph.

Thermocouple leads can be seen coming from the outer cylinder; readings of these couples are obtained by an expanded-scale electronic-type indicating potentiometer.

Just in front of the drive rod is a flexible coil of tubing which connects to the supply groove of the outer cylinder of the viscometer.

The cabinet at the lower left of the photograph supplies the temperature control liquid to the retaining cup and to the bore of the inner cylinder. Temperature control liquid can be supplied at any desired temperature from −20° to 250° F.

In operation of the viscometer, equilibrium thermal conditions are established at each test point; in practice a period of approximately 25 minutes is allowed. Owing to the heat generated in the film, a temperature differential exists between the temperature control liquid and the film. Either of two procedures can be followed: The temperature of the control liquid can be adjusted to produce a selected constant value of the film temperature, or the temperature of the control liquid can be held at a constant value and the viscosity can be related to the measured film temperature. The latter of these procedures has generally been employed.

CALIBRATION (NEWTONIAN FLUIDS)

For the instrument as constructed, viscosity is given by:

$$\mu = \frac{Th}{0.154N}$$

where μ = viscosity in pound seconds per square inch (reyns)
  T = torque in inch-pounds
  h = film thickness in inches
  N = revolutions per minute

By making tests with Newtonian fluids of known viscosity, it is possible to calculate an effective clearance for each set of inner and outer cylinders and this value serves as a calibration constant of the instrument. Systematic calibration measurements have been made at high and low rates of shear using high and low viscosity index Newtonian liquids at several levels of temperature. Sample results of these tests are illustrated in Table I. For each combination of cylinders the constancy of the effective clearance with rate of shear, viscosity index, and temperature level constitutes good evidence of the generally

Table I. Sample Calibration Results

Calibration Oil	Nominal Radial[a] Clearance, In.	Measured Film Temperature, ° F.	Calculated Rate of Shear, Sec.⁻¹	Calculated Film Thickness, In.
Calculated Film Thickness for Several Rates of Shear				
97 V.I.-Oil	0.00020	142.0	45,700	0.000235
97 V.I.-Oil	0.00020	143.0	157,000	0.000234
97 V.I.-Oil	0.00020	148.0	196,000	0.000237
97 V.I.-Oil	0.00020	149.0	243,000	0.000237
Calculated Film Thickness at Several Test Temperatures with High and Low Viscosity Index Oils				
97 V.I.-Oil	0.00010	130.0	430,000	0.000122
97 V.I.-Oil	0.00010	145.2	412,000	0.000127
97 V.I.-Oil	0.00010	199.5	417.000	0.000126
11 V.I.-Oil	0.00010	134.5	427.000	0.000123
11 V.I.-Oil	0.00010	147.0	407,000	0.000128
11 V.I.-Oil	0.00010	210.0	417,000	0.000126

Comparison of Nominal and Effective Film Thicknesses

Nominal Radial[a] Clearances, In.	Effective Film Thickness, In.
0.00050	0.000528
0.00020	0.000234
0.00010	0.000124

[a] Specification for manufacture of cylinders, not actual measurement on cylinders.

satisfactory operation of the apparatus and of the fact that the film temperature problem is under control.

COMPARISON WITH PUBLISHED DATA

In a recent paper (14) Needs presented comparative data on the viscosity of four lubricants, two Newtonian and two non-Newtonian. Samples of the two non-Newtonian lubricants (API-103 and API-104) were obtained by the authors and viscosity determinations were made by the present apparatus and technique for comparison with Needs' results. The comparative results are shown by Figure 4.
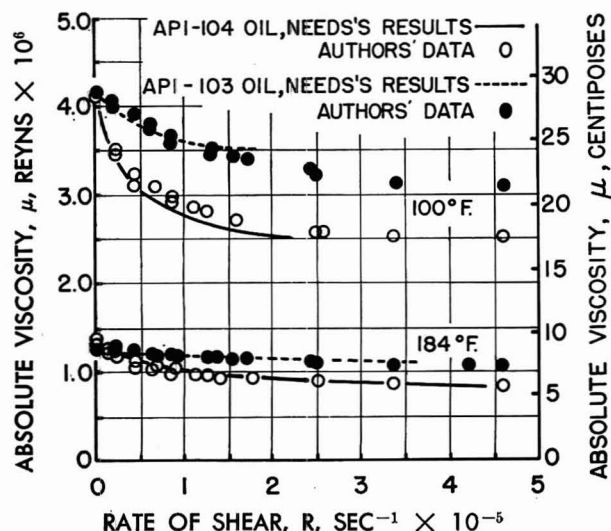
Figure 4. Comparison of high rate of shear rotational viscometer data with results of Needs (14)

In the method of test used in the present work, surface tension forces are sufficiently great so that under the gravity head on the film supply groove no detectable end leakage occurs. Thus it is thought that the same element of test liquid undergoes continuous shearing during the entire test. Furthermore, the practice is followed of starting a test at a low rate of shear, of taking points at progressively higher rates of shear to the maximum for the test cylinders, and then of taking points at successively lower shear rates so that the test is terminated at a low rate of shear near the initial one. A systematic variation between points obtained upon increasing and decreasing rates of shear would be evidence that the shear history to which the film has been subjected has produced a permanent change in viscosity; conversely, agreement between the increasing and decreasing rate of shear points is evidence that no permanent loss has occurred. In the work reported in this paper no permanent viscosity change could be detected for any of the test oils. Needs also stated that he did not detect any permanent loss of viscosity with shearing action for the two API oils.

There appears to be good agreement between these two investigations which employed different apparatus and techniques of operation.

TYPICAL TEMPERATURE, VISCOSITY, AND RATE OF SHEAR PATTERNS

Viscosities of several non-Newtonian lubricants have been determined over a range of temperatures and rates of shear. The typical pattern of these results is of considerable interest and the patterns exhibit several features which, if they turn out to be the common pattern of such fluids, will minimize the amount of
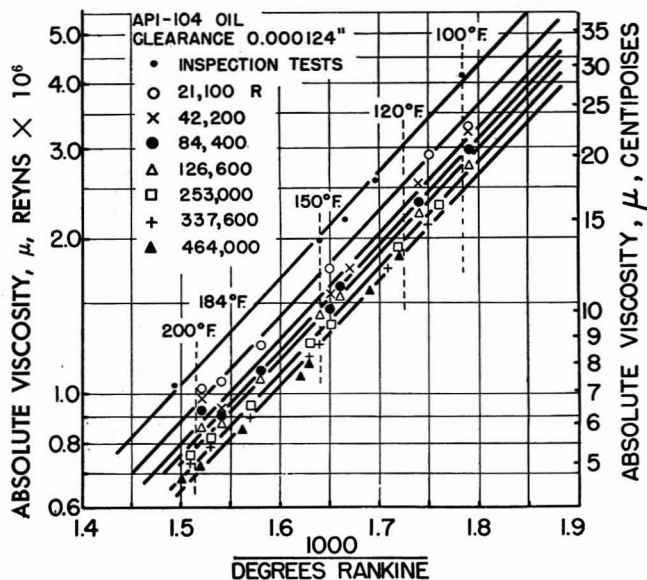
Figure 5. Typical observed data with high rate of shear rotational viscometer

data required to specify the shear behavior of non-Newtonian fluids.

Figure 5 is the typical pattern of measured viscosity vs. temperature at a range of rates of shear. On the coordinates, log $m\mu$ vs. the reciprocal of absolute temperature, the viscosity data for each rate of shear plot as a straight line, the viscosity level decreasing as the rate of shear increases.

From inspection of Figure 5 it appears that the percentage loss in viscosity with increasing rate of shear is independent of temperature, since the data can be fitted with parallel lines for constant rates of shear.

Figure 6 shows plots of the viscosity–rate of shear coefficient against rate of shear for six non-Newtonian oils which were tested. Viscosity–rate of shear coefficient is the ratio of the high rate of shear viscosity to the capillary viscosity at the same temperature. A two-parameter exponential or hyperbolic relationship appears to fit these λ vs. R curves reasonably well, suggesting that the entire rate of shear behavior of such oils may be described by the values of two parameters.
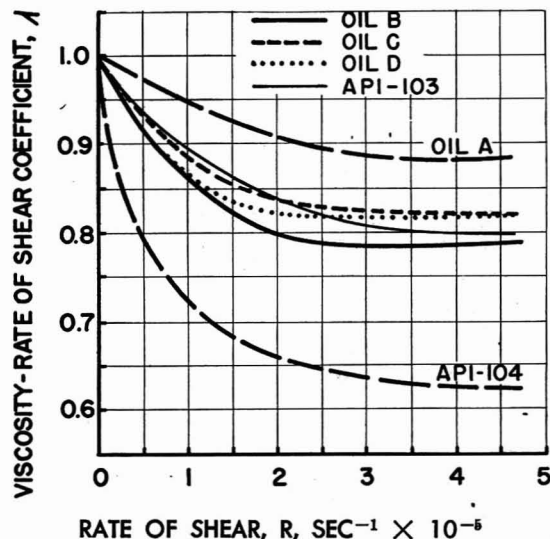
Figure 6. Viscosity–rate of shear coefficients for six polymer–mineral oil lubricants

Table II. Identification of Six Test Lubricants

	Tests on Base Oil				Tests on Blend		
Name	Gravity, ° API	Vis. at 100° F., cs	V.I.	Polymer Additive	Gravity, ° API	Vis. at 100° F., cs	V.I.
API-103	29.4	8.75	60	1	28.0	33.1	168
API-104	29.4	8.75	60	2	28.9	33.3	172
Oil A	...	...	..	3	30.3	43.1	121
B	31.3	32.3	98	4	31.1	52.3	144
C	31.3	32.3	98	5	31.2	59.8	127
D	31.3	32.3	98	6	31.3	60.0	127

Table II identifies these oils by gravity, viscosity at 100° F., and viscosity index of both the base and finished oils.

SUMMARY AND DISCUSSION

A high rate of shear rotational viscometer has been developed for studying the shear behavior of non-Newtonian materials. Temperature effects due to high rates of shear have been satisfactorily controlled by employing thin films and making equal heat paths from the film through the outer and inner working cylinders. Operation of the instrument has covered the temperature range of 100 to 200° F. and the rate of shear range of 5000 to 460,000 sec.$^{-1}$ on oils comparable in viscosity range to 10 grade motor oils. Data are given for six mineral oil–polymer blends, two of which were investigated by Needs (14). Agreement is shown for the two investigations. The rate of shear effects (the maximum reduction in viscosity was 38%) were found to be reversible; that is, no permanent loss in viscosity resulted from prolonged and high rates of shear. The viscosity-rate of shear coefficient, λ_R, appears to be independent of temperature for these oils.

Preliminary experiments were also made with an inner cylinder having a nominal clearance of 0.00005 inch, with which it was possible to reach rates of shear up to 1,200,000 sec.$^{-1}$

The authors believe that the apparatus can be extended advantageously to a variety of investigations of non-Newtonian materials such as the study of greases, where the yield point can be determined and where the viscosity can be determined as a function of the "shear history."

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Analyzer-Recorder for Measuring Hydrogen Sulfide in Air

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An instrument for measuring and recording hydrogen sulfide in air in the concentration range of 0 to 100 p.p.m. has been developed for testing refinery atmospheres. The instrument is based upon the use of special film prepared by coating blank 16-mm. motion picture film with buffered lead acetate. The sample is pumped continuously through an exposure hood, where the hydrogen sulfide reacts with the film coating to form a stain. A light beam through the stained film falls on a photoelectric cell and generates an electric current proportional to the hydrogen sulfide concentration. This current operates a conventional electronic recording potentiometer. A warning alarm sounds automatically if the hydrogen sulfide exceeds 25 p.p.m.

HYDROGEN sulfide always present in petroleum refining operations causes concern because it is a deadly poison. In concentrations over 20 p.p.m., it is considered unsafe (6). Its obnoxious odor reveals it at low concentrations, but the human nose soon becomes insensitive to the odor. The safety-mindedness of the petroleum industry demands that such a hazard be continuously recognized. Hence, a sensitive instrumental method is needed for continuous detection and measurement of hydrogen sulfide, particularly in the range from 0 to 100 p.p.m.

None of the instruments on the market monitors continuously and specifically the presence of hydrogen sulfide in this concentration range, and none has wide use. Some instruments measure hydrogen sulfide together with other gases present in refinery atmospheres (1, 2). Another measures only hydrogen sulfide (5, 7), but it was designed for analysis of gaseous streams low in oxygen content and is not continuously recording. The American Iron and Steel Institute sampler intermittently detects hydrogen sulfide by staining of lead acetate impregnated on a filter-paper tape (4). Laboratory determination of light transmittance is required. Most refiners have had to rely on spot checks made with a hydrogen sulfide detector (3); these provided information only for the moment at which they were made.

An instrument has been developed for continuously measuring small amounts of hydrogen sulfide in air. It can be equipped to operate an alarm, either visual or audible, should the concentration exceed a predetermined limit within the range of the instrument. The new hydrogen sulfide analyzer-recorder is based upon the reaction of hydrogen sulfide with buffered lead acetate on a transparent moving film. In operation, the continuously moving film is moisture-conditioned and exposed to the air streams under test. Any hydrogen sulfide present stains the film coating; the stain density is proportional to the hydrogen sulfide concentration. A light beam through the stained film falls on a photoelectric cell and generates an electric current, which operates a recording potentiometer. The recorder reads directly in parts per million of hydrogen sulfide.

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DESIGN AND OPERATION

Figure 1 shows the complete instrument contained in an upright metal cabinet mounted on casters. An electronic recording potentiometer of 0- to 10-mv. range is located at the top. The analyzer section is just below the recorder. Valves and the two large rotameters on the panel below the analyzer control the sample-carrier air stream and a moist air stream for conditioning the film. The rotameter tube at the center and the valves on the lower panel are used in checking the calibration. A water saturator for the moist air stream and the sample pump are in the lower compartment, to which access is gained by removal of covers on the rear of the cabinet.

Figure 2 shows the essential parts of the sample system and the analyzer section. The former consists of a blower, which continuously forces a large volume (50 to 60 cubic feet per minute) of the test air from the source location to the instrument, and a variable-volume sample pump which forces an aliquot of the test air into the carrier air line. The sample pump is a specially designed constant-speed, positive-displacement pump built of corrosion-resistant materials. Throughput may be adjusted from zero to maximum as required to compensate for minor variations in film sensitivity. The sample-carrier air stream is controlled at a fixed flow rate for an instrument of given range. Acting as a diluent, it carries the sample through an H-type humidifier in the analyzer chamber. Wicking, partially submerged in water, furnishes a large evaporative surface to aid humidification. The sample then is introduced into the exposure hood, impinging on the film through a jet. The spent sample is exhausted from the hood by a fan and passes from the instrument to a vent with the aid of eductor action.

The analyzer chamber is airtight. It is held under a slight vacuum by means of the eductor in the sample line. Thus, unexposed film does not become contaminated from the area in which the instrument is operated. Constant temperature is maintained by an electrically controlled heater mounted on the heavy aluminum panel to which the various parts of the analyzer mechanism are attached. Uniform heat distribution thus is achieved. The moist air stream (2000 ml. per minute) to condition the film just prior to exposure is brought to constant humidity while passing through a bubbler-type water saturator mounted on the panel. Water levels are adjusted by leveling bottles on the rear of the panel. Access to them is gained by moving the panel outward on a pair of telescoping slides.

The manner in which film stain forms and its intensity is measured is shown in Figure 3. Unexposed film passes through the exposure hood at the rate of $3^3/_8$ inches per hour. Moist air issuing from a broad jet softens the film coating to aid gas diffusion and stain formation. The sample stream containing hydrogen sulfide impinges on the film through a flat rectangular compartment with the upper surface sealed by a thin section of glass. Reaction with the film coating occurs over an area $^3/_8$ inch square, with the spent gas discharged around the edges of the film and exhausted from the hood with the surplus moist air.

A constant-intensity light source is housed in the upper portion of the exposure hood. A lens system focuses the light beam upon the film in the area where the stain is formed. The intensity of
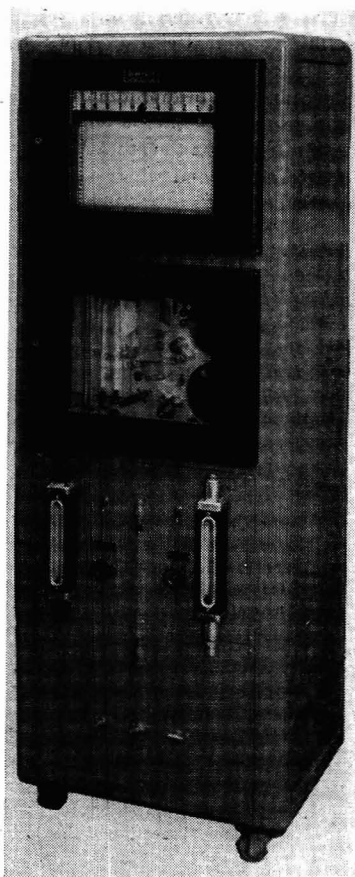
Figure 1. Hydrogen sulfide analyzer-recorder

the light beam transmitted by the film, inversely proportional to the hydrogen sulfide concentration, is picked up by the photoelectric cell just beneath the film. The electrical response from the cell is transmitted to the electronic recorder.

The film is the type used for 16-mm. sound motion pictures, but the silver is removed from the gelatin emulsion to make it completely transparent. It is prepared for the analyzer in equipment designed to apply a $^3/_8$-inch trace of sensitized lead acetate to the gelatin coating. The coating solution contains $1M$ lead acetate and $1M$ sodium acetate, maintained at a pH of 6.7. In the coating step, film passes over a trough containing the coating solution and a layer of reactant is taken up by the gelatin surface. The film is dried and rolled on reels, which are ready for installation in the analyzer. The coating, drying, and rolling operations are continuous. For storage, the film is hermetically sealed in plastic bags and kept below 70° F. The film coating is light-stable, an important factor for use in this instrument. Film aging at analyzer-chamber temperatures necessitates weekly changing of the film. A 50-foot reel lasts about a week.

CALIBRATION

Calibration of the instrument is based upon determination of light transmittance through film stains obtained with known concentrations of hydrogen sulfide in air. A standard mixture is prepared by transferring a measured portion of pure hydrogen sulfide into a stainless steel bomb holding about 1.25 cubic feet. The bomb is pressurized with dry air to dilute the hydrogen sulfide to the desired concentration. Loose strips of stainless steel sheet are placed in the bomb to mix the gas when shaken. Samples so prepared were found by chemical analysis to be stable for several
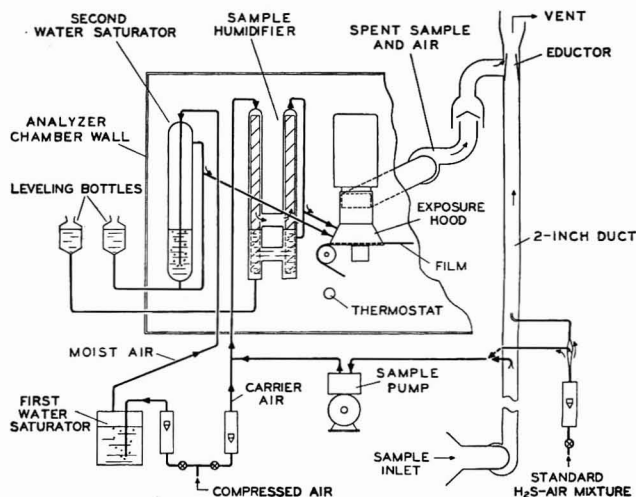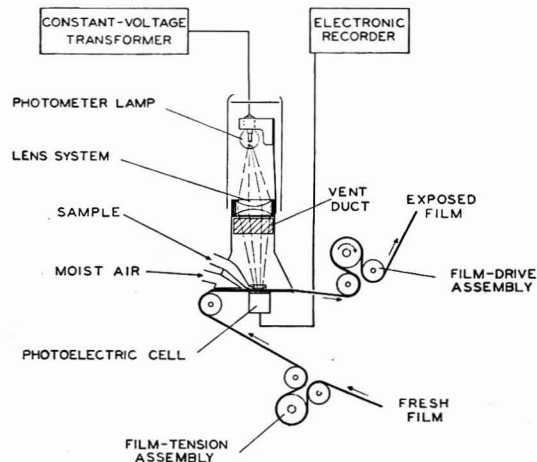

Figure 2. Essential parts of analyzer section

Figure 3. Means for film-stain formation and measurement

days. The bomb must be free from dust or finely divided rust be-cause such materials appear to promote decomposition of the hy-drogen sulfide.

In the calibration step, standard mixtures are fed to the sample pump, as indicated in Figure 2. The flow through the rotameter is adjusted within the range of 125 to 150 ml. per minute to pro-vide an excess over the 50 to 100 ml. per minute normally required by the pump. The sample is taken from the side arm of a special Pitot-tube T through which the excess of the mixture is being passed. Several known mixtures are passed through the instru-ment; the recorder positions obtained from the resulting film stains determine the instrument scale.

Typical flow rates are 2000 ml. of moist air, 275 ml. of carrier air, and 60 ml. of test sample per minute. After calibration, all tests are made with the same moist air and carrier air rates. The test sample rate may be varied to compensate for slight dif-ferences between rolls of film. This adjustment may be made by a single check with a standard mixture containing 25 p.p.m. of hydrogen sulfide; the throughput of the sample pump is varied until the recorder indicates 25 p.p.m. of the scale, other rates be-ing identical with those used in the original calibration.

Although the instrument has a scale limit of 100 p.p.m., it will
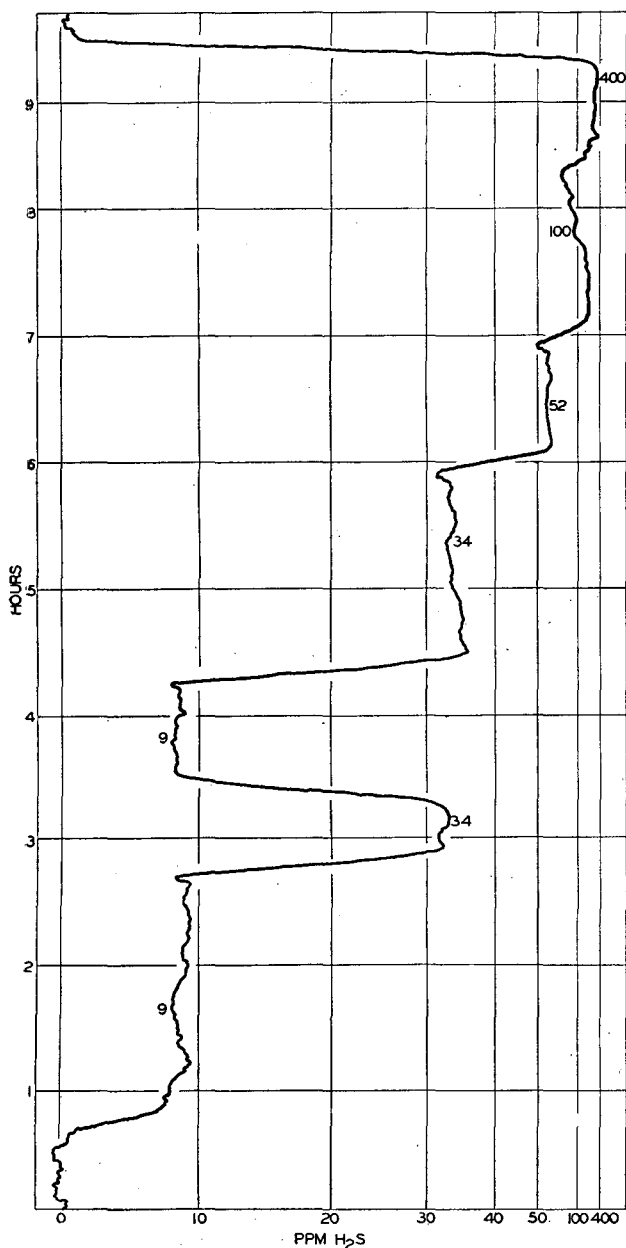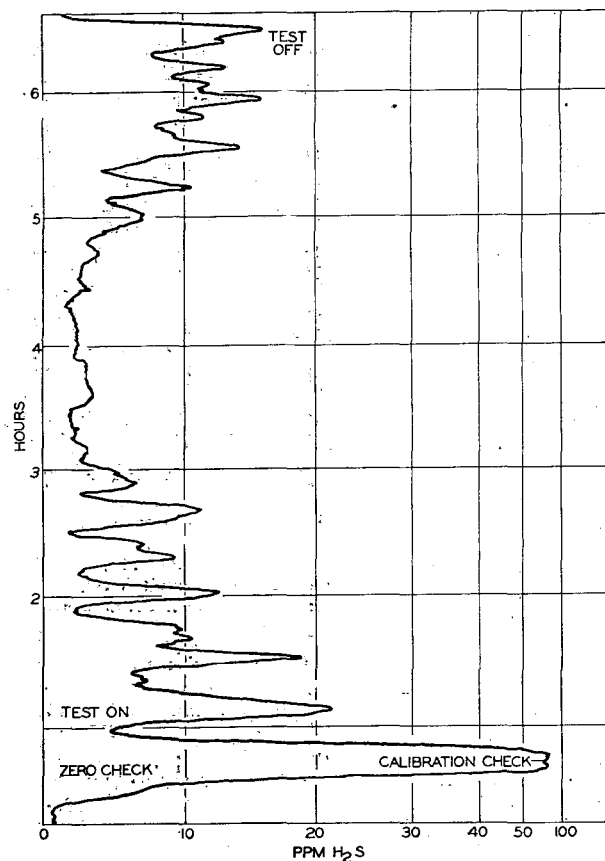
Figure 5. Typical field test record

detect up to 400 p.p.m. as limited by the sensitivity of the re-corder. Through control of the carrier air rate, concentrations of hydrogen sulfide as high as 2000 p.p.m. have been estimated.

PERFORMANCE

Figure 4 is a section of a typical recorder chart obtained from measurements of standard mixtures. It illustrates the precision of the instrument. In the range of 1 to about 25 p.p.m. of hydro-gen sulfide in air, the instrument is sensitive to a change of 1 p.p.m.; in the range of 25 to 50 p.p.m., to about 2 p.p.m.; and in the range of 50 to 100 p.p.m., to about 5 p.p.m.

The speed of response of the instrument is related to the volume of the sample system and to the time required for film stain to form and be picked up by the recorder. If the change in con-centration is from a low value to a high one, the recorder responds in 10 seconds. If the change is from high to low, the response is slower. With an increase in hydrogen sulfide from 0 to 400 p.p.m., the instrument will actuate the alarm set to operate at 25 p.p.m. in 40 seconds. The instrument requires several min-utes to level out at each new concentration.

The hydrogen sulfide analyzer-recorder has been used for con-tinuous tests in several locations about the refinery. In a typical location, the test air stream was brought 50 feet to the instrument from a pump room suspected of containing dangerous amounts of hydrogen sulfide. A section of the recorder chart obtained from this installation is shown in Figure 5. Hydrogen sulfide was present intermittently. At times the concentration was as high as 20 p.p.m., which is the maximum allowable concentration for prolonged exposure. The source of the gas was traced by a flexible hose to a valve that leaked intermittently.

The instrument has performed satisfactorily in hot, smoky, and dusty locations and is considered suitable for general plant use.

Figure 4. Response and precision

Routine service consists of daily inspection of instrument operation and addition of water to the saturator and humidifier; weekly changing of the film and inspection of the sample pump; and monthly changing of the recorder chart and lubrication of certain parts. Servicing the recorder normally requires an additional 15 minutes daily, an extra hour once a week, and an extra 2 hours once a month.

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Spot Reaction for Acidic Polynitro Compounds

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When enolizable nitro compounds react with rhodamine B, they give red-violet salts, whose red solutions in benzene fluoresce orange. This finding has been made the basis of a new, fairly sensitive spot reaction for the detection of enolizable polynitro compounds.

EGRIWE (*1*) discovered that the violet precipitates formed in strong hydrochloric acid solution by the amphoteric water-soluble dye rhodamine B with antimony(V), gold(III), and thallium(III) ions can be made the basis of sensitive tests for these metals. According to Kuznetsov (*7*), these reactions involve the production of salts of the dye, or its quinoidal zwitter ions, with the complex $[SbCl_6]^-$, $[AuCl_4]^-$, or $[TiCl_4]^-$ ions. The production of the antimony compound, whose formation may also be used for quantitative microdeterminations (*8, 9, 11*), can be represented as:
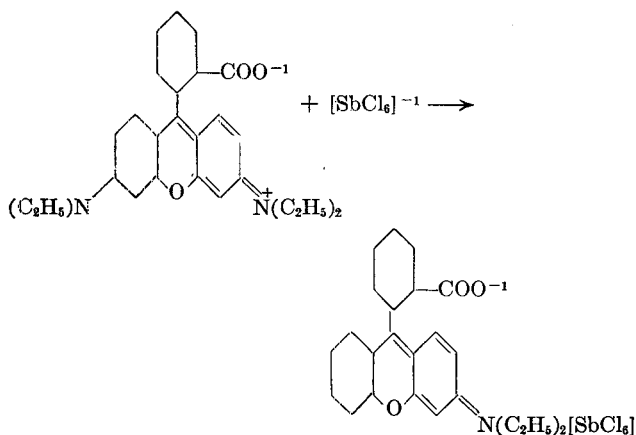
Analogous reaction schemes apply for the other two metal chloride ions and likewise for $[SbI_4]^-$ (*4*). Most of the water-insoluble salts of rhodamine B with metal-halogen acids dissolve in benzene (toluene) to give red-violet solutions (*5*), a finding that was first reported by Webster and Fairhall (*10*) in the case of the antimony salt. The benzene solutions of most of the rhodamine salts exhibit an orange-red fluorescence in ultraviolet light.

The behavior of rhodamine B toward organic acidic compounds with respect to the production of colored benzene-soluble salts has been studied in the present investigation. Enolizable nitro compounds are outstanding in this regard. When not too dilute solutions of such compounds are allowed to react with aqueous

neutral or mineral acid solutions of rhodamine B, violet precipitates appear in many cases. These products are soluble in benzene and the resulting red solutions display an intense orange-red fluorescence in ultraviolet light. The color and the fluorescence reaction can be observed at dilutions that are too slight to produce a visible precipitate. This behavior, which is completely analogous to that of complex metal halogen acids, is a strong indication that the reaction involves the formation of benzene-soluble salts of rhodamine B with the *aci-* form of the nitro compounds.

When other acid groups are absent, the acidic character of organic nitro compounds is due to the formation of the so-called nitroxy acids (*6*)—in other words, to the enolization of the NO_2 to the NO_2H group. In the case of aliphatic primary and secondary nitro compounds, there is an equilibrium between the tautomeric forms:

$$-CH_2-NO_2 \rightleftharpoons -CH=NO_2H$$

$$CH-NO_2 \rightleftharpoons C=NO_2H$$
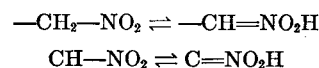
In the case of aromatic nitro compounds, the acidification results from the rearrangement into quinoidal compounds with development of NO_2H groups. For example, the following equilibria are established in the case of *p*-nitrophenol and hexanitrodiphenylamine, respectively:
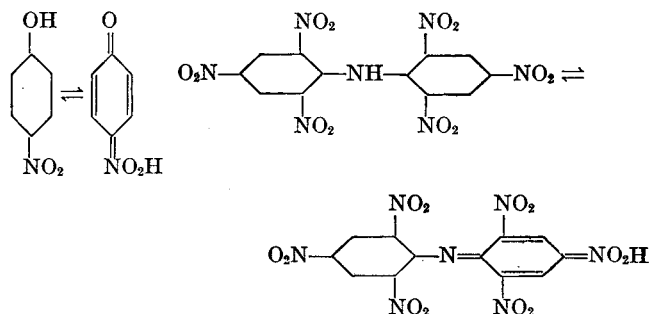
Enolizable aliphatic and aromatic nitro compounds give yellow solutions in caustic alkali because formation of the water-soluble alkali salt removes the *aci-* form from the equilibrium. This removal resulting from salt formation with rhodamine B can also occur in the absence of water. This is proved by the fact that the colorless solution of rhodamine B in benzene (toluene), which contains the *lacto-* form of the dyestuff, immediately turns red on the addition of nitro compounds which are able to produce nitroxy acids on enolization. This salt formation, beginning with the *lacto-* form, can be represented schematically as:
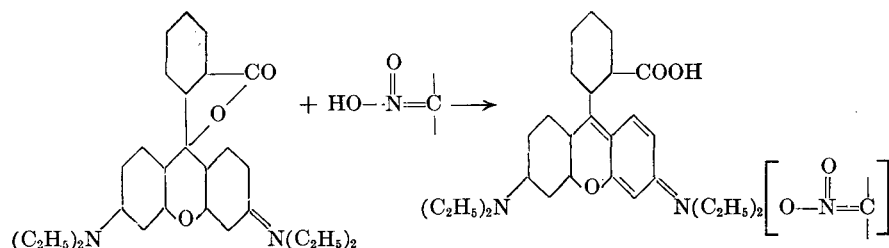
Because most nitro compounds are far more soluble in benzene than in water and the colorless *lacto-* form of the dyestuff can be extracted by benzene from the water solution of rhodamine B, it is probable that the reaction given above is likewise involved— i.e., the reaction theater is transferred to the benzene solution when neutral or acid suspensions of nitro compounds in water solutions of rhodamine B are shaken out with benzene. The possibility that the color and fluorescence reaction with rhodamine are due to the formation of molecular compounds with participation of NO_2 groups can be excluded, because nitro compounds which are not capable of an enolization do not react with rhodamine B. This was demonstrated by the negative results obtained with *o*-nitrophenol, *p*-nitrotoluene, *m*-nitroaniline, 6-nitroquinoline, nitroguanidine, *p*-nitrobromobenzene, *p*-nitroacetamide, *p*-nitromandelic acid, nitrobarbituric acid, *β*-nitroalizarin, and trinitrotoluene.

Very small amounts of many enolizable nitro compounds can be detected directly through the salt formation on treatment with a benzene solution of rhodamine B. However, this procedure is not reliable. Some phenols, mercapto compounds, carboxylic acids, and sulfonic acids give red solutions; others are tinted red when treated with a benzene solution of rhodamine B. Obviously, here again there is some production of a rhodamine B salt. Acidic compounds which are not soluble in benzene nevertheless produce benzene-insoluble salts with rhodamine B via topochemical surface reactions.

Solid monobasic fatty acids are not colored by benzene solutions of rhodamine B. The aliphatic dicarboxylic acids—oxalic, malonic, maleic—give a pronounced reaction, whereas succinic, glutaric, and adipic acids show no reaction. An intense color is obtained with *α*-hydroxycarboxylic acids such as tartaric and (anhydrous), mandelic acid and its derivatives, malic acid, and citric acid (including the hydrated variety). The following are colored irreversibly: 2,7-dihydroxynaphthalene, gallic acid, dimethylglyoxime, benzoinoxime, alizarin, nitroalizarin, 8-quinolinol 5-sulfonic acid, and barbituric acid. Powdered phenol resins are tinted red on contact with benzene solutions of rhodamine B. Frequently the tinted products give the same fluorescence color in ultraviolet light as the salts of rhodamine B.

Many inorganic compounds likewise yield red or red-violet colors when treated with a colorless benzene solution of rhodamine B. Instances of this behavior are: sulfates of the alkaline earths, anhydrous sulfates of copper, manganese, and cobalt, cuprous iodide, silver halides, and oxides of aluminum, zirconium, titanium, columbium, and tantalum. In these cases it is likely that there is an irreversible adsorption of the dye, whose *lacto-* form is thus rearranged into its corresponding quinoidal form. Perhaps the adsorptive binding of the dye molecule involves its trivalent nitrogen atom or the oxygen of the pyrone ring.

Furthermore, some enolizable nitro compounds do not react directly with rhodamine B dissolved in benzene, because the quantity of the *aci-* form in the tautomerism equilibrium is insufficient. The red solutions of rhodamine salts of *aci*-nitro compounds in benzene can be lightened in tone considerably by adding an equal volume of ether. This result is due solely to dilution; the red benzene solutions of rhodamine salts with phenols and the like are decolorized by this treatment. It may be assumed that the ether causes a splitting of the rhodamine B salts into their lactone colorless phenol components. The utilization of this finding together with the fact that alkaline solutions contain only ions of the *aci-* form of the nitro compounds, from which the respective nitroxy acids are liberated transiently on the

addition of an acid (*6*) makes possible a new test for *aci*-nitro compounds with rhodamine B as reagent.

In the procedure, it is necessary to bring together a weak alkaline solution or suspension of the test material and a mineral acid solution of rhodamine B and to extract the mixture at once with ether-benzene. A red color in the supernatant layer is characteristic of enolizable nitro compounds. The sensitivities attained are adequate for micro or semimicro tests, especially for enolizable nitro compounds. More than 80 acidic compounds of the most varied classes were subjected to this test in amounts from 5 to 10 mg. per ordinary drop. Only thio compounds, such as mercaptobenzothiazole and thioglycolic acid aminonaphthalide, show a behavior with this reagent analogous to that of acidic polynitro compounds. However, thio compounds are easily revealed by the iodine-azide test (*2*) and furthermore they are readily oxidized to noninterfering disulfides by evaporation with hydrogen peroxide, a treatment which leaves nitro compounds unchanged. Accordingly, the test with rhodamine B in the form given here can be regarded as characteristic for polynitro compounds (*3*).

EXPERIMENTAL

Reagent. A 0.1% solution of rhodamine B in 4% hydrochloric acid.

Procedure. One drop of the weakly alkaline test solution is placed in a micro test tube and 5 drops of the rhodamine B reagent solution is added. Five drops of 1 to 1 ether-benzene mixture is introduced and the system shaken vigorously. A red or pink color in the upper layer and an orange fluorescence in ultraviolet light signify the presence of enolizable polynitro compounds.

DISCUSSION RESULTS

The following mononitro compounds, which are capable of enolization, were tested with the colorless reagent solution: nitromethane, nitroethane, the three isomeric nitrophenols, *p*-nitroaniline, and 5-nitrosalicylic acid. With the exception of the latter compound, the color and fluorescence reaction were not evident with amounts below 2500 γ. *o*-Nitrophenol does not show any reaction below 0.5 gram. Furthermore, and again with exception of 5-nitrosalicylic acid, the stability of the red benzene solution toward ether is distinctly less than in the case of enolizable polynitro compounds. Consequently, it appears that nitro and carboxyl groups in enolizable nitro compounds exert a positive influence on the occurrence of the reaction with rhodamine B.

IDENTIFICATION LIMITS

0.25	γ of dipicrylamine (2,4,6,2′,4′,6′-hexanitrodiphenylamine)
0.5	γ of picric acid (2, 4, 6-trinitrophenol)
1	γ of picrolonic acid [3-methyl-4-nitro-1-(*p*-nitrophenyl)5-pyrazolone]
4	γ of 5-nitrosalicylic acid
10	γ of Martius yellow (alkali salt of 2,4-dinitrophenol)
10	γ of naphthol yellow (alkali salt of 2,4-dinitro-1-naphtholsulfonic acid)
500	γ of 2,4-dinitrophenol
2000	γ of *p* (*m*)-nitrophenol

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Colorimetric Estimation of Ultramicro Quantities of Calcium in Human Serum as the Complex with Alizarin

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A rapid colorimetric method suitable for the routine clinical laboratory is described for estimating calcium by extraction of the calcium alizarin complex in *n*-octyl alcohol. Practical or technical grades of triethanolamine are used to adjust to alkaline pH so as to avoid turbidity in the alcohol phase. Purer grades of triethanolamine are not satisfactory for this purpose. With inorganic alkali iron interference is noted but magnesium, barium, and strontium do not interfere. With organic bases iron does not interfere but interference is noted from magnesium, strontium, and barium. The procedure described for human serum subtracts a constant factor for magnesium interference which is of the order of 7%. The calcium may be precipitated as the oxalate and then determined colorimetrically as the calcium alizarinate in *n*-octyl alcohol.

THERE is a need in the clinical laboratory for a rapid, simple method for calcium estimation on fingertip blood. The method should analyze from 0.01 to 0.02 ml. of serum or 1 to 2 γ of calcium. In the search for such a method, a number of procedures which are in use for larger quantities of calcium were investigated.

Methods studied include analysis of precipitated calcium oxalate oxidimetrically *(2, 5, 33, 36, 38)*, acidimetrically *(34)*, colorimetrically *(37)*, and by ethylenediaminetetraacetic acid titration *(9, 11, 19)*. Precipitation as the molybdate *(16, 39)*, tungstate *(12, 18, 24, 27)*, phosphate *(25)*, and sulfate *(3)*, and direct titration with sodium ethylenediaminetetraacetic acid in the presence of Eriochrome Black T *(32)* or murexide *(7, 8, 10, 21–23, 28, 40)* were also studied. Colorimetric estimation with 1,8-dihydroxy-2-(3-chloro-6-hydroxybenzene azo) naphthalene-3,6-disulfonic acid *(4)* and calcium analysis by flame photometry *(13, 17, 30, 41)* were also investigated.

These methods were found unsuited to the purpose of the present work because of one or more undesirable features, such as the need for too large a sample, long or tedious procedure, or interference caused by icteric or even slightly hemolyzed serum or magnesium.

Alizarin and related compounds were then investigated, since these compounds form intensely colored complexes with calcium as evidenced by their use in staining bone *(6)*. Further, calcium had been assayed by precipitating the oxalate, dissolving in acid, and reprecipitating with alizarin and dissolving the precipitate to read the alizarin colorimetrically *(14)*. A method was finally devised which was suitable for analyzing 1 to 2 γ of calcium rapidly from serum.

METHOD

Reagents and Materials. SCREW-TOP TUBES (Kimble No. 45066, 16 × 125 mm.). The tubes have a 15-ml. capacity and the caps should be fitted with liners cut from Tygon sheeting. Tygon tubing (5-mm. bore) slit lengthwise may be flattened after gentle warming on the hot plate to obtain the sheeting. A No. 7 cork borer is used to cut disks to fit snugly inside the caps.

1.0*N* TRIETHANOLAMINE. Triethanolamine (141 ml. of 95% Eastman Kodak No. P 1599) is diluted to 1000 ml. with distilled water.

ALIZARIN REAGENT (4 mg. per 100 ml.). Alizarin (Eastman Kodak No. P 1599) is purified by heating 1 gram with 100 ml. of 95% alcohol, filtering while hot with suction, filtering off the crystals obtained on cooling, and washing with 25 ml. of cold alcohol. The recrystallized alizarin (40 mg.) is dissolved with warming and made up to 1 liter with *n*-octyl alcohol (Eastman Kodak No. P 871).

CALCIUM STANDARD (10 mg. per 100 ml.). Calcium carbonate (reagent grade) is dried overnight at 100° C.; 250 mg. is transferred to a 1000-ml. volumetric flask. Distilled water (100 ml.) and concentrated hydrochloric acid (1 ml.) are added and the mixture is shaken until solution is complete. The solution is diluted to 1000 ml.

Procedure. Into the screw-cap tubes is introduced 1.0 ml. of distilled water. A fresh serum sample (0.02 ml.) is washed into the water. Serum should be fresh, as calcium phosphate precipitates on standing *(1)*. Solutions of 2.0 ml. of 1.0*N* triethanolamine and 3.0 ml. of the alizarin-octanol are added from burets. The standard contains 0.02 ml. of the calcium standard solution washed into the 1.0 ml. of water from the same pipet. The blank comprises 1 ml. of water, 2 ml. of 1.0*N* triethanolamine, and 3 ml. of the alizarin solution. The tightly capped tubes are shaken for 20 minutes in the shaking machine. After removal from the shaker the tubes are centrifuged at 2000 r.p.m. for 5 minutes. Approximately 2 ml. of the upper layer are transferred to another tube and recentrifuged to ensure clarity. The color is read in the Klett-Summerson colorimeter with the 56 filter. For the Coleman spectrophotometer 2 ml. is diluted to 5 ml. with *n*-octyl alcohol and the color is read at 560 mμ on the optical density scale.

Calculations.

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times$$
$$10 = \text{mg./100 ml. (Ca + Mg interference)}$$

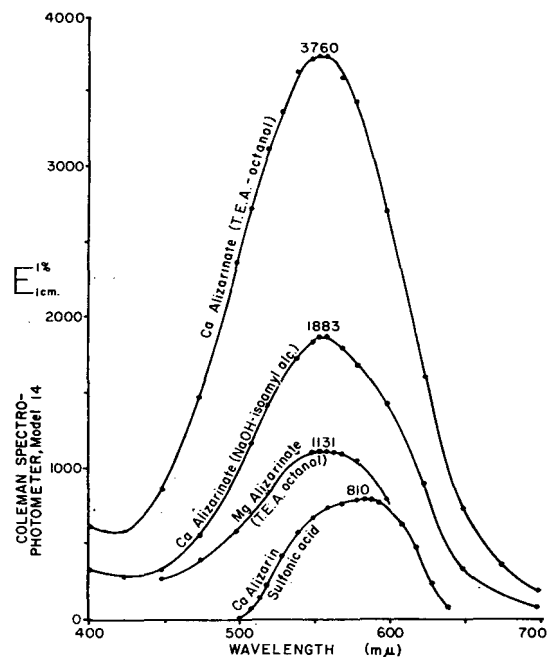$$\text{Mg./100 ml.} - 0.7 = \text{mg./100 ml. calcium}$$

Figure 1. Extinction coefficients of calcium and magnesium complexes with alizarin and alizarin sulfonic acid plotted against wave length. T.E.A. triethanolamine

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Table I. Comparison of Ultramicromethod and Method of Clark and Collip (5) for Human Serum

Ultramicro					Clark and Collip (5)				
Serum No.	No. of detns.	Mean, mg./100 ml.	$\Sigma x^2 - \frac{(\Sigma x)^2}{N}$	Corrected value (mean − 0.7)	Serum No.	No. of detns.	Mean, mg./100 ml.	$\Sigma x^2 - \frac{(\Sigma x)^2}{N}$	
1	2	10.73	0.0000	10.0	1	2	10.26	0.0040	
2	2	11.11	0.0450	10.4	2	2	10.10	0.0008	
3	2	10.11	0.0648	9.4	3	2	9.70	0.0068	
4	2	10.29	0.0000	9.6	4	2	9.86	0.0040	
5	2	10.82	0.0000	10.1	5	2	10.26	0.0364	
6	2	9.55	0.0000	8.9	6	2	8.92	0.0264	
7	2	10.44	0.0392	9.7	7	2	9.88	0.0032	
8	2	10.69	0.0018	10.0	8	2	10.13	0.0100	
9	2	10.22	0.0128	9.5	9	2	9.36	0.0242	
10	2	9.67	0.0024	9.0	10	2	9.59	0.0040	
11	4	9.81	0.1130	9.1	11	2	9.65	0.0012	
12	2	10.30	0.0924	9.6	12	2	10.15	0.0084	
13	2	10.33	0.0000	9.6	13	2	9.89	0.0040	
14	2	10.60	0.1405	9.9	14	2	9.89	0.0012	
15	2	10.40	0.0338	9.7	15	2	9.50	0.0008	
	Av.	10.31		9.63				9.81	
		Std. dev. ±0.18					Std. dev. ± 0.10		

The correction factor may be avoided by adding magnesium to the calcium standard (2 mg. of magnesium per 100 ml. or 204 mg. of magnesium sulfate heptahydrate per liter). This system (suggested by R. E. Mosher) has been found to have advantages for the routine laboratory.

ALIZARIN METHOD USING OXALATE PRECIPITATE

Serum (or urine) (0.2 ml.) is measured into a 3-ml. centrifuge tube with a mark at 2 ml. Saturated ammonium oxalate solution (0.2 ml.) is added and the well-mixed solution is allowed to incubate at 37° C. for 2 hours. After centrifuging at 2000 r.p.m. for 5 minutes the supernatant is aspirated. Then 0.3 ml. of 1.2% ammonium hydroxide (concentrated ammonium hydroxide, 58%, diluted 1 to 50) is added and the solution is mixed and centrifuged as before, aspirating the supernatant. The precipitate is redissolved in 0.2 ml. of 0.1N hydrochloric acid at 100° C. and diluted to the 2-ml. mark; 0.2 ml. is transferred to the culture tube, 0.8 ml. of distilled water is added, and one proceeds as described.

Calculations.

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 10 = \text{mg. Ca/100 ml.}$$

DISCUSSION

Alizarin sulfonic acid (alizarin red S), which was first investigated, was found unsatisfactory because of relatively low absorptivity (Figure 1), overlapping of its absorption spectrum with that of the calcium complex, magnesium interference, linearity of absorbance-concentration plot being maintained over a narrow range of concentration (Figure 2), and because pH had to be controlled critically (10.1 to 10.6). It was felt that greater specificity would be obtained if the complex could be extracted preferentially by an organic solvent. Because of the polar nature of the sulfonic acid group, the alizarin sulfonate complex could not be extracted by an organic solvent. Alizarin was therefore investigated for the same purpose.

It was found that calcium alizarin complex may be preferentially extracted from an alkaline (0.1 to 2.0N sodium or potassium hydroxide) solution of alizarin, leaving the excess alizarin in the aqueous phase. 1-Butanol, n-amyl, isoamyl, n-hexyl, n-heptyl, n-octyl, 2-methylcyclohexyl, and n-decyl alcohol would extract the calcium alizarin complex from the aqueous phase while diethyl ether, diisopropyl ether, di-n-butyl ether, 4-methyl-2-pentanone, 3-heptanone, chloroform, carbon tetrachloride, toluene, heptane, benzene, 4-methyl-2-pentanone, 2,4-dimethyl-3-pentanol, methyl n-hexyl carbinol, and caprylic alcohol were unsatisfactory. It is of interest that 4-methyl-2-pentanol and 4-methyl-2-pentanone were found to extract the iron alizarin complex but not the calcium complex. This preferential solubility of alizarin metal complexes in organic solvents may be useful for metal determinations other than calcium. From isomers of alizarin, 1,4-dihydroxyanthraquinone and 1,5-dihydroxy-

anthraquinone, a calcium complex was not extractable into the organic phase. 1,2,5,8-Tetrahydroxyanthraquinone has properties similar to alizarin but oxidizes in air when alkaline.

With potassium or sodium hydroxides, magnesium, barium, and strontium are not extractable, as the alizarin complexes, into the organic phase. Iron is extractable. However, because of persistent turbidity in the alcohol layer, the inorganic alkali was abandoned in favor of organic base. Triethanolamine (practical or commercial grade) as the base in the aqueous phase resulted in a clear alcoholic phase with high absorptivity (Figure 1). In this system iron was not extractable but magnesium, barium, and strontium could be extracted. The absorptivity of the magnesium alizarin complex is 0.3 times that for the calcium complex and magnesium is normally present in serum at 0.2 times the calcium concentration (32, 35). This should introduce an error of 6%. Table I is a study designed to determine the correction factor for magnesium experimentally.
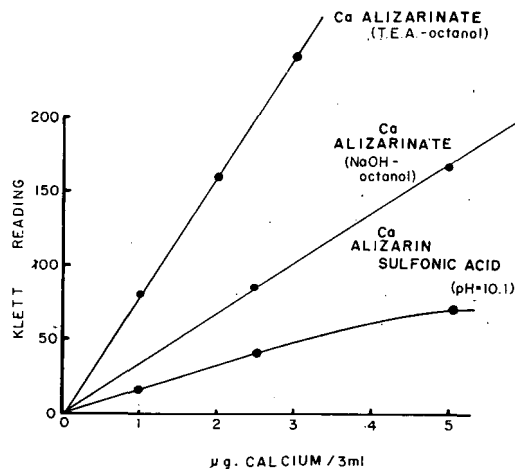

Figure 2. Klett reading (absorbance × 500) plotted against concentration for calcium complexes with 560-mμ filter

The observed difference between the ultramicromethod and the Clark and Collip method (5) was 0.50. The t value to be exceeded at the 1% level was 2.74, with an observed t value of 13.38 comparing the averages of the two methods. Since the value of 0.6 was not observed, it was suspected that the Clark and Collip method was giving high results as claimed by others (31). Table II compares the results by the Clark and Collip method with that obtained by analyzing the precipitate from the Clark and Collip method by the ultramicromethod for its actual calcium content. This procedure is listed as the absolute procedure in Table II because magnesium interference has been essentially eliminated by oxalate precipitation.

A significant difference between the two procedures was observed. The t value for differences between averages (8.79) exceeded the t value for differences between averages (2.85) at the 1% level. Adding this difference (0.38) to that observed in Table I a total difference of 0.88 mg. per 100 ml. was obtained.

Table II. Comparison of Absolute Procedure and Method of Clark and Collip (5) for Human Serum

Absolute Procedure				Clark and Collip (5)			
Serum No.	No. of detns.	Mean, mg./100 ml.	$\Sigma x^2 - \frac{(\Sigma x)^2}{N}$	Serum No.	No. of detns.	Mean, mg./100 ml.	$\Sigma x^2 - \frac{(\Sigma x)^2}{N}$
18	4	9.09	0.0727	18	2	9.65	0.0012
19	2	10.10	0.0018	19	2	10.15	0.0084
20	4	9.87	0.0827	20	2	9.89	0.0040
21	2	9.27	0.0338	21	2	9.89	0.0012
22	2	8.91	0.0000	22	2	9.34	0.0162
23	5	9.00	0.0207	23	2	9.50	0.0008
24	7	9.70	0.1289	24	7	9.94	0.0941
		Av. 9.43				9.81	
		Std. dev. ± 0.15				Std. dev. ± 0.10	

Table III.   Recovery of Calcium Added to Human Serums

No. of Serums	Ca in Serums (Mean), γ	Ca Added, γ	Mean Recovery	Std. Dev. ±	Mean Recovery, %
11	2.095	1.00	1.015	0.051	101.5

as the effect of magnesium interference, as compared to a calculated value of 0.60 mg. per 100 ml. One reason for this difference is the fact that oxalate does not precipitate all the calcium (26, 29). Another reason came from an unexpected source.

It was noticed that recoveries of magnesium added to calcium solutions were high. The readings obtained for magnesium were higher in the presence of calcium than in its absence. Thus to the calculated figure of 0.60 had to be added a correction factor for this auxochrome effect. Statistical analysis revealed this factor to be 0.14. The total factor, to be subtracted from the calcium value obtained experimentally, calculated to 0.74 or 0.7 in significant figures. One explanation for this auxochrome effect is the possible formation of a combined magnesium–calcium alizarin complex. In Table I this factor is subtracted from the observed values to obtain the corrected values in the last column of the ultramicro section. These values are lower than that obtained by the Clark and Collip method.
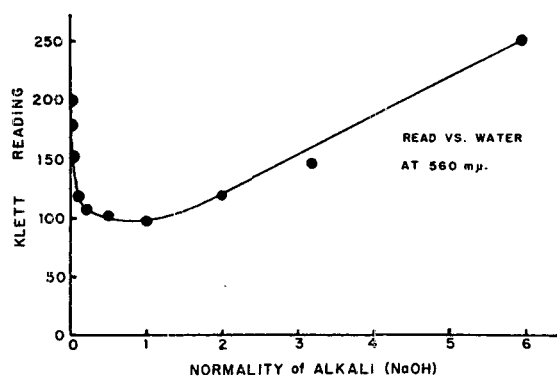
Figure 3.   Influence of alkali concentration on color density of solvent blanks

The extent of error introduced by variations in serum magnesium may be estimated from the extremes of magnesium concentration observed in human serum. The lowest value observed is reported as 0.6 mg. per 100 ml. (15). In this seven-month old infant, 1 gram of magnesium sulfate administered daily resulted in a level of 2.6 mg. per 100 ml. after two weeks. The highest serum magnesium level reported by Sobel (32) was 2.27 mg. per 100 ml. and by Orange (20) was 2.3 mg. per 100 ml. Assuming extremes of 0.6 to 2.6 mg. of magnesium per 100 ml., possible errors of +0.49 to −0.21 mg. per 100 ml. could be made when using the 0.7 mg. per 100 ml. correction factor.

When larger amounts of serum are available and when speed is not essential the authors prefer to precipitate the calcium as the oxalate. For serum obtained from experimental animals a magnesium interference factor needs to be determined for the particular animal. In rabbits the interference is of the same order, percentagewise, as in humans (7%), the absolute factor being higher since total calcium is higher.

Analytical grade triethanolamine (Eastman Kodak No. 1599) may not be used in this procedure since it is not sufficiently basic to convert the alizarin to the purple color. Small amounts of diethanolamine (5%) added to pure triethanolamine results in complete extraction of the alizarin from the organic phase but a decrease in sensitivity as for inorganic alkali (Figure 1). Thus pH is not the sole factor in producing the results observed with impure grades of triethanolamine. Addition of 5% monoethanolamine to the triethanolamine gave the high sensitivity of the impure triethanolamine. Thus the results observed with practical triethanolamine are probably due to a calcium alizarin complex formed with monoethanolamine or some impurity present in monoethanolamine other than diethanolamine. Commercial triethanolamine (Carbon & Carbide) is also satisfactory. Matheson, Pract. No. 2885 is not satisfactory for this purpose. Tests of different shipments of Eastman's practical triethanolamine produced consistent results and this material is recommended.

High concentrations of alkali or salts in the aqueous phase should be avoided since it tends to drive the alizarin into the organic phase (Figure 3). Concentrations of phosphate, fluoride, and oxalate in the aqueous layer exceeding 0.1N will interfere with complete extraction of the calcium alizarin complex into the aqueous layer. The amount of oxalate remaining in the procedure where calcium is precipitated as the oxalate does not measurably interfere.

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Applications of Curved-Crystal X-Ray Spectrometers

Microanalysis and Simultaneous Analysis

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Curved, reflection-type, focusing crystals allow fluorescent x-ray spectroscopy to be applied to analysis of microgram quantities of specimen material. The intensity, resolution, and line-background ratio from a 1-mg. specimen on a curved-crystal spectrometer were found to be as good as those from a 10-gram specimen on a commercial flat-crystal spectrometer. Parts per million of niobium, hafnium, tantalum, thorium, and uranium in iron were measured by extracting these elements from 10 grams of iron, the residue containing 5 to 20 γ of each of the five elements. Standard deviations for these measurements were of the order of 13% of the amount present. Curved crystals also simplify the instrumentation for simultaneous analysis of several elements in a specimen. An example is shown for the analysis of chromium, nickel, and molybdenum in steel with a very compact arrangement of fixed detectors and curved crystals.

THE use of a curved, reflection-type, focusing crystal for fluorescent x-ray spectroscopy has been described (*3*). In the focusing arrangement shown in Figure 1, an ordinary flat specimen is located outside the focusing circle and a slit on the focusing circle acts as a line source of radiation. In another arrangement, the large specimen may be removed, and the slit can be replaced by a capillary-type specimen located on the focusing circle. Two applications in which the curved-crystal arrangement has advantages over other x-ray spectrometers (*3*) are: analysis of microgram quantities of specimen material and repeated simultaneous analysis for a particular group of elements. For simultaneous analysis, a number of appropriate crystals and detectors are used corresponding to the number of elements being detected.

MICROANALYSIS

For many small samples, the constituents are not known, and a qualitative analysis is required before a quantitative analysis can be performed. Therefore a scanning, curved-crystal spectrometer was constructed as shown in Figure 2. The lead shielding has been removed in the photograph, so that the components are more easily shown. The capillary-type specimen, consisting of powder cemented to a fine glass fiber, is mounted at *A* on the focusing circle. The crystal, *C*, and detector, *D*, move along the periphery of the focusing circle centered at *B*. Gearing of the crystal and detector arms is such that the detector

moves at twice the angular velocity of the crystal, so that the Geiger-counter slit will always be at the focus of the diffracted radiation. Furthermore, it is necessary to keep the detector pointed at the crystal rather than tangent to the circle. This is accomplished by the wire and pulley system, *EFG*, which turns the detector mounting table, *E*, through the proper angle as the detector arm rotates. Both manual and motor drive are provided for the spectrometer. The range in wave length that can be covered by the spectrometer is limited at the short wave-length end by the crystal touching the specimen and at the long wave-length end by the spacing of the crystal. With lithium fluoride the wave-length range is from 0.46 to 3 A. By substituting
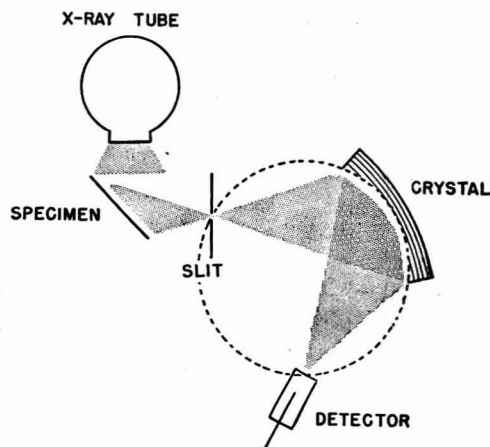

Figure 1. Principle of curved, reflection-type focusing-crystal arrangement for x-ray spectroscopy

Table I. Analysis of Iron and Manganese in an Aluminum Alloy Containing 0.52% Iron, 0.81% Manganese

	Curved Crystal[a]		Flat Crystal[b]	
	Fe	Mn	Fe	Mn
Intensity, counts/sec.	390	160	280	190
Breadth of line at half maximum	0.5°	0.4°	0.5°	0.5°
Line/background ratio	19.5	16.0	18.7	19.0

[a] 1-mg. aluminum alloy sample containing less than 10 γ each of iron and manganese.
[b] Ordinary flat sample containing several grams of aluminum alloy.

sodium chloride, the long wave-length end may be extended to 4 A. For wave lengths less than 0.86 A. (krypton absorption edge), it is of advantage to substitute a krypton-filled Geiger tube for the usual argon-filled tube.

A test of the analysis of small quantities of specimen material was conducted on an aluminum sample containing fractional percentages of iron and manganese along with other elements. The aluminum merely acts as a matrix and does not interfere with analysis for the desired elements. A specimen of about 1 mg. was scraped from the aluminum block and mounted on a capillary as described above; the actual amounts of iron and manganese present in the milligram sample were less than 10 γ each. Results from this small specimen were compared with results from the large aluminum block analyzed on a commercial flat-crystal spectrometer and are shown in Table I. As can be seen from the table, the intensity, resolution, and line-background ratio are as good from the 1-mg. specimen as from the specimen containing several grams of material.
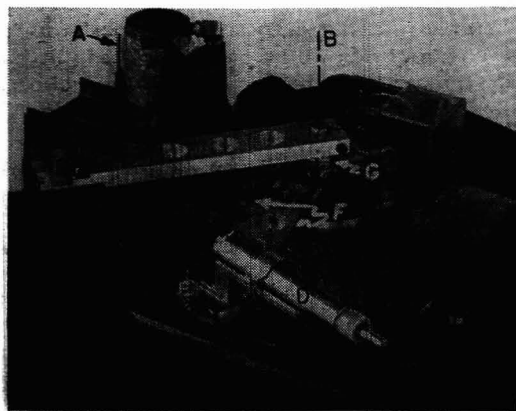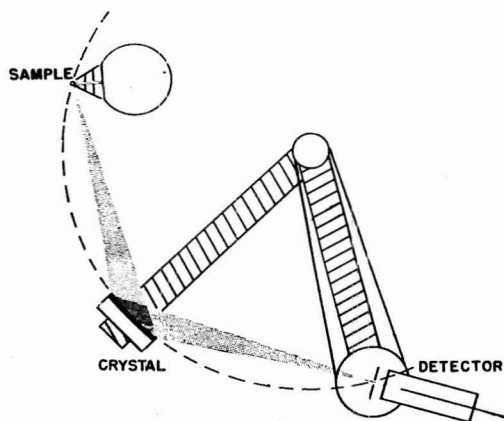
Figure 2.   Scanning-type curved-crystal spectrometer

A. Capillary specimen
B. Axis of focusing circle
C. Curved crystal on brass mount
D. Geiger-counter detector
E. Detector mounting table pulley
F. Pulley wire
G. Drive pulley

As a further illustration of the application of the curved-crystal arrangement to microanalysis, a particular problem arose in which it was desired to determine some 50 elements in iron when the concentrations were about 1 p.p.m. Although most of the elements were conveniently handled by conventional emission spectroscopy, six of the elements—niobium, hafnium, tantalum, thorium, uranium, and cerium—were not readily amenable to that method. Of these six elements, only cerium could not be detected by x-ray spectroscopy; the wave lengths of its K series lines were

too short, and those of its L series lines were too long. The remaining five, along with possibly some 15 other elements, were separated from the iron by a mercury-cathode, electrolysis extraction. As some of the 15 undesired elements would interfere with x-ray spectroscopy of the desired five, it was necessary to remove them by standard chemical procedures, the procedures used being determined by which of the 15 elements appeared with emission spectrocopy. The final residue from an initial 10-gram iron sample (containing of the order of 10 γ of each of the five desired elements) was ideally suited to the scanning, x-ray spectrometer described above. Because one could not be sure of collecting all the residue on the capillary, an internal standard (5)—thallium—was added at the end of the extraction process, so that the intensities of each of the five elements could be compared with the intensity of the known percentage of thallium. As the same fraction of thallium as for the other five elements would be lost in collecting the residue, the composition ratio of each of the five to thallium remained unchanged.

When a residue was prepared in the manner described above, it was indeed possible to measure the x-ray fluorescence from the five desired elements and to determine the ratio to the thallium intensity. However it is not possible to state, *a priori*, what the content of each of the elements is from the measured intensity ratios without known standards for comparison. Five known compositions were prepared to illustrate how the "unknown" thallium intensity ratio varied for the five elements and also to determine the accuracy that might be expected from analyses of such residues taking into consideration how the intensity from one element might be affected by changing the relative concentration of another element, in this case the internal standard thallium.

Table II lists the composition of the five standards which were prepared by dissolving appropriate amounts of the elements in sulfuric acid and carrying through the steps in the extraction process. As shown in the table, both the absolute amount of a given element and the amount relative to thallium are varied. In order to compare results from the five combinations in Table II it was necessary to normalize the data as follows: For a given unknown, say niobium, the intensity ratio niobium-thallium was determined for each of the five combinations in Table II. These five intensity ratios were normalized to equal concentrations of niobium and thallium by multiplying by the inverse of the actual concentration ratio. For instance, in the combination 1 p.p.m. of niobium to 2 p.p.m. of thallium, the observed intensity ratio for niobium-thallium was multiplied by 2/1, etc. All the niobium-thallium ratios should then be the same and standard and maximum deviations from the average could be determined.

Table II.   Combinations of Unknowns and Thallium

(Quantities equivalent to parts per million in 10 grams of iron)

Unknowns, P.P.M.	Thallium, P.P.M.
0.5	1
1	0.5
1	1
1	2
2	1

Table III.   Intensity Ratio of Unknowns to Thallium

Unknown Element	Wave Length, A.	Average[a] Intensity, Counts/Sec.	Average[a] Intensity Ratio, Unknown Tl	Standard Deviation, %	Maximum Deviation, %
Nb	0.745	353	4.02	18.9	26.9
Hf	1.57	114	1.29	8.5	13.2
Ta	1.52	92	1.05	11.2	18.1
Th	0.95	84	0.96	12.9	16.7
U	0.91	67	0.77	14.9	18.6
			Average	13.3	18.8

[a] Average after normalization to 1 p.p.m. of unknown and 1 p.p.m. of thallium of five combinations given in Table II. Wave length of Tl $L\alpha$ = 1.21 A.
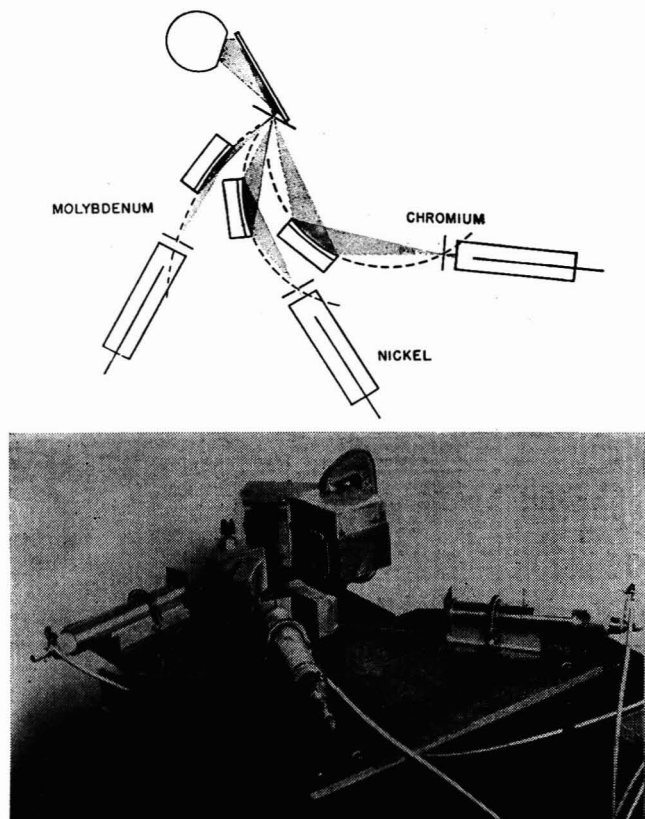
Figure 3. Fixed crystal-detector arrangement for simultaneous analysis of chromium, nickel, and molybdenum steel

Table IV.   Composition of Chromium-Niobium-Molybdenum Steels

Specimen	Cr, %	Ni, %	Mo, %
1	10–18	10–14	2–3
2	0.53	0.55	0.2
3	1.25	3.44	0.28
4	11.5	0·	0.55
5	11.4	0	1.08

Figure 4.   Fluorescent x-ray intensity vs. composition for chromium, nickel, and molybdenum steel

Table III lists results from the normalized data for each of the unknown elements. Standard and maximum deviations are of the order of 13 and 19% of the amount present, respectively. It is felt that these represent the errors which would be observed in practice due to inaccuracies in adding the internal standard and the relative effect of one element upon another. From the intensity obtained with 5 γ of an element (0.5 p.p.m. in Table II), it is estimated 1 γ would be detectable.

As shown in Table III, with the exception of niobium, the intensity ratio of unknown thallium decreases as the wave length of the unknown decreases as would be expected, since the sensitivity of the detector decreases with wave length. The niobium-thallium ratio cannot be compared with the others because a krypton-filled Geiger tube was used to detect the niobium radiation. To illustrate the advantage of the curved crystal, a residue similar to those used above was collected on a 0.25-mil Mylar film and examined in a commercial, flat-crystal spectrometer. The intensity was less than 0.1 that with the curved crystal. For reasonable counting times, this made the total count so low that large statistical errors were introduced.

For small samples such as these, almost all of the material is subject to excitation and fluorescence. Such is not the case in ordinary fluorescence analysis, where the specimen is effectively of infinite thickness. Calculation procedures such as that described by Beattie and Brissey (2) cannot be applied to small samples.

SIMULTANEOUS ANALYSIS OF SEVERAL ELEMENTS

Simultaneous analysis of several elements using flat analyzing crystals has been described (1). Curved-crystal arrangements have several advantages over flat crystals: No collimators are required, thus allowing a more compact instrumentation; either a large or small sample may be used, and with a large specimen,
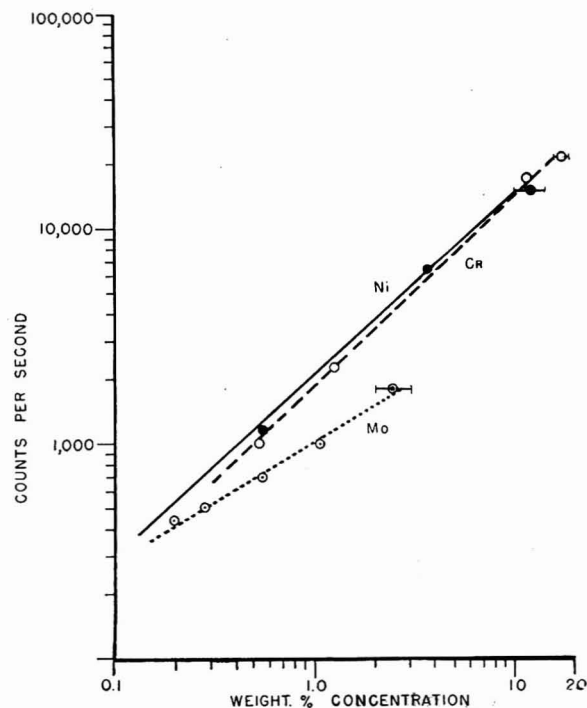
the slit on the focusing circle controls resolution and intensity and may be opened to increase intensity when resolution can be sacrificed.

Figure 3 shows the instrumentation of simultaneous analysis of chromium, nickel, and molybdenum in steel using three crystals and three detectors in fixed positions. Curved lithium fluoride (4) analyzing crystals were used for all three elements, the radii of curvature being 40 cm. for molybdenum and 20 cm. for nickel and chromium. A krypton-filled detector was used for the molybdenum radiation; argon-filled detectors were used for nickel and chromium.

Five nickel-chromium-molybdenum steels were chosen with compositions covering a large variation in each of the three elements as shown in Table IV. Ordinary sized specimens were used and yielded the intensity vs. composition curves shown in Figure 4 (no background corrections were made). Because of the large variation in composition, it was necessary to use absorbers in front of the detectors for some of the measurements; the counting rates shown in Figure 4 were corrected for this, which explains the counting rates of 20,000 counts per second.

The three crystals and detectors may be repositioned to detect three other elements, or more crystals may be added within reason for simultaneous analysis of more than three elements.

CONCLUSIONS

Curved-crystal spectrometers for fluorescent x-ray analysis have some advantage over flat-crystal spectrometers even for

large specimens. With a slit acting as a line source of radiation, the slit width controls resolution. By opening the slit, intensity may be increased where resolution can be sacrificed. To accomplish the same purpose with flat-crystal spectrometers, it is necessary to have several interchangeable collimators. For very small amounts of specimen material, the curved-crystal instrument has its greatest advantage. It yields appreciably higher intensities (by an order of ten times) without loss of resolution. It offers the possibility of analyzing microgram quantities of material with accuracy comparable to the analysis of gram samples. The curved crystal is applicable to either a scanning-type instrument or multichannel analysis. For multichannel analysis, elimination of any collimating device allows more compact instrumentation.

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Colorimetric Determination of Phosphorus by Modified Phosphomolybdate Method

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A modified colorimetric procedure for orthophosphate employs a 4% solution of molybdic acid in 10N sulfuric acid and a medium which is at least 25% acetone by volume. As most water-insoluble alkyl phosphates are acetone-soluble, the method serves well in determining the amount of uncombined phosphoric acid in these compounds. The method is specific for orthophosphate and is not affected by moderate concentrations of pyro-, meta-, or polyphosphates. It may be used to determine orthophosphate in commercial condensed phosphate compounds, and in organic and inorganic phosphorus compounds after conversion of the phosphorus to orthophosphate by acid digestion or alkaline fusion in a semimicro Parr bomb.

THE yellow complex formed by phosphate with vanadate and molybdate in acid solution has been shown to be capable of high precision in the quantitative colorimetric determination of phosphorus (1, 2). The mixed reagent of vanadate and molybdate must be allowed to age a few days before use, as it tends to precipitate. In this laboratory free phosphoric acid in organic phosphates (some of which are insoluble in aqueous solution) and orthophosphates in polyphosphates have been determined by the molybdenum blue procedure. The investigation of the vanadomolybdate method led to the development of a simplified procedure.

The method presented here makes use of only molybdate in acid solution and acetone or acetone-water solutions as the solvents. The former eliminates the need to age the reagent. The latter intensifies the phosphomolybdate color and allows complete solution of the organic phosphates. Although the omission of the vanadate is not entirely new (3), the use of a water-soluble solvent such as acetone simplifies the extraction procedure using butyl alcohol and may be used in aqueous solutions. For complete color development the final volume of solution may be as low as 25% or as high as 95% acetone by volume with very little change in color intensity taking place between these concentrations. The color forms immediately and is stable for at least 5 hours. As low as 0.0025 mg. and as high as 3.00 mg. of phosphorus pentoxide may be detected by this procedure using a Klett-Summerson photoelectric colorimeter at 430 mμ. Using this instrument at that particular wave length, standard curves which varied slightly from being linear were obtained at concentrations of from 0 to 0.3 mg. of phosphorus pentoxide and from 0 to 3 mg. of phosphorus pentoxide using a 40-mm. cell and 10-mm. test tube, respectively. When C.P. ammonium molybdate and commercial grade acetone were used, the blanks were practically undetectable with the Klett-Summerson photoelectric colorimeter.

Orthophosphates may be determined in commercial polyphosphates, since the latter, in moderate concentrations, do not affect the color intensity and do not hydrolyze to orthophosphate rapidly enough to vitiate the results. Table I shows the accuracy achieved in determining orthophosphates in both condensed phosphates and alkyl phosphates.

The total phosphorus pentoxide contents of various organic and inorganic phosphate compounds were determined by this procedure following conversion to orthophosphate by either acid digestion or oxidation in a semimicro Parr bomb. Table II shows a satisfactory agreement between this method and the volumetric ammonium phosphomolybdate method which is commonly used in this laboratory.

Table I. Determination of Orthophosphates in Condensed and Alkyl Phosphates

Compound	Weight of Sample, Gram	H₃PO₄, Mg. Added	H₃PO₄, Mg. Found
Dibutyl phosphonate	1	0.100	0.099
		0.200	0.200
		0.300	0.302
Mono-octyl phosphonate	1	0.100	0.100
		0.200	0.198
		0.300	0.303
Sodium tripolyphosphate	0.01	0.100	0.100
		0.200	0.200
		0.300	0.302
Tetrasodium pyrophosphate	0.01	0.100	0.100
		0.200	0.201
		0.300	0.300
Sodium hexametaphosphate	0.01	0.100	0.101
		0.200	0.203
		0.300	0.301

Standard deviation, 7 parts per thousand.

Table II. Comparison of Colorimetric and Volumetric Methods

Compound	Total P_2O_5 Content, %	
	Volumetric	Colorimetric
Monosodium phosphate	57.5	57.5
Tetrasodium pyrophosphate	53.6	53.5
Sodium tripolyphosphate	57.5	57.2
Sodium metaphosphate (hexameta)	67.3	67.5
Trisodium phosphate	19.9	19.8
Diammonium phosphate	53.8	53.8
Dicalcium phosphate	41.1	41.3
Tricalcium phosphate	39.5	39.7
Calcium tripolyphosphate	46.2	46.2
Trimagnesium phosphate	35.9	36.1
Trioctyl phosphate, wet ashed	16.4	16.4
Trioctyl phosphate, Parr bombed	16.4	16.3

REAGENTS

Ammonium Molybdate Solution. Dissolve 18.8 grams of ammonium molybdate, reagent grade, in 300 ml. of water. Add 150 ml. of sulfuric acid (98%) carefully. Cool and dilute to 500 ml. with water.

Standard Phosphate Solution A. Dissolve 1.9157 grams of monopotassium phosphate, previously dried at 105° C. for 1 hour, in water and dilute to 1 liter; 1 ml. = 1.0 mg. of phosphorus pentoxide.

Standard Phosphate Solution B. Dilute 10 ml. of the solution A to 100-ml. volume with water; 1 ml. = 0.1 mg. of phosphorus pentoxide.

PROCEDURE

Free Phosphoric Acid in Organic Phosphates. Dissolve 1 gram of sample in acetone and dilute to 100 ml. with acetone. Transfer a 5-ml. aliquot to a 25-ml. volumetric flask, add 2 ml. of ammonium molybdate solution, dilute to 25 ml. with acetone, and mix thoroughly. Prepare a blank with the same quantities of reagents and adjust the colorimeter to zero at 430 mμ. Determine the absorbance of the sample and calculate the free acid content from a standard curve prepared with the same quantities of reagents and 0, 1, 2, and 3 ml. of standard phosphate solution B.

Orthophosphate in Polyphosphates (Pyro-, Tripoly-, Meta-). Dissolve 1 gram of sample in water and dilute to 100 ml. Transfer a 1-ml. aliquot to a 25-ml. volumetric flask, add 2 ml. of ammonium molybdate and 10 ml. of acetone, and dilute to volume with water. Prepare a blank with the same quantities of reagents and adjust the colorimeter as before. Determine the absorbance and calculate as in the free acid method above using the same standard curve.

Total Phosphorus Pentoxide Content. INORGANIC PHOSPHATES. Convert 0.5 gram of sample to orthophosphate by fuming with 10 ml. of perchloric acid (if the sample is an orthophosphate dilute to 100 ml. with water, add 10 ml. of perchloric acid, and boil for 5 minutes). Dilute to 500 ml. with water. Transfer an aliquot containing 1.5 to 2.5 mg. of phosphorus pentoxide to a 50-ml. volumetric flask (a 5-ml. aliquot is appropriate for most phosphates). At the same time, transfer a 2-ml. aliquot (2 mg. of phosphorus pentoxide) from standard solution A to a second 50-ml. volumetric flask. Add 10 ml. of ammonium molybdate solution and 25 ml. of acetone to each flask. Mix well and set the colorimeter by adjusting the reading to match that of the standard curve at 2 mg. with the standard containing the same amounts of reagent and 2 mg. of phosphorus pentoxide. This curve is prepared by using the same volume of reagents as above with 0, 1, 2, and 3 ml. of standard solution A. Obtain the absorbance of the sample and calculate the phosphorus pentoxide content from the sample weight and the standard curve.

ORGANIC PHOSPHATES. Oxidize a 50- to 100-mg. sample in a semimicro Parr bomb or fume 0.5 gram with a mixture of 25 ml. of nitric acid and 10 ml. of perchloric acid. Use the latter procedure only if the phosphorus is linked to carbon through oxygen. Dilute to appropriate volume and transfer an aliquot containing 1.5 and 2.5 mg. of phosphorus pentoxide and continue as indicated above for inorganic phosphates.

CONCLUSION

The method is simple, rapid, and accurate. The reagent is more stable than the mixed vanadate-molybdate reagent. The acetone solution intensifies the color and makes possible the complete solution of organic phosphate for the colorimetric determination of free phosphoric acid.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of Esther Paciorek in obtaining the data presented in this paper.

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Infrared and X-Ray Diffraction Studies of Digitonin

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Commercial digitonin preparations gave three types of x-ray diffraction patterns: a noncrystalline type, and two distinct crystalline ones. Differences also occurred in infrared absorption, but spectra of lots which gave different x-ray diffraction patterns may be indistinguishable. Digitonin preparations which differed with respect to these optical properties proved to be equally useful in several analytical applications.

MANY analysts who used the original procedure of Schoenheimer and Sperry (4) for determination of cholesterol searched diligently for a consistently satisfactory source of digitonin. In the more recent methods of Sobel and Mayer (5) and Sperry and Webb (6), earlier difficulties have been overcome by dissolving the glucoside in 50% alcohol instead of in water. Nevertheless, it must be kept in mind that commercial digitonin has a number of components, since new analytical procedures involving its use continue to appear. For example, the color reaction which digitonin gives with anthrone has recently been used by Beher and Anthony (1) to estimate total β-steroids in a digitonide precipitate. Dihydrocholesterol is precipitated by digitonin but does not give the Liebermann-Burchard reaction, and can thus be detected in the presence of cholesterol.

In the present study it was proposed to determine whether infrared spectra and x-ray diffraction patterns could serve as criteria for selection and preparation of digitonin suitable for determination of cholesterol and other β-steroids. It was found that lots of the glucoside obtained from a number of suppliers were equally useful in present methods, although they differed considerably with respect to infrared absorption and x-ray diffraction patterns. It is possible, however, that these criteria may be of value in the problem of purifying digitonin.

EXPERIMENTAL

Eight digitonin preparations from four different firms were examined. Each preparation was powdered to 200-mesh size for

Table I. X-Ray Diffraction Powder Pattern d Values and Relative Intensities for Types B and C Digitonin

Type B		Type C	
d, A.	I/I_1	d, A.	I/I_1
13.19	7	13.50	6
10.78	4	11.87	5
9.77	3	10.22	2
8.42	1	9.26	8
7.86	1	8.35	2
6.83	6	7.08	5
6.63	6	6.07	10
5.83	5	5.64	7
5.45	10	5.11	9
5.10	8	4.88	3
4.93	5	4.19	4
4.73	1	3.82	8
4.56	2	3.59	7
4.18	3	3.41	2
3.97	1	3.08	4
3.87	1	2.93	2
3.74	3	2.86	2
3.56	4	2.75	1
2.87	2	2.68	1
2.75	2	2.49	1
2.52	1	2.35	1
2.32	1	2.28	1
2.11	1	2.15	2
		2.06	1
		1.81	2
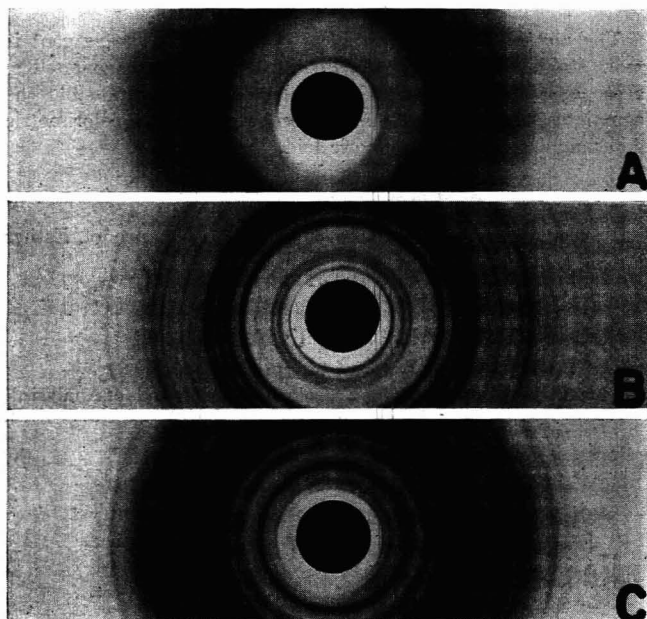
Figure 1. X-ray diffraction patterns of digitonin preparations

x-ray diffraction analysis, and mounted in thin walled plastic tubes as described by Beu and Claassen ($2, 3$). These tubes have a wall thickness of 0.01 mm. and an inside diameter of 0.2 mm. The mounted samples were exposed for 5 hours in Norelco (North American Philips Co., Inc.), 114.59-mm. diameter, Debye-Scherrer cameras. Eastman no-screen x-ray film was used. Nickel-filtered copper radiation produced at 35-kv. peak and 20-ma. current was employed.

For infrared spectroscopy, paraffin oil suspensions were prepared by rubbing 10-mg. samples of powdered digitonin with 0.1 ml. of Nujol in a small agate mortar. The suspension was spread between clear polished plates of sodium chloride separated by shims 0.0025 inch in thickness, secured in a holder provided with suitable clamping plate and screws. A Beckman IR-2 infrared spectrophotometer was used. Readings were made potentiometrically throughout the range from 7.5 to 11.5 microns at intervals of 0.04 micron or less when necessary. As the zero setting before each reading was made without cell or sample in the radiation path, absorption of the sodium chloride plates is included, but this does not alter the nature of the extinction curves.

Color production with anthrone was measured with a Coleman Junior spectrophotometer. Two milliliters of glacial acetic acid solution of digitonin, which contained 0.2 mg. of the sample to be tested, were placed in a colorimeter tube, and 3 ml. of

freshly prepared 0.2% solution of anthrone in glacial acetic acid were added. While the solution was vigorously agitated, 5 ml. of concentrated sulfuric acid were added rapidly. It is important to remove the stirring rod immediately after this addition. The blank and a series of standards prepared from various lots of digitonin were placed in a dark closet for an hour, after which readings were made at 750 mμ. The period of color development in determinations can be shortened to 30 minutes, but in this case should be kept uniform by making the preparations and subsequent readings at 1-minute intervals. Completeness of recovery of cholesterol with various lots of digitonin was tested by using conditions of precipitation similar to those of Sperry and Webb (6), and by determining cholesterol in the precipitate with the Liebermann-Burchard reaction.

RESULTS

Examination of the x-ray diffraction patterns revealed two distinct crystalline types and one which was relatively noncrystalline. In Figure 1 pattern A is noncrystalline while patterns B and C represent the two crystalline types. The term noncrystalline has come to mean those very poorly crystalline materials such as silicate glasses, certain organic polymers like polystyrene, and unstretched rubber. These substances do show one or two broad bands, the centers of which do indicate some degree of repetition such as average chain spacing. The band centers of the A type patterns agree well with the strong line positions of type B.

The d values and relative intensities, I/I_1, of the lines in the patterns of types B and C are given in Table I. The accuracy of d value measurement varies from ±0.004 A. at 2 A. to ±0.20 A. at 13 A. The relative intensities, I/I_1, were measured by visual estimation with the strongest line rated 10. Classification of the eight samples of digitonin as to the source of their manufacture and x-ray diffraction pattern type is presented in Table II.

Table II. Source of Digitonin Sample and Type of X-Ray Diffraction Pattern

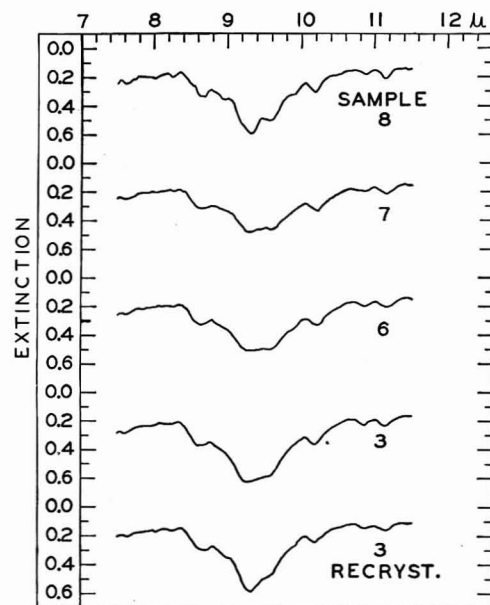| Sample	Supplier	Lot No.	Type Pattern
1	Fisher	502467	C
2	Fisher	454193	B
3	Hoffmann-La Roche	B511080	A
4	Hoffmann-La Roche	512092	B
5	Penick	No number	B
6	Penick	1588-LJA	A
7	Penick	2329-LGB-1	B
8	Merck	2495-A52340	C

Figure 2. Infrared spectra of various samples of digitonin

Table III. Absorbance of Color Produced by Various Samples of Digitonin (Anthrone Method)

Sample	Absorbance, 0.2 Mg. Digitonin	Standard Deviation
3	0.193	0.010
5	0.198	0.011
6	0.199	0.005
7	0.200	0.019
8	0.199	0.010

Table IV. Precipitation of Cholesterol by Digitonin (Liebermann-Burchard Reaction)

Sample	Cholesterol Recovery, Mg.	Theory, Mg.
3	201	200
5	199	200
6	200	200
7	202	200

Three recrystallizations were made of poorly crystalline sample 3 (Table II). Rapid recrystallization from 95% ethyl alcohol produced no change in the pattern obtained. When the same alcohol solution was allowed to crystallize more slowly, the pattern observed began to take on the appearance of type C. The third recrystallization, from an ethyl alcohol–benzene mixture controlled so as to proceed at a slow rate, produced a type C diffraction pattern sharper than the previous ones, but not nearly so distinct as that of samples 1 and 8. Although no chemical determinations of the structures of the samples before and after recrystallization were made, an examination of the infrared curves in Figure 2 indicates no chemical change occurred during crystallization.

Infrared absorption spectra of five of the eight samples are presented in Figure 2. Below 9.2 and above 9.7 microns the spectra are virtually identical. Differences between 9.2 and 9.7 microns appear to be due to the relative intensity of absorption at about 9.3 and 9.6. Sample 8, at the top of Figure 2, shows the sharpest "peak" at 9.32. This was the sample which also gave the most clearly defined x-ray diffraction pattern of type C. Sample 7, which gave a crystalline type B pattern, sample 6 from the same supplier but noncrystalline in type, and the noncrystalline sample 3 from another manufacturer, differed very little in absorption characteristics. Absorptions at 9.3 and 9.6 were more nearly equal in these samples than in sample 8. When sample 3 was recrystallized, the infrared absorption shown at the bottom of Figure 2, became similar to that of sample 8. That the peak at 9.32 microns became sharper was actually due to diminished absorption at 9.6. Sample 3, during recrystallization, approached sample 8 with respect both to infrared absorption characteristics and type of x-ray diffraction pattern.

Data on the intensity of color production with anthrone are presented in Table III. Samples 3 and 6 were of the noncrystalline type C. Absorbance at 750 mμ was essentially the same for all samples. Recovery by precipitation of cholesterol as the digitonide was equally good when digitonin samples of various types were used, as indicated by the data in Table IV.

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Phenol-Indo-2,6-Dichlorophenol as a Spray Reagent

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Indophenol dye may be used as a spray reagent, in the paper chromatography of organic acids. This dye was better than the usual indicator sprays because of its ease of preparation and its usefulness in differentiating certain acids. Separation of maleic and malonic acids is also reported.

SEVERAL spraying materials have been introduced by Buch and coworkers (2) for the identification of organic acids on paper chromatograms. Similarly, mercurochrome has also been used to locate the positions of organic acids on filter paper disks (1). Indophenol dye may also be used as a spray reagent in the paper chromatography of organic acids.

The organic acids were separated on paper strips, as well as on circular disks, using a refluxed mixture of 1-butanol, formic acid, and water—a solvent that has been used by Wiggins and Williams (3) for the separation of amino acids and sugars. When a chromatogram was treated with an alcoholic solution of the indophenol dye, organic acids usually developed dark pink spots immediately after spraying against a blue background, thus making the distinction between the spots and the background well pronounced; certain acids bleached the dye, thereby aiding in differentiating these acids from the rest. The spray reagent was prepared in ethyl alcohol, and it did not require the adjustment of pH as is usually necessary for indicator sprays.

MATERIALS AND REAGENTS

Paper. Whatman filter paper No. 1 strips (32 × 28 cm.) and circular disks (36 cm. in diameter).

Solvent. A solvent mixture of 1-butanol, formic acid, and water was prepared by refluxing for an hour a mixture of 10 ml. of 85% formic acid, 120 ml. of 1-butanol, and 10 ml. of water. A further 60 ml. of water were added in small quantities, the mixture being shaken from time to time during cooling. After having been allowed to stand for 24 hours, the upper layer was used.

Spray reagent. Phenol-indo-2,6-dichlorophenol (0.1 gram) in neutral ethyl alcohol (100 ml.).

METHOD AND RESULTS

An ascending strip chromatogram (32 × 28 cm.) containing 25 γ of test acid per spot, was developed in a cylinder (height 36 cm., radius 6.5 cm.) in the usual manner. After development, the chromatogram was dried in air for about 3 hours, until it was free from formic acid fumes, and it was then sprayed with the dye solution. The organic acids developed dark pink spots against a blue background, except for ascorbic acid and gallic
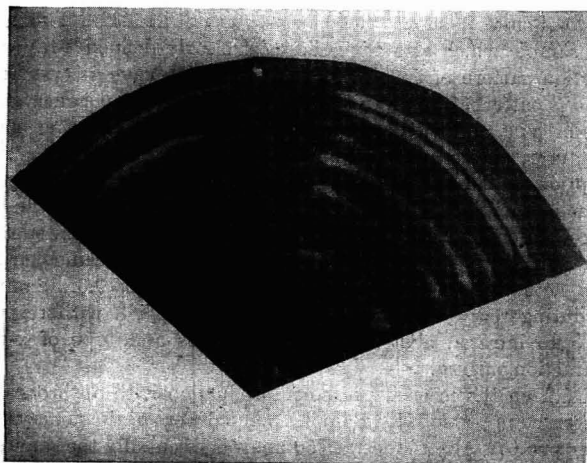
Figure 1. Circular paper chromatogram of organic acids

C.	Citric	*MLe.*	Maleic
F.	Fumaric	*M.*	Malic
GLu.	Gluconic	*S.*	Succinic
MLn.	Malonic	*T.*	Tartaric

acid, which bleached the dye immediately. With time, lactic acid, maleic acid, and malonic acid showed a tendency to bleach, the bleaching being complete within 4 hours. The positions of the acids in a mixture were determined by running mixed chromatograms, as well as by applying the color tests proposed by Buch and coworkers (*2*).

A circular paper chromatogram (36 cm. in diameter) containing 50 γ of test acid per spot was prepared in the manner described [Airan and coworkers (*1*)]. After being air-dried, the chromatogram was treated with the spray reagent, and was photographed (Figure 1). Maleic and malonic acids, which have an identical R_f value in a pentanol–formic acid system, were separated.

CONCLUSION

Indophenol dye is a better spray reagent than the usual indicator sprays because of its ease of preparation and use and its usefulness in differentiating certain organic acids.

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Colorimetric Determination of Chloride Ion via Ion Exchange

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Chloride ion is determined colorimetrically over the range 0 to 180 p.p.m. after exchanging for iodate ion with granular silver iodate in a column. The released iodate ion reacts with cadmium iodide–linear starch reagent to form the blue linear starch–triiodide ion complex, the absorbancy of which at 615 mμ is proportional to the concentration of chloride ion. No serious interferences were found among ions commonly found in natural waters, within the limits of their usual concentrations. Bromide and iodide ions react in the same manner as chloride ion but are not commonly present.

COLORIMETRIC procedures for the determination of chloride ion involving ion exchange with a solid phase reagent, such as silver chromate or silver ferrocyanide, have been described (*3*). Chloride ion exchanges for chromate or ferrocyanide ions, and is determined indirectly by the colorimetric determination of the released ion or the reaction products of the released ion.

In the method described here, chloride ion exchanges for iodate ion with granular silver iodate, and the concentration of chloride ion is determined by the absorbancy of the blue linear starch–triiodide ion complex formed by the reaction of the released iodate ion with cadmium iodide–linear starch reagent in acid solution. The relatively low melting point of silver iodate permits its preparation in massive form, from which particles of uniform size suitable for column reactions are obtained by grinding and sieving. Its use in a column ensures the attainment of equilibrium with chloride ion in solution.

REAGENTS AND EQUIPMENT USED

Silver iodate, granular, 100- to 200-mesh.
Cadmium iodide–linear starch reagent (*1*), 11.00 grams of cadmium iodide and 2.50 grams of twice-recrystallized linear potato starch fraction per liter of solution.
Hydrochloric acid, 1.0N.
Standard chloride ion solution, 200 p.p.m., 0.330 gram of sodium chloride per liter of solution.
Buret, glass stopcock, 50-ml.
Volumetric flasks, 100-ml. and 250-ml.
Pipets, 1-ml., 10-ml., and 20-ml.
Pressure bulb assembly.
Spectrophotometer, Beckman Model DU, 10-mm. cells.
Pure silver iodate may be purchased, or prepared by reaction of 0.1 mole each of silver nitrate and potassium iodate in dilute solution. It may be prepared under nearly homogeneous conditions by dissolving each salt in 500 ml. of distilled water and allowing both solutions to drip slowly at the same rate into 1 liter of rapidly stirred water. After precipitation is complete, the product is washed well and dried. Small batches are carefully melted and poured into cold water, forming hard masses which can be ground and sieved to the desired 100- to 200-mesh size. Silver iodate decomposes slightly on melting, but the resulting silver iodide does not affect the reactions of the granular silver iodate. The pure salt is white, but turns yellowish white on melting owing to the silver iodide formed. Its melting point is above 200° C.
The silver iodate column is prepared by firmly tamping a small plug of borosilicate glass wool into the constriction of the buret above the stopcock, filling the buret half full of water, and pouring in a slurry of the granular silver iodate to form a column 6 cm. high. A second borosilicate glass wool plug is packed firmly on top of the column to keep the silver iodate particles firmly in place. The column is never allowed to run dry, and distilled water is kept in the buret at all times when the column is not in use.
The pressure bulb assembly for forcing wash water through the column is very convenient. A short length of flexible tubing is

attached to a double-acting rubber bulb in such a manner that the bulb acts as a source of air pressure. A No. 00 one-hole rubber stopper, which fits the top of the buret, is attached to the other end of the rubber tube by means of a short length of glass tubing. If the column is accidentally allowed to run dry, it may be reverse-flushed by reversing the rubber bulb and using it to suck distilled water up through the column.

PROCEDURE

The column in the buret is washed twice with the solution to be analyzed by filling the buret (with the stopcock closed) and inverting to empty, taking care to keep liquid around the silver iodate particles. The glass wool plug on top of the column effectively prevents liquid from draining out of the column, if the rinsing solution is poured off quickly. A buretful of the solution is allowed to run through at normal speed (about 80 drops per minute) or, alternatively, two buretfuls are forced through by the use of the pressure bulb.
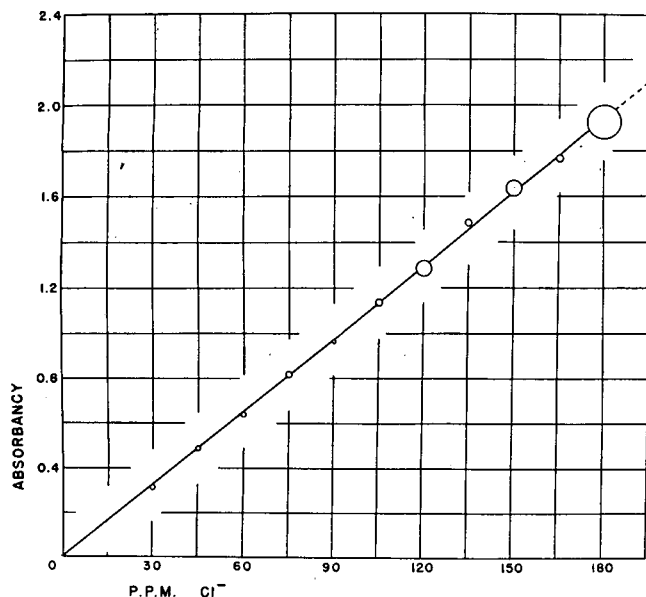
Figure 1. Absorbancies of linear starch–triiodide ion complex produced by chloride ion solutions of known concentrations

At 615 mμ

The buret is filled approximately to the zero mark, and the solution is allowed to run out until the meniscus is exactly on the 10-ml. mark. The next 10.0 ml. are very carefully measured into the 100-ml. volumetric flask and diluted up to the calibration mark with distilled water. After thorough mixing, 10.0 ml. of this solution are pipetted into the 250-ml. volumetric flask, and diluted up to the calibrated volume. From this, 20.0 ml. are taken as a sample, and 1.0 ml. each of the 1.0N hydrochloric acid and cadmium iodide–linear starch reagent are added. The absorbancy of the solution at 615 mμ is determined 5 minutes from the time the acid and the starch-iodide reagent are added. The absorbancies produced at all the concentrations studied were practically constant between 5 and 20 minutes after addition of the acid and starch-iodide reagent.

DISCUSSION

From data given in the literature (2), the solubility product constant of silver iodate at 25° C. is calculated to be 3.57 × 10^{-8}, and that of silver chloride to be 1.85 × 10^{-10} at the same temperature. At equilibrium, the ratio of iodate ions to chloride ions in solution would be 193. Equilibrium is apparently reached quickly in the column, as the dark band of silver chloride is seen to form at the very top of the column and progress downward slowly, as long use of the column exhausts the silver iodate. Less than 0.25 inch of the column was darkened as a result of all the determinations made in this study, indicating a long useful life for the column. Silver iodate is not very light-sensitive but should be protected from direct light by an opaque cylinder around the buret.

The calibration curve, Figure 1, was determined using pure sodium chloride solutions of known concentrations. The vertical

Table I. Effect of Possible Interferences

Substance		Concentration, P.P.M.	Color
F$^-$	NaF	50	Equals blank[a]
NO$_3^-$	KNO$_3$	500	Equals blank
HCO$_3^-$	NaHCO$_3$	500	Equals blank
C$_2$H$_3$O$_2^-$	NaC$_2$H$_3$O$_2$	500	Equals blank
SO$_4^{--}$	ZnSO$_4$	500	Equals blank
HPO$_4^{--}$	Na$_2$HPO$_4$	50	Equals blank
K$^+$	KC$_2$H$_3$O$_2$	500	Equals blank
Na$^+$	NaC$_2$H$_3$O$_2$	500	Equals blank
NH$_4^+$	NH$_4$C$_2$H$_3$O$_2$	300	Appreciable[b]
		200	Equals blank
Ca^{++}	Ca(C$_2$H$_3$O$_2$)$_2$	500	Appreciable
		400	Equals blank
Mg^{++}	Mg(C$_2$H$_3$O$_2$)$_2$	300	Appreciable
		200	Equals blank
Zn^{++}	Zn(C$_2$H$_3$O$_2$)$_2$	500	Equals blank
Al^{+++}	Al(NO$_3$)$_3$	400	Appreciable
		300	Equals blank
Fe^{+++}	Fe(NO$_3$)$_3$	300	Appreciable
		200	Equals blank
H$^+$	H$_2$SO$_4$	10^{-3}N	Equals blank
OH$^-$	NaOH	10^{-3}N	Equals blank

[a] Color approximately as intense as that obtained with distilled water by the regular procedure.
[b] Significantly more color than is produced by distilled water.

Table II. Analysis of Typical Water Samples

Sample	No. of Detns.[a]	Cl$^-$ Added, P.P.M.	Cl$^-$ Found, P.P.M. Mohr method	Cl$^-$ Found, P.P.M. This method
I[b]	5	0	68	
	4	0		70
	4	16		84
	4	32		100
II[c]	5	0	29	
	2	0		28
III[d]	4	0		28
	4	25		55
	4	50		79
	4	100		130

[a] Good agreement between results of multiple determinations.
[b] Kansas river water collected near Manhattan, Kan., with suspended solids removed by filtration and/or centrifugation.
[c] Manhattan, Kan., city water supply, obtained from wells in Blue River valley.
[d] Manhattan, Kan., city water supply, different date.

diameter of each circle indicates the range of four determinations made at that concentration. The relationship apparently would be linear over a greater range than is shown, but practical difficulties in reading the absorbancies at high concentrations limit the concentrations that can be determined directly. The loss of precision at higher concentrations may be due partly to errors in reading absorbancy values. The optimum concentration range for analysis without resort to dilution is apparently 0 to 150 p.p.m. of chloride ion.

A faintly colored blank is obtained when distilled water is run through the column and determined in the regular manner. This could perhaps be reduced by greater dilution, but at a loss of sensitivity of the method. The absorbancy of the blank is very small (about 0.015) but constant, and is due to the very slight solubility of silver iodate, which is 1.89 × 10^{-4} mole per liter at 25° C. The solutions analyzed in Figure 1 were compared with such blanks obtained at the time each series of samples was determined. Another calibration curve was obtained by comparing the absorbancies against distilled water as a reference. This line was displaced upward at all concentrations by the optical density of the zero chloride ion blank. The constant value of this blank would permit use of distilled water as a reference in spectrophotometric determinations. No attempt was made to control temperature for any of the determinations.

Potential interferences were studied up to a maximum of 500 p.p.m., with the results shown in Table I. The colors produced by the various substances shown which were approximately the same as the zero chloride ion blank are listed as "equals blank," and are considered not to interfere. From these data, it is evident that very few ions would interfere at the concentrations usually found in natural waters or drinking water supplies. The inorganic salts of several of the cations showed interference at slightly lower concentrations than the acetate salts of the same cations, probably because of trace amounts of chloride ion present as an impurity in the inorganic salts. Bromide and iodide ions would give the same reactions as chloride ion, but they are not usually present in natural waters in concentrations that would give high chloride ion values.

Chloride ion concentration in two representative waters was analyzed with the results shown in Table II. Determinations were made on the raw samples and on samples to which known amounts of chloride ion were added. The river water, after removal of suspended matter, was diluted with 200 p.p.m. of

standard chloride ion solution to give the desired increase in chloride concentration. Solid sodium chloride was added to one batch of the city water to give the increased chloride ion concentrations. The values obtained by this method agree well with those obtained by the Mohr method.

ACKNOWLEDGMENT

The research of which the development of this analytical procedure was a part was made possible through the aid of a grant from Research Corp.

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Determination of Lead in Lead Drosses and Lead-Base Alloys

Application of Ethylenediaminetetraacetic Acid Method

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The rapid and accurate determination of lead in lead drosses and similar materials is often difficult owing to the high lead content and numerous impurities. The purpose of this investigation was to develop a simple, rapid, and accurate determination of lead in lead drosses by use of a Versenate titration. The average relative error of the method in the determination of pure lead was less than 0.5 part per thousand, and the standard deviation in the analysis of a series of typical drosses was 0.106%. The method should be applicable to the determination of lead as a major constituent in lead drosses and lead-base alloys.

THE rapid and accurate determination of lead in lead drosses and similar materials is difficult owing to the high lead content and the varying amounts of other materials such as silica, rubber, and compounds of arsenic, antimony, tin, copper, iron, and zinc. Of the many methods suggested, the molybdate titration (7) is perhaps the most widely used, even though it requires an outside indicator. Schwarzenbach (6) first suggested the use of ethylenediaminetetraacetic acid (Versene) for titration of solutions containing lead. This method has been further developed by Flaschka and his coworkers (2-4). Kinnunen and Wennerstrand (5) have applied the method to the determination of small amounts of lead in nickel sulfate.

The purpose of this investigation was to develop a rapid, simple, and accurate method for the determination of lead in lead drosses and alloys by titration with disodium dihydrogen ethylenediamine tetraacetate (Versenate) using Eriochrome Black T (F241) as the indicator. The proposed method is rapid and the results are accurate and precise.

REAGENTS AND SOLUTIONS

Reagents. All reagents used were C.P. or analytical grade chemicals which conformed to AMERICAN CHEMICAL SOCIETY specifications. Analytical reagent grade disodium dihydrogen ethylenediamine tetraacetate (disodium dihydrogen Versenate) was used to prepare the titrant solutions. The indicator was the sodium salt of 1 (1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid, which is also known as Eriochrome Black T and as Indicator F241.

Solutions. Standard Versenate. Approximately 18.6 grams of disodium dihydrogen Versenate were dissolved in water and diluted to 1 liter. This solution was standardized against pure lead by the recommended procedure used in sample analysis. One milliliter of this solution equals approximately 10.0 mg. of lead.

Indicator Solution. Approximately 0.20 gram of Indicator F241 was dissolved in 50 ml. of ethyl alcohol. This solution was not stable and was discarded after 48 hours.

Ammonium Acetate. Approximately 454 grams of ammonium acetate were dissolved in water and diluted to 1 liter.

EXPERIMENTAL

Owing to the fact that drosses and similar materials contain relatively large amounts of lead and varying amounts of materials which interfere in Versenate titrations, the recommended titration method was developed using lead sulfate. Accurately weighed samples were dissolved by boiling with 30 ml. of ammonium acetate solution to which 2.0 grams of tartaric acid were added to keep the lead in solution upon dilution and adjustment of pH. After dilution to 100 ml., the pH was adjusted to 9.5 with concentrated ammonium hydroxide and the solutions were titrated warm using 7 drops of indicator. The color change of the indicator from pink to sky blue at room temperature occurred over a range of 1.0 ml. of the titrant, but with experience could be reproduced satisfactorily. It was noted, however that the color change was much sharper at elevated tempera-

Table I. Determination of Lead Metal and Lead Sulfate

Lead Metal				Lead Sulfate			
Taken, mg.	Found, mg.	Error, mg.	Relative error, %	Taken, mg.	Found, mg.	Error, mg.	Relative error, %
450.1	450.2	0.1	0.02	658.4	658.1	0.3	0.05
454.1	454.0	0.1	0.02	602.0	601.6	0.4	0.07
449.6	449.0	0.6	0.13	602.2	601.9	0.3	0.05
453.8	453.6	0.2	0.04	611.7	611.6	0.1	0.02
449.6	449.6	0.0	0.00	605.4	605.6	0.2	0.03
450.6	450.5	0.1	0.02	607.6	607.8	0.2	0.03
				604.5	604.1	0.4	0.07
Average		0.18	0.038			0.27	0.046

Table II. Determination of Lead in National Bureau of Standards Alloys

	N.B.S. 127a Solder (65/35)			N.B.S. 53c Lead-Base Bearing Metal	
N.B.S.[a] value, %	Found, %	Error, %	N.B.S.[a] value, %	Found, %	Error, %
[69.01	69.16	0.15	84.28	84.22	0.06
	68.97	0.04		84.28	0.00
	69.14	0.13		84.26	0.02
	69.08	0.07		84.30	0.02
	69.14	0.13			
Average	69.10	0.104		84.27	0.025
Standard[b] deviation	0.082			0.039	
Confidence[c] limits, 95%	69.10 ± 0.10			84.27 ± 0.06	

[a] By difference.
[b] Calculated from range and deviation factor.
[c] Calculated from range and confidence factor.

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tures. Titrations were tried at several temperatures and the optimum was found to be between 70° and 80° C. At these temperatures, a complete change from pink to sky blue occurs with 0.10 ml. or less of the Versenate.

Several methods of dissolving the samples were tried using various types of materials encountered in lead smelter control laboratories. Most of these methods were satisfactory for some samples but not for others. In some cases the time required for solution of the sample was excessive, while in others there was incomplete removal of or introduction of interfering materials. The recommended procedure of solution in sulfuric acid containing filter paper and destruction of organic matter by concentrated nitric acid is rapid and has proved satisfactory for all types of materials encountered. Interferences are removed by the digestion, filtration, and thorough washing of the lead sulfate.

Preparation of Samples. All dross samples were prepared for analysis by grinding with a Wiley mill to pass a National Bureau of Standards No. 40-mesh screen and, after thorough mixing, reduced to laboratory sample size with a Jones riffle. Reduction of particle size to pass a No. 100-mesh screen is preferable when such reduction is feasible.

Recommended Procedure. Sample weights were selected, which contained 0.40 to 0.48 gram of lead and were weighed into Erlenmeyer flasks. A small piece of filter paper was added to aid in reduction and solution of antimony, and the sample was covered with 20 ml. of sulfuric acid and heated until solution was complete. A few drops of concentrated nitric acid were added to the hot solution to destroy the filter paper and other organic matter. The solution was cooled and diluted to 200 ml. Approximately 2 grams of tartaric acid were added, and the solution was boiled to digest the precipitate and to facilitate separation of iron and antimony. It was then cooled, filtered, and washed thoroughly with cold sulfuric acid (1 to 9). The precipitate and paper were returned to the original flask and boiled with 30 ml. of ammonium acetate solution to break up the paper and to ensure complete solution of the lead sulfate. Two hundred milliliters of water, 2 grams of tartaric acid, and 25.0 ml. of concentrated ammonium hydroxide were added, and the solution was heated to 70° to 80° C. The hot solution was titrated with standard Versenate using 7 drops of the indicator. The end point is a sharp change from pink to pure blue.

RESULTS

The results obtained in the determination of pure lead and pure lead sulfate by the recommended procedure are shown in Table I. The average relative error in both sets of data is less than 0.5 part per thousand, and the maximum relative error is 1.3 parts per thousand. As a further check on the accuracy and precision of the method, two National Bureau of Standards standard samples were run by the recommended method; the results are shown in Table II. The standard deviations and the confidence limits were calculated from the range as suggested by Dean and Dixon (1) for sets of data with up to 10 measurements. In the case of sample 127a, solder (30/70), the average absolute error from the accepted value was 0.10%, and the standard deviation of the individual values from the average of the set was 0.082%. The accepted value as listed by the National Bureau of Standards lies within the 95% confidence limits of the average. For sample 53c, lead-base bearing metal, the average absolute error from the accepted value was 0.025%, and the standard deviation of the individual values from the average of the set was 0.039%. The accepted value in this case also is within the 95% confidence limits of the average. These results show that the method is both precise and accurate.

A series of typical dross samples was determined by the recommended procedure with the results shown in Table III. The standard deviations shown were calculated by the method of Dean and Dixon (1). The average standard deviation is 0.11%. The standard deviation of all the determinations in this table is 0.106% as calculated by the usual statistical procedures.

A series of duplicate determinations on 43 samples representing an average week showed a standard deviation of 0.137% for lead values in the same range as those in Table III. The 99% confidence limits on this set of determinations was ±0.04%. The

Table III. Determination of Lead in Lead Smelter Samples

| Sample | | Percentage of Lead | | |
Number	Type	Found	Average	Standard deviation[a]
3961	Battery mud	66.97 67.20	67.09	0.20
3951	Breakdown dross	72.39 72.48 72.50 72.52 72.63	72.50	0.10
3986	Battery scrap dross with battery mud and rubber	74.13 74.25 74.36	74.25	0.14
3973	Battery scrap dross	77.33 77.40 77.45 77.46 77.50	77.43	0.07
3970	Battery scrap dross	78.10 78.13	78.12	0.03
3971	Battery plate dross	80.62 80.64 80.74	80.67	0.07
3963	Sump mud	86.51 86.53 86.70 86.72 86.88	86.67	0.16

Average standard deviation 0.11

[a] Calculated from range and deviation factor.

maximum difference between duplicates was 0.46%, and the average difference between duplicates was 0.16%. These represent a maximum difference between duplicates of approximately 6 parts per thousand and an average difference between duplicates of 2 parts per thousand. All of these samples were handled by four different technicians working on two different shifts.

DISCUSSION

The proposed method is simple, rapid, accurate, and precise and has proved satisfactory for all samples analyzed over a period of several months.

The sharpness of the end point at elevated temperatures overcomes the usual difficulties associated with the use of the F241 indicator for Versenate titrations of metals. A fading of the end point color to purple or pink while the solutions are still hot usually indicates incomplete solution of the lead sulfate in the hot ammonium acetate solution due to incomplete disintegration of the filter paper.

Calcium and barium both interfere in the determination, as calcium produces high values and barium low values. However, these two metals are seldom contaminates of lead drosses and lead-base alloys.

The use of potassium cyanide to complex interfering metals is not necessary, as the digestion and thorough washing of the precipitate with cold dilute sulfuric acid satisfactorily remove all interfering substances with the exception of barium and calcium.

The method should be applicable to the determination of lead in lead-base alloys and lead drosses.

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Determination of Micro Quantities of Cyanide in Presence of a Large Excess of Sulfide

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The Aldridge procedure for the determination of cyanide has been modified to minimize interference from the sulfide ion and thus to eliminate the necessity for its prior removal from the sample. Sulfide is oxidized to sulfuric acid with bromine at the same time that the cyanide is brominated to form cyanogen bromide. Cyanogen bromide then reacts with a pyridine-benzidine mixture to form a colored compound which may be measured colorimetrically. This method is sensitive to less than 0.5 γ of cyanide in the presence of 2500 γ of sulfide and is accurate to $\pm5\%$ at a concentration of 10 γ of cyanide. This procedure has been used for the analysis of refinery dry gases and separator water from the distilling units.

T HE determination of micro quantities of cyanide in the presence of a large excess of sulfide in refinery dry gases and in certain aqueous streams has been handicapped by the lack of a sensitive procedure that does not require sulfide removal. Since casutic solutions from scrubbing of dry gas and separator water from distillation columns may contain several thousand times as much sulfide as cyanide, there is a definite risk of losing the cyanide if prior sulfide removal is necessary.

Several sensitive procedures have been developed (3, 6) for cyanide determination for similar applications, but sulfide must first be removed. The pyridine-benzidine procedure of Aldridge (1) and the pyridine-pyrazolone procedure of Epstein (5) are sufficiently sensitive, and it appeared that sulfide would not seriously interfere with either. Both methods have been successfully applied to the analysis of sewage and industrial wastes (4, 7–9), where the sulfide content was apparently very low. Because of the availability of reagents, the Aldridge procedure was chosen for this work. However, when applied to cyanide solutions containing as little as 5 γ of sulfide, results were 8 to 10% low, and more serious interference was encountered at high sulfide concentrations. This procedure was therefore modified to minimize the interference from sulfide and thus to eliminate the necessity for its removal. Aldridge (1, 2) brominates cyanide and thiocyanate in neutral or acid solution to form cyanogen bromide. This reacts with a mixture of pyridine and benzidine to form a red colored compound which may be measured colorimetrically. Cyanide can be distinguished from, and determined in the presence of, thiocyanate owing to the difference in volatility of the two acids. Hydrogen cyanide is removed by aeration of an acidified aliquot of the solution, and the difference before and after aeration gives the cyanide content.

APPARATUS

Spectrophotometer, Beckman Model B or DU or equivalent, or colorimeter equipped with green filter such as Corning 401.

REAGENTS

The reagents used in these experiments were the same as those described by Aldridge (2), except for the benzidine solution and the benzidine-pyridine reagent. The benzidine was not found to be soluble in dilute hydrochloric acid to the extent indicated by Aldridge. Baker and Adamson's reagent pyridine gave a clear color without being redistilled.

Benzidine Solution. Add 0.5 gram of benzidine to 50 ml. of 0.5N hydrochloric acid. Heat to boiling, cool, and filter the solution. Store the solution in a dark bottle.

Benzidine-Pyridine Reagent. Dissolve 18 ml. of pyridine in 12 ml. of water and add 3 ml. of concentrated hydrochloric acid. Add 10 ml. of benzidine solution and shake until any precipitate dissolves. This solution must be prepared daily.

Sulfide Solution. Dissolve 0.75 gram of sodium sulfide (Na$_2$S·9H$_2$O) in water and dilute to 100 ml. This solution contains 1 mg. per ml. of sulfide.

PROCEDURE

Calibration. Pipet into 25-ml. volumetric flasks, containing 1 ml. of 1% by weight of sodium hydroxide, appropriate volumes of standard cyanide solution to give concentrations of 0, 1, 3, 5, 7, and 10 γ of cyanide, and add 2.5 ml. of sulfide solution to each flask. Add a small (3 × 3 mm.) piece of Alkacid paper to each flask, acidify with glacial acetic acid, then add 0.5 ml. in excess. Immediately after acidification, add 2 ml. of saturated bromine water, and swirl the flask to mix thoroughly. Let the solutions stand with occasional shaking for 10 minutes. If a precipitate of elemental sulfur remains or if the bromine is consumed, add bromine water in 0.2-ml. increments until an excess is present as shown by the color. When the solutions clear, add arsenious acid solution dropwise and gently swirl until the mixtures are free from bromine, then add 0.2 ml. in excess. Add 4 ml. of pyridine-benzidine reagent, and swirl to mix thoroughly. Wait 30 seconds, then add 5 ml. of ethyl alcohol, and dilute to volume with water.
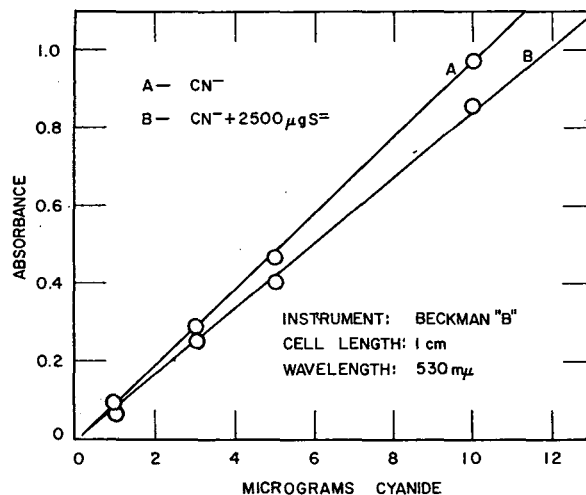

Figure 1. Calibration with and without added sulfide

After 15 minutes measure the absorbance of each solution at 530 mμ. The red color formed is stable for approximately 30 minutes. Prepare a calibration curve of concentration versus absorbance. A new calibration curve should be made for each batch of reagents. Typical calibration curves with and without added sulfide are shown in Figure 1.

Unknown. Determine the sulfide concentration of the unknown by an appropriate method such as electrometric titration (10). If the sulfide content is above 2500 γ per milliliter, take an aliquot for analysis in which the sulfide is below this amount. If the sulfide is less than 50 p.p.m., add sufficient sulfide solution to the volumetric flask to give 100 to 2500 γ of sulfide in the reaction mixture. Failure to add this sulfide will cause a high cyanide value. Pipet 1 to 5 ml. of the aqueous solution or aliquot into a 25-ml. volumetric flask and proceed as described above. Measure the absorbance at 530 mμ against a reagent blank containing approximately the same sulfide concentration as the sample tested. Determine the cyanide concentration by reference to the calibration curve.

Table I. Effect of Added Sulfide on Absorbance

| CN⁻, γ | S⁻⁻ Added, γ | S⁻⁻ in Blank, γ | | |
| | | 200 | 1000 | 2500 |
		Absorbance		
4	200	0.335	0.320	0.300
4	1000	0.350	0.330	0.310
4	2500	0.370	0.350	0.330

EXPERIMENTAL

Determination of Wave Length of Maximum Absorption. The color was developed in a solution containing 10 γ of cyanide. The absorption spectrum was determined between 380 and 700 mμ with a Beckman Model B spectrophotometer. No sharp peaks were found, but the maximum absorbance occurred at 530 mμ. All subsequent measurements were made at this wave length.

Optimum Concentration Range. Since varying amounts of bromine water must be added, depending on the amount of sulfide present, it is necessary to dilute the reaction mixture to a definite final volume before absorbance measurement. When 30 γ of cyanide in 25 ml. of final solution was exceeded, a red precipitate formed. This was prevented by the addition of 5 ml. of ethyl alcohol immediately after the addition of the benzidine-pyridine reagent.

With a Beckman Model B spectrophotometer using a 1-cm. cell, the absorbance approaches 1 when the color is developed from 10 γ of cyanide in 25 ml. of solution. As reasonably accurate measurements can be made up to an absorbance of 2.5 with this instrument, about 25 γ of cyanide in 25 ml. of final solution is the maximum amount which can be determined without dilution. However, 10 γ in 25 ml. is probably the upper limit for most colorimeters unless smaller cells are used.

Application to Thiocyanates. Solutions of ammonium thiocyanate were substituted for potassium cyanide and the calibration was repeated. Calculation of the observed data into terms of equivalent cyanide concentrations gave a series of points which were on the previous calibration curve. Accordingly, the method is equally applicable to either ion if the concentration levels are properly adjusted.

Effect of Sulfides. Solutions containing various concentrations of cyanide (0 to 10 γ) and sulfide (0 to 2500 γ) were analyzed by the Aldridge procedure, except that the solutions were diluted to 25 ml. before measurement of absorbance. The results are shown in Figure 2. Even 5 γ of sulfide caused a significant error, while larger amounts of sulfide caused up to 40% decrease in absorbance. At the higher sulfide concentrations much of the sulfide was not oxidized beyond the elemental state when the bromine color disappeared. Therefore, a series of experiments was undertaken in which an excess amount of bromine required to oxidize the sulfide to sulfuric acid was added in addition to the 0.2 ml. required for the bromination of the cyanide present. These tests were made on solutions containing 4 γ of cyanide and from 200 to 2500 γ of sulfide in a final volume of 25 ml. After standing with occasional shaking until all of the sulfur was dissolved, the color was developed as described above. When measured against reagent blanks containing the same concentration of sulfide, a nearly constant absorbance was obtained for a given cyanide concentration. Therefore, if the same amount of sulfide is added to the reagent blank as is present in the sample to be tested, a single calibration curve may be used. The results of these experiments are shown in Table I.
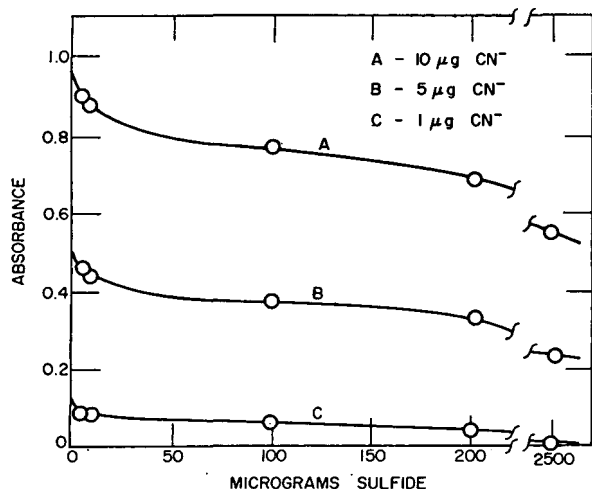
A similar experiment on a cyanide solution containing 5000 γ of sulfide was unsuccessful, as no definite red color developed. It appears, therefore, that the upper tolerance for sulfide under these conditions is between 2500 and 5000 γ.

Applications. Synthetic samples containing varying amounts of sulfide and cyanide were analyzed by this modified procedure. These results are presented in Table II. Cyanide has been determined in waste water which is separated from various refinery streams. Several examples of these determinations are presented in Table III.

Table II. Cyanide Determination in Synthetic Samples

CN⁻⁻ Added, γ	S⁻⁻ Added, γ	CN⁻⁻ Found, γ
0.4	1000	0.3
0.6	1000	0.6
1.0	1000	0.98, 1.04
2.0	1000	2.00, 2.08
3.0	2500	2.90, 2.99
5.0	2500	5.00, 5.11
10.0	2500	10.3, 9.95

Table III. Cyanide Determination in Water Separated from Various Distillates

Source of Water	Sulfide, P.P.M.	Cyanide, P.P.M.
Cat. cracker fractionator accumulator	2500	5.4
Propane, propylene absorber	400	23.0
Straight-run naphtha accumulator	0.0	0.7
Pressure distillate tops	270	1.2

CONCLUSIONS

The presence of sulfide inhibits the color development in the original procedure. At high sulfide concentration this may be due in part to competition of the sulfide for the available bromine. However, as shown in Figure 2, very small amounts of sulfide cause a significant drop in absorbance, although an excess of bromine may still be present. The modified procedure minimizes this interference and has been successfully applied to the analysis of synthetic and contaminated water samples. Less than 0.5 γ of cyanide can be detected in the presence of 1000 γ of sulfide. At the 10-γ level, this method is repeatable to ± 5% of the cyanide present.

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Figure 2. Effect of sulfide on absorbance with fixed bromine addition

A – 10 μg CN⁻
B – 5 μg CN⁻
C – 1 μg CN⁻

Improvements in Karl Fischer Method for Determination of Water

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A substantial gain in stability can be achieved by substituting methyl Cellosolve for methanol in the formula for Karl Fischer reagent. In addition to its greater stability, the modified reagent extends the applicability of the method by permitting an appropriate choice of sample solvent. Methanol is an undesirable solvent in some cases because of interfering side reactions with the sample. A mixture of ethylene glycol and pyridine permits the direct titration of water in ketones and some aldehydes and improves the direct titration of water with Fischer reagent to the reverse dead-stop end point.

FOR the quantitative determination of water in various materials, the Karl Fischer reagent (5) has found continually expanding application. The principal advantages of this versatile reagent lie in the broad applicability and the rapidity with which titrimetric determinations can be made. Because substances which normally interfere in the method often react quantitatively or can be converted to inert derivatives, and because many compounds other than water can be determined by means of stoichiometric water-producing or water-consuming reactions with other substances, the method has a wide scope. One disadvantage of the reagent is its relative instability, which necessitates frequent standardization.

It has become common practice to prepare Fischer reagent with methanol, which serves the dual purpose of acting as diluent and reacting with the pyridine–sulfur trioxide formed in the primary reaction of water with iodine, sulfur dioxide, and pyridine (7).

Although this methanolic reagent is satisfactory for most purposes, a reagent prepared with the monomethyl ether of ethylene glycol (methyl Cellosolve) in place of methanol has several advantages, whereas no difficulty attributable to this substitution of diluents has been observed in 15 years of use by the authors. Reagent prepared with methyl Cellosolve is appreciably more stable than that prepared with methanol, is less subject to interfering side reactions, and has some advantage in the direct titration with Fischer reagent to the reverse dead-stop end point. Simple, but effective, apparatus for dispensing the reagent and conducting the titrations also have been thoroughly tested.

MODIFIED FISCHER REAGENT

The reagent suggested here is essentially the same as that employed by Karl Fischer (5), except that methyl Cellosolve is substituted for methanol and the quantity of solvent used is decreased so that the effective strength of the reagent is approximately three times as great. The molar ratio of iodine to pyridine to sulfur dioxide is maintained at 1:10:3. It is essential that the ingredients of the reagent be pure and free of water. However, commercial methyl Cellosolve (Carbide & Carbon Chemicals Co.) and c.p. pyridine (J. T. Baker Chemical Co.) can generally be used without further purification. If the water content of the methyl Cellosolve is appreciably greater than 0.1%, it may be decreased by distilling off about 5% through a small column and using the remaining 95%, but this has seldom been necessary.

For each liter of solution, dissolve 133 grams of c.p. iodine in 425 ml. of c.p. pyridine in a dry, glass-stoppered bottle, add 425 ml. of anhydrous methyl Cellosolve, and cool in an ice water bath.

Add, in small increments and with constant swirling, 70 ml. of anhydrous liquid sulfur dioxide from a graduated cylinder and mix thoroughly. The water equivalent of this reagent is approximately 6 mg. of water per ml. of reagent.

The relatively greater stability of Fischer reagent prepared with methyl Cellosolve compared with that prepared with methanol is shown in Figure 1. The curves show the percentage decrease in strength from the original standardization on standing.
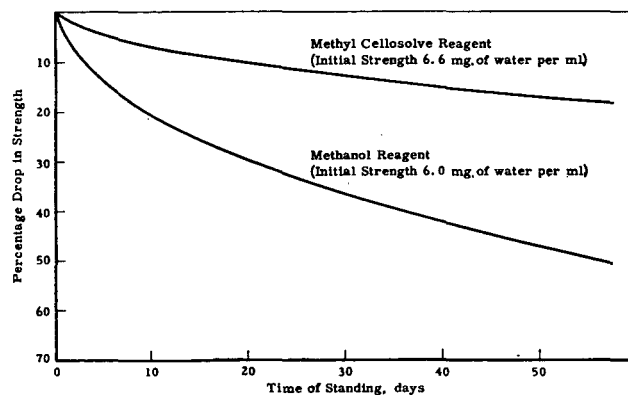

Figure 1. Stability of Karl Fischer reagent prepared with methanol and methyl Cellosolve

The stability of both the methyl Cellosolve reagent and the methanol reagent is improved by decreased concentration. Stability measurements on more dilute reagents, prepared with larger amounts of alcoholic solvent than recommended above, are given in Table I. In these tests, the water contents of the components of both reagents were identical, such that the theoretical strength of each reagent was calculated to be 4.3 mg. per ml., based on the weight of iodine added and allowing for the known water contents of the solvents employed. The results clearly show the greater stability and more nearly theoretical strength of the methyl Cellosolve reagent. Although the more dilute reagent is somewhat more stable than the 6 mg. per ml. reagent described above (compare Table I with Figure 1), the stronger reagent tends to give sharper end points and readily permits the titration of as much as 0.2 to 0.3 gram of water in samples and in standardizations against weighed amounts of water. However, the choice of reagent strength is largely dependent upon personal preference and the particular application for which the reagent is to be used.

Table I. Stability of Dilute Fischer Reagent Prepared with Methanol and Methyl Cellosolve

Age, Days	Methanol Reagent			Methyl Cellosolve Reagent		
	Mg./ml.	% of theory[a]	% drop from first value	Mg./ml.	% of theory[a]	% drop from first value
0.3	2.84	66	..	3.63	84	..
3	2.69	63	5	3.54	82	2
6	2.66	62	6	3.52	82	3
10	2.53	59	11	3.45	80	5
14	2.43	57	14	3.39	79	7
20	2.33	54	18	3.35	78	8
30	2.21	51	22	3.31	77	9

[a] Theoretical strength of each reagent = 4.3 mg./ml.

Mitchell and Smith (8) have found it advisable to prepare a very stable stock soluton of iodine, pyridine, and methanol, and to add the sulfur dioxide only a few days prior to use; they further recommend that the mixture be prepared in small batches—e.g., 3 liters at a time. However, owing to the greater stability of the reagent prepared with methyl Cellosolve, it is practical to prepare 9-liter quantities of finished reagent. This reagent usually decreases in strength over a period of several months only to the extent of 1 mg. of water per ml. of reagent. Because of the increased stability of the reagent, it need be standardized only once during an 8-hour period of use for highest accuracy demands. Moreover, the reagent prepared with methyl Cellosolve permits the elimination of methanol from the titration mixture; methanol is sometimes undesirable because of side reactions with components of the sample.

BURET ASSEMBLY

While several commercial, all-glass, automatic buret assemblies are suitable for storing and dispensing Fischer reagent, the specially constructed system illustrated in Figure 2 is suggested for long, continuous service. This assembly consists of a 9-liter serum bottle, a modified 50-ml. precision buret, and connecting glass tubings fitted with spherical joints, constructed to afford ease of assembly and considerable flexibility. All stopcocks in the connecting lines are spring-loaded with light tension springs in order to minimize leakage; a large drying tube containing Indicating Drierite affords adequate protection against atmospheric moisture. Sisco 300 lubricant (Swedish Iron and Steel Co., 17 Battery Place, New York, N. Y.) and Apiezon N (Shell Oil Co., New York, N. Y.), are satisfactory as lubricants for the ground-glass surfaces.
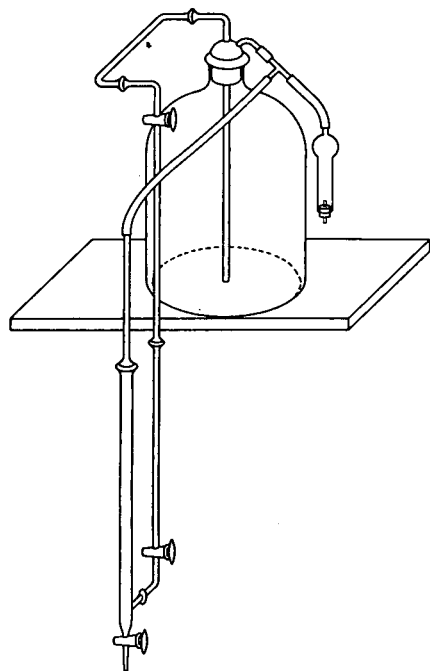
Figure 2. All-glass, siphon-type buret assembly

Modification of the buret involves the addition of a spherical joint to the top of the buret and addition of a side arm below the 50-ml. graduation of the buret; the side arm also terminates in a spherical joint.

In this laboratory it has been found convenient to mount burets by means of two spring-loaded clamps, one above and one below the graduated portion of the buret. The lower one is clamped to the buret in the area below the graduation but above the side arm; to accomplish this without modification of the buret requires that the clamps be trimmed to avoid masking the extreme lower portion of the graduations. The clamps in turn are fastened to horizontal metal strips which extend over the

Table II. Effects of Solvents on the Determination of Water in Ketones with Fischer Reagent

(Visual end point procedure)

Sample Analyzed	Solvent[a] Reagent	Solvent[a] Sample	Titration Temp., ° C.	Recovery, %	Nature of End Point Color change	Nature of End Point Stability
1.17% water in acetone	M	M	25	130	Good	Fades very rapidly
	M	M	0	107	Good	Fades rapidly
	M	P	25	104	Poor	Fades
	M	P	0	98	Poor	Fades slowly
	MC	P	0	101	Very poor	Stable
	MC	G-P	25	101	Good	Fairly stable
	MC	G-P	0	100	Good	Stable
1.15% water in methyl ethyl ketone	M	M	25	115	Good	Fades rapidly
	M	M	0	105	Good	Fades
	M	P	25	103	Poor	Fades slowly
	M	P	0	101	Poor	Fairly stable
	MC	P	0	98	Very poor	Stable
	MC	G-P	25	101	Good	Fairly stable
	MC	G-P	0	100	Good	Stable

[a] M = methanol, MC = methyl Cellosolve, P = pyridine, G-P = ethylene glycol + pyridine (4:1).

length of the titration stand. Behind each buret and fastened to the horizontal metal strips there is a vertical strip of white plastic or metal with a white enamel finish to aid in reading the buret.

TITRATION SOLVENT

Although methanol usually is a highly satisfactory solvent for the sample, as well as for the reaction products, in the titration of water with Fischer reagent, it has certain disadvantages. By a study of several sample solvents it was found that a mixture of ethylene glycol and pyridine (4 to 1, by volume) is satisfactory and widely applicable. Pyridine reduces the viscosity of the ethylene glycol to a considerable extent; it also has good solvent properties and in some cases avoids side reactions which may otherwise occur.

When methanol is used as a solvent for samples containing carbonyl compounds it is necessary to convert these compounds to the relatively inert cyanohydrins (10) in order to avoid interfering water-producing reactions between methanol and the carbonyl compound.

To determine water directly in the presence of ketones, other investigators have recommended the use of a reagent high in pyridine content (10, 12) or an excess of pyridine instead of methanol as solvent for the sample (10). Results obtained by these modifications are reasonably accurate, but the end points still fade slowly and are rather indistinct.

When the glycol-pyridine solvent mixture is employed in conjunction with Fischer reagent prepared with methyl Cellosolve, the titration can be performed in the presence of free ketones without interfering side reactions (provided the titration mixture is maintained near 0° C. throughout the titration), and the color change at the end point is good. Results with various solvents in the presence of ketones are shown in Table II

Large amounts of the lower aldehydes (except formaldehyde) interfere even when glycol-pyridine sample solvent at 0° C. is employed with the methyl Cellosolve reagent. Such samples must first be reacted with hydrogen cyanide to form the cyanohydrins (10). However, the interference from aromatic aldehydes and from C_6 and higher aliphatic aldehydes is slight when less than 1 or 2 grams of such aldehydes in 10 ml. of glycol-pryidine at 0° C. are titrated with Fischer reagent prepared with methyl Cellosolve.

Although most organic acids do not cause serious errors in water titrations by virtue of the relatively slow reaction between the acid and methanol, possible interference by acids is also eliminated by means of the reagent and solvent described. If ketones and organic acids are known to be absent, the titration may be carried out at room temperature, and methanol or other suitable solvents for the sample may be employed as the titration solvent.

Table III.   Solubility of Water in Hydrocarbons Determined by Means of Fischer Reagent

(Visual end point procedure, using 15 ml. of ethylene glycol as the lower phase and side-well flask)

Temp., °C.	Toluene[a] Sample size, g.	Water, wt. %	Diisobutylene[a] Sample size, g.	Water, wt. %	Propylene Tetramer[a] Sample size, g.	Water, wt. %
0	257	0.0293	152	0.0052	148	0.0091
	267	0.0293	157	0.0053	156	0.0099
			170	0.0051		
25	..	..	149	0.0149	..	..
			200	0.0154		
27	172	0.0605	..	..	154	0.0176
	176	0.0599			161	0.0175
50	80	0.113	163	0.0388	123	0.0281
	81	0.114	200	0.0384	127	0.0287
75	62	0.239	110	0.079	75	0.072
	62	0.243	115	0.078	81	0.068
	63	0.246	154	0.081		
	64	0.247				
	64	0.231				
	69	0.231				

[a] Saturated with water at temperature indicated.

ELECTROMETRIC DEAD-STOP TITRATION

In the reverse dead-stop method for detecting the end point in the direct titration of water with Fischer reagent, temporary drifting deflections of the galvanometer are frequently observed before the true end point when methanol is employed as the sample solvent. To avoid this difficulty, the addition of excess Fischer reagent and back-titration with standard water in methanol solution to the dead-stop end point is frequently recommended (9, 12). Modifications of the dead-stop apparatus, employing increased potential across the electrodes (2) or increased current (3), have been reported to be satisfactory for the direct titration with Fischer reagent, and an automatic titration apparatus incorporating a timer mechanism which distinguishes between transitory currents produced by temporary excesses of unconsumed Fischer reagent and the true end point has been described (6) and marketed.

Although these instruments appear to be satisfactory for most direct titrations, a further decrease in tendency for temporary currents in advance of the true end point can be obtained by the use of the glycol-pryridine solvent mixture and the modified Fischer reagent described above. Under these conditions, the true end point can be quickly determined by direct titration, even when a dead-stop apparatus with low potential (20 mv.) across the electrodes and a low current (10 μa.) at the end point is used. Apparently, a slightly larger concentration of Fischer reagent (and consequently larger concentrations of iodine and sulfur dioxide resulting in more rapid reaction with water) is required to depolarize the electrodes in glycol-pyridine solution than in methanol. The sensitivity of the end point is slightly lower in glycol-pyridine than in methanol, but is adequate for nearly all analytical purposes, giving more precise results than the visual end point procedure.

An alternative solvent mixture, consisting of 1M sulfur dioxide and 1M pyridine in methanol, also permits direct titration with Fischer reagent to the dead-stop end point when the presence of methanol is not objectionable because of side reactions. The use of this solution as the titration solvent gives just as high end-point sensitivity as the use of methanol alone and decreases the tendency for temporary, drifting deflections before the end point by increasing the rate of the reaction with water.

A convenient titration vessel for reverse dead-stop titrations is shown in Figure 3 (left) (this apparatus is available from Rankin Glassblowing Co., 3920 Franklin Canyon Road, Martinez, Calif.). An interchangeable platinum electrode pair is inserted into a 250-ml. volumetric flask through a side arm carrying a standard-taper ground-glass joint. A volumetric flask is used because the long, narrow neck provides a gradient between the dry air in the flask and the moist atmosphere outside. When the moisture content of the atmosphere is not too high, the flask also protects

the buret tip while it is inserted in the neck. As a precaution, any moisture which collects on the buret tip while it is exposed to the atmosphere during a titration should be frequently removed with a dry cloth or tissue by wiping with a downward motion. A flexible closure, such as a rubber dam with a hole for introducing the buret tip, between the neck of the flask and the buret is desirable in humid climates. The flask is swirled by hand during titration. In laboratories which employ Fischer reagent for various purposes—for example, determination of organic functional groups by means of water-producing or water-consuming reactions—these titration vessels are more convenient than stationary titration vessels in permanent setups because they can be used as the containers in which preliminary reactions are carried out in water baths, ovens, etc., and they permit simultaneous preparation of a number of samples. One electrode pair is sufficient for many flasks, as the side arm can be kept closed with a standard-taper glass plug until just before the titration step.

For the highest accuracy, Fischer reagent should be standardized in the same manner (visual or dead-stop end point procedure) as it is to be used. The reverse dead-stop technique has been found to yield a standardization value approximately 1% higher than the value by the visual end-point procedure. Choice of titration solvent appears to make very little, if any, difference in standardization values, provided the reagent (or the solvent) contains a reactive alcohol.

TITRATION IN TWO-PHASE SYSTEM

For the determination of water in materials such as hydrocarbons which, because of low water content, require a large sample, a procedure whereby all of the water in the sample is concentrated in a relatively small volume is obviously desirable. Solvent systems which give a large quantity of homogeneous solutions (11) do not yield the highest accuracy because of the indefinite nature of the end point obtained in a highly diluted titration mixture. Extraction of the water from the sample with methanol or ethylene glycol (11) is generally satisfactory. Ethylene glycol (without pyridine) is to be preferred because it is less miscible with hydrocarbons than is methanol and is an efficient water-extracting agent. A marked improvement in results and greater simplicity are attained by performing the extraction and titration in the same vessel.
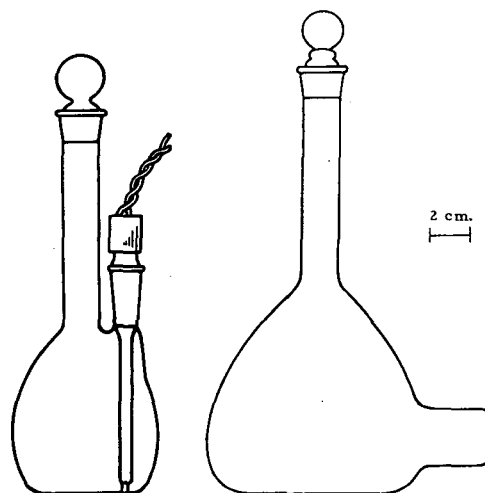

2 cm.

Figure 3.   Titration flasks

Left.   Dead-stop titration flask
Right.   Side-well titration flask

For this purpose, the special titration flask shown in Figure 3 (right) has been employed (this apparatus is available from Rankin Glassblowing Co., 3920 Franklin Canyon Road, Martinez, Calif.). This flask is made from a borosilicate glass 500-ml. volumetric flask by adding a side well of 20-ml. capacity as shown in the figure. The iodine color is retained in the lower (glycol) phase and is easily observed in the side well. The glycol phase is pre-

titrated with Fischer reagent to the end-point color of a matching standard prepared in a similar flask. The hydrocarbon sample is then added and shaken thoroughly in the stoppered flask to extract the water into the glycol phase, with care that the upper portion of the neck of the flask does not become wet with glycol. The mixture is then titrated with Fischer reagent until the lower phase is near the end point color. The flask is again stoppered and shaken as before. The phases are allowed to separate and the titration is then completed with small increments of titrant until the color of the lower phase, as observed in the side well, matches the original color standard. When dense or viscous oils are analyzed, the addition of a light petroleum fraction before the pretitration of the glycol phase assists in the separation of phases.

The application of this procedure for the determination of solubility of water in hydrocarbons is shown in Table III. Replicate determinations indicated excellent precision of the method, except for the experiments at 75° C., where difficulties in sampling caused somewhat poorer precision. The reverse dead-stop end-point procedure also can be employed, using the dead-stop titration vessel (Figure 3, *left*). As long as the electrodes are immersed in the lower (glycol) phase, the end point can be readily determined when the flask is gently swirled.

CONCLUSIONS

The usefulness of the reagent, solvents, and apparatus described has been demonstrated in the laboratories of the authors and others who have adopted several of these techniques but have not included complete details in their publications (1, 4). It is felt that these techniques will be found to be of general value to others employing Karl Fischer reagent for aquametric analysis.

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Apparatus for the Pyrohydrolytic Determination of Fluoride and Other Halides

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An apparatus for the pyrohydrolytic determination of fluoride and other halides is constructed entirely of nickel and stainless steel rather than platinum and quartz. The apparatus is economical, compact, and easily manipulated. Approximately 5000 determinations were made in 22 months before replacement of the reactor tube was necessary. The tube had been maintained at 1000° C. for approximately 3500 hours with daily cooling and reheating.

THE method of pyrohydrolysis for the determination of fluorides was first exploited by Warf and coworkers (1), who developed a method for the determination of fluoride in which steam is passed over a heated sample in a platinum apparatus. The volatile products of the pyrohydrolysis are then condensed and titrated:

$$MF_2 + H_2O \rightarrow MO + 2HF$$
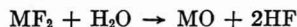
This reaction, in the case of many fluorides, is slow and is usually conducted at temperatures of the order of 1000° C. This high temperature and the known corrosive action of solutions of hydrochloric, hydrofluoric, and hydrobromic acids on metals dictates the use of extremely resistant structural materials. Warf and coworkers (1) used platinum and quartz in the fabrication of their apparatus. This report concerns the use of nickel for this purpose.

It was desired that the apparatus be constructed of material of similar durability to platinum and require less initial expenditure. Nickel was chosen as the metal most likely to meet these requirements. In order to reduce the cost of the apparatus still further, stainless steel was to be used wherever possible. In the

design of the apparatus, prime consideration was given to these four factors: cost, durability, ease of manipulation, and compactness.

DESCRIPTION OF APPARATUS

The apparatus, in its final design, is shown in Figure 1. Its over-all dimensions are 28 inches high, 21 inches deep, and 15 inches wide. Thus, it is possible to place two sets of apparatus in a 6-foot fume hood and still have ample space for titration and any other necessary operation. Steam is generated in a 1-liter flask, which is heated by an electric hot plate. The steam is passed through a line made of 1-inch (inside diameter) 316 stainless steel, which is heated by a 420-watt, 5-inch furnace operated at 1000° C. A ball joint is used to connect the steam line to the reaction tube, which is made of nickel. The ball joint was fabricated by the machine shop and was patterned after the familiar glass ball joints. Fabrication was from nickel metal.

Pertinent dimensions for the apparatus shown are: male joint, $1^9/_{16}$-inch outside diameter in width; female, $1^5/_{16}$-inch outside diameter broadening to $1^5/_8$-inch outside diameter. The seal is made tight by the weight of the reactor tube. The reaction tube is heated by a 9-inch, 580-watt furnace. The condenser jacket is of 316 stainless steel, as is the handle. The joint is made leakproof by means of the position and weight of the handle which is 7.25 inches in over-all length, 4 inches of which is a 1-inch bar and 3.25 inches of 0.25-inch 316 stainless steel rod. The handle weighs approximately 1.4 pounds. A photograph of the ball joints and ball joint lock is shown in Figure 2.

The apparatus is positioned so that the handle of the door lock is directly in front of the operator. Thus, the receiver vessel for the condensate is located directly to the rear. This position is the most advantageous, as it makes for simplicity in introducing the sample into the tube and permits a full view of the ball joint and thus virtually eliminates any inadvertent loss of samples due to escape of hydrolysis products through an improper fitting of the lock.

The apparatus was designed to use steam either generated by a water boiler or obtained from the plant steam line. A condensing

trap may be viewed at the bottom of the preheater. A water boiler was found to be the more reliable source of steam, as steam taken from steam lines was often contaminated with titratable acidic constituents.

DISCUSSION

This apparatus has proved entirely satisfactory in all respects over a period exceeding 2 years of almost constant use. With
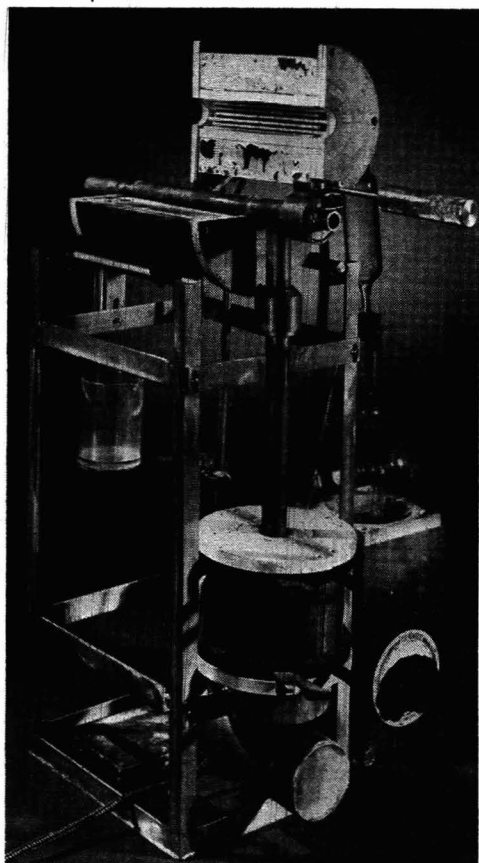

Figure 1. Front view of apparatus for the pyrohydrolysis of halides, showing nickel reactor tube and ball joints

respect to durability, it has performed beyond original expectations. Approximately 5000 determinations were made over a period of 22 months before replacement of the reaction tube was necessary. It was estimated that the tube had been maintained at 1000° C. for approximately 3500 hours with daily cooling and reheating. No rupture of the tube was noted despite this treatment. When the tube was removed from the furnace and cooled in air, spalling occurred, probably due to the high cooling rate. A nickel insert was welded to the tube and the apparatus has been used for 8 months since replacement.
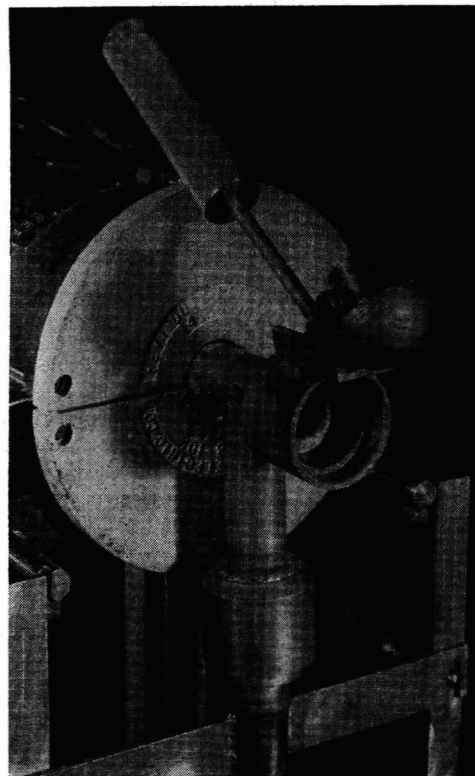
Figure 2. Close-up of sample entrance port and ball joint lock

A new tube is conditioned by passing preheated steam through the reactor tube for several hours. This treatment removes any constituents that may be titratable with base. Nearly 1500 determinations have been performed during this period. The replacement of the nickel reaction tube in direct contact with the furnace has been the only maintenance required in this period, which attests to the durability and economy of the apparatus.

Monel metal has also been suggested as a possible material for this apparatus, but has not been tested in this laboratory. Stainless steel is not satisfactory because of its nonresistivity to steam. The original model of the nickel apparatus was built with a stainless steel condenser line. Subsequent testing of the apparatus revealed that this was not satisfactory because significant amounts of iron are dissolved by the acid condensate which interferes with the titration of the acid with standard alkali solution.

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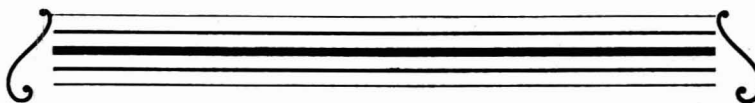
Determination of Phosphate in Perchloric and Sulfuric Acid Solutions of Uranium Phosphates

Ion Exchange Separation and Amperometric Determination

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The amperometric titration of phosphate with uranyl acetate has been applied to the determination of phosphate in sulfuric and perchloric acid solutions of uranium phosphates. Uranium is removed by cation exchange separation with Dowex 50 resin. The coefficient of variation was 0.6% for solutions containing 65 to 150 mg. of phosphate and 180 mg. of uranium (as UO_2^{++}) and having acid concentrations as high as $8M$ perchloric acid and $11M$ sulfuric acid.

THIS investigation was undertaken to study the applicability of an amperometric method for the determination of phosphate in solutions of uranium phosphates which are strongly acidic with respect to sulfuric and perchloric acids. The range in concentration of phosphate considered in this report is of the order of 0.1 to $0.4M$.

Kolthoff and Cohn (2) have shown that phosphate in low concentrations (0.01 to $0.0002M$) can be determined by amperometric titration with uranyl acetate. The method depends upon the precipitation of a slightly soluble alkali uranyl phosphate such as:

$$UO_2^{++} + K^+ + PO_4^{---} \longrightarrow KUO_2PO_4 \qquad (1)$$

The alkali uranyl phosphates are very slightly soluble in weakly acidic solutions, and their solubility may be further decreased by the addition of alcohol. Kolthoff and Cohn (2) carried out the titration in acidic solutions (pH 3.5) having an ethyl alcohol concentration of 20%, and used either potassium chloride or nitrate as the supporting electrolyte. The end point was found by measuring the diffusion current of uranyl ion at an applied voltage of about -0.7 volt (vs. S.C.E.). They were able to determine phosphate in concentrations as low as $0.0002M$ in solutions of pure potassium dihydrogen phosphate with an accuracy of 1% or better.

This report describes the application of a combination of the ion exchange separation of phosphate from interfering substances and the amperometric titration of phosphate with uranyl acetate, to the determination of phosphate in sulfuric and perchloric acid solutions of uranium phosphates.

REAGENTS AND APPARATUS

Reagents. Uranyl Acetate, Stock Solution, $0.105M$. Dissolve 45 grams of uranyl acetate dihydrate and 8 ml. of glacial acetic acid in about 800 ml. of water. The salt dissolves slowly and may require several hours' standing, with occasional mixing. Dilute to 1 liter with water.

Uranyl Acetate, Titrant Solution, $0.035M$. Dilute 1 volume of the stock $0.105M$ solution with 2 volumes of water. Each milliliter is equivalent to 3.3 mg. of phosphate. Standardize the solution by the amperometric titration of aliquots of a solution of potassium dihydrogen phosphate as follows: Take an aliquot containing about 10 mg. of phosphate and transfer it to the titration cell. Dilute to 50 ml. with water, add 5 drops of bromocresol green solution, and adjust the pH with dilute sodium hydroxide and $0.1M$ hydrochloric acid until the solution has a shade of yellow green. Add 0.5 ml. of $0.1N$ acetic acid, 7 ml. of $1M$ potassium chloride, and 17 ml. of ethyl alcohol, then titrate. Diffusion current readings are conveniently taken at 2.0, 2.5, 3.25, 3.50, 3.75, and 4.00 ml. of standard solution.

Standard Phosphate Solution, 1 mg. per ml. Dissolve 1.433 grams of potassium dihydrogen phosphate—dried at 120° C.—in water, and dilute to 1 liter.

Potassium Chloride, $1M$. Dissolve 75 grams of potassium chloride in 1 liter of water.

Bromocresol Green Indicator, 0.1 w./v. %. Dissolve 50 mg. of the solid indicator, Eastman Organic Chemicals Co., No. 1782, in 50 ml. of ethyl alcohol.

Sodium Hydroxide, $2M$. Dissolve 80 grams of solid sodium hydroxide in 1 liter of water.

Hydrochloric Acid, $0.1M$. Dilute 1 ml. of concentrated hydrochloric acid to 120 ml. with water.

Acetic Acid, $0.1M$. Dilute 1 ml. of glacial acetic acid to 170 ml. with water.

Absolute Alcohol. Reagent grade ethyl alcohol, 95 volume %, may be substituted.

Apparatus. ION EXCHANGE COLUMN. Leach overnight the contents of a 1-pound bottle of Dowex 50 (Nalcite HCR) resin, 50- to 100-mesh, with about 2 liters of $2M$ sodium hydroxide. Wash with water by decantation and leach for 1 hour with three successive, 1-liter portions of $6M$ hydrochloric acid. Wash several times, by decantation, with water to remove fines.

Shorten the outlet tubing of a Jones reductor tube of the usual dimensions (body of tube approximately 21 inches long and $7/8$ inch in diameter) by cutting it off about 1.5 inches below the stopcock. Fit the outlet tube with a short length of tubing, carrying a Hoffman pinch clamp and a short glass delivery tip. Half-fill the tube with water, then expel the air bubbles from the outlet tube. Insert a plug of glass wool into the body of the tube and pack it tightly at the bottom, working it with a glass rod to expel entrapped air. Mix the resin with water and with the aid of a wash bottle, wash it into the column. Continue to add resin until a bed 24 cm. deep is obtained. Pass 200 ml. of 5 to $6M$ hydrochloric acid through the column, followed by several hundred milliliters of water. The column is then ready for use.

In operation, the flow rate of liquid through the column can be regulated by the pinch clamp with the stopcock wide open. The maximum flow rate is determined by measuring the column of water delivered per minute when the liquid level is at the top of the tube. The preadjusted setting of the pinch clamp may then be left undisturbed while a sample is being put through the column and the flow can be stopped or started at will with the stopcock without altering the flow rate. This permits shutting off the flow just when the liquid level has reached the top of the resin bed, so that each portion of wash liquid can be drained completely before the next portion is added. This aids the effective elution of the column with a minimum of wash liquid, and is a convenience when several columns are operated simultaneously.

After separation of uranium, the column should be eluted with about 200 ml. of 5 to $6M$ hydrochloric acid, at a flow rate of approximately 10 ml. per minute, and finally washed with water.

POLAROGRAPH. A manually operated Fisher Elecdropode was used.

TITRATION ASSEMBLY. The titration cell consisted of a cylindrical glass vessel, 4.8 cm. in diameter and 10 cm. high, attachable to the buret assembly by a 35/55 spherical ball joint. The usable volume of the cell was about 100 ml. The cell carried a side arm, 8 mm. in diameter, through which the gas inlet tube was inserted, and a wide side arm near its bottom in which was mounted a 20-mm. fritted-glass disk. This arm was filled with saturated potassium chloride–agar gel up to the fritted disk, the gel extending through a flexible bridge-tube of Tygon tubing into a saturated calomel electrode.

SOURCE OF INERT GAS. Helium (nitrogen or argon may be used) was freed from oxygen by passing it through a series of three gas-scrubber bottles half-filled with Oxsorbent solution (Burrell Corp., Pittsburgh, Pa.), $2M$ sodium hydroxide, and an aqueous solution containing 20 volume % of alcohol, respectively.

PROCEDURE

Procedure Adopted. Transfer a 5-ml. aliquot which contains at least 25 mg. of phosphate to a 50-ml. Erlenmeyer flask and

Table I. Polarographic Reduction of Uranyl Ions in Presence of Certain Anions

| Concentration, Molar | | | | | $E_{1/2}$, Volt | | Region of $I_{Constant}$, Volt |
Acetate	Chloride	Per-chlorate	Sulfate	Nitrate	First wave	Second wave	
0.002	0.1	...	...	...	-0.25	-1.27	-0.33 to -0.80
0.003	...	0.27	...	...	-0.26	-1.28	-0.35 to -0.95
0.003	...	...	0.27	...	-0.53	-1.3	-0.85 to -1.15
0.003	0.067	0.067	...	0.10	-0.28	-1.2	-0.40 to -1.00

Table II. Precision of Amperometric Titration of Phosphate with Uranyl Acetate in Various Supporting Electrolytes

Composition of Supporting Electrolyte	KH_2PO_4 Taken, Ml.	Titrant $UO_2(C_2H_3O_2)_2$, Ml.	Phosphate, Mg.	Ratio $UO_2(C_2H_3O_2)_2$ per Ml. of KH_2PO_4	Deviation from Over-all Average Ratio
0.1N Cl	4.00	2.90	9.60	0.725	-0.002
	5.00	3.65	12.00	0.730	+0.003
	6.00	4.33	14.40	0.723	-0.004
				Av. 0.726	
0.07M ClO₄	4.00	2.91	9.60	0.728	+0.001
0.10M NO₃		2.90		0.725	-0.002
0.07M Cl	5.00	3.63	12.00	0.726	-0.001
		3.62		0.724	-0.003
		3.67		0.734	+0.007
		3.62		0.724	-0.003
				Av. 0.727	
0.07M SO₄	4.00	2.92	9.60	0.730	+0.003
0.10M NO₃		2.91		0.728	+0.001
0.07M Cl	5.00	3.62	12.00	0.724	-0.003
		3.62		0.724	-0.003
		3.63		0.726	-0.001
		3.61		0.722	-0.005
				Av. 0.726	
Over-all average volume ratio of standard solutions				0.727	
Standard deviation				0.0033	
Coefficient of variation				0.5%	

neutralize with concentrated ammonium hydroxide to a methyl red end point. The contents of the flask should be cooled in an ice bath and swirled constantly to prevent spattering. Add 5 ml. of concentrated nitric acid, and boil gently for 1 or 2 minutes to oxidize the uranium to the hexavalent state. Dilute the solution with about 300 ml. of water. Pass the solution through a 24 × 2 cm. column of Dowex 50 resin at a maximum flow rate of 5 ml. per minute. Catch the effluent in a 500-ml. volumetric flask. Rinse the beaker with water, and pour into the column. When the level of liquid in the column reaches the top of the resin bed, shut off the flow and wash the column with 25-ml. portions until 500 ml. have been collected. Transfer a 50-ml. aliquot of the effluent to the titration cell, add 5 drops of bromocresol green solution, then neutralize with 2M sodium hydroxide. Add 0.1N hydrochloric acid dropwise until the solution is yellowish green. Add 0.5 ml. of 0.1N acetic acid. Stirring may be effected by passing a slow stream of oxygen-free inert gas, nitrogen, helium, or argon, through the solution. Add 7 ml. of 1M potassium chloride and 17 ml. of ethyl alcohol. Allow the solution to cool to room temperature. Attach the titration cell to the buret assembly, then pass inert gas through the solution for 10 minutes. Set the applied voltage at -0.7 volt (vs. S.C.E.). Titrate with the standard solution of uranyl acetate by the usual amperometric technique.

Alternative Procedure. A slightly different method from that described above was tested in which the solutions were diluted to a known volume of 500.0 ml. before passage through the resin column. The first 400 ml. of the effluent were then rejected, and the 50-ml. aliquot to be titrated was taken from the last 100-ml. portion of effluent without further dilution. This technique permits maximum dilution of the influent and obviates the time-consuming elution of the column which is necessary if the solutions are to be made up to exact volume after the ion exchange separation. It does not, however, consume appreciably less time than the usual procedure. A dilution of 300 ml. of the influent appears to be adequate for efficient action of the exchange resin.

RESULTS AND DISCUSSION

Polarographic Reduction of Uranyl Ion. Current-voltage curves for the reduction of hexavalent uranium at the dropping mercury electrode are markedly affected by the composition of the supporting electrolyte (3). Especial interference is exerted by the nitrate ion (which is catalytically reduced in the presence of uranyl ions) and by anions, such as acetate and sulfate, with which the uranyl ion forms complex anions.

No attempt was made in this work to study the polarography of uranium, except in so far as it was necessary to select the proper applied voltage for the amperometric titration under certain specified experimental conditions. Only the diffusion current plateau corresponding to the first stage of the reduction of uranyl ion is of interest; the voltage selected must lie within the region of constant diffusion current between the first and second waves. As the half-wave potentials of these waves may change with varying concentration and kind of indifferent electrolyte, it was necessary to determine the shape of the polarograms of uranyl ion in media similar to that in which the titration was to be carried out. For this purpose, current-voltage curves were recorded for uranyl acetate in solutions having concentrations of chloride, sulfate, perchlorate, and nitrate approximating the highest concentrations of these ions to be expected in an actual determination of phosphate. Similar polarograms were made with solutions in which the supporting electrolyte was potassium chloride, sodium sulfate, or sodium perchlorate. An ORNL Model Q-1160 recording polarograph (1) was used in these experiments. Pertinent data from the polarograms obtained are shown in Table I. With the exception of the first solution in Table I (0.1N potassium chloride, pH 4) which contained no alcohol, the ethyl alcohol concentration of all solutions was 20 volume % and the pH was approximately 3.5.

The data show that, of the anions considered, sulfate causes the greatest displacement of the first wave of the uranyl reduction from its position in a supporting electrolyte which is predominantly potassium chloride. The region of constant diffusion current extends from 0.45 to -1.00 volt and any applied voltage between these values should be suitable for the amperometric titration. The value of -0.7 volt was therefore adopted, and used in this work.

Precision of Titration. The precision of the titration was determined by titrating aliquots of a standard solution of potassium dihydrogen phosphate both in a supporting electrolyte of 0.1N potassium chloride and in supporting electrolytes containing perchlorate, sulfate, and nitrate ions. The results of these determinations are given in Table II.

The average volume ratios (0.726, 0.727, 0.726) of the standard solutions of uranyl acetate and potassium dihydrogen phosphate which were found in the experiments involving three different supporting electrolytes are for practical purposes identical, thus indicating no interference, in the concentration ranges studied, with the titration of perchlorate, sulfate, or nitrate ions. The experiment reveals that the coefficient of variation is of the order of 0.5%.

Separation of Uranium by Ion Exchange. Samuelson (4) showed that phosphate can be separated quantitatively from cations by means of a cation exchange resin. This technique was applied to the separation of phosphate from uranium in perchloric and sulfuric acid solutions of uranium phosphates. The optimum conditions that were selected for this separation

Table III. Determination of Phosphate by Amperometric Titration with Uranyl Acetate after Cation Exchange Separation

[Dowex 50, 24 × 2.1 cm. column. Composition of original solution, uranium, 180 mg. (UO_2^{++}), $HClO_4$, 0.66M, H_2SO_4, 10.9M]

Volume of Influent Solution, Ml.	Flow Rate, Ml. per Min.	Phosphate, Mg.		
		Taken, A	Found, B	Difference, B − A
100	3.5	99.1	99.7	0.6
	3.1	100.8	100.6	−0.2
300	4.7	64.8	68.8	0.4
	4.4	68.5	69.2	0.7
	4.6	100.6	101.7	1.1
	4.3	100.1	99.5	−0.6
300	3.7	148.1	147.8	−0.3
	3.5	148.1	148.0	−0.1
500	4	100.1	99.9	−0.2

were: size of resin column, 24 × 2.1 cm., and flow rate, 3 to 5 ml. per minute. The longer resin column effectively eliminated breakthrough of uranium (in strong sulfuric acid solution) as the anionic uranyl sulfate complex. Phosphate in solutions as concentrated as approximately 11M with respect to sulfuric acid was successfully determined. Perchloric acid presented no difficulty in the separation. The longer columns were also satisfactory with regard to holdup of phosphate in the column. The volume of influent was not critical.

Precision of Method. Synthetic samples of uranium phosphate in volumes of 5 ml. were prepared which contained varying amounts of sulfuric and perchloric acids. Each contained 1 ml. of 0.67M uranyl sulfate (180 mg. of UO_2^{++}) and 65 to 150 mg. of phosphate. The latter was added as a solution of potassium dihydrogen phosphate containing 92.56 mg. of phosphate per gram of solution. The portion of the standard solution of phosphate used to prepare each sample was measured by weighing it in a stoppered flask. Typical data, which were obtained under the optimum conditions, are shown in Table III.

When the removal of uranium by the ion exchange resin appeared to be complete, in no case was the amount of phosphate found to differ from that taken by more than 1.1%. The average difference of 14 samples from the known values (68 to 100 mg.) was 0.5% and the coefficient of variation was 0.6% on a 95% confidence level. Neither precision nor accuracy appear to have been affected adversely with changes in flow rates ranging from 3 to 5 ml. per minute.

Concentration Range of Applicability. The phosphate content varied from 65 to 150 mg.; hence, the quantities of phosphate in the aliquots finally taken for titration lay between 6.5 and 15 mg. No difficulty should be expected in applying the method to more concentrated samples, because the phosphate content of the solution which is to be titrated can be brought within this range by taking a smaller aliquot either of the original sample or of the effluent from the ion exchange step. At the lower extreme, the limiting factor should be the titration itself. Kolthoff and Cohn (2), titrating with 0.01M uranyl acetate, were able to determine phosphate in solutions as dilute as 0.0002M with an accuracy of 1%. This dilution corresponds to 1 mg. of phosphate in a 50-ml. aliquot. At two to four times this dilution, results (using 0.005M titrant) were high by 2 to 11%.

Under the experimental conditions applied here, and using 0.035M uranyl acetate as the titrant, 3 mg. of phosphate can be determined within 1 %; for smaller quantities, the results are high.

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Weighing Pipet Method for Preparing Infrared Gas Standards for Ether and Alcohol

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A method is described for the preparation of infrared gas standards of ether and alcohol using a weighing pipet (micro). The method was found satisfactory when applied to synthetics containing known amounts of ether and alcohol. It should be applicable also to any reasonably volatile substances such as the numerous organic solvents which have widespread commercial application.

IN THE manufacture of solid propellants, ether and alcohol are widely used as solvents for the nitrocellulose. The finished propellant is then usually placed in a solvent recovery house where the concentration of ether and alcohol may reach appreciable proportions. It was in connection with the infrared analysis of the air for ether and alcohol in such a solvent house that the weighing pipet method for preparing infrared gas standards was developed.

The infrared analysis of a gas (1), in essence, consists of obtaining the infrared absorbance of the sample and relating such absorbance (usually at some selected absorption band) to concentration from a previously established relationship (working curve) of absorbance versus concentration. The establishment of the working curve necessitates the preparation of standards containing known and varying amounts of the gas. Such gas standards are usually prepared by the introduction of controlled amounts of gas into an infrared gas cell, making accurate measurements of their pressure, and relating such pressure measurements to concentration. This method is lengthy, very difficult to control accurately, and in the case of ether and alcohol it would also be very difficult to apply as these substances are not normally in the gaseous state. For these reasons a more suitable method for preparing standards was considered desirable.

In the field of organic quantitative microanalysis (2), volatile liquids are handled in capillaries called weighing pipets. It therefore appeared reasonable to anticipate that a suitable technique could be developed to introduce known amounts of ether and alcohol in an infrared gas cell using similar weighing pipets. This paper describes the developed technique and presents some

Table I. Results Obtained on Synthetics of Ether and Alcohol

Sample No.	Ether, %		Alcohol, %	
	Added	Found	Added	Found
1	6.9	7.0	1.3	1.3
2	2.2	2.4	2.1	2.0
3	1.1	1.0	0.58	0.55
4	1.0	1.0	3.0	3.1
Av.	2.80	2.85	1.75	1.76
Std. deviation of differences	0.13		0.082	

data obtained in the application of this method to prepared samples containing known amounts of ether and alcohol.

EXPERIMENTAL PROCEDURE

Preparation of Weighing Pipets. Thin-walled soft glass tubing 1 to 3 mm. in inside diameter was sealed at one end with a flame. At about 2 cm. from the sealed end the tube was heated in a flame, drawn out to a fine capillary, and cut at about 3 cm. from the sealed end. (Weighing pipets were purposely made in different sizes ranging from 1 to 3 mm. in inside diameter, so as to be able to prepare standards of ether and alcohol ranging in concentration from about 1 to 8%. Using a 10-cm. gas cell, the weighing pipet with a 1-mm. inside diameter, when filled to 1 cm. from the sealed end, produced a concentration of about 1%.)

Filling and Weighing the Pipet. The pipet, previously weighed empty on a microbalance or a good semimicrobalance, was placed with the open end down in a 10-ml. beaker containing about 1 ml. of the ether or the alcohol. The beaker was placed in a Fisher Filtrator in which the upper opening was stoppered. The vacuum was turned on for a few seconds and then turned off. In this

process some of the air within the pipet was withdrawn in the evacuation process and, upon shutting off the vacuum, the ether or alcohol moved into the pipet to replace the air withdrawn. The pipet was placed in a small centrifuge tube with the sealed end in the outward direction and centrifuged to move the liquid to the sealed end. The open capillary end was sealed in a flame and the sealed pipet was weighed on the same balance which was previously used. The gain in weight of the pipet was considered as ether or alcohol. (The pipet should be filled to no more than 1 cm. from the sealed end. To get varying amounts of ether or alcohol, pipets of different inside diameters are used together with variation in the level of the liquid in the pipets. A preliminary weighing of the pipet can be made prior to sealing its tip to ensure that it contains the desired amount of liquid. To place additional liquid in a pipet, after centrifuging and prior to sealing, repeat the evacuation process described above. If too much liquid has been introduced in a pipet, some of it may be removed before centrifuging by warming the pipet slightly, by holding it between two fingers or even by centrifuging with the tip of the pipet in the outward direction.)

Preparation of Standard Samples and Working Curves. The pipet containing a known amount of ether or alcohol was introduced through one of the threaded openings into a Perkin-Elmer 10-cm. gas cell having a metal body. With a thin metal rod heated to about 50° C. and inserted through the threaded opening, the pipet was fragmented inside the cell. The end of the rod was held in the cell for about a minute to allow evaporation of any ether or alcohol which may have deposited on the tip during the fracturing process. (The rod was heated to about 50° C. to facilitate this evaporation.) The rod was then withdrawn, and the threaded opening of the cell was suitably closed. (The other threaded opening was kept closed during the complete operation.) The cell was allowed to stand for about 0.5 hour to allow the ether or alcohol to evaporate completely and to disperse itself inside the cell. The percentage of ether or alcohol in the
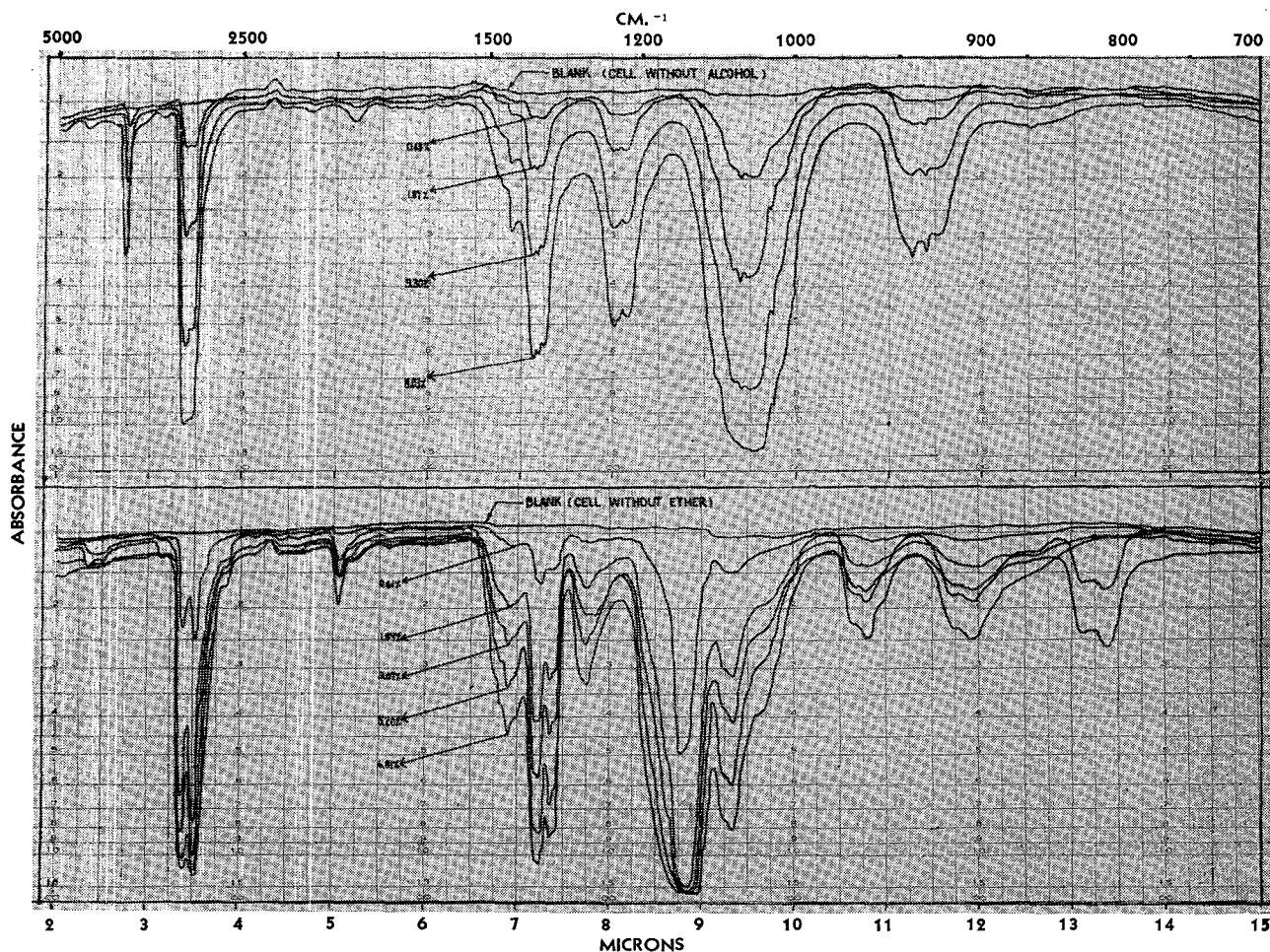

Figure 1. Absorbance of alcohol and ether in 10-cm. cell
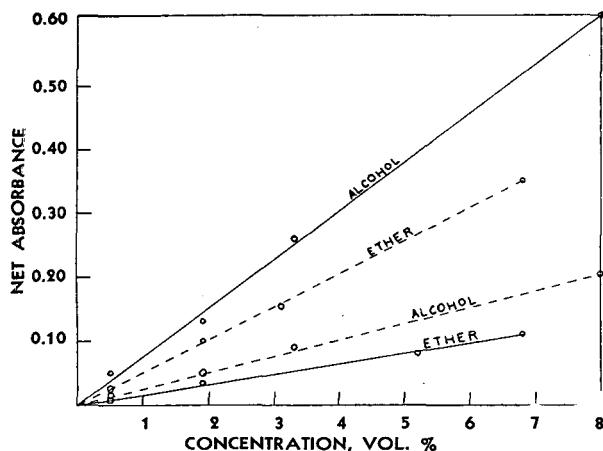
Upper. Alcohol. Lower. Ether

Figure 2. Infrared absorbance of alcohol and ether

— At 8.00 microns in 10-cm. cell
--- At 6.90 microns in 10-cm. cell

cell was calculated as follows (all work was done at 760 mm. of pressure and 25° C. or 298° K.):

$$\text{Per cent of ether or alcohol by volume} = \frac{\text{wt.} \times 22{,}400 \times 100 \times 298}{\text{M.W.} \times V \qquad 273}$$

where

Wt. = weight of ether or alcohol in pipet in grams
M.W. = gram molecular weight of ether or alcohol
V = volume of gas cell in cubic centimeters calculated from its geometry (found to be 147.6 cc. for the cell used)

In this way various standards of ether and alcohol were prepared. The infrared spectrograms of the gas cell without ether or alcohol, and of the prepared standard samples were obtained on paper with absorbance markings using a Perkin-Elmer double-beam infrared spectrophotometer (Figure 1). The 6.9-micron ether band and the 8.0-micron alcohol band were selected for

analysis. The net absorbance (total absorbance minus cell absorbance) of the various standards at the two selected positions was plotted against concentration to obtain the working curves (Figure 2).

DISCUSSION AND APPLICATION OF METHOD

The relationship of concentration to absorbance for both ether and alcohol at both 6.9 and 8.0 microns appear to follow a straight line and therefore the working curves have been drawn as straight lines. The various points lie very close to the lines, indicating that the weighing pipet method of preparing infrared gas standards for ether and alcohol is valid.

To test the applicability of the method, four known samples of ether and alcohol in air were prepared by introducing known amounts of both ether and alcohol in the gas cell as described above. The infrared absorbance of the prepared samples was measured at 6.9 and 8.0 microns and the values were calculated to per cent ether and alcohol using the method of successive approximations (3). The results obtained are listed in Table I and show good agreement between the values added and the values found.

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Instrumental Variability of a Model 7 Coleman Photonephelometer

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Instrumental variability is attributed to variations in the blanks used to set the instrument sensitivity and to a tendency for the readings to drift to lower values. By modifying the manufacturer's operations and making frequent checks against standards, a standard deviation for individual measurements of 0.28 reading unit at the normal instrument sensitivity has been estimated.

DURING the course of a study on the nephelometric determination of sulfate (4), the need arose for an evaluation of the variability contributed to the measurements by the instrument alone. The Model 7 Coleman Photonephelometer used in the study measures the light scattered at right angles to the direction of illumination by means of two barrier layer cells. The instrument, which is linear in its response to scattered light, may be operated as either a direct or a null-reading device.

Unknown turbidities may be compared with standards, supplied by the manufacturer, which are suspensions of an inorganic salt in a highly viscous organic polymer (3). Each one is labeled with a particular Nephelos number which has been assigned with reference to a master standard. Thus, the light-scattering prop-

erties of the standards, and the samples subsequently measured with reference to them, are empirically related. In this way, a correlation of readings in Nephelos units between laboratories should be possible.

Essentially, the standards provide a means of reproducibly establishing the instrument sensitivity, which is defined as the slope of the linear relationship between the instrument reading and the 90°-scattered light intensity. This relationship is referred to below as the instrument-response curve.

RESULTS

Using the procedure outlined by the manufacturer for the null method of operation (1), the instrument was adjusted using a standard 38 and a distilled water blank. [A modification (2) of this procedure is now recommended by the manufacturer.] Five other standards were then run as "samples" in a random order which was determined by a chance selection of a 5 × 5 Latin square (5). A different Latin square was used for the data in each table.

Table I indicates the values taken after a single initial adjustment of the instrument with standard 38. The readings for each

Table I. Readings Obtained after Single Initial Adjustment of Instrument Sensitivity at Normal Value with Standard 38

(Distilled water used as blank)

Nominal Value of Standard[a]	Individual Readings					Average	Standard Deviation[b]	Coefficient of Variation[c]
4	3.7	3.6	3.6	3.9	3.7	3.7	0.12	3.3
9	8.5	8.2	8.4	8.4	7.8	8.2	0.28	3.4
18	19.9	19.6	19.6	19.6	19.4	19.6	0.20	1.5
37	41.7	41.9	41.4	41.1	41.1	41.4	0.36	0.9
78	84.2	83.4	82.8	82.1	81.8	82.9	0.52	0.6

[a] Nominal values are Nephelos numbers assigned to standards by manufacturer.

[b] Estimated standard deviation of individual measurements, $s =$

$$\sqrt{\frac{\sum_i (X_i - \bar{X})^2}{N - 1}}.$$

[c] Coefficient of variation, $v = \frac{s\,100}{\bar{X}}$.

standard have been recorded in their chronological order of measurement; but, because of the Latin square arrangement, the time intervals between individual values for a given standard were not necessarily constant. A drift toward lower values is observable, particularly for the high standards. This drift could be due to photocell fatigue and/or decrease of the intensity of light source. The power supply was a 6-volt storage battery. A recheck of the standard 38 at the completion of the series showed that its value read 35.9.

Table II. Readings Obtained Using Repeated Adjustment of Instrument Sensitivity at Normal Value with Standard 38 and Distilled Water for Blank

(Each group of readings taken on different day)

Group	Nominal Value of Standard	Individual Readings					Average	Standard Deviation	Coefficient of Variation
A	4	4.6	4.3	4.2	4.2	4.5	4.4	0.18	4.1
	9	8.6	8.9	9.2	9.4	3.6	8.9	0.36	4.0
	18	21.3	20.8	20.9	20.9	20.6	20.9	0.26	1.2
	37	42.2	42.8	42.3	42.5	42.8	42.5	0.28	0.6
	78	86.1	86.7	86.1	86.6	87.1	86.5	0.42	0.5
B	4	3.4	3.4	3.4	3.2	3.4	3.4	0.09	2.6
	9	8.6	8.3	8.4	8.5	8.5	8.5	0.11	1.3
	18	20.6	20.2	19.9	20.1	20.0	20.2	0.27	1.3
	37	42.9	42.7	42.2	42.4	43.1	42.7	0.37	0.9
	78	87.9	87.4	87.4	87.8	87.6	87.6	0.23	0.3

In Table II, each measurement was preceded by a check or readjustment of the instrument sensitivity with standard 38, with the result that the drifting of the readings toward lower values was avoided. However, when the average values obtained for the same standard on different days were compared by means of the t-test (5), the difference between them, with the exception of standard 37, was shown to be greater than could be accounted for by the variability within the data taken on a particular day. With standards 4 and 78, the differences were "very highly significant"—i.e., the probability that the differences were due to chance variations alone was less than 0.1%.

These results suggested that the blank adjustment, which was made with unfiltered distilled water, was influencing the slope of the instrument-response curve. To show the effect of a high blank, standard 4 was substituted for the distilled water as the blank, and, as before, standard 38 was made to read 38.0. Table III indicates the measurements taken. It is evident that the high blank caused a tilting of the instrument-response curve about the value of the standard chosen for setting the instrument sensitivity.

In order to make a precise adjustment of the instrument sensitivity, a blank with a constant light-scattering ability is required. Zero would have been ideal, but in lieu of such a blank, the sensitivity level was adjusted with two standards using the following procedure.

With the blank adjustment completely inoperative, standard 38 was made to read 38.0 with the STD knob. Next, the second standard, which had a nominal value of 9 (not the same one included in the tables), was adjusted to read 10.0 with the BLK knob. This could be done because its value was slightly higher than 10 before this adjustment. A second adjustment with each standard was usually all that was required to provide an instrument-response curve which passed through the values 10.0 and 38.0. Table IV shows the values obtained for the five "samples" when using the above procedure for setting the instrument, together with an adjustment with the STD knob using standard 38 before each reading. The BLK knob needed no further attention.

With the instrument in the authors' laboratory, the slope of the instrument-response curve was varied from 0.3 to 3.2 times the normal value, on the basis of the fact that standard 38 could be made to read any value from 11.4 to 122. These values were only approximate because of the tendency to drift, which lowered both limits simultaneously. By reducing the galvanometer sensitivity (maximum galvanometer setting was used with the null method of operation), the lower limit could be reduced still further. However, for the measurement of low turbidities the gain in sensitivity over the normal—i.e., 1X—value was of principal interest. Therefore, the standards were read at 3X sensitivity and then converted to normal sensitivity. The resulting average values of 4.2, 9.3, 20.4, and 41.8 show good agreement with the data in Table IV. Standard 78 could not be read because the full scale limit of the instrument is 130.

DISCUSSION

The null method of operation was used in preference to the direct-reading method because, even at the normal sensitivity, it was twice as sensitive as the direct-reading method at full galvanometer sensitivity. For example, standard 38, which could be

Table III. Readings Obtained Using Repeated Adjustment of Instrument Sensitivity at Normal Value with Standard 38 and Standard 4 Used as Blank

Nominal Value of Standard	Individual Readings					Average	Standard Deviation	Coefficient of Variation
4	0.0	0.0	0.0	0.0	0.0	...	...	...
9	7.0	7.4	6.9	7.4	7.1	7.2	0.23	3.2
18	19.2	18.6	18.4	18.2	18.6	18.6	0.37	2.0
37	42.2	42.2	42.2	42.4	42.2	42.2	0.09	0.2
78	89.4	90.9	90.4	90.9	90.2	90.4	0.62	0.7

Table IV. Readings Obtained Using Two-Standard Method for Establishing Instrument Sensitivity and Hypothetical Blank

(Each group of readings taken at normal sensitivity on different days)

Group	Nominal Value of Standard	Individual Readings					Average	Standard Deviation	Coefficient of Variation
A	4	4.0	4.2	4.1	4.4	4.6	4.3	0.24	5.6
	9	8.9	9.3	9.4	9.2	9.9	9.3	0.36	3.9
	18	19.9	19.9	20.2	20.1	20.4	20.1	0.21	1.1
	37	41.7	41.9	42.0	41.9	42.4	42.0	0.26	0.6
	78	85.2	85.6	85.4	85.6	85.4	85.4	0.17	0.2
B	4	4.3	3.9	4.6	3.9	3.6	4.1	0.39	9.5
	9	9.5	9.1	9.1	9.4	9.3	9.3	0.18	1.9
	18	20.6	20.4	20.1	20.0	19.9	20.2	0.29	1.4
	37	41.7	41.8	42.2	42.1	42.4	42.0	0.29	0.7
	78	85.4	85.8	85.9	86.3	85.9	85.9	0.32	0.4

adjusted to read 38.0 by the null method, gave a maximum value of only 20.3 by the direct-reading procedure; after 0.5 hour of instrument operation, the maximum reading was 16.6. Only when the null method was used at the 3X sensitivity (standard 38 made to read 114.0) was maintenance of the sensitivity difficult, owing to the downward drift of the readings. The cause of this drifting was not investigated, but it was noted that an initially high maximum value could always be obtained after the instrument had been inoperative for a short time.

A comparison of the instrument with three other Model 7 Photonephelometers was made, using standard 38 and the null method of operation. Each instrument had a widely different inherent maximum sensitivity. The instrument used in this study proved to be capable of four times the sensitivity of one of the instruments tested. The condition of each instrument was not investigated, but each was relatively new and apparently undamaged.
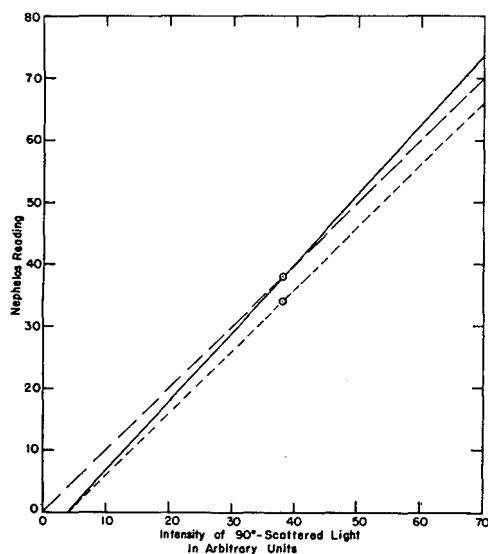
Figure 1. Hypothetical instrument-response curves established with standard 38 at normal sensitivity

--- Ideal curve obtained with zero blank
——— Effect of unknown blank; not subtracted from standard 38
---- Effect of subtraction of known blank from standard 38

It is evident from the tables that the nominal values of the standards as assigned by the manufacturer are not exact. Obviously, standard 37 had a greater light-scattering power than standard 38, which was used to adjust the instrument. Thus, correlation of data, even within a particular laboratory, will be poor if the instrument sensitivity is adjusted with different standards without regard for their true values relative to one another. The linearity of the instrument-response curve has been assumed without investigation, and subsequent use of the instrument with chemical systems (4) has brought forth no reason to doubt this assumption.

One year after the data in Table IV were taken, a replication of the work was done with the same five standards. The average values obtained were 3.8, 9.1, 20.5, 42.1, and 84.5, respectively. For each standard, a "between and within treatments" analysis of variance (5), involving all the data used to obtain the three averages—for example, the averages 20.1, 20.2, and 20.5 for standard 18—showed no significant difference between them— i.e., the probability was greater than 5% that variations were due to chance alone. The standards used above had been in frequent use by several workers during the 1-year interim period. This indicates the very satisfactory stability of the Coleman Nephelos standards.

In order to limit the instrumental variability to a minimum, the light-scattering power of the blank must be known relative to the standard used to establish the instrument sensitivity. The dashed line in Figure 1 represents an ideal instrument-response curve at 1X sensitivity as obtained with standard 38 and a blank with zero scattered-light intensity. The full line in Figure 1 shows the effect of using a blank, the value of which is assumed to be unknown and therefore cannot be subtracted from standard 38. As in Table III, where standard 4 was used as the blank, the slope of the curve has been increased. Because of the negative intercept, samples with light-scattering abilities less than those of standard 38 have readings which are lower than those on the ideal instrument-response curve, whereas those with more light-scattering ability are higher. As indicated by the dotted line in Figure 1, the subtraction of a known blank from standard 38 has the effect of "nulling" out its light-scattering power without changing the sensitivity (slope) of the instrument. Unfortunately, in chemical systems, a prior knowledge of the value of the blank relative to the standard used to establish the slope of the instrument-response curve is unavailable. As shown in Table II, slight variations in the turbidity of distilled water caused significant changes in the sensitivity setting. The procedure, using two standards to establish the instrument-response curve as described for Table IV, is really a method for defining a hypothetical blank the value of which is unknown but constant with respect to standard 38. Later work (4) with optically clear solutions, which were obtained by filtration, showed this hypothetical blank to be essentially zero.

In order to show that the separate variances (square of standard deviations) calculated for each standard might be combined to provide an estimate of the instrument error, the Bartlett test (5) for the homogeneity of variances was applied to the data in Table IV. This test indicated that, regardless of the level of the light-scattering ability of the standards, the variances did not differ significantly among themselves $[P\%(\chi_0) \simeq 82]$. Therefore the standard deviations in Table IV were pooled, and provided an estimated standard deviation for individual measurements of 0.28 reading (or Nephelos) units at the normal (1X) instrument sensitivity.

In general, the instrumental variability is independent of the level of turbidity being measured, and depends upon the precision with which the slope of the instrument-response curve can be established. However, as indicated by the coefficients of variation shown in the tables, the percentage of error contributed by the instrument depends upon the level of turbidity being read. Where the instrument sensitivity was three times the normal value, an improvement in precision was achieved in the measurement of very low turbidities at instrument sensitivities greater than normal. However, for turbidities with values greater than 10 Nephelos units at 1X sensitivity, no particular advantage seems to accrue from the use of greater sensitivities than normal.

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Extraction of Metal Thiocyanate Complexes with Tributyl Phosphate

Copper(II) Thiocyanate

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The study of the extraction of metal thiocyanate complexes with tributyl phosphate has been extended to include effects of temperature, pH, thiocyanate to copper ratio, and concentration of copper on the extraction of copper. Spectral transmittance data were obtained for copper thiocyanate in tributyl phosphate. Under the proper conditions copper(II) thiocyanate can be extracted completely from aqueous solution.

TRIBUTYL phosphate has previously been found to be a useful solvent for extracting iron(III) thiocyanate (*1, 6*). Preliminary results indicated that copper(II) thiocyanate could also be extracted with tributyl phosphate. Although copper(II) is reduced by thiocyanate in basic solution (*4*), reduction in acid solution does not normally occur (*9*). The extraction of copper(II) thiocyanate from acid solution was studied with regard to its analytical potentialities.

REAGENTS AND APPARATUS

Unless otherwise stated, all reagents are c.p. or reagent grade.
All pH measurements were made with a Beckman pH meter.
Ammonium thiocyanate. Stock solutions were standardized with silver nitrate.
Tributyl phosphate obtained from Commercial Solvents Corp., and used without further purification.
Stock copper solutions prepared with pure copper.
Calibrated weights and buret.
Beckman DU spectrophotometer.

EXPERIMENTAL PROCEDURE

The extraction of copper(II) thiocyanate from aqueous solution is affected by ratio of thiocyanate to copper, volume of tributyl phosphate, copper concentration, and temperature. After each extraction, copper in the raffinate was determined colorimetrically as the diethyldithiocarbamate (*3*). Each raffinate was diluted to 100 ml. in a volumetric flask. An appropriate aliquot was placed in a second 100-ml. volumetric flask, and diluted to about 50 ml. The following additions were made, the solution being mixed after each addition: 5 ml. of 20% citric acid, concentrated ammonia added dropwise until the solution was basic to litmus, 2 ml. of 1% gum arabic, and 10 ml. of 0.1% sodium diethyldithiocarbamate. After diluting to the mark, spectrophotometric measurement was made at the absorption maximum of 445 mμ.

In order to find the thiocyanate to copper ratio at which maximum extraction occurs, ammonium thiocyanate was added in varying amounts to 25-ml. aliquots of 0.001285M copper sulfate solution so that the mole ratio of thiocyanate to copper varied from 5 to 1 to 25 to 1. The pH values of the samples were adjusted to 3.20 $\pm$ 0.05 with dilute sodium hydroxide, the total volume of each solution being approximately 45 ml. Copper(II) thiocyanate was extracted from each solution with 25 ml. of tributyl phosphate, and the raffinate was collected as previously described (*6*). The temperature rise on shaking the aqueous and organic phases was noted, and the pH of the raffinate was measured. Copper in the raffinate was then determined spectrophotometrically.

To determine the optimum volume of tributyl phosphate needed, a series of extractions was carried out in which the volume of tributyl phosphate was varied from 5 to 30 ml. in 5-ml. increments. Twenty-five-milliliter aliquots of the copper solution were taken, the ratio of thiocyanate to copper being constant at 25 to 1. The pH values of the samples were 3.20 $\pm$ 0.05. After extraction, copper was determined in the raffinate.

To find the affect of acidity on the extraction of copper, samples were run in the pH range 1.46 to 5.13 with the ionic strength constant. Twenty-five-milliliter aliquots of 0.0001285M copper were taken, and the pH values were adjusted by the addition of dilute sodium hydroxide. The thiocyanate to copper ratio was maintained constant at 20 to 1. Extractions were made with 25 ml. of tributyl phosphate, and the copper remaining in aqueous solution was determined.

The effect of copper concentration on the extraction of copper was studied next. Twenty-five-milliliter aliquots of two copper solutions, 0.001285M and 0.005140M copper, were taken. Thiocyanate was added to each aliquot so that the ratio of thiocyanate to copper for each group of samples ranged from 5 to 25 to 1 in increments of 5. The pH values of the solutions were adjusted to 3.20 $\pm$ 0.05 with dilute sodium hydroxide, the final volume of the samples being approximately 45 ml. Extractions were made with 25 ml. of tributyl phosphate. Copper was then determined in the raffinate.

Extractions of copper were next made at four different temperatures to find the temperature coefficient of extraction. Extractions were made using 25-ml. aliquots of 0.001285M copper solution with the thiocyanate to copper ratio maintained constant at 25 to 1. The pH values of the samples were 3.20 $\pm$ 0.05, and the volume of tributyl phosphate for each extraction was 25 ml. The extraction temperatures, measured after the phases were mixed for 30 seconds, were varied from 10° to 55° C. Copper was determined in the raffinate, and the per cent copper extracted was calculated.

RESULTS AND DISCUSSION

The smallest ratio of thiocyanate to copper to give maximum extraction is between 23 and 24 to 1 (see Figure 1). Assuming that the compound extracted is [Cu(SCN)$_2$]$_x$, the theoretical optimum ratio of thiocyanate to copper should be 2 to 1. Hence,
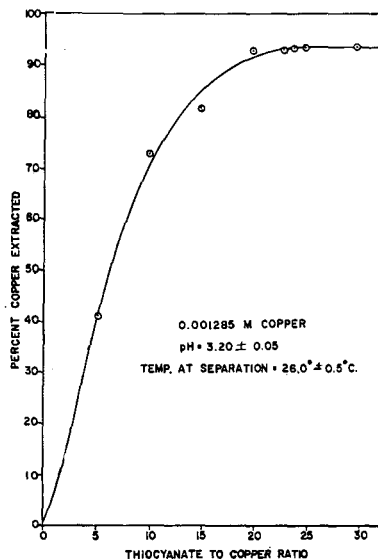

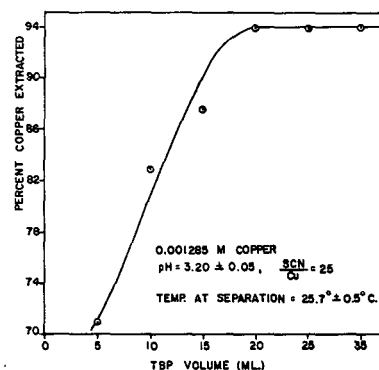**Figure 1. Extraction of copper as a function of ratio of thiocyanate to copper**

Figure 2. Extraction of copper as a function of volume of tributyl phosphate

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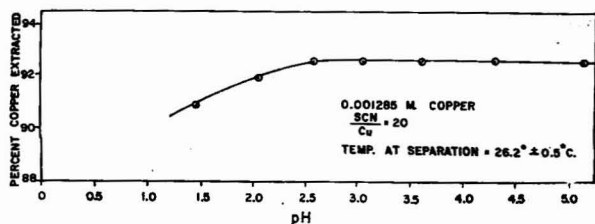
Figure 3.   Influence of pH on extraction of copper

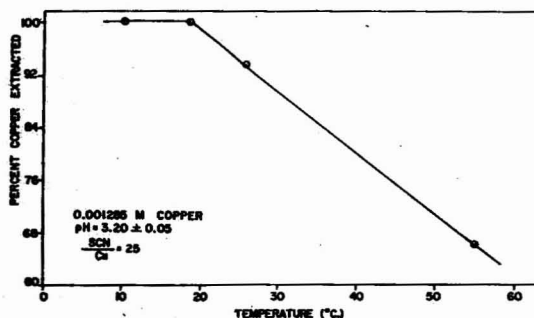Figure 4.   Effect of copper concentration on extraction of copper

Figure 5.   Effect of temperature on extraction of copper

Figure 6.   Spectral transmittance curve for copper(II) thiocyanate in tributyl phosphate

the bracketed quantities referring to activities. Then the association number, n, would be equal to the slope of a plot of the logarithm of the tributyl phosphate copper activity versus the logarithm of the aqueous copper activity. From calculation using the above equation it was found that the association number varied from 5 to 17 depending on the thiocyanate to copper ratio. However, the following should be taken into consideration:

Each curve consisted of only two points.
Molarities were used instead of activities.

The ionic strength for the samples of one copper concentration differed from the ionic strength of the samples at the other copper concentration.

Further study would therefore be necessary to determine whether the dependence of extraction of copper on copper concentration is due to the extraction of a polymer or to a self-salting-out phenomenon.

Decreasing the temperature resulted in increased extraction of copper (see Figure 5). For the 0.001285M copper solution, 100% extraction was obtained at 18.5° C. At a still lower temperature, 10.2° C., extraction again was complete. The temperature coefficient of extraction over the range 18.5° to 55° C. was −0.9% per 1° C.

A spectral transmittance curve for copper(II) thiocyanate in tributyl phosphate (see Figure 6) showed this compound to have an absorption maximum at 382.5 mμ and a molecular extinction coefficient of 620. For comparison, the molecular extinction coefficients for other colored copper complexes are 61.2 for the copper ammonia complex (5), 95,500 for copper dithizonate in carbon tetrachloride (8), and 9100 for copper diethyldithiocarbamate in aqueous solution. The spectrophotometric determination of copper(II) thiocyanate in tributyl phosphate would therefore be a relatively insensitive method.

one may conclude that the effect of tributyl phosphate extraction of copper(II) thiocyanate upon the formation of this complex is far less significant than it seemed to be upon the analogous iron(III) case (6). After the aqueous and organic phases had been mixed, the temperature was about 1° higher than before mixing. Only a 30-second mixing period was needed to establish equilibrium. Samples mixed for 3 minutes with tributyl phosphate showed no improvement in separation. Also, 20 ml. of tributyl phosphate were sufficient to obtain good extractions for the samples and conditions involved in this work (see Figure 2). There were no discernible volume changes of the phases upon equilibration.

Figure 3 shows that the extraction of copper, at constant ionic strength, is independent of pH over the range 2.58 to 5.13. Below a pH of 2.58 there is a slight decrease in extraction of copper. The pH independence of extraction is to be expected since thiocyanic acid is a strong acid.

From Figure 4 it may be seen that the fraction of copper extracted at a given thiocyanate to copper ratio increases with increasing copper concentration. This same anomaly was found on extraction of iron(III) chloride from hydrochloric acid solution (2). This was attributed to the formation of a tetramer (2) and also to a self-salting-out phenomenon (7). If a polymer of copper(II) thiocyanate is extracted, then the distribution of copper may be expressed by

$$\frac{(Cu)_{nTBP}}{(Cu)^n_{aq}} = K$$

or

$$\log (Cu)_{n\ TBP} = \log K + n \log (Cu)_{aq}$$

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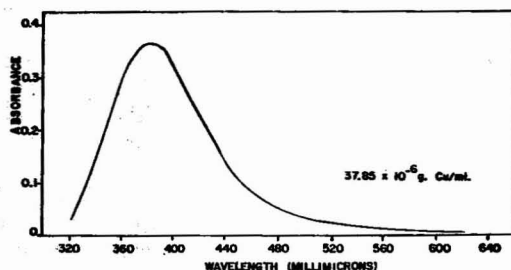
Microchemical Detection of Fluorides

Sodium Fluosilicate Crystal Test

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A modification in composition of the hanging drop solution in the sodium fluosilicate crystal test for the microchemical detection of fluorides renders the test considerably more sensitive. A detailed procedure is given for detection in a variety of organic and inorganic substances. The test is applied to distinguish between inorganic fluorides and monofluoroacetic acid.

THERE are a number of microchemical tests described in the literature for the detection of soluble fluorides, wherein the fluoride is converted into hydrofluoric acid or fluosilicic acid, which is then distilled, entrapped, and identified by various means. The common devices used are a hanging drop of liquid reagent suspended on a glass slide or in an open tube of small diameter, or identification by the etching action on the glass slide itself.

Of these the best known and most commonly used are the etch test (*3*), in which hydrofluoric acid evolved from a soluble fluoride is detected by its etching action on a dry glass slide; and the silicic acid hanging drop test (*9, 10*), in which fluosilicic acid is evolved and absorbed by a drop of water hanging in a small glass tube, where it is hydrolyzed into silicic acid and detected by means of the cloudy effect produced by the latter. Less known and rarely used are the sodium fluosilicate crystal test (*1*), in which evolved fluosilicic acid is trapped in a drop of sodium chloride solution hanging from the surface of a glass slide, with subsequent identification of characteristic sodium fluosilicate crystals microscopically; and the barium fluosilicate crystal test (*2*) identical in performance with the latter except for the substitution of barium chloride solution in the hanging drop.

A search of the literature failed to disclose any single work in which the sensitivities of these or the numerous colorometric methods for the detection of fluorides had been evaluated comparatively, evidence on this question being only fragmentary and not definitive.

Probably the etch test is the most frequently used today, because of its simplicity. As ordinarily performed, its sensitivity is not of a high order, various reports indicating the amount of fluoride required to produce a visible etching as ranging between 10 and 0.1 mg., with an average of about 0.5 mg. Greatly increased sensitivities were achieved by Woodman and Talbot (*14, 15*) and later by Gautier and Clausmann (*5*), who with modified techniques were able to detect a few micrograms without, however, obtaining consistent results. Williams (*13*), in an attempt to develop a quantitative method for the determination of minute amounts, was able to detect as little as 0.1 γ, using flanged platinum distillation tubes embedded in a specially designed heating block. He obtained consistent results in qualitative detection, but the apparatus required is not readily available to the average analytical laboratory.

In a comparative appraisal of some of the outstanding qualitative tests Gettler and Ellerbrook (*6*) concluded that the sodium fluosilicate crystal test was the most sensitive, and adapted it for detection in blood and tissues. Fluorides were isolated by coprecipitation of lanthanum fluoride with the hydroxide, followed by distillation and entrapment in a hanging drop of 5% sodium chloride solution and microscopical identification of sodium fluosilicate crystals. These investigators stated that 10 γ of fluoride in 50 grams of normal tissue could be detected, but the precise degree of sensitivity was not made clear, as they found that 20 grams of normal tissue, which according to their tables contained approximately 10 γ of fluoride, always gave a negative result, whereas 50 grams of normal tissue containing 25 γ of fluoride produced a positive result, with 3 or 4 sodium fluosilicate crystals appearing in the entire microscopic field. No indication was given as to whether the lanthanum fluoride precipitation was quantitatively complete. Harrigan (*7, 8*), comparing the above procedure with the Feigl method (*4*) of quenching aluminum oxine fluorescence in the presence of fluoride ion, decided in favor of the latter because the size and number of sodium fluosilicate crystals were too small. Harrigan found the lowest limit of sensitivity of the Feigl test to be circa 50 γ of fluoride.

In the present investigation a study was made of the sodium fluosilicate crystal test, and by minor variations in conditions, principally a modification in the composition of the hanging drop, it was possible to obtain a positive test with as little as 0.2 γ of fluoride. With increasing concentrations of fluoride larger numbers of crystals could be observed, until with the subjection of 1.0 γ of fluoride to the test several thousand crystals of assorted sizes appeared in the field.

Along with the increased sensitivity the modified test has the additional advantage of enabling the sodium fluosilicate crystals, which appear in characteristic hexagonal form (Figure 1) or as six-pointed stars, to stand out individually and more distinctly from the larger sodium chloride crystals. They are furthermore tinted a deeper shade of pink and are more easily recognized than those produced in the Gettler and Ellerbrook procedure.

REAGENTS

Standard sodium fluoride solution. Dissolve 0.2210 gram of pure sodium fluoride in water and dilute to 2000 ml. Each milliliter of this solution contains 50.0 γ of fluorine.

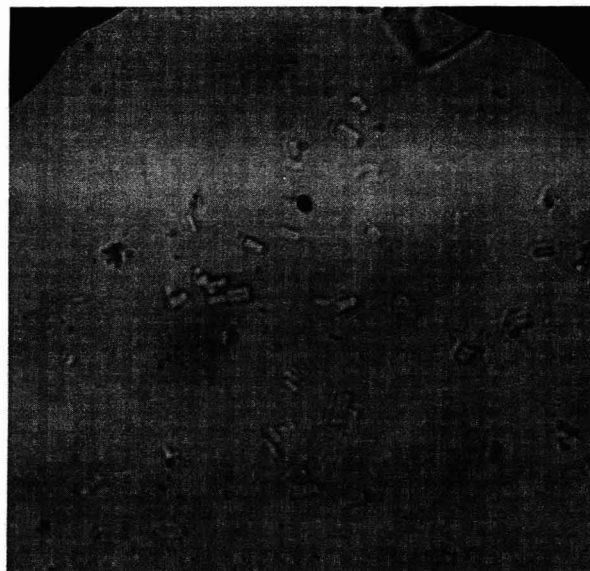Standard sodium monofluoroacetate solution. Dissolve 0.05

Figure 1. Photomicrograph of sodium fluosilicate crystals (440×)

gram of sodium monofluoroacetate in water and dilute to 250 ml. Each milliliter of solution contains 0.2 mg. of the salt; 0.05 ml. of solution contains 1.9 γ of fluorine.

Standard sodium fluosilicate solution. Dissolve 0.1650 gram of pure sodium fluosilicate in water and dilute to 2000 ml. Each milliliter of solution contains 50 γ of fluorine.

Sodium chloride hanging drop solution. Dissolve 1.0 gram of pure sodium chloride and 3.0 grams of pure glycerol in water, add 2 drops of 40% formaldehyde to preserve, dilute to 100 ml., and filter through paper into a glass reagent bottle. Insert a 3-mm. diameter glass rod with fire-polished ends, and of suitable length, through a rubber stopper and keep bottle well stoppered. This apparatus serves very conveniently for the transfer of a small drop of solution to the surface of the glass slide in the crystal test.

Silver sulfate. Pure crystalline silver sulfate stored in a brown bottle.

Saturated silver sulfate solution. An excess of silver sulfate suspended in water and stored in a brown dropping bottle.

Silica. Fluorine-free powdered silicon dioxide.

APPARATUS

Heating block. A metal block approximately 2.5 cm. thick and large enough to hold four 10-ml. porcelain crucibles is suitable. A well to hold the bulb of a thermometer is drilled into the block. A few drops of mineral oil are placed in the well to cover the bulb. The block is set on a tripod and preferably heated with a multiple-jet gas burner. A satisfactory block may be constructed by melting sufficient printer's type metal in an aluminum pie plate. A small test tube 1 cm. in diameter is set and held in the molten metal by a clamp on a ring stand, then the metal is allowed to cool slowly and to solidify.

Glass slides. Microscope slide glass is cut into pieces 4 × 4 cm.

Pipets. Pipets, 0.2 ml., graduated into 0.01-ml. divisions are used.

Standardized micropipet. For convenient delivery of uniform drops of standard fluoride solutions a satisfactory pipet may readily be constructed. A length of thin-walled glass tubing of 5-mm. diameter is drawn out into a fine capillary, which is broken off at a point where its diameter is less than 1 mm. It is standardized by allowing water to flow from it, drop by drop, at a uniform rate into a microburet filled with water exactly to the 1.00-ml. mark. If the zero mark is not reached by addition of 50 drops, the individual drops are too small, and a short length of capillary is cut off and the trial repeated. The procedure is repeated until a uniform drop of exactly 0.02 ml. is delivered. The pipet is dried and inserted through a rubber stopper fitted to a test tube or small reagent bottle containing standard fluoride solution, from which definite quantities of fluoride may be accurately delivered when required.

Crucibles. A number of high-form glazed porcelain crucibles of 10-ml. capacity.

Dropping bottle. T.K. type of 30-ml. capacity for delivering small uniform drops of concentrated sulfuric acid.

EXPERIMENTAL

Modified Sodium Fluosilicate Crystal Test. Using a standardized pipet, 1 drop (0.02 ml. containing 1.0 γ of fluorine) of standard sodium fluosilicate solution was transferred to a 10-ml. porcelain crucible. Approximately 0.5 mg. of powdered calcium carbonate was added, the crucible was dried on a hot plate until free of moisture and then cooled to room temperature. To the residue were added 2 small drops of sulfuric acid (specific gravity, 1.84); the crucible was placed on a metal block maintained at 170° C. It was immediately covered with a glass slide, on the undersurface of which had been placed a small drop (diameter 0.4 cm.) of modified hanging drop solution. A 50-ml. beaker containing an ice cube was firmly set on top of the slide and the distillation was allowed to proceed for 20 minutes, after which the slide was carefully removed, its upper surface was blotted dry with filter paper, and it was then put in a warm place for a few minutes until the hanging drop was dry. Microscopic examination (440×) revealed the presence of several thousand decidedly pink crystals of various sizes, either in hexagonal form or as six-pointed stars. These crystals were not uniformly distributed throughout the field but were mainly concentrated along the periphery of the drop. Viewed very slightly out of exact focus they appeared opaquely black. The limit of sensitivity was reached when 0.2 γ of fluorine was subjected to the test, producing a few tiny crystals, the number increasing to over 100 when 0.3 γ was used. To perform the test on quantities less than 1.0 γ the standard solution was diluted to one tenth its fluoride content and the appropriate number of drops was taken. When standard sodium fluoride solution was used instead of the

fluosilicate, the procedure was not changed except for the addition of circa 2 mg. of silica powder in the microdistillation; this converted the hydrofluoride into fluosilicic acid. Tests indicate that the recovery in the form of sodium fluosilicate crystals was not quantitative, only part of the fluoride being trapped in the hanging drop.

INTERFERENCES

During the course of the work it was found that a number of common negative ions such as chlorides, nitrates, borates, carbonates, and sulfates influenced in varying degrees the formation of sodium fluosilicate crystals in the hanging drop test. The presence of these ions tended to inhibit the quantity of fluoride recovered, and in general the more negative ions present, the fewer crystals appeared in the microscopic field. The ions mentioned above are listed in the descending order of their capacity to interfere. When the test was performed on 1.0 γ of fluoride, to which had been added 1 mg. of sodium chloride or nitrate, interference was complete and no crystals could be observed in the field. Some of these ions influenced the shape of the crystals, tending to round off the corners of the hexagon, so that they were more nearly circular. In the distillation, the negative ions were volatilized along with the fluosilicic acid and were absorbed in the hanging drop, where they influenced the formation of the crystals.

It was essential that conditions of absolute cleanliness be maintained in preparing and handling microscope slides.

APPLICATIONS

Detection of Fluorides in Foods, Drugs, and Biologicals. The sensitivity of the modified crystal test lends itself to the detection of minute amounts of fluoride in foodstuffs, drugs, biological materials, tissues, and other organic and inorganic substances. This procedure is designed to eliminate the interferences of carbonates and chlorides, which are usually present in the ash of such substances.

Alkalize a few grams of the material to be tested with a slight excess of sodium carbonate solution, dry in an oven at 100° C., cautiously burn off the organic matter over a Bunsen flame, then continue heating in a muffle furnace held below 500° C. until a gray or white ash is obtained. Transfer about 20 mg. of the ash to a 15-ml. test tube, add 10 ml. of distilled water, shake until all soluble matter is dissolved, and then transfer half of the solution to another 15-ml. test tube. To the second tube, which serves as a control, add 2.0 γ of fluoride, and heat both tubes in a beaker of boiling water. Add a small pinch of silver sulfate powder to each, and shake occasionally until the silver precipitate formed coagulates. Test the clear supernatant liquid by adding a drop of saturated silver sulfate solution, and if additional precipitation occurs, add more powdered silver sulfate; continue to heat, shake, and test until precipitation is complete. Cool tubes in an ice bath and filter through small paper filters into 10-ml. porcelain crucibles, washing with two successive small portions of water. To each crucible add circa 0.5 mg. of calcium carbonate powder and circa 2 mg. of powdered silica, then evaporate gently (to avoid spattering) on a hot plate to dryness, allowing the crucibles to bake for a few minutes. Cool to room temperature and proceed with the modified crystal test. If fluoride is present in the sample tested it is indicated by the presence of the characteristic fluosilicate crystals, the control being, of course, positive.

Distinction between Inorganic Fluorides and Sodium Monofluoroacetate. With the discovery of the powerful rodenticidal action of sodium monofluoroacetate, designated in the trade as Compound 1080, and its introduction into the exterminating industry, it became necessary to devise methods for the detection and estimation of this compound. Ramsey and Clifford (11) published a quantitative method for its estimation in the presence of inorganic fluorides involving a chromatographic separation followed by an alkaline fusion of the isolated monofluoroacetic acid and subsequent estimation of the released fluorides by a standard method. Ramsey and Patterson (12) followed later with a qualitative test based on the formation of thioindigo, a red dye, by the interaction of monofluoroacetic and thiosalicylic acids; both procedures are rather lengthy and complicated.

It was found that the modified crystal test afforded a means of distinguishing between inorganic fluorides and sodium monofluoroacetate. The test is based on the stability of the carbon-fluorine linkage in monofluoroacetic acid in contact with hot concentrated sulfuric acid, under which condition no free hydrofluoric acid is released. Therefore, the modified crystal test performed on sodium monofluoroacetate will be negative, as no hydrofluoric acid is evolved. If, however, monofluoroacetic acid is first fused with sodium carbonate, the fluorine is converted into sodium fluoride, which with the addition of silica will produce a positive crystal test.

Transfer 0.05 ml. (0.01 mg. of the salt or 1.9 γ of fluorine) of a standard solution of sodium monofluoroacetate to each of two 10-ml. porcelain crucibles. To the second crucible add a drop of phenolphthalein solution and a small drop of $0.01N$ sodium hydroxide solution, and dry both crucibles on a steam bath. Fuse the contents of the second crucible over a low Bunsen flame or in a muffle furnace below 500° C. for a short time. Allow the crucibles to cool, add circa 2 mg. of powdered silica to each, and perform the crystal test on both. The unfused sodium monofluoroacetate will give a negative test, while the fused salt will produce large numbers of sodium fluosilicate crystals. If the volume of standard solution is increased to 0.2 ml. (7.6 γ of fluorine) great masses of pink crystals are formed in the hanging drop. Commercial sodium monofluoroacetate usually contains traces of free sodium fluoride and a few crystals are sometimes observed when the test is performed on the unfused salt, but the contrast in numbers between the latter and the fused salt is so sharp that no doubt exists in the interpretation of results. The test is not specific for sodium monofluoroacetate, as other organic fluorine compounds will be converted to sodium fluoride by alkaline fusion. Furthermore the test is inapplicable to those organic fluorine compounds in which the carbon-fluorine linkage is unstable when in contact with sulfuric acid

This test was put to practical application when Compound 1080 began to be employed as a rodenticide in New York City. The practice among exterminators was to distribute a number of shallow paper cups containing about 10 ml. of a 0.4% solution of the salt throughout a rodent-infested cellar. Because of the high toxicity of the compound to humans, the Sanitary Code required that the cups be collected and burned after sufficient time had elapsed for the rodents to partake of the bait. Instances occurred where the Code was violated, and in order to prove legally the presence of sodium monofluoroacetate rather than sodium fluoride, the cups were collected and subjected to the crystal test. The bait had usually evaporated to dryness by the time they were collected, and the crystal test was performed on a drop of infusion of the cup in 10 ml. of water.

APPLICATION AS QUANTITATIVE METHOD

The sensitivity of the crystal test suggested the possibility of its application to the quantitative estimation of the fluoride content of potable waters with a very low fluoride content. A study was made of the recovery from such waters by evaporating measured volumes of water, applying the crystal test to the residue, and microscopically estimating the number of sodium fluosilicate crystals formed in the hanging drop. Many tests were performed on measured volumes of tap water and also on volumes of distilled water to which had been added known quantities of fluoride.

Where the volume of sample evaporated was small—that is, up to 30 ml.—the number of crystals counted in the hanging drop was fairly consistent and it was possible to make quantitative comparisons. However, evaporation of larger volumes of water, up to and beyond 100 ml. led to highly inconsistent results, and numerical comparisons were of little use.

From this work the author concluded that the failure in the quantitative recovery probably stems from the varying adsorption of fluorides on the surface of the glass container during evaporation. Evaporation in porcelain or platinum containers gave results no more consistent, nor did the use of various alkalizing agents such as sodium, calcium, and magnesium hydroxides and carbonates. If this failure is indeed due to adsorp-

tion, this factor is present and is a source of error in any of the accepted methods for the microestimation of fluorides where evaporation is necessary. The addition of a drop of silver sulfate solution served to prevent the adsorption to some extent, but not sufficiently to create a condition of complete recovery. If this difficulty can be overcome, this approach to the problem of estimating fluoride in potable waters of very low content has the possibility of producing a method more accurate than those currently in use.

DISCUSSION

The modified crystal test produces greater sensitivity in the microchemical detection of fluorides, although no claim is made that it represents the complete solution to the problem of fluoride determination. The interference of negative ions, particularly nitrate, requires further study. The procedure for the distinction between inorganic fluorides and monofluoroacetic acid is not intended as a replacement for the methods of Ramsey and coworkers, but is presented as a quick preliminary sorting test, useful and time saving, where the analysis of a number of samples is required. It may also serve as a confirmatory test.

In the suggested application for quantitative estimation it would be necessary to design and experiment with a microchemical apparatus in which all the evolved fluosilicic acid is entrapped. This might be accomplished by an apparatus in which a thin stream of air passes through a heated reaction chamber into a trap of cold hanging drop solution, followed by microscopic examination of an aliquot of the latter.

During the distillation the heating block was maintained at a temperature of 170° C., but this does not mean that the reaction took place at this level, as measurements indicated that the actual reaction temperature was in the region of 110° C. It was found that 170° C. was the optimum condition for producing the maximum number of crystals in the hanging drop, although some distillation takes place when the block is held at lower temperature levels.

The technique of applying the hanging drop to the microscope slide requires a further word of instruction. The purpose of restricting the diameter of the drop is to facilitate easier examination of the slide. It is desirable to obtain maximum convexity approaching hemispherical shape in the drop, so as to prevent spreading during the distillation. This is best accomplished by cooling the slide in a refrigerator prior to transferring the drop, and then giving the glass applicator rod, wet with hanging drop solution, a quick touch to the slide, a technique easily acquired with a few practice attempts. It is well to restrict the alkalinity of the residue in the reaction crucibles to a minimum, as the addition of sulfuric acid generates water which distills and condenses, thereby increasing the diameter of the hanging drop.

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High Frequency Induction Furnace in the Determination of Radiocarbon

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Assay of materials labeled with carbon-14 commonly involves their oxidation to carbon dioxide, which is then measured directly by a Geiger counter or in a carbon dioxide ionization chamber. Alternatively, the material may be converted to a solid, such as barium carbonate, before radioassay. The use of high frequency induction heating in an oxygen atmosphere provides a fast, reliable method of oxidizing radioactive carbon to carbon dioxide, which can then be determined in the usual way. The method has been successfully applied to the combustion of radiocarbon in mixtures of labeled lauric acid and quartz and in radioactive solutions. The radioactive carbon dioxide has been assayed with internal Geiger counters to prove the reliability of the combustion method.

BOTH dry and wet combustion techniques have been used in tracer work to oxidize carbon to carbon dioxide. Dry combustion, as in the Liebig and sodium peroxide fusion methods, and wet combustion, as in the persulfate and Van Slyke–Folch methods, are described in the literature.

The use of a high frequency induction furnace provides a fast and convenient method of dry oxidation, securing a reproducible and reliable combustion.

Induction heating has been used for the determination of carbon in high-melting alloys (6) as well as in low-carbon iron and steel (8). A rapid method of determining minute quantities of carbon in metals using the high frequency induction furnace has been described (7). Recently, electrolytic iron powder has been used to induce heat in a nonconductor such as soil (4). In this way, rapid and accurate determinations of carbon in soils are being made.

In the present investigation the induction furnace has been applied to the combustion of radiocarbon. Mixtures of labeled lauric acid and quartz and a radioactive solution were chosen to illustrate the method. Using the gas counting technique described by Miller and Brown (1, 5) adopted in this laboratory (3), the reproducibility of the oxidation procedure was ascertained.

The carbon dioxide evolved was purified in a leakproof train, using chemical absorbents for water and sulfur dioxide. A special procedure was used for humid and liquid samples from which much water vapor was evolved.

APPARATUS

A Fisher induction carbon apparatus, originally designed for the determination of carbon and sulfur in steels, was used as a high frequency induction furnace. It is commercially available with a leakproof train containing chemical absorbents for water and sulfur dioxide.

The outlet of the instrument was sealed to a vacuum system which has five liquid nitrogen traps. Three of them suffice to recover the carbon dioxide when the water content of the sample is low. When water is present, the two extra traps are used to distill the carbon dioxide from the water.

Figure 1 is a schematic drawing of the apparatus.

SAMPLE

Because the method is intended primarily for tracer use in mineral engineering, quartz was chosen as a suitable mineral to carry a labeled organic agent. To induce heat in a nonconducting mineral, electrolytic iron powder was mixed with the sample, as suggested by Jackson (4) for the determination of organic carbon in soil. Actually a mixture of iron and tin is used to lower the melting point of the iron. The sample should fuse completely during oxidation. Smith and Hockenyos (6) remark that failure to obtain fusion results in incomplete removal of carbon as carbon dioxide in the determination of carbon in high-melting alloys.
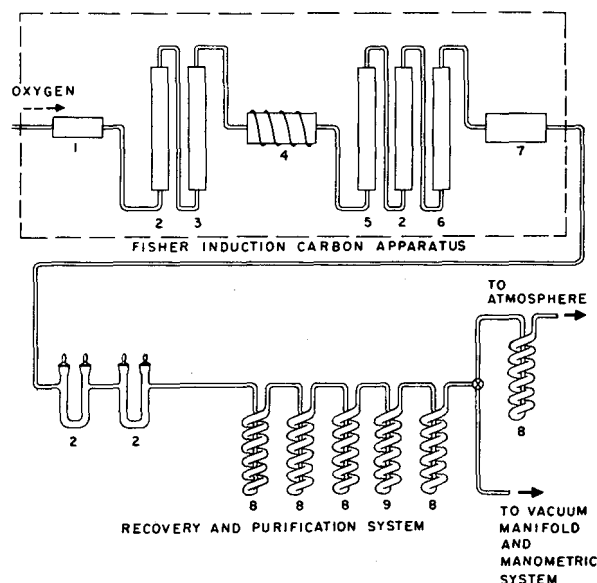

Figure 1. Apparatus for burning sample and isolating active carbon dioxide

1. Solenoid valve
2. Magnesium perchlorate
3. Caroxite
4. Combustion tube and coil
5. Manganese dioxide
6. Flowmeter
7. Platinum oxidizer
8. Liquid nitrogen trap
9. Liquid nitrogen and isohexane mixture at $-140°$ C.

If the powder containing the sample, iron, and tin is placed on and then covered by a layer of electrolytic iron, satisfactory conditions are obtained with the use of the Fisher furnace, which is credited by the manufacturers with reaching temperatures of 1600° C.

Samples containing quartz and 3, 4, and 5 mg. of radioactive lauric acid were prepared in the following way.

An Alundum boat was one-half filled with granular Alundum and approximately 0.5 gram of powdered electrolytic iron was then spread evenly over the Alundum. One gram of quartz was thoroughly mixed with the powdered radioactive lauric acid and approximately 0.5 gram of a 50–50 mixture of granular tin and electrolytic iron powder. This mixture was placed on the bed of electrolytic iron powder in the boat and covered with additional electrolytic iron and granular Alundum.

As adsorbed coatings of radiocarbon-marked organic agents on minerals have been measured by depending on low-temperature

combustion methods (2), it is reasonable to expect that even more reproducible results are obtained at the high temperature obtained in the induction furnace. Accordingly, special tests were not made to determine whether adsorbed coatings could be measured as readily as mixtures.

Radioactive solution samples were prepared in the following way.

An Alundum boat was one-half filled with granular Alundum and approximately 0.5 gram of powdered electrolytic iron was spread evenly over the Alundum as in the preparation of solid samples. Next, 1 ml. of radioactive solution was evenly added dropwise to the bed of electrolytic iron powder in the boat, which was left overnight in a desiccator with anhydrous magnesium perchlorate. When the concentration of the solution so required, enough solid inactive lauric acid was added to the boat to carry the isotopic carbon. A layer of granular Alundum was then added to the boat and the sample was ready for determination (see Figure 2).

EXPERIMENTAL PROCEDURE

The boat and sleeve were pushed into position in the induction furnace. The apparatus was fired and the effluent gases passed through three traps cooled by liquid nitrogen and an extra safety trap cooled by liquid nitrogen and open to the air. The complete cycle lasted 2 minutes, after which the three traps where gases and water were frozen were isolated from the induction apparatus and the atmosphere by closing appropriate taps in the vacuum system. The oxygen was then pumped out.

When the chemical absorbents were sufficient to prevent water contamination of the carbon dioxide, the latter was allowed to evaporate from the liquid nitrogen traps, and was then ready for measurement.
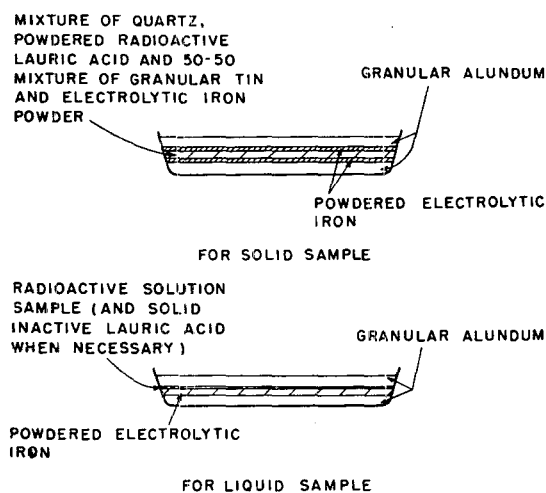
MIXTURE OF QUARTZ,
POWDERED RADIOACTIVE
LAURIC ACID AND 50-50
MIXTURE OF GRANULAR TIN   GRANULAR ALUNDUM
AND ELECTROLYTIC IRON
POWDER

POWDERED ELECTROLYTIC
IRON

FOR SOLID SAMPLE

RADIOACTIVE SOLUTION
SAMPLE (AND SOLID
INACTIVE LAURIC ACID
WHEN NECESSARY)   GRANULAR ALUNDUM

POWDERED ELECTROLYTIC
IRON

FOR LIQUID SAMPLE

Figure 2.   Preparation of combustion boat

When water was not completely absorbed by the chemicals as in the case of humid or liquid samples, it remained in part frozen with the carbon dioxide in the three traps. In this case the contents of these traps were allowed to warm up and diffuse through a glass spiral at $-140° \pm 2°$ C. (boiling solution of isohexane in liquid nitrogen) to another trap cooled with liquid nitrogen. These two traps were otherwise not used. The operation was performed under vacuum with the stopcocks to the pumps closed. Fifteen minutes sufficed for the operating volume of carbon dioxide to diffuse and be recovered entirely free of water in the end liquid nitrogen trap.

Assay of the radioactive carbon dioxide has been made with internal Geiger counters using a technique already described (3).

It is not important if all the ingredients in the boat give off carbon dioxide, provided it is not radioactive carbon dioxide, but the technique of measuring radioactivity with an internal Geiger counter includes a blank to determine the background.

RESULTS

Results obtained determining carbon-14 in mixtures of quartz and radioactive lauric acid are shown in Table I, which presents the data of 14 different determinations. For each of the three amounts of lauric acid, the standard deviation of a single observation is under 3.5%. This value is considered satisfactory for the kind of counters used.

Table I.   Results Obtained with Mixtures of Quartz and 3, 4, and 5 Mg. of Radioactive Lauric Acid

Counting Rate, Counts per Minute per Mg.		
3-Mg. sample	4-Mg. sample	5-Mg. sample
254	255	250
272	260	265
247	262	271
259	243	270
259		
250		
Average 257	255	259

An experiment in duplicate was also made using a dilute aqueous lauric acid solution instead of powdered solid agent. The solution contained 20 mg. per liter and 1.080 ± 0.003 ml. of this solution was used in each test. The total net activity of each sample was 494 ± 10 counts per minute in both cases. The agreement is excellent.

APPLICATIONS

The high frequency induction furnace may be used for the oxidation of carbon-14 to carbon dioxide in the determination of labeled collectors for tracer work in mineral engineering. Minerals with the radioactive substance adsorbed, as well as humid samples or radioactive solutions, may be conveniently oxidized using this combustion method. An internal carbon dioxide counter or a carbon dioxide ionization chamber can be used for the final counting of radioactive carbon dioxide. Alternatively the carbon dioxide may be converted to a solid, such as barium carbonate, before radioassay.

Samples other than those encountered in mineral engineering research work can probably be treated in the same way.

It is believed that radioactive sulfur may be also determined using induction heating to convert the sulfur to sulfur dioxide, but experiments to support this opinion have yet to be made.

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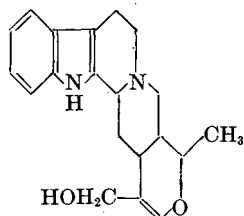## 92. Ajmalicine, Ajmalicine Hydrate, and *py*-Tetrahydroserpentinol

HARRY A. ROSE, Lilly Research Laboratories, Indianapolis 6, Ind.
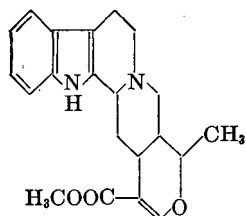
Structure of ajmalicine

Structure of *py*-tetrahydroserpentinol

AJMALICINE is one of the alkaloids found in the plant *Rauwolfia serpentina* benth. The compound has been given several names by various investigators: ajmalicine, alkaloid F, δ-yohimbine, and *py*-tetrahydroserpentine (for the hydrate) (*1–4*). Discussions of the chemistry and structure of the compound have been published by these investigators and independently by Norbert Neuss of this laboratory.

Anhydrous ajmalicine is obtained by crystallization from absolute methanol, while the hydrate is obtained by crystallization from methanol-water solutions. The hydrate on heating to 110° to 120° C. loses water and the resulting powder gives the x-ray pattern of the anhydrous material.

Tetrahydroserpentinol is obtained by reduction of ajmalicine with hydrogen in the presence of lithium aluminum hydride.
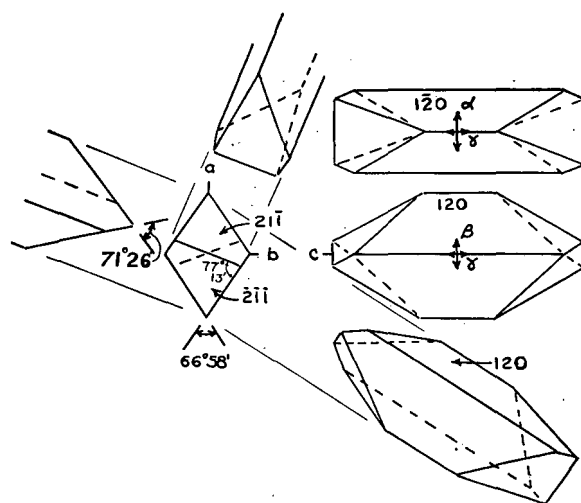

Figure 1. Projections of ajmalicine

FUSION DATA. On heating, ajmalicine melts in the range 253 254° C. with decomposition.

AJMALICINE. HYDRATE

CRYSTAL MORPHOLOGY

Crystal System. Monoclinic.

Form and Habit. Blades lying on 100 elongated parallel to and showing the prism {110}, positive orthodome {$\bar{2}$01}, ortho pinacoid {100} and basal pinacoid {001}.

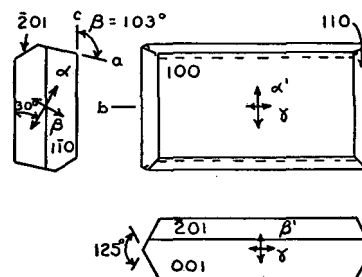Axial Ratio. $a:b:c$ = 1.9822:1:0.8233 (x-ray).

Figure 2. Orthographic projection of ajmalicine hydrate

X-Ray Powder Diffraction Data for Ajmalicine

d	I/I_1	hkl	d(Calcd.)
7.53	0.40	011	7.52
7.08	0.32	120	7.11
6.53	0.60	111	6.49
6.07	0.16.	210	6.09
5.42	1.00	121	5.42
5.11	0.16	220	5.14
4.93	0.08	211	4.89
4.65	0.40	031	4.70
4.38	1.00	221	4.38
4.20	0.04	002	4.19
4.11	0.04	310	4.16
4.02	0.04	140	4.05
3.91	0.08	102	3.98
3.88	0.40	112	3.88
3.61	0.32	122	3.61
3.26	0.20	222	3.24
3.02	0.12	420	3.04
2.99	0.12	042	2.99
2.57	0.08	440	2.57

AJMALICINE

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Blades elongated parallel to *c* and showing the prism {120} and right bisphenoid {$\bar{2}$11}.

Axial Ratio. $a:b:c$ = 0.7557:1:0.4917.

Interfacial Angles (polar). 120 $\wedge$ $\overline{1}$20 = 66° 58'. 21$\bar{1}$ $\wedge$ $\bar{2}\overline{1}\overline{1}$ = 108° 34'.

X-RAY DIFFRACTION DATA

Cell Dimensions. a = 12.88 A.; b = 17.04 A.; c = 8.38 A.

Formula Weights per Cell. 4(4.011, x-ray).

Formula Weight. 352.4.

Density. 1.276 grams per cc. (flotation), 1.273 grams per cc. (x-ray).

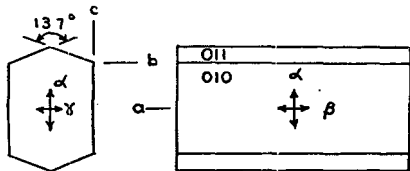OPTICAL PROPERTIES

Refractive Indices (5893 A., 25° C.). α = 1.59, β = 1.678, γ = 1.686.

Optic Axial Angles. $2V$ = (−) 18° (calcd. from β and Mallard's constant), $2E$ = 30°.

Optic Axial Plane. 100.

Acute Bisectrix. α = *b*.

X-Ray Powder Diffraction Data for Hydrate Ajmalicine

d	I/I_1	hkl	d(Calcd.)
16.31	0.20	100	16.28
8.08	0.03	200	8.14
7.50	0.20	110	7.57
6.76	0.20	001	6.76
5.85	1.00	210	5.86
5.43	0.07	300	5.43
5.32	0.07	11$\bar{1}$	5.29
4.82	0.67	21$\bar{1}$	4.82
4.56	0.33	310	4.56
4.16	0.53	31$\bar{1}$	4.16
4.09	0.13	400	4.07
3.89	0.07	40$\bar{1}$	3.89
3.78	0.03	220	3.78
3.59	0.07	12$\bar{1}$	3.58
3.53	0.07	41$\bar{1}$	3.53
3.43	0.27	22$\bar{1}$	3.42
3.17	0.13	102	3.17
3.02	0.20	31$\bar{2}$	3.02
2.86	0.07	202	2.90

Interfacial Angles (polar). 110 Λ Ī10 = 53° 32' (x-ray), 2̄01 001 = 45°.

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 16.71$ A.; $b = 8.43$ A.; $c = 6.94$ A.

Formula Weights per Cell. 2(2.03, x-ray).

Formula Weight. 370.4 (monohydrate).

Density. 1.277 grams per cc. (flotation), 1.292 grams per cc. x-ray).

OPTICAL PROPERTIES

Refractive Indices. (5893 A., 25° C.). $\alpha = 1.580, \beta = 1.680$, = 1.683.

Optic Axial Angles. $2V = (-) 16°$ (calcd. from α, β, and γ), 8° (calcd. from β and Mallard's constant), $2E = 31°$.

Optic Axial Plane. Perpendicular to 010.

Acute Bisectrix. α.

Extinction. $\alpha \Lambda c = 30°$ in obtuse β.

Dispersion. $v > r$, large.

FUSION DATA. On heating, ajmalicine hydrate loses water in the ange 110° to 120° C. to give the anhydrous form.

py-TETRAHYDROSERPENTINOL

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Blades lying on 010 elongated parallel to a and showing the forms macropinacoid {100}, brachydome {011}, and brachypinacoid {010}.

Cleavage. Good, parallel to 100.

Axial Ratio. $a:b:c = 0.4138:1:0.3929$ (x-ray).

Interfacial Angle (polar) 011 Λ 0Ī1 = 42° 54'.

X-RAY DIFFRACTION

Cell Dimensions. $a = 11.50$ A.; $b = 27.79$ A.; $c = 10.92$ A.

Formula Weights per Cell. 8(7.99, x-ray).

Formula Weight. 324.4.

Density. 1.233 grams per cc. (flotation), 1.235 grams per cc. x-ray).

Figure 3. Orthographic projection of *py*-tetrahydroserpentinol

OPTICAL PROPERTIES

Refractive Indices (5893 A., 25° C.). $\alpha = 1.590, \beta = 1.613$, $\gamma = 1.68$ (est.).

Optic Axial Angle. $2V = (+) 60°$ (est.)

Optic Axial Plane. 100.

Acute Bisectrix. $\gamma = b$.

Dispersion. $r > v$.

X-Ray Powder Diffraction Data for *py*-Tetrahydro-serpentinol

d	I/I_1	hkl	d(Calcd.)
13.90	0.53	020	13.90
10.15	0.20	011	10.15
8.86	0.66	120	8.86
8.57	0.20	021	8.59
7.61	0.27	111	7.62
6.88	0.07	040	6.95
5.95	0.13	140	5.91
5.78	0.13	200	5.75
5.63	0.27	210	5.63
5.32	0.53	220	5.31
5.22	0.53	141	5.19
4.84	1.00	112	4.86
4.76	0.20	221	4.78
4.63	1.00	060	4.63
4.45	0.13	240	4.41
4.35	0.20	132	4.35
4.08	0.07	241	4.09
3.92	0.13	212	3.92
3.80	0.66	222	3.81
3.67	0.20	232	3.64
3.53	0.03	..	..
3.44	0.03	..	..
3.36	0.13	..	..
3.06	0.03	..	..
3.01	0.07	..	..
2.967	0.03	..	..
2.860	0.03	..	..
2.633	0.03	..	..

FUSION DATA. On heating, tetrahydroserpentinol melts at 244° C. with decomposition.

X-RAY POWDER DIFFRACTION DATA. All powder diffraction data were obtained using a camera 114.6 mm. in diameter and chromium radiation with vanadium pentoxide filter. A wave-length value of 2.2896 A. was used in the calculations.

ACKNOWLEDGMENT

The author is indebted to Norbert Neuss of these laboratories for supplying the crystals used in this work.

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MEETING REPORT

Society for Analytical Chemistry

THE Western Section of the society met November 13 at Cardiff, Wales, when the following paper was presented and discussed.

Gas-Phase Chromatography as an Analytical Technique. C. J. HARDY, Department of Chemistry, University of Bristol, Bristol.

The development of gas-phase chromatography as a technique for the separation and estimation of volatile substances was described. The basic principles were briefly outlined and apparatus and practical methods of analysis described in detail. Separations of substances by differential adsorption on, or desorption from charcoal and similar adsorbents were compared with those obtained by the newer James and Martin technique on gas-liquid partition columns.

Intensive research by many workers during the last 2 years on the development of apparatus and the semimicroanalysis of gases and liquids was summarized. Examples to illustrate the methods and results were given from the author's own work on the separation and determination of halogenated hydrocarbons, aliphatic hydrocarbons, and alkyl esters. Gas-phase chromatography has been shown to be applicable to specific problems such as the analysis of intermediates and final products in gaseous reactions. An example of the analysis of products in a gaseous reaction between ethyl nitrite and nitrogen dioxide was given in detail.

Gas-phase chromatography was compared with fractional distillation and mass and infrared spectrometry as a technique for the separation of closely related compounds and complex mixtures. Its use for the preparation of certain pure substances was considered. The advantages of gas-phase chromatography over other analytical methods and its potential applications in many fields were discussed.

The tenth annual general meeting of the Physical Methods Group, held November 30 in London, presented a discussion on possibilities in the establishment of standard samples for the determination of some trace elements. Topics mentioned included the use of square wave polarography and radioactivation analysis, involving lead in foodstuffs, and minor elements in analytical reagents, pure metals, ferrous and nonferrous alloys, and rocks and minerals.

A joint meeting of the Society for Analytical Chemistry and the Oils and Fats Group of the Society of Chemical Industry was held December 1 in London, devoted to the subject of methods for the chemical determination of vitamin A.

Determination of Vitamin A in Natural Products and Especially Cod Liver Oil. R. A. MORTON, Department of Biochemistry, The University, Liverpool, AND F. BRO-RASMUSSEN, State Vitamin Laboratory, Copenhagen.

In view of the lack of official recognition of vitamin A_2 it is not perhaps desirable to express the results of spectrophotometric assays in terms of total vitamin A activity without indicating how much is due to vitamin A_2. Liver oils from salt fish in general have at least 90% of the total activity supplied by vitamin A_1.

The analytical procedures of the British and United States Pharmacopeias were discussed in relation to the historical setting and the newer problems arising out of the presence of three vitamin A–active substances (all-trans and neovitamin A_1 and vitamin A_2) in fish liver oils.

The properties of the three active substances were noted and the combined effects of their intrinsic biological activities and their absorption spectra were worked out with special reference to conversion factors.

The geometrical correction procedure using the fixation points for all-trans vitamin A_1 overcorrects the neovitamin A contribution to the total absorption but this is very nearly balanced by the lower biological activity of neovitamin A compared with the all-trans form.

Three cod liver oils and two rich oils have been examined by the new chromatographic method of Bro-Rasmussen, Hjarde, and Porotnikoff, which permits quantitative estimate of how the total vitamin A absorption at 325 mμ is distributed between the three active substances. The same oils tested on the "unsap." without chromatography but corrected by the "geometrical" procedure lead to much the same estimate of total vitamin A_1 potency.

Chromatographic Separation of Vitamin A–Active Compounds in Cod Liver Oil. F. BRO-RASMUSSEN, W. HJARDE, AND OLGA POROTNIKOFF, State Vitamin Laboratory, Copenhagen, Denmark.

A method was described for preparing dicalcium phosphate for chromatography of fish liver oil unsaponifiable matter. The adsorbent permits a separation of all-trans vitamin A, neovitamin A and vitamin A_2. The method recommended for cod liver oil consists of determining the absorption spectrum of the total vitamin A fraction obtained by a single chromatographic separation and determining the proportions of the three vitamin A–active materials by a separate chromatography.

Modified Method for Spectrophotometric Estimation of Vitamin A in Margarine. J. W. LORD AND PAULINE M. BRADLEY, Research Department, J. Bibby & Sons, Ltd., Liverpool.

In principle, the modified method is similar to the official method, but does not require specially activated adsorbents. Commercially available defatted bone meal of appropriate particle size is the selected adsorbent. Experiments on margarine show that it is possible to recover from the column a fraction giving spectral absorption substantially the same as that of vitamin A. After a correction for slight irrelevant absorption, results on commercial margarines using the official and modified methods are, for practical purposes, in good argument.

At the 349th meeting of the Physical Methods Group, held January 18 in London, four papers were presented.

Solvent Extraction. Introductory Survey. H. M. N. H. IRVING, Inorganic Chemistry Laboratory, Oxford.

The distribution of a substance between two immiscible phases lends itself to procedures for enrichment or separation; these can be conducted by batch or continuous operation. It is convenient to distinguish the solvent extraction of inorganic materials under four classes: neutral substances which obey the Nernst partition isotherm (iodine or osmium tetroxide); uncharged inner complexes of metals with chelating agents (oxine dithizone, T.T.A.); mineral acids, their metallic salts, and metal acido complexes; salts or ion pairs incorporating bulky anions or cations (tetraphenylarsonium perrhenate or pertechnetate). Some of the difficulties encountered in the practical application and quantitative theoretical treatment of solvent extraction were indicated.

Laboratory Apparatus for Solvent Extraction. I. WELLS, Atomic Energy Research Establishment, Harwell.

Laboratory equipment for solvent extraction is used to achieve separation for analytical work and to investigate and develop new types of solvent extraction processes. Simple separating funnel techniques are suitable when the partition coefficients are large in favor of the solvent. When the partition coefficients are small, countercurrent flow is necessary to keep to a minimum the number of operations and the volume of extract. The advantages and disadvantages of various types of countercurrent equipment were discussed. Two types of laboratory apparatus were demonstrated.

Fractionation of Crude Fumagillin by Distribution Methods. R. R. GOODALL AND JUSTUS K. LANDQUIST, Imperial Chemical·(Pharmaceuticals), Inc., Manchester.

From a strain of *Aspergillus fumigatus* C. T. Calam isolated fumagillin and a noncrystalline product which also had amebicidal activity. The purification of this product was difficult and we submitted it to a countercurrent fractionation to ascertain whether the biological activity was due to contamination with fumagillin or to the presence of a new amebicide. Distribution in the system benzene petrol, ethanol-water, demonstrated the presence of fumagillin and two other fractions, neither of which had amebicidal activity.

There followed the development of distribution methods for the bulk purification of crude fumagillin. In this case advantage was taken of the acidic properties of fumagillin for separation from neutral or less acidic impurities by partition between a dilute aqueous buffer solution and a solvent only partly miscible with water. Fumagillin and accompanying impurities are decomposed in aqueous solution above pH 10, so that operating conditions were kept below this level. For example, distribution between pH 9 aqueous borax buffer (0.05M) and butyl acetate effected substantial purification, so that subsequently fumagillin of purity higher than 90% was isolated in good yield by a single crystallization from acetone.

Solvent Extraction in the Analysis of Precious Metals. W. A. McBRYDE, University of Toronto, Toronto, Canada.

Present-day applications of solvent extraction for the analytical separation of gold and the six platinum metals were summarized. The types of compounds whose solvent extraction was discussed include halogen complexes, oxides, complexes with stannous chloride and organic complexes. The choice of method in these cases usually governed by the environment of the metal being separated and by the subsequent operations in the over-all analysis. Another case of solvent extraction discussed is that of the metals themselves from reducing fluxes into collecting buttons of lead or other metals a for instance, in the fire assay.

At a joint meeting of the Western Section with the local sections of the Royal Institute of Chemistry, the Society of Chemical Industry, and the Chemical Society, held January 27 at Bristol E. Windle Taylor, Metropolitan Water Board, spoke on "Recent Advances in the Bacteriological Examination of Water."

Until about 75 years ago the assessment of purity of a water depended upon the chemical analysis, but with the discovery of bacteria and the association of some of them as being the causative agents of disease, a more delicate test for purity became available.

The chief object of the bacteriological analysis of water is to ensure that the water is free from pathogenic bacteria by the time it passes into supply to consumers. The criterion of purity from the bacteriological standpoint has been based on the isolation of normal intestinal organisms from water. If it can be shown that a sample is free from bacteria normally present in the human or the animal intestine, it can be assumed that the water is free from disease-producing bacteria.

Correct sampling procedure is of importance; otherwise the results of laboratory investigation are worthless. The bacteriological analysis is only a part of the investigation of a water supply and the result must be interpreted in conjunction with the chemical analysis, inspection of the site, and its history.

Improvements in the bacteriological examination of water have been concentrated in recent years on speeding up the examination and the present practice for the control of water supplies is to sample more frequently, but limit the scope of the examination on each sample.

New bacteriological procedures for the isolation of *coliaerogenes* and other microorganisms from water were outlined and the significance of the results was discussed. Finally a plea was made for uniformity in analytical procedure. Report 71, "The Bacteriological Examination of Water Supplies" published by the Ministry of Health has done much toward this end. Uniformity of methods means uniformity of interpretation and leads to greater confidence in their value when the results and opinions therefrom are submitted and explained to lay persons.

A lecture on "The Complexones and Their Analytical Application," accompanied by practical demonstrations was given February 2 in London by G. Schwarzenbach, Zurich University.

Ethylenediaminetetraacetic acid (EDTA, Versene, ENTA, sequestric acid) is probably the most important and versatile organic reagent ever introduced into analytical practice. Yet it is only one of the large group of polyaminocarboxylic acids developed at Zurich by Schwarzenbach and known collectively as the "complexones," which have found widespread use as complexing (masking) agents in gravimetric and volumetric analysis, in polarography, in separations of rare earths, and in many other fields. In conjunction with "metal indicators," many of which were first developed at Zurich the complexones provide new volumetric procedures for many metals among which the simple and rapid determinations of calcium and magnesium hardness in water is the most revolutionary.

Ionbumping Digestion Heater

ndrejs Steinbergs, Division oi Plant Industry, Commonwealth Scientific nd Industrial Research Organization, Canberra, A. C. T., Australia.

WHEN solid particles are boiled for a prolonged period with a liquid on the normal type of electric heater (as in Kjeldahl igestion for determination of nitrogen in soils), bumping usually ccurs, accompanied frequently by loss of some of the contents ·om the container. The liquid enclosed among the heavy solid articles is prevented from free movement and when heating is ·om beneath, this leads to local superheating and consequent umping.

A	ASBESTOS CEMENT OUTER BODY
B	PORCELAIN SUPPORT FOR HEATING ELEMENT
C	NI-CHROME HEATING ELEMENT
D	PORCELAIN SUPPORT FOR FLASK
E	ASBESTOS CEMENT BOTTOM PLATE
F	ANGLE IRON FRAME
G	BUSBARS
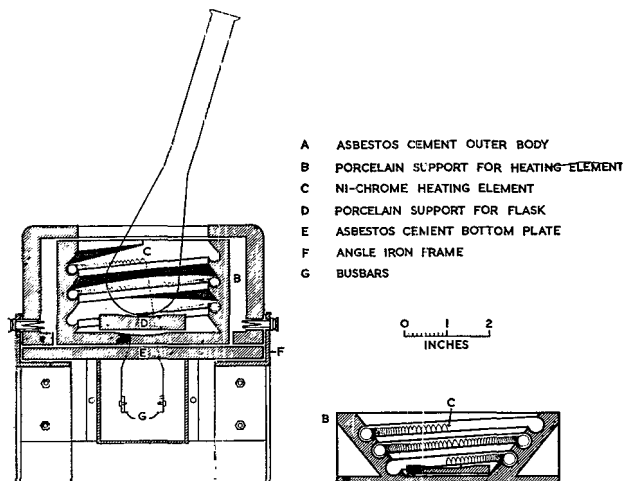
Figure 1

A new type of digestion heater eliminates this effect. The main eating is directed toward the sides of the flask, with only slight eating from the bottom. Accordingly, the bulk of the liquid ecomes heated, and readily circulates.

Two types of heater have been used: one with cylindrically haped walls for use with Kjeldahl flasks up to 30-ml. volume or or test tubes, the other with conical shaped walls for Kjeldahl asks up to 100-ml. volume. Details of the heaters are shown in igure 1.

The heating element, C, is wound twice around the inside of a reclay support, B, commencing 1.0 to 1.5 cm. from the bottom. The flask is placed on a removable concave support, D, inside the eater. By placing asbestos rings underneath the support or by emoving it and placing the flask directly on the concave bottom f the heater, it is possible to adjust the heating of the flask to the esired zone.

An assembly of six heaters is mounted on an asbestos-cement ase plate, E, lying in an angle-iron frame, F. The asbestos-ement outer cover, A, for each of the heaters is fastened to the rame by means of wedge-tailed clips. Thus all three parts (outer over, heater, and base plate) are easily firmly assembled or dis-nantled for repairs.

All Nichrome wires of the heaters are connected to bus bars, G, ocated under the base plate. These run along the length of the ssembly under the heaters, their ends resting freely in slots in sbestos-cement sheets which are attached to the inside faces of he end panels of the heater support. The bus bars are protected ·y a safety casing clipped to the asbestos-cement side sheets. Thus the bus bars are readily accessible.

In several hundred nitrogen determinations, with a wide range ·f soil types, no bumping whatsoever occurred during the diges-ions. Because of more efficient and uninterrupted heating, the itrogen analyses gave results in many cases up to 10% higher han those obtained with the usual type of digestion stand. In itrogen determinations on plant materials, frothing in early

stages of the digestion has been reduced by this improved heating arrangement and creeping of the charring material into the neck of the flask eliminated.

Salt Bridges of Porous Glass and Ion Exchange Membranes

W. N. Carson, Jr., C. E. Michelson, and Karl Koyama, Hanford Atomic Products Operation, General Electric Co., Richland, Wash.

ALTHOUGH salt bridges are widely used in the analytical labora-tory in polarography, titrimetry, and pH measurement, and their use is increasing as more electrometric methods of analysis are employed, the present forms and materials leave much to be desired in mechanical stability, small solution flow, and low electrical resistance. Work in this laboratory has shown that porous glass and ion exchange resin membranes can be used to make salt bridges that are superior in most respects to conventional bridges.

Porous glass bridges are made from Corning Glass Works glass 7930, which is leached but unfired Vycor glass. The glass is available in a variety of forms, such as sheet, tubing, and rod. It cannot be handled easily by glassblowing techniques (This glass burns, when touched to the lips or other moist skin areas, and will cause severe dehydration and excoriation in the manner of a burn.), as it shatters when placed in a flame. Special shapes, closed tubes, etc., must be fabricated before leaching; these can be supplied by Corning. Some properties of Corning porous glass 7930 are (Corning Glass Works technical brochure): void space (dry), 28% of volume; average pore diameter, 4 mμ; composition: 96% SiO_2, 3% B_2O_3, 0.4% R_2O_3, traces of alkalies and arsenic; flow through 2-mm. thickness, 0.00065 ml. of water per sq. cm. of area per atmosphere of pressure per hour.

The large void space permits a large amount of electrolyte to be held in the glass, and thus give low electrical resistance to the bridge, while the small pore size prevents more than a very small flow of solution. As the glass is electrically inert, the junction potential of a bridge is determined by the electrolyte used in the bridge. Nonaqueous solutions may be used as bridge electrolytes for nonaqueous systems. The bridges are suitable for use at elevated temperatures.

Fluoride and caustic attack the glass, as would be expected for finely divided silica. The high specific surface gives rise to ad-
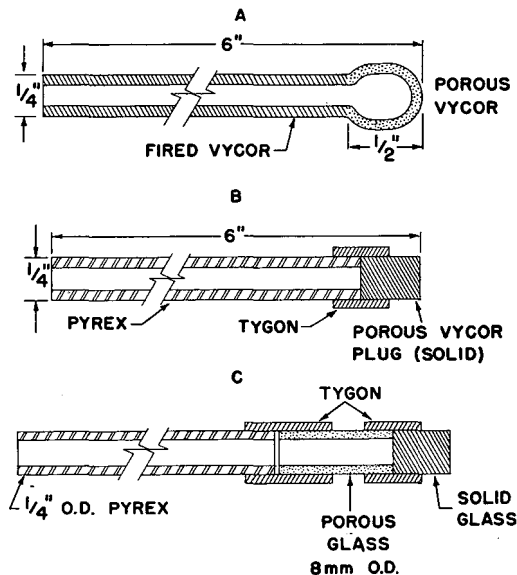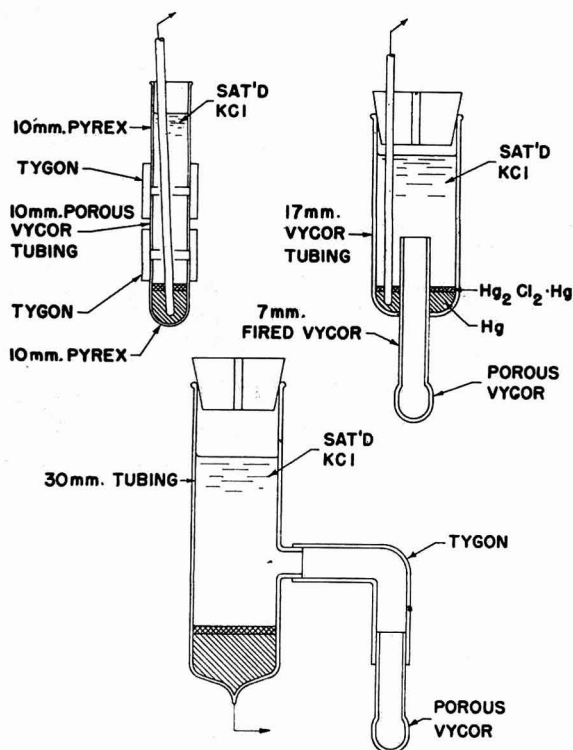

Figure 1. Porous glass salt bridges

Figure 2. Reference electrodes

sorption of materials such as dyestuffs; desorption is difficult. Mechanically, the bridges are rugged. Care must be taken to avoid drying porous glass with salt solution inside, as the material shatters when the salts crystallize.

Figure 1 gives bridge designs used in this laboratory.

Bridge A, which can be supplied by Corning, consists of a closed tube unfired at the closed end, but fired over the remaining length; the fired portion may be worked like ordinary Vycor. Bridge B is made from porous glass rod. The sleeve must fit very tightly to prevent creep of salts between the sleeve and porous glass. Tygon, or other relatively stiff tubing, is suitable for the sleeves. Bridge C is made from porous glass tubing. Ordinary glassworking techniques will not work on this glass; hence seals and junctions must be of plastic or other material. For most work, type A is recommended. All the bridges illustrated operate satisfactorily with up to about 1 atmosphere pressure difference across them. Flow rates of electrolytes with such a pressure difference are very low; about 0.1 ml. per week is typical.

Figure 2 gives some designs for reference half cells using porous glass bridges.

The other bridge materials used in this study which have great promise are the ion exchange membranes, which are sold under the trade name Amberplex by Rohm & Haas, Philadelphia, Pa., and under the trade name Nepton by Ionics, Inc., Cambridge, Mass. The membranes are available in cationic or anionic forms. The sheets are $1/16$ to $1/8$ inch thick, and handle, when wet, like medium hard gasketing materials; when dry they are fragile. Neither form can be allowed to dry under constraint, although they may be wet and dried repeatedly if no constraint is applied.

When these membranes are used in salt bridges, they do not give low junction potentials, as conduction through them is almost exclusively either cationic or anionic. The advantage of the membranes is that they do not pass any solution per se, and can be used to block unwanted ions—e.g., mercury—from passing from the reference half cell to the other half cell. Figure 3 gives two forms of bridges using these membranes; both utilize the gasketing properties of the material. Bridge B is made from a small screw-cap vial with a hole drilled in the cap. Bridges of

large area can be made in a similar fashion from wide-mouthed bottles.

The electrical resistance of bridges made from the new materials was measured, employing saturated potassium chloride as electrolyte, by coupling the bridge in series with two mercury pool electrodes by means of potassium chloride solutions of known resistance. A 60-cycle alternating current Wheatstone bridge was employed. To obtain the resistance contributed by the bridge materials, the resistance of a column of potassium chloride solution of the same cross section and length as the bridge was measured. The difference was assigned to the bridge material. Although the total resistance values obtained in the measurements were easily reproducible to within 0.1% of the total value, the differences are uncertain to 1 to 5 ohms. Hence, the values given should be considered orders of magnitude rather than absolute values.
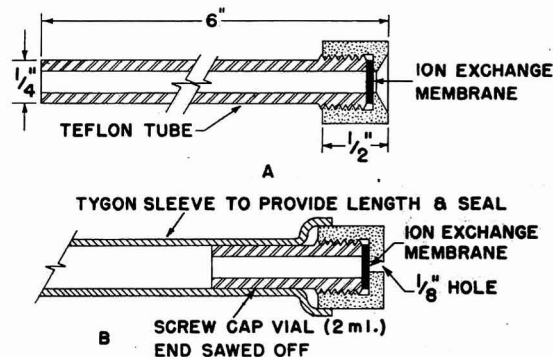

Figure 3. Ion exchange membrane salt bridges

The total resistance of the A type of porous glass (of full tube length as shown in Figure 1) is about 300 ohms; of this 15 to 20 ohms are in the porous glass section. Porous glass rods $1/4$ inch in diameter, such as those used in bridge B, give an average resistance of 9 to 10 ohms per mm. of length. The resistance of disks of porous glass $1/4$ inch in diameter and $1/16$ inch thick (employed in a bridge similar to membrane bridge A) averaged 15 to 20 ohms. Cation exchange membrane (Amberplex C-1) salt bridges of type A have resistances of 25 to 40 ohms across the membrane; similar bridges of anion exchange membrane (Amberplex A-1) have resistances of 40 to 100 ohms across the membrane. For comparison, the resistance of some 1% agar-agar, saturated potassium chloride bridges was measured. The total resistance of a bridge made with tubing 7 mm. in outside diameter and 11.75 inches long was 620 ohms. For a bridge made from tubing 4 mm. in outside diameter and 11.75 inches long, the resistance was 2059 ohms. These may be compared to the 480-ohm total resistance of a bridge of porous glass Type A, $11^3/4$ inches long (7 mm. in outside diameter). Although bridges of the same cross section show similar resistances, the mechanical properties of the porous glass bridge make it possible to use a much shorter length than would be feasible for agar-agar bridges. Bridges of 50 ohms or less are easily made with porous glass.

The limited experience with these new bridges to date has shown them to outlast conventional bridges many times, especially in applications requiring evacuation of the measuring cell, and have shown little change in characteristics for several months, if the bridge was not allowed to dry. Their superior mechanical and flow resistance should make them popular, and their electrical characteristics are very satisfactory for all uses. The only adverse observation has been the severe leaching of salt from porous glass bridges stored in distilled water. This leaching is observed to a lesser extent with agar-agar bridges.

Determination of Phosphorus in Organic Compounds

Barbara C. Stanley, Sadie H. Vannier, Leon D. Freedman, and G. O. Doak, Venereal Disease Experimental Laboratory, U. S. Public Health Service, School of Public Health, University of North Carolina, Chapel Hill, N. C.

A MODIFICATION of the method described by Bachofer and Wagner (1) for the semimicrodetermination of phosphorus has been used in this laboratory for over 3 years. With this modification satisfactory values for phosphorus have been obtained with such compounds as aromatic phosphonic and phosphinic acids, and their esters and amides. The principal change consists in the use of a Parr electric ignition macrobomb (22 ml.) instead of the flame ignition semimicrobomb used in the original method. The electric ignition bomb does not require the experience or skill demanded by flame ignition methods. Furthermore, the 22-ml. bomb is the most widely distributed size (3). The modification is applicable to both solids and nonvolatile liquids. The sample size used is the same as in the original procedure. The accuracy obtainable is illustrated by the analyses reported in a recent communication (2), in which 27 aromatic phosphonic diamides gave phosphorus values with a median deviation from the theoretical of 0.07% and a maximum deviation of 0.14%.

A fusion cup of 98% nickel and a head gasket of lead are used. Fusion cups of 30% nickel-steel and rubber gaskets give less satisfactory results. The charge for the bomb consists of the sample (whether solid or liquid) intimately mixed with 0.40 gram of potassium nitrate, 4 grams of sodium peroxide (from a scoop), and powdered sucrose in such amount that the combined weight of sample and sucrose is 0.50 gram. The bomb cup cover is threaded with a 10-cm. length of ignition wire. After ignition, the melt is dissoved and acidified as described by Bachofer and Wagner. The solution is boiled for 15 minutes and then cooled to about 40° before the molybdate reagent is introduced. After 2 hours the precipitated ammonium phosphomolybdate is transferred to the filter with the aid of about 50 ml. of 2% ammonium nitrate solution. The precipitate is finally washed with an ice-cold solution of 1% potassium nitrate in 30% ethyl alcohol. The determination is completed exactly as described by Bachofer and Wagner.

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Simple Adaptation of the Beckman DU Spectrophotometer as a Spectrofluorometer

Allan G. Gornall and Harold Kalant, Department of Pathological Chemistry, University of Toronto, Toronto, Canada

R ECENT developments in a number of analytical fields have demonstrated the usefulness of spectral distribution measurements of the radiation emitted by fluorescing materials. Such measurements cannot be made with the fluorescence accessory set as supplied by Beckman Instruments, Inc., for the Model DU spectrophotometer, as the set makes no use of the excellent monochromator which the instrument contains. By constructing special accessory equipment, Burdett and Jones (1), Lauer and Rosenbaum (3), and Huke and coworkers (2) have used the Beckman monochromator to study fluorescence spectra. The method of Huke and coworkers is unique, in that the emitted radiation is passed through the monochromator in reverse of the usual direction. The attachments supplied with the regular Beckman fluorescence accessory set can be adapted very easily in a similar optical arrangement.

When the cuvette compartment of the fluorescence accessory is in its usual position, the incident-exciting radiation enters from behind and is reflected upward through the bottom of the cuvette. After traversing about 10 mm. of solution, the light reaches a por-
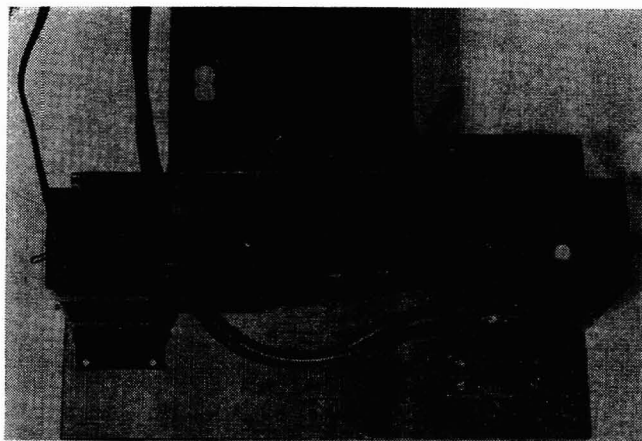

Figure 1.   Beckman DU spectrophotometer adapted for use as spectrofluorometer

tion of the cuvette directly opposite the photocell and the upper slit of the monochromator. Fluorescent radiations can pass through this slit, travel through the monochromator, and emerge from the lower slit. Here the light is reflected toward the rear by the plane mirror contained in the mounting block. The only difficulty in placing the phototube housing to receive this light, is that the lamp housing is in the way. Fortunately, the cuvette compartment can be reversed in position on the mounting block without any structural alteration, and without disturbing the optical alignment between the cuvette and the monochromator. The light source can then be placed in front, mounted on a fixed base, and aligned with the lens opening of the fluorescence accessory by means of a connecting collar.

The assembled system is illustrated in Figure 1.

Incident light from a 500-watt tungsten lamp passes through the quartz lens of the accessory and is reflected upward from a front-surfaced mirror through the bottom of the square Corex cuvettes. Suitable filters can be fitted in the horizontal slide beneath the cuvette holder. Fluorescent radiations from the cuvette enter the upper slit of the monochromator compartment; selected wave lengths emerge from the lower slit and are reflected toward the rear. This light is shielded in a rectangular channel and directed through the opening of the phototube housing onto a high-sensitivity photocell such as the IP28 tube supplied with the Beckman photomultiplier accessory set.

In order to keep the light source and phototube housing in proper alignment, the whole instrument has been mounted on a
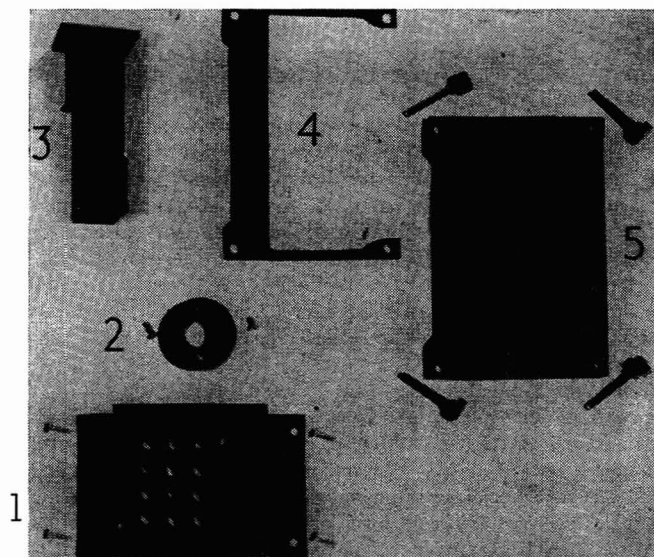

Figure 2.   Extra parts necessary to adapt Beckman Fluorescence accessory as spectrofluorometer

plywood base. Two wooden blocks accommodate the feet of the monochromator housing in fitted holes and raise the instrument to the height desired. A platform with angle irons and set-screws holds the phototube housing. The light source is mounted on a metal platform and secured to the base by screws. The instrument can readily be converted from spectrophotometer to spectrofluorometer in less than 5 minutes.

The parts necessary to make the adaptation of the fluorescence accessory are shown in Figure 2.

Part 1 is the base for the light source, made from $1/32$-inch sheet brass. This base can be modified for different light housings and its height is determined by the position of the fluorescence accessory lens. Part 2 is a brass ring collar, machined to fit the lamp housing and into the lens opening. This collar holds in position the metal washers which determine the size of the incident beam. Part 3 is a light channel made from $1/8$-inch brass plate cut to size and soldered. The channel is designed at one end to fit the aperture at the back of the mounting block and at the other to fit the opening of the phototube housing. A projecting frame of thin metal sheet and a collar at each end help to provide light-tight connections. In order to facilitate optical alignment, the face plate of the monochromator serves as one wall of the light channel for part of its length. A layer of felt interposed between the cuvette holder and the channel helps to hold the latter tightly against the face plate. Part 4 is a gasket made of heavy blotting paper or pressed cardboard, placed between the mounting block and the fluorescence cuvette holder. Part 5 is an end plate of $1/8$-inch brass made to fit the outer side of the cuvette holder. A layer of blotting paper and a gasket similar to part 4 should be cemented to the inside of this plate. The gaskets help to provide light-tight connections and clearance for the cuvette carriage to be racked in and out. All parts are painted in a dull black finish to minimize reflected radiation.
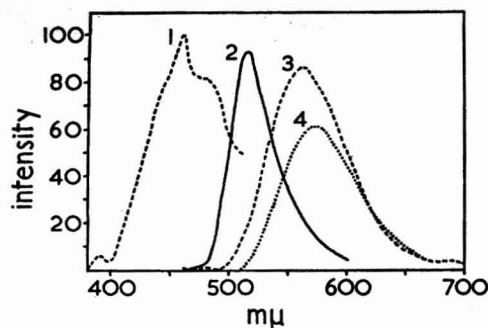

Figure 3. Fluorescence spectra as determined with modified Beckman spectrofluorometer

1. Quinine sulfate in 0.1N sulfuric acid
2. Fluorescein in aqueous sodium hydroxide
3. Pyronine in ethyl alcohol
4. Rose bengal in ethyl alcohol

When the instrument is assembled for the first time, it is essential to make certain that the light source is in alignment with the mirror in the base of the fluorescence accessory and that the beam passes vertically through the cuvette. The angle of the mirror in the mounting block of the monochromator may require some adjustment, to ensure that light emerging from the lower slit passes through the center of the channel to the photocell.

RESULTS

The general principles of procedure described in detail by Huke and coworkers (2) apply equally to the above arrangement. The incident light reaches the cuvette from below rather than above and the light path through the solution is about 10 mm. rather than 2 mm., factors which, in the case of quinine in dilute sulfuric acid, reduce sensitivity by about 20%. Other systems would be affected similarly unless they absorbed the exciting radiation strongly. In practice there has been no difficulty due to light scattering or fluorescence by the cell. In some applications there is an advantage in being able to use covered cuvettes.

Because in many analytical situations fluorescence may be of a low order of intensity, the over-all sensitivity of the instrument is a major consideration. For comparison with other instruments the following calibration was carried out with a solution of quinine sulfate in 0.1N sulfuric acid. The incident light was passed through a 3-mm. Corning 9863 filter. With the photomultiplier at maximum sensitivity and a sulfuric acid blank set at zero, a concentration of 0.15 γ of quinine sulfate (U.S.P.) per ml. of solution gave a scale reading of 100 at a slit width of about 1.8 mm. A straight-line relationship held for lower concentrations.

The higher sensitivity of the modification described by Huke and coworkers can be attributed mainly to the higher intensity of incident radiation supplied by the hydrogen arc and larger quartz lens which they used. Replacing the tungsten lamp in the present instrument with an Allen hydrogen arc operating at 0.5 ampere gave a 30% increase in fluorescence with quinine sulfate. A higher sensitivity could also be achieved with a mercury or xenon arc light source.

Figure 3 illustrates the spectral distribution curves of fluorescence emitted by extremely dilute solutions (less than 1 γ per ml.) of quinine sulfate in sulfuric acid, fluorescein in aqueous sodium hydroxide, pyronine in ethyl alcohol, and rose bengal in ethyl alcohol. These substances were used simply to demonstrate the application of the instrument at different wave lengths of fluorescent light.

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Automatic Micromuffle for Determination of Ash in Carbonaceous Material

Robert Meyrowitz and C. J. Massoni, U. S. Geological Survey, Washington 25, D. C.

THE mineralogy and geochemistry of carbonaceous rocks are being studied as part of a program of research on the geochemistry of uranium that the U. S. Geological Survey is conducting on behalf of the Atomic Energy Commission. To help solve certain phases of the problem, an organic microanalytical laboratory has been organized. This paper describes an automatic microcombustion apparatus that has been designed and used in this laboratory for the determination of the ash content of small amounts of carbonaceous materials. Often the amount of material available for analysis is small, necessitating the use of micromethods. Norton, Royer, and Koegel (1) have shown that there is a saving of time without loss of precision when the microtechnique is used for ash determinations.

Two automatic micromuffles have been described (1, 2). These micromuffles have been made to order and require a great deal of machine-shop work in their construction. The micromuffle described by Norton, Royer, and Koegel (1) has platinum heating coils, and the temperature is regulated by varying the voltage with a variable autotransformer. Either one or two samples can be burned at one time; the furnace is stationary, and the tubes are drawn through the furnace. There are two rates of travel, the slowest speed being 2.5 cm. per 10 minutes. Steyer-

nark's micromuffle (2) is heated by means of small Nichrome wire, and temperature regulation is by means of a variable autotransformer. Two samples can be burned at one time; the tubes are stationary and the furnace is moved. There is one rate of travel, 2.5 cm. per 10 minutes.

The micromuffle described here can be assembled with a minimum of labor, using parts available from certain scientific laboratory apparatus companies.
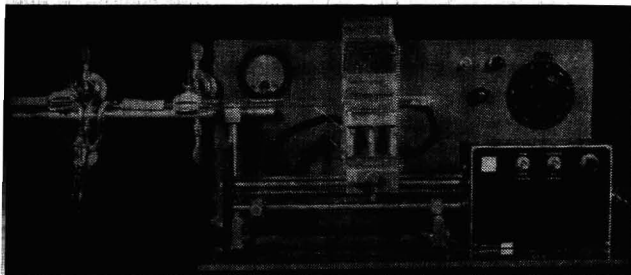

Figure 1.   Micromuffle

The micromuffle pictured in Figure 1 consists of two parts: (1) a furnace drive, single speed (2.0 cm. per 10 minutes), electric, having adjustable limit stops to arrest the advance of the furnace automatically, and (2) the moving, short-furnace section of an automatic, micro- and semimicro- combustion apparatus.

To the best of the authors' knowledge, part 1 is available only from Arthur H. Thomas Co., Philadelphia 5, Pa. (Catalog No. 5683K), and part 2 is available only from E. H. Sargent and Co., Chicago 30, Ill. (Catalog No. S-21580).

This furnace is of the radiant-heating type, with hinged shell and open-sided heating elements, and operates on a 115-volt, 60-cycle, alternating current circuit. The furnace unit consists of six parts: (1) voltage-reducing transformer, designed to operate on 100-volt, 50/60-cycle, single-phase service; the secondary is rated 10 volts at 28 amperes; (2) variable autotransformer; (3) thermocouple and direct-reading pyrometer for temperature control; (4) fuse; (5) pilot light; and (6) switch. The maximum temperature for continuous operation is 900° C.

To calibrate the pyrometer, the thermocouple of a second pyrometer is inserted into the combustion tube until it reaches the position to be occupied by the combustion boat. While a stream of oxygen is flowing at the rate to be used in the ash determination, the position of the thermocouple in the furnace is adjusted so that the reading of the pyrometer of the micromuffle is the same as that of the second pyrometer.

LITERATURE CITED

(1) Norton, A. R., Royer, G. L., and Koegel, R., IND. ENG. CHEM., ANAL. ED., 12, 121–3 (1940).
(2) Steyermark, Al, "Quantitative Organic Microanalysis," pp. 48–9, Blakiston, Philadelphia, 1951.

Apparatus for Automatically Changing Solvent Polarity during Chromatography

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IN the separation of organic dibasic acids by partition chromatography [Higuchi, T., Hill, N. C., and Corcoran, G. B., ANAL. CHEM., 24, 491 (1952)] the mobile phase is a series of solvent mixtures progressively increasing in concentration of n-butyl alcohol. An apparatus has been designed that adds the solvents automatically in the correct proportions and, when coupled with a mechanical fraction collector, makes the whole separation procedure automatic. Also, constant pressure in the apparatus permits more even elution of the acids.

The apparatus consists of two glass containers, A and B, connected at the bottom by rubber tubing through a T-tube to a

mercury container, C. Near the tops of A and B, capillary side arms lead to a distributor head, D, to which the chromatograph tube is connected by a ball joint. Small funnels are joined through pressure stopcocks to the tops of A and B. The container, A, which contains chloroform, is a straight tube but B, which contains butyl alcohol, is made up of a series of progressively larger tubes sealed together.

In operation the sample of acids is washed into the stationary phase in the chromatograph tube. The tube is filled to the joint with the first eluting solvent mixture, the stopcock at the bottom is closed, and the tube is clamped to the distributor head. With the mercury container lowered, A is filled with chloroform and B with butyl alcohol, and the stopcocks are closed. The mercury container, C, is then raised to a position so that the desired total volume of eluent will be obtained. The stopcock on the chromatograph tube is opened to obtain the desired rate of flow. As the effluent passes from the chromatograph tube, the mercury rises in A and B and forces both chloroform and butyl alcohol into the distributor head. The ratio of butyl alcohol to chloroform changes as the mercury rises into each new segment of B.

The diameter of each segment of B is calculated from each desired ratio of butyl alcohol to chloroform, and the length is determined by the total volume of each composition. For example, if 3% volume of solvent B in solvent A is desired, the ratio of the diameters of B and A would be $3^{1/2}$ to $97^{1/2}$. If the specific gravities of the liquids in A and B are equal, a segment of B containing 3 ml. would be the same length as a segment of A containing 97 ml. However, because the specific gravities of butyl alcohol and chloroform are different, the mercury level in B will be higher than that in A. The length of each segment of B may be corrected by the equation

$$\Delta h = \frac{(\text{Sp. gr. } A - \text{sp. gr. } B)}{(\text{Sp. gr. Hg} - \text{sp. gr. } B)} hA$$

where Δh is the difference in mercury levels in A and B, and hA is the distance from the mercury level in A to the capillary-tube outlet in the distributor head.
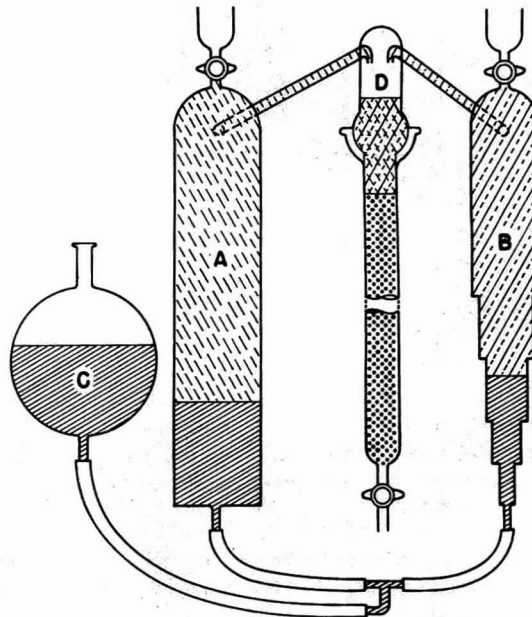
In the construction of the apparatus, a tube of sufficient volume is selected for A, and the length necessary for the volume of chloroform in each composition is calculated. This length corrected for the difference in mercury levels is the length of each segment of B. The diameter of each segment of B is then determined to obtain the required amount of butyl alcohol for each mixture.

Some space above the stationary phase in the chromatograph tube is necessary to obtain thorough mixing of the liquids as they are delivered. The change of compositions is smooth and does not give rise to false shoulders on the chromatographic peaks.