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The Economics of Analysis

We have just completed a series of articles written by people in various industrial positions as to the place and importance of analysis in modern industry. The authors seem to be unanimous with respect to the need for the large expenditures required to maintain quality control in present-day manufacturing operations.

We do not have to go back many years to find that manufacturing gave little heed to analysis or quality control as we know it today. The plant manager and his staff, steeped in the tradition of secrecy, knew by intuition when production was satisfactory. With scientific study of processes over the years, we now take for granted the fact that careful scientific control is necessary and that a control laboratory, backed up by a research group on methods, is part of every manufacturing operation.

Even with expanded analytical staffs and increased precision and speed in chemical methods, analytical laboratories can seldom turn out the work fast enough. Management, therefore, is beginning to question the high cost and efficiency of analytical operations.

The change from batch to continuous process operations in industry has created a need for continuous analysis. The old-fashioned chemical wet methods, while useful and relatively fast, have been outmoded. In their place we have expensive automatic equipment. Although it cannot do without them, the high cost of these instruments disturbs management. Also disturbing is the fact that analytical staffs, instead of decreasing with automation, seem to increase. Changes authorized in a manufacturing process are usually made with the idea of lowering costs. If this goal is not achieved, someone generally has failed in his assignment.

Mounting costs of quality control plus the analytical research necessary to develop and maintain a high standard are beginning to be studied in more detail to see if the best use of talent and equipment is being achieved in the light of manufacturing objectives.

We think it pertinent, therefore, to discuss some of the background against which analysis has developed over the past few years.

Analysis is actually part of research, and so its contribution to the profit picture at any one time is difficult to assess. If, however, over a period of time definite progress and contribution to efficiency cannot be shown, this position is hard to justify. As we have noted, in earlier days the production man used the chemist, with his analytical methods and quality control tests, to prove what he already knew—namely, that the product was satisfactory. With more rigid specifications and speed-up of process, the production man was proved in error far too often and so, reluctantly, he welcomed the help of the chemist. The chemist soon found his methods too slow to keep up with production. This led to expanded analytical research staffs to speed up procedures. During this period all samples were brought to a central laboratory for analysis and the results relayed to the production department for its guidance.

It is to the credit of the control laboratory that it met the challenge of production and did a commendable job during this period. However, the pressure of work mounted and demand for increased speed saw the introduction of such instruments as faster balances, spectrophotometers, and automatic burets. Pneumatic tube systems for sending samples and information were introduced at the same time. The next development was the introduction of physical methods to meet demands of production. These methods permitted production operations to follow and adjust conditions so that a final product meeting rigid specifications was produced.

We are presently in the stage where these instruments are being moved from the laboratory to the production department where they are locked into the production machinery. They can actually monitor and automatically change conditions so that a final acceptable product is produced continuously. Because such systems are extremely expensive and lead to increased personnel, management will be forced to take a sharp look at all aspects of analysis.

In studying this problem, management should keep in mind that it is demanding far more information about its products than ever before. Newer instruments, even though expensive, do give more information and permit faster and more precise control of production. Management should also keep in mind that the quality of analytical personnel must be higher than ever before in order to carry out analytical research and maintenance on these newer instruments.

Those responsible for analysis should remember that they should question whether the analyses they are requested to do are necessary. They should be sufficiently qualified about analysis and processes that they are in a position to suggest the most economical and expedient means of controlling process and product. They should use the simplest instrument or method to achieve their goal, and they should study all methods which are applicable so that the least expensive one is used. Lastly, they should be sure that they have not fallen into that easy habit of hiring more analysts to solve their problems when a critical survey might show they have the wrong type of personnel, or that further instrumentation or research on methods might solve their problems better and more cheaply.

Paper Chromatography of a Petroleum Porphyrin Aggregate

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Paper-strip and paper-sheet chromatographic methods have been developed to separate the porphyrin aggregate of a California crude oil into several groups. The first two groups are characterized by spectra of the etio type, in which peak III (532 mμ) is stronger than peak II (565 mμ). Peaks I (620 mμ) and IV (500 mμ) are displaced toward longer and shorter wave lengths, respectively, in groups 1 and 2. The other groups have phyllo-type spectra; peak II is stronger than peak III. The wave length of the main fluorescence band changes about 2 mμ toward shorter wave lengths proceeding from group 1 to 6, in line with the changes in the absorption spectra.

THE presence of remnants of plant and animal life in petroleum was established several years ago (*13*), and a quantitative method for the extraction of the porphyrin contents of crude oils was published recently (*6*). This method, with modifications in the colorimetric procedure (*3*), has been used to determine the porphyrin contents of various petroleum extracts (*2, 4*). These investigations indicated that the porphyrin aggregates were of a heterogeneous nature, as would be predicted from their biological origin. A better knowledge of the nature of these porphyrins would allow more exact evaluation of their significance in the origin of and exploration for petroleum.

Paper chromatography has proved to be a rapid, effective method for resolving naturally occurring mixtures of porphyrins. However, this method has been used primarily for the separation of carboxylated porphyrins and their esters which are of clinical importance (*1, 8–11*). Much of the porphyrin aggregate of crude oils consists of decarboxylated porphyrins (*3*), for which established methods are generally inapplicable.

Paper-strip and -sheet chromatographic methods have been developed to allow the separation of the porphyrin aggregate of a California crude oil into five groups of porphyrins. This aggregate was derived principally from the nickel-porphyrin complex, of which this oil is a relatively rich source (*2, 3*). Larger quantities of the aggregate were chromatographed on paper pulp to obtain sufficient quantities of these, and two additional groups, for spectrophotometric studies. The groups were investigated further by paper-sheet chromatographic methods to estimate their purity and to identify them with the groups isolated in the preliminary paper-strip and -sheet methods. Two groups of porphyrins which moved more rapidly through the paper columns had spectra of the etio (*3, 5*) type. The remaining groups, including the major constituent, had spectra of the phyllo type.

Table I. Specifications

Material	Source	Specifications
Paper, strips	Whatman	No. 1, 1-inch roll
Paper, sheets	Whatman	No. 1, 16 × 19 inches
Paper, sheets	Munktells	Chromatographic, 18 × 18 inches
Paper, pulp	Whatman	Ashless cellulose powder, coarse
Petroleum ether	Merck & Co., Inc.	Reagent grade, boiling range 30–60° C.
Chloroform	Mallinckrodt Chem. Co.	Analytical reagent grade
Methanol	Merck & Co., Inc.	Reagent grade
Benzene	Merck & Co., Inc.	Reagent grade
2,4-Lutidine	Eastman Kodak Co.	Technical grade
2,6-Lutidine	Eastman Kodak Co.	Practical grade
2,4,6-Collidine	Eastman Kodak Co.	Technical grade

MATERIALS

The chemicals used in extracting the porphyrin aggregate from the crude oil have been described (*2, 3*). The specifications of the materials used in the separation procedures are listed in Table I.

The crude oil from which the porphyrin aggregate was isolated was a heavy, asphaltic oil from the North Belridge field, Kern County, California (*2*).

EXPERIMENTAL METHODS

Paper-Strip Method. The ascending method of development was used because of the simplicity of the apparatus employed and the better definition of the zones.

The apparatus consisted of a glass tube, 7.5 cm. in inside diameter and 61 cm. in length, mounted vertically with the lower end immersed in the solvent container. The upper end was sealed by a waxed cork fitted with a metal support for the paper strip. A 50-cm. strip of paper, to which the sample had been added, was suspended above the developer. Twelve to 24 hours were required for the system to reach equilibrium. Then the paper was lowered and development proceeded until the solvent front reached the desired height.

Freshly prepared samples of 4 to 10 μl., containing about 0.5 γ of porphyrin aggregate in chloroform solution, were put on the paper strip at a reference mark near the bottom.

Paper-Sheet Method. A new method of paper-sheet chromatography was developed because saturation of the available cabinet was difficult with the solvents employed and several hours normally are required to attain equilibrium.

The apparatus consisted of a large bell jar, inverted in a framework. A vacuum desiccator lid was used for the cover, and the ground surfaces were lubricated with high-vacuum silicone grease. The opening in the standard-taper joint of the desiccator lid was fitted with a vented cork. Two 6-inch wooden disks were supported in the bell jar by means of a $^3/_4$-inch dowel rod centered in the cork.

The samples were placed on a reference line near the bottom of the paper sheet about an inch apart; the same spotting methods and concentrations were used as with the paper strips. The paper then was tacked to the wooden disks and placed in the jar containing the solvent, and the system was rapidly evacuated. The solvent was allowed to boil briefly, the chamber sealed by rotating the sleeve on the desiccator lid, and development allowed to proceed.

A second apparatus of similar design also has been used in the paper-sheet studies. This apparatus allowed the sheet to be lowered into the solvent after equilibrium was established. Therefore, it was used for both vacuum saturation studies and studies in which the atmosphere was saturated by allowing the closed system to stand for several hours.

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Paper-Pulp Method. The columns were packed by filling with dry cellulose powder and frequently tamping the powder tightly in place. The one-piece columns consisted of three sections: an upper section 4.1 by 44 cm., a center section 2.5 by 44 cm., and a lower section 1.1 by 25 cm.

The column was prewet with petroleum ether and, in the early studies, the sample was introduced in a petroleum ether solution. However, as the porphyrin aggregate was not entirely soluble in this solvent, quantitative studies were complicated by this method. In the final separation, paper pulp was added to a chloroform solution of the porphyrin. Then the chloroform was evaporated, with constant mixing, to leave the sample on a small amount of paper. This impregnated paper pulp was tamped tightly in the top of the prewet column and the column developed with a 0.1 volume % solution of 2,4-lutidine in petroleum ether. This mixture also was used in the strip and sheet work, after it was found to give optimum resolution of the aggregate.

The porphyrin zones on the various chromatograms were detected by their fluorescence under ultraviolet light. Except for this necessary illumination during location of the zones, the chromatograms were developed in darkness. The zones on the strips and sheets were barely detectable after the paper was dried. Therefore, their location and movements were determined in the apparatus with a cathetometer, or immediately upon removal from the apparatus.

R_f Values. Rates of movement are expressed relative to the rate of movement of a pure sample of etioporphyrin II determined under identical conditions. The reproducibility of this relative rate was considerably greater than the individual R_f values in the various systems used and between the two laboratories involved. The R_f values were observed to be relatively insensitive to small differences in amounts of porphyrin in the spots, but varied somewhat with different methods of obtaining saturation in the cabinet. The relative values were insensitive to differences in concentration, types of paper, saturation methods, and operators. The average R_f value of the etioporphyrin II sample, with the petroleum ether–lutidine solvent and the cabinet allowed to become saturated by standing overnight, was 0.56 ± 0.04.

Approximate R_f values and porphyrin contents of the zones were determined by colorimetric analysis of each of the effluent fractions from the paper-pulp columns. Quantitative measurements of the porphyrin contents of the zones were accomplished by combining the effluent fractions to form the various groups and applying the colorimetric method of Dunning, Moore, and Myers (*3*).

RESULTS AND DISCUSSION

A petroleum ether–2,4-lutidine mixture was found most effective for the separation of the porphyrin aggregate. Mixtures containing 0.05, 0.1, 0.2, and 0.3 volume % of lutidine were tested. The mixture containing 0.1 volume % lutidine was optimum. Mixtures in which 2,6-lutidine or 2,4,6-collidine was substituted for 2,4-lutidine were used without materially altering the results. Hexane was a satisfactory substitute for petroleum ether and because of its more constant composition is preferred.

Although not so efficient as petroleum ether–lutidine mixtures, petroleum ether–chloroform mixtures also were effective as developers. Several compositions of the mixture were tested and a 3 volume % solution of chloroform was the most effective. Several zones were obtained, but separation was not as good as with the petroleum ether–lutidine mixture and the leading zones were difficult to detect. Another deterrent to using chloroform is that the porphyrins are less stable in chloroform than in the other solvents (*3*).

Other developers that were tested, together with a brief statement of their effects in resolving the mobile zones, are summarized in Table II.

The systems listed in Table II were evaluated for their effectiveness in resolving the mobile porphyrins. However, part of the porphyrin aggregate is not moved by the petroleum ether–lutidine mixture. Lutidine, saturated with water and in the presence of ammonia vapors, was effective as a developing solvent for this part of the porphyrin aggregate.

With the 0.1 volume % solution of 2,4-lutidine in petroleum ether, and paper strips, four distinct zones were observed, in addition to a portion that did not move from the point of application. The rates of movement of these zones, relative to the movement of a pure sample of etioporphyrin II, were 1.0, 0.8, 0.7, and 0.5. There was an indication of further resolution in the leading and trailing zones, but the concentrations were too low to make positive identifications.

Similar results were obtained by the paper-sheet method. The use of sheets instead of strips allows the simultaneous separation of several samples. Also, because the experimental conditions are identical for any one sheet, the movement and separation of different samples may be compared accurately.

Table II. Developers for Paper Chromatograms

Components			
Major	Minor	Volume ratio	Effects
Butanol	Acetic acid	9 to 1	Too strong
Butanol	Water	Saturated	Too strong
Water	Butanol	Saturated	Too weak
2,6-Lutidine	Water	Saturated	Too strong
2,4-Lutidine	Water	Saturated	Too strong
Water	2,4-Lutidine	Saturated	Too strong
Methanol	Water	10 to 1	Good movement, no resolution
Methanol	None		Too strong
Petroleum ether	Methanol	1 to 1	Good movement, no resolution
Water	Methanol and 2,4-lutidine	5, 5, and 3	Good movement, no resolution

The behavior of the porphyrin aggregate was compared to that of several pure porphyrin samples in this manner. The esterified forms of coproporphyrin I and uroporphyrin I, the acidic form of these porphyrins, and the acidic form of mesoprophyrin IX did not move perceptibly with the petroleum ether–lutidine solvent. The dimethyl ester of mesoprophyrin IX had an R_f value of 0.04. In the upper region of the chromatograms, the esterified and unesterified portions of the porphyrin aggregate moved identically, having zones with rate values relative to etioporphyrin II of 1.0, 0.8, 0.7, and 0.5. However, near the origin, the esterified porphyrin aggregate formed a zone not observed with the unesterified aggregate. This is attributed to the presence of porphyrin containing a single carboxylic acid group. The high rates of movement of the four leading zones indicate that they contain decarboxylated types of porphyrins. The portion of the aggregate remaining at the origin is indicated to contain carboxylic acid groups.

The porphyrin aggregate was chromatographed on paper pulp to obtain samples large enough for further studies. The petroleum ether–lutidine mixture used in strip and sheet work was used as the developer. In the final column chromatogram, 28 ml. of solution containing 140 p.p.m. of porphyrin aggregate was fractionated. A total of 1800 ml. of eluate was collected in 9-ml. portions over a period of 72 hours with an automatic fraction collector. Every fifth fraction was concentrated to an absorbance of about 0.7 to 620 mμ and 8 μl of each was spotted on a paper sheet for chromatographic study of purity and R_f value.

Figure 1 illustrates the rate of movement of the several fractions on paper sheets relative to the movement of etioporphyrin II. These studies show that the fractions form six main groups. Accordingly, the fractions were recombined to form the indicated groups. The seventh group remained at the top of the pulp column. It was extracted with ethyl ether and separated from impurities of similar adsorption characteristics by extraction into 10 weight % hydrochloric acid, neutralization to a pH of 5.2, and extraction by ether. In this manner, a homogeneous portion was obtained which contained about half of the porphyrin in group 7. This portion of group 7 will be referred to as group 7a.

The rates of movement of groups 1, 3, 4, 5, 6, and 7 on the paper-pulp column, relative to the rate of group 2 which had been observed to move at the same rate as etioporphyrin II, were 1.1, 0.9, 0.8, 0.65, 0.45, and 0.0, respectively.

The solutions containing the porphyrin groups were diluted to have an absorbance of 0.7 at 620 mμ. Then 8 μl. of each solution was spotted on a paper sheet and their movement, relative to etioporphyrin II, measured for accurate establishment of rates of movement.

The absorption spectra of the seven groups were determined with a Beckman DU spectrophotometer. The wave lengths of maximum absorption and fluorescence (under ultraviolet light) were determined with a Hartridge Reversion spectroscope, with which the bands can be determined more precisely, although not necessarily more accurately, than with the spectrophotometer.

Average values of the properties of the porphyrin groups are summarized in Table III.

Table III. Properties of Porphyrin Groups Isolated by Paper-Pulp Chromatography

Group	Fractions (Incl.)	Movement (Rel. to Etio II)	Spectral Type (5)	% of Porphyrin Aggregate
1	1– 12	1.18	Etio	7.9
2	13– 27	0.99	Etio	16.1
3	28– 40	0.85	Phyllo	14.2
4	41– 70	0.74	Phyllo	21.6
5	71– 97	0.62	Phyllo	12.6
6	98–200	0.53	Phyllo	16.0
7	...[a]	0.00	Phyllo	7.6
			Total	96.0

[a] Remained at top of column and was extracted later.

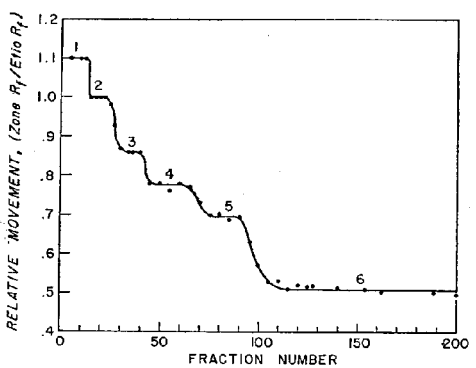

Figure 1. Rate of movement of porphyrin fractions on paper sheet relative to movement of etioporphyrin II

R_f of etioporphyrin II 0.56

Direct comparison of the R_f values with those obtained in earlier paper-strip work is invalidated by differences in concentration of the samples as well as impurities. However, relative observations indicate that groups 2, 3, 4 to 5, and 6 correspond to the paper-strip zones in order of decreasing rate of movement.

Group 1 moved faster than did a sample of pure etioporphyrin II. Because previous work showed there were present decarboxylated porphyrins which moved at various rates, this is entirely possible. However, the faster rates may possibly be due to the presence of solubilizing impurities in the aggregate, which increase the rates with which its zones move (4).

The spectra of the groups are shown in Figures 2 and 3. The solutions were diluted to one tenth of the concentrations used for determinations in the visible region for measurements at wave lengths below 470 mμ.

The absorbance peaks of porphyrins are designated I, II, III, and IV, proceeding toward shorter wave lengths through the visible region. The first two groups (Figure 2) have spectra of the etio type (5); peak III is stronger than peak II. This type of spectrum is typical of animal porphyrins and a few plant porphyrins. The spectra of the other groups (Figures 2 and 3) are of the phyllo type, as peak II is stronger than peak III in each spectrum. This type of spectrum is typical of plant porphyrins in general.

The etio-type spectra (groups 1 and 2) have peak I displaced about 2 mμ toward the red and peak IV about 2 mμ toward the blue, compared to the same peak in the phyllo spectra (groups 3 to 7).

The fluorescence of porphyrins irradiated by ultraviolet light affords an extremely sensitive means for their detection and, to a limited extent, for their identification. Porphyrins in neutral or slightly basic solutions typically have a strong fluorescence band at about 620 to 630 mμ and secondary bands at longer wave lengths (12, 14). Fluorescence may be measured at concentrations far below those detectable by absorbance studies (7).

The Hartridge revision spectroscope, calibrated with a mercury lamp, was used to locate the position of the major fluorescence band in the groups of porphyrins separated by paper pulp chromatography. The positions of the main fluorescence band and of the four absorption peaks in the visible region were determined several times and the results averaged. Although it is difficult to locate an unsymmetrical peak—e.g., peaks 2 and 3—exactly with this instrument, the differences among corresponding peaks of the several groups were reproducible. The locations of these bands are summarized in Table IV.

These results show a progressive decrease in the wave length of peak I and the increase in wave length of peak IV from group 1 through group 5, in agreement with the spectrophotometric measurements. Corresponding shifts of the fluorescence band corroborate these slight changes. This kind of spectral change generally indicates a simplification of the side chains attached to

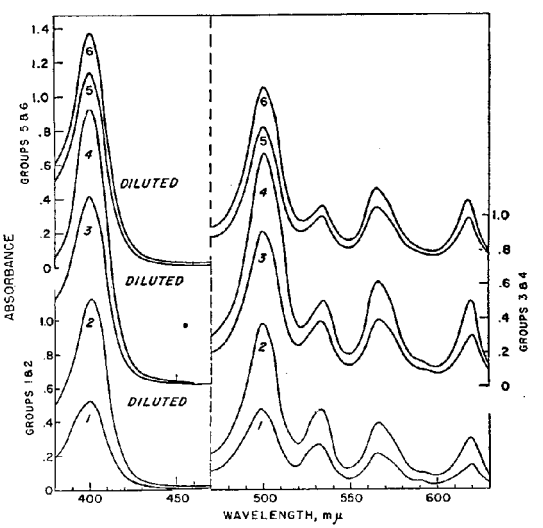

Figure 2. Absorption spectra of porphyrin groups

In 15 ml. of chloroform

the porphyrin ring or ring closure as in derivatives of deoxophylloerythrin (*5, 7, 12, 14*).

The spectrum of the etioporphyrin II sample was not detectably different from that of group 1. The higher R_f values of the more mobile groups are attributed to the presence of more, or larger, aliphatic side chains in the porphyrins of these groups. The

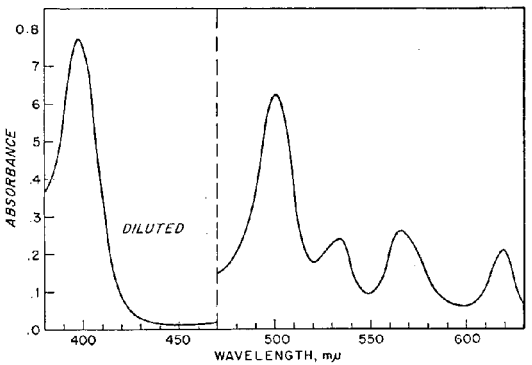

Figure 3. Absorption spectrum of porphyrin group 7

In 40 ml. of chloroform

decarboxylated animal porphyrins—e.g., mesoetioporphyrin—contain four methyl and four ethyl side chains. The decarboxylated plant porphyrins contain three ethyl side chains and either four (pyrroetioporphyrin) or five (phylloetioporphyrin) methyl side chains or an isocyclic ring (*5*). Therefore, the high R_f values of groups 1 and 2 indicate that the etio-type spectra of these groups are due to the presence of decarboxylated porphyrins similar to the etioporphyrin II sample used as a standard. Porphyrins of the "animal" type sometimes occur in plant systems (*7*). Therefore, the identification of etio-type porphyrins in the aggregate is not positive proof that animal matter was involved in the genesis of the crude oil. Correlations of data show that groups 3 through 6 contain decarboxylated porphyrins, presumably of the phylloetioporphyrin or deoxophyllerythroetioporphyrin type. No attempt has been made to distinguish among the spatial isomers of the various types of porphyrins.

The early paper-sheet chromatographic investigations indicated the presence of a porphyrin species containing one carboxylic acid group. Therefore, group 7a (from the acidic portion of the aggregate) was investigated by the method of Nicholas and Rimington (*10*). The solvent was 2,4-lutidine saturated with water used in the presence of ammonia vapor. Saturation of the atmosphere was obtained by allowing the sytems to stand for 16 hours. The results are shown in Figure 4. The R_f values of the standards (coproporphyrin, uroporphyrin, and mesoporphyrin) are in good agreement with data reported in the literature (*8, 10*). The R_f value of group 7a shows that this component of group 7 consists of porphyrins containing one carboxylic acid group. This corroborates observations with the esterified porphyrin aggregate.

Peaks I and IV in the spectrum of this group were located at longer and shorter wave lengths, respectively, than the corresponding peaks in the middle groups. The paper chromatograms show that group 7a contains a carboxylic acid group not present in the earlier groups, supporting the assumption that groups 1 and 2 contain more, or larger, aliphatic side chains or side chains of a more aliphatic nature than do the middle groups (3 to 6). The locations of absorption bands, their relative intensities, and the R_f value of group 7a indicate that the porphyrin involved is a monocarboxylic porphyrin, probably deoxophylloerythrin, which was identified in oil shale extracts by Treibs (*13*).

CONCLUSIONS

Paper-strip and paper-sheet chromatographic methods have been developed to separate the porphyrin aggregate of a California crude oil into several groups. Columns packed with paper pulp were used to prepare large enough samples of seven such groups for further study.

The absorption spectra of the porphyrin groups have been investigated. The first two groups are characterized by spectra of the etio type, in which peak III (532 mμ) is stronger than peak II (565 mμ). Peak I (620 mμ) and peak IV (500 mμ) are displaced toward longer and shorter wave lengths, respectively, in groups 1 and 2. The other groups have phyllo-type spectra; peak II is stronger than peak III.

Table IV. Wave Lengths (mμ) of Absorption Bands and Main Fluorescence Band of Porphyrin Groups in Chloroform

Group	Absorption I	II	III	IV	Fluorescence (Main)
1	620.3	566.5	532.8	499.9	620.9
2	619.9	566.4	533.6	500.5	620.5
3	618.9	565.5	533.7	501.2	619.7
4	618.3	565.3	536.6	502.4	619.3
5	618.2	564.9	535.4	502.5	619.2
6	618.1	565.3	535.9	502.6	619.3
7	618.7	565.2	533.5	501.5	620.0

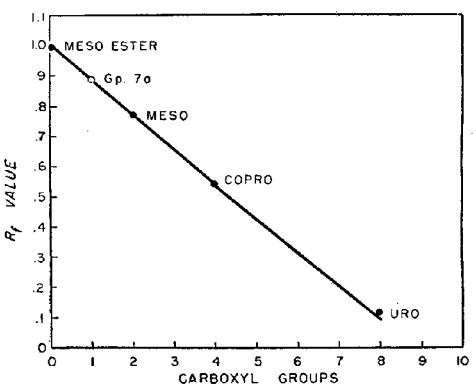

Figure 4. Relation of R_f values to number of carboxyl groups in paper-sheet chromatogram

The wave length of the main fluorescence band changes about 2 mμ toward shorter wave lengths proceeding from group 1 to 6, corroborating the changes in the absorption spectra.

Representative samples of the porphyrin groups have been compared by paper-sheet chromatography. These studies verify the separations accomplished and give rates of movement relative to that of etioporphyrin II of 1.18, 0.99, 0.85, 0.74, 0.62, 0.53, and 0.00 for the seven zones.

Correlations of spectral and chromatographic data indicate that the etio-type spectra of groups 1 and 2 actually represent the presence of porphyrins similar to etioporphyrin III, rather than pyrroporphyrin, a plant porphyrin having a spectrum of the etio type.

Paper-sheet chromatography, using a water-saturated lutidine solvent in the presence of ammonia vapors, and spectral measurements, show that group 7a (a major portion of group 7) consists of monocarboxylic porphyrins similar to deoxophylloerythrin.

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Selection of Solvent Proportions for Paper Chromatography

Ethyl Acetate–Acetic Acid–Water System

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Variation of composition within the system ethyl acetate–acetic acid–water has been related by statistical methods to the effect on the rate and the degree of separation of monosaccharides. By selection of acetic acid content and ethyl acetate–water ratio, solvents may be tailored to suit the mixture to be separated. The chromatographer is relieved of guesswork by being able to calculate spot position, distance between spot centers, and time to accomplish the separation without danger of losing the most mobile component. Because one-phase systems far removed from the two-phase region are used, temperature changes do not affect the results.

SINCE its first adaptation to sugars by Partridge and Westall (*8*) paper chromatography has received wide application in the study of carbohydrate polymers and derivatives. Despite the many references to be found in this field, only a limited number present the results of investigations on the separating power of solvent systems (*2-5*). A new worker must usually choose between a solvent producing a high degree of separation at a slow rate or one which moves the sugars rapidly while sacrificing separation.

A systematic method for developing a solvent combining high rate and high degree of separation was sought in this investigation. Two previous findings served as the basis for this work. Jermyn and Isherwood (*5*) reported that improved separation for pairs of sugars was obtained by use of three-component solvents, which produced R_f values of 0.2 for sugars. This conclusion indicated an optimum range in the ratio between rate of sugar movement to rate of solvent front movement. These authors also noted that rate of development was inversely related to solvent viscosity. If the best separation were to be obtained by a solvent which must travel five times the desired displacement distance of the sugars, then an obvious method for obtaining an analysis in a reasonable time would involve lowering solvent viscosity. Among the possibilities for doing this was increasing the temperature or varying the nature of the solvent.

More recently, Müller and Clegg (*7*) reported that solvent front movement rate was related to the diffusion coefficient of the components. This value was defined as the ratio $D = \gamma/\eta d$,

where γ = surface tension
η = viscosity
d = specific gravity

This useful relationship indicated that other physical properties of solvent components could be selected in order to obtain a high development rate, which led to the exploitation of the conclusions of Jermyn and Isherwood. Evaluation of 10 common solvents by their diffusion coefficients indicated that ethyl acetate, acetic acid, and water should be a promising mixture.

EXPERIMENTAL

From a three-component plot of molar compositions, 12 mixtures were so chosen that the results could be expressed as functions of the composition variables. To this end the factorial, a statistical design, was employed to determine the number of runs and to evaluate the significance of results and factors

Table I. Composition of Test Solvent Mixtures

Solvent Mixture	Mole % Acetic acid	Ethyl acetate	Water
1	15	57	28
2	35	43	22
3	55	30	15
4	75	16.7	8.3
5	15	45.5	39.5
6	35	35	30
7	55	24	21
8	75	13.3	11.7
9	15	34	51
10	35	26	39
11	55	18	27
12	75	10	15

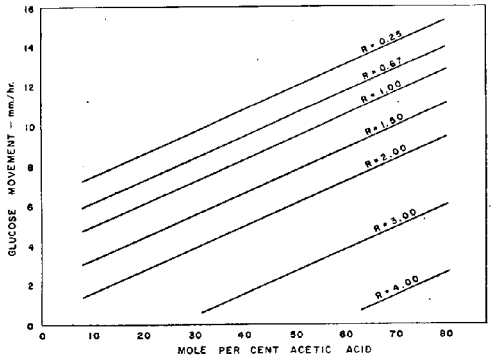

Figure 1. Development rate *vs.* solvent composition

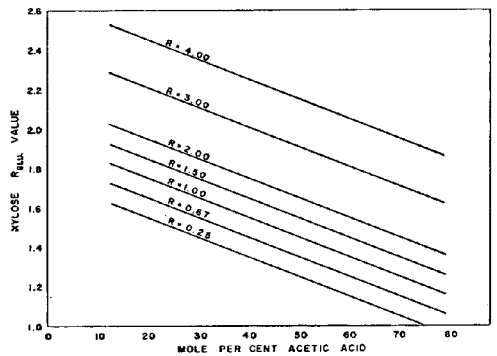

Figure 2. Xylose separation *vs.* solvent composition

studied. Acetic acid content was chosen as one independent variable. Because the other two factors, ethyl acetate and water contents, would then be dependent, they would not fill the requirements of the statistical experiment. The requirement was choice of factors of such nature that values for either might be chosen independently of values assigned to the other. Therefore, the other independent variable selected was the molar ratio of ethyl acetate to water. The levels used for molar ratio of acetate to water were 2 to 1, 7 to 6, and 2 to 3. The compositions of the 12 mixtures used in this work are presented in Table I. The 12 solvents cover more than half of the area above the two-phase region of this system. Any other ordered arrangement of compositions would have been equally suitable if the major portion of the area were experimentally investigated.

Whatman No. 1 filter paper was spotted with 2.5-μl. aliquots of a mixture of equal parts of glucose, galactose, mannose, arabinose, and xylose at a total concentration of 5 γ per μl. Solvent mixtures were made up from reagent grade acetic acid and ethyl acetate, and distilled water. The paper was cut into strips 7 × 23 inches with machine direction in the 7-inch dimension. This was done to reduce the length of the spots. For each solvent duplicate papers were irrigated in standard descending chromatographic equipment consisting of sealed glass jars 12 inches in diameter, 24 inches high, and containing stainless steel solvent assemblies. Runs were made at room temperature without attempt to maintain constant conditions, since one purpose of this work was to ensure reproducibility of results but at the same time to eliminate operator attention from elaborate schemes. After irrigation the papers were dried overnight in a hood and sprayed with 10% ammoniacal silver nitrate to develop the spots. The position of maximum density for each spot was located with a Photovolt densitometer, Model 501A. The results were tabulated as glucose movement rate in millimeters per hour and degree of separation for each of the other sugars as the ratio of sugar displacement to glucose displacement.

These results were evaluated by variance analysis to determine the significant factors. The statistical methods for calculation of polynomial coefficients were then employed to determine the form of the relationship between effect and variable. For molar acid content the significance of linear, quadratic, and cubic functions was tested while only the first two were fitted to the acetate-water ratio variable. A discussion of statistical methods is beyond the scope of this report and the reader is referred to any of the basic texts in this field.

RESULTS AND DISCUSSION

The equations derived for expressing rate and degree of separation in terms of the variables are as follows.

$$\text{Glucose rate, } r = 7.2 + 0.11A - 3.38R \quad (1)$$

$$\text{Galactose } R_{glu.} = 0.94 \quad (2)$$

$$\text{Mannose } R_{glu.} = \frac{5.29 - 0.03A + 0.0002A^2}{4} \quad (3)$$

$$\text{Arabinose } R_{glu.} = 1.50 - 0.007A + 0.22R \quad (4)$$

$$\text{Xylose } R_{glu.} = 1.69 - 0.01A - 0.24R \quad (5)$$

where r = glucose movement, millimeters per hour
$R_{glu.}$ = ratio of sugar displacement to that of glucose
R = molar ratio, ethyl acetate to water
A = molar content of acetic acid in per cent

By arbitrary choice of A and R values, these equations lead to families of curves showing the effect of each independent variable. Plots for Equations 1 and 5 are shown in Figures 1 and 2, respectively.

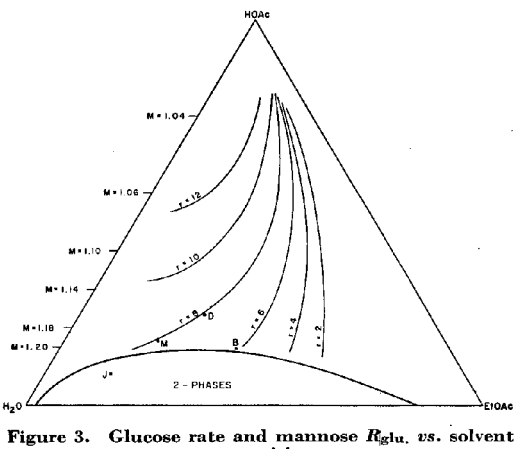

Figure 3. Glucose rate and mannose $R_{glu.}$ *vs.* solvent composition

B,J,M. **Solvent compositions of others (*1, 4–6*).**
D. **Volume ratio of 6 to 3 to 2, ethyl acetate–acetic acid–water.**

Rate of development as typified by glucose movement is directly related to the amounts of acetic acid and water, and inversely related to ethyl acetate content. Choosing ethyl acetate–water ratio and acid content values producing the same rate of movement leads to the curves shown in Figure 3. Here the relationship of result to each of the components is presented graphically.

Degree of separation of the sugars is affected in a manner opposite to rate of movement by the same solvent composition changes. The typical curves obtained for Equations 4 and 5 by arbitrary substitution of A and R values are shown in Figure 2. Selection of values for an equal degree of separation then leads to the plots shown in Figures 4 and 5.

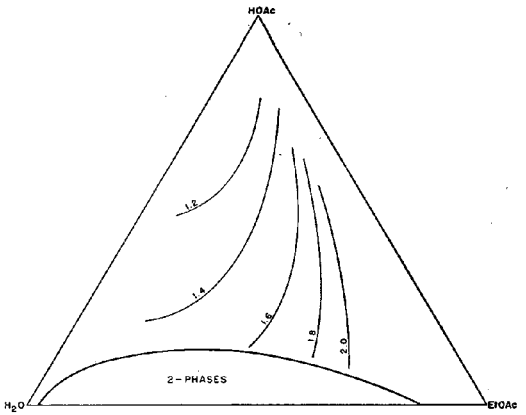

Figure 4. Arabinose $R_{Glu.}$ *vs.* solvent composition

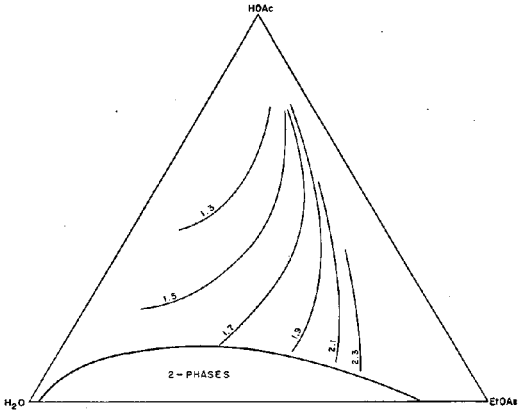

Figure 5. Xylose $R_{Glu.}$ *vs.* solvent composition

Table II. Characteristics of Solvent Mixture

(6 to 3 to 2 parts ethyl acetate–acetic acid–water)

Glucose rate	8 mm./hour
$R_{Glu.}$	
Galactose	0.94
Mannose	1.16
Arabinose	1.45
Xylose	1.55

Glucose, arabinose, and xylose values are linearly related to changes in solvent composition, while the mannose relationship requires a quadratic equation in acetic acid content only. The ratio of galactose to glucose movement is fixed for this solvent system at 0.94. The mannose values, shown as M values on Figure 3, decrease as acid content increases but are fixed for any given acid content regardless of acetate-water ratio.

The points labeled B, J, and M on Figure 3 refer to the solvent compositions used by Boggs, Jeannes and others and Jermyn and Isherwood, and McCready, respectively (*1*, *4–6*). The general applicability of the findings reported here is attested to by the fact that the results of these other workers, in terms of rate and degree of separation, can be accurately predicted from Figures 3, 4, and 5.

CONCLUSIONS

This is a generally applicable method for study of chromatography solvent systems. For selection of any particular solvent, Figures 3, 4, and 5 are superimposed. The point where curves of selected rate and degree of separation cross is the mixture desired. For work in this laboratory, the mixture chosen consists of 27.3, 23.3, and 49.4 molar quantities of ethyl acetate, acetic acid, and water. In volumes the ratios are 6 to 3 to 2 (point D, Figure 3). In a period of 20 to 24 hours, this solvent produces separations sufficient for quantitative work without interference from temperature variations as great as ±10° from a normal temperature of 25° C.

The rate and degree of separation obtained are summarized in Table II. This solvent mixture has been used to separate mixtures ranging in composition from 99 parts of glucose, 0.5 part of mannose, and 0.5 part of xylose to those containing essentially equal parts of the five sugars studied. Because the papers are irrigated crosswise to machine direction, the spots are only slightly elliptical—that is, they are essentially homogeneous round spots. This, of course, is necessary in order to permit use of the densitometer for quantitative work.

Through choice of aliquot size and time of development, the spots are completely separated. This finding applies to all of the experimental points with the exception of those mixtures containing 75% acetic acid. In these, some tailing up to the starting point was observed.

An example of the application of Figures 3, 4, and 5 is provided as follows. A mixture of xylose and arabinose will not separate if one uses a solvent composition defined by a glucose movement rate of 8 mm. per hour and a ratio of 1.06 for mannose to glucose displacement. However, the 6 to 3 to 2 mixture cited above, while moving the sugars at the same rate, separates xylose and arabinose in a satisfactory manner. Other aspects of the system become apparent when one prepares transparencies of these figures and compares by superimposing them.

Rather than recommend this system or the selected mixture as a panacea for all chromatographic problems, the authors wish to emphasize the method of evaluating solvents. With a small number of ordered experiments, one can develop desired compositions in less time than trial-and-error work in which variations by volume ratio may appear large but in molar composition are actually insignificant. It is hoped that, by stimulating others to study their favorite mixture and present their results in a similar fashion, some progress may be made in reducing chromatography to a science.

ACKNOWLEDGMENT

The authors wish to express their appreciation to A. F. Johnson for his aid in the statistical aspects of this work.

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Separation of Gases by Gas Adsorption Chromatography

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Gas adsorption chromatography was investigated as a method of separating some low-boiling gases and hydrocarbons. Adsorbent columns containing activated charcoal and alumina were continuously heated during elution. This method is advantageous for analyzing gas mixtures with wide boiling ranges, as it sharpens peaks, improves peak symmetry, and reduces time for analysis.

THE various techniques of gas chromatography have resulted in very useful methods for the separation and quantitative analysis of gaseous mixtures. In particular, Patton and coworkers (*3*) have shown good separations of some gases; the mixtures were separated on thermostated columns packed with charcoal, alumina, or silica gel, by elution with an inert carrier gas. They state that continually increasing column temperature during elution permits a more efficient separation of materials with widely different affinities for adsorbents, but were unable to take advantage of this technique because of the experimental difficulties attending reproduction of heating and carrier gas flow rate. For gas-liquid partition columns, Lichtenfels and coworkers (*1*) found that increasing column temperature during elution is advantageous for analysis of gas mixtures containing compounds with wide boiling ranges, but the reproduction of temperature rise and carrier gas flow rate is too poor for quantitative analysis. This paper describes the separation and quantitative analysis of some low-boiling gases and hydrocarbons by elution from columns packed with activated charcoal or alumina, while the column temperature is continually increased.

EXPERIMENTAL WORK

Continuously increasing the column temperature during elution results in a decreased flow to the flow-sensitive channels of a detector thermal conductivity cell. To maintain constant flow, the pressure upstream of the column should be continually increased. If reference and sample channel are connected in series, increased pressure in the reference channel will result in an annoying base-line drift of the recording potentiometer, used in conjunction with the thermal conductivity cell (*2*).

This difficulty was obviated by splitting the carrier gas, and controlling gas flow to both reference and sample channels by means of pressure regulators (Conoflow Corp., Philadelphia, Pa.). Gas flows were manometrically measured by the pressure drops (approximately 8 inches of water) across two variable orifices (micrometer needle valves) located downstream of the thermal conductivity cell. The apparatus is schematically shown in Figure 1. Once a sample was being analyzed and the column heated, visual inspection of the manometer (which could be read to ±1 mm. of water) indicated decreasing pressure drop across the orifice and thus decreasing flow through the sample channel. The correct pressure drop (and flow) was continually restored by suitable adjustments of the pressure regulator.

The columns were fabricated from copper tubing 0.25 inch in outside diameter, and after being filled with the adsorbent were wound into a coil 3 inches in inside diameter. Columns were immersed in a stirred oil bath (initially at ambient temperatures), and at the beginning of the run immersion heaters were plugged into a 110-volt source. Resultant heating rates were extremely reproducible. When a chart speed of 1 inch per minute was used, the position of peaks for the same gases during different runs did not vary in position on the chart paper by more than ±0.5 inch. A detector (Gow-Mac thermal conductivity cell) was used with a 0 to 5-, 10-, and 25-mv. recording potentiometer. The helium carrier gas flow rate was 100 ml. per minute. Gas samples (approximately 10 ml.) were intro-

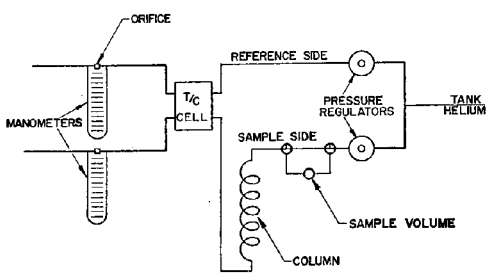

Figure 1. Schematic diagram of apparatus

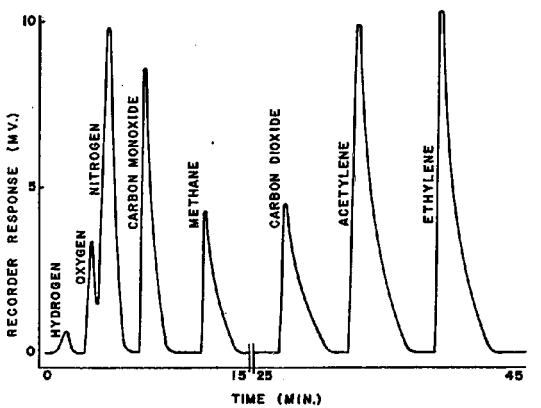

Figure 2. Separation of gas mixture on a 9-foot charcoal column, heated to 170° C.

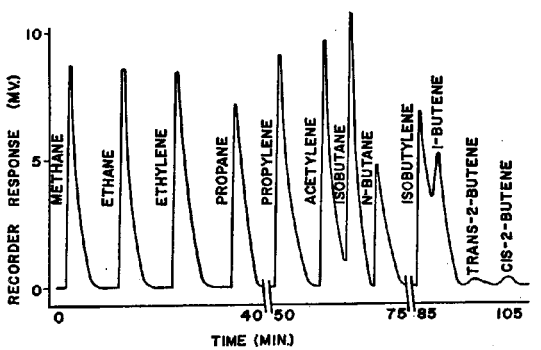

Figure 3. Separation of hydrocarbon gases on a 20-foot alumina column, heated to 150° C.

duced through a metal sample system similar to that of Patton and coworkers (*3*).

RESULTS AND DISCUSSION

Figure 2 shows the separation of a mixture of hydrogen, carbon monoxide, air, methane, carbon dioxide, ethylene, and acetylene on a 9-foot column packed with 40- to 60-mesh activated charcoal. The sensitivity to hydrogen could have been increased by elution with nitrogen rather than helium, but this would have resulted in a large loss in carbon monoxide and air sensitivity. Column temperature at the end of the run was 170° C. Figure 3 shows the results obtained with some hydrocarbons below C_5 on a 20-foot column packed with 60- to 80-mesh activated alumina, and heated to 150° C. The sample size was 10 ml., and all components were completely separated when a sample size of 0.3 ml. was used.

Figure 4 illustrates the quantitative determination of four hydrocarbons by plotting the planimetered areas of peaks against the pressures in a constant-volume sample tube which contained the mixtures. Precision of the determinations was within ±2%. The adjustment of pressure during heating and elution does not seem to interfere with quantitative measurements.

Aside from column length, the most critical factor in the separation of hydrocarbon mixtures is the rate of column heating. The results of Figure 2 were obtained using a single 250-watt heater; two 250-watt heaters resulted in a single peak for isobutylene and 1-butene and a double peak for acetylene, isobutane, and *n*-butane. Where increased heating rates can be tolerated, the symmetry of the peaks will be improved.

ACKNOWLEDGMENT

The writers express their appreciation to the Air Force for financial assistance.

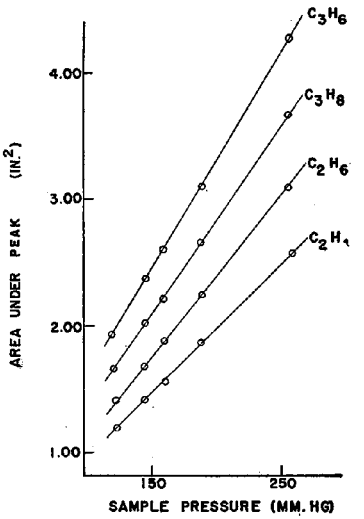

Figure 4. Plot of planimetered areas of peaks against pressures

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Qualitative Gas Chromatographic Analysis Using Two Columns of Different Characteristics

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Information as to the time required for a member of a given homologous series to pass through a gas chromatographic column is often sufficient for identification of the compound. However, if members from other series are present, the time of emergence from a single column does not positively identify a compound. This paper reports the results of experiments using two columns for the systematic identification of volatile materials. Elution times of known compounds from two gas-liquid partition columns having different characteristics were plotted against each other on logarithmic paper. Each compound studied had a definite location on this plot, which was somewhat comparable to a two-dimensional paper chromatogram. This method was found to be simple and effective for the identification of alkanes, cycloalkanes, esters, aldehydes, ketones, and alcohols. It is probable that the method could be easily extended to include other classes of compounds.

A WIDESPREAD interest has recently developed in the use of gas chromatography for the separation of gases and volatile liquids. The gas-liquid partition method in particular has been shown to be exceptionally versatile and effective for the resolution of mixtures that are difficult to separate by other means. This method was first applied to the analysis of volatile fatty acids by James and Martin (*15–17*). It has since been used by a number of workers to separate a variety of compounds (*1–26*).

In general, a separated component is identified by the time required for it to pass through the chromatographic column. However, this is sometimes insufficient information for characterization, particularly of fractions from very complex mixtures which may contain a variety of molecular types. Although members of a homologous series are generally separated with ease, the characteristic times for members of different series may not be sufficiently different to avoid confusion.

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In many cases separated fractions can be identified by studying them by infrared (*8, 22*), ultraviolet (*22*), or mass spectrometry (*2, 3, 22*). However, it is not possible to take advantage of these methods if the amount of material isolated is too small or if the necessary instruments are not available. Hence it was considered worth while to investigate means of reducing the uncertainty associated with identification by gas chromatographic methods alone.

An approach to the problem was suggested in the literature. James and James and Martin reported that primary, secondary, and tertiary amines could be distinguished by comparing their times of elution from two columns having different hydrogen-bonding tendencies (*12, 15, 17*). A similar scheme involving two columns with different affinities for different hydrocarbon types was shown by James (*14*) and James and Martin (*17*) to be an aid in the identification of aliphatic, aromatic, olefinic, halogenated, and naphthenic hydrocarbons as well as alcohols and ketones.

The purpose of this work was to investigate further the method described by James and by James and Martin and to extend it to include other homologous series. Two columns having different separatory characteristics were used to study alkanes, cycloalkanes, esters, aldehydes, ketones, and alcohols. Application of the recommended procedure to a mixture usually results in rapid and positive identification of its components.

EXPERIMENTAL

Apparatus. The gas chromatographic apparatus used was essentially the same as that previously described by the authors (*23*). Since publication of this earlier work, helium was tried as a carrier gas and found to give results similar to those for hydrogen. Because of the explosion hazard with hydrogen and the possibility of reaction between hydrogen and sample components, helium was used for all the work reported here. The flow rate of carrier gas was 40 ml. per minute for all cases.

Preparation of Gas-Liquid Partition Columns. Gas-liquid partition columns were prepared using a procedure described by Patton and Lewis (*22*). In order to minimize the pressure drop across the column, the kieselguhr (Johns-Manville, Celite 545) was screened and only that portion retained on a 100-mesh sieve was used. This material was washed with water to remove the dust and dried in an oven overnight at about 130° C. The high-boiling liquids used to prepare the columns were tricresyl phosphate and Cenco Hyvac oil (a vacuum pump oil distributed by the Central Scientific Co.).

The high-boiling liquid was not mixed with Celite directly, as suggested by James and Martin (*16*), but was applied from solution in a volatile solvent. A solution of 2.0 grams of the high-boiling liquid in 12 ml. of acetone was poured onto 4.7 grams of Celite with constant stirring. Ideally, the Celite should be completely wet, with no excess solution. The acetone was then allowed to evaporate, leaving the Celite uniformly impregnated with high-boiling liquid.

The materials thus prepared were packed into glass columns, 0.5 cm. in diameter and approximately 115 cm. in length, by agitation from an electric vibrator. Glass wool plugs were used to keep the packing in place.

Calibration and Analysis. The columns were operated at room temperature (25° ± 2° C.). Although the results were satisfactory for the work reported here, the change of elution time with changes in room temperature was perceptible in some cases.

Volatile alkanes, cycloalkanes, esters, aldehydes, ketones, and alcohols were used to explore the method. Compounds of each of these types were put through the tricresyl phosphate and oil columns, respectively, and their elution times were determined. The homologs used for calibration are listed in Table I.

Table I. Homologs Used for Calibration

Class	Compounds
Alkane	Butane, pentane, hexane, heptane
Cycloalkane	Cyclopropane, cyclopentane, cyclohexane
Ester	Methyl, ethyl, propyl, and butyl acetates
Aldehyde	Acetaldehyde, propionaldehyde, butyraldehyde
Ketone	Acetone, 2-butanone, 2-pentanone
Alcohol	Methyl, ethyl, propyl, and butyl alcohols

Figure 1 shows these data plotted according to the method suggested by James (*14*) and James and Martin (*17*). The points for members of a given homologous series lie on a smooth curve which is almost a straight line in each case. The lines converge toward the origin, and the experimental points are crowded in this region. This crowding is eliminated and some additional advantages are gained by plotting the data on logarithmic paper, as shown in Figure 2. Again a straight line can be drawn through the points for each series. However, on this plot the lines are parallel and points for consecutive members are approximately equally spaced, as would be expected from the linear relationship between

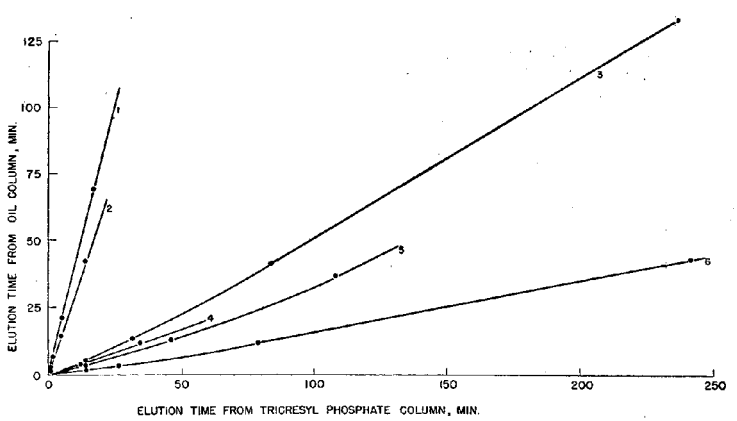

Figure 1. Linear relation of elution times

1. **Alkanes**
2. **Cycloalkanes**
3. **Esters**
4. **Aldehydes**
5. **Ketones**
6. **Alcohols**

logarithm of elution time (or retention volume) and number of carbon atoms per molecule. The regular spacing facilitates interpolation or extrapolation to include compounds of the same homologous series for which experimental calibration data are not available. In many respects the plot shown in Figure 2 is comparable to a two-dimensional paper chromatogram.

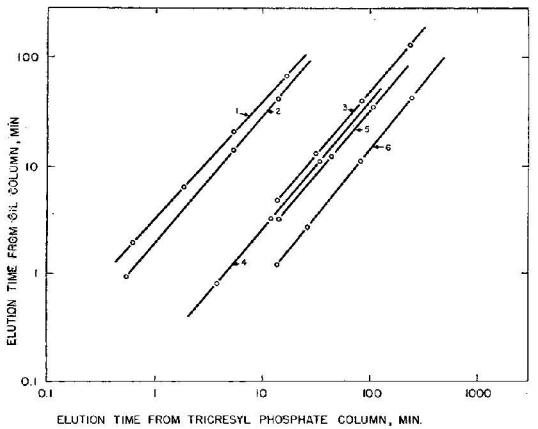

Figure 2. Logarithmic relation of elution times

1. Alkanes
2. Cycloalkanes
3. Esters
4. Aldehydes
5. Ketones
6. Alcohols

As a check of its usefulness, the method was used to identify the components of a synthetic mixture of unknown composition. The elution curve of this material from the tricresyl phosphate column is shown in Figure 3. Fractions corresponding to each of the three peaks on this curve were collected in a manner previously described (*22*) and put through the oil column. The three curves shown in Figure 4 resulted. When the elution times obtained from these curves were compared with those for reference compounds by means of the calibration curve in Figure 2, the components of the mixture were identified as methanol, acetone, ethyl acetate, and cyclopentane. Methanol and acetone could not be distinguished by elution from the tricresyl phosphate column alone. Likewise, elution of the original mixture from the oil column alone would not have separated cyclopentane and ethyl acetate.

Miscellaneous Experiments. Two identical columns were prepared in order to determine the accuracy of reproducing the packings. Materials having elution times covering the range of from 0.6 to 115 minutes were put through the columns. The average deviation was found to be ±4%. Therefore, in case a particular column is destroyed, another column can be prepared and used without having to calibrate the system with the new column.

As an aid in the identification of volatile organic materials, it is sometimes desirable to remove a particular class of compounds completely from a mixture. This can be accomplished by specially prepared columns. For instance, a short column (about 0.5 cm. in diameter by 10 cm. in length) packed with Celite 545 impregnated with 95% sulfuric acid was used for removing aldehydes, esters, ketones, and alcohols from mixtures with saturated hydrocarbons. A short column of sodium hydroxide on asbestos (Ascarite, A. H. Thomas Co., Philadelphia, Pa.) may be used for the removal of aldehydes alone.

While investigating the effect of various parameters, such as boiling point and number of carbon atoms, on the elution time, it was found that a linear relationship existed between the elution time and the reciprocal of the vapor pressure for most of the series studied.

DISCUSSION AND CONCLUSIONS

The logarithmic plot shown in Figure 2 has certain advantages over the linear curves of James (*14*) and James and Martin (*15–17*). First, it has been reported that for a given homologous series, a straight line is obtained when the logarithm of the retention volume (or elution time, which is a function of the retention volume) is plotted against the number of carbon atoms (*2, 13–17, 21, 23, 26*). Therefore, consecutive members of a given series should be equally spaced. As shown in Figure 2, this is approximately true for the oil and tricresyl phosphate columns. Second, because the curves for the various homologous series do not converge as they do for a linear plot, the more vola-

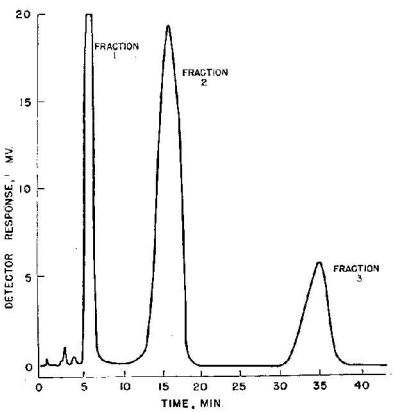

Figure 3. Elution of synthetic mixture from tricresyl phosphate column

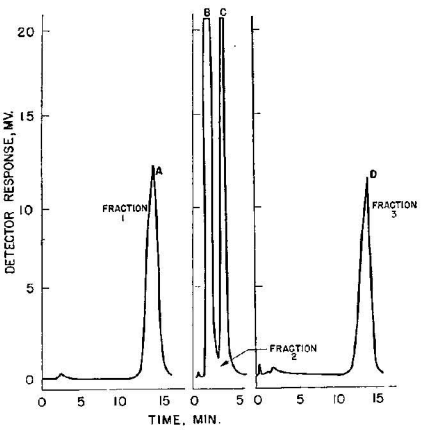

Figure 4. Elution of separated fractions of synthetic mixture from oil column

A. Cyclopentane
B. Methanol
C. Acetone
D. Ethyl acetate

tile members of a series can be identified as easily as the less volatile.

As the columns were not insulated, changes in room temperature affected the elution times of a given column. A 5% variation was perceptible in some cases. When more accuracy of reproducing the elution time is needed, such as in identifying the isomers of a homologous series, the columns should be insulated and the temperature regulated. The elution time of a given component can be measured to ±1% by incorporating an internal standard in the samples analyzed (*21*).

Techniques of gas chromatography alone can be used for the systematic identification of volatile organic materials. A characteristic graph was obtained by plotting against each other the elution times of known compounds passed through two different columns. Each compound studied had a definite location on this plot, which was somewhat comparable to a two-dimensional paper chromatogram. Components of a synthetic mixture were identified by observing their elution times when passed through two different columns having different separatory characteristics. The method was used for the identification of alkanes, cycloalkanes, esters, aldehydes, ketones, and alcohols. Higher boiling members of these series can probably be characterized by using heated columns. It seems likely that the method can be easily extended to include other classes of compounds.

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Chromatography of Organic Bases on Multibuffered Paper

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When a filter paper strip is impregnated in individually marked zones with various buffers in sequence of decreasing pH, and a mixture of organic bases is chromatographed on such a paper, using a single solvent in presence of water vapor, the bases tend to form salts at zones, depending upon their pK values. Separations are thus obtained which are otherwise difficult to achieve. This procedure eliminates the tedious search for a suitable solvent mixture and permits the immobilization of individual bases at predetermined pH levels. Complete separation can be made of many compounds, for which only minor differences in R_f values had been previously reported. A semiquantitative technique is described for the reflectance measurement of the developed spots.

FILTER paper impregnated with buffers has been widely used for the separation of a variety of compounds. Partridge and Swain (*9*), Blackburn (*1*), Felix and Krekels (*4*), McFarren (*6*), and many others have improved the separation of amino acids by the use of buffered paper chromatograms. Iwainsky (*5*) separated the 2,4-dinitrophenyl derivatives of various amino acids on buffered paper, adding the same buffer solution in place of water to the solvent mixture.

The separation of strongly basic solanaceous and ergot alkaloids on buffered papers was described by Carless and Woodhead (*3*). Brossi, Häfliger, and Schnider (*2*) reported the separation on filter paper uniformly buffered at pH 6.3 or 8.1 of morphinan and a number of its derivatives, such as (−)-3-hydroxy-*N*-methylmorphinan (levorphan, Dromoran), (+)-3-methoxy-*N*-methylmorphinan (dextromethorphan, Romilar), and (−)-3-hydroxy-*N*-allylmorphinan (levallorphan, Lorfan). On paper buffered at pH 6.3, these authors were able to effect a good separation of Dromoran (R_f, 0.37 to 0.41) and Romilar (R_f, 0.47 to 0.52), and a better separation between these two compounds and Lorfan (R_f, 0.67 to 0.70). On paper buffered at pH 8.1 only fair separation of these compounds was obtained. The R_f values at pH 8.1 were Dromoran, 0.84 to 0.88; Romilar, 0.88 to 0.92; and Lorfan, 0.94 to 0.96. The spots were identified by spraying the paper with the platinum chloride–iodide reagent of Munier and Macheboeuf (*8*). With paper buffered at pH 6.3, excellent separation of morphine (R_f, 0.13) from the various morphinan derivatives was obtained (R_f values ranging from 0.37 to 0.70).

While a successful separation can thus often be achieved, the detection of very small quantities, such as 1 to 2% of one component in the presence of another one of closely related structure, may cause great difficulties due to the long tailing effect of the major component.

The purpose of this work was to find a paper chromatographic method which would overcome these difficulties, avoid the search for a suitable combination of solvents, and result in a better separation of certain basic organic compounds of related structure for which only minor differences in R_f values have been reported.

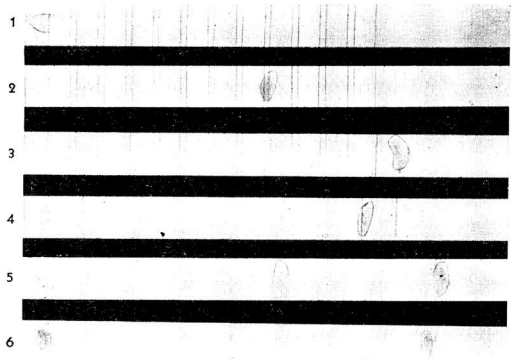

Figure 1. Separation of opium alkaloids

1. Morphine
2. Codeine
3. Narcotine
4. Morphine plus codeine
5. Morphine plus codeine plus narcotine
6. 1 part narcotine in 100 parts morphine

The first step of the procedure consists of the preparation of a multibuffered filter paper strip. This is accomplished by the application in marked zones of small quantities of buffers at various acidic pH levels in the sequence of their decreasing pH. When a number of organic bases is placed on such a paper and chromatographed by descending procedure, using a single organic solvent as the mobile phase in the presence of water vapor, the bases are likely to form salts with the buffers at various levels, depending upon their pK values. Consequently, a strong organic base may form a salt at a pH just below 7 and will not travel any farther on the paper, while a weaker base may proceed to a zone buffer at a pH of 4 or 5. This was found to be the case with a large number of substances.

EXPERIMENTAL

Reagents. Chloroform, reagent grade.

MacIlvaine buffers (*7*), double strength.

Platinum chloride–potassium iodide reagent (*8*). Dissolve 0.2 gram of platinum chloride in about 2 ml. of water, mix with a solution of 1 gram of potassium iodide in 25 ml. of water, and dilute to 50 ml. with water.

Paper Preparation. A strip of Whatman No. 1 filter paper, 1.5 inches wide and 22 inches long, is marked in the following manner. About 10 cm. from the end of the paper a horizontal pencil line is drawn, marking the starting zone. A parallel line is then drawn 2 cm. below this, designating an unbuffered space. This line is followed by another one, 2 cm. down, marking the first buffered zone. The next unbuffered zone, 1 cm. below, is again marked with a pencil line. From then on the paper is divided along the length into 2-cm. wide buffered areas, followed by 1-cm. wide zones.

The purpose of the unbuffered areas is to prevent intimate mixing of the adjacent buffers. However, at times a certain amount of migration of buffer from one zone to the neighboring one may take place.

A simple procedure for applying the buffers is to tack the ends of the suspended paper strip to two blocks of wood, appropriately spaced. Double strength MacIlvaine buffers (*7*), ranging from pH 6.4 to pH 4.2, are applied to the paper within the 2-cm. zones only, by dipping a heavy glass rod into the particular buffer solution and rolling the rod within the marked area. Thus, the zones start with pH 6.4 and decrease in pH at intervals of 0.2. The paper is allowed to dry in air.

Chromatography. About 15 γ of the organic bases in 10 μl. of chloroform or other suitable solvent are applied to the starting line with a micropipet. The paper is allowed to remain overnight in a chromatographic chamber partially lined with moistened filter paper and containing water in the bottom in addition to two beakers filled with chloroform. Chloroform or some other water-immiscible solvent is used as the mobile phase, and the chromatogram is developed until the solvent front moves past the last buffered zone. It usually takes about 3 hours for the chloroform solvent front to move down the strip.

The papers are dried at room temperature, then sprayed with the platinum chloride–iodide reagent to make the organic bases visible. The excess of reagent is removed by washing with water and the strip is again air-dried.

SEPARATIONS

Certain Opium Alkaloids. A multibuffered paper was prepared, starting with buffer pH 6.4 and decreasing to pH 4.2.

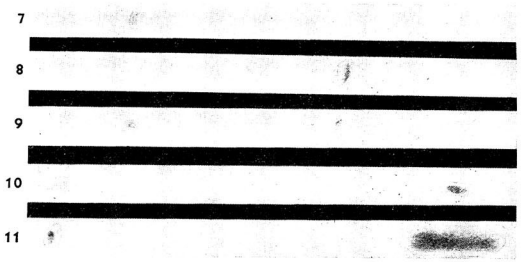

Figure 2. Separation of Dromoran and Romilar

7. Dromoran
8. Romilar
9. 10. Romilar plus Dromoran
11. 1 part Dromoran in 100 parts Romilar

Each of the bases morphine, narcotine, and codeine was individually applied in 15-γ amounts on three separate multibuffered strips. Figure 1 shows that morphine (R_f, 0.1) was immobilized at pH 6.4; codeine (R_f, 0.56) moved to a pH of about 4.8, while narcotine (R_f, 0.94) traveled below pH 4.2. To obtain wider separation between morphine and codeine, strip 4 was prepared by buffering an upper zone at pH 5.8 and a lower zone at pH 4.2. When a mixture of the two alkaloids was applied to this strip, morphine (R_f, 0.03) remained at the top while codeine (R_f, 0.8) moved to the zone buffered at pH 4.2.

Although an equally good separation can be obtained by applying pH 5.8 buffer to the upper part of the strip, allowing the rest of the paper to remain unbuffered, the use of two widely separated buffered zones has the following advantages: (*a*) It permits the concentration of each component within a narrow band; and (*b*) it enables the migration of each component to be stopped at a predetermined area of the paper. This makes it possible to cut out the desired zone for quantitative determination.

Strip 5 was prepared by buffering the upper zone at pH 5.8 and a center zone at pH 4.2. Morphine (R_f, 0.03) was immobilized on the upper zone, codeine (R_f, 0.57) traveled to the pH 4.2 buffer, while narcotine (R_f, 0.94) moved just behind the solvent front. In order to separate a small quantity of narcotine (15 γ) in the presence of morphine (1.5 mg.), strip 6 was arranged with an upper area buffered at pH 5.8 and the remainder of the paper unbuffered. All of the morphine (R_f, 0.05) was concentrated at the top of the paper, while the narcotine (R_f, 0.97) moved almost with the solvent front.

Dromoran and Romilar. Dromoran (R_f, 0.21), when chromatographed on a multibuffered strip, traveled to a pH of about 6.0, while Romilar (R_f, 0.63 to 0.69) moved to a pH of approximately 4.6 (Figure 2). A mixture of both compounds behaved as shown on strip 9. Strip 10 was prepared by applying pH 5.8 buffer to the upper zone and allowing the rest of the paper to remain unbuffered. On such a chromatogram, Dromoran (R_f, 0.06) stopped at the top while Romilar (R_f, 0.8 to 0.9) traveled well down the paper.

Strip 11 was prepared in a similar manner. On this strip Dromoran (15 γ) was concentrated at the top, permitting the Romilar (1.5 mg.) to move to the bottom.

Strychnine and Papaverine. Munier and Macheboeuf (*8*) determined the R_f values of strychnine and papaverine in two different solvent systems. One system indicated a fairly good separation (papaverine R_f, 0.80; strychnine R_f 0.73), while the other one gave rather close R_f values (papaverine R_f, 0.73; strychnine R_f, 0.69). When strychnine (R_f, 0.61 to 0.63) was placed on a multibuffered paper, it was immobilized at a pH of about 4.8 and papaverine (R_f, 0.94) traveled past pH 4.2 (Figure 3). With a mixture of these two on a similar paper (strip 14), strychnine and papaverine stopped at the same levels as the individual components. To separate these two compounds more widely, strip 15 was prepared with an upper buffered zone at pH 4.2 and the remainder of the paper unbuffered. Strychnine (R_f, 0.00) did not move, while papaverine (R_f 0.96) traveled almost along with the solvent front.

Lorfan and Dromoran. When Lorfan was applied to a multibuffered strip (Figure 5, strip 19) a spot was observed at a pH of about 5.2.

Strips 16 and 17 (Figure 4) were prepared by buffering an upper zone at pH 5.6 and a lower one at pH 4.4. Dromoran (R_f, 0.24) did not move past pH 5.6 (strip 16), while Lorfan (R_f, 0.85) stopped at pH 4.4 (strip 17).

A wide separation between Dromoran and Lorfan, its allyl derivative and narcotic antagonist, was easily achieved (strip 18) by chromatography of a mixture of both compounds on paper having two zones buffered as described for strips 16 and 17.

Codeine and Romilar. Codeine (R_f, 0.39), chromatographed on a paper buffered from pH 5.7 down to pH 4.3, stopped at a pH of about 4.9. Because Romilar stops at pH 4.6, an attempt was made to separate these two compounds more widely, although there was only a difference in pH of 0.3 between their respective zones. When a paper was prepared with an upper zone at pH 4.8 and a lower one at pH 3.4, and a mixture of Romilar and codeine was applied, the codeine appeared to have moved with tailing slightly past the first zone, while all of the Romilar was concentrated in the area of pH 3.4. The fact that codeine was not completely stopped at pH 4.8 may have been due to the rapid initial movement of the chloroform solvent front. This condition could be eliminated by placing the pH 4.8 buffered area about 6 inches below the initial point of application of the sample and another buffered zone at pH 3.4 further down the strip. On such a chromatogram the codeine (R_f, 0.47) stopped at the first buffered zone with Romilar (R_f, 0.7) moving to the second one.

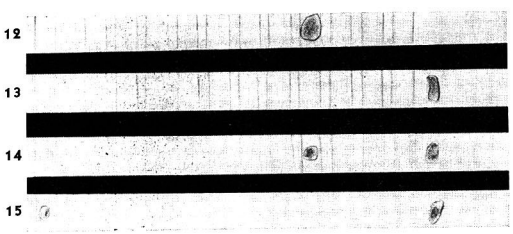

Figure 3. Separation of strychnine and papaverine

12. Strychnine
13. Papaverine
14, 15. Strychnine plus papaverine

Organic Bases. Five organic bases, morphine, papaverine, Romilar, Lorfan, and Dromoran, were dissolved in chloroform and the equivalent of about 15 γ of each was placed on the paper at the starting line.

Five different bands were observed (Figure 5 strip 19) at pH levels 6.4, 6.0, 5.2, 4.8, and below 4.4.

When the five compounds were chromatographed individually in a similar manner, their locations were:

	pH Zone	R_f
Morphine	6.4	0.05
Dromoran	6.0	0.15
Lorfan	5.2	0.53
Romilar	4.8	0.67
Papaverine	Below 4.4	0.95

The same five compounds were separated on another strip (strip 20), using four different buffers, each one selected for its affinity to the particular compound. The upper zone, buffered at pH 6.4, immobilized morphine. The second zone was buffered at pH 5.6, past which Dromoran did not move. The next zone, buffered at 5.0, stopped Lorfan, while the the fourth zone at pH 3.8 held Romilar. Papaverine traveled past the pH 3.8 buffer.

In this manner five different compounds can be separated on one strip of paper at predetermined locations, which may then be utilized for quantitative determinations if desired.

Limitations. The method is limited to compounds which possess at least small differences in basic strength. Compounds having the same pK values could not be separated by this technique. Thus far isomers have not been separated. The opium alkaloids, narcotine and papaverine, could likewise not be separated, and strychnine could not be distinguished from brucine successfully.

The extension of this technique to the separation of compounds of acidic nature, using buffers in the alkaline range, is being considered.

SEMIQUANTITATIVE ESTIMATION BY REFLECTANCE MEASUREMENT

Apparatus. A Beckman Model B spectrophotometer, with reflectance accessory (Beckman Catalog No. 12400), was used. A removable spindle fitted with a slit for winding the paper strip was attached on each side of the reflectance unit by means of a flexible bracket (Figure 6). A plastic or glass plate, 80 × 35 × 6 mm., was pressed on top of the three coil springs in the unit. The paper is inserted between this plate and the three small metal covers of the unit and the ends of the paper are wound onto the spindles.

Procedure. A paper strip, 1 inch wide, is developed in the manner previously described and placed in the reflectance unit.

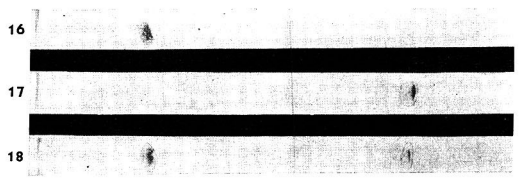

Figure 4. Separation of Lorfan and Dromoran

16. Dromoran
17. Lorfan
18. Lorfan plus Dromoran

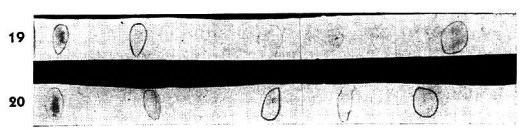

Figure 5. Separation of organic bases

19, 20. Morphine, Dromoran, Lorfan, Romilar, and papaverine

The unit is then inserted into the spectrophotometer. At the desired wave length the absorbance is set at zero with a part of the paper not containing the spot. The paper is then moved to the first outline of the spot and absorbance readings are taken every 2 mm. as the paper is moved through the unit. The absorbance readings are plotted against distance and the total area covered by the resulting curve is calculated.

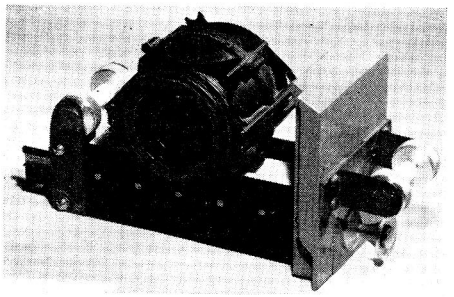

Figure 6. Reflectance unit with adapter for paper strips

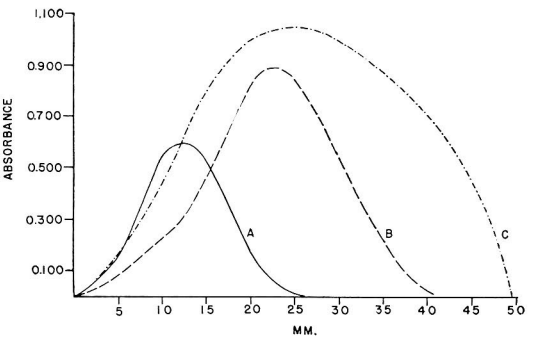

Figure 7. Area of zones obtained with different quantities of Nisentil

γ of Nisentil; *A*, 10; *B*, 20; *C*, 40

The estimation of Nisentil (alphaprodine or α-1,3-dimethyl-4-phenyl-4-propionoxypiperidine) in the presence of Dromoran serves as an example. On a multibuffered paper, Nisentil is immobilized at pH 5.2. Because Dromoran does not move below pH 6.0, good separation between these two compounds can be achieved.

Three separate paper strips were prepared, 1 inch wide, each having a small upper zone buffered at pH 5.8, with the remainder of the paper unbuffered. A sample containing 20 γ of Dromoran was placed on each strip and 10, 20, and 40 γ, respectively, of Nisentil were added at the starting line. After the run the strips were sprayed with platinum chloride–iodide. The excess reagent was removed by washing and the paper was allowed to dry at room temperature. The lower part of the paper containing the Nisentil spot was then placed in the reflectance unit, absorbance measurements were taken and plotted on graph paper, and the area was determined (Figure 7). The resulting areas were:

Sample, γ	Area, Sq. Cm.	Curve
10	35	*A*
20	80	*B*
40	167	*C*

The reproducibility is of the order of $\pm 15\%$.

ACKNOWLEDGMENT

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Gas Partition Analysis of Light Ends in Gasolines

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The technique of gas-liquid partition chromatography is rapidly establishing itself as a fast and accurate method for analyzing gases and volatile liquids. A modification was developed to provide rapid separation and identification of the light components in gasolines and similar products. The modified apparatus consists of two chromatographic columns in series. The first column acts as a stripping column to separate the light fraction and the second provides a more complete analysis of this fraction. The heavier components retained in the stripping column are removed or prevented from interfering with the light components in subsequent runs by backflushing with gas during the regular chromatographic separation of the light fraction.

ONE of the most important analytical problems in the petroleum industry is the determination of the light components in gasolines and similar samples. A frequently used technique is a combination of low temperature distillation to obtain a C_5 and lighter fraction plus an additional mass or infrared spectrometer analysis of this fraction. These procedures require considerable operator and instrument time and are subject to errors and delays in obtaining the desired analytical data. The solution to this problem was the development and application of a modified gas-liquid partition chromatographic apparatus.

The modified apparatus consists of two chromatographic columns in series. The first is a short column used for stripping the light fraction from the heavier components. The second column then provides a more complete analysis of the lighter

fraction. The use of the stripping column prevents the heavy components of the gasoline from entering the second column, from which they would be very slow to emerge. The results obtained by this technique in less than an hour per sample are comparable to those from the former method of distillation plus mass spectrometer analysis, which required 2 to 3 hours per sample.

The technique of gas-liquid partition chromatography was first suggested by Martin and Synge in 1941 (*3*). This suggestion was neglected until 1951, when James and Martin developed the technique for the analysis of gases and volatile liquids (*1*). This technique has been pursued in Europe for the past few years, but only recently have similar works been published in the United States (*2*).

The method involves separation of components with a narrow column packed with an inert granular support which has been coated with a high boiling organic liquid. When a small amount of a mixture to be analyzed is injected into the end of this column, each component will partially go into solution in the high boiling organic coating and partially exist as a gas phase in the pore spaces. The column is then eluted with an inert carrier gas, which causes the components to move forward with individual velocities less than that of the carrier gas. The velocity with which a component moves is dependent on its partition coefficient. As the partition coefficient varies for different components, a separation into zones results within the column. The different components are detected with a thermal conductivity cell as they emerge from the column.

For this problem of analyzing gasolines, the technique of gas-liquid partition chromatography was first applied for the analysis of the C_5 and lighter fraction as obtained by low temperature distillation of the original sample. The application of this technique provided a rapid and frequently more complete analysis of this fraction than could be obtained by other techniques. However, the man-hours per sample were still excessive, because of the time required for the low temperature distillation.

An attempt was made to analyze gasoline samples by injecting them into a single chromatographic column. This provided a determination of the light components but required excessive time to elute the heavier components from the column before another sample could be injected. The modified chromatographic apparatus eliminated these difficulties.

APPARATUS

A schematic diagram of the modified apparatus is shown in Figure 1.

Carrier Gas Accessories. Accessories include a high pressure tank of helium, coarse and fine pressure regulators, a surge tank, a rotameter, and a wet-test meter.

Detector. The detector is a double-pass thermal conductivity cell, connected electrically to form a Wheatstone bridge. The output of the bridge current is connected directly to a modified Brown recorder having a 0- to 5-mv. range.

Chromatographic Columns. The chromatographic columns are constructed from copper tubing $^1/_4$ inch in outside diameter and approximately $^3/_{16}$ inch in inside diameter. The granular support used in this work consisted of a crushed insulating firebrick, Sil-O-Cel C-22 (Johns-Manville Co., Pittsburgh, Pa.), that was screened to a 40-80 mesh and coated with approximately 30% by weight of a high boiling organic liquid. Column I is the short stripping column and column III provides a more complete resolution of the lighter fraction. Column I is equipped with a self-sealing serum bottle cap through which the sample to be analyzed is injected.

Air Bath. The chromatographic columns, detector, preheater, etc., are all enclosed in a constant-temperature air bath. This bath is equipped with a mercury-type thermoregulator, circulating fan, and heater for maintaining these components at the desired operating temperature.

Sampling Syringe. The sample to be analyzed is injected into the chromatographic column by means of a modified microsyringe (Figure 2), which is made by sealing a No. 27 hypodermic needle to a short length of fine-bore glass tubing containing an enlarge-

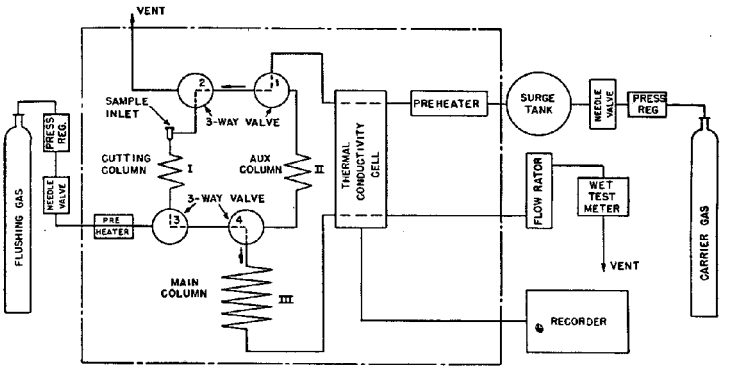

Figure 1. Schematic diagram of apparatus

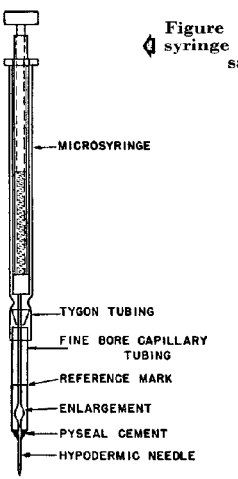

Figure 2. Microsyringe for charging samples

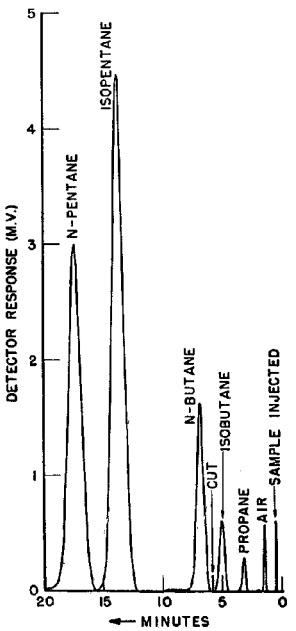

Figure 3. Chromatogram of light components in unstabilized gasoline sample

ment as shown. This pipet arrangement is attached to a microsyringe equipped with a micrometer screw and metal plunger. A sample is drawn into this special pipet by turning the micrometer screw until it is filled to the reference mark. The liquid is injected into the column by pressing the plunger.

PROCEDURE

In operation, a constant flow of carrier gas is passed through the system in the direction indicated by the arrows in Figure 1.

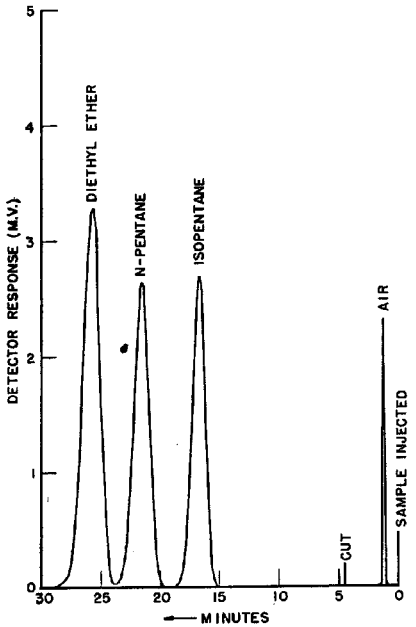

Figure 4. Chromatogram of light components in gasoline using diethyl ether as internal standard

The carrier gas is drawn from a cylinder through the reducing valves, surge tank, and preheat coil before passing through the reference side of the thermal conductivity cell. The carrier gas passes through columns I and III in series, then the sample side of the thermal conductivity cell, and finally the rotameter and wet-test meter, before it is vented. The flow of carrier gas is indicated by the rotameter and is accurately measured by the wet-test meter. After equilibrium has been established with carrier gas passing through the system, the electrical bridge of the thermal conductivity cell is balanced and the recorder pen base line is established.

Before the sample is charged to the apparatus, the three-way valve, 2, is turned to shut off the flow of carrier gas momentarily. This also vents the column to atmospheric pressure to prevent loss of sample during charging. By means of the modified microsyringe, a calibrated volume of the gasoline sample is injected into column I through the rubber serum bottle cap. Valve 2 is then opened to allow carrier gas to flow through the system in the direction indicated by the arrows, until the faster moving components are eluted from column I. Valves 1 and 4 are turned to bypass column I and allow carrier gas to flow through columns II and III. The flow continues in this direction until all of the light fraction that entered column III is eluted.

In order to maintain the same carrier gas flow rate through both paths, it was necessary to install column II, which has the same pressure drop as column I. The installation of a surge tank in the carrier gas line also provided a means of smoothing out the pressure disturbances resulting from turning the valves. At the same time the light fraction is eluted from column III, valves 2 and 3 are turned to allow carrier gas from another cylinder to backflush through column I. In this manner, the heavier components that are retained in the short column I are eluted, while the regular chromatographic separation of the light fraction is continuing in column III. As soon as the light fraction is eluted from column III, the flow of carrier gas is again passed in the direction indicated by the arrows. The apparatus is then ready to be charged with another sample.

Because some samples may contain components that are not eluted from the stripping column by the backflushing procedure, it may be necessary to change this column occasionally to prevent variations in retention times. However, more than 100 gasoline samples having distillation end points of approximately 400° F. have been analyzed by this technique in the same stripping column with no apparent change in retention times.

The choice of column lengths, liquid coatings, and operating conditions will depend on the particular analytical problem.

As an illustration, a unit was operated at a temperature of 45° C. with a carrier gas flow rate of 30 ml. per minute using di-2-ethylhexyl sebacate (Octoil S, Consolidated Vacuum Corp., Rochester, N. Y.) as the liquid phase in both columns. Column I was approximately 1 foot long and column III was 7 feet long. It was determined by calibration that *n*-pentane and lighter components were eluted from column I in 4.5 minutes under these conditions. Thus, the flow of carrier gas was passed in the direction indicated by the arrows in Figure 1 for at least 4.5 minutes. Then valves 1 and 4 were turned and carrier gas was passed through columns II and III for approximately 20 more minutes to elute the *n*-pentane and lighter components. For this type of sample, the entire run is complete in less than 30 minutes' instrument time and approximately 10 to 15 minutes' operator time.

RESULTS

A chromatogram of the C_5 and lighter components for an unstabilized gasoline is shown

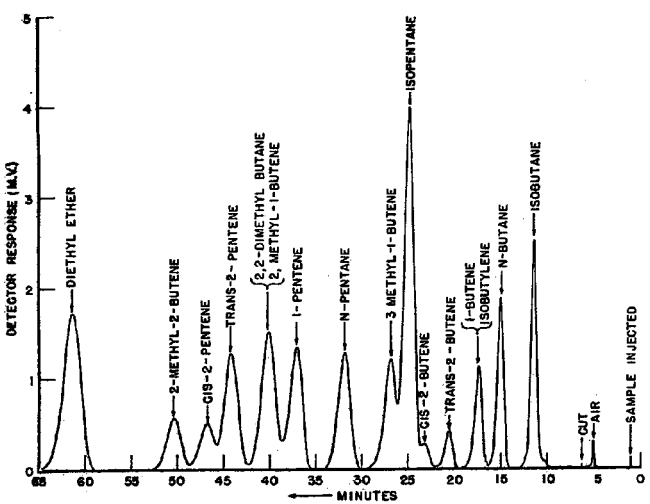

Figure 5. Chromatogram of light components in unstabilized cracked gasoline sample

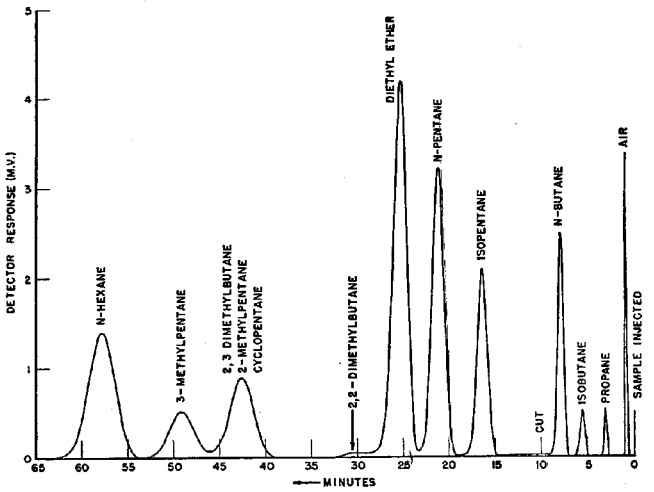

Figure 6. Chromatogram of *n*-hexane and lighter components in unstabilized gasoline sample

in Figure 3. This curve shows a continuous plot of the signal from the thermal conductivity cell sampling the gases leaving the column *vs.* time of elution. From previous calibration data for retention times, it is evident that the sample contained propane, isobutane, *n*-butane, isopentane, and *n*-pentane. The heavier components are retained in the stripping column. The concentration of each eluted component is calculated from the area under the peaks. The peak areas are usually determined with a planimeter or by multiplying peak heights by the half-band widths. Recently the application of a continuous integrator is providing a fast and accurate method for determining peak areas. In order to relate these calculations to the total sample, the practice of adding a known amount of internal standard is used. The line marked "cut" indicates the time when the flow of carrier gas was discontinued through the stripping column.

One of the first applications of this technique was the determination of isopentane and *n*-pentane in a typical gasoline sample. The chromatogram of one of these samples containing a known amount of diethyl ether as an internal standard is shown in Figure 4. Using the weight and peak area of the internal standard, the percentages of the other eluted components can be calculated from their peak areas. For this calculation, known weights of sample and ether are first blended. From the weight of ether added, the number of moles can be calculated. The moles of ether are then related to the moles of the different hydrocarbons by use of the peak areas. From the moles of each hydrocarbon, the weight of each hydrocarbon in the original sample is calculated. This weight divided by the weight of the original sample yields the results in weight per cent.

The accuracy of this method was tested using synthetic samples similar in composition to the regular gasoline samples. In order to prepare these synthetic samples, several regular gasoline samples were distilled to remove the *n*-pentane and lighter components. Using the residues from these distillations, several blends containing different amounts of isopentane and *n*-pentane plus the internal standard were prepared and analyzed. The results obtained by this method were comparable to those obtained by distillation techniques.

Table I shows the comparative results obtained on three unstabilized straight-run gasoline samples analyzed by this technique compared to the former method of low temperature distillation plus mass spectrometer analysis of the C_5 and lighter fraction.

Figure 5 shows the application of this technique for the determination of the C_5 and lighter components in a cracked gasoline sample. This sample was analyzed in an apparatus containing tri-*m*-tolyl phosphate saturated with silver nitrate as the liquid coating in the second column.

Although this technique was developed mainly for the determination of C_5 and lighter components, the same technique can be used for hydrocarbons of higher molecular weight. This is illustrated in Figure 6, which shows the determination of the *n*-hexane and lighter components in a gasoline sample.

The advantage of speed makes this technique very useful for control purposes. By maintaining a constant temperature, flow rate, and sample size, it is possible to analyze routine samples for control purposes without the addition of an internal standard to each sample. For these samples, the amount of each component is determined by a comparison of peak areas for the unknown with the areas obtained on previously analyzed synthetic samples of known composition.

Table II shows the results of four separate determinations on a synthetic sample analyzed over a period of several days.

Table I. Comparison of Results by Different Methods

(Weight per cent)

Component	Sample 1		Sample 2		Sample 3	
	A[a]	B[a]	A[a]	B[a]	A[a]	B[a]
Propane	0.22	0.21	0.14	0.16	0.05	0.06
Isobutane	0.43	0.35	0.72	0.59	0.07	0.10
n-Butane	1.09	1.27	1.48	1.74	0.29	0.40
Isopentane	2.22	2.06	5.37	5.60	4.19	4.04
n-Pentane	1.93	2.06	4.80	4.96	3.50	3.59
Hexane + (by diff.)	94.11	94.05	87.49	86.95	91.90	91.81

[a] A. Analysis by distillation plus mass spectrometry.
B. Analysis by gas-liquid partition method.

Table II. Results for a Synthetic Sample without Use of an Internal Standard

(Weight per cent)

Component	% by Blend	% Determined			
		Run 1	Run 2	Run 3	Run 4
Isopentane	4.25	4.24	4.06	4.21	4.21
n-Pentane	4.64	4.69	4.77	4.74	4.70
Hexane +	91.11	91.07[a]	91.17[a]	91.05[a]	91.09[a]

[a] Determined by difference.

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Analytical Separation and Identification of Sulfur Compounds in a Petroleum Distillate Boiling to 100° C.

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The object of the work reported was to identify and quantitatively determine the sulfur compounds present in a crude oil in the boiling range 0° to 100° C. A naphtha was isothermally distilled from Wasson, Tex., crude oil to avoid thermal degradation and by adsorption and distillation processes and infrared examination of the final fractions the following compounds were identified: methanethiol, ethanethiol, 2-thiapropane, 2-propanethiol, 2-methyl-2-propanethiol, 2-thiabutane, 1-propanethiol, 3-methyl-2-thiabutane, 2-butanethiol, 2-methyl-1-propanethiol, 3-thiapentane, 2-thiapentane, 1-butanethiol, 3,3-dimethyl-2-thiabutane, and 2-methyl-2-butanethiol. Thiophene was not found, although repeated tests were made for this compound. Knowledge of the sulfur compounds and their concentration in crude oil is of considerable theoretical interest and should aid in refining processes.

IMPORTANT phases of American Petroleum Institute Research Project 48A are the identification and quantitative estimation of the sulfuric compounds in a selected crude oil. The prosecution of this research necessitates developing methods of separating the sulfur compounds and of identifying and determining them quantitatively. This paper presents methods developed for and applied to the investigation of an uncracked distillate boiling to 100° C., prepared from Wasson, Tex., crude oil, and lists the 15 sulfur compounds, boiling below 100° C., in the crude oil identified by the methods described.

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THE PROBLEM

In essence, the problem was to concentrate, without appreciable loss, the sulfur compounds (approximately 40 grams) from 9000 grams of distillate, to identify these sulfur compounds, and to estimate the concentration in which each is present. Theoretically 21 compounds of carbon, hydrogen, and sulfur exist whose boiling point is below 100° C., in addition to hydrogen sulfide and carbon disulfide. However, thiacyclopropane (*5*), its methyl and dimethyl derivatives, and thiacyclobutane (*15*) are very unstable and not likely to be present in petroleum. Carbon disulfide (*7*, *13*) and thiophene (*13*) have been reported present in straight-run petroleum distillates. The identification of the first is generally discounted, while some uncertainty surrounds that of thiophene. This investigation failed to identify either. [Since this was written three of the present authors and coworkers working in APIRP 48 have identified thiophene and 2-methylthiophene in a Wilmington, Calif., crude oil (*17*).] It is conceivable that thiophene may be present in Wasson crude oil in a quantity too small to be detected by the methods of separation and identification used in this investigation.

In addition, the problem involved consideration of the stability of the sulfur compounds during the separation processes. Previous work (*2*, *3*) had indicated considerable difference in the type of sulfur compounds obtained by distillation at atmospheric and at reduced pressure. Other studies (*4*, *6*) had shown that elemental sulfur reacts with crude oil to form hydrogen sulfide

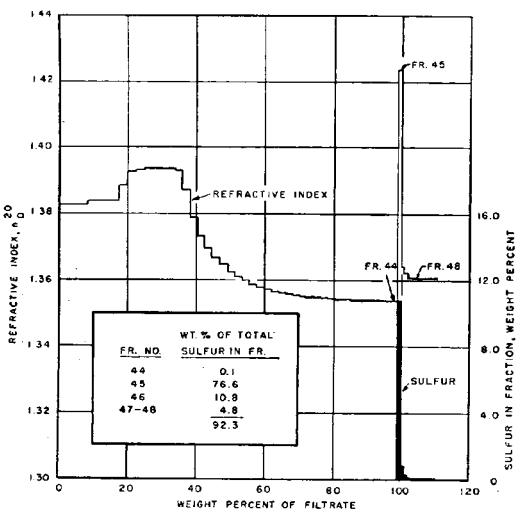

Figure 1. Alumina adsorptogram of Wasson distillate

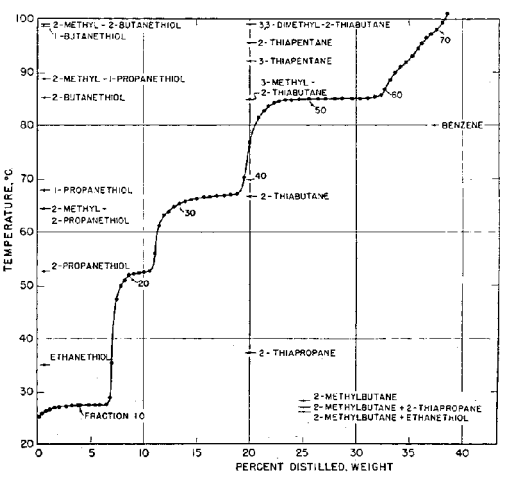

Figure 2. Fractionation of sulfur concentrate from Wasson distillate

and other sulfur compounds at temperatures near 125° C. Elemental sulfur, known to occur naturally in certain crude oils (*6, 14*), has been found in some low boiling distillates (*3, 10*), but was not detected in the crude oil sample investigated.

THE INVESTIGATION

The several processes used in this study were isothermal distillation, fractionation, concentration by adsorption on alumina, and semimicrofractionation of the concentrate. Identification and quantitative estimation of the individual compounds were based on infrared spectra. A description of each procedure is given below and its application in the problem discussed.

Isothermal Distillation. The isothermal distillation system utilized the principle of flash evaporation of the distillate from a heated, descending film of crude oil, with the vapor being removed continuously by a countercurrent stream of inert gas. In practice, the crude oil was pumped into the top of a steam-heated column so constructed as to distribute the oil in a film as it progressed downward. A countercurrent nitrogen stream provided a scrubbing action, while a short dephlegmator, packed with glass helices, gave some fractionation of the ascending vapors. The condensing system consisted of an ice water–cooled condenser with an appropriate receiver followed by a liquid air–cooled trap. This method of stripping crude oil has several advantages: The temperature is easily maintained at 100° C., and no excessive heating can occur; the contact time is short, particularly as compared to a batch distillation; the process is continuous, so that the large quantities of crude oil required to obtain adequate quantities of sulfur compounds can be easily handled; and the quantity of distillate produced can be regulated to some extent by changing the flow rates of the oil or gas, or both. Thus it fulfills the need for a method eliminating, to a large degree, the possible effects of heat on the sulfur compounds and the reaction of elemental sulfur with crude oil to produce sulfur compounds.

This method has some disadvantages: It is difficult to condense the low-boiling compounds completely from the gas stream; a product of wide boiling range is obtained; and, although some improvement can be effected by use of a reflux condenser, the distillate must be fractionated if it is desirable to obtain sharp cuts in a desired boiling range. Fractionation is not a serious objection, as most of the heavy, more thermosensitive material has been removed.

To provide the necessary quantity of distillate, 107.70 kg. of crude oil (33 gallons) was processed, yielding 2.05% gas plus loss, 8.45% distillate, and 89.5% residuum.

Thermal Stability of Distillate. It was thought advisable to determine whether distillates prepared in this way could be distilled by a batch process without significant changes in the content of sulfur compounds. Accordingly, a portion of distillate boiling from 35° to 240° C. (85% between 65° and 165° C.) was subjected to a stability test consisting of refluxing at different temperature levels up to about 200° C. and determining the amounts of hydrogen sulfide and thiols (mercaptans) evolved. A complete description of this apparatus and procedure has been given (*4*). A group sulfur analysis (*1*) of the distillate was made before the test, and of the combined residuum and fractions after the test.

The results, given in Table I, indicated negligible thermal effects, as only a trace of hydrogen sulfide was evolved, and the

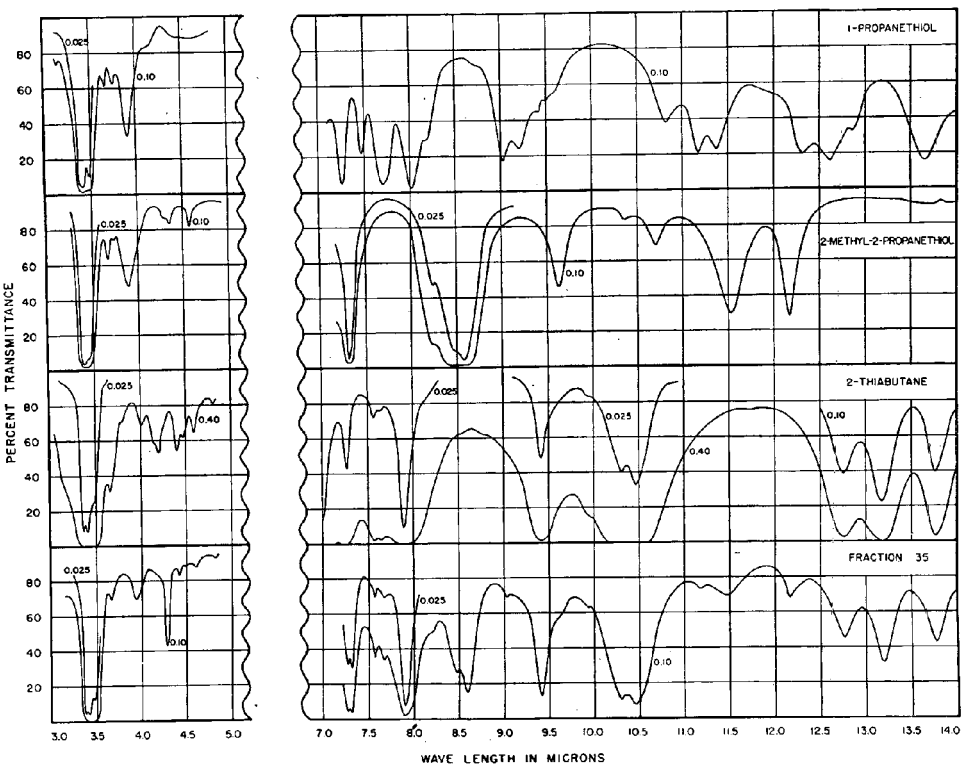

Figure 3. Infrared spectra of fraction 35 and its components

two analyses agree within the limits of analytical error. It was concluded, therefore, that batch distillation of a Wasson naphtha could be conducted with freedom from thermal reactions of the sulfur compounds if a temperature of about 200° C. were not exceeded. However, a deliberate effort was made to hold temperatures as low as possible. Experimental temperatures throughout this investigation never exceeded 100° C. in any operation except in the fractionation of the sulfur concentrate, where material boiling up to 100° C. was removed overhead.

Fractionation. Because of the high volatility of methanethiol, ethanethiol, and 2-thiapropane, it was decided that these three compounds should be determined independently, and, therefore, that the distillate from the isothermal stripping of the crude oil should be "topped" to 38° C. to remove them. This was accomplished in a high-temperature Heli-grid packed column at atmospheric pressure. From this operation a light fraction (below 38° C.) of 1.54%, a residual topped distillate of 6.85%, and a loss of 0.06% were obtained, all percentages based on the original crude oil charge. Following a few preliminary experiments, approximately 7100 grams of "topped" Wasson distillate was available for percolation through alumina.

Table I. Group Sulfur Analyses of Wasson Distillate before and after Stability Test

Compound	% Sulfur Based on Weight of Sulfur in Original Charge	
	Before stability test	After stability test
Hydrogen sulfide	0.00	0.03[a]
Elemental sulfur	0.0	0.0
Thiols (mercaptans)	46.47	2.90[a] / 44.07 } 46.97
Disulfides	0.00	0.34
Sulfides I	48.42	47.07
Sulfides II	0.67	0.67
Residual sulfur	4.04	3.33
Total sulfur	100.00	98.41
Loss		1.59

[a] Evolved during stability test.

Concentration by Adsorption. Extensive experimentation in concentrating sulfur compounds by percolation through solid adsorbents had shown that grade H-41 activated alumina was effective in preferentially adsorbing the sulfur compounds. The technique was to percolate the distillate through the alumina, using a ratio by weight of about 1 to 1. The distillate was followed by an equal volume of isopentane, and when the refractive index of the eluate reached that of isopentane, ethyl alcohol was added to displace the sulfur compounds. During these operations the absorption column was cooled with ice water.

The small amount of material resulting from this separation precluded the expenditure of any portion to obtain analytical data for establishing material balances. However, results, derived from the percolation of a similar sample are shown in Figure 1, and indicate a recovery of 92.3% of the sulfur compounds. The sulfur compounds recovered in this first percolation step were contaminated with a considerable quantity of benzene and toluene, and the resultant concentrate required several successive percolations through alumina to reduce the quantity of aromatics to a negligible value. Some sulfur compounds were contained in the alcohol eluate and were removed by combining the eluate with salt solution and extracting with isopentane. These isopentane extracts were used as part of the diluting material in the next rerun through the alumina. In all, four reruns were made, using adsorption columns having the sizes and shapes necessary to meet the requirements of the samples. The final product was a sulfur concentrate weighing 37.6 grams, equivalent to 0.0349% of the original crude oil. This concentrate still contained a trace of aromatic hydrocarbons, but in the boiling range of 38° to 100° C. benzene would be the only one present and would not interfere in the identification studies. This material accordingly was charged to a semimicro fractionation column.

Semimicrodistillation of Concentrate. The column used was a semimicro, Heli-grid packed column 8 mm. in inside diameter and 300 mm. long. The charge, 37.6 grams, was distilled at a take-off rate of 0.67 gram per hour. On the distillation curve, shown in Figure 2, the plateau at about 26° C. is caused by isopentane used in the adsorption separation. The numbers along the curve indicate fraction numbers. Selected fractions were subjected to infrared analysis.

Infrared Analysis. Through the efforts of that section of APIRP 48A at the Bureau of Mines, Laramie, Wyo., and of APIRP 48B at Northwestern University, the spectra of all the sulfur compounds expected to be present in the boiling range to 100° C. have been made available, as well as pure reference samples. Thus it was possible to identify all the compounds present. Figure 3 is typical of the data obtained, showing the spectra of fraction 35 and of 1-propanethiol, 2-methyl-2-propanethiol, and 2-thiabutane. The cell lengths, in millimeters, are noted on each of the curves. The spectrum for fraction 35 shows the presence of all three compounds—for example, bands characteristic of 1-propanethiol appear at about 7.7, 9.1, and 11.2 microns; of 2-methyl-2-propanethiol at 8.5, 11.5, and 12.2 microns; and of 2-thiabutane at 9.3, 10.4, 12.7, and 13.2 microns. From these data it is possible also to make quantitative estimates of the amounts of the components present.

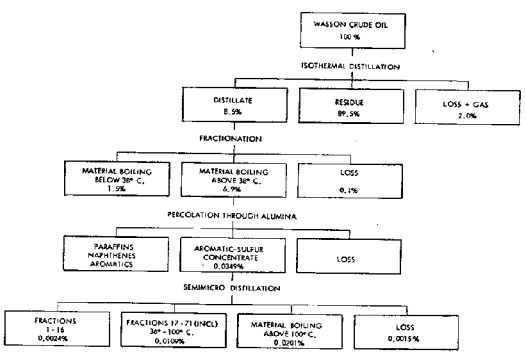

Figure 4. Outline of sample treatment

Quantitative Determination of Methanethiol, Ethanethiol, and 2-Thiapropane. The three very volatile sulfur compounds, methanethiol, ethanethiol, and 2-thiapropane, have been left out of the procedure described above and were determined in a separate and independent analysis. Several procedures were tried for determining them independently. The one that gave the most promise was to distill about 3 liters of crude oil in a high-temperature Heli-grid packed column, taking fractions at 9° and 28° C. The fractions were collected in special aluminum LPG "bombs" and analyzed for thiols by amperometric titration and for sulfides by the ultraviolet absorption of the iodine complex, as described by Hastings (*9*). The only sulfur compound in fraction 1 was methanethiol, 0.0024% of the crude oil. The second fraction containing ethanethiol, 0.0053%, and 2-thiapropane, 0.00088%. Ethanethiol and 2-thiapropane distill as azeotropes (with *n*-pentane) boiling at about 26° to 27° C., instead of at the normal boiling points of the compounds, 35.0° and 37.3°, respectively.

A flow chart giving in simplified outline the several procedures used to concentrate and separate the sulfur compounds is shown in Figure 4.

THE RESULTS

Compounds Identified in Material Boiling below 38° C. As indicated above, methanethiol, ethanethiol, and 2-thiapropane were identified and determined in this part of the crude oil. No hydrogen sulfide was found (this probably varies with sampling and storage conditions).

Compounds Identified in Distillate at 38° to 100° C. The results of the infrared studies of the fractions from the semimicrodistillation are shown in Figure 5. In this figure the graph for each component shows, on the vertical scale, the percentage of a given sulfur compound in one or more fractions. The horizontal scales at the bottom show weight per cent distilled and fraction numbers, and the horizontal scale at the top shows the cut points of the fractions in ° C. The figure shows, by shaded areas, the distribution of a given sulfur compound through several fractions and the overlapping of several sulfur compounds in the same fraction. Dotted lines represent reasonable extrapolation based on the data. The predominant compounds are 2-thiabutane and 2-butanethiol.

Summary of All Sulfur Compounds Found Boiling to 100° C. Table II lists all the compounds identified in the fraction of Wasson crude oil boiling below 100° C., and gives an estimate of their concentration in the crude oil. Several compounds have not been identified previously as being present in crude oil, so far as the authors can ascertain. All of the theoretically possible compounds of carbon, hydrogen, and sulfur except thiacyclopropane, 2-methylthiacyclopropane, 2,2-dimethylthiacyclopropane, *trans*-2,3-dimethylthiacyclopropane, thiacyclobutane, and

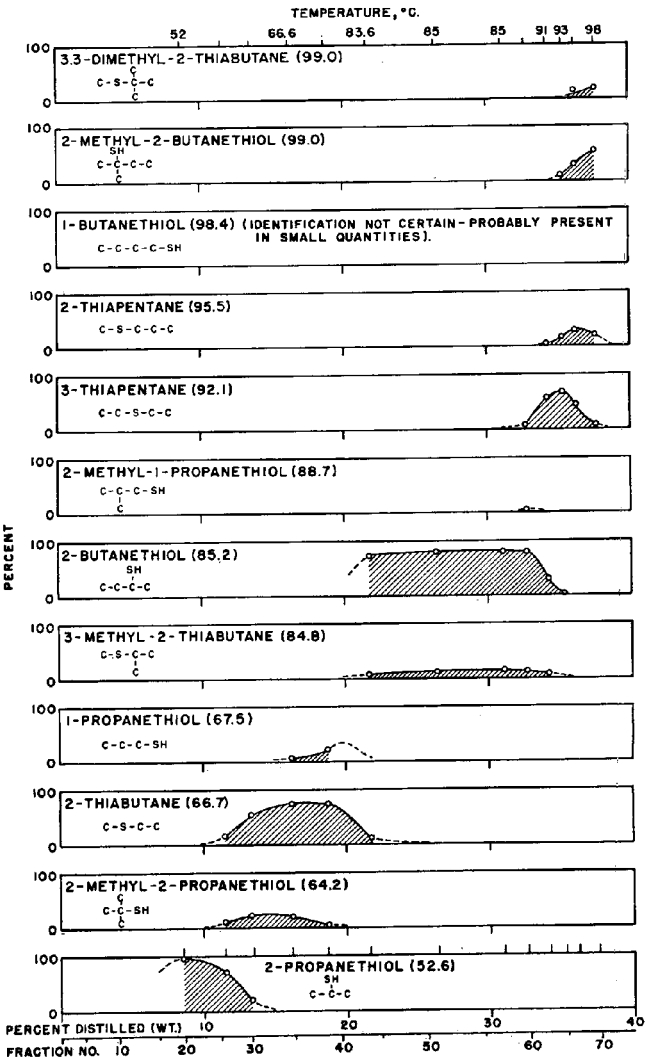

Figure 5. Distribution of sulfur compounds in sulfur concentrate prepared from Wasson crude oil

thiophene were found. The three- and four-membered cyclic sulfides are unstable and were not expected. In this investigation, no evidence of thiophene absorption bands was present in the infrared spectra of appropriate boiling range cuts. Negative results were also obtained with the isatin reagent when applied to selected fractions from the adsorption column, including those eluted before the sulfur concentrate. This test can be expected to fail in the presence of large amounts of thiols.

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

Name	Boiling Point, ° C.	Weight % in Wasson, Tex., Crude Oil[a]	Literature References to Compounds Found in Petroleum
Methanethiol (methyl mercaptan)	5.96	0.00240	(*18*)
Ethanethiol (ethyl mercaptan)	35.0	0.00530	(*3*)
2-Thiapropane (methyl sulfide)	37.31	0.00088	(*11*)
2-Propanethiol (isopropyl mercaptan)	52.56	0.00199	(*3*)
2-Methyl-2-propanethiol (*tert*-butyl mercaptan) [b]	64.22	0.00055	
2-Thiabutane (methyl ethyl sulfide)	66.65	0.00222	(*11, 16*)
1-Propanethiol (*n*-propyl mercaptan) [b]	67.5	0.00041	
3-Methyl-2-thiabutane (methyl isopropyl sulfide) [b]	84.81	0.00064	
2-Butanethiol (*sec*-butyl mercaptan)	85.15	0.00386	(*3, 12*)
2-Methyl-1-propanethiol (isobutyl mercaptan)	88.72	0.00003	(*3*)
3-Thiapentane (ethyl sulfide)	92.10	0.00075	(*11*)
2-Thiapentane (methyl *n*-propyl sulfide)	95.52	0.00030	(*16*)
1-Butanethiol (*n*-butyl mercaptan) [b]	98.4	Trace	
3,3-Dimethyl-2-thiabutane (methyl *tert*-butyl sulfide) [b]	99.0	Not determined	
2-Methyl-2-butanethiol (*tert*-amyl mercaptan) [b]	99.0	0.00126	

[a] Minimum values.
[b] Not previously reported in literature.

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Identification of Thiophene and 2-Methylthiophene in Virgin Petroleum

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Whether thiophene as such exists in virgin petroleum or is produced by pyrolytic breakdown or rearrangement of other sulfur compounds has been of interest to petroleum chemists for many years. This paper discusses the concentration and spectroscopic identification of thiophene and 2-methylthiophene in a Wilmington, Calif., crude oil. The crude oil was exposed to a maximum temperature of only 100° C. for less than 30 seconds in the preparation of the distillate from which the thiophene was concentrated. The knowledge that thiophene exists in petroleum is of importance not only to the practical chemist interested in the removal of sulfur compounds from petroleum distillates, but also to the fundamental chemist and geologist who are concerned with the origin of petroleum and of the sulfur compounds found in it.

THIOPHENE was first discovered by Victor Meyer (*15*) in coal-tar light oil in 1882. This discovery initiated intensive research in thiophene chemistry, and attempts to associate the compound with certain natural products followed.

As a result of this research, its presence in products of pyrolytic origin, such as coal tars, shale oils, and "cracked" petroleum, is firmly established. For example, Saladini (*20*) and Heusler (*10*) state that thiophene is present in lignite tar oils; Challenger, Haslam, Bramhall, and Walkden (*4*) isolated it and several alkyl derivatives from shale oil; Dodonov and Soshestvenska (*6*) reported it and two alkyl derivatives present in a Russian shale oil; and Scheibler and Rettig (*21*) found thiophene and three alkyl derivatives in the bituminous shales of Tyrol. More recently Kinney, Smith, and Ball (*12*) have identified thiophene, 15 alkylthiophenes, and 2,3-benzothiophene (thianaphthene) in a Colorado shale oil. Weissgerber (*26*) and Weissgerber and Kruber (*27*) isolated thianaphthene, biphenylene sulfide (dibenzothiophene), and two isomeric methylthianaphthenes from coal tars, and Weissgerber (*25*) identified tetramethylthiophene in the same material. Boes (*3*) found thianaphthene in brown coal tar and dibenzothiophene in coal tar.

The presence of thiophene in products of nonpyrolytic origin does not appear to be as soundly established. Charitschkoff (*5*) in 1899 described the isolation and identification of thiophene in a Grozny crude oil by precipitation of the mercuric chloride complex from a fraction of the distilled oil. Upon decomposing the complex with boiling hydrochloric acid and re-

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covering the product in thiophene-free benzene, he obtained a positive color reaction with isatin. Charitschkoff estimated the quantity of thiophene in the Grozny "benzine" at 1 p.p.m. or 0.0001%. In 1900 Edeleanu and Filiti (*7*) reported the possible presence of thiophene derivatives in a Romanian petroleum. On the other hand, Nametkin and Sosnina (*16*), using essentially the same procedure as that of Charitschkoff on distillate fractions from an Ishembaevsk crude oil, isolated mercaptans but indicated that the isatin test for thiophene was negative on all fractions. In 1944 Postovskiĭ, Bednyagina, and Mikhailova, (*18*) reported that in fractions from the silica gel filtration of crude oil the presence of thiophene was not indicated but that if the experiment were repeated, using heated crude oil, thiophene was found in the fractions. Emmott (*8*) reported the presence of thiophenes in sulfur dioxide extracts of Iraq and Texas middle distillates. Birch (*1*, *2*) identified several alkylthiophenes in a sulfur dioxide extract of Agha Jari crude oil, including 2,3,4-trimethylthiophene, 2,3-dimethyl-4-ethylthiophene, 2,3,4,5-tetramethylthiophene, and 2,3,4-trimethyl-5-ethylthiophene, but did not mention finding the parent compound. Other investigators (*13*, *19*, *24*) have reported the presence of alkylated and condensed ring thiophenes in crude oil.

The most recently reported evidence of thiophene in crude oils is that of Hastings (*9*). This identification was made by mass spectral examination of vacuum distilled and silica gel-filtered fractions from a West Texas crude oil. His paper did not specifically mention "thiophene," but the boiling range of the fraction analyzed (24.6° to 79.4° C.) leaves little doubt that the "thiophenes" identified must be the parent, thiophene. The concentration reported by Hastings (0.004%) is very near the detection limit of the mass spectrometer.

The identification of thiophene in petroleum as reported in

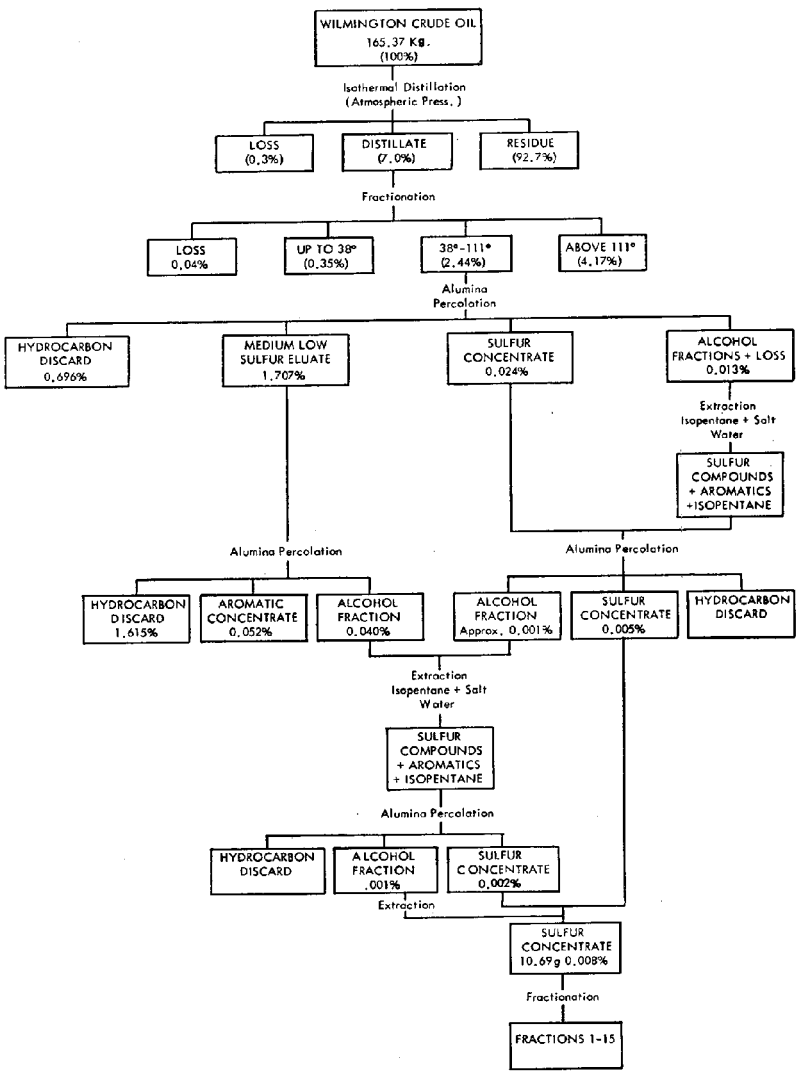

Figure 1. Summary of processing of Wilmington crude oil

the literature has been based, in most instances, on the thiophene-isatin color reaction obtained with distillate fractions. No thorough investigation of this reaction has been reported, but interferences are known. Both 2-methyl- and 3-methylthiophene give a color reaction with isatin, although the colors developed with the pure compounds are of a different hue than that obtained with thiophene. It has been shown that thiols in sufficient concentration will cause the isatin test for thiophene to fail. Unsaturates interfere with the test and even cyclic hydrocarbons in straight-run fractions (*14*) are thought to give a color reaction, although in this instance the color development (red) is tardy in comparison with that of thiophene. Furthermore, E. C. Craven, in the discussion of the work of Challenger and associates (*4*), states that he believes the indophenin reaction to be "utterly unreliable for the detection of thiophene compounds in any sort of oil; one could prepare a mixture to which thiophene had been added and get no colour at all."

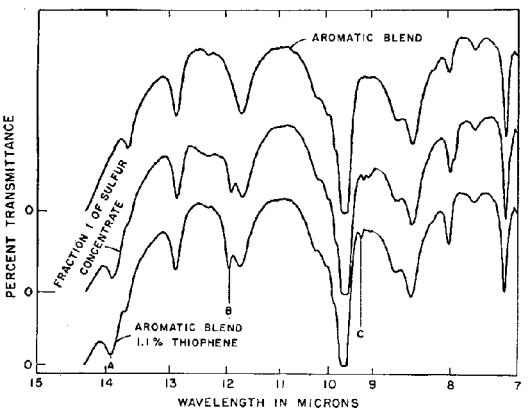

Figure 2. Infrared spectra of fraction 1 of Wilmington sulfur concentrate and of aromatic and aromatic-thiophene blends

Karr (*11*) investigated various chemical tests, including a sulfuric acid reaction, on 15 sulfur compounds, among them thiophene. He did not discuss the isatin test, but as this reagent contains sulfuric acid it may be assumed that any sulfur compound reacting positively in Karr's sulfuric acid test could confuse or interfere with the isatin reaction.

Some confusion exists in the chemical literature between thiophene, C_4H_4S, and "thiophane," C_4H_8S (thiacyclopentane), and their derivatives. There is no doubt that thiacyclopentane exists in petroleum (*1, 17, 22–24*).

EXPERIMENTAL

As it is well known that low-boiling sulfur compounds are produced by thermal decomposition of heavy sulfur-containing molecules present in the high-boiling portion of the crude oil, it was the aim of this investigation to prepare the distillates used at a low temperature and with a short contact time.

To accomplish this an all-glass isothermal still (*24*) was used to separate the "primary distillate" from the crude oil. In this process the crude oil is in contact with a glass drum, heated to 100° C., for about 30 seconds, during which the distillate is flashed from the crude oil and carried from the system by a stream of inert gas. As the isothermal still is constructed entirely of glass, except for Teflon bearings and gaskets, possible catalytic effects induced by metals are eliminated. The "primary distillate" recovered in this process was 7% of the crude oil and contained all material in the crude oil boiling below 111° C. The loss in the operation was 0.3%, which probably was made up entirely of uncondensable material. In accordance with general plans for treating the crude oil, the distillate was divided by fractionation through a 30-plate Oldershaw column (about 20 theoretical plates) into three fractions of the following boiling ranges: below 38° C., 38° to 111° C., and above 111° C. In this process still-pot temperatures were maintained below 100° C. by reduction of pressure to 200 mm. during distillation of the 38° to 111° C. fraction.

Fifty gallons (165.4 kg.) of Wilmington crude oil was processed as indicated in Figure 1. The 38° to 111° C. fraction, which is the concern of the present paper, constituted 2.4% of the crude oil. Although Wilmington crude oil contains 1.53% sulfur, most of this occurs in the high boiling range, and the 38° to 111° C. fraction contains only 0.010%. To concentrate the sulfur compounds in this fraction it was percolated batchwise through spray-dried, activated, H-41 (Alcoa) alumina. After appropriate retreatment of selected fractions from this percolation, a final aromatic-sulfur compound concentrate, which represented 0.008% of the original crude oil and contained 1.6% sulfur, was obtained.

A preliminary examination by mass spectrometry revealed that the final aromatic-sulfur compound concentrate described above contained as its major components approximately 29% benzene and 69% toluene. Also indicated as possible components were 2.0% methylthiophenes and 0.4% thiophene. This was not considered to be proof of the presence of thiophene, because of the possibility of interference in the mass spectrum. The infrared spectrum of the concentrate gave no further information about thiophene, but the presence of the methylthiophenes was confirmed. 2-Methylthiophene was found to be the predominant isomer, with possible traces of 3-methylthiophene.

To increase further the concentration of the sulfur in the sample, 5 ml. was separated in an all-glass concentric-tube column into 15 fractions. Although the column was normally of high efficiency (rated at 150 plates), it had partially lost its vacuum insulation without the knowledge of the operator. Despite the column's impaired efficiency, satisfactory resolution was achieved, permitting the positive identification of thiophene in the low boiling fractions.

Table I shows the partial mass spectrum of fraction 1 from this fractionation compared to that of thiophene.

The peaks at *m/e* 45 and 84 were corrected for the contributions of benzene and toluene and the concentration of thiophene in fraction 1 was calculated to be 1.1%. In addition, it contained 0.5% toluene and 98.4% benzene.

Table I. Partial Mass Spectra of Fraction 1 and Thiophene

	Relative Intensity	
m/e	Fraction 1[a]	Thiophene
45	58.2	55.3
58	64.2	65.4
84	100.0	100.0

[a] Adjusted for contributions of benzene and toluene.

Figure 2 compares the infrared spectrum of fraction 1 with that of a synthetic blend of the composition indicated above. The top curve of Figure 2 is the spectrum of a blend of 99.5% benzene and 0.5% toluene. The bottom curve is the spectrum of this same blend, to which 1.1% thiophene had been added. The center curve is the spectrum of fraction 1 of the sulfur-concentrate distillation. The bands indicated at *A*, *B*, and *C*, which appear when thiophene is added to the toluene-benzene

blend, are also present in the spectrum of fraction 1. This confirms the presence of thiophene in this fraction.

Further confirmation of the presence of thiophene in fraction 1 was obtained with the isatin and alloxan color tests. Both were positive. For these tests a 1% blend of thiophene in benzene was prepared and treated in the same manner as fraction 1.

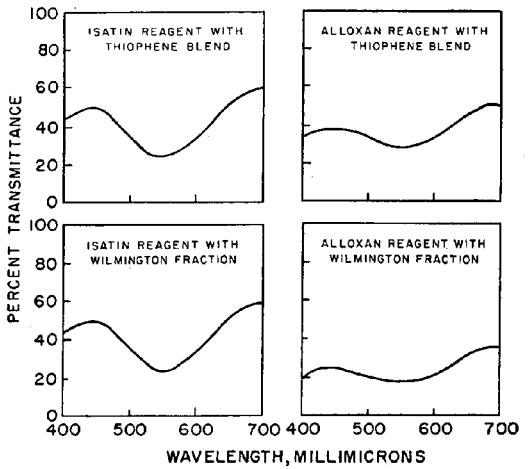

Figure 3. Visible absorption spectra of complexes of Wilmington fraction and thiophene blend

Both the thiophene blend and fraction 1 were diluted 1 to 250 with iso-octane and then further diluted 1 to 5 with the alloxan and isatin reagent. In the case of the isatin reagent the color developed was too dark for satisfactory observation and a further dilution 1 to 1 with 92% sulfuric acid was made. The absorption spectra in the visible wave length range were taken with a rapid scanning spectrophotometer. A comparison of the spectra of the isatin and alloxan complexes of a thiophene blend and of fraction 1 is shown in Figure 3. The curves shown, with other data presented above, establish beyond reasonable doubt the presence of thiophene in fraction 1 and hence in Wilmington crude oil.

A summary of the mass spectrometer analyses of the fractions from the distillation of the aromatic-sulfur compound concentrate is given in Table II.

Table II. Mass Spectrometry Analysis of Distillation Fractions

Fraction No.	Concentration, Mole %	
	Thiophene	Methylthiophenes
1	1.1	0.0
2	1.3	0.1
3	1.1	0.3
4	1.0	0.5
5	0.7	0.7
6	0.5	1.0
7	0.3	1.2
8	0.1	1.5
9	0.1	1.6
10	0.0	1.8
11		1.7
12		1.2
13		1.2
14		0.9
15		0.8
Residue		0.9

On the basis of the total column charge, the thiophene concentration was calculated to be 0.4% and the methylthiophene concentration 1.3%.

CONCLUSIONS

Thiophene has been found in a 38° to 111° C. boiling range distillate from Wilmington, Calif., crude oil in a concentration of no less than 0.000032%. In addition, 2-methylthiophene was identified in the same distillate and its concentration in the crude oil estimated at 0.00010%. The presence of 3-methylthiophene in the crude oil is probable, but this could not be established with certainty.

ACKNOWLEDGMENT

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Infrared Identification of Fumarates and Maleates

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Seven absorptions in the region of 7.7 to 15 microns are shown to be relatively constant in wave length and intensity for the fumarates. By contrast, the maleic esters have only four peaks at constant wave lengths in the same region. One of these is at about 8.2 microns; it proves to be generally useful in the measurement of maleates in the presence of fumarates.

QUALITATIVE recognition of maleic and fumaric esters has been accomplished by many "wet chemical" procedures, all of them less convenient than the spectroscopic method presented here. A convenient quantitative method, developed first by Kistiakowsky and Smith (*3*), depended upon miscibility of the esters with paraffin oil, the fumarates being miscible at lower temperature than the corresponding maleates. It is applicable only where the esterifying alcohol is known and the pure esters have been prepared and their mixtures studied. Spectroscopic methods have been applied by Gauthier in the study of maleoid-fumaroid isomerization of simple esters (*2*). Here isomeric structures were recognized directly on the esters, but only the methyl and ethyl esters (and inconveniently in the gas phase).

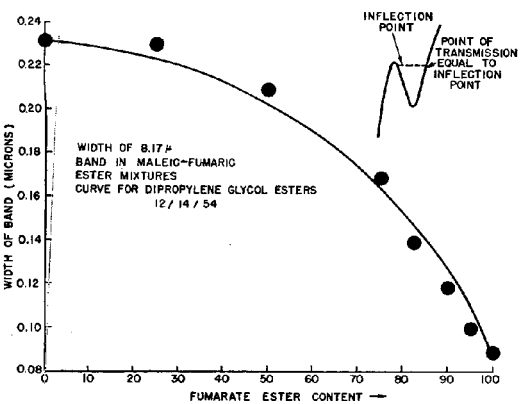

Figure 1

That Gauthier's work does not constitute a satisfactory, direct, spectroscopic method is further indicated by the reports of more recent workers (*4, 5*), who apparently abandoned direct methods in favor of converting the ester to the ethyl ester (by way of the acid), or to a potassium salt, or to an *N*-benzylamide, followed by spectroscopic examination of these derivatives. These methods suffer from the fact that an organic reaction yield is introduced before the identification step, as well as the possibility that some of the material may be isomerized.

RESULTS

It was found that a series of absorptions at 7.7, 7.9, 8.5, 8.6, 10.2, 12.9, and 15.0 microns is highly constant in wave length in the fumarate esters. (The combination of all these absorptions in an unknown constitutes adequate identification of fumarate.) A less prominent peak is also found at 8.17 microns; this is always weak by comparison to the strong absorption of maleates at about 8.2 microns. The possibility of this can be read from the Colthup (*1*) chart. What is offered here goes farther, in that there have been no exceptions to the generalizations that a strong maleate absorption is always observed in the part of one maleate range which is not overlapped by a range given for fumarates in the Colthup (*1*) chart.

The maleates have a strong absorption in the 11- to 12.5-micron range, which will be useful in measurement of maleate structure where the alcohol or polyol is known and working curves have been plotted. This peak (or group of peaks) is too variable in position to afford much value in qualitative identification of maleates. The peaks which are of value from this standpoint are found at 7.75, 8.00, 8.20, and 8.60 microns. The one at 8.2 to 8.3 microns is first or second strongest of the reliable ones. Because of this strength this peak can be used for measuring the cis structure, even in low concentrations in the presence of much of the fumarate. By measuring the width of the 8.17-micron peak of such mixtures a rough (about ±5%) measure of maleate fumarate percentage can be made. This is a case of measuring the apparent "broadening" of the 8.17-micron absorption of the fumarate by superposition of the unresolved 8.2 to 8.3-micron absorption due to the smaller amount of maleate ester.

This quick and facile measurement is of particular value, as many commercial polyesters prepared from maleic anhydride are actually largely isomerized to the fumarate structure. It has the further practical advantage that in the commercially important mixtures other materials used do not particularly interfere with this measurement. Styrene and vinyl toluene are transparent at this wave length, whereas phthalates (though more highly absorbing because of their ester linkage) do not interfere too severely.

Figure 1 gives a plot of "width of the 8.17-micron peak" *vs.* composition in known mixtures of polyesters of dipropylene glycol maleate and fumarate. Figures 2 and 3 give evidence that such a plot could be developed for mixtures of the esters in general.

The characteristic absorption of fumarates have intensities which are constant to within a factor of about 5. Table I shows the absorbances based on estimated background interpretations for the three absorptions of longest wave length for some of the esters. Widths at half density and product of absorbance and half density widths (relative integrated intensities) are also given. All values are relative to the arbitrarily chosen standard, the 12.9-micron absorption.

From Table I it is clear that intensities can be used as approximate confirmation of the identity of the absorptions for qualitative analytical purposes.

EXPERIMENTAL

Figures 2 and 3 show the spectra of the maleate and fumarate esters, recorded by means of a Perkin-Elmer 21 spectrophotometer. In general, a cell of 0.0268-mm. thickness was used and both a dilution in carbon disulfide (at stated concentration) and the pure ester were run. The exceptions were: The dimethyl fumarate curve was run in a cell of about 0.165-mm. thickness with carbon disulfide in a cell of 0.175-mm. thickness in reference beam (this introduces a small error of negative absorption at about 11.7 microns). The curves of isobutyl, cyclohexyl, and *sec*-butyl

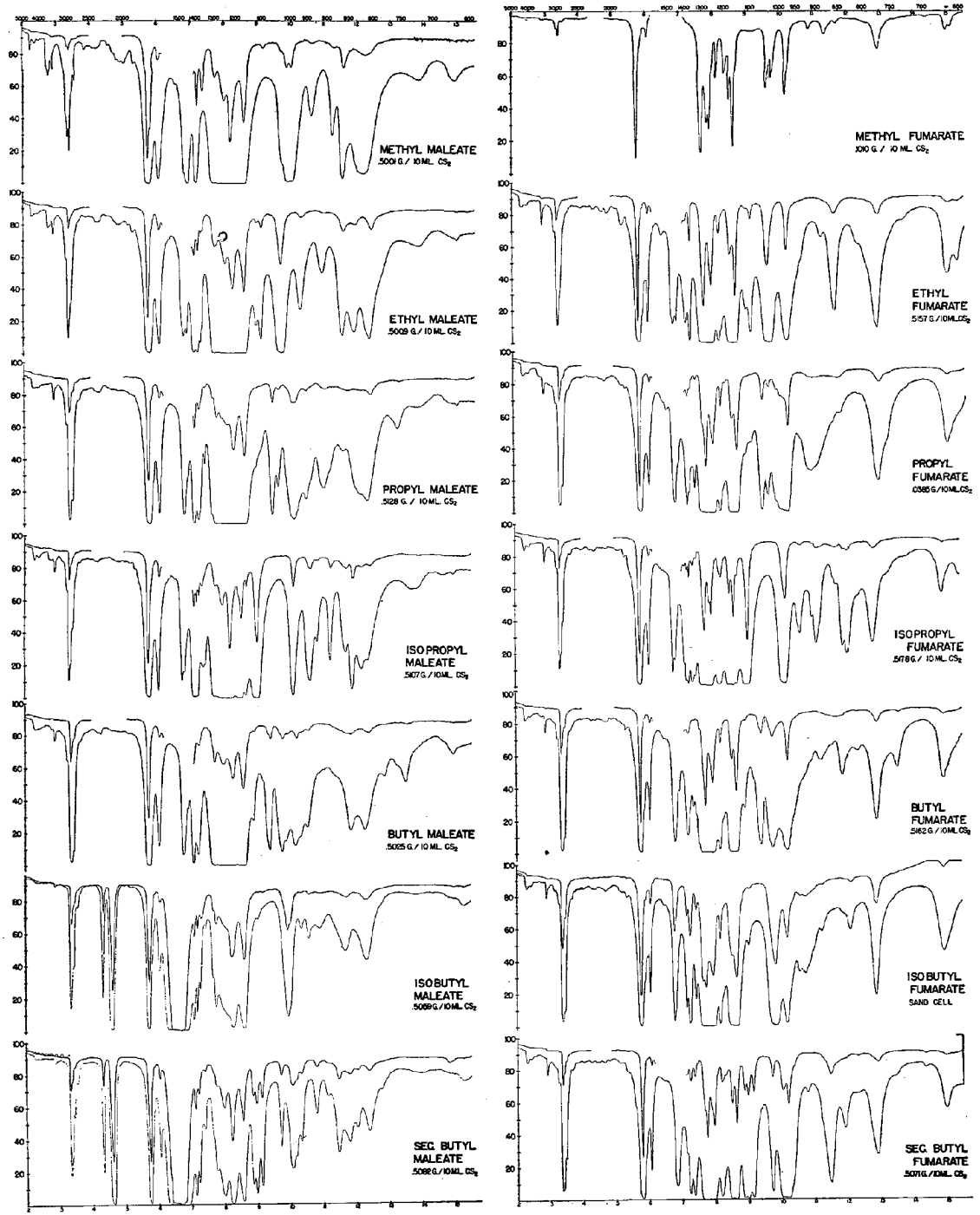
METHYL MALEATE
.5001 G. / 10 ML. CS2
ETHYL MALEATE
.5009 G. / 10 ML. CS2
PROPYL MALEATE
.5128 G. / 10 ML. CS2
ISOPROPYL MALEATE
.5107 G. / 10 ML. CS2
BUTYL MALEATE
.5025 G. / 10 ML. CS2
ISOBUTYL MALEATE
.5059 G. / 10 ML. CS2
SEC. BUTYL MALEATE
.5082 G. / 10 ML. CS2
METHYL FUMARATE
.1010 G. / 10 ML. CS2
ETHYL FUMARATE
.5157 G. / 10 ML. CS2
PROPYL FUMARATE
.0385 G. / 10 ML. CS2
ISOPROPYL FUMARATE
.5786 G. / 10 ML. CS2
BUTYL FUMARATE
.5162 G. / 10 ML. CS2
ISOBUTYL FUMARATE
SAND CELL
SEC. BUTYL FUMARATE
.5071 G. / 10 ML. CS2

Figure 2

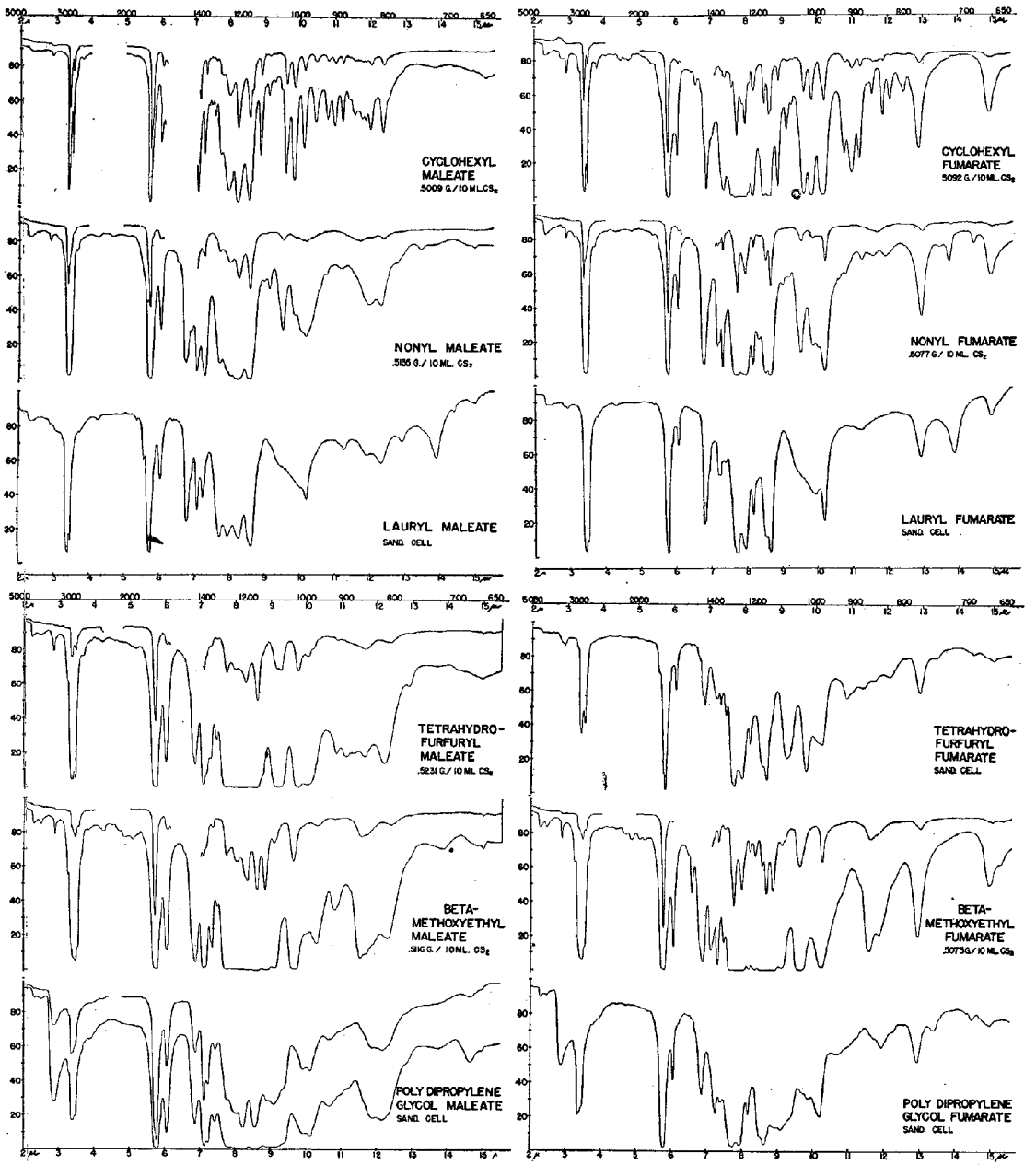
CYCLOHEXYL MALEATE
.5009 G./10 ML. CS_2
CYCLOHEXYL FUMARATE
.5092 G./10 ML. CS_2
NONYL MALEATE
.5136 G./10 ML. CS_2
NONYL FUMARATE
.5077 G./10 ML. CS_2
LAURYL MALEATE
SAND. CELL
LAURYL FUMARATE
SAND. CELL
TETRAHYDRO-FURFURYL MALEATE
.5231 G./10 ML CS_2
TETRAHYDRO-FURFURYL FUMARATE
SAND. CELL
BETA-METHOXYETHYL MALEATE
.5116 G./10 ML. CS_2
BETA-METHOXYETHYL FUMARATE
.5073 G./10 ML CS_2
POLY DIPROPYLENE GLYCOL MALEATE
SAND. CELL
POLY DIPROPYLENE GLYCOL FUMARATE
SAND. CELL

Figure 3

Table I. Characteristic Fumarate Absorptions

Ester	Absorbances 10.2	12.9	15.0	Half Density Widths, μ 10.2	12.9	15.0	Relative Integrated Intensities 10.2	12.9	15.0
Methyl	3.10	1.00	0.51	0.10	0.17	0.13	1.83	1.00	0.39
Ethyl	3.56	1.00	0.28	0.10	0.23	0.33	1.55	1.00	0.40
n-Propyl	5.35	1.00	0.21	0.11	0.32	0.30	1.85	1.00	0.26
Isopropyl	8.15	1.00	0.36	0.16	0.23	0.21	5.66	1.00	0.33
n-Butyl	4.67	1.00	0.55	0.09	0.18	0.41	2.33	1.00	0.125
sec-Butyl	7.4	1.00	0.36	0.12	0.30	0.25	2.96	1.00	0.30
Isobutyl	1.57	1.00	0.45	0.14	0.19	0.43	1.16	1.00	1.02
Cyclohexyl	5.7	1.00	0.54	0.13	0.18	0.30	4.12	1.00	0.90
Nonyl	4.7	1.00	0.40	0.08	0.20	0.30	1.88	1.00	0.60
Lauryl	2.8	1.00	0.45	0.12	0.22	0.30	1.53	1.00	0.61

Table II. Physical Properties of Experimental Materials

Alcohol or Glycol Group	M.P., ° C.	B.P., ° C.	n_D
Maleates			
Methyl	...	...	1.4390/27
Ethyl	...	...	1.4374/27
Propyl	...	102–3/3	1.4404/26
Isopropyl	...	...	1.4342/27
Butyl	...	...	...
sec-Butyl	...	90/1	1.4391/26
Isobutyl	...	103/2	1.4393/27
Cyclohexyl	86	...	...
Nonyl[a]	...	190/2	1.4518/27
Lauryl	...	...	1.4434/27
Tetrahydrofurfury	...	...	...
β-Methoxyethyl	...	...	...
Dipropylene[b]	...	...	1.4815/27
Fumarates			
Methyl	103	...	...
Ethyl	1.6	...	1.4380/27
Propyl	...	...	1.4380/27
Isopropyl	2.1	208/760	...
Butyl	−13.5	...	1.4450/27
sec-Butyl	...	...	1.4396/27
Isobutyl	...	...	1.4408/27
Cyclohexyl	35–36	...	1.4891/27[c]
Nonyl[a]	...	...	1.4540/27
Lauryl	32–33	...	1.4508/27[c]
Tetrahydrofurfuryl	...	...	...
β-Methoxyethyl	...	...	1.4541/27
Dipropylene	...	...	1.4842/27

[a] 3,5,5-Trimethylhexyl.
[b] From commercial dipropylene glycol.
[c] On supercooled melt.

maleate were run on the same solution, the less intense in the usual 0.0268-mm. cell and the more intense in the cell of about 0.165 mm. The spectra of lauryl maleate, poly(dipropylene glycol maleate), lauryl fumarate, tetrahydrofurfuryl fumarate, and poly(dipropylene glycol fumarate) were run by the "sandwich" technique (film of unknown thickness between optical salt blocks).

Some of the compounds studied were obtained as commercial materials; others were prepared from maleic anhydride and the appropriate alcohol by azeotropically removing the water liberated when 50% excess of alcohol (over theory) and anhydride were heated with toluenesulfonic acid and benzene. Isomerization of maleates to fumarates was found to be essentially quantitative on heating at 200° C. for 1 hour in the presence of a trace of iodine. Sealed tubes were used. After the tube was opened, the mixture was heated to about 180° C. with sparging with nitrogen to remove the iodine and yield colorless fumarate product in essentially quantitative yield. In general, purification procedures applied to these products resulted in negligible changes in their infrared spectra.

In Table II are given data on the materials used for the spectra in Figures 2 and 3.

The presence of some minor impurities is evident from the spectra of lauryl maleate (in which traces of maleic anhydride and lauryl fumarate are evident) and tetrahydrofurfuryl maleate (in which a trace of tetrahydrofurfuryl fumarate is evident).

In the preparation of poly(dipropylene glycol maleate), toluene was employed as azeotroping agent and the temperature in the esterifying mixture was maintained below 125° C. (Thiophene-containing benzene has been avoided in these experiments because of the known catalytic effect of sulfur compounds on the isomerization of maleates.)

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Systematic Approaches to Continuous Infrared Analyzer Sensitization

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Several years' experience with sensitization of negative, nondispersive-type infrared analyzers for the continuous analysis of field sample streams has led to the formulation of a system by which a satisfactorily compensated cell filling may be readily determined. The basis of the system lies in the logarithmic relationship between the concentration of a gas in a compensator cell and the analyzer response to that gas in the sample cell. The problem is complicated by component inconsistencies of the analyzers and by pressure-broadening effects. Pressure-broadening effects appear to be a severe limitation to the application of infrared analyzers to many sample streams.

IN RECENT years there has been an increased demand by industry for instruments that can continuously determine a specific component of a sample stream at the point of operation. The infrared analyzers are at present the major analytical instrument for process analysis. This is principally because they are more versatile than analyzers based on such techniques as thermal conductivity, density, and pressure. There is, however, the problem of sensitizing the infrared analyzer in such manner that it is sensitive only to a specific gas in a background of other similar infrared-absorbing gases. Several methods of obtaining such a sensitization have been developed for the negative, nondispersive (*2*) and the selective detector types of analyzers (*5*). A method which has proved successful for sensitizing the negative, non-

dispersive-type analyzer and which should be applicable to the selective detector type has been developed.

Figure 1 is a schematic diagram of a nondispersive infrared analyzer. The energy beam passes through the interference cell and the sample cell, and is then split, with one part passing through the filter cell and the other through the compensator cell. The interference, filter, and compensator cells are permanently filled and sealed. This arrangement provides two paths of infrared energy through the analyzer. The objective is to make one of these paths insensitive to absorption by a certain compound, while the other path is left sensitive to this absorption.

Table I. Composition of Sample Stream

(Infrared analyzer sensitized specifically for ethylene)

Component	Concentration Range, %
CH_4	28 ± 4
C_2H_4	27 ± 7
C_2H_6	5.5 ± 2
C_3H_6	15 ± 3
C_3H_8	17 ± 6

The effects of change in source output or of other absorbing compounds in the sample are made equal between the two paths (4).

Assume that an analyzer is to be sensitized for methane in the presence of ammonia. The filter cell will be filled with methane. This will remove essentially all radiation absorbed by methane in the sample gas. The energy beam which passes through the compensator cell is to be left sensitive to methane absorption in the sample stream. Varying concentrations of methane in the sample will cause varying absorption of the energy of the compensator beam and will result in changes of energy balance proportional to the methane concentration in the sample. The interference cell will be filled with ammonia to remove essentially all radiation absorbed by ammonia. This will make both energy beams insensitive to ammonia in the sample stream. If the analyzer remains somewhat sensitive to ammonia after the interference cell is filled, a small amount of ammonia in the compensator cell will compensate for this interference.

The cell fillings in cases of one or two interference materials are often simple enough to be determined by a systematic trial and error approach. This method is extremely cumbersome for even moderately complex streams and a simple systematic approach is therefore necessary.

CELL FILLING DETERMINATION

The first step in systematically determining the cell filling for a specific application is to determine the concentration ranges of all infrared-absorbing components that occur in the sample. The infrared absorption spectra of these compounds should be studied and compared to the spectrum of the sensitive gas (for which the analyzer is to be sensitized) to determine the extent of overlapping absorption and the wave lengths at which this occurs. The most desirable material for the cell windows and the principal interfering components may be determined in this manner.

Windows. The common window materials—quartz, lithium fluoride, and calcium fluoride—effectively absorb all radiation of wave lengths greater than 5, 7, and 9 microns, respectively. Figure 2 shows the absorption spectra of hydrogen cyanide and methane. If an analyzer were to be sensitized for methane in the presence of hydrogen cyanide, it is immediately obvious from a study of their absorption spectra that almost all hydrogen cyanide interference would be due to its absorption in the 6.5- to 7.5-micron range. This is almost completely covered by a similar absorption range for methane. Therefore, the use of quartz windows for the cells would remove almost all hydrogen cyanide interference by simply cutting out the overlapping range.

The actual cell filling may be determined by setting up the cell assembly in the laboratory so that the gas in the various cells may be readily changed. Such an assembly was set up to determine an optimum cell filling for an analyzer sensitive to

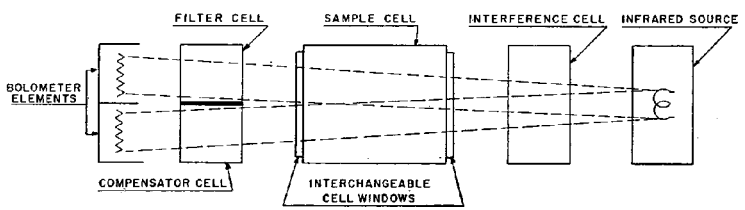

Figure 1. Schematic diagram of nondispersive infrared analyzer

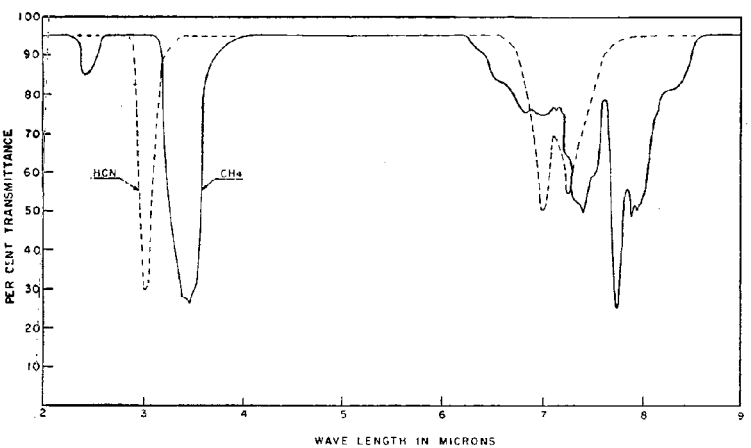

Figure 2. Absorption spectra of hydrogen cyanide and methane

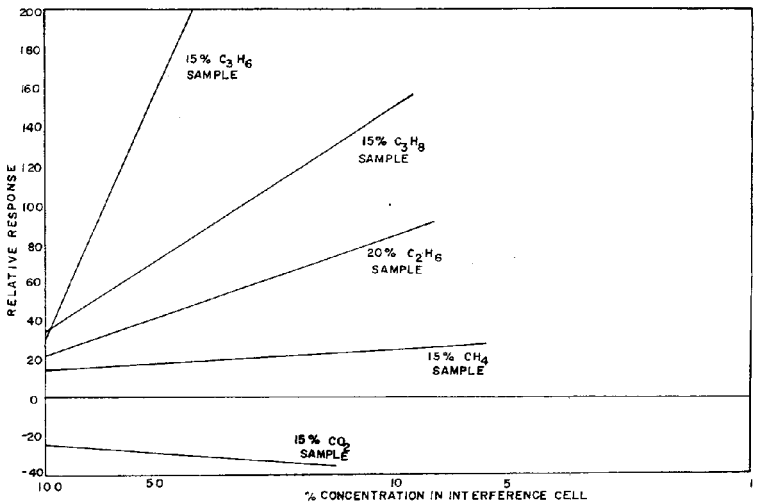

Figure 3. Effect of interference cell on gases

Analyzer sensitized for ethylene

ethylene in methane, ethane, propane, and propylene (see Table I). The interference, compensator, and filter cells were 1 inch long, with calcium fluoride windows. The sample cell was 3 inches long, with lithium fluoride windows. The problem was approached by the system outlined below and a satisfactory cell filling developed (see Table II).

Interference Cell. The filter cell is filled with the gas for which the analyzer is to be sensitized. The compensator cell is filled with nitrogen or some other nonabsorbing gas. The interference cell is set up to determine the effects of the use of this cell to remove the interference.

The concentration of a gas in the interference cell is logarithmically related to the response of the instrument to the presence of that gas in the sample cell. Each interfering gas is separately studied by plotting a series of concentrations in the interference cell against the response to an average anticipated concentration in the sample. The gases most affected by their presence in the interference cell give curves with the greatest slopes with respect to the zero axis.

Figure 3 shows the results of such an interference study of methane, ethane, propane, propylene, and carbon dioxide in an analyzer sensitized to ethylene. The positive direction of the signal is taken to be the direction of response to ethylene. The composition of the gas in the final filling of the interference cell is chosen to be either the most affected gas alone—in this case propylene—or a combination of the two or three most affected gases. In the case shown in Figure 3 the interference cell would be wasted if it were filled with methane. The other interferences would have to be removed by using the compensator or the filter cell. The filling of these cells is a great deal more exacting than the filling of the interference cell.

For the specific problem mentioned, the interference cell filling chosen was 40% (30.4 cm.) propylene and 60% (45.6 cm.) propane. Propylene and propane were chosen because they are more affected by use of the interference cell than the other interfering gases and occur in the sample in relatively large concentrations. The larger portion of propane was chosen because it occurs in larger and more variable concentrations than propylene. Such a choice is, of course, rather arbitrary.

Compensator Cell. The compensator and filter cells are used for positively and negatively responding interfering gases, respectively. The cell filling is determined by the same procedure in both cases, except that the sensitive gas is used for dilution in the filter cell and a nonabsorbing gas is used in the compensator cell.

The compensator cell filling is determined by setting up an analyzer with the sensitive gas in the filter cell and the interference cell filled as outlined above. The strongest interfering gases should be compensated first, since, because of overlapping spectra, the weaker interferences may be compensated in the process of eliminating the stronger interferences. The compensator cell is filled with a series of concentrations of each interfering gas and the response to an average sample of the interfering gas is noted.

Table II. Cell Filling for Ethylene Analyzer for Stream of Table I

Cell Filling (Partial Pressures CM. of Mercury)

Filter cell	Compensator cell	Interference cell
6.1 cm. C_2H_6	26.6 cm. C_2H_6	30.4 cm. C_2H_6
3.8 cm. C_3H_8	22.8 cm. CH_4	45.6 cm. C_3H_8
66.1 cm. C_2H_4	26.6 cm. N_2	

Results (Bridge Voltage = 1.5 volts)

	Signal, μv.
30% C_2H_4 + N_2	124
20% C_2H_4 + N_2	98
15% CH_4 + N_2	1
8% C_2H_6 + N_2	−2
25% C_3H_8 + N_2	0
25% C_3H_8 + N_2	0
20% C_2H_4 + 20% C_2H_6	...
+ 20% C_3H_6	...
+ 20% C_3H_8	...
+ N_2	99

The gas concentration in the compensator cell is logarithmically related to the response to the sample. The point at which the curve bisects the zero signal axis indicates the concentration that should compensate for the interfering gas. If the interfering gas concentration is known to vary widely, it should be studied by using more than one concentration of the synthetic test sample. This will result in a logarithmic curve for each sample concentration. Normally, these curves will intersect at the zero signal point. In some instances no one concentration in the compensator cell will reduce the interference to zero over the entire range. This is believed to be primarily due to pressure-broadening effects. These effects are usually small and a cell filling may be chosen to give negligible interference over the range of concentrations expected in the sample.

Figure 4 is a plot of the concentration of ethane in the compensator cell against the response to sample of an analyzer sensitized for ethylene. The ethane was studied at three sample concentrations; the three curves intersect at zero signal point.

Figure 5 is a plot of the concentration of carbon dioxide samples of an analyzer sensitized for ethylene. The carbon dioxide was studied at three sample concentrations, the curves for which do not intersect at the zero signal point. In this case the concentration of carbon dioxide chosen for the filter cell must produce a minimum response to varying concentrations of carbon dioxide in the sample. Figure 5 was obtained in a problem different from the ethylene sensitization problem outlined in Tables I and II, but is an example of a phenomenon occasionally encountered, where no single compensating gas concentration will reduce an interference to zero over all sample concentration ranges.

If several interfering materials are to be compensated, the concentration of each material in the compensator or filter cell is determined successively; each time with the dilution gas are included the compensation gases previously determined.

Because the compensation of a complex sample with several interfering materials is a usually delicate balance of small concentrations of materials in the compensator and filter cells, it is subject to several problems. It is unusual for a cell filling which is corrected for one analyzer to be exactly correct for a different analyzer in the same service. This is due to differences in infrared energy sources and slight variations in the light absorption characteristics of the windows employed. This is a rather minor problem, as a cell filling developed for one analyzer is nearly correct for another analyzer and can be perfected with only a little effort by slightly varying one or more components of the compensator or filter cell filling.

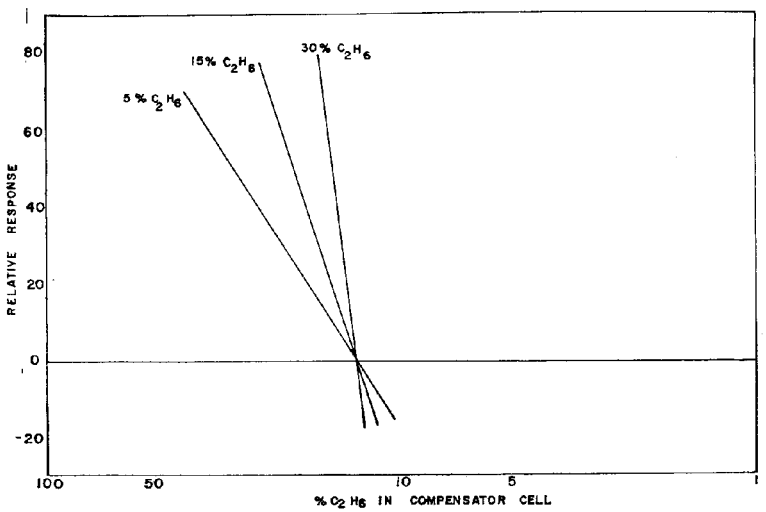

Figure 4. Ethane compensation in ethylene analyzer

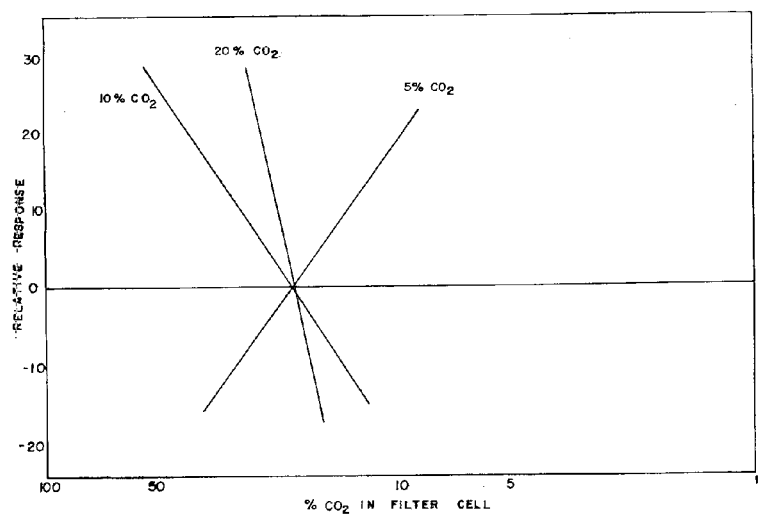

Figure 5. Carbon dioxide compensation in ethylene analyzer

PRESSURE BROADENING

The problem of pressure-broadening effects appears to be serious in many cases, especially in the low concentration applications. In view of the lack of control parameters for correcting for pressure-broadening effects, this problem may limit the application of infrared analyzers.

Lambert's law of absorption states that when a parallel beam of the monochromatic radiation of intensity I_0 enters a homogeneous absorbing material of thickness l, the radiation transmitted will have the intensity.

$$I = I_0 e^{-kl}$$

where k is the absorption coefficient of the material. Beer's law of absorption states that if the absorbing material is a mixture of several components, the absorption coefficient is given by the linear equation

$$k = c_1 e_1 + c_2 e_2 + \text{etc.}$$

where c_1, c_2, etc., are the concentrations of the various components and e_1, e_2, etc., are the extinction coefficients for these components. However, the observed absorption in many cases, particularly with lighter gases and vapors, deviates from these simple absorption laws. It may be readily demonstrated that pressure has an effect on the absorption by many materials. In the case of a material absorbing a single component, if the pressure is increased by decreasing the volume so that the quantity of absorbing material remains constant, the absorption increases as a nonlinear function of the pressure (I). Similar results may be obtained with a material absorbing several components by varying the partial pressure of a single nonabsorbing component. These deviations from the simple absorption laws caused by pressure are called pressure-broadening effects.

The pressure-broadening effects are probably caused by several factors. However, they are principally related to the mean free path of the molecule and hence appear to result from molecule collisions. The vibrations of the electrons of a molecule are interrupted or undergo a change of phase when molecules collide. The absorption spectrum is interpreted to be composed of a series of fine lines partially overlapping. These fine structural

lines are somewhat shortened and broadened by these changes in electron vibration. The over-all result is an increase in absorption as the pressure is increased (*2*).

Figure 6 shows the pressure broadening of methane caused by varying the partial pressure of nitrogen, a nonabsorbing material. The absorption bands are shorter and broader at the higher pressure. The extent of the broadening appears to vary with the partial pressure and with the type of molecule of the foreign gas. Oxygen, nitrogen, carbon monoxide, etc., cause approximately the same amount of pressure broadening of the methane spectrum, but ethylene causes a different amount of broadening at the same partial pressure (*3*).

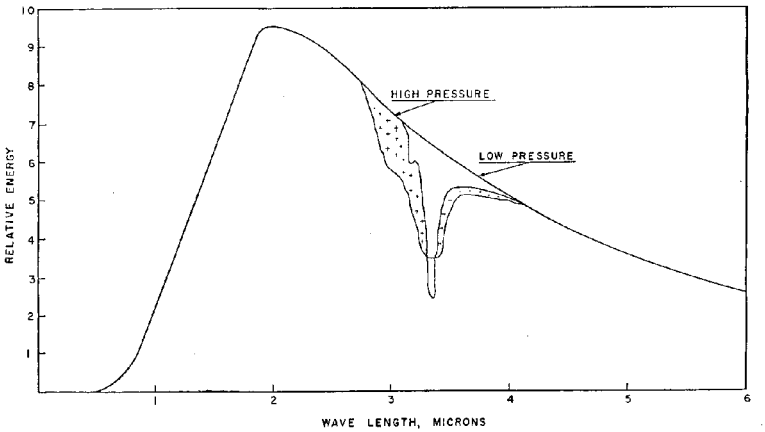

Figure 6. Energy change with methane pressure broadening

This phenomenon affects the cell filling of an infrared analyzer, because the interferences result from overlapping absorption spectra. If the absorption bands of one or more compounds are changed in shape from that for which the instrument has been set up, the instrument may well not be properly compensated, owing to an increase or decrease in the overlap of the absorption spectra.

Table III compares the data obtained with an analyzer sensitized for ethylene and compensated for ethane, propane, and propylene. When a 3-inch sample cell was used, with two extra windows in the radiation path, the analyzer did not respond to the 20% concentrations of ethane, propane, and propylene in nitrogen and gave the expected response to a sample composed of all three plus 20% ethylene. The 3-inch sample cell was replaced with two 0.6-inch sample cells arranged in series, but with the same windows used with the 3-inch cell. One of these cells filled with a pure gas should give the same results as 20% of that gas in the 3-inch cell. However, each interference gas gave a response nearly 40% that given by ethylene, the gas for which the analyzer was sensitized.

Table III. Interference Effects with Series and Single Sample Cells

Cell Fillings		
No. 1	No. 2	Response
Two 0.6-Inch Sample Cells in Series		
100% C_2H_4	100% N_2	100
100% C_2H_6	100% C_2H_6	140
100% C_3H_8	100% C_3H_8	140
100% C_2H_4	100% C_3H_8	142
Single 3-Inch Sample Cell, with Blends		
Sample Blend		
20% C_2H_4 + N_2		98
20% C_2H_6 + N_2		0
20% C_3H_8 + N_2		0
20% C_2H_4 + 20% C_2H_6 + 20% C_3H_8 + N_2		0
20% C_2H_4 + 20% C_2H_6 + 20% C_3H_8 + N_2		99

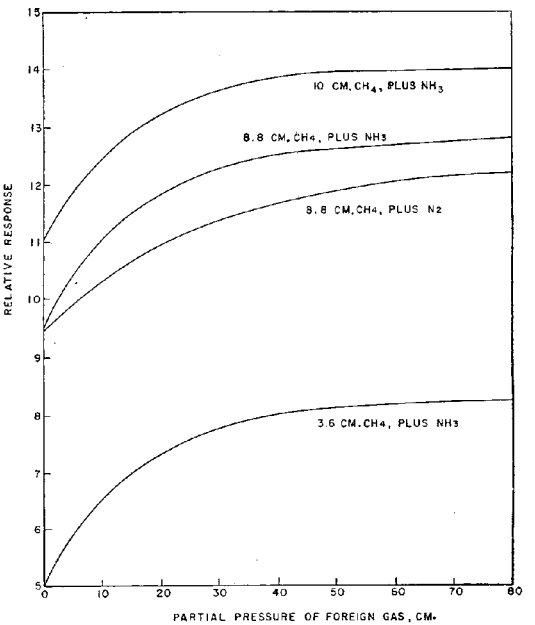

Figure 7. Methane pressure broadening

Figure 7 was obtained with an analyzer sensitized for methane and insensitive to ammonia in all concentrations. Each curve represents one partial pressure of methane held constant while the partial pressure of a foreign gas—in this case ammonia or nitrogen—is varied over a range of about 70 cm. of mercury pressure. The partial pressure of the foreign gas is plotted against the relative response. The greatest changes in response are in the lower ranges, with the response apparently leveling out at about 0.5 atm. Nitrogen causes only about 80% as much change as ammonia.

Figure 8 shows the pressure-broadening effects of nitrogen on six common gases. These effects appear to diminish with higher molecular weights for molecules of similar configuration. The pressure broadening does not appear to be related to degree of saturation of a hydrocarbon, as evidenced by the similar curves for acetylene, ethylene, and ethane. The greatest effects are at low concentrations.

Because the pressure-broadening effects tend to become constant as the partial pressure becomes higher, it is probable that a cell may be filled and operated at a pressure sufficiently high to make the pressure-broadening effects essentially con-

stant. This appears to be the best way to reduce the pressure-broadening compensation problem to a point where it can be solved.

CONCLUSION

A systematic approach has been developed for determining a fully compensated infrared analyzer cell filling. As one becomes skilled in the art, refinements in techniques will improve the approach. However, at best several days' work are often required to achieve a fully compensated cell filling. This same filling may be used again without refinement in the same analyzer cell at a later time, unless a window or the source has been changed.

Pressure broadening is important, particularly in applications requiring high sensitivity and accuracy. This may well prove to be a limiting factor in the application of infrared analyzers.

In general, the method outlined gives dependable cell fillings. It grows more difficult with additional interfering materials and a stream of a dozen interfering compounds might be very difficult to adapt to an analyzer. For the average stream of two to six interferences, however, this approach permits the determining of a reasonably compensated cell filling in a comparatively short time.

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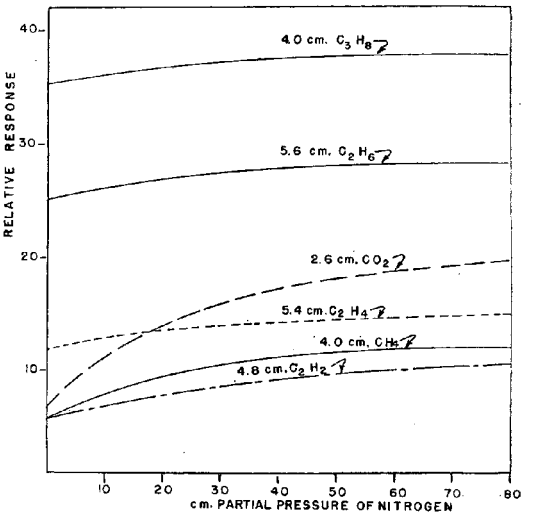

Figure 8. Pressure broadening of several gases

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Modification of Bolometer and Bolometer Circuits of an Infrared Analyzer

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Field experience with a negative-type process-monitor infrared analyzer indicated that improved performance was desirable. The detector is a two-element bolometer operated in an electrically balanced bridge. Improved temperature stability has been achieved by making the two elements very nearly identical and matching thermal properties at assembly. The factors to consider are element heating due to bridge current and source radiation and heat losses due to conduction, convection, and radiation. Changes in construction and mounting of the elements have increased sensitivity to sample gas. As the bridge supply voltage is 60-cycle, the recorder amplifier is tuned to this frequency and will respond to 60-cycle pickup. The noise due to pickup signals has been reduced by addition of shielding to the bolometer bridge circuit. The transmission line between the analyzer and the recorder is a part of the bridge network and thus in the low level signal circuit. The two leads of the amplifier input are particularly critical. Relatively small changes in reactive impedance between the leads or to ground unbalance the bolometer bridge. The effect is eliminated by inserting an isolation transformer into this circuit adjacent to the bolometer. The changes have materially improved the accuracy and usefulness of the more sensitive analyzers.

THE use of infrared analyzers in monitoring process streams has increased rapidly in the past few years (*2*), accompanied by the demand for more accurate and sensitive analyzers. While the basic principles are the same for the process stream and the laboratory, the practical requirements are different—in plant use stability and trouble-free operation are paramount, and versatility is of relatively less value.

One of the first commercial analyzers designed primarily for plant process use was of the nondispersive, negative-filter type. The block diagram of the optical system of this analyzer is shown in Figure 1. Radiation from the infrared source passes through the interference cell and the sample cell, and then is split so that parallel beams pass through the filter and the compensator cells to the bolometer elements (*1*). This analyzer, the Baird-BJI, has been successfully applied to many applications. For streams in which a typical infrared-adsorbing gas is present in concentrations greater than 5%, the operation is usually satisfactory. In the range 1 to 5% success depends on the interfering gases present, and for full scale response of 1% or less the accuracy is seriously impaired. It appeared that the bolometer was the limiting factor and the signal-to-noise ratio, and hence the ultimate usable sensitivity, could be greatly improved by changes in this part of the system.

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BOLOMETER

The bolometer of this analyzer consists of a pair of temperature-sensitive elements connected electrically with two precision resistors into a bridge circuit. The bolometer elements are made of nickel wire wound on an insulating form and are placed so that each element receives radiation from one of the split-beam paths. A change in gas in the sample cell results in a relative change in the energy received by the bolometer elements and, consequently, a resistive unbalance of the bridge. This unbalance is a measure of the change of sensitized gas in the sample cell.

The limitation of this type of bolometer detector is its response to ambient temperature change. Ideally, if the two elements are identical in construction and mounting, there will be no such response to uniform temperature changes. The bolometer element is heated above its surrounding by energy received in two different ways. The radiation from the source delivers about 0.1 cal. per second to each bolometer, with a resultant temperature rise of about 10° C. The electrical power supplied by the bridge voltage is 0.002 cal. per second and contributes another few tenths of a degree temperature rise. With mechanical shutters the source radiation in the two paths can be closely balanced and by matching resistances of the two bolometer elements the electrical power can be made equal. The heat loss parameters are considerably more complex. The general forms of the equations of heat transfer for support conduction, air conduction, convection, and radiation are as follows:

Support Conduction

$$\frac{\Delta Q}{\Delta t} = \frac{kA\Delta T}{l} \qquad 0.2 \times 10^{-3} \text{ cal./second} \qquad (1)$$

Air Conduction

$$\frac{\Delta Q}{\Delta t} = \frac{kA\Delta T}{l} \qquad 0.5 \times 10^{-3} \text{ cal./second} \qquad (2)$$

Convection

$$\frac{\Delta Q}{\Delta t} = hA\Delta T \qquad 10^{-5} \text{ cal./second} \qquad (3)$$

Radiation

$$\frac{\Delta Q}{\Delta t} = A_3\epsilon\sigma(T^4 - T_0^4) \qquad 0.2 \times 10^{-3} \text{ cal./second} \qquad (4)$$

$$\simeq A_3 g \Delta T$$

For small temperature differences, all equations can be written as the product of a geometry factor, a heat coefficient and temperature difference. The geometry factor is the area, A, divided by the length, l, for conduction, or only the area for convection and radiation. The coefficients k, h, and g—appropriate for the respective heat loss processes—are not independent of temperature, but over a moderate range may be treated as such.

Because all the equations are linear with temperature difference, ΔT, two important conclusions may be drawn: The adjustment for balancing the sum of the three heat loss parameters can be made with any one of them, and at temperatures other than the final operating temperature difference. The rate of heat flow for a typical bolometer with $\Delta T = 1°$ C. is shown and indicates that convection losses are small, while the other three processes are of approximately equal importance.

Another quantity of interest is the thermal time constant, which in analogy with electricity is the total heat capacity divided by the total rate of heat loss. A typical value for a bolometer element is 10 seconds; any appreciable drift lasting more than 1 minute after changing power level into bolometer elements is due to secondary effects such as strain relief.

The original bolometer elements in this analyzer consisted of separate coils of nickel wire wound on an insulating strip. The new elements are different, in that the nickel wire is wound on a cylindrical metal foil form. The best operation was obtained when gold foil was used, although aluminum was also satisfactory.

The foil cylinder is supported on a spindle and a layer of insulating varnish is applied. Before the varnish is completely dry, the nickel wire is wound in a single layer, with care not to strain the wire. Another coat of insulating varnish is applied and allowed to dust dry. As shown in Figure 2, the foil cylinder is pressed flat onto two support wires. Then the element is mounted in a rigid frame and can be handled readily. The electrical resistances are matched in pairs by selection and finally by stripping small lengths of wire from one element, so that in a 1300-ohm element the difference is less than 0.5 ohm. The insulating varnish is very important, as the original enamel insulation on the wire cannot be relied upon to maintain the high resistance to the support form and between adjacent turns which is essential. A final clear varnish layer and a coat of dull black lacquer complete the element. All paint layers are thin and as uniform as possible.

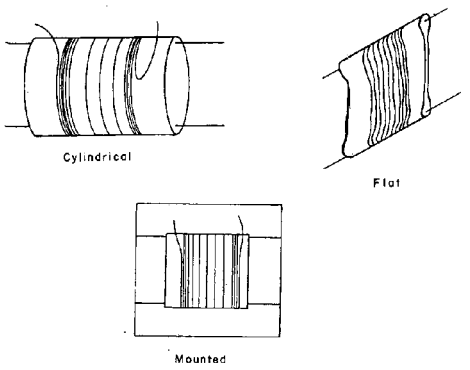

Figure 2. Steps for bolometer element fabrication

The completed elements are mounted with a conical light-gathering block and a thermal balance block, as shown in Figure 3. The entire unit is surrounded by a copper band and finally a copper cover to assure uniform temperature conditions at the element location. To achieve the final thermal balance of the bolometer, the air conduction term of heat loss is varied. The thermal balance screws behind the bolometer elements are adjusted so that no change in signal results from a change in bridge voltage. As the resistances have been previously matched, this is an adjustment to give equal thermal response to equal power changes. One indication of the uniformity of construction is that only very slight differences in thermal balance screw positions are necessary to achieve this final balance.

The sensitivity of these bolometers to change in sample gas concentration is about twice that of the original bolometer supplied with the analyzer. This is due largely to the elimination of the insulating support strip and consequent use of all the nickel wire rather than only the half facing the radiation, and to the polished cones which improve the light-gathering efficiency. The improvement in thermal stability is more difficult to measure.

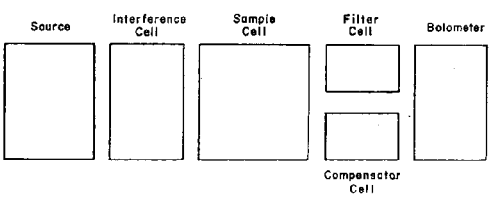

Figure 1. Schematic diagram of nondispersive negative-filter infrared analyzer

Based on both laboratory tests and field experience, a conservative estimate of the average improvement over the original bolometer is a factor of 2.

BOLOMETER CIRCUIT

The electrical circuit for the bolometer is a conventional bridge with 60-cycle alternating current as the bridge supply voltage. Figure 4 shows that any unbalance in the bridge is fed to the recorder amplifier and then to the slide-wire motor. The slide-wire introduces a resistance change which rebalances the bridge.

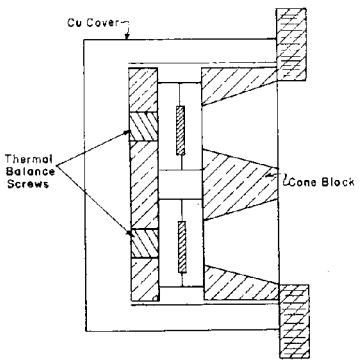

Figure 3. Assembled bolometer

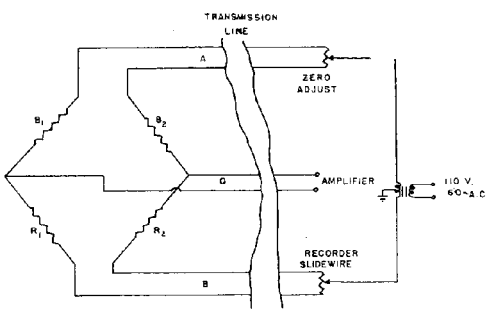

Figure 4. Bridge circuit of bolometer

This balanced bridge or closed loop operation makes the analyzer insensitive to changes in gain and aids materially the long-term stability of the analyzer. However, in this particular system two features present serious circuit problems. One arises from the operation of the bridge on 60-cycle voltage. Any pickup from nearby electrical apparatus or circuits appear to the amplifier as an unbalance of the bolometer bridge. The full scale deflection of the recorder corresponds to a voltage change of about 0.5 mv. Thus to keep these effects less than 1% of full scale of the recorder, the pickup voltages must be less than 5 μv. To reduce the pickup in the bolometer circuit, the terminal strips in the analyzer case and in the recorder have been replaced by Amphenol connectors. In the recorder, the six leads of the bolometer circuit were shielded in pairs and kept to a minimum length. The result was a noticeable reduction in signal due to nearby 60-cycle circuits.

The other circuit problem arises from the separation of the bolometer and the recorder with the resulting lengthy transmission line in the low signal level circuit. Measurements on such a system show that a change in capacity from either lead at C, in Figure 5, to ground will unbalance the bolometer bridge. Because the leads at A and B are very close to ground through the center tap of the supply transformer, the capacity change at C adds an impedance in parallel with the bridge element. A 200-$\mu\mu$f. capacity change in this location gives a bridge unbalance equivalent to full scale recorder response. A typical capacity to ground of a shielded lead in this circuit is 0.1 μf., so that only 0.002% change in line capacity gives a zero drift of 1% full scale. In some of the more sensitive analyzers, the change of capacity of the transmission line with atmospheric temperature change was large enough to give a very troublesome zero drift.

The importance of line capacity to ground also shows up in the operation of the so-called "hum signal." This is a voltage out of phase with the bridge supply that is introduced into the amplifier input circuit. Some out of phase 60-cycle will be present, owing to pickup, and the amplitude of the hum signal is varied to obtain a complete null to the amplifier. In the different plant analyzer installations it was observed that variations in the amplitude of hum signal required were too wide to be explained on the basis of 60-cycle pickup. The high capacity in the lead line introduces a phase shift into 60-cycle signal, as seen by the amplifier, and it is the out of phase component that must be balanced with the hum signal.

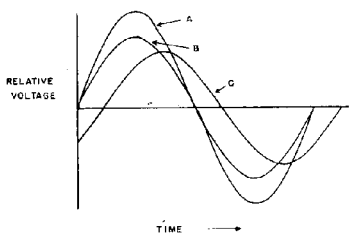

Figure 5. Bridge unbalance voltage

A. Input voltage
B. Amplitude change only
C. Amplitude and phase change

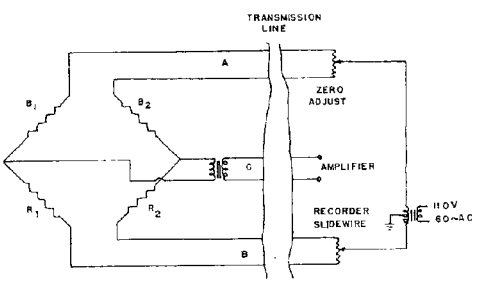

Figure 6. Bolometer bridge circuit with isolation transformer

A solution to this capacity difficulty has been incorporated into several analyzers. As shown in Figure 6, this consists of adding a special isolation transformer to the amplifier input circuit, located adjacent to the bolometer. This removes the most critical pair of transmission lines from the bridge circuit and puts them in a circuit which is very insensitive to capacity or resistance changes. In every case to which this transformer has been

added, the zero drifts with atmospheric temperature were eliminated and the hum signal required was very small. Thec haracteristics of a transformer (supplied by the Southwestern Industrial Electronics Co., Houston, Tex.) for this purpose are: approximate matching of circuit impedances, electrostatic shielding, and excellent electromagnetic shielding.

CONCLUSION

The bolometers described improve the signal-to-noise ratio by about a factor of 4. Modifying the bolometer circuit brings the over-all signal-to-noise ratio improvement to about a factor of 10. This is significant and in many cases has materially improved the usefulness of an analyzer.

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Identification of Some Polynuclear Aromatic Hydrocarbons in the Atmosphere

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The separation and identification of individual aromatic hydrocarbons in a mixture of air contaminants are described for an investigation that was carried out on samples of air-borne dust collected in Windsor as part of the Windsor-Detroit air pollution investigation. Samples were collected on fiber glass filters or from the air intake of an air-conditioning system, depending on the size of sample required. The separation of single aromatic hydrocarbons from other interfering compounds was effected by extracting the sample with organic solvents and fractionating the extract by chromatographic adsorption analysis. The identification of the hydrocarbons in the small fractions of eluate was made by measuring the ultraviolet absorption. The polynuclear aromatics thus far identified in this way comprise pyrene, fluoranthene, benz[a]anthracene, chrysène, and benzo[e]pyrene.

ENVIRONMENTAL and health aspects of air pollution in the greater Windsor-Detroit area have been studied for a number of years under the terms of an international reference. The nature and scope of this problem have been discussed by Katz (*11, 12*) and information on the composition of inorganic particulate pollutants has been presented (*16*). However, the composition of the organic material is largely unknown because of its complexity.

These organic constituents of atmospheric pollution have been receiving increasing attention in recent years because of their importance as possibly harmful agents from a health standpoint. In the Los Angeles study it has been established that hydrocarbons represent one of the largest single groups of pollutants discharged into the atmosphere; a number of saturated and unsaturated hydrocarbons in the C_2 to C_{10} range have been individually determined by mass spectrometer analyses (*15*).

The condensed polycyclic aromatic hydrocarbons in the organic fraction have attracted widespread interest because it is known that some of these compounds are potent carcinogens and their presence as pollutants of city air has been associated by some workers (*13*) with the increase in the incidence of lung cancer. Benzo[a]pyrene was detected in domestic soot by Goulden and Tipler (*7*), who utilized a method of separation by chromatographic adsorption and subsequent identification by fluorescence spectrography as described by Berenblum and Schoental (*1*). In a similar study (*18*) the presence of this carcinogen in the air of a number of English towns was demonstrated. The utilization of ultraviolet spectroscopy permitted the extension of the analysis of city air to include a number of other polynuclear hydrocarbons (*4, 14*), because these substances possess distinctive and sufficiently different absorption spectra to permit identification by this means.

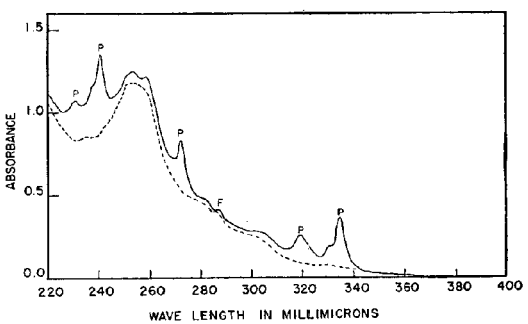

Figure 1. Ultraviolet absorption spectrum of fraction containing pyrene

P. Characteristic peaks for pyrene
F. Slight peak for fluoranthrene
– – – – Absorbance of pyrene-containing fraction of eluate minus absorbance of pure pyrene in cyclohexane

The present investigation was undertaken to identify individual condensed ring polycyclic aromatic hydrocarbons present in the Windsor atmosphere.

REAGENTS AND APPARATUS

Aluminum oxide (Merck & Co., Inc.) suitable for chromatographic adsorption was used for most of the chromatograms. Silica gel (28 to 200 mesh) was also used but appeared to give an inferior separation of the aromatic fractions. This separation might have been improved, however, by suitably adjusting the conditions. The activity of the alumina in the weakly active columns was grade IV as determined by the method of Brockmann and Schodder (*2*). The adsorbent was activated by

heating in an oven at 110° C. for 24 hours and cooling in a desiccator to produce an alumina of activity II for the separation of the aliphatic compounds.

A grade of cyclohexane was used which showed a transmittance greater than 90% in the spectral region from 2260 to 4000 A.

At first the collection of fractions from the chromatographic column was manipulated by hand, but in later experiments the use of a Shandon automatic fraction collector greatly facilitated the collection of the large numbers of fractions required for the analysis.

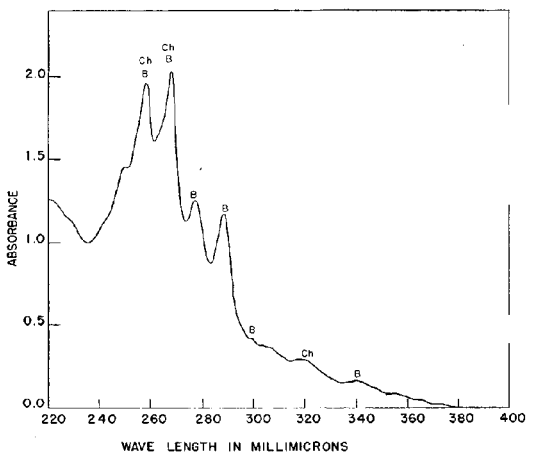

Figure 2. Ultraviolet absorption spectrum of fraction containing benz[a]anthracene and chrysene

B. Characteristic peaks for benz[a]anthracene
Ch. Characteristic peaks for chrysene

All the ultraviolet absorption measurements were made on a Cary automatic recording spectrophotometer with two fused silica cells of 1-cm. length.

EXPERIMENTAL

Most samples consisted of the total particulate matter removed from approximately 2500 cubic meters of air during a 20-hour period by a high volume sampler adapted to use flat sheets of a flash-fired fiber glass filter medium. Larger samples comprised material collected over a period of 2 months on fiber glass filters at the air intake of an air-conditioning unit, which was located in a high pollution area and had a capacity of 340 cubic meters of air per hour.

Samples of approximately 2 grams were extracted with chloroform and the extract was transferred to 20 cc. of cyclohexane. The loss in weight of the sample on extraction was approximately 5%, but this was partly accounted for by small particles of carbon which penetrated the Soxhlet thimble.

The chromatographic column was prepared by filling a tube (11 × 400 mm.) to a depth of 200 mm. with alumina poured as a slurry in cyclohexane. The extract was added and, when it had been adsorbed, the column was developed with cyclohexane. On the developed column four bands could be distinguished in ultraviolet light. These consisted of: band 1, a colorless band at the bottom of the column which showed a very faint blue fluorescence; band 2, a colorless band which showed a strong blue-violet fluorescence; band 3, a yellow band possessing green fluorescence; and band 4, a dark brown band at the top of the column.

The column was eluted with cyclohexane. Fractions of 4 cc. were collected and the absorption of each was measured in the spectral region from 2200 to 4000 A. When the ultraviolet absorption indicated that very little material was being eluted, the eluent was changed to a mixture of benzene and cyclohexane (1 to 1) and additional 4-cc. fractions were collected, in the course of which the yellow band was completely eluted. The separation was slightly improved by rechromatographing on small columns (8 × 65 mm.) individual fractions of particular interest. For each extraction 50 to 100 fractions were collected.

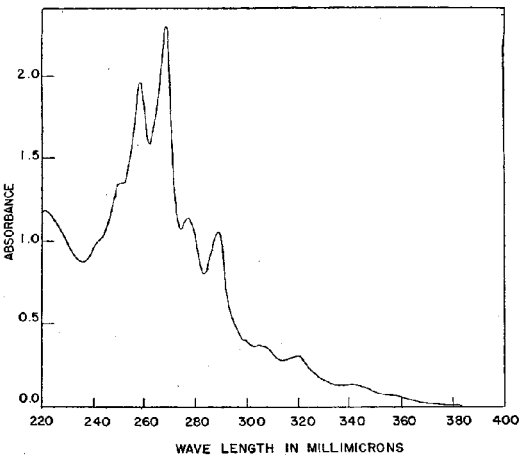

Figure 3. Ultraviolet absorption spectrum of fraction showing increasing chrysene concentration

To improve the purity of the fractions in later experiments the extract was first adsorbed on a column prepared from strongly active alumina and washed with cyclohexane until no further material was eluted. The alumina was then extruded from the tube and the aromatics were extracted from it with chloroform. This solution was then rechromatographed on weakly active alumina.

The ultraviolet absorption spectra of the fractions were compared where possible with standards of pure hydrocarbons in cyclohexane. Where specimens were not available reference was made to a collection of spectra of aromatic compounds (*6*).

The analysis is greatly aided by a knowledge of the order of adsorption on alumina of these polynuclear hydrocarbons. This was elucidated by Winterstein (*20*), who separated pairs of known compounds on alumina by elution with petroleum ether, and by Wedgwood and Cooper (*19*), who determined the following order of adsorption of hydrocarbons from cyclohexane solution onto alumina: naphthalene, acenaphthene, 9,10-dihydroanthracene, fluorene, phenanthrene, anthracene, pyrene, fluoranthene, benz[*a*]anthracene, chrysene, naphthacene and perylene, benzo[*a*]pyrene and benzo[*ghi*]perylene, anthanthrene, and coronene.

RESULTS

The early fractions from the chromatogram show the ultraviolet absorption characteristic of substituted benzenes, but are not sufficiently specific to permit identification. The first fraction to be identified is that containing pyrene (Figure 1). The five absorption maxima at 231, 241, 273, 319, and 335 mμ are clearly indicated although superimposed on a curve of interfering absorbance. A trace of fluoranthene is present in this fraction as indicated by the small band at 288 mμ. The absorption peaks of this and other pyrene fractions all agree to within 5 A. with the spectrum obtained from a sample of pure pyrene in cyclohexane. In an analysis of this nature there is insufficient material to permit derivative formation or melting point determinations of purified material, so that, although it is possible that the fractions identified as pyrene may contain some alkyl pyrenes, this is unlikely because of the absence of a bathochromic shift associated with these compounds (*5*, *6*, *9*). The three strongest bands of fluoranthene are at 236, 276, and 288 mμ in cyclohexane solution; these increase in succeeding fractions while the pyrene rapidly decreases in concentration. No spectra of alkyl derivatives of fluoranthene were available for comparison but there was no evidence of a bathochromic shift in the recorded peaks.

The concentration of fluoranthene then decreases and benz[*a*]-anthracene appears in the eluate. There is a little chrysene

present with the benz[*a*]anthracene and these two do not separate well (Figure 2). However, the gradually increasing concentration of chrysene and decreasing concentration of benz[*a*]-anthracene is apparent from Figure 3, which is the absorption curve of the immediately succeeding fraction of eluate. It will be observed that the absorption at 268 mμ, the strongest band of chrysene, is increasing while the absorption at 288 mμ, the strongest band of benz[*a*]anthracene, is decreasing. Rechromatographing these fractions on a small column, however, failed to separate the two components. Of all the alkyl derivatives of benz[*a*]anthracene only the 1-methyl compound has a bathochromatic shift of less than 10 A. for the absorption maximum at 288 mμ (*6*, *10*). In the spectrum of the material from the chromatogram this peak suffers no interference from the chrysene and corresponds exactly in wave length to that obtained from a sample of pure benz[*a*]anthracene in cyclohexane measured under the same experimental conditions. Similarly the spectra of alkyl chrysenes available for comparison all indicate fairly large bathochromic shifts, especially for the band at 320 mμ (*6*). None of these was observed in the spectra of the main chrysene fractions, all the peaks of which corresponded very closely with those of pure chrysene. Therefore, it seems reasonable to assume that the spectra recorded here refer to the parent hydrocarbons.

Succeeding fractions possessed absorption bands at 237, 277, 289, 317, and 332 mμ which correspond to the absorption maxima of benzo[*e*]pyrene. There was an indication of the presence of benzo[*a*]pyrene, but the separation was not sharp enough to permit unambiguous identification of this compound.

DISCUSSION

Band 1 on the chromatogram can be eluted with cyclohexane from alumina of high activity and would be expected to contain the paraffins, naphthenes, and olefins in the sample, as these compounds are not strongly adsorbed on alumina. The ultraviolet absorption spectra of the early fractions show in many cases a large absorption maximum around 220 mμ and a small amount of absorption between 250 and 280 mμ, indicating that some conjugated diolefins or benzene derivatives are also present.

Band 2 comprises the polynuclear condensed ring aromatic hydrocarbons, some of which have been identified.

Band 3 may consist of higher members of the series containing five and six condensed rings. The absorption throughout the whole spectral range of the ultraviolet is extremely strong for fractions from this band, but it is for the most part less distinctive than that of fractions from Band 2 and no clear-cut identifications have been made thus far.

Band 4 will be expected to contain any nitrogen or oxygen compounds together with any acids, phenols, or bases which have been extracted from the dust.

By subtracting the absorbance of a solution of pure pyrene in cyclohexane from the absorbance of the pyrene-containing fraction of eluate, a difference curve was obtained. This is indicated by a broken line in Figure 1 and represents the interfering absorbance. This impurity was not removed by alkaline permanganate treatment or by rechromatographing the fractions on small columns of alumina or silica gel. The interfering absorbance persisted in this form throughout all the fractions of eluate, but in later fractions the peak appeared to move to slightly longer wave lengths. In the analysis of petroleum for benzene and naphthalene derivatives this problem of interfering compounds which cannot be removed by chemical means has also been encountered (*17*).

INFRARED DATA

A sample of dust collected from the air intake of an air conditioning unit was extracted with carbon tetrachloride to give a specimen sufficiently concentrated for infrared analysis. The spectrum obtained is shown in Figure 4,*A*. The presence of

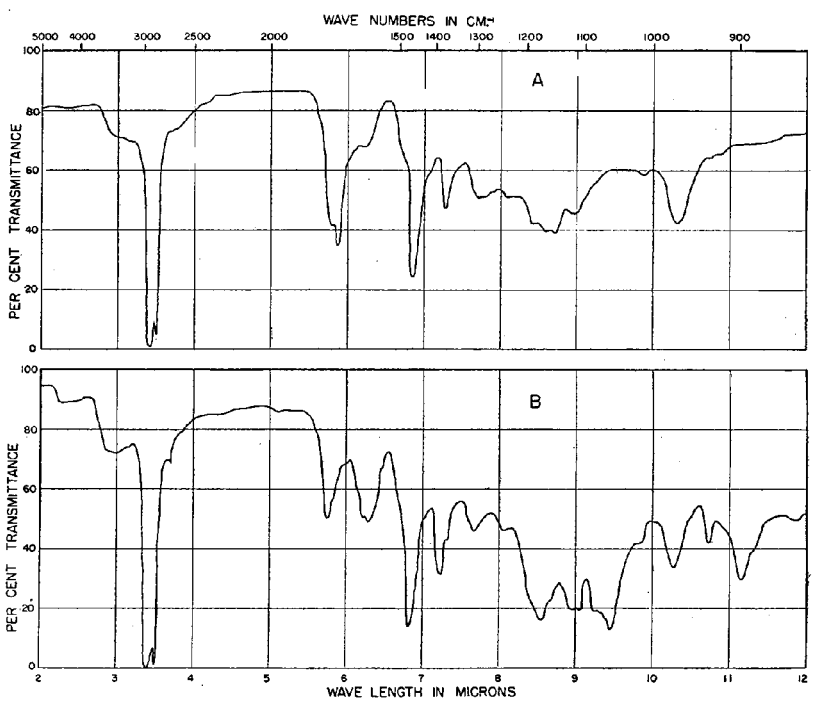

Figure 4. Infrared absorption spectra

A. Carbon tetrachloride extract
B. Carbon tetrachloride extract after saponification with aluminum isopropoxide

aliphatic and aromatic C—H bonds is indicated by the strong absorption bands at 3.4 and 3.5 microns. The band at 5.8 microns may be due to aldehyde, ketone, or ester and that at 5.85 microns to carboxylic acid. The presence of this acid was confirmed by saponifying the extract with aluminum isopropoxide and obtaining the characteristic soap band at 6.3 microns (Figure, 4,*B*). The spectrum of the extract (Figure 4,*A*) is similar to others which have been obtained for the cities of Philadelphia, Houston, and Detroit (*3*), except for the presence in the Windsor chart of a strong band at 10.3 microns. In the analysis of gasoline a band in this region has been assigned to olefins with an internal double bond (*8*), but in the present instance no change was observed after treatment of the extract with iodine monochloride.

CONCLUSION

Two well-known techniques, chromatography and absorption spectroscopy, have been applied to the investigation of the organic constituents of air pollution in Windsor and have proved successful in identifying a number of condensed ring polycyclic aromatic compounds. It should be possible by improved separation to put these results on a quantitative basis and by suitable chemical treatment to extend the range of compounds which can be determined by this method.

ACKNOWLEDGMENT

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Spectrophotometric Study of the Thorium-Morin Mixed-Color System

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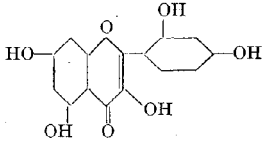
A spectrophotometric study was made of the thorium-morin reaction to evaluate the suitability of morin as a reagent for the determination of trace amounts of thorium. At pH 2, the equilibrium constant for the reaction is 1×10^6, and a single complex having a thorium-morin ratio of 1 to 2 is formed. The complex shows maximum absorbance at a wave length of 410 mμ, and its absorbance obeys Beer's law. The absorbance readings are highly reproducible, and the sensitivity is relatively high, an absorbance difference of 0.001 being equivalent to 0.007 γ of ThO_2 per sq. cm. The effects of acid, alcohol, and morin concentration, time, temperature, and age of the morin reagent as well as the behavior of morin with zirconium(IV), iron(III), aluminum(III), ytterbium(III), yttrium(III), uranium(VI), praseodymium(III), lead(II), lanthanum(III), and calcium(II) ions are discussed. A method is presented for the determination of thorium in pure solutions. Appropriate separations for the isolation of thorium may extend the usefulness of the method and permit the determination of trace amounts of thorium in complex materials.

THE color and fluorescent systems resulting from the reaction between thorium and morin were studied to obtain basic information concerning the system and to evaluate the suitability of morin as a reagent for the quantitative determination of trace amounts of thorium. The spectrophotometric study of the color system and a method for the determination of thorium in pure solutions are discussed here. This information will be fundamental to any specific adaptation of the reaction for the determination of thorium in rocks or other materials.

Morin, 5,7,2′,4′-flavanol, has the structure:



It is obtained as $C_{15}H_{10}O_7 \cdot 2H_2O$ with a molecular weight of 338.26, or as $C_{15}H_{10}O_7$ with a molecular weight of 302.23, depending upon the method of preparation. It reacts with a number of metallic ions under various conditions of acidity to produce fluorescent or colored complexes or both (*1, 3, 4, 12*). The reaction between

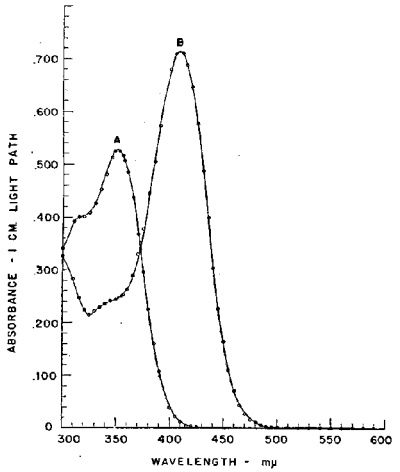


Figure 1. Spectrophotometric curves

A. 600 γ of pure morin per 50 ml. pH 1.96
B. 600 γ of morin + 5.0 mg. of ThO_2 per 50 ml. pH 1.96

morin and thorium takes place under slightly acid conditions to give a yellow solution, which exhibits a green fluorescence when exposed to ultraviolet light. Figure 1 shows a spectrophotometric curve for the yellow solution (curve *B*) and one for a solution of pure morin (curve *A*). The absorption spectra in Figure 1 represent a system highly favorable for quantitative analysis; the peak of the curve for morin plus thorium occurs at a point where the absorbance for the morin is very low, and the peaks of the two curves are well separated (350 mμ for morin and 410 mμ for morin plus thorium).

APPARATUS AND REAGENTS

A Beckman DU quartz spectrophotometer was used in this study. A comparable instrument would be satisfactory. A pH meter was also used.

Morin. Morin, $C_{15}H_{10}O_7 \cdot 2H_2O$ (molecular weight 338.26), of high purity was obtained from Dr. Theodor Schuchardt, München, Germany. Solutions of morin of the strength desired were prepared by dissolving the solid material in 95% ethyl alcohol. The reagent adopted for use in analysis contained 1 mg. of morin per milliliter of alcohol.

Standard Thorium Solutions. Stock solutions of thorium chloride and thorium nitrate were prepared by dissolving the respective salts (C. P. grade) in 3% hydrochloric or nitric acid. These solutions, which contained the equivalent of 100 mg. of ThO_2 per milliliter, were standardized gravimetrically by precipitation of the hydroxide with ammonium hydroxide that had been freshly prepared from tank ammonia gas; after precipitation, the thorium hydroxide was ignited to ThO_2 and weighed. Working solutions were prepared from the stock solutions by dilution with distilled water and, when necessary, by the addition of more acid. The acidity of all working solutions was adjusted to give a pH of 1.9 to 2.0; thorium solutions are stable at this pH (7).

Hydroxylamine Hydrochloride. C. P. grade was a 10% solution in distilled water.

Materials Tested as Possible Interferences. Stock solutions of the nitrates (C. P. grade) of aluminum, calcium, iron, lanthanum, lead, uranium, zirconium, yttrium, ytterbium, and praseodymium were prepared in nitric acid and diluted as required to give working solutions of the proper concentration and at a pH of 2.0. The working solutions were used immediately after dilution.

EFFECT OF EXPERIMENTAL VARIABLES ON SENSITIVITY OR STABILITY

Optimum conditions for the reaction between thorium and morin were established by evaluating the individual effects of the more important experimental variables. In studying these effects, the reagents were added in the order shown in the standard procedure.

Acidity. In Figure 2, curve 1 shows the effect of acidity on the absorbance at 410 mμ for nitric acid solutions of pure morin, and curve 2 shows the effect on solutions that contain both thorium and morin. On the basis of these curves, a pH of 1.9 to 2.0 was selected for all subsequent work, because in this pH range the sensitivity is near the maximum (maximum sensitivity occurs at a pH of 2.4), the reagent shows constant absorbance, and other ions should interfere less than at lower acidities.

Other experiments showed no essential difference in the reaction when either hydrochloric or nitric acid was used. Although perchloric acid may be used, sulfuric acid cannot because sulfates complex thorium to such an extent that no color develops.

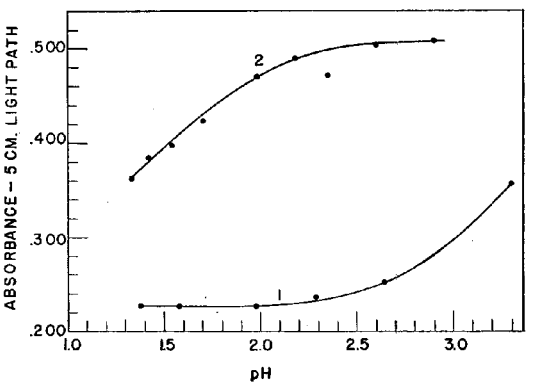


Figure 2. Effect of acidity on absorbance at 410 mμ

2.0 mg. of pure morin per 50 ml.
2. 2.0 mg. of morin + 15 γ of ThO_2 per 50 ml. 2 ml. of alcohol per 50 ml. in all solutions

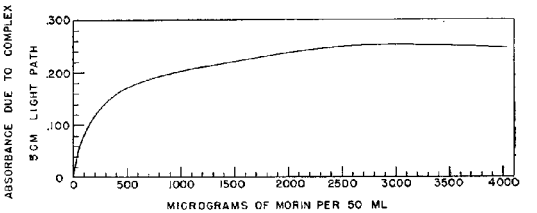


Figure 3. Effect of morin concentration

15 γ of ThO_2 per 50 ml. plus varying amounts of morin

Alcohol. The absorbance (410 mμ) of the pure complex remains essentially constant when the alcohol concentration of the solution varies from 2 to 16 ml. of alcohol per 50 ml. of solution. However, in this range of alcohol content, the absorbance of pure morin increases steadily with the alcohol content. For greatest sensitivity only the 2 ml. of alcohol per 50 ml. of solution added with the morin reagent was used in the standard procedure.

Time of Standing. The yellow color develops almost instantly in the solutions and essentially constant absorbance readings are obtained over a period of 7 hours. In the standard procedure, half an hour was allowed for the reaction to reach equilibrium before the absorbance was measured.

Morin. Figure 3 shows net absorbance (410 mμ) due to the

complex formed from 15 γ of thorium dioxide as a function of the original morin content of the solutions and indicates essentially complete reaction when 2500 γ or more of morin are present.

In other similar experiments, a precipitate formed in solutions containing 80 γ of thorium dioxide when the morin content was as low as 2000 γ; precipitation also occurred when the thorium dioxide was reduced to 50 γ if the morin content was increased to 2800 γ or more. In the standard procedure 2000 γ of morin was selected as the best compromise, because this amount gives practically complete reaction and also insures against precipitation for as much as 60 γ of thorium dioxide.

Age of Morin Reagent Solution. Three solutions of morin reagent that had been standing in the laboratory for varying periods of time were tested to determine whether the reagent deteriorates with age. Each of the three reagents was used to prepare a standard blank containing 2 mg. of morin, and each was also used to prepare a solution of the complex using 400 γ of morin plus 50 mg. of thorium dioxide. The absorbance values at 410 mμ for the solutions of blanks and complex as well as the age of the morin reagent are presented in Table I. Solutions of morin in ethyl alcohol are essentially stable for at least 4 months under usual laboratory conditions, and use of morin solutions several months old will introduce no gross errors.

Temperature. The temperature of the solutions affects the absorbance readings somewhat. The absorbance of a solution containing the equivalent of 15 γ of thorium dioxide and 2 mg. of morin per 50 ml. at 12° C. is about 9% greater than the absorbance of the solution at 30° C. However, the differences in absorbance corresponding to the differences generally found in room temperature are small and can usually be neglected.

On the basis of the results of these tests, the following standard procedure was adopted for the determination of thorium in pure solutions.

STANDARD PROCEDURE FOR DETERMINATION OF THORIUM IN PURE SOLUTIONS

The colored solutions are prepared in 50-ml. glass-stoppered graduates or volumetric flasks.

1. Add 1.0 ml. of 0.63N hydrochloric or nitric acid.
2. Add the solution containing not more than 60 γ of ThO_2 and free from other ions. (The pH of thorium solution should be previously adjusted to 2.0.)
3. Adjust volume to about 20 ml. with water, and mix.
4. Add 2.0 ml. of morin reagent (0.10% in ethyl alcohol).
5. Mix.
6. Adjust volume to exactly 50 ml. with distilled water.
7. Stopper and mix thoroughly.

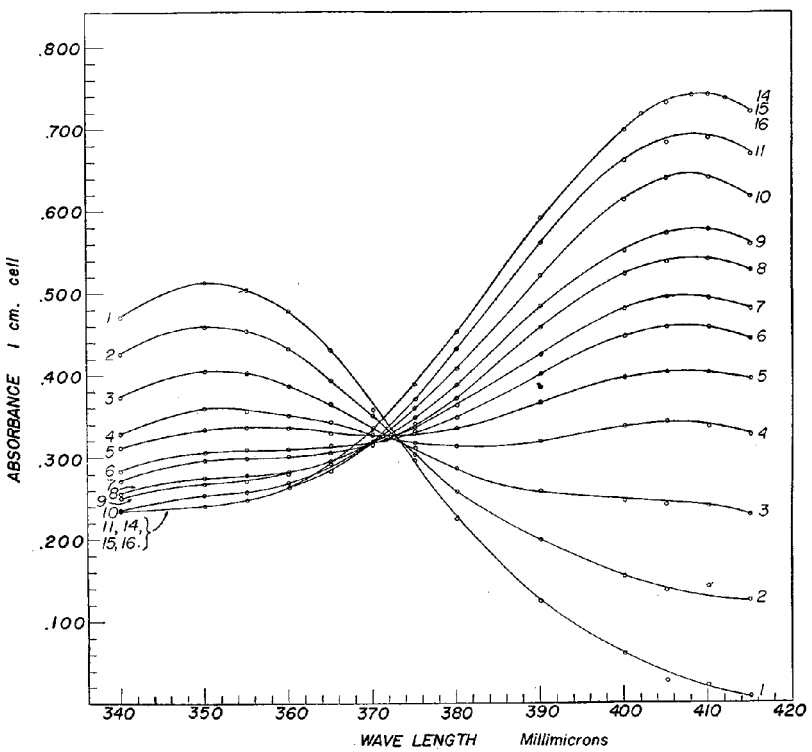


Figure 4. Spectrophotometric curves showing isoabsorptive point

Curve	ThO_2, γ	Curve	ThO_2, Mg
1	0	10	1.01
2	50.3	11	2.01
3	100.6	12	4.02
4	154.5	13	8.05
5	201.2	14	15.5
6	251.5	15	30.2
7	301.8	16	50.3
8	402.4		
9	503.0		

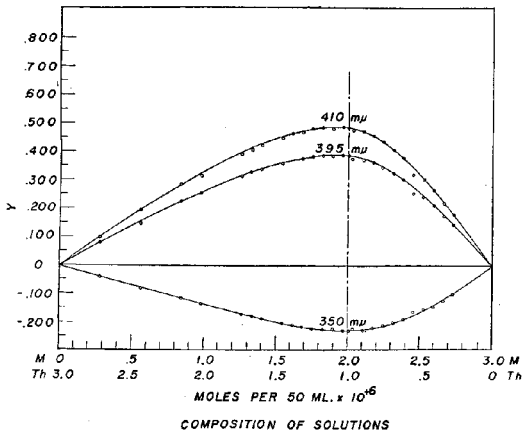


Figure 5. Determination of composition of complex by method of continuous variations

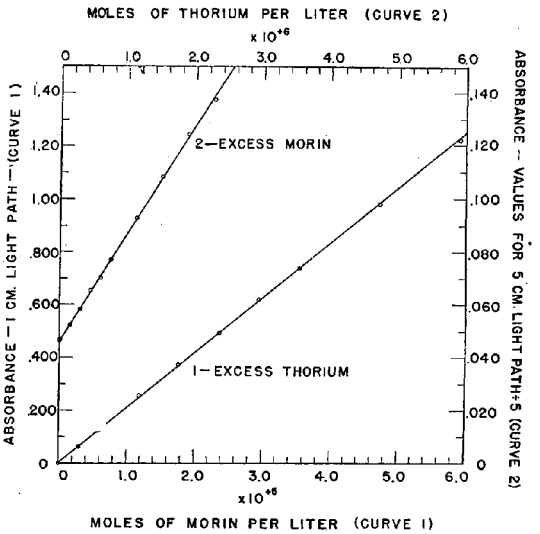


Figure 6. Determination of composition of complex by slope-ratio method

Table I. Effect of Age of Morin Reagent on Absorbance Measurements

Age of Morin Solution, Months	Absorbance: Morin, 2.0 mg./50 ml. solution (5-cm. cell)	Absorbance: Complex, 400 γ morin plus 50 mg. ThO_2/50 ml. solution (1-cm. cell)
1 day	0.235	...
3	0.235	...
4	0.242	...
3 weeks	...	0.492
3.5	...	0.481
4.5	...	0.481

8. After half an hour measure absorbance at 410 mμ, using a slit width of 0.04 mm. and water as the reference solution.

SENSITIVITY

A standard curve derived from known amounts of thorium dioxide in solutions prepared according to the above procedure is linear from 0 to 60 γ of thorium dioxide per 50 ml. The sensitivity of the reaction, as obtained from this curve using Sandell's criterion (*8*) of an absorbance difference of 0.001 as the limit of detection, is 0.0070 γ of thorium dioxide per sq. cm. This means that 0.0014 γ of thorium dioxide per milliliter can be detected using a 5-cm. light path.

NATURE OF THE REACTION

Using the standard procedure for the preparation of the colored solutions, but varying the concentrations of the thorium and of the morin reagent as indicated, tests were made to determine whether the reaction is stoichiometric and to ascertain the number and composition of the complexes that might be formed. Sixteen solutions were prepared; each contained 600 γ of morin and an amount of thorium nitrate that ranged from 0 to 50.3 mg. of equivalent thorium dioxide. The absorbances of these solutions were measured for wave lengths between 340 and 420 mμ, and spectrophotometric curves were prepared. The resulting family of curves is shown in Figure 4.

The occurrence of an isoabsorptive point (*2*), which is shown at a wave length of approximately 372 mμ, in Figure 4, indicates that only one complex is formed; such a family of curves with an isoabsorptive point is very strong evidence that only two light-absorbing components—in this case morin and complex—are present and in equilibrium (*10, 11*).

The changes that occur in the shape of the curves as the composition of the solutions varies again indicate that only one complex is formed. Curve 1 (Figure 4) is the curve for pure morin, and the other curves, in numerical order, are for solutions containing larger and larger amounts of thorium. As the amount of thorium was increased, more and more complex was formed in the reaction represented by the equation

$$x\ Th^{4+} + y\ M.H \rightleftharpoons Th_xM_y + yH^+$$

where M.H = morin.

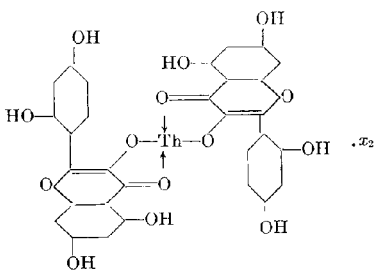
Thus, with each additional increment of thorium, there was a corresponding decrease in the amount of free morin, which was revealed by the diminishing absorbance of the peak at 350 mμ. Simultaneous with the diminution of this peak was the development of another peak at 410 mμ. The second peak is characteristic of the complex, and the value of its absorbance grew larger as the amount of complex increased. When the thorium content reached about 7 mg. of equivalent thorium dioxide, further increases up to 15 mg. of thorium dioxide resulted in less than a 2% increase in the absorbance at 410 mμ. A single curve (14, 15, and 16, Figure 4) resulted from 15, 30, and 50 mg. of thorium dioxide, and this curve represents the spectrum for the pure complex, as excess thorium dioxide has no absorbance at these wave lengths.

DETERMINATION OF COMPOSITION OF COMPLEX

Job's method of continuous variations (*6, 9*) was used to determine the composition of the complex. Twenty-eight solutions were prepared, each containing thorium and morin in different proportions; but in each solution the combined concentration of thorium and morin was always 3×10^{-6} mole per 50 ml. The absorbances of these solutions were measured at three different wave lengths; and for each measurement, a value Y was calculated that was equivalent to the difference between the measured absorbance and the absorbance the solution would have had if there had been no reaction. These values for Y, plotted as a

function of the composition of the solutions, are given in Figure 5; a separate curve was prepared for each of the three wave lengths. Job (*6*) and Vosburg and Cooper (*9*) have shown that the value of Y at each wave length is at a maximum when the greatest amount of complex is formed. This occurs when the ratio of the molar amounts of the two reactants is exactly equal to their combining ratios.

All the curves in Figure 5 reach a peak at the same point on the abscissa. At this point the moles of morin are essentially twice as great as the moles of thorium. The ratio of thorium to morin in the complex is therefore 1 to 2, and an abbreviated formula for the complex may be written as ThM_2. A possible structure of this complex is



where X is a univalent negative ion such as Cl^- or NO_3^-.

In addition to giving the mole ratio of thorium to morin in the complex, the curves in Figure 5 also substantiate the earlier conclusion that only one complex is formed. The curves for three different wave lengths all show peaks at the same molar ratio; moreover, all the curves are smooth and continuous, no secondary peaks appear, and the curves are concave with respect to the abscissa. These characteristics all indicate the formation of a single complex (*9*).

The conclusion that the mole ratio of the thorium to morin in the complex is 1 to 2 was substantiated according to the slope ratio method (*5*) by absorbance measurements at 410 mμ on two series of solutions. The first series contained amounts of morin that ranged from 0 to 1000 γ and an excess of thorium (50 mg. of thorium dioxide), which ensured complete reaction of the morin to form complex. The second series contained amounts of thorium dioxide that ranged from 0 to 30 γ and an excess of morin (2.0 mg.) that was sufficient to ensure essentially complete reaction for the small amounts of thorium employed.

In Figure 6 the absorbance and corresponding moles of morin per liter for the first series of solutions are shown in curve 1, whereas the absorbance and corresponding moles of thorium per liter for the second series are shown in curve 2.

The slope of curve 1 is 2.092×10^4 and represents the rate of change in the absorbance of the complex per mole of morin complexed. The slope of curve 2 is 4.105×10^4 and represents the rate of change in the absorbance of the complex per mole of thorium complexed. But absorbance is proportional to the moles of complex formed; therefore

$$\frac{\text{Slope 1}}{\text{Slope 2}} = \frac{2.092 \times 10^4}{4.105 \times 10^4} = \frac{1}{1.96} = \frac{\Delta \text{ absorbance/mole of morin}}{\Delta \text{ absorbance/mole of thorium}}$$

$$\text{and } \frac{\Delta \text{ moles of complex/mole of morin}}{\Delta \text{ moles of complex/mole of thorium}} = \frac{1}{2}$$

thus substantiating the earlier conclusion.

The linear relationships shown in Figure 6 indicate that light absorption by the complex follows Beer's law:

$$P = P_o 10^{-abc} \quad (1)$$

where
P = transmitted radiant power
P_o = incident radiant power
a = absorptivity or specific absorbance
ϵ = absorptivity when c is expressed in moles per liter
b = internal cell length
c = concentration of complex

Thus, the absorbance

$$A = abc \text{ or } \epsilon bc \quad (2)$$

and the absorptivity

$$a = A/bc \quad (3)$$

and

$$\epsilon = A/bc \quad (3a)$$

when c is expressed in moles per liter.

The absorptivity for the complex was calculated from the data in curve 1, Figure 6, and that for pure morin was determined from the slope of a curve (not included here) in which the absorbance values for a series of pure morin solutions were plotted as a function of morin content.

The absorptivity values at 410 mμ are as follows:

For the Complex	For Pure Morin
$\epsilon_{ThM_2} = 4.184 \times 10^4$ liter/mole × cm.	$\epsilon_{M.H} = 3.767 \times 10^3$ liter/mole × cm.
$a_{ThM_2} = 5.013 \times 10^{-2}$ sq. cm./γ	$a_{M.H} = 1.120 \times 10^{-3}$ sq. cm./γ

DETERMINATION OF COMPONENTS OF ANY SOLUTION AND CALCULATION OF EQUILIBRIUM CONSTANT

In any solution composed of several components that absorb light, the total absorbance of the solution is equal to the sum of the individual absorbances. In the morin-thorium system there are only two components that absorb light: morin, M.H, and the complex, ThM_2.

If X = moles of complex per liter

M.H = total moles of morin added per liter

Y = moles of uncombined morin per liter

the absorbance, A, of any given solution can be expressed as

$$A = \epsilon_{M.H} Yb + \epsilon_{ThM_2} Xb \quad (4)$$

The morin originally added is distributed between the combined and uncombined states, and each mole of complex contains 2 moles of combined morin.

Thus, the total morin

$$\text{M.H} = Y + 2X \quad (5)$$

and

$$Y = \text{M.H} - 2X \quad (6)$$

Substituting in Equation 4

$$\frac{A}{b} = \epsilon_{M.H}\,(M.H - 2X) + \epsilon_{ThM_2}\,X \qquad (7)$$

and

$$X = \frac{A - \epsilon_{M.H}\,(M.H)\,b}{b\,(\epsilon_{ThM_2} - 2\,\epsilon_{M.H})} \qquad (8)$$

If the original constituents of the solution are known, Equation 8 may be used to calculate the moles of complex, and moles of uncombined morin and thorium present per liter in any solution. These values may then be used to calculate the apparent equilibrium constant for the reaction

$$Th^{+4} + 2\ M.H \rightleftarrows ThM_2^{++} + 2H^+$$

according to the equation

$$K_{equil.} = \frac{(ThM_2^{++})\,(H^+)^2}{(Th^{+4})\,(M.H)^2}$$

In this study, the concentration of the hydrogen ion, (H), was constant at (10^{-2}) because a pH of 2.0 was maintained in all solutions.

Table II. Sensitivity of Reaction

(Weight of various substances in 50 ml. of solution, equivalent to an absorbance difference of 0.010 at 410 mμ when measurements are made in a 5-cm. cell)

Substance	Micrograms Equivalent to 0.01 Absorbance
ThO_2	0.60
ZrO_2	0.79
Fe_2O_3	1.3
Al_2O_3	15.6
Fe_2O_3 (in presence of hydroxylamine)	28.6
Yb_2O_3	69.4
Y_2O_3	81.3
U	89
Pr_2O_3	161
Pb	500
La_2O_3	700
CaO	37×10^3

Equilibrium constants were calculated in this manner from the data from 80 solutions used in this study. These solutions had widely varying compositions and included those used to obtain the data for Figures 4 and 6. Besides these, there was a set of solutions containing 50.3 γ of thorium dioxide with 0 to 1000 γ of morin per 50 ml., and also a set with moles of thorium, morin, and (thorium + morin) all continuously varying. The constants calculated from this experimental data ranged from 0.13×10^5 to 6×10^5; the low figures were obtained when fairly large amounts of excess morin were present, and the large figures were obtained from solutions containing excess thorium. However, most of the values fell between 0.5×10^6 and 1×10^6 and the average of all values was 1×10^6.

REACTION OF MORIN WITH OTHER IONS

Zirconium, aluminum, ferric, and ferrous iron ions, which were considered as possible serious interferences because of their tendency to form complexes with morin; calcium and lanthanum, which might be useful as carriers in separations of thorium from other ions; and also uranium, lead, yttrium, ytterbium, and praseodymium were tested under the standard conditions for their reaction with morin in the absence of thorium. The sensitivity of the reaction for each of these substances with morin is given in Table II.

Zirconium and aluminum are serious interferences and must be virtually absent when trace amounts of thorium are determined. Interference from ferric iron is serious also, but the effect is appreciably decreased if the iron is reduced with hydroxylamine. As much as 400 mg. of hydroxylamine may be added to the solutions with little or no effect on the reaction between thorium and morin; in fact, hydroxylamine is now added to all solutions as a standard practice. (In the standard procedure 4 ml. of 10% hydroxylamine hydrochloride is added between steps 2 and 3—i.e., before the volume is adjusted to 20 ml. with water.) The interference from relatively large amounts of calcium and lanthanum is negligible and these ions can, therefore, be used as carriers in separations of thorium from other ions. At present, separations to isolate thorium from other ions have not been investigated nor has any attempt been made to apply the method to complex materials, as such studies are beyond the scope of this investigation.

CONCLUSIONS

The reaction between thorium and morin is stoichiometric, only one complex is formed, and morin is a suitable reagent for the determination of trace amounts of thorium in pure solutions. As little as about 0.2 γ of thorium dioxide in 50 ml. can be determined. However, the reaction can be adapted to the analysis of complex materials only after suitable separations of thorium from other ions have been made.

ACKNOWLEDGMENT

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Automatic Derivative Spectrophotometric Titrations

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An automatic derivative spectrophotometric titrator is described which is capable of rapid, precise, and accurate titrations. The method utilizes a derivative technique by which a relatively simple electronic circuit develops a voltage signal proportional to the third derivative of the photometer output. The derivative signal is ideally suited to trigger a relay system and terminate the flow of titrant at the break (end point) in the curve of absorbance *vs.* milliequivalents. This automatic system requires a minimum of pretitration considerations and instrument adjustments. It is applicable to automatic titration of solutions where there is a relatively sharp change of absorbance at the end point, even though the absolute absorbance is unknown and differs considerably from sample to sample. Quantitative results for a few common acid-base and redox titrations show precision within 0.1%, even at relatively fast flow rates of titrant (about 5 ml. per minute).

THE detection of the end point in a titration by measuring the changes of absorbed narrow band-pass radiation, often referred to as spectrophotometric or photometric titration, has received considerable attention in the past few years because of certain advantages over other methods. These advantages have been discussed in recent articles (*2, 5, 6, 16*). In the general spectrophotometric titration procedure the titrant is added to the reactant contained in a suitable titration vessel, the solution is mixed, and absorbance values are read from a spectrophotometer or filter photometer after the addition of each increment of titrant. A plot of absorbance *vs.* milliliters of titrant is then made. The shape of the titration curve depends on the combination of reactant, reaction product, titrant, and/or indicator that undergoes changes of absorbance at the preset wave length during the titration.

The manual technique is time-consuming, however, and Malmstadt and Gohrbandt (*9*) and Malmstadt and Roberts (*10*) have shown that many spectrophotometric titrations can be facilitated by automatically plotting the titration curve while continuously adding the titrant, either by electrolytic generation or from a constant-flow buret, with efficient stirring of the solution by a motor-driven paddle stirrer. The reactant and titrant must, of course, react rapidly if this method is to be successful, but a great many useful titrations meet this requirement. However, recording equipment and synchronized buret systems are expensive, and the operator time required per titration is somewhat more than desirable for routine titrations.

A few investigators (*3, 4, 12, 17*) have used specially constructed apparatus for automatic photometric titrations in which there are large changes of absorbance at the end points. These titrators usually have been tested with titrations using the classical visual color indicators. With this automatic technique the flow of titrant is terminated at an absolute absorbance value which is preset to correspond closely to the equivalence point of the titration. Such a method is applicable in many cases, but it has disadvantages in certain titrations. For example, it is difficult or impossible to set a definite absorbance value corresponding to the equivalence point if there are substances present in solution which absorb light in the same wave length region as the titration species, but do not take part in the titration reaction and vary in concentration from sample to sample. The same would be true for solutions in which turbidity varies from sample to sample, or scattered light varies from beaker to beaker, or the reaction products absorb in the preset wave length ranges.

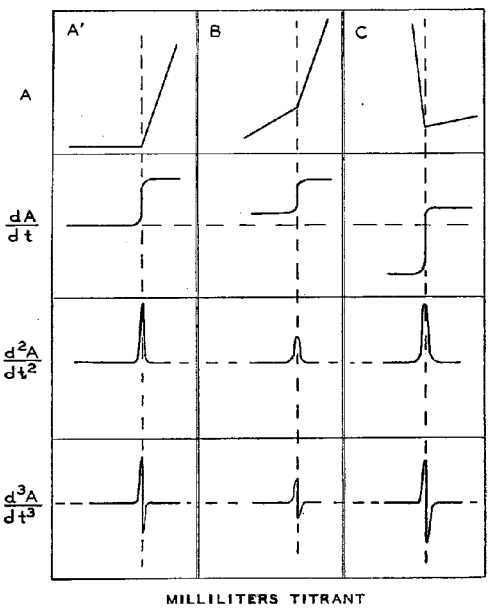

Figure 1. Spectrophotometric titration curves with respective first, second, and third derivative curves

The problems inherent in knowing and presetting an absolute value in automatic potentiometric titrations have been circumvented by a derivative technique described by Malmstadt and Fett (*7*). An automatic derivative system with the other necessary equipment has now been devised to facilitate the performance of spectrophotometric titrations; it does not have the inherent problems of other systems, in which it is necessary to know and preset an equivalence point absorbance value. The break in the curve of absorbance *vs.* milliliters must be sharp, but this is the case for nearly all titrations using visual color indicators and for many titration systems which have useful absorption bands in the ultraviolet. The design and construction of the equipment and the analytical results of a few applications illustrating some characteristics of the system are presented here.

The automatic derivative potentiometric titrator electronically produces a voltage which is proportional to the second derivative of the ordinary electrode potential curve; the second derivative voltage is ideally suited for triggering a relay system which turns the buret off at the inflection point (end point) of the titration (*7*). The second derivative system is also directly applicable for some spectrophotometric titration systems in

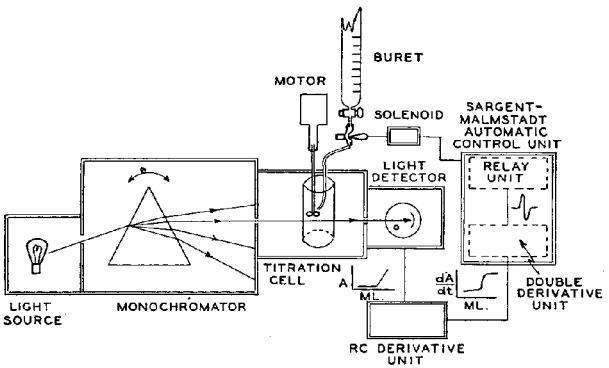

Figure 2. Basic instrumental components for derivative spectrophotometric titrations

which the titration curves are similar to the sigmoid potentiometric curves. A specific example of this type is described in another paper (*11*). However, the shapes of most spectrophotometric titration curves resemble conductometric titration curves, involving the intersection of two straight lines at the end point (Figure 1). The theoretical first, second, and third derivatives of the ordinary spectrophotometric titration curves of the three examples are also given in Figure 1; it is seen that the third derivative of the ordinary curve is required to yield an output signal ideally suited for triggering a relay system which terminates the flow of titrant at the break in the titration curve. Equipment for electronically producing an output voltage curve proportional to the third derivative of a curve of absorbance *vs.* milliliters curve is described below.

INSTRUMENTATION

The basic components for automatic third derivative spectrophotometric titrations are illustrated in Figure 2. With the elimination of the first derivative stage, the same equipment is obviously applicable for those cases where the second derivative output is suitable for automatic termination of the titration.

The output of the photometer is fed into a simple resistance-capacitance network to obtain a first derivative signal which is then fed into the commercially available Sargent-Malmstadt automatic titrator control unit (*14*). This control unit has two sections. One section amplifies and electronically computes the second and third derivatives of the curve of absorbance *vs.* milliliters; the other section contains a relay system which utilizes the derivative output to activate the buret solenoid or current relay (in the case of electrolytic generation of titrant) and terminate the flow of titrant at the break in the titration curve.

Specific Components. In some cases it should be possible to modify the photometer circuits of various spectrophotometers for the derivative titration technique, but it is probably best to construct a special photometer circuit—one that is especially suited for the derivative titration technique. The circuit should be stable and sensitive and, preferably, should yield an output signal proportional to the solution absorbance or the logarithm of transmitted light. There are several possible circuits for producing a log T or absorbance response. The specific instrument for the spectrophotometric titration work in this laboratory was devised to be versatile and to make use of components not tied up on other spectrochemical problems. Many other designs are possible to simplify the system for certain routine titrations.

Titration Cell Compartment. The titration cell compartment is fixed between the monochromator and photometer units. It should be designed to permit simple and rapid insertion and removal of the titration vessel. Also, because continuous and efficient stirring is a prerequisite of the method, the above design objectives should be met while providing the required stirring.

The titration cell compartment consisted of a large 10-cm. cell unit from the Beckman DU spectrophotometer, modified as illustrated in Figure 3 to facilitate the titration manipulations. A cell with quartz windows, described by Malmstadt and Gohrbandt (*9*), could be set in the compartment for titrations requiring ultraviolet radiation. Glass beakers are suitable for titrations in the visible range down to about 350 mμ. All of the titrations described herein were performed in 200-ml. electrolytic beakers which had about $^3/_4$ inch cut off the top so they would fit in the compartment.

A polystyrene cell holder, *A*, was fastened securely in position in the compartment, *B*, by a plug-in arrangement consisting of two banana plugs and jacks, *C*. The bore of the holder was of a slightly larger diameter than the 200-ml. beakers, *D*, so that they fit easily but snugly into correct position for the light beam.

A special cover was made from a Lucite block, *E*, screwed to a flanged metal lid, *E'*, which made a lighttight fit to the top of the cell compartment. A small 1500-r.p.m. motor, *M*, was mounted on the top of the cover. The motor shaft and paddle stirrer, *F*, were connected by a metal coupling, *G*. The coupling and shaft were protected from corrosive solutions and fumes by spraying with plastic spray (Krylon).

The stirring efficiency was greatly increased by running the motor at maximum speed and in the presence of a baffle, *H*, to prevent a vortex from forming and drawing air bubbles into the solution. A large cylindrical platinum gauze electrolysis electrode ($1^1/_2$ inches in diameter, $1^1/_2$ inches high) was suitable for this purpose, and it was also convenient as the generator electrode for titrations utilizing electrolytic generation of titrant. No troubles from gas bubbles were encountered with this system, even with the stirrer at maximum speed, and the excellent efficiency of stirring is apparent from the analytical results obtained for relatively fast flow rates of titrant. Both stirrer and baffle were adjusted to be directly above but not in the light path.

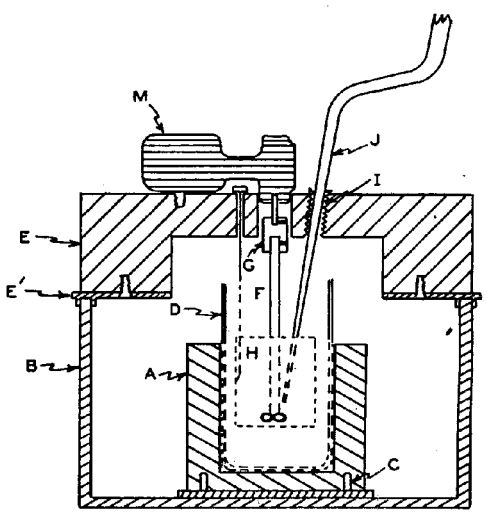

Figure 3. Cross-sectional end view of titration cell compartment and stirring arrangement

A $^3/_8$-inch hole, *I*, was drilled in the cover at a slight angle so that the buret tip, *J*, would come close to the stirrer shaft just above the stirrer paddle. The careful positioning of the buret tip with relation to the stirrer is essential for efficient stirring without streaming of titrant. A small section of rubber tubing on the buret tip provided a convenient lighttight seal for the hole, *I*, and also held the buret tip in position. The hole was

tapped so that a threaded polystyrene tube with a sintered-glass disk sealed on the end could be screwed into position instead of the buret tip. This was used as the isolated electrode compartment for electrolytic generation of titrant.

Light Source and Monochromator. The light sources and monochromator depend on the requirements of a particular laboratory. A tungsten bulb with selected filters is suitable for many titrations, but for a versatile instrument it is desirable to have prism or grating monochromators of good resolution and both visible and ultraviolet sources.

Any available monochromator can be connected to the one end of the titration cell compartment if a flat metal plate is firmly connected at the exit slit of the monochromator and drilled with holes for the light beam and the four aligning holes for connecting to the titration cell compartment and photometer housing. For expediency in this laboratory the Cenco-Sheard grating monochromator was used for all titrations described in the following sections. The solution and barrier-layer cell compartments were removed from the Cenco-Sheard spectrophotometer and replaced with an end plate of proper size to make a lighttight fit around the edges at one end of the titration cell compartment. The light source compartment consisted of the old-type housing from the Beckman DU, which was firmly fixed at the entrance slit of the monochromator. An ordinary 6-volt, 6-ampere constant voltage transformer provided sufficiently stable power for the light source.

Photometer Circuit. Although various photometer circuits are available, a unique circuit was chosen because of its high sensitivity and stability and an output voltage response which is closely proportional to absorbance over a wide range of light values. The basic circuit was originally described by Sweet (*15*) and modified by Gilford and coworkers (*1*). These investigators presented detailed circuits and described their characteristics. Therefore, only those sections of the circuit necessary to illustrate the modifications for the derivative titrations are presented in Figure 4. The other circuits are given only as blocks with specific functions, which facilitates explanation of the basic circuit.

The phototube housing was an old unit from a Beckman DU spectrophotometer, discarded when a photomultiplier attachment was installed. The old circuits of the housing were removed and replaced by a 931-A photomultiplier tube, V_2, a 12AX7 twin triode tube, V_1, and associated circuit, and a 200-megohm resistor, R_1. A five-conductor cable was used for the leads from the low voltage power supply section, and a shielded cable for the leads from the high voltage power supply section. All of the other components for operation of the photometer circuit were compactly mounted on one chassis and fit into a cabinet 8 × 12 × 12 inches.

The operation of the circuit is readily understood by reference to Figure 4. If the absorbance of a solution increases, the light incident on the photomultiplier tube decreases and the photocurrent tends to decrease. However, a small decrease in photocurrent causes a relatively large decrease in voltage across the 200-megohm resistor, R_1. A decrease in voltage across R_1 decreases the grid bias of one half of the twin-triode tube, V_1, and

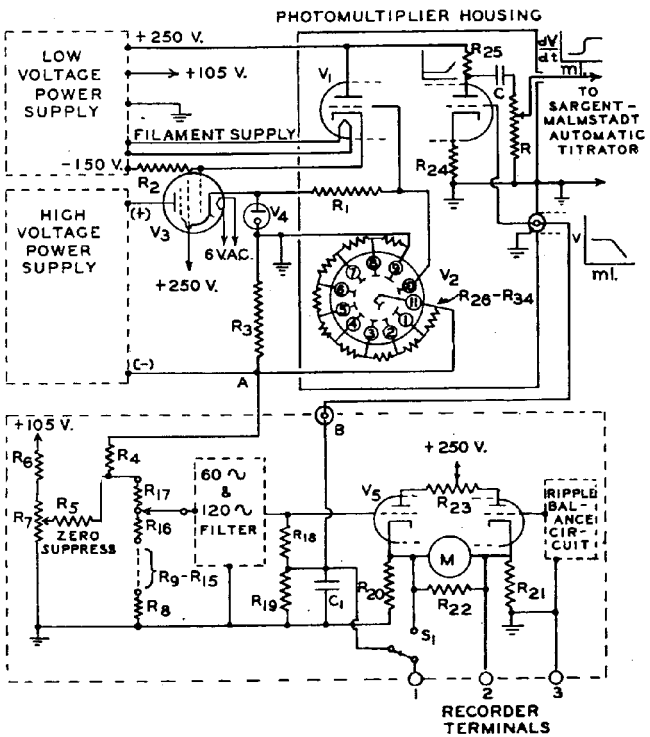

Figure 4. Photometer and first derivative circuits

R. 100-kilo-ohm helipot
R_1. 200 megohm, 1/2 watt
R_2. 500 kilo-ohm, 1/2 watt
R_3. 100 kilo-ohm, 25 watt
R_4. 1 megohm, 1 watt
R_5. 100 kilo-ohm, 1 watt
R_6. 10 kilo-ohm, 2 watt
R_7. 20-kilo-ohm helipot
R_8. 1 kilo-ohm, 1/2 watt
R_9. 5 kilo-ohm, 1/2 watt
R_{10}. 50 kilo-ohm, 1/2 watt
R_{11} to R_{17}. 130 kilo-ohm, 1/2 watt
R_{18}. 2 megohm, 1/2 watt
R_{19}. 500 kilo-ohm, 1/2 watt
R_{20}, R_{21}. 5 kilo-ohm, 1 watt
R_{22}. 100 ohm
R_{23}. 10-kilo-ohm potentiometer
R_{24}. 2 kilo-ohm, 1/2 watt
R_{25}. 1 megohm, 1/2 watt
R_{26} to R_{34}. 110 kilo-ohm, 1/2 watt
C. 1/2-μfd. oil condenser, 300 volt
C_1. 1/2-μfd. oil condenser, 100 volt
V_1. 12AX7 twin-triode
V_2. 931-A photomultiplier tube
V_3. 6BG6 tube
V_4. VR 75 tube
V_5. 6SN7 twin-triode tube
S_1. Double-pole, single-throw switch
M. 100-μa. zero-center meter

consequently increases the current through the tube. An increase of current through V_1 increases the voltage drop across the cathode resistor, R_2. An increase in voltage across R_2 causes the grid of the series regulator tube, V_3, in the high voltage power supply circuit to become more positive; the current through V_3 and the series load resistor, R_3, increases. The voltage across R_3 is the voltage applied across all the dynodes of the photomultiplier tube, and the increased current through R_3, therefore, provides an increased voltage across the dynodes and tends to increase the photomultiplier current. The net result is that the photomultiplier current remains essentially constant for changing incident radiation, but the voltage on the dynodes changes in the direction opposite to the incident light intensity changes. Sweet (*15*) has shown that the voltage on the dynodes is approximately proportional to the absorbance.

A voltage divider and zero suppression circuit in the measuring circuit permit small changes of the dynode voltage to be measured over a wide range of light intensity. The meter, M, in the balanced twin-triode circuit can be used for regular absorbance measurements; a filter network is inserted between the voltage divider and measuring circuit. However, for automatic derivative titrations the meter and filter circuits could be eliminated and a small fraction of the dynode voltage could be taken across R_{19} and fed by shielded cable to the grid of the second half of the twin triode, V_1, in the photomultiplier housing. Tube V_1 amplifies and inverts the input voltage signal, which is differentiated with the simple resistance-capacitance network. This provides an output signal proportional to the first derivative of the titration curve, and it is fed directly to the Sargent-Malmstadt automatic control unit (*14*).

The amplification in tube V_1 is not needed, but the tube provides the necessary phase inversion in consideration of the subsequent control unit. The Sargent-Malmstadt automatic control unit requires the input voltage to swing in the positive direction at the end point.

For titrations in which the absorbance increases at the end point, such as ferrous iron *vs.* permanganate, the voltage across the dynodes increases, and point A, Figure 4, becomes more negative with respect to ground potential. In such titration cases the curve of voltage *vs.* milliliters at the input to tube V_1 will be as illustrated in Figure 4. The phase inversion in V_1 with subsequent differentiation gives a signal with the required positive swing at the end point for operating the control unit.

It is possible to overdrive V_1 with too large an input signal from the photometer; this can distort the voltage signal fed to the differentiator. However, this generally does not cause any difficulty if tube V_1 is in an operating range in the end point region.

It would not be necessary to go through V_1 for titrations in which the absorbance remains constant during the titration but decreases sharply at the end point. It is convenient in such cases to attach a resistance-capacitance network to one end of a shielded lead and connect point B, Figure 4, to the network, with the other end of the lead going directly to the Sargent-Malmstadt control unit. For such titrations the zero suppress control must be positioned at the start so that meter M is about midscale. This prevents a large bias on the input tube to the Sargent-Malmstadt automatic titrator, permitting it to operate and detect the end point. If the solution is too opaque to permit a meter reading by rotating the zero suppress control, the wave length must be changed to a region of lower absorbance.

The ordinary spectrophotometric titration curve can be recorded by utilizing a small fraction of the voltage across the recorder terminals 1 and 3. The derivative curve can be simultaneously recorded from the recorder output of the control unit.

It is not necessary to close the shutter on the photometer while opening the cover and removing the titration cell because of the inverse feedback arrangement on the photomultiplier tube.

Automatic Control Unit. The Sargent-Malmstadt control unit can be used directly by feeding the output of the first derivative resistance-capacitance circuit into the input lead, which is normally connected to the electrodes for automatic potentiometric titrations. One small modification in the control unit is necessary because the positive voltage swing at the end point of some spectrophotometric titrations is of relatively short duration. The usual values in the relay circuit discriminate between long and short duration pulses, because this prevents difficulties with short period "noise" which is occasionally present on certain electrodes for potentiometric titrations. The discrimination time should be reduced, however, for some spectrophotometric titrations by changing the value of resistor R_9 (*13*) from 1 to about 5 megohms. The delay time on the original relay circuits can be decreased as discussed in the original publication (*7, 8*).

The buret solenoid from the Sargent-Malmstadt titrator can be connected directly into the control unit for starting and stopping the flow of titrant. Also, other type buret valves, syringe motor drives, or current supply relays (for electrolytic generation of titrant) can be connected directly to the output of the automatic control unit if they operate on 110 volts alternating current.

EXPERIMENTAL RESULTS

Some of the characteristics of the automatic derivative spectrophotometric titrator are illustrated by the results of a few common titration systems. These systems are not investigated to provide a critical evaluation of the automatic derivative spectrophotometric technique, but rather to demonstrate the feasibility of the third derivative method. Practical and theoretical limitations and a thorough evaluation of both the second and third derivative spectrophotometric end point technique will be presented in the near future. The precision of the automatic second derivative spectrophotometric technique is demonstrated by the determination of iron with electrolytically generated titanous ion (*11*).

Acetic Acid–Sodium Hydroxide. The third derivative technique was first tested by titrating aliquots of standard acetic acid with standard sodium hydroxide using phenolphthalein indicator. The wave length dial of the Cenco-Sheard monochromator was set for a region of high absorptivity for the basic form of phenolphthalein, about 555 mμ.

The concentration of any one-color indicator such as phenolphthalein is important for visual titrations as well as for spectrophotometric titrations. The concentration of indicator will not always be the same for spectrophotometric as for visual titrations, but in this specific case the concentration used in either method can be about the same.

The acetic acid and sodium hydroxide solutions were 0.1000N and the phenolphthalein stock solution was 0.03M. Aliquots of 25.00 ml. of acetic acid were diluted in the titration beaker with distilled water to about 130 ml. and 4 drops of 0.03M phenolphthalein was added. The titration beaker was inserted in the titrator, and the start button pushed to initiate the stirring and delivery of sodium hydroxide at a flow rate of about 5 ml. per minute. The titration was automatically terminated by the third derivative technique. In order to check the reproducibility of the method, the pH of the solution was checked at the end point with a pH meter, and the volume of titrant delivered was recorded. The results in Table I show that there was no significant overshooting of the end point, even at this relatively fast flow rate.

Table I. Titration of 25-ml. Aliquots of 0.1000N Acetic Acid with 0.1000N Sodium Hydroxide

	Ml. NaOH Required	pH of End Point Solution
Automatic Method	25.05	8.6
	25.00	8.8
	25.04	8.5
	25.00	8.6
	24.97	8.5
Av.	25.01	8.6
Manual (Visual) Method	25.00	8.6

Ferrous Iron–Permanganate. The titration of ferrous iron with potassium permanganate was performed at 547 mμ, the region of maximum absorbance for permanganate. The shape of the ordinary spectrophotometric titration curve is similar in this case, as in the previous case, to A', Figure 1.

The results of four determinations of 25-ml. aliquots of approximately 0.1N ferrous ammonium sulfate with 0.1000N potassium permanganate by the automatic derivative technique are given in Table II. All of the solutions when removed from the titrator were a faint purple color and required only 0.01 to 0.03 ml. of 0.1N ferrous iron to discharge the color. This indicates that

there was no significant overshooting of the end point at a titrant flow rate of about 5 ml. per minute.

Ferrous Iron–Dichromate. This system provides an interesting example because it was performed without added indicator. It is, of course, impossible to observe precisely by visual means the first excess of dichromate in the presence of the green chromic color. However, the absorptivity of a dichromate solution is sufficiently greater than a chromic solution at about 420 mμ so that the curve of absorbance *vs.* milliliters of dichromate appears as *B*, Figure 1. This illustrates a case where the absolute absorbance value changes depending on the sample size.

Table II. Titration of 25-ml. Aliquots of Approximately 0.1*N* Ferrous Iron with 0.1000*N* Permanganate or Dichromate

	Automatic Method	
	Ml. Required	
	$KMnO_4$	$K_2Cr_2O_7$
	25.10	25.15
	25.15	25.15
	25.15	25.17
	25.18	
Av.	25.15	25.16
	Manual (Visual) method	
		25.17

The results for three automatic titrations of 25-ml. aliquots of ferrous iron with dichromate are also given in Table II. In addition, one titration by the standard visual color indicator (diphenylamine sulfonate) method is listed. This titration could be performed automatically with added indicator, but the concentration to be used would have to be determined in consideration of the sensitivity of the photometer circuit.

Permanganate–Titanous. The titration of potassium permanganate with standard titanous solution gives a spectrophotometric titration curve in the end point region similar to *C*, Figure 1. Several automatic derivative titrations were run with this system, and in all cases the addition of a fraction of a drop of 0.1*N* permanganate to the end point solutions restored the purple permanganate color. The titrations were run at a wave length of 660 mμ.

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Determination of Iron in Titanium Sponge, Alloys, and Ores

Automatic Derivative Spectrophotometric Titration with Electrolytically Generated Titanous Ion

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Iron in titanium sponge, alloys, and ores can be determined rapidly, precisely, and accurately by a new and completely automatic titration procedure. The sample is dissolved in either sulfuric or a fluoboric-sulfuric acid mixture, and the iron is oxidized to the ferric state, with leuco methylene blue added as an indicator. The ferric iron is automatically titrated in the derivative spectrophotometric titrator with electrolytically generated titanous ion supplied by the titanium in the samples. A generator electrode is described which provides 100% efficient generation of titanous ion in the acid solutions, with no electrode care after it is once prepared. The method is useful over a wide concentration range of iron—0.01 to 20% or more. The precision is within 0.1% relative for iron concentrations greater than 3%, and the average absolute error is about 0.003%. It is possible to run the complete determination in about 35 minutes, most of which is consumed in dissolving the sample. The method has excellent possibilities for rapid automatic titration of other materials which contain constituents readily reduced by titanous ion.

THE Metallurgical Advisory Committee on Titanium has recently recommended two methods for the determination of iron in titanium materials (*4*). One method involves a sulfide separation and dichromate titration and the other is the well-known colorimetric method using 1,10-*o*-phenanthroline. It was considered worth while, however, to investigate the possibilities of an accurate and completely automatic titration system which would be directly applicable to the determination of iron in titanium sponge, alloys, or ores.

The method developed consists of solution of the sample, oxidation of iron to the ferric state, and titration of ferric iron with electrolytically generated titanous ion at constant current and with automatic derivative spectrophotometric end point detection. This method provides rapid, precise, and accurate results over a wide concentration range of iron. Also, the technician's time, which is normally spent on titration manipulations and standard solution preparation, is released for other laboratory functions. The method is conservative of standard reagents because the titrant (titanous ion) is generated from the major constituent of the samples.

The electrolytic generation of titanous ion at constant current was first described by Arthur and Donahue (*1*) for the determina-

tion of iron in iron ores. They used manual potentiometric end point detection and a gold-plated platinum electrode as their titanous generator electrode, performing the titrations in relatively dilute acid solutions. Unfortunately, they experienced difficulty and need for frequent treatment with their gold-plated electrode, and this detracted from the merits of the method. Malmstadt and Roberts (*2*) found 100% efficient generation of titanous ion with a platinum electrode in concentrated hydrochloric acid solutions (greater than 7*M*) and with titanium rod in 1*M* or greater hydrochloric acid solutions. However, it was not possible to use either of these electrodes in the sulfuric or fluoboric-sulfuric acid solutions which are usually used for dissolution of the titanium samples. Fortunately, it was possible to treat a platinum gauze electrode so that, once treated, it provided 100% efficient generation of titanous ion with no care over an indefinite period of time.

Potentiometric end point detection with a calomel reference electrode and platinum indicator electrode did not give good results, especially at low concentrations of iron. The response of the indicator electrode was erratic in the strong acid solutions, and there was apparently some interaction with the generator electrode. Also, the electrode responded slowly—a disadvantage for rapid automatic titrations. Therefore, spectrophotometric end point detection was attempted.

The ferric iron was at first titrated with electrolytically generated titanous ion at constant current and automatic recording of the spectrophotometric titration curve (*2*). When the wave length was set at 490 mμ, there was a decrease in absorbance with decreasing ferric iron concentration up to the equivalence point, after which there was a steady increase of absorbance with addition of excess titanous ion (*2*). The results by this technique were good, but it was desirable for routine titrations to eliminate the recording of the titration curve and to terminate the titration automatically at the equivalence point.

In order to provide for a completely automatic titration of the iron, the derivative spectrophotometric titrator (*3*) was employed. In this particular case it was advantageous to use leuco methylene blue as the indicator and to use the second derivative of the ordinary spectrophotometric titration curve. This technique provides accurate results for titanium samples containing from about 0.01% to high percentages (20% and more) of iron. The method is also simple, rapid, and trouble-free. After oxidation of the iron to the ferric state, the titration vessel is inserted into the titrator and the titration manipulations are automatically performed.

Quantitative results were obtained for pure titanium samples to which known amounts of iron were added and also for five Watertown Arsenal titanium alloy samples issued to the iron panel.

END POINT DETECTION SYSTEM

If a small amount of leuco methylene blue (colorless) is added to a solution containing ferric iron, it is quantitatively oxidized to methylene blue (blue) which in turn can be quantitatively reduced by titanous ion. The sum of the milliequivalents of ferric iron remaining plus milliequivalents of methylene blue (oxidized form) is equal to the milliequivalents of ferric iron originally in solution.

If this solution is titrated with titanous ion, it remains dark blue until the region of the end point is reached, and then continually decreases in absorbance until both the ferric iron and methylene blue are reduced, after which the curve levels off. This is shown by the recorded curve of absorbance *vs.* milliequivalents of titanous ion (Figure 1). The inflection point of the curve was nearly coincident with the intersection of the two extrapolated lines which would normally be considered the end point. Similar titration curves were recorded for a wide range of concentrations of ferric iron and methylene blue at several practical rates of generation of titanous ion. The inflection points of the recorded curves were always coincident with the intersection of the extrapolated lines.

From the shape of the titration curves it was considered feasible to terminate the titration at the inflection point by the second derivative technique, and thereby benefit by its features as previously described (*3*). The output of an absorbance photometer circuit was sent directly to the input of the commercially available Sargent-Malmstadt automatic derivative control unit (*8*).

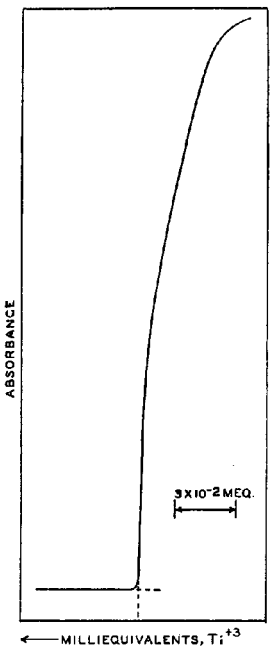

Figure 1. Recorded titration curve of absorbance *vs.* milliequivalents of trivalent titanium

665-mμ, 100-ma. electrolysis current

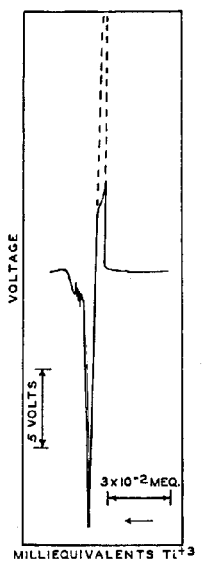

Figure 2. Recorded spectrophotometric second derivative curve from output of Sargent-Malmstadt control unit

The electronically computed second derivative curve operates a relay system and current relay to terminate the generation of titanous ion at the end point. A recorded second derivative curve from the recorder outlet of the Sargent-Malmstadt control unit is shown in Figure 2. The positive peak is cut off by grid current from the first relay thyratron tube (*7*), and dotted lines are used to show that a large positive pulse would be recorded if the first relay thyratron were removed. The titrations were invariably terminated at the same point of the titration curve.

APPARATUS

Automatic Titrator. The automatic derivative spectrophotometric titrator consisted of essentially the same basic components as described previously (*3*). As just noted, however, the second derivative technique was applicable for this specific titration so

that the resistance-capacitance differentiator between the photometer and control unit was not necessary (*3*).

The 10-second plug-in delay relay, *K*3, in the Sargent-Malmstadt standard control unit (*7*) can be changed to a 2-second relay for titration of small quantites of iron at relatively high currents, when the titration time may be less than 10 seconds. The delay tube, which prevents termination by the relay system until 10 seconds after the start of a titration, is useful in some potentiometric titrations where initial voltage changes at the start of a titration would falsely stop titrant flow; but for many spectrophotometric titrations the delay time can be cut down to 2 seconds, thereby permitting short duration titrations.

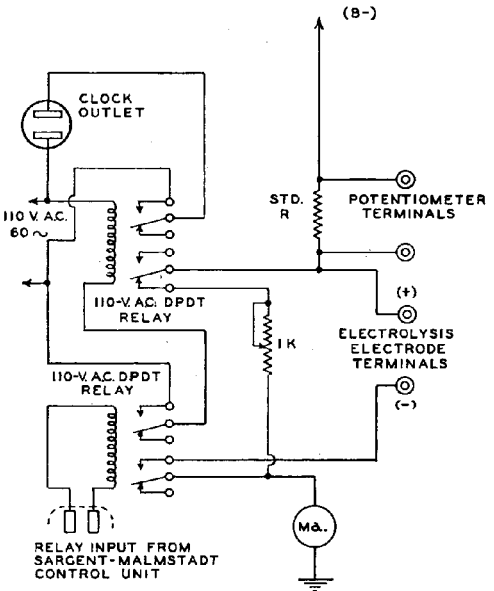

Figure 3. Current and clock relays in constant current source

The dual 110-volt alternating current output from the control unit, which normally goes to the stirring motor and buret solenoid unit of the Sargent-Malmstadt titrator, was fed to the stirring motor mounted on the cover (*3*) and the current and clock 110-volt alternating current relay input on the constant current source (Figure 3). Therefore, when the start button on the control unit is pushed, the stirring motor, the electrolysis current, and the timer clock are started. All three are automatically terminated at the end point by the second derivative signal, which causes inactivation of the 110-volt alternating current output from the control unit. However, for short duration titrations with the 2-second delay in the circuit, it is best to start the stirring motor with the switch at the rear of the automatic titrator a few seconds before pushing the automatic titration button. This provides complete mixing of the solution before starting the titration and eliminates interference from an initial voltage pulse which might occur because of incompletely mixed absorbing species in the solution at the start of the titration.

Constant Current Source. The constant current source used for this work was similar to one described by Reilley, Cooke, and Furman (*6*). The double-pole, double-throw switch in the original design was replaced by two double-pole, double-throw 110-volt alternating current relays, as shown in Figure 3. These relays could also be replaced by one special shorting-type relay, but the two inexpensive 110-volt alternating current double-pole, double-throw relays were readily available and worked well. All of the components in Figure 3 were enclosed in the cabinet with the power supply components. The potentiometer and electrode terminals were mounted on the front panel with the other controls. The 110-volt alternating current relay input and clock output sockets also were mounted on the front panel.

A modification of the original source (*6*) by Reilley, Adams, and Furman (*5*) provides continuously variable current from 0.6 to 150 ma. This is advantageous for routine titrations, because it is possible to adjust the current to an exact value so that the titration time is an even multiple of the concentration or percentage of the constituent being sought. In other words, the timer becomes essentially direct reading in terms of the answer.

The standard resistance was a calibrated 3.502-ohm resistor; the voltage across it was measured with a Leeds & Northrup K-2 potentiometer. The voltage across this standard resistance was measured during each titration for very accurate work, but only once or twice a day for routine work.

Generator Cathode. A platinum gauze electrode could be conditioned so that it would develop and maintain indefinitely a higher hydrogen overvoltage than that of clean platinum surface. The conditioned electrodes provided 100% efficient generation of titanous ion for the titration of ferric iron in sulfuric and fluoboric-sulfuric acid solutions.

The conditioning of the electrode was simple. A 50-ml. aliquot of 3*M* sulfuric acid solution containing about 20 mg. of titanium per ml. was oxidized to the +4 state with potassium permanganate, and the excess permanganate was reduced by sodium azide. The solution was made up to about 150 ml. with 4*M* sulfuric acid and boiled for 5 minutes to destroy excess azide. It was cooled to 75° C. and 1 ml. of methylene blue was added. The platinum gauze electrode was then placed in the solution and made the cathode in an electrolysis circuit, and a current of 100 ma. was maintained for a few hours.

During the conditioning process the surface of the electrode became coated with a dull gray-colored deposit, probably caused by methylene blue. Electrolysis of the acid titanium solution for 5 hours without methylene blue did not decrease the visible evolution of hydrogen from an unconditioned electrode, and a titration of a standard iron solution showed only 95% current efficiency. When methylene blue was added for a 1-hour electrolysis, no visible hydrogen was evolved and the electrode had a current efficiency of 99%. Electrolysis was continued for another hour, after which the electrode was 100% efficient for the titration of ferric iron with generated titanous ion. A current-voltage curve was made in 2.8*M* sulfuric acid with a microelectrode taken from a conditioned gauze electrode. The coating was then destroyed by anodic oxidation and another current-voltage curve was made in the same solution. A comparison of the two curves showed that evolution of hydrogen began 0.2 volt more positive with the unconditioned electrode than with the conditioned one.

A new platinum gauze electrode visibly evolves hydrogen gas and initially has a titanous generation efficiency of about 92%. At the end of 1 hour the titanous generation efficiency is about 99%, and at the end of 2 hours, 100%. Electrodes conditioned in this way maintain their higher hydrogen voltage indefinitely (one has been used daily for about 1 year) and continuously provide 100% efficient generation of titanous ion with no further care. Three different electrodes have been treated in this way and all have developed the higher hydrogen overvoltage. The increase in hydrogen overvoltage due to adsorption of organic material on the platinum electrode seems to be responsible for the increased efficiency of the conditioned electrode. The gray-colored deposit on the generator cathode can be scraped off with a knife blade or can be destroyed by using as a cathode in the electrolysis of chromic or permanganate solutions.

The cylindrical gauze electrode also provides a good stirring baffle to prevent gas bubbles from being whipped into the solutions at the fast stirring rate previously described (*3*). It was not necessary to rinse the electrode between titrations because the titrations were terminated at the end point.

Isolated Anode. The anode, a piece of platinum wire 0.5 mm. in diameter wound in a helix about $^3/_{16}$ inch in diameter, was inserted into an isolated anode compartment containing 0.1*N* sulfuric acid. The compartment was made from a piece of polystyrene tubing, $^3/_8$ inch in outside diameter, $^3/_{16}$ inch in inside diameter. A glass tube, 1 cm. in outside diameter and $^7/_8$ inch long, with a medium porosity sintered-glass disk on one end, was fastened with cement to one end of the polystyrene tube. The compartment was made in this way so that it could be screwed into the titration compartment cover (*3*) and fit inside the cylindrical gauze cathode.

Timer. A Time-It 110-volt alternating current stop clock (Precision Scientific Co., Chicago, Ill.), reading to the nearest 0.1 second, was used to time the titrations.

Inert Gas Tube. A hole ($^3/_8$ inch) was made in the top of the cell compartment and threaded so that a polystyrene tube of the same size could be screwed through the cover until it protruded slightly on the inside. Nitrogen gas was passed through this and into the cell compartment during the titration with electrolytically generated titanous ion. However, without an inert gas a relative positive error of only 0.2 to 0.3% was caused

by air oxidation with large amounts of iron, while with small amounts of iron the error was insignificant. For routine work the inert atmosphere would probably not be necessary.

REAGENTS

Titanium Sponge. Titanium sponge with low concentrations of iron and other impurities was obtained from Cramet, Inc., Chattanooga, Tenn.

Iron Solutions. Standard iron solutions containing 2.895, 2.940, and 8.370 mg. of iron per ml. were prepared by dissolving iron wire (99.97% pure, Mallinckrodt) in hydrochloric acid and diluting to volume.

Sulfuric Acid, reagent grade.

Fluoboric Acid, 45% reagent grade. The quantity of fluoboric acid used in dissolving the titanium samples contained an amount of iron equivalent to about 0.03% of the titanium sample. A blank correction should be determined for each bottle of acid.

Potassium Permanganate. A saturated solution was prepared from reagent grade potassium permanganate.

Sodium Azide, reagent grade.

Leuco Methylene Blue. A 0.02*M* solution of methylene blue in 2*M* hydrochloric acid was prepared. To obtain the reduced form, this solution was passed through a silver reductor, preferably just before use, to prevent air oxidation.

PROCEDURE

Sample Size. The weight of the titanium sample depends somewhat on the homogeneity of the sample, and also whether aliquots of one master sample are to be used for several different determinations. The sample size is generally 1 gram (*4*). However, for relatively nonhomogeneous samples larger amounts should be used. The larger samples are prepared to contain 20 mg. of sample per ml. of solution, so that a 50-ml. aliquot of these solutions contains 1 gram of sample. If the titanium ores or alloys contain large percentages of iron it is desirable to use smaller samples or aliquots in order to prevent long titration times at the currents available from the source.

Dissolution of Sample. Titanium sponge and alloys can be dissolved by adding about 30 ml. of 5*M* sulfuric to about 1 gram of sample, heating, and maintaining at a temperature just under boiling until all the sample is dissolved (3 to 5 hours). A more rapid dissolution is realized by adding 30 ml. of 5*M* sulfuric acid and 10 ml. of 45% fluoboric acid to a 1-gram sample and heating gently until the sample is dissolved (15 to 20 minutes). The fluoboric acid should, however, be tested for significant quantities of iron impurity. Sample solution can take place in the 200-ml. electrolytic beakers which are used for the titration.

Pretitration Treatment. After dissolution of the sample, 2 ml. of concentrated hydrochloric acid was added and the titanous ion and iron were oxidized by adding a saturated solution of potassium permanganate until a pink color appeared. Excess permanganate was destroyed by adding from a spatula about 0.3 gram of sodium azide, and the sample was diluted with an acid solution (4*M* in sulfuric acid) to within about ¾ inch from the top of the beaker. A glass hook and watch glass were placed on top of the cell and the solution was boiled gently for 5 minutes to expel oxygen or chlorine that might be present. The solution was cooled slightly (to about 75° C.) in a water bath, and 1 to 2 ml. of 0.02*M* leuco methylene blue was added. The outside of the titration beaker was wiped dry and placed in the titration cell compartment; the cover, containing the mounted stirrer and electrodes, was replaced.

Titration. The wave-length dial was set for 665 mμ. The start button on the Sargent-Malmstadt control unit was pressed, starting the generation of titanous ion, the timer, and the stirring. All three were automatically terminated at the end point.

Calculation.

$$\% \text{ Fe} = \frac{i \text{ (amp.)} \times t \text{ (sec.)} \times 55.85 \times 100}{96{,}494 \times \text{wt. of sample (grams)}}$$

For routine titrations it is desirable to select the current and/or the sample size so that the time in seconds on the timer is some simple multiple of the percentage concentration of iron.

EXPERIMENTAL RESULTS

Samples with Known Amounts of Iron. The precision and accuracy of the method were tested both by adding known amounts of iron to a titanium solution already containing a known small quantity of iron and by checking titanium samples issued by Watertown Arsenal. The results of the latter samples were checked against the results obtained by other laboratories using other methods.

Samples of 40 grams of titanium sponge were dissolved in sulfuric or fluoboric-sulfuric acid solution and diluted to 2 liters. A 50-ml. aliquot, therefore, corresponded to 1 gram of sample. The sponge contained 0.03% iron, which had been determined by the colorimetric procedure (*4*). Aliquots from four different batches of dissolved samples were automatically titrated by the derivative technique; the results are presented in Table I.

Currents of either 50 or 70 ma. were used, but at the lower current sharper end points and more precise results were obtained by adding 5 ml. instead of the usual 1 to 2 ml. of 0.02*M* leuco methylene blue. The additional quantity of leuco methylene blue was used only for the sponge samples containing small amounts of iron. The 0.3 mg. (approximate) of iron in the sponge samples was in about 150 ml. of solution, so that the concentration was about 2 p.p.m.

Known amounts of iron were then added to 50-ml. aliquots of the sponge solutions for which the residual iron had been determined. The solutions were given the usual pretitration treatment. In all determinations 1 to 2 ml. of leuco methylene blue was used with an electrolysis current of 100 ma. The automatic titration results for solutions to which various amounts of iron were added are shown in Table II.

Table I. Determination of Iron in Titanium Sponge Samples[a]

	% Fe in Solution[b]			
Aliquot	1	2	3	4
1	0.029	0.034	0.029	0.035
2	0.028	0.035	0.027	0.032
3			0.036	0.028
4				0.027
5				0.028
6				0.032
7				0.029
8				0.035
Av.	0.029	0.035	0.031	0.031
Std. dev.				0.003

[a] Colorimetric determination (*4*) gave 0.03% iron.
[b] 1, 2, and 4 dissolved in sulfuric acid; 3 dissolved in fluoboric-sulfuric acid.

Table II. Automatic Titration of Titanium Solutions Containing Added Iron

Iron, Mg.		
Added	Found	Error
2.94	2.95	+0.01
2.94	2.89	−0.05
2.94	2.93	−0.01
5.88	5.90	+0.02
5.88	5.90	+0.02
14.67	14.66	−0.01
14.67	14.66	−0.01
58.80	58.72	−0.08
58.80	58.78	−0.02
58.80	58.75	−0.05
58.80	58.80	0.00
167.30	167.24	−0.06

If it is assumed that this quantity of iron was present in 1 gram of titanium sample, the range covered represents about 0.3 to 17% iron.

The precision and accuracy of the method were also tested by adding successive 10-ml. aliquots of a standard ferric solution to a titanium sponge solution which had been automatically titrated to the end point of the residual iron. After each end point, about 10 ml. of solution was withdrawn and another 10-ml. aliquot of standard ferric solution was added and then auto-

matically titrated. The results are shown in Table III. The precision obtained is comparable to the precision of delivery from the 10-ml. pipet.

Watertown Arsenal Titanium Samples. Five titanium samples prepared by Watertown Arsenal and issued to the panel on methods for iron were obtained and determined by the automatic derivative titration procedure described. In addition to the results shown in Table IV, three 1-gram samples of WA-29 were determined and gave an average of 0.100% iron.

Table III. Automatic Titration of Successive Aliquots of Standard Ferric Iron Added to Same Titanium Solution

Iron, Mg.	
Added	Found
28.95	28.98
28.95	29.00
28.95	28.83
28.95	28.92
28.95	28.86
28.95	28.93
28.95	29.00
Av.	28.93
Std. dev.	0.05
Coef. of var., %	0.2

Table IV. Determination of Iron in Watertown Arsenal Samples

(All aliquots are 50 ml.)

Determination No.	WA 20[a]	WA 21[b,c]	WA 29	WA 35	WA 36[a]
	% Iron in Sample[d]				
1	0.369	4.832	0.111[e]	2.267[a]	1.717
2	0.375	4.869	0.108[e]	2.263[a]	1.714
3	0.372	4.856	0.110[e]	2.269[a]	1.715
4			0.116[e]	2.273[a]	
5			0.111[e]	2.274[b]	
6			0.111[b]	2.265[b]	
7			0.109[b]	2.290[b]	
Av.	0.372	4.852	0.111	2.271	1.715
Std. dev.	0.003	0.017	0.003	0.009	0.002

[a] Aliquot of 10-gram sample.
[b] Aliquot of 5-gram sample.
[c] Pretreated for removal of molybdenum.
[d] Samples dissolved in fluoboric-sulfuric acid.
[e] Aliquot of 20-gram sample.

The results of the 1,10-o-phenanthroline and the sulfide separation–dichromate titration procedures as performed by several other companies were made available after the results shown in Table IV had been obtained. The averages of the average results from the other laboratories are as follows:

WA Sample	Av. % Fe	No. of Laboratories
Sulfide Separation–Dichromate Titration (4)		
20	0.36	3
21	4.87	3
29	0.105	3
35	2.27	4
36	1.714	2
Colorimetric Procedure		
20	0.35	4
21	5.04	4
29	0.092	3
35	2.34	4
36	1.73	5

Interferences. Many of the elements sometimes present in titanium sponge or alloys were checked as possible interferences in the determination of iron. Of the elements checked, cobalt, nickel, tungsten, chromium, manganese, magnesium, zirconium, tin, and aluminum did not interfere, even in quantities as large as 5 to 10%. Aluminum lowered the iron results by about 0.3% relative when present in large amounts, but this was considered as negligible interference. Colored ions such as chromic do not interfere in the derivative technique, even though the absorbance at the end point varies from sample to sample.

Vanadium, molybdenum, and copper interfered in the rapid automatic iron titration procedure. Of these three, vanadium and copper are seldom present in titanium alloys, but molybdenum is present in some alloys. Fortunately, both molybdenum and copper can be separated effectively by a simple and relatively rapid sulfide precipitation.

The titanium solution was oxidized by potassium permanganate, and excess oxidant reduced with sodium azide. Hydrogen sulfide gas was bubbled vigorously through the solution for 5 minutes to precipitate the molybdenum and copper sulfides. The solution was boiled 1 minute to make the precipitate more suitable for filtering, cooled slightly, and filtered through a 65-mm. Büchner funnel using vacuum. The filter paper was washed with three portions of 1*M* sulfuric acid saturated with hydrogen sulfide, and the filtrate was boiled an additional 3 minutes to remove most of the hydrogen sulfide. The sample was cooled, then oxidized with potassium permanganate, and titrated by the regular automatic derivative spectrophotometric iron procedure.

The acid strength at which the molybdenum sulfide was precipitated was not ideal for quantitative separation of the molybdenum. That some molybdenum remained in solution was indicated by the slightly high results for the iron determinations after molybdenum separations. Several titanium samples containing 14.80 mg. of iron were repeatedly 0.20 mg. too high. The error was reproducible; therefore, a correction factor was applied for the iron determinations when the molybdenum separation was required. A titanium alloy (WA 21) containing about 4% molybdenum was treated by the rapid sulfide precipitation procedure; the iron results were close to the average of those obtained in other laboratories by different procedures.

Because no alloy containing copper was available, copper sulfate was added to the solution in an amount corresponding to 1.5% of the sample. The result obtained after copper removal by sulfide precipitation was 0.16 mg. greater than the theoretical amount of 14.80 mg. of iron present, indicating a small positive error in this case also.

No method was found for determining iron with good accuracy in the presence of significant amounts of vanadium. The few alloys which at present contain vanadium have such small amounts that it would not interfere in the iron determination. If iron must be determined in the presence of vanadium, it would probably be best to use another procedure (*4*).

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Flame Photometric Determination of Lithium, Rubidium, and Cesium in Silicate Rocks

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For the flame photometric determination of the trace alkali elements, lithium, rubidium, and cesium, in silicate rocks, the alkali metals and magnesium are separated from the other rock constituents by sulfuric acid and hydrofluoric acid decomposition followed by precipitation of the ammonia group with solid calcium carbonate. Calcium is removed from the filtrate by precipitation of the sulfate in 50% ethyl alcohol. The alcohol is removed from the final filtrate by evaporation and the salts are dissolved in water. Flame interference from sodium and potassium is compensated by use of appropriate standard solutions. The method shows a precision and accuracy within 10% in the range from 10 to 200 p.p.m. of the alkali metal and is sensitive to 5 p.p.m. of lithium, 10 p.p.m. of rubidium, and 5 p.p.m. of cesium.

THE determination of the trace alkalies in silicate rocks has been restricted to optical spectrographic techniques until the recent application of radioactivation (*5, 6*), mass spectrometer (*4*), and flame photometric methods. Flame spectrophotometric techniques for the determination of lithium have been discussed by Ellestad and Horstman (*2*). Williams and Adams (*7*) determined rubidium and cesium in glasses in amounts and associations quite different from natural silicate materials. The purpose of the present investigation was to develop a rapid flame photometric procedure for the determination of lithium, rubidium, and cesium on a single sample with accuracy suitable for geochemical interpretation. The method described here has been used for such determinations in a large number of igneous and sedimentary rocks. Results of this study will be published elsewhere.

An acid decomposition method is preferred because of rapidity. The removal of the combined oxide group and calcium from the solution obtained from the decomposition is essential when determining traces of alkali metals. Difficulty in removing ammonium sulfate from the final solution eliminates any method using an ammonium hydroxide precipitation. The ammonium ion has a serious quenching effect on alkali emission.

The method of Ellestad and Horstman (*2*), although satisfactory for lithium, shows serious losses of sodium, potassium, and rubidium caused by occlusion and adsorption of the alkalies by the lead sulfate precipitate. To prevent these losses calcium carbonate is substituted for the lead carbonate, the more soluble precipitate preventing much of the contamination. Aging the precipitate further reduces loss of the alkali metals.

Use of calcium carbonate to precipitate the ammonia group raises the calcium content of the filtrate to the point where it interferes spectrally with the determination of lithium. Calcium is removed from the solution by precipitation as the sulfate in 50% ethyl alcohol. The small amount of alkali sulfates lost through coprecipitation is reduced by aging overnight. This precipitation must be carried out at a pH of 7.6 to 7.8 to minimize coprecipitation of the less soluble alkali sulfates. Magnesium sulfate accompanies the alkali sulfates in the final solution, but does not interfere with the determination of the alkalies in the low-temperature flame.

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APPARATUS AND REAGENTS

A Beckman Model DU spectrophotometer was used with an air–natural gas flame attachment described by Ellestad and Horstman (*2*).

Standard Solutions. LITHIUM SULFATE. This was prepared and standardized according to Ellestad and Horstman (*2*), except that the quantities were halved. The solution prepared in this manner contains approximately 700 p.p.m. of lithium.

RUBIDIUM SULFATE. Weigh out 1.4425 grams of rubidium sulfate, dissolve in water, and dilute to 500 ml. Determine the potassium content of the solution by adding known amounts of standard potassium sulfate solution to the rubidium solution. Determine an emission value for each potassium concentration and extrapolate the resultant emission–potassium concentration curve back to zero. Then determine the rubidium content of the solution by subtracting the weight of potassium found from the original weight of the salt. This solution contains approximately 1800 p.p.m. of rubidium.

CESIUM SULFATE. Weigh out approximately 1.2 grams of cesium nitrate in a 50-ml. platinum dish, dissolve in distilled water, and add a slight excess of sulfuric acid. Evaporate to dryness and bring the sulfate to constant weight by repeated heating to dull redness over a low flame. Dissolve the salt in distilled water and dilute to 500 ml. Determine the weight of the sulfate by drying and weighing the empty dish. It is extremely difficult to remove the excess sulfuric acid from the large amount of salt during the conversion from nitrate to sulfate, and some losses by decrepitation are likely to occur. For this reason it is necessary to know the final weight of the converted salt. The solution prepared as described contains approximately 1900 p.p.m. of cesium.

Calcium Carbonate. Analytical reagent, low in alkalies.

Ethyl Alcohol, 95%.

PROCEDURE

Weigh out a 0.5-gram sample and transfer to a 50-ml. platinum dish. Moisten the sample with water and add 0.8 ml. of 18*N* sulfuric acid. Add 2 drops of 16*N* nitric acid and 10 to 15 ml. of 48% hydrofluoric acid. Evaporate, with occasional stirring, to fumes of sulfuric acid. Cool the residue and take up in a few milliliters of water. Add 0.1 ml. of 36*N* sulfuric acid, and evaporate the solution to fumes of sulfuric acid. Add 5 ml. of water and carry out a third evaporation. Multiple evaporations are required to remove the last traces of fluoride. Cool the residue and dissolve in 25 ml. of water. Transfer to a 150-ml. beaker and dilute to 70 to 80 ml. Add several drops of bromothymol blue indicator and neutralize the solution by the addition of solid calcium carbonate. Allow the resulting precipitate to stand overnight to minimize contamination and consequent loss of the alkalies.

Heat the solution with the precipitate to boiling and filter through an 11-cm. medium-grade filter paper, washing the precipitate with hot water until the volume of the filtrate and washings is 150 ml. Evaporate the combined filtrate and washings to 50 ml. Cool and add 50 ml. of 95% ethyl alcohol to precipitate calcium sulfate. Allow the precipitate to stand overnight and filter on an 11-cm. medium-grade filter paper. Wash the precipitate with a 1 to 1 solution of ethyl alcohol and water until the volume of the filtrate and washings is about 150 ml. Evaporate the filtrate and washings to dryness on a steam bath. Bring the residue into solution with a few milliliters of water, transfer to a 50-ml. volumetric flask, and dilute to volume.

INTERFERENCES

In the determination of the trace alkalies three important types of interference are encountered. Continuum or background interferences are positive and arise from continuous spec-

Table I. Recovery of Trace Alkali Metals

Lithium, P.P.M.				Rubidium, P.P.M.				Cesium, P.P.M.			
Present	Added	Total	Found	Present	Added	Total	Found	Present	Added	Total	Found
17	7	24	22	205[a]	18	223	218	0	10	10	10
	14	31	30		37	242	257		21	21	21
	71	88	87		55	260	267		31	31	25
				154	4	158	158		42	42	43
					4	158	151		52	52	56
					18	172	174		62	62	68
					37	191	188				
					55	209	220				
					73	227	204				
					110	264	263				
					146	300	302				
					256	410	424				
					366	520	513				

[a] Granite, G-1.

Table II. Comparison of Results by Different Decomposition and Separation Procedures

Lithium, P.P.M.		Rubidium, P.P.M.	
Present method	Acid decomposition (2)	Acid decomposition	J. Lawrence Smith method
23	24	119	146
29	19	137	165
28	31	119	110
143	147, 159	150	147
59	59	204[a]	205[a]
212	216	18[b]	18

[a] Average of six determinations.
[b] Average of five determinations.

tral emission by the major alkalies and the solvent. Radiation interference involves the depression or enhancement of the emission from the measured constituent by another constituent and results in either a positive or a negative error. Line spectral interference is caused by the proximity of the spectral lines of two elements, the radiation from each contributing to the other and giving a positive error. The interference of sodium and potassium in the lithium determination has been investigated previously (*2*). Approximate determinations of the alkali elements are made by using calibration curves and correcting for continuum interference. Continuum and line spectral interferences and radiation effects for lithium, rubidium, and cesium are compensated by adding appropriate amounts of interfering elements to the standard solutions. The sample is compared directly to these standards.

PRECISION AND ACCURACY

The sensitivity of the instrument at the settings used (full sensitivity; 0.4-mm. slit, 671 mμ for lithium; 0.15-mm. slit, 795 mμ for rubidium; 0.2-mm. slit, 852 mμ for cesium) is 0.02 p.p.m. of the metal in solution, corresponding to 0.0002% metal in the sample. Because of the magnitude of errors in the decomposition and separation, this sensitivity is of use only in the determination of lithium.

No standard samples were available for analysis, and the only check on the method was the recovery of known amounts of the metal added to a sample on which replicate determinations had been made. The average value obtained without addition of the metals sought was used as the true value. Rubidium values obtained by a J. Lawrence Smith decomposition and a chloroplatinate separation were also compared to those obtained by acid decomposition. Lithium values were compared with those obtained by a slightly different method (*2*). These results are given in Tables I and II.

A granite (G-1) and diabase (W-1) (*3*) were run as an additional check. These samples have been analyzed by a number of investigators using different methods (*1, 2, 4, 5*). As shown in Table III the flame and optical spectrographic determinations of lithium compare favorably, but serious differences were found for rubidium. The flame determinations of rubidium compare favorably with the results obtained by the isotope dilution and neutron activation methods. The precision of the flame spectrophotometric determinations with different methods of separation (Table II) and in recovery of known amounts of rubidium added (Table I) indicates a serious error in the determination of rubidium with the optical spectrograph. No figures are available for comparison of cesium determinations.

Table III. Determinations of Lithium and Rubidium in Granite (G-1) and Diabase (W-1) by Different Methods

Analyst	Method	G-1	W-1
		Lithium, P.P.M.	
Present method	Flame spectrophotometer	22	14
Ellestad, Horstman (*2*)	Flame spectrophotometer	24	14
Mitchell (*1*)	Optical spectrograph	19	9
Gorfinkle, Ahrens (*1*)	Optical spectrograph	23	9
Nockolds (*1*)	Optical spectrograph	25	20
		Rubidium, P.P.M.	
Present method	Flame spectrophotometer	205[a]	19[c]
		204[b]	25[d]
Ahrens (*1*)	Optical spectrograph	550	64
Mitchell (*1*)	Optical spectrograph	590	15
Nockolds (*1*)	Optical spectrograph	250	20
Herzog, Pinson (*4*)	Stable isotope dilution	215, 218	27.9, 29.1
Smales (*5*)	Neutron activation	221, 254, 243	27, 29, 26

[a] Average of six determinations, chloroplatinate separation.
[b] Average of six determinations, acid decomposition.
[c] Average of five determinations, acid decomposition.
[d] Single determination, chloroplatinate separation.

The maximum deviation of a result from the average of two or more results is about 10% of the amount present in the range up to 200 p.p.m. of the metal. Above this range the maximum deviation drops to less than 5% of the amount present. The method is considered accurate to 5 p.p.m. of lithium and cesium and 10 p.p.m. of rubidium over the range from 10 to 200 p.p.m. of the metal.

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Direct Photometric Determination of Aluminum in Iron Ores

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Gravimetric determinations of aluminum in iron ores are inaccurate, slow, and tedious. A specific spectrophotometric method is described which is fast, direct, and accurate. A sodium carbonate fusion of the sample is dissolved in hydrochloric acid, diluted to volume, and an aliquot is taken for a blank and sample. Sodium mercaptoacetate is added to the sample to form a colorless complex with the iron and sodium Versenate is added to the blank. Ammonium acetate buffer and stabilized Eriochrome Cyanine R dye are added to both solutions. The red Eriochrome Cyanine R–aluminum complex which forms in the sample is measured against the blank at 535 mμ. Beryllium, background color of the dye, and other interfering elements are compensated for in the blank. When measured in a narrow-band instrument, the complex obeys Beer's law up to 10% aluminum or higher.

A RAPID and specific method is needed for the determination of aluminum in iron ores and other ferrous metallurgical products. The shortcomings of the usual phosphate gravimetric procedure for aluminum in iron ores have been pointed out by Saxer and Jones (*18*). Numerous photometric methods have been described (*9, 10, 15, 16*), but tedious and time-consuming separations of iron and other interfering elements make these procedures unattractive for routine use.

Eegriwe (*3*) used a dye, Eriochrome Cyanine R, synthesized by Conzetti (*2*) for the colorimetric determination of aluminum. This method was critically studied by Millner and Kunos (*13, 14*); their improved procedure has been widely applied. Iron and other interfering elements must be removed, as demonstrated by a recent application of the method to the determination of aluminum in galvanized coatings and steel by Ikenberry and Thomas (*9*).

Mayr and Gebauer (*12*) employed thioglycollic acid to hold iron in solution when making an ammoniacal separation of aluminum. Their gravimetric procedure has been studied by Hutchinson and Wollack (*8*) and Hummel and Sandell (*7*). The latter point out that both titanium and phosphate accompany aluminum, but do not mention other possible interferences. Chenery (*1*) employed thioglycollic acid in an aurintricarboxylic acid method for aluminum, but limited his study to the interference of iron, magnesium, and phosphorus. Chenery's method has been applied to the determination of aluminum in bronzes and other nonferrous alloys by Sherman (*20*), Luke and Braun (*11*), and Pellowe and Hardy (*15*). The earlier work of Richter (*16, 17*) with thioglycollic acid has been overlooked by Chenery (*1*) and others. Richter, using Eriochrome Cyanine R, was unsuccessful in eliminating interfering elements except by a chemical separation. Richter's procedure has been applied to refractories by Glemser, Raulf, and Giesen (*4*).

In the method described, the chemical properties of Eriochrome Cyanine R have been controlled so that the reagent is made specific for aluminum by the combined use of sodium mercaptoacetate, sodium Versenate, 8-quinolinol, or a fluoride. The method is accurate and simple to apply. A single aluminum determination, including sample preparation, may be carried out in 20 minutes or less.

EXPERIMENTAL

Apparatus. Beckman DU spectrophotometer.

Reagents. Eriochrome Cyanine R, 0.075%, General Dyestuff Corp. A 0.0075% solution at pH 6.0 should have an absorbance of 1.0 in a 1-cm. cell at 438 mμ. Dissolve 0.75 grams of Eriochrome Cyanine R in about 200 ml. of water, add 25 grams each of sodium chloride and ammonium nitrate, add 2 ml. of nitric acid (specific gravity 1.42), dilute to 1 liter, and mix.

Ammonium acetate buffer solution, pH 6.0, 32%. Dissolve 320 grams of ammonium acetate in sufficient water, add 5 ml. of glacial acetic acid, and dilute to 1 liter.

Sodium mercaptoacetate solution, 0.3%. Eastman Kodak Co. Dissolve 1.5 grams of sodium mercaptoacetate in water, add 50 ml. of 95% ethyl alcohol, and dilute to 500 ml. The solution deteriorates on standing and should be renewed every 3 days.

Standard aluminum solution. Dissolve 0.5291 gram of pure aluminum in 15 ml. of hydrochloric acid, and dilute to 1 liter. One ml. = 8 γ of Al_2O_3.

Sodium Versenate solution, 0.04%, Hach Chemical Co. Dissolve 0.4 gram of disodium dihydrogen Versenate [(ethylenedinitrilo)tetraacetate] in water, add 50 ml. of 95% ethyl alcohol, and dilute to 1 liter.

Standard iron blank solution. Dissolve 0.100 gram of pure iron in hydrochloric acid and fuse 2 grams of sodium carbonate in a platinum crucible. Take the iron salts down to dryness and take up in 30 ml. of 1 to 2 hydrochloric acid. Dissolve the fused carbonate in this solution and boil out the carbon dioxide. Cool and dilute to 250 ml.

Sodium fluoride solution, 2.4%. Dissolve 24 grams of sodium fluoride in water and dilute to 1 liter.

8-Quinolinol, 1%. Dissolve 1 gram of 8-quinolinol in 100 ml. of carbon tetrachloride.

Procedure. Transfer 0.2000 gram of iron ore (100-mesh) to a platinum crucible, add 2 grams of sodium carbonate, and mix and fuse for 10 or 15 minutes over a Meker burner at full blast. Place the cooled crucible in a 250-ml. beaker and add 30 ml. of 1 to 2 hydrochloric acid. When the fusion is dissolved, remove the crucible and rinse into the beaker. Boil to liberate carbon dioxide, cool, and dilute to 250 ml.

Place two 1-ml. aliquots (2 ml. for aluminum concentrations below 0.5%) in two 50-ml. volumetric flasks. Add to the sample 25 ml. of 0.3% sodium mercaptoacetate solution, mix well, and to the blank add 25 ml. of 0.04% sodium dihydrogen Versenate solution. To the sample and blank add 5 ml. of 0.075% Eriochrome Cyanine R solution and mix. To each flask add 5 ml. of ammonium acetate buffer (pH 6.0), dilute to the mark, and mix. Measure the absorbance at 535 mμ in a 1-cm. cell with a slit width of 0.025 to 0.04 mm. To estimate vanadium, prepare a sample as for aluminum, add 2 ml. of 2.4% sodium fluoride, dilute to the mark, and compare against the iron blank. Alternatively destroy the vanadium complex in the sample with a few drops of 8-quinolinol, using mercaptoacetate solution containing no alcohol.

From a previously prepared calibration curve obtain the percentage of aluminum or aluminum oxide in the sample. Determine a reagent blank using the iron blank in place of the sample, and correct if necessary. As the three interfering elements are usually present in trace amounts, the aluminum complex in the sample may be measured against the iron blank directly.

Preparation of Calibration Curve. Transfer 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 ml. of standard aluminum solution in duplicates to 50-ml. volumetric flasks. To each flask add 1 ml. of standard iron solution. Determine aluminum as in the procedure for iron ores.

DISCUSSION

Absorption Spectra and Combining Ratio. The absorption spectrum of the Eriochrome Cyanine R–aluminum complex is shown in Figure 1. Eriochrome Cyanine R dyes differ in the concentration of the chromophore group, which has an absorption maximum at 438 mμ. The Eriochrome Cyanine RA dye used in this work had an absorbance index of 12.5 at pH 5.85, compared to 0.92 for a dye similar to one employed by Richter. The dye with the low chromophore concentration formed weakly bonded metal complexes at low pH values. The iron complex, for instance, was quickly destroyed with a few milliliters of 0.33% sodium mercaptoacetate, while it was difficult to destroy the iron complex formed with a dye having a higher concentration

of a chromophore, even with a large excess of mercaptoacetate. When these dyes were added in the ratio of their chromophore concentration, analytical results were identical.

The chromophore group enters into a stoichiometric combination with the aluminum ion to form a complex having an absorption maximum at 535.5 mμ, as is shown in Figure 1. Figure 2 compares the absorption spectra of the dye and the aluminum complex at 440 mμ. The chromophore group is depleted to form the aluminum complex.

Figure 3 shows calibration curves for the Eriochrome Cyanine R dye and the aluminum complex at 435 mμ, both plotted in micromoles at pH 6.0.

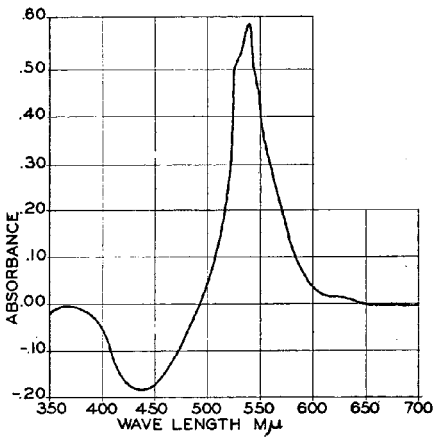


Figure 1. Absorption spectrum of aluminum–Eriochrome Cyanine R complex measured against iron blank

The dye curve was obtained by pipetting aliquots from a standard dye solution, adding 5 ml. of ammonium acetate buffer, and measuring the absorbance against water at 435 mμ in a 1-cm. cell at a slit width of 0.025 mm. The aluminum calibration curve was obtained by pipetting standard aluminum solutions into a 50-ml. volumetric flask, adding 5 ml. of 0.075% Eriochrome Cyanine R solution and 10 ml. of 0.33% alcoholic mercaptoacetate solution, diluting to the mark, and mixing. Like amounts of dye and mercaptoacetate were added to a separate flask to serve as a blank. Absorbance of the blank was measured against the aluminum complex at 435 mμ, using a slit width of 0.025 mm. and 1-cm. cells. Appropriate sensitivity settings and a photomultiplier tube were used. The absorbance difference between the aluminum complex and the blank is a measure of the amount of dye combined with the aluminum.

Thus, 1.11 μmoles of aluminum, 7 μmoles of dye, 10 ml. of 0.33% alcoholic mercaptoacetate, and 5 ml. of ammonium acetate were placed in a 50-ml. volumetric flask, diluted to the mark, and mixed. Like amounts of reagents were placed in another 50-ml. volumetric flask to serve as a blank. The two solutions were placed in matched 1-cm. cuvettes, the instrument was balanced against the aluminum complex, and an absorbance reading of 0.495 was obtained on the cuvette containing the blank solution.

From the aluminum calibration curve it may be seen that this reading is equal to 1.11 μmoles, the expected value; referring the same reading to the dye calibration curve it may be seen to represent 3.30 μmoles of Eriochrome Cyanine R. This is in rather good agreement with the theoretical value of 3.33 μmoles for $Al(ER)_3$ and substantiates the combining ratio for this complex suggested by Millner (*13*). ER is used to designate one mole of dye.

This 1 to 3 ratio of aluminum to dye is maintained only when an excess of the dye is present. It may be seen from Figure 4 that when the dye concentration is lowered below 0.005%, equivalent to 4.65 μmoles in 50 ml. in equilibrium with 10 γ or 0.37 μmoles of aluminum, the absorbance of the aluminum complex is lowered in straight-line segments. Millner (*13*) has obtained three such segments by maintaining a constant dye concentration and varying the amount of aluminum. These he interpreted as being $Al(ER)^{++}$, $Al(ER)_2^+$, and $Al(ER)_3$ complexes of Eriochrome Cyanine R. By measuring the amount of dye depletion at 435 mμ at the two inflections of the absorption curve of Figure 4 it was determined that only the $Al(ER)_2^+$ and $Al(ER)_3$ complexes are formed under the present condition. An absorption spectrum of $Al(ER)_2^+$ did not show any significant differences when compared to the $Al(ER)_3$ complex, except that the absorption of $Al(ER)_2^+$ is slightly higher per mole of combined ER. This may be seen in Figure 4. If equal absorbance per mole of combined ER is assumed, the value of the inflection point for $Al(ER)_2^+$ would be two thirds of that for $Al(ER)_3$. It may be seen to be 0.457 rather than the theoretical 0.402.

Four inflection points on an absorbance curve, such as those shown in Figure 4, have been obtained with the yellow silicomolybdate complex when it was stabilized with cane sugar (*5*), apparently in harmony with the four valences of silicon.

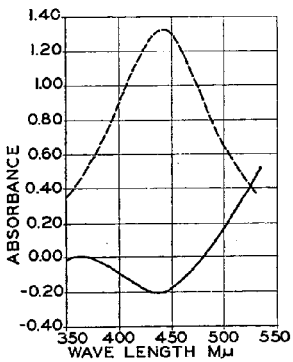


Figure 2. Absorption spectra of Eriochrome Cyanine R and aluminum complex

Showing depletion of dye by aluminum at 435 mμ
– – – Eriochrome Cyanine R at pH 5.85 in iron blank against water
—— Aluminum complex against blank

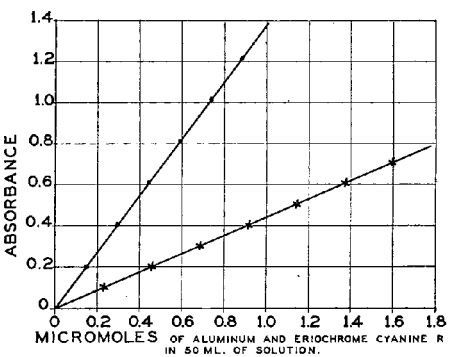


Figure 3. Molar relationship of aluminum and combined Eriochrome Cyanine R at pH 6.0

● Aluminum present, negative absorption at 435 mμ
× Eriochrome Cyanine R × 10 at 435 mμ and pH 6.0

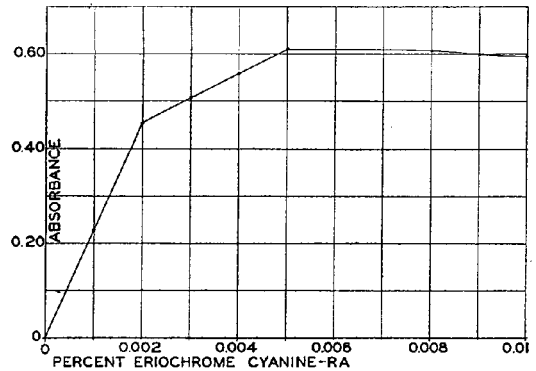


Figure 4. Effect of Eriochrome Cyanine R concentration on absorbance of aluminum complex

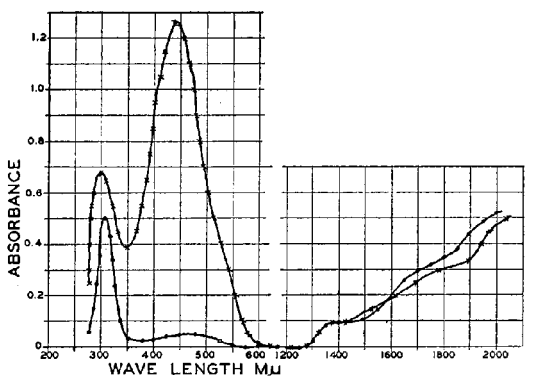


Figure 5. Absorption spectra of reduced and oxidized Eriochrome Cyanine R at pH 6.0

● Reduced Eriochrome Cyanine RA
× Oxidized Eriochrome Cyanine RA

Chemical Properties of Eriochrome Cyanine R. The essential chromophore group is readily destroyed by numerous reducing compounds. Figure 5 shows the effect of sodium bisulfite on the chromophore group. The essential portion of the dye has been almost completely destroyed by the sulfite. Only trace amounts of sodium hydrosulfite are required for the same effect, while other reducing compounds such as sodium mercaptoacetate, hydroxylamine hydrochloride, or hydrogen sulfide have a lesser effect, depending upon the pH level of the solution. The reduction is more rapid at the lower pH values. Some dyes, such as those reported by Richter (*16*), undoubtedly contain reducing compounds as impurities, requiring long aging periods for stabilization of the chromophore group. The reduction of the chromophore group is a reversible reaction. Thus, when an aluminum complex was destroyed by the addition of sodium hydrosulfite, it was completely restored by the addition of a drop or two of hydrogen peroxide. It is obvious that the preparation of an Eriochrome Cyanine dye having little or no background color is not possible, as has been anticipated by some investigators (*9*, *21*).

The chromophore group is most stable in an oxidizing medium and, as the stability of the aluminum complex was found to be directly dependent upon maintaining a constant concentration of the chromophore, numerous methods were investigated for obtaining a stabilized solution.

By the reduction of the dielectric constant of the solution with ethyl alcohol, some improvement in stability of the dye was obtained. The most satisfactory method, however, was the addition of sodium chloride, ammonium nitrate, and nitric acid to the dye solution. At the concentration suggested in the procedure, no oxidation of iron or destruction of mercaptoacetate took place in amounts sufficient to disturb the formation of a reproducible aluminum complex.

Interfering Elements, Chemical Equilibrium, and Dye Concentration. The formation and the absorbance of many metal–Eriochrome Cyanine R complexes were found to be related to the ratio of the concentration of the chromophore group to the metal at a given pH value. Thus, at a 12.5 absorbance index of the dye, zirconium formed a complex which was stable in the presence of excess sodium Versenate; at one half of this concentration of the chromophore, no complex was formed even in the absence of Versenate. Under the above conditions the absorbance of the vanadium complex proved to be directly proportional to the dye concentration.

Even zinc was found to form an Eriochrome Cyanine complex, if the ratio of the metal to the dye was high enough. This formation of Eriochrome Cyanine R–metal complexes at high equilibrium constants limited the determination of small amounts of aluminum in large amounts of iron at a high dye concentration. In such cases it became expedient to reduce the amount of dye to obtain a satisfactory aluminum complex without resorting to a chemical separation.

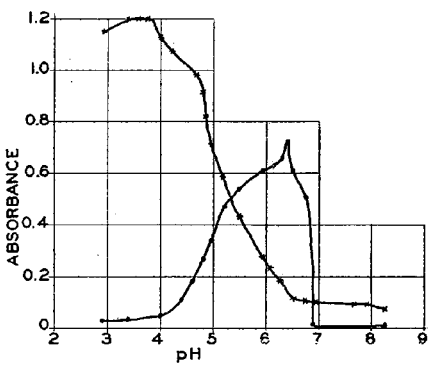


Figure 6. Effect of pH on absorbance of aluminum complex and Eriochrome Cyanine R at 535.5 mμ

● Aluminum complex
× Eriochrome Cyanine R

The dye concentration chosen for the final procedure was such that only beryllium, aluminum, vanadium, and zirconium formed complexes. Only the beryllium and zirconium complexes formed in the presence of sodium Versenate, while only the vanadium complex formed in the presence of a fluoride. By varying the dye concentration it has been possible to differentiate between beryllium and zirconium. The vanadium complex is unique, in that it has twice the absorbance in the presence of aluminum or iron as in solution free of these elements. Addition of a fluoride has the same effect on the vanadium complex as the removal of both aluminum and iron.

The following oxides, representing 10% of the sample weight, were added as impurities to a standard ore and carried through the procedure as given, except that a 0.1% Chromoxane Cyanine R reagent was used with no added stabilizer or alcohol.

WO_2, ThO_2, NiO, CoO, UO_2, ZrO_2, TiO, B_2O_3, BeO, TeO_2, CsO_2, CeO_2,

CaO, MgO, As_2O_5, Sb_2O_5, CuO, TaO_2, InO, Li_2O, Mo_2O_3, BaO, Cr_2O_3, GeO, SnO_2, CdO, BiO_2, V_2O_5, SeO_2, P_2O_5, SiO_2 (24%), Mn_2O_3 (28%), NbO, PbO, ZnO.

They produced no deviations greater than ±0.03%. Niobium caused a negative error of 0.10% by coprecipitation, but the aluminum was recovered by filtering, igniting, and re-fusing the residue. Vanadium produced a colored complex, which was quantitatively destroyed by the addition of 8-quinolinol dissolved in chloroform. Beryllium and zirconium produced colored complexes which, for analytical purposes, could be destroyed with a fluoride. Addition of Versenate destroyed all known complexes except those of beryllium and zirconium.

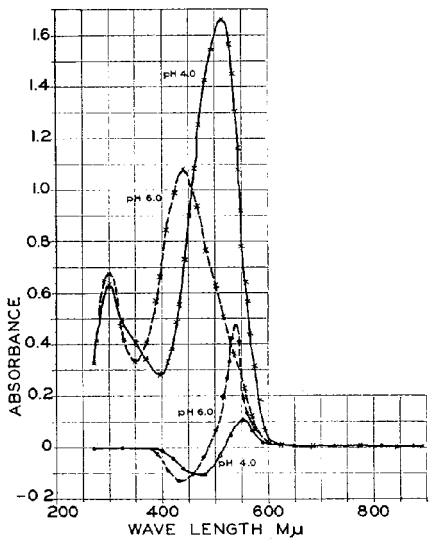


Figure 7. Absorption spectra of aluminum complex and Eriochrome Cyanine R at pH 4.0 and 6.0

● Aluminum complex
× Eriochrome Cyanine RA

These complexes were then used as a compensating blank, as well as a means of determining beryllium and zirconium. A fluoride blank was used for estimating vanadium. Alternatively the vanadium complex was destroyed by a carbon tetrachloride solution of 8-quinolinol without disturbing the aluminum complex. Phosphate in high concentrations (5% or more) precipitated aluminum when the diluted sample solution was allowed to age. If, however, the sample was processed immediately on dilution, the aluminum complex was strong enough to resist the effect of phosphorus when permitted to form before the aluminum phosphate precipitated. Addition of 1 ml. of 1% lead perchlorate dissolved in 5% perchloric acid permitted the determination of aluminum up to 50% phosphorus pentoxide concentration. Chromium in high concentrations inhibited the color formation. Addition of ferrous sulfate and 8-quinolinol aided aluminum complex formation in the presence of large amounts of chromium. Because beryllium, zirconium, and vanadium are present in trace quantities in iron ores, the complex may be measured against an iron blank, thus making it possible to avoid the employment of compensating blanks.

Effect of pH. The selection of an optimum pH level for a reproducible and stable aluminum complex has been the subject of previous investigations (*9*, *13*, *16*, *17*). The maximum absorbance of the aluminum complex using ammonium acetate as a buffer is at a pH of 6.4. Figure 6 shows the absorbance of the aluminum complex with varying pH for a 5-mμ band width. The curve was obtained with a stabilized dye, a pure aluminum solution, and buffer. The sharp maximum obtained at pH 6.4 was not observed when the effect of pH was studied using an iron ore, sodium mercaptoacetate, and an unstabilized dye solution.

Figure 7 compares the absorption spectra of an Eriochrome Cyanine R dye at pH 6.0 and 4.0. The maxima of both the chromophore and the aluminum complex have shifted toward the longer wave length and the absorbance of the chromophore has increased, while that of the aluminum complex has decreased at the lower pH value.

The effect of pH is not limited to the changes in the properties of the dye alone, but it is also related indirectly through the accelerated reduction of the chromophore group by reducing compounds at the lower pH values. Thus, mercaptoacetate will

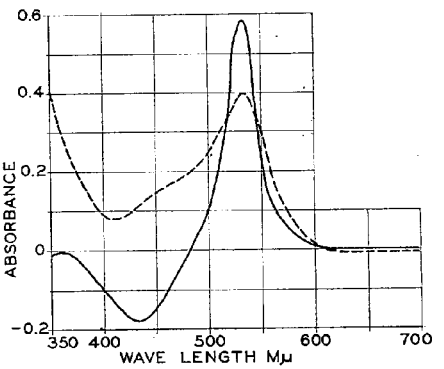


Figure 8. Comparison of aluminum complexes of Eriochrome Cyanine R and aurintricarboxylic acid

—— Eriochrome Cyanine R complex, slit width 0.02 mm.
– – – Aurintricarboxylic acid complex in 20% ethyl alcohol pH 5.35, slit width 0.02 mm.

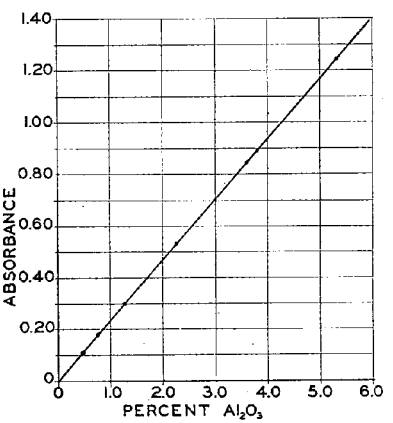


Figure 9. Calibration curve of aluminum oxide based on Eriochrome Cyanine R complex at 535 mμ

1-cm. Corex cell, slit width 0.04 mm.

reduce the chromophore group at low pH values, while at the higher values it has very little effect upon the dye. For this reason, it is best to add the mercaptoacetate after the addition of a buffer or, in acid solution, to limit the reaction time and dilute the solution. The adverse effect of mercaptoacetate upon the chromophore group is least when dissolved in 10% ethyl alcohol.

Metals forming strongly bonded hydrates, such as thorium, formed complexes only at low pH values and high dye concentrations. Such complexes once formed were stable at the higher pH levels, but were eventually destroyed above pH 7.0. This is contrary to the theory of hydrate absorption on the surface of the dye, which has been advanced in explaining lake formations.

Probable Chelate Structure. The structural formula of Eriochrome Cyanine R is given by Schultz (*19*) as:

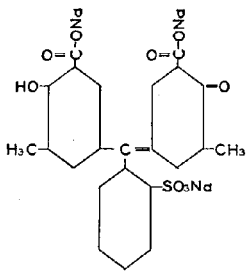
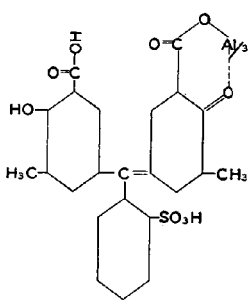


Figure 8 compares the absorption spectrum of the aluminum–aurintricarboxylic acid complex with that of the aluminum–Eriochrome Cyanine R complex. In order to accentuate the maximum of the aurintricarboxylic acid complex, alcohol was added; otherwise the maximum tended to decrease and shift to the left. The ultraviolet spectrum of the aurintricarboxylic complex was about 25 times more sensitive at 330 mμ than the conventional spectra usually measured between 520 and 450 mμ. On the basis of molecular structure of the dye and the similarity of the absorption spectra, the following structural formula of the aluminum complex is proposed.



Conformity to Bouguer-Beer Laws. Linear relationship of absorbance and concentration was found to hold up to 80 γ of aluminum in 50 ml., the highest measurable in a 2-mm. light path obtained by using 8-mm. inserts in a 10-mm. cell and a sufficient excess of Erichrome Cyanine R. Aluminum concentrations beyond the calibration curve may be brought into range by diluting the sample with a solution of the dye and buffer at the concentration contained in the sample. Conformity to the Bouguer-Beer law was obtained only with a narrow-band instrument.

Table I. Analysis of Standard and Miscellaneous Ores

NBS Samples	Name	Al_2O_3 Present, %	Al_2O_3 Found, %	Deviation, %
26	Crescent	1.02	1.02	0.00
27B	Sibley	0.59	0.60	+0.01
29	Magnetite	1.91	1.93	+0.02
29A	Magnetite	0.46	0.45	−0.01
No. 1	Sinter	2.49	2.52	+0.03
2	Hematite	2.37	2.40	+0.03
3	High manganese	1.54	1.54	0.00
4	High phosphorus	2.25	2.22	−0.03
5	Hematite	2.54	2.52	−0.02
6	Magnetite	3.33	3.34	+0.01
7	Synthetic	4.72	4.72	0.00
8	Hematite	2.14	2.14	0.00
9	Hematite	1.48	1.48	0.00
10	Hematite	0.68	0.70	+0.02

Table II. Repeat Analyses

Sample	Al_2O_3, %	Deviation from Mean	Standard Deviation
NBS 29a	0.46	0.00	
	0.46	0.00	
	0.48	+0.02	
	0.45	−0.01	
	0.44	−0.02	
	0.47	+0.01	
	0.46	0.00	
	0.45	−0.01	
	0.46	0.00	
Mean	0.46	±0.008	0.0149
NBS 29	1.92	+0.02	
	1.91	+0.01	
	1.88	−0.02	
	1.90	0.00	
	1.93	+0.03	
	1.92	+0.02	
	1.88	−0.02	
	1.89	−0.01	
Mean	1.90	±0.016	0.0208
Hematite ore	2.38	+0.03	
	2.34	−0.01	
	2.35	0.00	
	2.33	−0.02	
	2.37	+0.02	
	2.36	+0.01	
	2.35	0.00	
	2.32	−0.03	
Mean	2.35	±0.015	0.0208

Figure 9 shows the calibration curve obtained by adding known amounts of aluminum to standard iron solutions.

RESULTS

Agreement between established gravimetric results and those obtained by the direct photometric method is excellent, as has been demonstrated by analysis of available ores, including those from South America and Canada.

Determination of aluminum oxide in standard ores is shown in Table I. Table II shows the results of repeat analysis on three standard ores carried out over a period of 2 weeks. Trace analysis of ores for the past 7 years indicates that the method should be applicable to types of iron ores commercially available.

OTHER APPLICATIONS

The method has been applied, with modifications, for the past 3 years to steels, ferroalloys, galvanized coatings on steel, spelter, spelter dross, and other metallurgical products (*6*). In each instance it has been accurate and specific for aluminum.

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X-Ray Diffraction Powder Patterns of the Calcium Phosphates

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The x-ray diffraction powder patterns of 15 calcium phosphates are reported. The monocalcium and dicalcium orthophosphates used as starting materials were of commercial origin. The tricalcium orthophosphate was obtained by precipitation from solution.

THE calcium phosphates are important commercially as fertilizers and mineral supplements. The common naturally occurring form is phosphate rock, but numerous other calcium phosphates are known. The literature on these is voluminous (*1, 2, 5, 9, 10*) and sometimes contradictory. X-ray diffraction has proved a convenient method for identifying the crystalline calcium phosphates. Because the different polymorphs of the same compound can vary greatly in degree of availability to animals and plants, the distinction may be of considerable economic importance (*11, 14*).

Crystal structure studies have been reported on only a small number of the calcium phosphates, probably because of the difficulty of obtaining single crystals. Apatite (*3*), α-tricalcium orthophosphate (*13*), β-tricalcium orthophosphate (*7, 12*), and dicalcium orthophosphate dihydrate (*4*) have been investigated by single-crystal methods. In most cases, however, only the powder diffraction data are available.

The x-ray method requires a file of reference standards, and x-ray data on 15 calcium phosphates have been prepared (Tables I and II). The American Society for Testing Materials Index of X-Ray Diffraction Data lists 24 calcium phosphate patterns, but as there are two or more sets of data for several of the phosphates, only 11 different compounds are represented. Data on these compounds are included here. Of the additional four, two (β-calcium metaphosphate and γ-calcium pyrophosphate) appear to have no published x-ray powder data.

X-RAY APPARATUS AND TECHNIQUE

A photographic technique was employed, the cameras being 14.32 cm. in diameter. The finely ground sample was mounted either in collodion capillaries or in wedge form. When the material was formed by heating, the x-ray pattern was obtained after quenching to room temperature. Copper radiation (λ = 1.5418 A.) filtered through nickel foil was employed throughout. The intensities were estimated visually by comparison with a standard intensity scale, which was prepared by exposing film to the x-ray beam for accurately timed periods. A slit collimating system, approximately 0.5 × 1.0 mm. in aperture, was used.

PREPARATION OF MATERIALS

The starting materials for the preparation of the mono- and dicalcium phosphates were obtained from commercial sources. The tricalcium phosphates were obtained from tricalcium orthophosphate, which was prepared from ammonium phosphate and calcium chloride. The calcium oxide–phosphorus pentoxide ratios of some samples of these starting materials were as follows:

	CaO/P_2O_5	
	Measured	Theoretical
Monocalcium orthophosphate monohydrate (Mallinckrodt)	0.84	1.0
Monocalcium orthophosphate monohydrate (Monsanto)	1.10	1.0
Dicalcium orthophosphate (Allied Chemical)	2.15	2.0
Tricalcium orthophosphate hydrate	3.06	3.0

These ratios illustrate the variations from theoretical composition that occur even in reagent grade calcium phosphates. Except in the instance described below, no evidence was found that the structures of the ignition products were changed by the presence of impurities.

MONOCALCIUM PHOSPHATES

Monocalcium orthophosphate hydrate, when heated at 130° C. changes to the anhydrous form. When heated in air at 250° C., the anhydrous monocalcium phosphate becomes noncrystalline. Further heating to 500° C. forms β-calcium metaphosphate.

Some samples of monocalcium orthophosphate when heated at 400° C. form δ-calcium metaphosphate. It is believed that the formation of this compound is due to the presence of dicalcium phosphate as impurity. When a portion of the sample of monocalcium orthophosphate that formed δ-calcium metaphosphate was heated at 300° C., the x-ray pattern of dicalcium orthophosphate was obtained. At this temperature, the monocalcium phosphate is noncrystalline. When some of the same sample of monocalcium orthophosphate was recrystallized, the pattern of δ-calcium metaphosphate could not be obtained. When δ-calcium metaphosphate is heated to 700° C., β-calcium metaphosphate is obtained.

If monocalcium orthophosphate is heated at 270° to 280° C. in the presence of steam, calcium acid pyrophosphate is formed. If this is heated in steam at 325° C., a compound described by Hill and coworkers (*10*) as tetracalcium dihydrogen hexaphosphate forms. When the acid pyrophosphate is heated in air at 340° to 360° C., γ-calcium metaphosphate results. Heating this in air at 450° to 500° C. gives β-calcium metaphosphate. The α-calcium metaphosphate is reported (*11*) to form at above 963° C., but this compound, which melts at 984° C., was not obtained.

DICALCIUM PHOSPHATES

Dicalcium phosphate dihydrate was obtained as a commercial sample (Monsanto) and also by crystallization from a 25% acetic

acid solution of dicalcium phosphate (Allied Chemical). The dihydrate occurs naturally as the mineral brushite. When dicalcium phosphate dihydrate is heated at 180° C., the anhydrous form is obtained. Heating at 320° to 340° C. gives γ-calcium pyrophosphate. At 700° C. the β form is obtained. Heating this at 1200° C. gives α-calcium pyrophosphate.

A hydrated calcium pyrophosphate can be prepared from calcium chloride and sodium pyrophosphate. This is noncrystalline and is described by Hill and coworkers (*11*) as the tetrahydrate. When heated at 600° C., it forms β-calcium pyrophosphate. The reaction between excess sodium pyrophosphate and calcium chloride gives a crystalline product, but when heated at 600° C. this does not form anhydrous calcium pyrophosphate. The chemical analysis indicates that this compound is a calcium sodium pyrophosphate.

TRICALCIUM PHOSPHATES

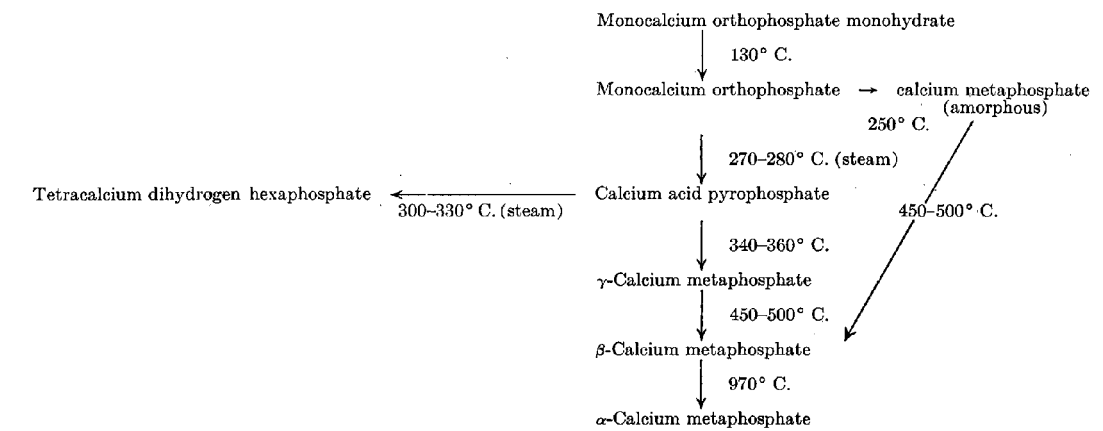
The starting material for the tricalcium phosphates was tricalcium orthophosphate hydrate. This was prepared by precipitation from a solution of diammonium hydrogen phosphate and calcium chloride, the pH being controlled with aqueous ammonia so that at the end of the reaction the preparation was at pH 9. The analysis of the solid shows a calcium oxide–phosphorus pentoxide ratio of approximately 3.0, corresponding to that of tricalcium orthophosphate. The existence of tricalcium orthophosphate hydrate as a distinct compound has been questioned (*8*). It has been suggested that it is hydroxyapatite with adsorbed or occluded calcium phosphate of one of the acid forms.

Tricalcium orthophosphate hydrate has the apatite structure which is possessed also by fluorapatite, chlorapatite, and hydroxyapatite. The isomorphism of these compounds makes it difficult to distinguish them by x-ray methods. However, there are minor differences in the unit cell parameters, so that a precise technique is capable of discriminating between the isomorphs (*6*, *15*).

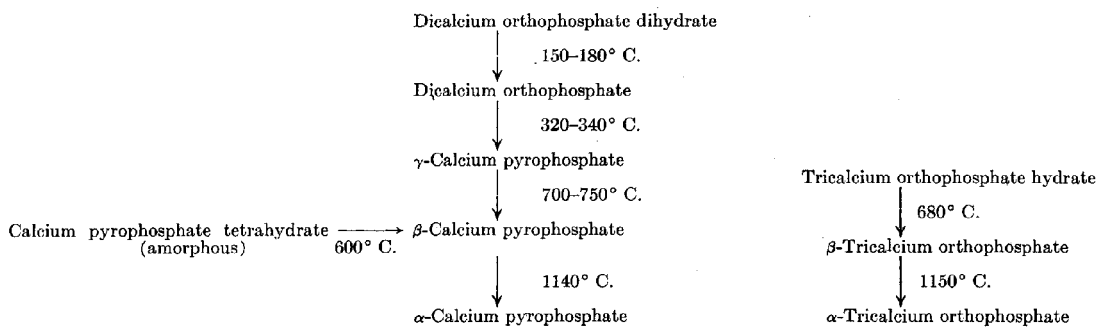
Table I. Three Strongest and Innermost Lines of the Calcium Phosphates

(Intensities given in parentheses)

Compound	1st	2nd	3rd	Innermost
Monocalcium orthophosphate monohydrate	3.90 (100)	11.75 (81)	3.71 (81)	11.75 (81)
Monocalcium orthophosphate	3.63 (100)	3.50 (64)	3.06 (51)	7.23 (11)
Calcium acid pyrophosphate	3.35 (100)	3.19 (58)	3.74 (47)	5.42 (4)
Tetracalcium dihydrogen hexaphosphate	3.14 (100)	3.58 (58)	2.79 (24)	11.8 (3)
δ-Calcium metaphosphate	3.54 (100)	2.83 (30)	3.15 (17)	5.54 (2)
γ-Calcium metaphosphate	3.49 (100)	2.76 (65)	4.76 (46)	5.77 (8)
β-Calcium metaphosphate	3.74 (100)	3.52 (100)	4.58 (69)	7.05 (19)
Dicalcium orthophosphate dihydrate	4.23 (100)	3.04 (91)	7.62 (75)	7.62 (75)
Dicalcium orthophosphate	3.38 (100)	2.99 (88)	1.74 (57)	6.83 (24)
γ-Calcium pyrophosphate	2.94 (100)	3.11 (65)	3.35 (42)	4.55 (7)
β-Calcium pyrophosphate	3.04 (100)	3.23 (62)	2.76 (57)	6.02 (9)
α-Calcium pyrophosphate	2.00 (100)	3.33 (80)	2.11 (63)	11.5 (3)
Tricalcium orthophosphate hydrate	2.80 (100)	3.44 (57)	1.94 (43)	8.19 (9)
β-Tricalcium orthophosphate	2.88 (100)	2.61 (71)	3.21 (53)	8.13 (11)
α-Tricalcium orthophosphate	2.90 (100)	3.89 (43)	3.67 (43)	7.28 (7)



Monocalcium Phosphates and Derivatives



Dicalcium Phosphates and Derivatives **Tricalcium Phosphates**

Table II. X-Ray Powder Diffraction Data

d, A.	I/I_1
Monocalcium Orthophosphate Monohydrate	
11.75	81
5.70	9
4.93	50
4.45	9
4.34	9
4.17	12
3.90	100
3.71	81
3.60	7
3.38	42
3.20	35
3.01	17
2.96	50
2.86	8
2.80	8
2.74	8
2.69	31
2.58	17
2.46	13
2.42	13
2.16	13
2.09	13
2.03	13
2.00	35
1.96	13
1.95	17
1.86	9
1.84	9
1.80	35
1.77	13
1.75	13
1.73	13
1.71	17
1.67	13
1.60	17
1.58	5
1.55	9
1.53	5
1.48	7

d, A.	I/I_1
Calcium Acid Pyrophosphate	
5.42	4
4.78	9
4.44	27
4.07	9
3.74	47
3.35	100
3.19	58
2.95	4
2.85	4
2.81	4
2.78	4
2.70	20
2.61	4
2.52	6
2.42	3
2.34	8
2.31	8
2.25	8
2.23	10
2.18	24
2.14	3
2.07	9
2.04	4
1.94	16
1.93	13
1.88	14
1.84	24
1.77	33
1.72	5
1.68	10
1.65	13
1.60	16
1.59	13
1.53	4
1.50	22
1.44	4
1.40	10
1.35	6
1.34	7

d, A.	I/I_1
δ-Calcium Metaphosphate	
5.54	2
4.72	2
4.30	2
3.93	2
3.54	100
3.33	2
3.15	17
3.05	4
2.92	4
2.83	30
2.69	4
2.60	2
2.50	6
2.31	2
2.22	2
2.15	2
2.08	4
2.01	6
1.87	6
1.81	2
1.77	6
1.69	2
1.66	2
1.64	2
1.61	2
1.58	4
1.57	3
1.50	2
1.47	3
1.30	2
1.27	1
1.15	1

d, A.	I/I_1
β-Calcium Metaphosphate	
3.01	42
2.90	23
2.85	65
2.76	7
2.66	9
2.62	7
2.54	42
2.49	11
2.40	11
2.32	11
2.25	7
2.21	13
2.13	17
2.03	17
1.97	42
1.92	13
1.84	9
1.78	13
1.72	7
1.69	7
1.68	7
1.65	23
1.62	7
1.60	23
1.57	7
1.56	10
1.52	13
1.48	35
1.39	17
1.37	5
1.30	8
1.28	11

d, A.	I/I_1
Dicalcium Orthophosphate	
6.83	24
4.99	14
4.32	4
4.08	4
3.76	5
3.52	5
3.38	100
3.32	4
3.16	12
2.99	88
2.78	33
2.74	33
2.51	18
2.37	3
2.33	5
2.27	22
2.22	14
2.18	9
2.14	5
2.08	3
2.04	5
2.01	9
1.93	25
1.87	41
1.81	12
1.77	7
1.74	57
1.70	7
1.69	7
1.66	7
1.65	12
1.62	12
1.58	7
1.55	7
1.52	9
1.51	5
1.49	7
1.46	4
1.42	7
1.40	4
1.37	18
1.35	5
1.32	5
1.30	7
1.28	8
1.27	5
1.25	5
1.24	5
1.22	12
1.19	8

d, A.	I/I_1
β-Calcium Pyrophosphate	
6.02	9
4.72	13
4.47	11
4.19	9
3.71	9
3.33	48
3.23	62
3.08	52
3.04	100
3.02	21
2.91	33
2.80	33
2.76	57
2.68	13
2.54	17
2.39	11
2.35	17
2.28	11
2.25	9
2.22	9
2.16	9
2.12	13
2.08	11
2.00	29
1.95	21
1.88	7
1.85	11
1.83	17
1.77	33
1.73	9
1.72	7
1.69	19
1.64	13
1.63	17
1.59	9
1.58	11
1.57	17
1.54	11
1.53	21
1.51	11
1.50	11
1.48	9
1.46	7
1.44	6
1.42	13
1.37	6
1.33	9
1.31	29

d, A.	I/I_1
Monocalcium Orthophosphate	
7.23	11
7.05	11
6.43	11
4.34	14
4.04	14
3.87	9
3.75	9
3.63	100
3.50	64
3.19	26
3.06	51
2.97	21
2.92	16
2.86	21
2.81	9
2.72	9
2.63	9
2.58	33
2.54	8
2.33	16
2.30	6
2.27	6
2.24	9
2.15	6
2.12	51
2.07	4
1.94	16
1.91	9
1.87	11
1.85	16
1.82	14
1.78	14
1.76	8
1.72	26
1.68	14
1.61	9
1.60	9
1.59	9
1.57	8
1.53	8
1.51	8
1.49	8
1.48	6
1.44	11
1.41	14
1.38	11
1.35	14

d, A.	I/I_1
Tetracalcium Dihydrogen Hexaphosphate	
11.8	3
5.96	2
4.95	3
4.74	8
4.29	8
4.10	3
3.58	58
3.41	10
3.29	16
3.14	100
2.96	3
2.85	8
2.79	24
2.69	20
2.62	13
2.52	5
2.43	4
2.40	4
2.26	8
2.16	14
2.05	6
2.03	4
2.01	6
1.97	4
1.95	6
1.93	3
1.90	6
1.84	6
1.81	3
1.78	3
1.76	6
1.72	3
1.70	13
1.67	6
1.65	5
1.64	5
1.62	13
1.61	10
1.59	4
1.58	11

d, A.	I/I_1
γ-Calcium Metaphosphate	
5.77	8
5.54	8
5.18	7
4.76	46
4.29	7
4.18	46
4.02	7
3.61	35
3.49	100
3.40	35
3.12	7
3.02	42
2.87	35
2.76	65
2.66	7
2.61	11
2.58	7
2.46	35
2.29	8
2.22	8
2.13	11
2.09	9
2.07	11
1.98	9
1.94	11
1.86	7
1.83	11
1.75	9
1.72	7
1.70	13
1.63	13
1.62	11
1.60	17
1.57	7
1.51	7
1.50	9
1.43	9
1.41	7

d, A.	I/I_1
β-Calcium Metaphosphate	
7.05	19
4.58	69
4.23	65
3.84	17
3.74	100
3.52	100
3.38	11
3.30	42
3.13	65

d, A.	I/I_1
Dicalcium Orthophosphate Dihydrate	
7.62	75
4.72	3
4.23	100
3.79	2
3.04	91
2.92	47
2.85	5
2.61	60
2.42	24
2.26	33
2.17	16
2.14	13
2.09	8
1.99	11
1.97	4
1.88	13
1.85	6
1.81	24
1.80	6
1.77	3
1.71	8
1.66	3
1.63	3
1.60	3
1.57	3
1.55	8
1.53	3
1.52	5
1.45	7
1.43	5
1.41	2
1.39	3
1.38	3
1.37	6
1.34	4
1.30	3
1.28	3
1.26	3
1.22	4
1.20	3
1.19	2
1.15	4
1.14	3
1.13	5
1.10	3
1.08	3
1.06	2

d, A.	I/I_1
γ-Calcium Pyrophosphate	
4.55	7
4.19	5
3.35	42
3.25	13
3.11	65
3.06	35
2.94	100
2.78	7
2.70	17
2.56	9
2.42	7
2.34	7
2.24	35
2.18	27
2.15	7
2.10	23
2.02	7
1.96	8
1.88	5
1.86	11
1.82	35
1.79	27
1.74	9
1.70	5
1.67	17
1.57	13
1.51	13
1.47	11
1.40	5
1.38	5
1.34	5
1.31	7
1.28	5
1.27	5
1.22	4
1.20	4
1.12	5
1.09	5

d, A.	I/I_1
α-Calcium Pyrophosphate	
11.5	3
7.14	6
6.32	6
4.90	17
4.27	17
3.81	11
3.67	6
3.56	15
3.33	80
3.23	51
3.09	15
3.00	6
2.95	6
2.88	7
2.79	27
2.67	15
2.60	27
2.55	3
2.47	17
2.44	32
2.36	9
2.34	9
2.26	4
2.16	6
2.11	63
2.06	27
2.00	100
1.92	5
1.90	17
1.86	7
1.84	17
1.80	9
1.79	32
1.77	4
1.72	9
1.68	11
1.64	27
1.63	15
1.62	11
1.59	9

Table II. X-Ray Powder Diffraction Data (*Continued*)

d, A.	*I*/*I*₁	*d*, A.	*I*/*I*₁	*d*, A.	*I*/*I*₁
α-Calcium Pyrophosphate		Tricalcium Orthophosphate Hydrate		β-Tricalcium Orthophosphate	
1.58	11	1.61	11	1.56	16
1.55	9	1.54	10	1.51	7
1.53	17	1.51	10	1.47	7
1.50	15	1.47	10	1.45	7
1.48	4	1.45	21	1.43	7
1.45	7	1.43	11	1.41	7
1.43	6			1.22	11
1.42	4			1.15	7
1.41	7			1.13	8
1.39	11				
1.36	7	β-Tricalcium Orthophosphate			
1.35	17				
		8.13	11	α-Tricalcium Orthophosphate	
Tricalcium Orthophosphate Hydrate		6.51	14		
		5.25	21	7.28	7
		4.06	11	6.36	5
8.19	9	3.47	35	5.83	5
5.28	7	3.21	53	4.00	9
4.08	10	2.88	100	3.89	43
3.87	6	2.81	26	3.67	43
3.44	57	2.77	26	2.90	100
3.16	10	2.71	26	2.85	17
3.08	11	2.61	71	2.62	43
2.80	100	2.56	7	2.59	13
2.72	33	2.43	11	2.16	9
2.64	21	2.27	16	2.04	6
2.52	7	2.21	14	1.95	5
2.30	6	2.17	11	1.94	21
2.25	13	2.09	7	1.90	13
2.16	10	2.05	7	1.82	9
2.06	9	2.00	8	1.80	9
1.99	9	1.94	35	1.76	7
1.94	43	1.90	14	1.73	6
1.89	9	1.88	11	1.71	6
1.84	33	1.84	14	1.67	9
1.81	11	1.81	7	1.54	6
1.78	11	1.78	11	1.50	7
1.76	13	1.73	35	1.47	6
1.72	21	1.71	8	1.31	6
1.64	11	1.69	8	1.29	6
		1.61	8		

All the compounds with the apatite structure do not possess the same degree of stability. Tricalcium phosphate hydrate changes to β-tricalcium orthophosphate when it is heated above 680° C. The other members of the group are unchanged by this treatment thus providing a means of distinguishing tricalcium orthophosphate hydrate from the apatites. β-Tricalcium orthophosphate occurs naturally as the mineral whitlockite (*7*). When β-tricalcium orthophosphate is heated at 1200° C., it changes to α-tricalcium orthophosphate (*13*).

DISCUSSION

The x-ray powder patterns of these 15 calcium phosphates have been reported for their value in identification of phosphatic materials. Many of the compounds have not been characterized with exactness, but the authors feel that this is overweighed by the advantage of being able to recognize substances as calcium phosphates formed under known conditions.

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Index of Refraction as Supplement to X-Ray Analysis of Solid Solutions

Ternary Solid Solutions

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Constant unit cell edge contour lines can be drawn across the ternary diagram of the reciprocal system RbCl + KBr ⇄ RbBr + KCl. Constant refractive index contour lines can also be drawn across the same diagram; the intersection of these lines with one of the constant unit cell lines represents a unique determination of the composition of the ternary solid solution if the solution is homogeneous.

MEASUREMENTS of index of refraction can be used as a supplement to x-ray analysis in certain binary solid solution studies (*9*). It is shown here how ternary solid solutions of the reciprocal system RbCl + KBr ⇄ RbBr + KCl, which cannot be analyzed by either index of refraction or x-ray measurements alone, can be resolved by a combination of the two.

Thomas and Wood (*5*) have shown that, when any given mixture of this reciprocal system is melted and frozen, the resulting crystals are composed of a single solid solution of the four kinds of ions. The composition of any starting mixture can be represented by a point in a reciprocal diagram as shown in Figure 1, and the composition of the resulting solid solution can be readily expressed as Rb^+ to K^+ and Br^- to Cl^- ratios. For a given set of conditions—e.g., constant temperature—values of properties such as unit cell edge or refractive index can be projected onto the diagram.

The dotted lines in Figure 1 show the relation between composition and unit cell edge and the solid lines show the relation between composition and index of refraction. The positions of the dotted lines were calculated on the assumption that the unit cell edges are additive; x-ray analysis has shown that this assumption is closely approximated by experiment (*7*). These lines are parallel to the rubidium chloride–potassium bromide diagonal

because the unit cell edges of these two salts are very nearly equal to each other. The positions of the solid lines were calculated on the assumption that the refractive indices are additive; index of refraction measurements made by the Becke line immersion method have shown that this assumption is also closely approximated by experiment (*8*).

The calculations for Figure 1 are based on the two ternary systems rubidium chloride–potassium bromide–rubidium bromide and rubidium chloride–potassium bromide–potassium chloride, which have the rubidium chloride–potassium bromide diagonal as a common base. The agreement with both observed unit cell edges and refractive indices is excellent. Similar calculations have been made for the two ternary systems having the rubidium bromide–potassium chloride diagonal as a common base. These calculations give almost the same locations for the dotted lines of constant unit cell edge because the average unit cell edge of rubidium bromide and potassium chloride differs little from the unit cell edge of rubidium chloride or potassium bromide. The locations of the solid lines of constant index of refraction, however, are in important disagreement with observed values.

If starting mixtures of this reciprocal system are made up and heated until final equilibrium is obtained, the observed unit cell edges and the observed refractive indices are always in almost perfect agreement with the calculated positions of the lines in Figure 1. It is clear that refractive indices calculated in this manner can be used as a supplement to the x-ray analysis of the solid solutions of this reciprocal system as described below.

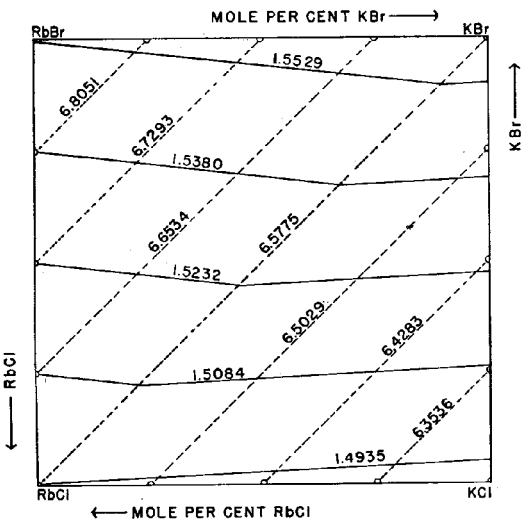

Figure 1. Composition diagram with unit cell edges in kX units

Refractive index constant along solid lines; unit cell edge constant along dotted lines

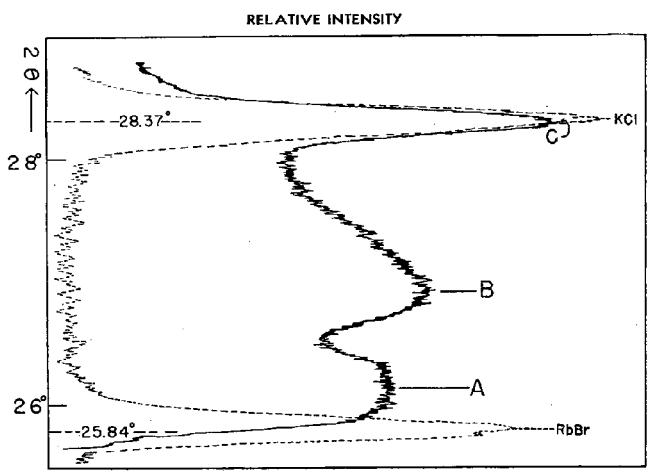

Figure 2. Portion of x-ray diffraction curve obtained on heating equimolecular mixture of rubidium bromide and potassium chloride powders for 12 minutes at 600° C.

Average particle size, 0.1 mm.; dotted curve indicates positions of peaks for pure rubidium bromide and pure potassium chloride

APPARATUS, MATERIALS, AND METHODS

The annealing oven, refraction oils, and apparatus for refractive index measurements were the same as used previously (*9*). For x-ray measurements a tube with a copper target was used in a General Electric XRD-3D recording unit. To facilitate comparison with earlier work, unit cell edges are recorded in kX units (1kX unit = 1.00202 A.). The precision is about ±0.0008 kX unit. The potassium salts used were Mallinckrodt analytical reagent grade, the potassium bromide containing 0.2 to 0.4% chloride. The rubidium salts were furnished by McKay Chemical Co., Inc., Brooklyn, N. Y., and were labeled as containing less than 0.1% potassium. Spectroscopic analysis indicated 0.02% or less potassium in each case.

In view of the nature of the mixtures examined in this work, high precision values for unit cell edges for the pure salts were not required. Direct comparison with aluminum as an internal standard gave unit cell edges as follows:

Compound	Unit Cell Edge Value, kX Units	
	Found	Reported
Potassium chloride	6.279	6.2804 (*3*)
Rubidium bromide	6.881	6.8797 (*1*)
Rubidium chloride	6.578	6.5677 (*2*)
Potassium bromide	6.577	6.5783 (*6*)
		6.5867 (*4*)

If the unit cell edge value for potassium bromide is extrapolated to 0% potassium by means of Vegard's law, the unit cell edge becomes 6.5784, in good agreement with Wasastjerna's value (*6*). An unheated, 50 mole % mixture of rubidium chloride and potassium bromide gave only one x-ray pattern. The pattern was well defined and the α doublet was resolved for the 400 and 420 peaks. It would be expected that a difference between the unit cell edges of rubidium chloride and potassium bromide of the magnitude reported by Swanson and coworkers would be detected by this comparison. The rubidium chloride used by Swanson contained an estimated 0.1 to 1% potassium, which would account, at least in part, for his lower value for the unit cell edge. Such small difference as exists between the unit cell edges of rubidium chloride and potassium bromide has been neglected in the preparation of the composition diagram of Figure 1. The unit cell edges shown in this diagram are calculated averages of the values obtained by direct comparison with aluminum as an internal standard.

EXPERIMENTAL

In the present work 50 mole % mixtures of rubidium bromide and potassium chloride powders were heated at below fusion temperatures for predetermined lengths of time, cooled quickly to room temperature, and examined for evidence of ion migration. Figure 2 shows a part of the x-ray interference pattern for such a mixture of fine rubidium bromide and potassium chloride powders (150 to 160 mesh) which had been heated at 600° C. for 12 minutes (point *X*,

Table I. Maximum Index of Refraction of Equimolar Mixtures of RbBr and KCl after Heating at 600° C.

(Average particle size, 0.1 mm.)

Sample	Heating Time, Hours	Maximum Index
RbBr	..	1.5529
X-A5	0.2	1.5460
X-B1	1	1.5415
X-A1	3	1.5330
X-A8	6	1.5316
KCl	..	1.4900

Figure 3; also mixture X-A5, Table I). The value of 2θ observed for peak A was 26.10°, which corresponds to the 200 peak of a rubidium bromide–rich solid solution having a unit cell edge of 6.822 kX units. This unit cell edge is considerably smaller than the unit cell edge of pure rubidium bromide, which is close to 6.881 kX units. The dotted peak at 25.84° is the 200 peak for pure rubidium bromide. Thus, as far as the x-ray results can show, it appears that no pure rubidium bromide remains in the mixture after this brief heating period of 12 minutes at 600° C.

Because the x-ray diffraction method requires a considerable number of powder particles for the formation of a diffraction peak, the question may well be raised as to whether or not there may be a few remaining particles of pure, or nearly pure, rubidium bromide which the x-rays have been unable to detect because of the small number present. A definite answer can be given to this question by the use of the Becke line method for the observation of refractive index, which is capable of detecting even a single particle of rubidium bromide. Table I shows that the maximum value for the refractive index observed for any particle in this mixture was 1.546, far below the refractive index value of 1.5529 for pure rubidium bromide. This maximum refractive index value of 1.546 then eliminates the possible existence of pure rubidium bromide particles.

It has already been pointed out that the middle value of 26.10° for peak A corresponds to a unit cell edge of 6.822 kX units (6.822 line, Figure 3). A binary solid solution containing rubidium bromide and rubidium chloride in the ratio of 81 to 19 (approximately) could produce peak A, as could a binary solid solution of rubidium bromide and potassium bromide in the same ratio. Any ternary solid solution having a composition anywhere on the 6.822 line could also produce peak A. It is clear that the x-ray results alone cannot fix a definite location for peak A. Because the maximum refractive index observed for any particle was 1.546, peak A must lie some place on line 6.822 below its intersection with the constant refractive index line 1.546. This is so because all of the solutions above this intersection have a refractive index greater than 1.546 and, therefore, cannot be present.

By the use of a special microscope slide with Neubauer rulings, the relative number of particles having a refractive index of about 1.543 was determined by direct count. Several fields of 40 to 50 particles were examined; about 20% of the total number had a refractive index in the range from 1.540 to 1.546. In order to account for the well-formed, intense peak at A, it is necessary to conclude that all or nearly all of these particles contribute to the formation of peak A. The particles contributing to this peak must have a composition somewhere along the 6.822 line of Figure 3, below the intersection of this line with the 1.546 constant index line.

Many of the particles in the refractive index range from 1.540 to 1.546 had indices between 1.541 and 1.542, which correspond closely to the refractive index of a binary solid solution of 80.7% rubidium bromide and 19.3% rubidium chloride. But peak A cannot be due entirely to this binary solid solution because many particles were observed with refractive indices between 1.542

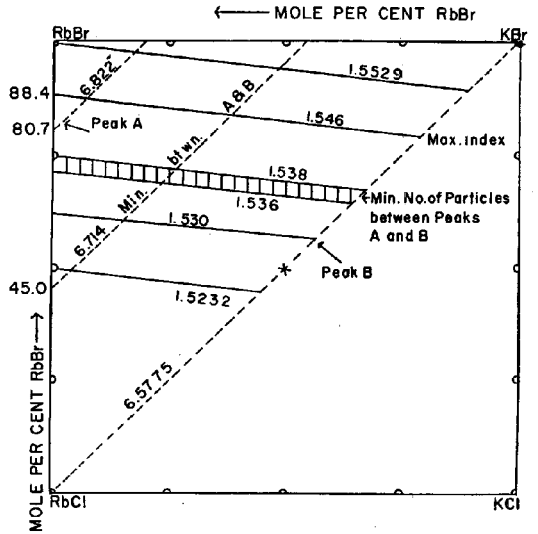

Figure 3. Correlation of refractive indices and x-ray diffraction measurements based on composition diagram

Equimolecular mixture of rubidium bromide and potassium chloride, particle size, 0.1 mm.; heated for 12 minutes at 600° C.

and 1.544 (but not greater than 1.546). Any particle in the index range from 1.540 to 1.546 could have a composition on the 6.5775 line and contribute to the production of peak B, but such particles would produce odd number index peaks. Because such peaks were not observed it seems safe to conclude that all or nearly all of the particles in this index range contribute to the production of peak A and that their compositions extend from that of the binary (marked 80.7) along the 6.822 line to its intersection with the constant index line of 1.546, and that the average of these compositions lies approximately midway between these two points.

The peak at B corresponds very nearly to the unit cell edge of pure rubidium chloride or pure potassium bromide as well as any mechanical mixture or solid solution of these two salts. This diffraction peak cannot be caused by either of the pure salts or any mechanical mixture of them because no odd number index peaks were observed. The absence of odd number index peaks also eliminates solid solutions near the ends of the rubidium chloride–potassium bromide diagonal and places peak B near the middle of this line (approximately the middle third). However, x-ray measurements alone are insufficient to fix the position of peak B more definitely. Unlike the unit cell edge which remains constant along the diagonal, the refractive index increases in the direction of potassium bromide. It can, therefore, be used to supplement the results of the x-ray measurements in locating peak B.

By the use of the special microscope slide, the relative number of particles having a refractive index in the range from 1.528 to 1.532 was found to be about 30% of the total number of particles. Because of this large number having an index near 1.530, it seems necessary to conclude that peak B is produced largely by solid solution crystals having a composition close to the intersection of the 1.530 index line with the rubidium chloride–potassium bromide diagonal as shown in Figure 3. A small but appreciable number of particles was found in the refractive index range of from 1.536 to 1.540. Few if any of these particles have compositions that lie in the area between lines 6.5775 and 6.714, as is

indicated by the fact that the intensity of peak *B* (Figure 2) falls off rather sharply in the direction of peak *A* and that the intensity reaches a minimum in line 6.714.

It is clear then that, in the top half of the composition diagram, Figure 1, there are relatively few particles that do not have compositions such that they contribute to the production of peak *A* or peak *B*. Particles not having such compositions must be too few in number to produce an x-ray peak. The few particles found in the refractive index range from 1.536 to 1.540 could actually all have compositions on the rubidium chloride–potassium bromide diagonal, in which case they would all contribute to the production of peak *B*. These particles could not contribute to the production of peak *A* because their refractive indices are too low.

The peak at *C* of Figure 2 represents pure or almost pure potassium chloride. This result is not surprising because the melting point of pure potassium chloride is at 770° C., which is 170° above the reaction temperature. Below an index of 1.530 (Figure 1) the refractive index observations were generally ambiguous. It is possible that in this region there was an initial small penetration of some of the potassium chloride crystals by rubidium ions and bromide ions, resulting in a thin coating of ternary solid solutions over a residual core of pure potassium chloride. Such crystals would give x-ray lines for pure potassium chloride, but it would be expected that the refractive index observations would be confused.

A rough estimate of the extent of the reaction of the starting mixture after 12 minutes at 600° C. can be made if it be assumed that particles not contributing to peaks *A*, *B*, or *C* can be neglected. Starting with 100 moles of rubidium bromide and 100 moles of potassium chloride, if reaction occurs to the extent of 50%, the equivalent of 50 moles each of potassium bromide and rubidium chloride will be produced, with 50 moles of each remaining. Because all of the rubidium bromide will be in the solution represented by peak *A*, which is about 80% rubidium bromide, there will be about 62.5 moles of this solution. Because the potassium bromide to rubidium chloride ratio is about 1 to 4, there will be about 2.5 moles of potassium bromide and about 10 moles of rubidium chloride in this solution. This estimate leaves the solution at peak *B* composed of the 47.5 moles of residual potassium bromide and the 40 moles of residual rubidium chloride for a total of 87.5 moles of this solution.

Actually the solution corresponding to peak *B* is somewhat richer in potassium bromide than indicated by this estimate. Calculation shows that an interaction to the extent of 43% would give 57 moles of pure potassium chloride and about 71.5 moles each of the two solid solutions. This calculation gives a potassium bromide to rubidium chloride ratio of about 56 to 44 which appears to be nearly the correct ratio. The extent of the reaction in 12 minutes at 600° C. appears to be somewhat less than 50%.

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X-Ray Powder Diffraction Patterns of Benzoic Acids

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X-ray powder diffraction data are given for the identification of 25 substituted benzoic acids. An interesting example is given of the ability of the x-ray pattern to differentiate between two compounds which have the same empirical formula and melting point.

X-RAY powder diffraction patterns have been made of 25 substituted benzoic acids using Cr$K\alpha$ radiation for those containing only light elements and Cu$K\alpha$ radiation for several compounds containing bromine.

This complication is of interest in the pharmaceutical industry because of the presence of substituted benzoic acid esters in plant extracts and physiologically active compounds.

PROCEDURE

The samples were finely ground and packed into thin-walled plastic capillaries about 0.5 mm. in diameter. The x-ray equipment used was the standard North American Philips unit with cameras 114.6 mm. in diameter. Vanadium-filtered chromium $K\alpha$ (λ = 2.2896 A.) radiation was used for the compounds containing only carbon, hydrogen, oxygen, and nitrogen; nickel-filtered copper $K\alpha$ (λ = 1.5405 A.) radiation was used for the bromine-containing compounds.

The intensities were estimated by comparison with a calibrated film strip.

Melting points were determined with a Kofler micro hot stage.

DISCUSSION

Table I lists the benzoic acid patterns in order of interplanar spacings as in the A.S.T.M. card file (*1*), with the melting point of the sample used as well as that given in the literature. A code number is given to relate the data of Table I to that of Table II.

The data for 2-hydroxy-3,6-dimethylbenzoic acid (No. 17) and *p*-ethoxybenzoic acid (No. 18) are interesting in that these two compounds have the same empirical formula, $C_9H_{10}O_3$, and the same melting point (197–9° C.). By these two commonly used identification criteria the two compounds are identical, yet the x-ray powder diffraction patterns are different. A similar condition exists for 2,3-dihydroxybenzoic acid (No. 5) (melting point, 204–5° C.) and 2,5-dihydroxybenzoic acid (No. 7) (melting point 203–5° C.), both having the empirical formula, $C_7H_6O_4$, but again having different x-ray patterns. The second case is not as striking, of course, because of the presence of the same

Table I. Melting Points and Three Most Intense Diffraction Lines of Benzoic Acids[a]

Pattern No.	Compound	Three Strongest Lines			Melting Point, °C. Found	Literature	
24	*p*-Isopropyl	12.94	5.19	4.14	112–5	115–7	(5)
25	3,4,5-Trimethoxy	7.75	3.47	3.38	..	168	(3)
17	2-Hydroxy-3,6-dimethyl	7.12	3.45	3.37	197–9	195	(3)
23	3,5-Dimethoxy	7.10	3.68	3.21	181–3	185–6	(3)
14	4-Hydroxy-3-methoxy	6.90	9.43	3.29	211–2	210	(5)
5	2,3-Dihydroxy	6.54	8.27	3.20	204–5	204	(3)
8	2,6-Dihydroxy	6.46	3.50	3.31	..	150–170	(3)
16	2-Amino-3-methyl	6.45	3.71	3.36	177–8	172	(3)
1	*o*-Bromo	5.90	3.68	3.29	149–50	147–50	(2)
b	2-Acetoxy[c]	5.66	3.92	3.30	..	135	(5)
b	*p*-Methoxy	5.55	3.53	3.09	..	184	(2)
7	2,5-Dihydroxy	5.08	3.63	3.56	203–5	200	(5)
4	*p*-Hydroxy	5.06	3.64	3.33	215–6	213–4	(5)
3	*p*-Bromo	4.68	3.28	3.71	255–7	251–3	(2)
10	3,5-Dihydroxy	4.13	3.16	3.96	238–41	237–40	(3)
b	2-Mercapto	3.81	3.15	7.74	..	164–5	(5)
6	2,4-Dihydroxy	3.76	3.57	3.36	..	218–9	(3)
21	2,6-Dimethoxy	3.66	5.18	6.34	188–91	186–7	(3)
18	*p*-Ethoxy	3.66	4.47	3.23	197–9	195–6	(3)
b	*o*-Hydroxy	3.52	8.20	5.20	..	157–9	(5)
20	2,5-Dimethoxy	3.42	3.30	3.64	71–3	76	(3)
2	*m*-Bromo	3.41	5.92	4.87	155–8	155	(2)
19	2,3-Dimethoxy	3.37	4.14	4.64	119–24	120–2	(3)
d	4-Amino-2-hydroxy	3.35	5.27	3.45	..	220	(4)
15	2-Hydroxy-5-methoxy	3.33	9.36	5.71	146–8	145–6	(3)
22	3,4-Dimethoxy	3.32	7.38	5.66	185–6	181–2	(3)
13	2-Hydroxy-4-methyl	3.30	7.35	6.65	182–3	177	(3)
12	5-Amino-2-hydroxy	3.28	3.67	3.14	..	283	(2)
9	3,4-Dihydroxy	3.21	6.04	3.37	204–5	199	(3)
11	3,4,5-Trihydroxy	3.20	5.48	3.51	..	220	(2)

[a] Arranged in order of first lines; pattern numbers from Table II.
[b] Pattern from (*1*) included for completeness.
[c] Aspirin.
[d] Pattern from (*4*) included for completeness.

Table II. Powder Diffraction Data[a]

1. *o*-Bromobenzoic Acid $C_7H_5BrO_2$

d, A.	I/I_1
11.32	0.20
6.55	0.80
5.90	1.00
4.28	0.40
3.86	0.50
3.68	1.00
3.54	0.40
3.39	0.10
3.29	1.00
3.17	0.10
3.10	0.10
2.97	0.20
2.84	0.50
2.77	0.10
2.67	0.30
2.54	0.05
2.50	0.20
2.45	0.10
2.38	0.40
2.28	0.20
2.19	0.05
2.13	0.05
2.04	0.10
1.941	0.05
1.866	0.10
1.816	0.05
1.786	0.10
1.754	0.10
1.734	0.10

2. *m*-Bromobenzoic Acid $C_7H_5BrO_2$

d, A.	I/I_1
12.70	0.50
6.39	0.10
5.92	0.80
4.87	0.80
4.41	0.30
4.17	0.10
3.81	0.40[b]
3.58	0.20
3.41	1.00[b]
3.19	0.50
3.07	0.10
2.98	0.10
2.82	0.05[b]
2.65	0.05
2.52	0.10[b]
2.42	0.05
2.35	0.05
2.26	0.10
2.18	0.05
2.11	0.05

2. *m*-Bromobenzoic Acid $C_7H_5BrO_2$

d, A.	I/I_1
2.08	0.10
1.953	0.05
1.856	0.05
1.824	0.10

3. *p*-Bromobenzoic Acid $C_7H_5BrO_2$

d, A.	I/I_1
13.90	0.40
7.25	0.04
5.60	0.04
5.18	0.32
4.68	1.00
3.71	0.60[b]
3.28	1.00
3.11	0.32
2.99	0.04
2.91	0.04
2.82	0.32
2.63	0.08
2.55	0.04
2.44	0.16
2.36	0.20
2.26	0.08
2.11	0.12
2.07	0.04
1.967	0.04
1.912	0.08
1.872	0.12
1.802	0.04
1.773	0.04
1.719	0.04
1.652	0.04[b]
1.595	0.04

4. *p*-Hydroxybenzoic Acid $C_7H_6O_3$

d, A.	I/I_1
5.06	1.00
4.59	0.40
4.01	0.20
3.64	0.80
3.44	0.10
3.33	0.60
3.05	0.10
2.992	0.60
2.689	0.10
2.502	0.05
2.413	0.10
2.276	0.10
2.239	0.05
2.081	0.10

5. 2,3-Dihydroxybenzoic Acid (*o*-Pyrocatechuic Acid) $C_7H_6O_4$

d, A.	I/I_1
8.27	0.60
7.39	0.12
7.03	0.16
6.54	1.00
5.43	0.40
4.82	0.08
4.55	0.08
4.31	0.12
4.11	0.12
3.74	0.08
3.66	0.20
3.37	0.16
3.31	0.16
3.20	0.60
3.04	0.08
2.897	0.08
2.638	0.12
2.532	0.12
2.413	0.12
2.323	0.12
2.182	0.12
2.116	0.12

6. 2,4-Dihydroxybenzoic Acid (*β*-Resorcylic Acid) $C_7H_6O_4$

d, A.	I/I_1
5.78	0.40
5.37	0.60
4.98	0.60
3.76	1.00[b]
3.57	1.00
3.36	0.80
3.13	0.40
2.979	0.10
2.880	0.20
2.570	0.20
2.423	0.20
2.328	0.40
2.200	0.20
2.120	0.10

7. 2,5-Dihydroxybenzoic Acid (Gentisic Acid) $C_7H_6O_4$

d, A.	I/I_1
8.18	0.53
5.62	0.33

7. 2,5-Dihydroxybenzoic Acid (Gentisic Acid) $C_7H_6O_4$

d, A.	I/I_1
5.26	0.27
5.08	1.00
4.63	0.53
3.98	0.03
3.73	0.07
3.63	1.00
3.56	0.67
3.17	0.67
2.906	0.53
2.817	0.07
2.674	0.07
2.498	0.07
2.399	0.27
2.319	0.20
2.266	0.07
2.137	0.07
2.113	0.07
2.058	0.07
1.982	0.07

8. 2,6-Dihydroxybenzoic Acid (*γ*-Resorcylic Acid) $C_7H_6O_4$

d, A.	I/I_1
11.28	0.20
6.46	1.00
6.15	0.40
5.43	0.60
4.95	0.10
3.90	0.20
3.75	0.20
3.50	0.80
3.40	0.10
3.31	0.80
3.16	0.80
3.08	0.20
2.909	0.10
2.793	0.10
2.717	0.10
2.586	0.10
2.150	0.10

9. 3,4-Dihydroxybenzoic Acid (Protocatechuic Acid) $C_7H_6O_4$

d, A.	I/I_1
12.11	0.04
7.50	0.08
6.04	0.60
5.72	0.12
4.53	0.04
4.40	0.04
4.18	0.04
3.69	0.04
3.51	0.02
3.37	0.32
3.21	1.00
3.09	0.02
2.973	0.04
2.851	0.04
2.777	0.02
2.724	0.02
2.557	0.08
2.376	0.02
2.327	0.02

10. 3,5-Dihydroxybenzoic Acid (*α*-Resorcylic Acid) $C_7H_6O_4$

d, A.	I/I_1
11.04	0.20
6.33	0.20
6.08	0.10
5.15	0.05
4.57	0.40
4.13	1.00
3.96	0.60
3.75	0.40
3.54	0.60
3.42	0.20
3.33	0.10
3.16	1.00
2.771	0.10
2.511	0.05
2.443	0.20
2.365	0.05
2.200	0.10

11. 3,4,5-Trihydroxybenzoic Acid (Gallic Acid) $C_7H_6O_5$

d, A.	I/I_1
10.95	0.20
7.46	0.40

11. 3,4,5-Trihydroxybenzoic Acid (Gallic Acid) $C_7H_6O_5$

d, A.	I/I_1
7.03	0.10
5.48	0.80
4.64	0.20
4.49	0.20
3.62	0.20
3.51	0.80
3.20	1.00
3.12	0.20
3.02	0.10
2.834	0.10
2.598	0.10
2.517	0.10
2.395	0.10

12. 5-Amino-2-hydroxybenzoic Acid (5-Aminosalicylic Acid) $C_7H_7NO_3$

d, A.	I/I_1
11.68	0.40
6.95	0.05
6.18	0.10
5.85	0.30
5.34	0.40
3.96	0.05
3.67	0.50
3.48	0.20
3.33	0.10
3.28	1.00
3.14	0.50
2.924	0.05
2.817	0.05
2.447	0.20
2.339	0.10
2.229	0.05
2.125	0.05
2.073	0.10

13. 2-Hydroxy-4-methylbenzoic Acid (2,4-Cresotic Acid) $C_8H_8O_3$

d, A.	I/I_1
11.3	0.40
8.2	0.20
7.35	0.80
6.65	0.60
4.99	0.40

[a] Patterns numbered in order of increasing empirical formula.
[b] Broad line.

Table II. Powder Diffraction Data[a] (*Continued*)

13. 2-Hydroxy-4-methylbenzoic Acid (2,4-Cresotic Acid) $C_8H_8O_3$

d, A.	*I*/*I*$_1$
3.75	0.60
3.51	0.20
3.40	0.20
3.30	1.00
2.23	0.20
2.19	0.20

14. 4-Hydroxy-3-methoxybenzoic Acid (Vanillic Acid) $C_8H_8O_4$

d, A.	*I*/*I*$_1$
9.43	0.63
8.74	0.50
6.99	1.00
5.68	0.06
5.38	0.25
5.15	0.25
4.05	0.25
3.90	0.13
3.71	0.25
3.53	0.06
3.45	0.13
3.29	0.63[b]
3.12	0.06
3.02	0.06
2.897	0.13
2.689	0.06
2.559	0.13
2.498	0.06
2.358	0.13
2.197	0.06
2.133	0.06
2.046	0.06

15. 2-Hydroxy-5-methoxybenzoic Acid $C_8H_8O_4$

d, A.	*I*/*I*$_1$
9.36	0.80
6.61	0.40
5.71	0.60
5.37	0.40
4.66	0.40
4.00	0.60
3.93	0.40
3.77	0.20
3.68	0.20
3.48	0.40
3.33	1.00
3.27	0.60

15. 2-Hydroxy-5-methoxybenzoic Acid $C_8H_8O_4$

d, A.	*I*/*I*$_1$
3.15	0.10
2.877	0.10
2.758	0.20
2.481	0.10
2.330	0.20
2.261	0.10

16. 2-Amino-3-methylbenzoic Acid (2-Amino-*m*-toluic Acid) $C_8H_9NO_2$

d, A.	*I*/*I*$_1$
11.45	0.50
6.45	1.00
5.70	0.13
3.92	0.06
3.71	0.63
3.36	0.63
3.30	0.63
3.23	0.13
2.628	0.06
2.559	0.06
2.467	0.06
2.423	0.06
2.337	0.06
2.228	0.06

17. 2-Hydroxy-3,6-dimethylbenzoic Acid $C_9H_{10}O_3$

d, A.	*I*/*I*$_1$
11.73	0.30
7.12	1.00
6.48	0.05
5.86	0.20
4.82	0.10
4.53	0.10
4.26	0.03
4.10	0.03
3.76	0.40
3.45	0.50
3.37	0.50
3.23	0.03
3.05	0.03
2.909	0.10
2.403	0.03
2.304	0.03
2.269	0.05
2.129	0.05
2.084	0.03

18. *p*-Ethoxybenzoic Acid $C_9H_{10}O_3$

d, A.	*I*/*I*$_1$
10.86	0.40
9.43	0.40
7.59	0.05
6.76	0.30
5.43	0.20
4.47	0.50
3.93	0.20
3.66	1.00
3.23	0.50
3.14	0.05
2.880	0.10
2.696	0.05
2.262	0.10
2.183	0.05
1.990	0.05

19. 2,3-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
7.63	0.30
6.85	0.40
6.34	0.20
5.26	0.30
5.15	0.10
4.86	0.20
4.64	0.50
4.14	0.80
3.86	0.30
3.46	0.20
3.37	1.00
3.04	0.30
2.900	0.05
2.777	0.05
2.628	0.05
2.563	0.10
2.395	0.10
2.197	0.05
1.977	0.05
1.912	0.05

20. 2,5-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
9.96	0.20
7.93	0.30
7.12	0.30
6.30	0.20
6.04	0.40

20. 2,5-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
5.74	0.10
5.36	0.05
4.98	0.20
4.73	0.05
4.12	0.05
3.89	0.10
3.64	0.50
3.53	0.10
3.42	1.00
3.30	0.80
3.23	0.10
3.19	0.10
3.02	0.10
2.915	0.05
2.640	0.05
2.311	0.05
2.220	0.05
2.119	0.05
2.008	0.05

21. 2,6-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
6.90	0.20
6.34	0.53
5.63	0.07
5.48	0.53
5.18	0.67
4.98	0.07
4.47	0.03
4.26	0.27
3.76	0.20
3.66	1.00
3.56	0.20
3.46	0.20
3.32	0.20
3.22	0.13
3.19	0.13
2.995	0.07
2.826	0.07
2.694	0.13
2.550	0.03
2.481	0.07
2.392	0.03
2.352	0.03

21. 2,6-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
2.284	0.03
2.233	0.03

22. 3,4-Dimethoxybenzoic Acid (Veratric Acid) $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
10.69	0.25
7.88	0.38
7.38	0.50
5.92	0.38
5.66	0.50
5.24	0.06[b]
4.70	0.06
4.53	0.06
4.07	0.03
3.95	0.06
3.79	0.13
3.70	0.03
3.62	0.03
3.52	0.06
3.46	0.06
3.32	1.00
3.07	0.03
2.745	0.06
2.667	0.06
2.570	0.06
2.401	0.06
2.273	0.03

23. 3,5-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
7.77	0.30
7.10	1.00
5.56	0.10
4.93	0.10
4.39	0.10
4.13	0.40
4.01	0.40
3.68	1.00
3.56	0.30
3.21	0.80
2.963	0.05
2.939	0.05
2.795	0.30
2.619	0.10
2.334	0.05

23. 3,5-Dimethoxybenzoic Acid $C_9H_{10}O_4$

d, A.	*I*/*I*$_1$
2.213	0.10
2.069	0.05

24. *p*-Isopropylbenzoic Acid (Cumic Acid) $C_{10}H_{12}O_2$

d, A.	*I*/*I*$_1$
12.94	1.00
9.67	0.02
7.30	0.04
6.51	0.04
5.19	0.60
4.56	0.04
4.14	0.32
3.92	0.20
3.81	0.04
3.26	0.20
3.05	0.02
2.960	0.02
2.588	0.02
2.486	0.02
2.428	0.02
2.288	0.04
2.226	0.02
2.150	0.02
2.109	0.02

25. 3,4,5-Trimethoxybenzoic Acid $C_{10}H_{12}O_5$

d, A.	*I*/*I*$_1$
8.86	0.12
7.75	1.00
5.87	0.12
5.66	0.08
5.09	0.04
4.79	0.04
4.46	0.04
3.65	0.12
3.57	0.12
3.47	0.60
3.38	0.16
3.23	0.12
2.982	0.04
2.704	0.04
2.528	0.04
2.247	0.04
2.207	0.04

[a] Patterns numbered in order of increasing empirical formula. [b] Broad line.

functional groups in the two compounds; however, in the first case, except for the carboxyl group, the functional groups differ.

These two cases point out the usefulness of the x-ray method in the study of organic compounds, in that it makes available a rapid and positive method of identification. This is true because the x-ray pattern depends on the kind and position of the atoms of the compound and on the bonding between the molecules of the compound. The melting point depends only on the strength of the bonding between the molecules while the empirical formula, and hence the chemical analysis, depends only on the kind of atom present. It is not surprising, therefore, that a condition such as mentioned above exists, although the authors have not seen it detailed in a publication.

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Determination of Platinum in Reforming Catalyst by X-Ray Fluorescence

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An x-ray fluorescence method has been developed and applied to the routine determination of platinum in petroleum reforming catalyst. The specimen is prepared for analysis by briquetting the sample with 5% of an organic binder. At the 0.6% level the precision of measurement is expressed as a standard deviation of 0.006% platinum. Excellent agreement with chemical methods is shown. Instrumental drift with time is minor but detectable; a method of correcting for it is described. Optimum tube conditions are selected for maximum peak-to-background ratio. The influence of carbon, iron, and water contamination in a catalyst is minor or negligible. Heat treatment does not affect the value obtained on a catalyst. Helium offers no marked advantage over air as an atmosphere in the optical path of the instrument.

THE initiation of plant reforming operations in the petroleum industry by means of platinum catalyst has made necessary the consideration of several methods of analysis for routine inspection of this catalyst. One of the inspections required is the determination of platinum content in the fresh catalyst. The platinum content of a commercially available fresh reforming catalyst is 0.50 to 0.60%. After depletion through plant use, the spent catalyst may be forwarded to a processor for reworking and recovery of platinum. Because platinum is an expensive commodity, an accurate evaluation of platinum content in both the fresh and spent inventory is very important. An inspection error of only 0.02% on a 100,000-pound quantity has monetary value equivalent to approximately $34,000 (platinum current market value of $120 per troy ounce is assumed). Thus the incentive is strong for employing the best analytical techniques available in the inspections. Several techniques are under investigation or have been developed (*5*). X-ray fluorescence has been demonstrated to be precise and accurate for the determination of trace metals in cracking catalyst (*3*). This article discusses the development and application of an x-ray fluorescence technique for the analysis of platinum reformer catalyst.

EXPERIMENTAL WORK

Calibration Standards. The x-ray fluorescence technique of analysis is dependent upon the use of reference standards of accurately known composition for calibration. The standards used for calibration should be very similar in composition to the unknown sample. The value of a result obtained in an analysis obviously is no better than the accuracy to which the components of the calibrating standards are known.

For the present purpose two types of standards have been employed in calibration: commercial catalysts, the platinum content of which is known, and synthetic samples containing known amounts of added platinum. Methods of analysis which have been employed on commercial catalysts or other platinum-bearing substances include the fire assay, gravimetric, and photometric methods. The difficulty of the technique or the state of the art of each chemical method is such at present that further development is necessary to adapt the method to as rapid routine use as is attainable with x-ray fluorescence. To select a "best" set of standards, judicious consideration must be given to the consistency as well as the quality of values obtained by several methods, including x-ray fluorescence. The fact that the sample is not expended in x-ray analysis enables the laboratory to accumulate a reliable set of reference standards.

Scope. The method is applicable to the determination of platinum in platinum-alumina catalyst in the range of 0.05 to 1%.

Sample Preparation. A portion of ground platinum catalyst is ignited in a muffle furnace for 3 hours at 1000° F., then cooled in a desiccator to room temperature. A 3.8-gram portion is admixed thoroughly with 0.2 gram of Sterotex, a hydrogenated corn oil product (Capital City Products Co., Columbus, Ohio), preparatory to briquetting. A 5% Sterotex addition helps prevent shattering of the pellet upon ejection from the briquetting press.

Apparatus. The pellets are fabricated in an Applied Research Laboratories Model 3502 briquetting press, using a 1-inch die and a pressure of approximately 40,000 pounds per square inch. These conditions yield pellets 1 inch in diameter and $^1/_8$ inch thick.

A North American Phillips (Norelco) x-ray fluorescence Geiger counter spectrometer is used for the analysis of the platinum reforming catalyst pellets. A lithium fluoride crystal (d = 2.0138 A.) is used in the goniometer diffracting the fluorescence spectrum. A leaf collimator with 0.02-inch spacings is used before the Geiger tube.

Conditions of Measurement. The catalyst pellet is positioned in the x-ray beam by means of a modification of the sample holder supplied with the instrument, as shown in Figure 1. X-irradiation is generated by a Machlett tungsten target source tube operated at 40 kv. and 20 ma. The Pt $L_{\beta 1}$ line intensity is measured at a fixed position of 32.2° 2θ, counting 102,400 Geiger impulses. Although the Pt $L_{\alpha 1}$ line is more intense than Pt $L_{\beta 1}$, the spectral coincidence of several tungsten L_β lines (*2*) at the Pt $L_{\alpha 1}$ position is rather troublesome, precluding the use of Pt $L_{\alpha 1}$. Background is read at 29.0° 2θ, counting 12,800 impulses, and subtracted from the platinum line to give net counts per second contributed by the platinum. The variance of counting statistics, introduced both in line and in background measurement, should be considered in selecting the number of counts to be taken. The exact positions or angles selected for measurement of maximum line intensity and for background are determined by manual operations of the specific instrument used for the analysis. Each face of two pellets or one face of four pellets is

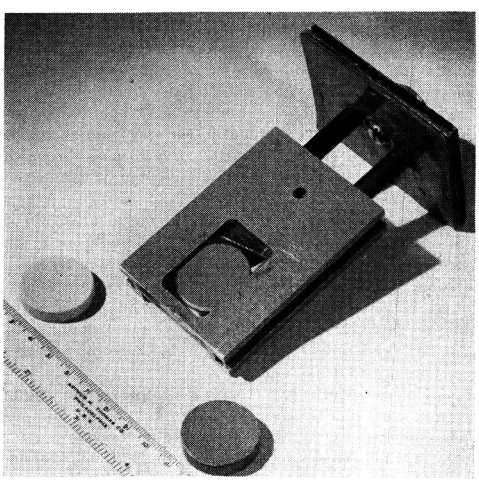

Figure 1. Modified sample holder

Table I. Analysis of Platinum Reforming Catalyst

Sample No.	Platinum, % Spectrophotometric	X-ray fluorescence	Difference
1	0.592	0.583	−0.009
2	0.587	0.590	+0.003
3	0.585	0.582	−0.003
4	0.581	0.583	+0.002
5	0.575	0.575	0.000
6	0.571	0.582	+0.011
7	0.586	0.575	−0.011
8	0.583	0.573	−0.009
9	0.587	0.581	−0.006
10	0.583	0.584	+0.001
11	0.586	0.574	−0.012
12	0.589	0.586	−0.003
		Av.	0.006

Table II. Influence of Specimen Preparation on X-Ray Fluorescence Measurement of Platinum in a Sample of Reforming Catalyst[a]

Specimen Preparation	Cell Fillings	Measurements	Standard Deviation, % Pt
Powder	12	12	0.0092
	1	11	0.0202
	1	9	0.0170
Briquet	1	7	0.0125
	1	12	0.0053
	1	6	0.0082
	1	6	0.0018
	1	6	0.0018
	1	6	0.0038
	1	6	0.0020

[a] Platinum content 0.632%.

measured and an average net value of counts per second is used for the interpretation for one determination.

RESULTS

Calibration. A straight-line regression curve was established to relate concentration of platinum with the intensity in counts per second of the fluorescent x-radiation. In this laboratory a working curve has been expressed by the equation

$$\% \text{ platinum} = 0.00164 \text{ (counts per second} - 60)$$

The curve passes through the ordinate rather than the origin for zero platinum. Several aluminas were tested to verify this property of the curve and the measurements were found to be in good agreement for different aluminas. A slight but characteristic "bump" above background in the spectrum at the position of the platinum peak causes the curve to pass through the ordinate. The occurrence of the $WL\gamma_1$ peak at 32° 2θ accounts for this observation (*2*).

The time requirement for measurement on an unknown normally is about 15 minutes per sample. The use of one or more reference samples with the unknowns gives greatest accuracy.

Precision. The estimated precision of fluorescence measurement may be obtained by replicate values on a given sample. The data of Table III, for example, are replicates on one sample. The standard deviation of four replicates is 3.2 counts per second; this is equivalent to 0.005% platinum.

Comparison of Methods. Table I compares spectrophotometric and x-ray fluorescence methods of analysis on typical commercial catalyst samples. The former method measures the intensity of the complex of chloroplatinic ion with stannous chloride in hydrochloric acid medium (*1, 5*); in this laboratory a wave length of either 490 or 403 mμ has been used. The maximum difference between methods is 0.012% and the average difference is 0.006% platinum.

DISCUSSION

In connection with the development of the x-ray fluorescence technique for the analysis of platinum reforming catalyst, an investigation was carried out on several variables which were believed to influence the accuracy of the method.

Powder *vs.* Pellet Specimen. Bulk density, surface, homogeneity, particle size, and area irradiated are physical factors that are known to influence the intensity of emitted x-ray fluorescence (*4*). The preparation of the sample specimen in a reproducible manner to minimize the effects of these factors is of utmost importance in an analysis. A comparison was made of preparing the specimen as a powder, which was hand-packed into a rectangular cell, and as a briquetted wafer. The smaller irradiated area for the pellet than for the hand-packed powder results in a slight decrease in peak-to-background ratio for the pellet as compared with the powder. The precision of a series of measurements is shown in Table II, in which the standard deviation is expressed as per cent platinum.

Table III. Variation of Measurement between Specimen Preparations

(0.632% Pt)

Specimen	Intensity Measurement, Counts per Second 1	2	3	4	5	6
A	450.3	448.2	449.4	450.5	446.6	461.4
B	448.6	438.8	466.4	440.0	440.8	443.1
C	443.4	442.7	440.8	443.7	447.3	450.0
D	444.1	449.4	450.0	441.5	442.0	443.2

Av. 446.76

Analysis of Variance

Source	Sum of square	Degrees of freedom	Variance
Within	810.7	20	40.54
			$F_{0.95}(3,20) = 3.10$
Between means	157.2	3	52.4
Total	967.9	23	$F = 1.29$

Conclusion. The difference between means on different specimen preparations is not statistically significant.

Table IV. Variation of Measurement on a Specimen with Time

(0.632% Pt)

Date of Measurement	Intensity Measurement, Counts per Second 1	2	3	4	5	6	7	8	Av.
4-13-1955	449.9	453.7	458.7	454.2	452.1	452.2	445.9	447.9	451.8
5-4-1955	450.6	450.3	452.1	449.3	453.3	451.3	...	...	451.2
5-19-1955	444.1	449.4	445.0	441.5	442.0	443.2	...	...	444.2

Analysis of Variance

Source	Sum of squares	Degrees of freedom	Variance
Within	227.6	2	113.8
			$F_{0.95}(2,17) = 3.59$
Means	161.6	17	9.51
			$F = 11.96$
Total	389.2	19	

Conclusion. The difference between means for a specimen measured at different times is highly significant.

With powder filling, the measurements tended to drift with time of residence in the instrument, although the data that illustrate this are not included in this paper. This effect was attributed to heating of the sample, which changed the level of the radiated surface. Actually the data of Table II indicate that replicates obtained on different fillings are more precise than replicates on the same filling, where the specimen remains in the instrument during measurements.

Table V. Influence of Tube Voltage and Amperage on Peak-to-Background Ratio for Six Samples[a]

Tube power setting	15 ma., 45 kv.	20 ma., 40 kv.	25 ma., 35 kv.
Av. intensity, c.p.s.[b]			
Background	219.9	219.5	226.6
Peak	277.8	278.7	258.8
Line/background	1.263	1.270	1.142

[a] Platinum concentrations. 0.0, 0.10, 0.25, 0.50, 0.60, and 0.632% Pt.
[b] Each value obtained by averaging measurements on six samples.

Table VI. Influence on Contaminants on Measurement of Platinum

Sample	N	C.P.S.[a] Av.	S.d.	t	Conclusion[b]
Reference[c] (0.632% Pt)	6	446.2	4.8		
Reference + 10% graphite	6	435.3	3.4	4.54	Statistically different
Reference + 1% Fe_2O_3	6	420.4	3.7	10.45	Statistically different
Reference (0.632% Pt)	6	443.0	1.7		
Reference + 10% H_2O	6	439.7	3.0	2.39	Statistically different

[a] 1 c.p.s. ~ 0.00165% Pt by calibration.
[b] At confidence level of 95%, $t = 2.23$ for 10 degrees of freedom represented by compared sets of values.
[c] Basis of analysis, 1000° F. preignition.

The precision for each of several pelleted specimens is better than for powder. The measurements did not drift with time of cell residence as they did with the powder specimen. The precision of a briquet measurement is indicated to be within about 0.006% platinum on this typical sample.

Repeatability between Specimens. Uniformity of the specimen is imperative for obtaining precise measurements. In a test of variation between specimen preparations (Table III), replicates were obtained on four specimens and an analysis of variance was made of the measurements. No difference in specimen preparation is detectable. This is significant, in that it demonstrates that the pelleted specimen can be prepared in a reproducible manner.

Repeatability with Time. The stability of the instrument with time was tested by three sets of measurements of the same specimen, taken over an interval of more than a month (Table IV). An analysis of variance showed that the difference in time of measurement reflects a significant difference in the results obtained. The maximum difference between averages for the first and last date of measurement is equivalent to 0.012% platinum. This indicates how a possible drift in calibration may be detected at a given time of analysis.

Optimum Excitation Source. In selecting the conditions of operation of the x-ray tube the criteria were that the intensity measurements be within the linear response region of the Geiger counter and that a maximum peak-to-background ratio be attained. A series of synthetic samples containing 0 to 0.6% platinum was selected for measurement. The tube voltage and current were changed simultaneously, but in opposite direction, so as to give approximately the same count on a given sample (Table V). A pair of measurements for both the net peak and background was taken for each sample at the tube voltage and current selected. An examination of the individual peak-to-background ratios showed that a setting of 20 ma. and 40 kv. was more favorable than the other settings. Therefore only the average of all measurements for peak and background at each tube condition is presented in Table V, inasmuch as the conclusion remains the same. This method makes possible a reasonable approximation of the optimum voltage-current setting, which gives maximum peak-to-background ratio.

Contaminant Effects. Contaminant substances which alter the composition of platinum reforming catalyst may interfere in the fluorescent measurement of platinum in two ways: by spectral coincidence—e. g., Pt L_{β_1} with SeK_{α_1} or GeK_{β_2}—and by matrix effects in which the platinum intensity is either enhanced or reduced by the presence of the extraneous element (*6*). All probable contaminants and their concentration in a used catalyst cannot be predicted with certainty. A test of the effects of a few likely contaminants can be made, however, by deliberately adding them to a fresh, clean reference catalyst. This has been done with carbon, iron, and water. The amount of contamination added in each case was presumed to be somewhat in excess of that normally found in a used catalyst. Sets of fluorescence measurements were made on the fresh and the contaminated portions.

In determining the differences between sets of measurements it has been necessary to resort to statistical interpretation, the t test, to define the reality of such differences with a given level of confidence. A 95% confidence level was arbitrarily selected for this purpose. The results of the statistical interpretation are summarized in Table VI.

The presence of 10% high-purity spectroscopic graphite produces a statistical difference when introduced by powder grinding into a reference sample—i.e., a slight reduction in count is observed. The difference produced by the carbon dilution is equivalent to 0.018% less platinum in an analysis. These measurements suggest that if about 1% of carbon were present as coke, the effect would not be detectable in a fluorescence analysis.

Ferric oxide contamination introduced by powder grinding in the amount of 1% produces a statistical difference. This amount of iron produces a reduction of 0.025% in the result for platinum. Where the amount of iron in a sample may be as much as several tenths per cent, an empirical correction factor, developed from the analysis of synthetic samples, should be applied.

Table VII. Influence on Heat Treatment on Measurements of Platinum

(0.632%)

Heat Treatment	N	C.P.S. Av.	S.d.	t	Conclusion[a]
1000° F.	6	446.2	4.8		
1800° F., 1 hr.	6	441.6	4.0	1.82	Not statistically different

[a] At confidence level of 95%, $t = 2.23$ for 10 degrees of freedom represented by compared sets of values.

Table VIII. Influence of Atmosphere upon Measurement of Platinum in Reforming Catalyst

(0.632% Pt)

Atmosphere	N	Intensity, C.P.S. Bg.	Net line	S.d.	Line/Bg.
Air	6	213.8	462.2	4.14	2.162
Helium	6	228.5	500.8	3.68	2.192

Ten per cent of water in a sample barely produces a statistical difference in a set of measurements where the reference sample is preignited at 1000° F. The difference is just significant in the sets of measurements compared in Table VI and is equivalent to 0.0055% platinum, which is approximately the standard deviation of measurement observed on other samples. As with carbon, the considerable dilution with 10% water has a rather minor effect on the results. This indicates that the dilution effect of the elements of low atomic number—i.e., carbon and water—is partially offset by the reduced absorption which allows more of the platinum radiation to be emitted from the sample. It is highly unlikely that, following ignition at 1000° F., the

sample would hydrate sufficiently during preparation and analysis to produce a measurable effect on the analysis value.

Effect of Ignition. The density of alumina normally is increased by strong ignition. To test the possible influence of ignition, a portion of sample previously ignited at 1000° F. was reignited at 1800° F. for 1 hour, then measured (Table VII). No difference due to degree of severity of ignition is shown. It is probable that this result is due in part to the briquetting operation in preparing the sample. The pellets are reduced to nearly the same bulk density, regardless of the effect of prior heat treatment. Thus no adverse effect of degree of heat experience in plant use between catalysts should be reflected in the platinum analysis by x-ray fluorescence.

Effect of Atmosphere. For elements of low atomic number, which emit long wave-length x-rays, the use of a vacuum or a gas of low atomic number, such as hydrogen or helium, is a great aid in fluorescence measurements. Although the Pt $L_{\beta 1}$ wave length is relatively short—i.e., 1.12 A.—it was considered that the use of helium might be an improvement over air in the quality of measurements obtained. Table VIII presents results for air and helium. No marked difference either in line-to-background ratio or in precision is achieved through the use of helium; the rather minor improvement does not appear to justify its use in the routine procedure.

ACKNOWLEDGMENT

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Stable Carbon Isotope Analysis by Optical Spectroscopy

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The use of C_2 radiation from an acetylene flame for the measurement of relative concentrations of the carbon isotopes, C^{12} and C^{13}, has been investigated. Carbon-13–enriched samples of acetylene were burned in premixed flames with air, and relative intensities of the Swan bands due to the isotopic C_2 radicals were measured photoelectrically. A representative calibration curve of the intensity ratio $C^{12}C^{13}/C^{12}C^{12}$ vs. C^{13} abundance in the fuel is presented and discussed.

SUCCESSFUL applications of optical spectroscopy to routine determination of hydrogen isotope concentrations (*1*, *2*) have encouraged the study of its applicability to the analysis of other stable isotope mixtures. The work reported here concerns the use of C_2 radiation from a flame for the measurement of relative concentrations of the carbon isotopes, C^{12} and C^{13}. It is not intended to describe a finished procedure suitable for routine application, but rather to indicate the potential usefulness of the method and to point out the precision which can be expected with the use of simple apparatus.

The C_2 Swan bands are well-known components of the visible radiation from the inner cone of hydrocarbon flames. At present the origin of the transient C_2 molecule is not known (*5*). These bands have been used previously (*6*) in studies of the natural abundance of carbon-13 in terrestrial carbon and in stellar atmospheres. The work described here differs from earlier studies in that a very small flame was the light source, photoelectric rather than photographic techniques were used, and a representative calibration curve covering a relative abundance range of interest in tracer work was obtained and is given for illustration.

In the procedure developed, several samples of acetylene with carbon-13 content in the range from 3.6 to 23 atom % of the total carbon were burned with air on a small burner, and the emission spectra of the flames (inner cone) were recorded with a photoelectric recording spectrometer. The relative intensities of the 1,0 band heads of the $^3\Pi - ^3\Pi$ (Swan) system due to the isotopic C_2 radicals $C^{12}C^{12}$, $C^{12}C^{13}$, and $C^{13}C^{13}$ in the region from 4735 to 4755 A. were measured; the data were used to make a plot of the $C^{12}C^{13}/C^{12}C^{12}$ band head intensity ratio against carbon-13 relative abundance in the fuel. The discussion which follows deals mainly with this curve and the possibilities which it indicates for determination of carbon-13 abundance in carbon-containing samples.

The 1,0 band was selected because of its relatively high intensity in a flame, the low intensity of background radiation at the head of the band, the favorable separation of the band heads of the isotopic C_2 radicals (about 7 A.), and because in this band the $C^{12}C^{13}$ head is clear of overlapping lines of the $C^{12}C^{12}$ spectrum.

An acetylene flame was chosen because of the relatively high intensity of C_2 emission from the inner cone with this fuel. Air rather than oxygen was used because with oxygen it is difficult to avoid flashback and at low flow rates the flame is inconveniently sensitive to adjustments. An acetylene-air flame gives little trouble in this respect.

EXPERIMENTAL

Acetylene–carbon-13 was prepared by the carbide method (*3*) from enriched barium carbonate (Eastman Kodak Co.). It was purified by three vacuum distillations from a trap at dry ice temperature into a trap at liquid nitrogen temperature. Ordinary tank acetylene, freed of acetone by vacuum distillation, was used to dilute samples of the acetylene–carbon-13 for the preparation of mixtures for analysis. The mixtures were prepared volumetrically on the vacuum line by first measuring acetylene–carbon-13 and ordinary acetylene separately and then condensing them together into a storage bulb. Volume measurements were made at the same pressure to avoid the necessity of compressibility corrections.

The starting sample (66.6 atom % carbon-13) and one diluted

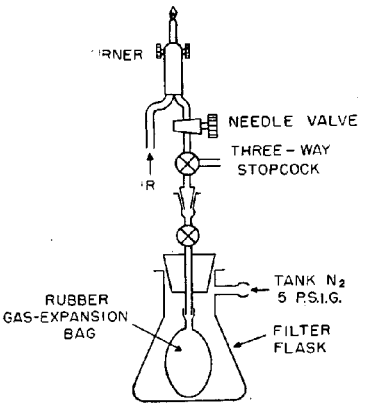

Figure 1. Burner assembly

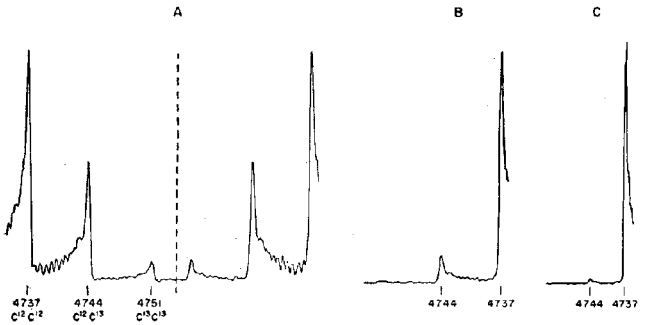

Figure 2. Recordings of the 1,0 band heads ($^3\Pi \rightarrow {}^3\Pi$ transition) of C in acetylene-air flame

A. Acetylene–carbon-13, 22.9 atom % carbon-13
B. Acetylene–carbon-13, 6.3 atom % carbon-13
C. Ordinary tank acetylene

sample (22.9 atom % carbon-13) were analyzed mass spectrometrically. The relative abundance of carbon-13 in the samples is given here as usual in atom per cent of the total carbon. The other mixture compositions, 11.9, 6.3, and 3.6 atom % carbon-13, were calculated from the results of these analyses and the volumetric dilution measurements. The mass spectrometric analyses showed a maximum of 1% impurity, most of which was ethylene.

The isotopic acetylene mixtures prepared by dilution of carbon-13–enriched acetylene with ordinary acetylene do not have the carbon isotopes distributed randomly among the acetylene molecules. For example, in the mixture containing 22.9 atom % carbon-13, the isotopic acetylenes $HC^{12}C^{12}H$, $HC^{12}C^{13}H$, and $HC^{13}C^{13}H$ are present in the concentration ratio of 69 to 16 to 15, respectively. If the acetylene sample had been prepared from homogeneous barium carbonate containing 22.9 atom % carbon-13, the respective concentrations would be approximately 60 to 35 to 5. The slight effect of the isotope distribution in the acetylene upon that in the C_2 in the flame is discussed below.

The acetylene mixtures were burned on a small welding torch with an orifice diameter of 0.5 mm., using the delivery apparatus shown in Figure 1. The acetylene and air were premixed in the torch in proportions (somewhat less than the stoichiometric amount of air) which gave the greatest intensity of the C_2 bands. Control valves were set using tank acetylene and were left in the same position for all mixtures burned. The sample was delivered from a rubber gas-expansion bag filled on the vacuum line; constant delivery was achieved by surrounding the bag with nitrogen at 5 pounds per square inch gage from a pressure-regulated tank. The three-way stopcock shown in Figure 1 was used for flushing or evacuating the upper part of the assembly between determinations. A stable and reproducible flame of sufficient intensity was obtained with an acetylene flow of 6 cc. per minute. The samples ranged from 50 to 100 cc. (normal temperature and pressure). Ten separate spectra could be obtained with a 100-cc. sample in a burning time of about 16 minutes.

The inner cone of the flame was focused on the slit of a monochromator with photomultiplier detection and pen recorder (*4*). (This instrument was constructed by the Research Department of Leeds & Northrup Co. and loaned to the National Bureau of Standards on a field trial arrangement.) Recorded intensities are directly proportional to the emitted light intensities. A slit height of 2 mm. and a slit width of about 0.02 mm. were used; this permitted the clear resolution of lines 0.6 A. apart. For the purpose of analysis considerably lower resolution would be adequate.

RESULTS

Figure 2 shows recordings of the 1,0 band heads of the isotopic C_2 radicals obtained with three acetylene samples of different carbon-13 content. The scanning rate was 12 A. per minute. The first recording, *A*, made with acetylene containing 22.9 atom % carbon-13 is shown to illustrate the reproducibility of the relative band head intensities. Just to the left of the dotted vertical line the direction of scanning of the spectrum was reversed and the tracing was repeated as a mirror image. Curve *B* is a recording of the same spectral region with a flame of acetylene containing 6.3 atom % carbon-13. In this case the $C^{13}C^{13}$ band head intensity is too low to stand out above the noise level, although the $C^{12}C^{13}$ head can still be measured accurately. Curve *C* was made with a flame of ordinary tank acetylene; the $C^{12}C^{13}$ band head is due to the natural abundance of carbon-13, which is about 1.1 atom %.

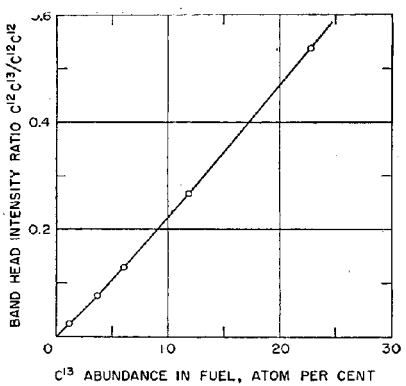

Figure 3. Plot of observed $C^{12}C^{13}/C^{12}C^{12}$ band head intensity ratios *vs.* C^{13} abundance in atom %

Figure 2 shows that the band heads are well separated and that the intensities can be measured easily. Only the $C^{12}C^{12}$ and $C^{12}C^{13}$ band head intensities are needed for the determination of the relative abundance of carbon-13. A calibration curve using these two band heads is shown in Figure 3, where the observed intensity ratio, $C^{12}C^{13}/C^{12}C^{12}$, is plotted *vs.* C^{13} abundance, expressed in atom per cent, in the acetylene burned.

This plot is not expected to be linear. If the carbon in the radiating C_2 is assumed to be a representative sample of that in the fuel, with random distribution of the isotopes among the C_2 pairs, a simple algebraic analysis will show that the curve is essentially a graph of $2(C^{13})/[1-(C^{13})]$ *vs.* (C^{13}), where (C^{13}) is the atom fraction of carbon-13 in the carbon.

Each ratio plotted is the arithmetic mean of the results of

three separate scans with the same sample and the same flame. In all cases, the standard deviation of the mean of the three measurements was less than 2.5% of the mean value. The corresponding error in the carbon-13 abundance, expressed in the same way, did not exceed 2.5% of the measured abundance. In the poorest measurement of the series (that for the sample containing 3.6 atom % carbon-13) the standard deviation of a single observation based on a group of three amounted to 4.3% of the mean value.

With samples containing less than 15 atom % carbon-13, the $C^{12}C^{12}$ and $C^{12}C^{13}$ intensities were very nearly proportional to the expected isotopic C_2 concentrations calculated by assuming a random distribution of the isotopes in C_2; thus, it is estimated that the measured intensity ratio alone could be used directly to determine the carbon-13 abundance to the nearest 1% in this range. For greater accuracy the use of a calibration curve is desirable; this eliminates possible difficulties caused by small isotope effects on reaction rates and differences in spectral characteristics of the isotopic C_2 radicals, and takes care of instrumental peculiarities as well.

The nonequilibrium composition of the isotopic acetylene mixtures burned was referred to earlier. Another study (*5*) has shown that the emission from the isotopic C_2 radicals in the flame depends to a slight extent upon the isotopic composition of the acetylene. For example, if the acetylene has a deficiency (compared with the statistical equilibrium concentration) of $HC^{12}C^{13}H$, the $C^{12}C^{13}$ intensity will be slightly lower than that calculated from the carbon-13 abundance alone. The effect is as if about one tenth of the C_2 comes directly from the carbon-carbon pairs of the acetylene introduced into the flame. It is necessary to take this effect into account for accurate measurements in the range above 15 atom % carbon-13 on samples prepared by dilution with ordinary acetylene. This can be done by using a calibration curve obtained with acetylene samples similar in composition to those to be analyzed. On the other hand, in undiluted acetylene prepared from diluted carbon dioxide or barium carbonate, the isotopic species are present in nearly equilibrium concentrations and the effect is absent.

DISCUSSION

These results show that optical spectroscopy can be used for rapid and reasonably accurate determinations of C^{13}/C^{12} ratios. The two major disadvantages of the method suggested here are that acetylene must be prepared from the carbon-containing sample and that the sample of acetylene is destroyed by the analysis. These disadvantages can be lessened by modifying the procedure.

In actual application of the method, the carbon in the compound to be analyzed can be oxidized to carbon dioxide. The carbon dioxide can then be converted to acetylene by the method of Monat and Robbins (*7*) with 98% yield. This is a simple procedure and can be used for the preparation of acetylene from most carbon-containing substances. In view of the high yield, isotope fractionation is probably negligible, although this has not been investigated experimentally.

To reduce the size of sample required for burning, the acetylene–carbon-13 can be diluted with ordinary acetylene, remembering that the natural abundance of carbon-13 is 1.1 atom %. The extent of dilution possible depends upon the optical instrumentation used and upon the accuracy desired. In addition, the dead space in the burner assembly can be reduced and the rate of burning might also be decreased. It should be possible, with care, to reduce the total sample to less than 10 cc. of acetylene.

The results reported here do not give basis for a reliable estimate of the ultimate accuracy of the method, which will be determined largely by the precision of the results. However, in the determination of hydrogen isotope ratios using the same instrument, a precision of 0.1% in the ratio of two intensities was obtained (*1*). This may be taken at present, with the instrument used, as a possible limit to the precision which can be achieved in such measurements under the very best conditions—i.e., with intense, well-resolved atomic line spectra and a very stable source.

The technique suggested does not compare favorably with the mass spectrometric method in regard to the time required for sample preparation (preparation of acetylene instead of carbon dioxide), size of sample used (10 cc. *vs.* 0.1 cc.), and in the extent of development of the procedure. However, it may be found useful, especially in laboratories which do not have access to a mass spectrometer but which are equipped with an optical spectrometer of moderate resolving power.

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Spectrophotometric Determination of Microgram Quantities of Disodium Dihydrogen Ethylenediamine Tetraacetate

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A rapid spectrophotometric method for the determination of microgram quantities of disodium ethylenediamine tetraacetate (EDTA) is based on the absorbance of the complex formed by the reaction of EDTA with an excess of cupric ion. The copper-EDTA complex is developed in a sodium dihydrogen phosphate buffer of pH 11. Absorbance is measured against a copper sulfate–buffer reference solution at a wave length of 250 mμ. The effects of several variables were studied and optimum operating conditions established. Nickel, cobalt, and chromate interfere; cations that form weaker complexes with EDTA than copper do not interfere. The relative standard deviation for samples containing 125 to 500 γ of EDTA was 8%; for 500 to 1500 γ, 2%.

DISODIUM dihydrogen ethylenediamine tetraacetate (EDTA) has, in recent years, found wide application in analytical and other branches of chemistry. The quantitative determination of this compound is, accordingly, of considerable interest to the analytical chemist. A limited number of methods for the determination of EDTA have been reported (*3–5*). Of these, a spectrophotometric method outlined briefly in a bulletin issued by the Bersworth Chemical Co. (*2*), which makes use of the color of a complex formed when EDTA reacts with cupric ions, was selected for the determination of microgram quantities of EDTA. As little or no detail is given, a number of variable factors affecting the procedure were studied. These included wave lengths best suited for measurement of absorbance, buffer concentration, reference solutions, and excess of chromogenic reagent. Studies were also made of interferences and of the stability of the complex with temperature. A satisfactory analytical procedure has been developed which incorporates a number of important modifications of the Bersworth procedure.

EXPERIMENTAL

Absorption Spectrum. EDTA reacts with cupric ion in a buffered solution of pH 11, to produce a colored complex. As shown in Figure 1, the absorbance of this complex, when measured against a blank consisting of a solution of the reagents in a buffer solution, exhibits a maximum in the ultraviolet region of the spectrum between wave lengths 230 and 260 mμ. Over this band, the absorbance is practically constant. Accordingly, a point near the center of this region, 250 mμ, was chosen for use in the determination of EDTA. At this wave length, the absorbance was found to be much greater than at the wave length recommended by Bersworth—280 and 340 mμ (*2*). Furthermore, small shifts in the wave length have little effect on the absorbance in the spectral region near 250 mμ, whereas at 280 or 340 mμ the absorbance changes rapidly with small changes in wave length.

Effect of Buffer Concentration. The complex is formed in a disodium hydrogen phosphate buffer solution adjusted to pH 11 with sodium hydroxide. The data presented in Table I indicate that minimum absorbance of the reagent blank and maximum precision are attained at 0.1*M* disodium hydrogen phosphate.

Calibration Graph. It was established that the absorbance of the EDTA-copper complex, when measured against a copper sulfate–buffer reference solution, conformed to Beer's law. When water, buffer, or EDTA solutions were used as reference solutions, the absorbance-concentration graphs were nonlinear and

Table I. Absorbance Index for EDTA as a Function of Buffer Molarity

Conditions. Buffer solution, Na_2HPO_4
EDTA, 20, 30, 40, and 60 γ per ml.
Final volume, 50 ml.
pH, 11
Reference solution, 4 ml. of 0.25% $CuSO_4$ diluted to 25 ml. with buffer solution

Buffer, *M*	Reference Solution, Absorbance	Absorbance Index,[a] Av., a_s	Standard Deviation, %
0.6	1.70	6.54	43
0.4	0.563	8.09	16
0.2	0.143	8.03	7
0.1	0.085	8.40	2
0.05	0.135	9.69	17

[a] Absorbance index = $a_s = \frac{A_s}{cb}$

where A_s = absorbance
c = concentration, g./1000 grams of solution
b = length of light path, cm.

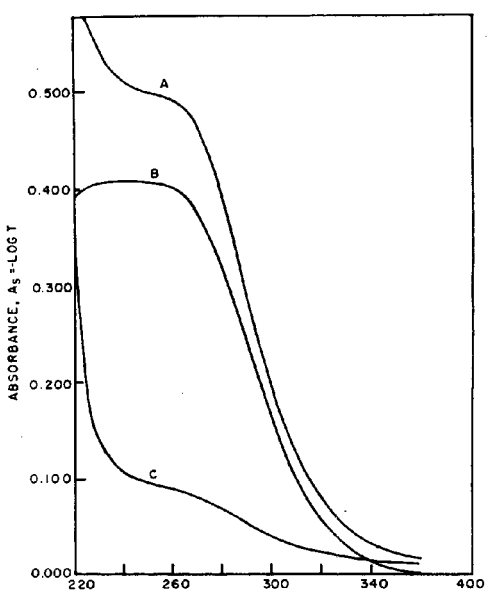

Figure 1. Absorption spectra of copper–ethylenediamine tetraacetate in ultraviolet region

	Absorbing Solution	Reference Solution
A.	1.49×10^{-4} *M* Cu-EDTA	H_2O
B.	1.49×10^{-4} *M* Cu-EDTA	$CuSO_4$–Na_2HPO_4
C.	$CuSO_4$ – Na_2HPO_4	H_2O

Table II. Effect of Excess Reagent on Absorbance of Complex and Reference Solutions

Conditions. EDTA, 50 γ per ml.
$CuSO_4$ solution, 0.25 w./v. %

Excess $CuSO_4$, 0.25% Soln., Ml.	Absorbance	
	Cu-EDTA complex[a]	Reference solution[b]
0.15	0.422	0.090
0.5	0.420	0.095
1.0	0.423	0.087
2.0	0.431	0.091
3.0	0.425	0.093
5.0	0.420	0.083

Relative standard deviation, 1%

[a] Reference solution, 0.25% $CuSO_4$ in 0.1*M* buffer solution.
[b] Reference solution, water.

a constant absorbance index could not be calculated. The data are presented in Figure 2. Accordingly, the copper sulfate–buffer solution was selected for use as the reference solution in subsequent studies and in the procedure that was finally adopted.

Effect of Excess Copper Sulfate Reagent. In the Bersworth procedure (*2*), only a very slight excess of copper sulfate is added over that required to complex all of the EDTA. Copper sulfate is added until a slight turbidity is observed by its Tyndall effect. An excess of 2 drops of 0.25% copper sulfate is then added. This method of regulating the excess copper sulfate is unsatisfactory, because the amount required to produce a detectable turbidity is dependent on the method of observation—i.e., whether observed by means of a narrow beam of light or by diffuse light—and, more important, a number of interfering cations will form precipitates that obscure the end point of the copper addition, since they are displaced from their EDTA complexes by copper.

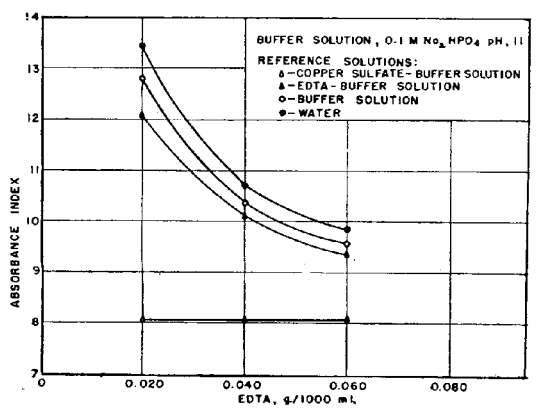

Figure 2. Absorbance index as a function of reference solution

Therefore, tests were made to determine whether excess reagent was detrimental. Excess copper sulfate, up to 5 ml. of a 0.25% solution, produced no significant effect on the absorbance of either the copper-EDTA complex or the reference solution (Table II). In fact, in the presence of a number of cations such as iron, calcium, or magnesium, an excess of copper sulfate was required to displace these cations from their complexes with EDTA; otherwise, the test results were low. In order to ensure a sufficient excess of copper for this purpose, 4 ml. of a 0.25% copper sulfate solution was added in the analysis of all samples. The excess copper precipitates as copper hydroxide at the pH at which the color is developed and is removed by filtration or centrifugation, together with hydroxides of any other metals that have precipitated after displacement from their complexes with EDTA.

Table III. Interference of Various Ions

Conditions. EDTA, 50 γ per ml.
Reference solution, water

Ion Added	Weight Ratio, Ion/EDTA	EDTA Found, γ	Error, %
Co^{++}	0.032	49.5	− 1
	0.13	49	− 2
	0.16	47	− 6
	0.32	45	−10
Ni^{++}	0.024	47	− 6
	0.12	33.5	−33
Cr^{+++}	0.32	48.5	− 3
	0.64	48.5	− 3
Ca^{++}	0.16	48.5	− 3
Mg^{++}	0.16	48.5	− 3
Fe^{++}	0.20	51	+ 2
Fe^{+++}	0.20	52	+ 4
	0.40	52.5	+ 5
Cr^{+6}	0.14	83.5	+67

Effect of Temperature. Tests were made to determine whether heating affected the absorbance of reference solutions and of copper-EDTA solutions after removal of excess copper as the hydroxide. The solutions were heated to 80° to 90° C. and cooled to room temperature, and the absorbance was measured. No difference was observed in the absorbance of heated and unheated solutions. However, the reproducibility was lowered for the reference solutions that had been heated.

Stability. Solutions of the copper-EDTA complex stored in polyethylene containers were stable over a 14-day period. Prior heating tended to increase the stability slightly. This was also true with the reference solution. However, the reference solution was more stable when stored in glass rather than polyethylene.

Interferences. A limited number of tests were made of interferences. Nickel and cobalt are more strongly complexed than copper by EDTA at pH 11 (*1*, *6*). Consequently, these two metals interfere in the determination of EDTA. Nickel interfered in all concentrations. Cobalt did not interfere at concentrations below one tenth of that of the EDTA. The chromate ion interfered because of its strong absorbance in the spectral region used for measuring the absorbance of the copper-EDTA complex. Ions which are displaced from their complexes by copper do not interfere, because they are precipitated as the hydroxides and removed with the excess copper. The extent of interference by various ions is shown in Table III.

PROCEDURE

The following procedure is simple and can be carried out rapidly without the use of special apparatus other than an ultraviolet spectrophotometer.

Transfer a test portion of sample containing from 125 to 1500 γ of EDTA in a volume of 5 ml. or less to a 25-ml. volumetric flask. Add 4 ml. of 0.25% copper sulfate reagent solution, and then add 0.1*M* disodium hydrogen phosphate buffer solution to the 25-ml. mark. Filter the solution through a dry filter paper. Prepare a reference solution by diluting 4 ml. of the copper sulfate reagent solution to 25 ml. with the buffer solution and filtering the diluted solution. Use the reference solution to set the spectrophotometer to zero at 250 mμ, and then measure the absorbance of the sample solution. For samples containing 500 γ of EDTA or less, use 5-cm. silica cells; for larger amounts of EDTA, use 1-cm. cells. Measure the absorbance of a series of standard

EDTA solutions, processed in the same manner as the sample, and compute the average absorbance index. Use the absorbance index to calculate the concentration of EDTA in the sample from its absorbance.

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Pyrohydrolytic Separation and Spectrophotometric Titration of Fluorides in Radioactive Samples

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Apparatus is described which is suitable for the pyrohydrolytic determination of fluoride in radioactive liquids or solids. Fluoride in the radioactive distillate from the pyrohydrolysis is determined by a remotely controlled titration in an apparatus of special design. The apparatus was evaluated by determining the fluoride content of solid uranium tetrafluoride, a mixture of solid fluoride salts, and a solution of fluoride salts. The relative standard deviation of the data for 2.5 to 15 mg. of fluoride was 3% for uranium tetrafluoride samples.

THE procedure described by Warf, Cline, and Tevebaugh (*5*) for the pyrohydrolytic determination of fluoride has been adapted to the analysis of semimicro quantities of highly radioactive liquids and solids. Modified apparatus was developed for the pyrohydrolysis of the sample and for spectrophotometric titration of fluoride by remote control. The fluoride contents of solid uranium tetrafluoride, of a mixture of fluoride salts, and of a radioactive liquid that contained fluorides were determined satisfactorily by use of the apparatus. The radiation level of samples was sometimes as high as 100 roentgens at contact, and radioactive fission products were present in the distillates.

PYROHYDROLYSIS APPARATUS

The unique feature of the pyrohydrolysis apparatus employed (Figure 1) is that it can be used for the analysis of either liquid or solid radioactive samples. It consists of a steam generator and superheater, a pyrohydrolysis unit, and a collector for the fluoride distillate.

The steam generator is constructed of standard borosilicate glassware. The superheater is a silica tube which passes through a furnace made from standard 8-inch-length heating elements. The furnace can be heated to a temperature of 1100° C. Control of the steam supply is critical because of the small volume of the pyrohydrolysis unit. Control is maintained by leaving the steam generator open to the atmosphere through a condenser. Steam passes through the system as a result of the reduced pressure that is maintained in the distillate receiver.

The pyrohydrolysis unit is constructed of platinum [or nickel (*3*)]; it incorporates a standard 20-ml. tubulated Gooch crucible.

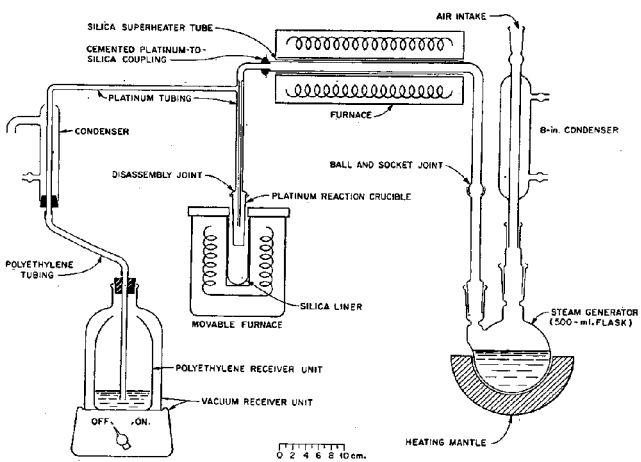

Figure 1. Apparatus for pyrohydrolysis

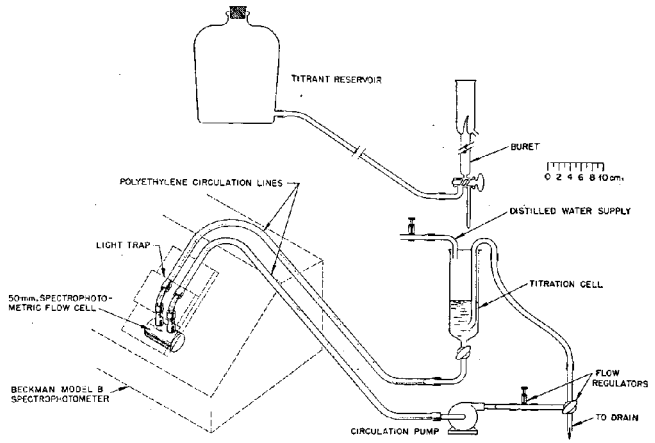

Figure 2. Apparatus for spectrophotometric titration

Use of this crucible was suggested to the authors by Vogel (*4*). The inlet tube of the crucible head is cemented to the silica tube of the superheater by means of Sauereisen paste No. 1. A side arm of platinum extends from the exit tube of the tubulated crucible to form the inner tube of the distillate condenser. The pyrohydrolysis crucible is heated by a movable furnace 1 inch in inside diameter, which is constructed from standard 6-inch heating elements, supplied as replacement units that are mounted in an appropriate refractory. The elements are mounted in a stainless steel beaker and are insulated with asbestos fiber. The flange of the crucible is supported by the top of the furnace; the entire lower section of the crucible extends into the hottest zone of the furnace. The tapered platinum-to-platinum seal of the crucible lid is held closed by the mild pressure of the supporting flange and is satisfactory without the use of a cemented seal, if care is taken to keep the contacting platinum surfaces clean and free of deformations. A thin sleeve of silica tubing is inserted between the furnace and the crucible in order to provide added protection to the platinum crucible.

The distillate is collected in a polyethylene container that is set inside a Fisher Filtrator, which is under moderate, regulated suction. The slightly reduced pressure (of the order of 1 to 2 inches of water) eliminates leakage at the crucible closure and surges that result from boiling phenomena. A condenser jacket is fitted to the platinum side arm to form the distillate condenser. The jacket is attached at the bottom to the platinum tube by means of a rubber stopper. The outlet port of the jacket is of larger diameter than the inlet tube, in order to allow discharge of the cooling water from the condenser before the water reaches the top of the jacket; it is therefore not necessary to support the jacket against the hot upper end of the platinum tube.

Table I. Fluoride in Solid Irradiated Uranium Tetrafluoride

Determination	Test Portion, Mg.	Fluoride[a], Wt. %
1	29.7	24.9
2	25.5	25.0
3	20.6	23.2
4	15.2	24.1
5	13.4	22.6
6	10.9	24.6
7	10.0	24.7
8	26.5	23.7
9	27.0	24.3
10	43.2	23.9
11	58.0	23.8
		Av. 24.1

[a] Theoretical fluoride content, 24.20 wt. %. An experimental value of 24.18 wt. % was obtained on the sample before it was irradiated. A macroapparatus, similar to that described by Warf, Cline, and Tevebaugh (*5*), and visual end point detection were used. The relative standard deviation was 1.07% for 15 consecutive analyses of test portions that weighed 35 to 60 mg.

SPECTROPHOTOMETRIC TITRATION APPARATUS

Fluoride in the radioactive distillate from the pyrohydrolysis apparatus is determined by a remotely controlled titration apparatus of special design (Figure 2).

The titration procedure is similar to that described by Nichols and Kindt (*2*). The titrant is thorium nitrate solution, the buffer is monochloroacetic acid–sodium hydroxide solution, and the indicator is alizarin red S. With the exception of the light-trap cover for the absorption-cell chamber of the Beckman spectrophotometer, the apparatus is constructed from stock items. The titration cell is made from an extraction cell or from the end of a large buret. The test solution is circulated from the titration cell through the absorption cell by means of an Eastern Model A-1 Monel centrifugal pump, available from Burrell Corp., Pittsburgh, Pa. In this way the radiation hazard to the operator is decreased, and the apparatus therefore has an advantage over the assembly described by Goddu and Hume (*1*). The radioactive solutions can be discarded with a maximum of safety through the drain provided in the line.

The radioactive, fluoride-containing distillate collected from the pyrohydrolysis in a solution of excess sodium hydroxide is titrated by transferring the distillate to the titration cell and neutralizing it to the phenolphthalein end point with hydrochloric acid. The centrifugal pump is used to stir the solution. The buffer solution and indicator are then added, followed by enough water to make a total volume of approximately 200 ml. The titrant is added in increments, and the solution is circulated for 10 to 20 seconds, and then allowed to rest for 30 to 40 seconds before the per cent transmittancy of the solution at 525 mμ is read and recorded. The point of maximum change in the per cent transmittancy is taken as the empirical equivalence point (*2*).

Air is trapped in the solution if the pump is not properly primed. After the titration is completed, the solution is drained from the

Table II. Fluoride in Radioactive Mixture of Solid Fluoride Salts

Determination	Test Portion, Mg.	Fluoride, Wt. %[a]
1	40.8	41.4
2	40.4	40.8
3	42.7	40.3
		Av. 40.8

[a] A value of 41.6 wt. % was obtained on the sample before it was irradiated. A macroapparatus, similar to that described by Warf, Cline, and Tevebaugh (*5*), and visual end point detection were used.

system, and the system is flushed clean by pumping distilled water through it.

EXPERIMENTAL EVALUATION OF APPARATUS

The apparatus was evaluated by determining the fluoride content of uranium tetrafluoride; no accelerator was used in the pyrohydrolysis. The results of 11 consecutive determinations on test portions that ranged in size from 10 to 58 mg. are given in Table I. The relative standard deviation of these test results is 3%.

A solid sample that was principally a mixture of uranium fluorides and alkali-metal fluorides, and had been shown by macroanalysis to contain 41.6 weight % fluoride, was also analyzed by use of the apparatus. Aluminum oxide was used as the accelerator for the pyrohydrolysis reaction; the test portions of the sample were bedded in 0.5 gram of alumina and hydrolyzed for 60 minutes. The results of the three determinations of fluoride are given in Table II. The average of these results agrees satisfactorily with the value obtained by use of the macroapparatus.

The apparatus was also evaluated by the analysis of a radioactive solution of fluoride salts that was prepared to contain 23 p.p.m. of fluoride.

The solution was 1*M* in nitric acid and also contained thorium, 7.5*M*; aluminum, 3.0*M*; vanadium, 1.5*M*; iron, 0.01*M*; nickel 0.01*M*; and chromium, 0.01*M*. The test portion of the sample was absorbed in the bed of aluminum oxide accelerator in the pyrohydrolysis crucible. A pellet of sodium hydroxide was also included in the crucible charge. This provided a sufficiently soluble residue from the high-temperature pyrohydrolysis reaction to permit subsequent cleaning of the crucible with a minimum of handling. The charged crucible was next positioned in the cold apparatus. After the superheater was heated to 1100° C. and the suction turned on, the temperature of the crucible furnace was elevated slowly. The volatile constituents of the test portion were thereby removed before the maximum supply of superheated steam came in contact with the test portion.

Table III. Fluoride in Radioactive Solution of Fluoride Salts

(5-ml. test portions)

Determination	Fluoride, P.P.M.[a]
1	23
2	21
3	20
4	20
5	25
6	25
	Av. 22

[a] Solution prepared to contain 23 p.p.m. of fluoride.

The results of the analysis of the sample are given in Table III. The agreement between the amount of fluoride added and that recovered is satisfactory, especially for the small fluoride concentration concerned.

ACKNOWLEDGMENT

The authors wish to acknowledge assistance received from D. J. Fisher, H. L. Hemphill, and P. F. Thomason.

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Rapid Photometric Determination of Magnesium in Electronic Nickel

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The photometric oxine method for the determination of magnesium in nickel has been modified to permit rapid determinations. Interference is eliminated by removing certain interfering metals with an ammonium hydroxide separation and by masking others with cyanide.

THE photometric oxine method for the determination of magnesium in nickel (*1*) gives reliable results, but is time-consuming because of the number of separations involved. Because most of the nickel samples submitted for analysis contain less than 0.01% calcium, and because interference due to this metal is not serious, it seemed probable that the previously published method could be greatly simplified if no attempt was made to remove the calcium. This has proved to be true. By removing manganese with the hydroxide precipitate, rather than with carbamate, and by masking with cyanide the interference due to nickel, cobalt, and the like, it has been possible to reduce the time of analysis to about 15 minutes.

REAGENTS

Nickel Metal. The nickel metal used in the preparation of the calibration curve should not contain more than 0.001% magnesium or 0.005% calcium, zinc, cadmium, or gallium. (Nivac supplied by Vacuum Metals Corp., Syracuse, N. Y., has proved to be satisfactory.)

Ferric Nitrate Solution. Dissolve 2 grams of pure iron in 25 ml. of nitric acid (1 to 1), heat to expel oxides of nitrogen, cool, dilute to 200 ml. with water, and mix.

Ammonium Persulfate Solution. Dissolve 1 gram of ammonium persulfate in 100 ml. of water. Prepare fresh each day as needed.

Sodium Cyanide Solution. Transfer 20 grams of sodium cyanide to a 500-ml. polyethylene bottle. Add 200 ml. of water and swirl to dissolve. Keep stoppered when not in use.

PROCEDURE

Preparation of Calibration Curve. Dissolve five 0.100-gram portions of pure nickel by warming gently in 2-ml. portions of nitric acid (1 to 1) in covered 125-ml. conical flasks. Avoid unnecessary loss of acid by evaporation. When solution is complete, blow the brown fumes from the flasks and then cool. Transfer 0, 2.0, 4.0, 6.0, and 8.0 ml. of standard magnesium solution (10 γ of magnesium per ml.) to the flasks and dilute each sample to 35 ml. with water. Add 1 ml. of ferric nitrate solution plus 1 ml. of ammonium persulfate solution (to oxidize manganese). Add ammonium hydroxide (specific gravity, 0.90) dropwise with swirling until the iron just precipitates, then add 1 ml. in excess from a graduate. (The ammonium hydroxide used here and subsequently must not be allowed to lose its strength before use by standing in an open container.)

Heat the solution to 65° C. and filter through a rapid paper into

Table I. Specificity of Method

No.	Other Metal Added	Magnesium Found, γ
1	Ca	56
2[a]	Ca	54
3	Zn	50
4[a]	Zn	46
5	Cd	46
6[a]	Cd	44
7	Ga	46
8[a]	Ga	44
9	P, Re, Mn	40
10	Be, In, Ag	39
11	Co, Cu, Zr, Au	40
12[a]	Co, Cr, Al	40
13	W, Ba, V	38
14	Pb, Sr, Mo	38
15	Tl, Hg, Sn, La, Bi	43
16	Ru, Ir, Pd, Os, Rh, Pt	40
17	K, Cs, Rb, Li, B, Sc, Y, U	41
18	Pr, Ce, Nd, Sm, Th	41
19	Te, Se, Sb, As	40
20	Ta, Nb, Si, Ge	39

[a] 1 ml. of ferric nitrate solution added.

a 125-ml. Squibb-type separatory funnel. When filtration is complete, force the solution from the filtering funnel stem by pressure from the hand, but do not wash the precipitate. Stopper the separatory funnel and cool under running water. Wash the separatory funnel, including the bore of the stem, with distilled water. Add 10 ml. of ammonium hydroxide, swirl, add 5 ml. of sodium cyanide solution, swirl, add 5.0 ml. of butyl Cellosolve solution (1 to 1) from a graduated pipet, and swirl. Add 20.0 ml. of oxine solution from a pipet and proceed to the extraction and photometric determination of the magnesium (*1*). The calibration curve should be linear.

Analysis of Nickel Sample. Depending on the magnesium content, transfer 0.010 to 0.100 gram of the sample to a 125-ml. conical flask. If less than 0.100 gram of sample has been taken, add sufficient pure nickel to make a total of 0.100 gram of metal present in the flask. Dissolve the sample in 2 ml. of nitric acid (1 to 1) and proceed as for the calibration curve. Dissolve 0.100 gram of pure nickel and carry it through the complete analysis as a reagent blank.

RESULTS

Experiments in the hydroxide separation have shown that loss of magnesium by coprecipitation or adsorption occurs if nickel and ammonium salts are not present and if the excess of ammonium hydroxide added is not limited. Apparently, the presence of nickel tends to eliminate the absorptive action of the ferric hydroxide on the magnesium.

Synthetic sample solutions were prepared by mixing aliquot portions of standard solutions of nickel and magnesium. The sample solutions contained various amounts of nickel dissolved in 2 ml. of nitric acid (1 to 1) plus 40 γ of magnesium. The sample solutions were analyzed for magnesium by the method described; the following results were obtained.

Nickel Present, Mg.	Magnesium Found, γ
None	26
25	36
100	40

Synthetic sample solutions were prepared from aliquots of standard solutions of nickel, magnesium, and other metals. Each sample was made up to contain 0.1 gram of pure nickel dissolved in 2 ml. of nitric acid (1 to 1), plus 40 γ of magnesium, plus 100 γ of one or more of 53 other metals. The metals added were in their highest normal valence states. The sample solutions were analyzed for magnesium by the proposed method, except that 0.1 ml. rather than 1 ml. of the ferric nitrate solution was added and the solution was neutralized to the methyl red end point before addition of the 1 ml. excess of ammonium hydroxide. The results obtained are shown in Table I.

Table II. Analysis of Composite Samples

No.	Magnesium, γ Added	Found
1	10.0	11
2	40.0	42
3[a]	40.0	56
4	80.0	84

[a] 0.1 ml. rather than 1 ml. of ferric nitrate solution added.

Synthetic sample solutions were prepared from aliquots of standard solutions to contain the maximum amount of metal impurities normally encountered in the analysis of electronic nickel samples. In addition to 0.1 gram of nickel dissolved in 2 ml. of nitric acid (1 to 1) and a measured amount of magnesium, each sample contained: cobalt, 7 mg.; silicon and manganese, 0.2 mg. of each; titanium, aluminum, and copper, 0.1 mg. of each; zinc, chromium, and calcium, 0.01 mg. of each. The sample solutions were analyzed for magnesium by the proposed method. The results obtained are shown in Table II.

Table III. Determination of Magnesium in Nickel and Nickel Oxide

Sample Analyzed	Magnesium Found, %
220	0.038
	0.039
220 (Melt H-1400)	0.040
	0.040
225	0.066
	0.066
999	0.0005
	0.0008
Cathaloy A-30	0.028
	0.027
Cathaloy A-31	0.038
	0.038
NBS standard sample 671, nickel oxide[a]	0.029
	0.029
NBS standard sample 672, nickel oxide[b]	0.021
	0.021

[a] 0.029% Mg (NBS provisional certificate).
[b] 0.020% Mg (NBS provisional certificate).

Several samples of electronic nickel plus two NBS standard samples of nickel oxide were analyzed in duplicate for magnesium by the proposed method. For the nickel oxide 0.127-gram samples were used. In order to obtain complete solution of the oxide sample, it was necessary to add a few drops of hydrochloric acid toward the end of the solution process. The results obtained are shown in Table III.

DISCUSSION

By removing precipitatable metals by coprecipitation with ferric hydroxide followed by masking of the nickel and various other metals with cyanide, the interference of small amounts of most metals can be eliminated in the photometric oxine-chloroform extraction method for magnesium.

An appreciable excess of iron must be present in order to coprecipitate interfering metals completely. When the amount of the latter present in the sample to be analyzed is small, as it is in the experiments shown in Table I, only about 1 mg. of iron is required to remove the interference completely. When larger amounts of interfering metals are present it is necessary to increase the amount of iron added. Oddly enough, gallium is not completely precipitated even in the presence of a large excess of

iron. This does not present any problem, however, because nickel does not usually contain gallium.

The interference due to nickel, cobalt, copper, silver, gold, and the platinum metals can be readily eliminated by masking with cyanide. On the other hand, the interference due to zinc and cadmium cannot be entirely suppressed by the limited amount of cyanide that can be tolerated in the oxine extraction separation (Table I). Fortunately the amount of zinc and cadmium present in nickel is usually less than 0.01%.

The results obtained by the new rapid method in the analysis of nickel samples (Table III) compare favorably with those previously obtained (*1*).

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Photometric Titration of Small Amounts of Barium with (Ethylenedinitrilo)tetraacetic Acid

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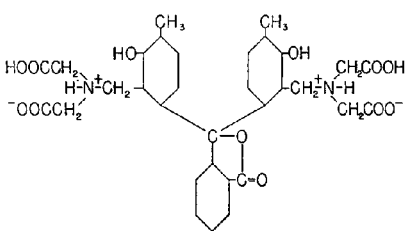
A method for the photometric titration of small quantities of barium with (ethylenedinitrilo)tetraacetic acid uses as indicator Phthalein Purpur, a phenolphthalein derivative having iminodiacetate functional groups. This forms a complex with barium which shows an absorbance maximum at 570 mμ. From 0.05 to 12 mg. of barium can be determined by titration with 0.01 or 0.002*M* (ethylenedinitrilo)tetraacetic acid.

ROWLEY, Stoenner, and Gordon (*4*) have proposed a photometric titration for the determination of from 0.1 to 5 mg. of barium. This is a modification of the method of Manns, Reschovsky, and Certa (*3*) for macro quantities of barium, which was determined by titration with 0.1*N* EDTA solution [(ethylenedinitrilo)tetraacetic acid] using the magnesium–Eriochrome Black T complex as an indicator for the detection of the end point.

Anderegg, Flaschka, Sallman, and Schwarzenbach (*1*) have recently proposed a compound which forms a complex with all the alkaline earths and which would appear to have some advantages as a direct indicator over the magnesium–Eriochrome Black T complex in the titration of barium.



The compound forms a purple-red complex with barium ions. The color of the indicator itself is a light pink in alkaline solution —i.e., the color of phenolphthalein. The indicator has been called "phtalein complexone" (*1*) and "metallphtalein" (*5*), and is commercially available as Phthalein Purpur.

Anderegg and associates have suggested the use of this indicator in a visual titration in 50% ethyl alcohol, so that the pink color of the free indicator will be suppressed. They used 0.1*N* EDTA in these titrations and claimed an accuracy within 0.2 to 0.3%.

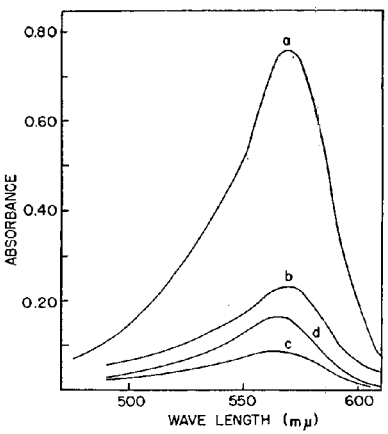


Figure 1. Absorption spectra of complex of barium with Phthalein Purpur during titration with EDTA

a. Start of titration (no EDTA added)
b. A portion of barium removed from complex by titration with EDTA
c. After end point
d. Untitrated blank (no added barium)

The present investigation was undertaken to study the applicability of Phthalein Purpur in photometric titrations of smaller quantities of barium, using more dilute solutions of EDTA.

MATERIALS AND EQUIPMENT

All reagents were of reagent grade quality, with the exception of triethanolamine, which was technical grade.

EDTA Solutions. Four grams of disodium dihydrogen (ethylenedinitrilo)tetraacetate and 0.6 gram of sodium hydroxide were dissolved in water and diluted to 1 liter. This solution was standardized against known amounts of barium and found to be 0.01340*M*. Approximately 120 ml. of this solution was then diluted to 1 liter. This solution was found to be 0.001610*M* as determined by subsequent standardization.

Buffer Solution. A solution was prepared by adding 10 grams of ammonium chloride to 1 liter of concentrated ammonium hydroxide and storing in a polyethylene bottle. A solution of pH 11 was prepared by diluting 10 ml. of this buffer to 60 ml.

Barium Solution. A solution was prepared from barium nitrate which had been twice recrystallized from water. The solution contained 124.0 mg. of barium per 50 ml., as was determined by evaporating an aliquot to which sulfuric acid has been added and weighing the ignited residue (900° C.).

Phthalein Purpur. This reagent was obtained from B. Siegfried, Zofingen, Switzerland. Sixty milligrams were added to 60 ml. of triethanolamine and the mixture was allowed to stand for several days until complete solution was effected. This solution appeared to be very stable; even after 4 months there were no signs of decomposition. A solution made by dissolving the reagent in dilute ammonium hydroxide showed signs of deterioration within 1 week.

Apparatus. The titration cell used was similar to that devised by Goddu and Hume (*2*), but was modified in that the cell could be capped with a ⅌ 55/50 cover provided with a precision Trubore shaft to which the helical stirrer was attached. The stirrer could thus be run at high speeds without hitting the sides of the titration cell. The cell fitted into an Evelyn colorimeter. An opening in the ⅌ cover permitted the insertion of a 10-ml. buret, the tip of which could be immersed in the solution so that small amounts of titrant could be easily added. Absorbances were read 1 to 2 minutes after the addition of an increment of titrant. Absorbance readings could be made without turning off the stirrer. The light path through the titration cell was 2.2 cm.

A 565 Evelyn filter was used with the colorimeter. The spectra of this filter *vs.* air as measured with a Beckman Model B spectrophotometer showed a transmittancy band from 547 to 575 mμ with a maximum at 557.

Absorption spectra were measured with a Beckman Model B spectrophotometer and pH measurements were made with a Beckman Model H pH meter.

PROCEDURE

Take a sample for analysis which contains from 0.05 to 12 mg. of barium in 1 to 50 ml. of a neutral solution (acid solutions should be neutralized with sodium hydroxide and not ammonia), which should be carbonate-free. Add 10 ml. of pH buffer and dilute the solution to approximately 60 ml. Adjust the colorimeter so that the absorbance of the solution is zero. Finally, add 10 drops of the Phthalein Purpur solution and stir the solution thoroughly before titrating. Titrate with 0.01*M* EDTA solution if more than 2.0 mg. of barium is present and with 0.002*M* EDTA if less is present. Near the end point take 0.050-ml. and 0.100-ml. increments for 0.01*M* and 0.002*M* EDTA solutions, respectively. Allow the solution to mix for 1 to 2 minutes before measuring the absorbance. Obtain the end point from a graph of milliliters *vs.* absorbance. Determine the blank correction (see Results and Discussion) by a similar titration in the absence of barium.

Standardize the EDTA solutions by titration against known quantities of barium.

Table I. Visual Titration[a] of Barium (*I*) with 0.01*M* EDTA

Barium Taken, Mg.		Mg. Barium/Ml. EDTA	Barium Found (Difference)[b], Mg.
9.92		1.872	+0.02
		1.851	+0.13
		1.852	+0.04
	Av.	1.858	
4.96		1.893	−0.05
		1.890	−0.04
	Av.	1.892	
2.48		1.879	±0.00
		1.900	−0.12
		1.905	−0.04
		1.908	−0.04
	Av.	1.913	

[a] One drop of triethanolamine solution of indicator, 13.8 mg./ml., used.
[b] Barium found was calculated using standardization data obtained in visual titration of 12.40 mg. of barium; titer value, mg. of barium per ml. of solution, was 1.875, average of three titrations.

Table II. Standardization of 0.01*M* EDTA Solution by Photometric Titration

Barium Taken, Mg.		Mg. Barium/Ml. EDTA[a]
12.40		1.838
		1.858
	Av.	1.848
9.92		1.848
		1.850
		1.841
	Av.	1.846
4.96		1.828
		1.839
		1.835
	Av.	1.834

[a] Molarity of EDTA solution found to be 0.01340, using over-all average of eight titrations.

RESULTS AND DISCUSSION

Results obtained with Anderegg's method (*1*) with 0.01*M* EDTA—i.e., titration in 50% ethyl alcohol with visual detection of the end point—are shown in Table I. The ratio, milligrams of barium per milliliter of titrant, varies to some extent with the amount of sample being titrated and thus introduces a slight error, unless the weight of barium used to standardize the EDTA solution is close to that in the unknown. When the more dilute, 0.001610*M* EDTA solution was employed as titrant, the end point could not visually be detected.

All photometric titrations were performed in the absence of alcohol. Figure 1 shows the spectral characteristics of the solutions at various stages of the titration. The maximum of the absorption peak of the barium complex is at 570 mμ. As is shown in Table II, the variation in the titer values with amount of barium taken is not as large as with the visual titration. The data of Table II were used to determine the molarity of the EDTA solution. This solution was then used in the titration of 2 to 12 mg. of barium, with the results shown in Table III. A more dilute solution of EDTA was then prepared, standardized by titration against known amounts of barium, and used in the titration of 0.05 to 2.00 mg. of barium. These results are also shown in Table III. Volume corrections for absorbance were not made in any of the photometric titrations. Figures 2 and 3 show that relatively sharper end points can be obtained by the present method than by the method of Rowley, Stoenner, and Gordon (*4*).

It was found that the blank correction was due to the presence of foreign materials in the 10 ml. of buffer solution used in each titration. A precise value of the blank correction was obtained by utilizing a tenfold quantity of the buffer solution in the blank titration. First, 90 ml. of the buffer was evaporated to less than 50 ml., 10 ml. of the buffer was added, and the resulting solution was titrated. The blank for a 10-ml. quantity of buffer amounted to 0.022 ml. for the 0.01340*M* EDTA solution and 0.183 ml. for the more dilute titrant. If the buffer was subjected to a batch treatment with Dowex 50 resin, in the ammonium ion form, to remove traces of alkaline earths, the values of the blank were reduced to 0.008 and 0.063 ml., respectively.

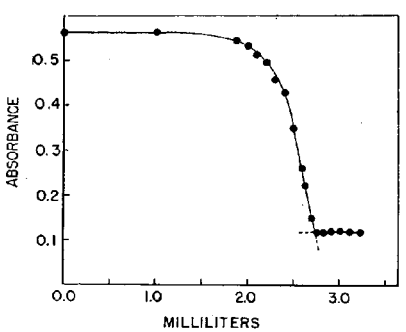


Figure 2. Titration of barium with 0.01340*M* EDTA

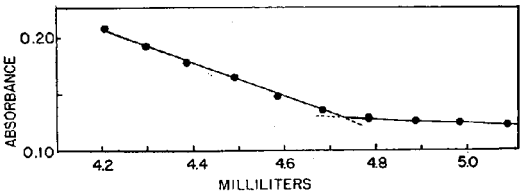


Figure 3. Titration of barium with 0.001610*M* EDTA

Preliminary work in the application of the colored complex to a spectrophotometric determination of barium indicated that color fading caused serious errors. The fading was apparently due to the conversion of the phenolphthalein part of the indicator molecule to a colorless form in alkaline solution. However, fading was no problem in the titration itself, which could be carried out in a reasonable length of time. The titration can be easily completed within 30 minutes.

The photometric method is recommended for the determination of 0.05 to 12 mg. of barium. Larger quantities can be determined readily by the visual method (*1*).

ACKNOWLEDGMENT

The authors wish to thank the Atomic Energy Commission for support of this investigation under Contract AT (30-1)-1213.

Table III. Photometric Titration of Barium

Molarity of EDTA	Barium Taken, Mg.	Barium Found (Difference), Mg.
0.01340	2.48	+0.03, +0.06, +0.03
	6.20	−0.02
	9.92	+0.02, +0.01, +0.01
	12.40	+0.01, ±0.00, −0.04
0.001610	0.050	+0.007, ±0.000, −0.009
	0.099	+0.002, +0.001, +0.003
	0.149	−0.001, −0.004, −0.004
	0.248	+0.002, +0.002, +0.002
	0.496	±0.000, +0.003, +0.034
	0.99	−0.01, −0.01, ±0.00
	1.24	±0.00, ±0.00, −0.01
	1.98	−0.01, −0.01, −0.01

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Effect of Iron on Determination of Tin in Brass and Bronze

Application of Radioisotope Techniques

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Radioisotope tracer techniques have been utilized for quantitative evaluation of the effect of iron on the nitric acid precipitation of tin in brass and bronze. Two digestion temperatures were studied, with iron-59 and tin-113 used as tracers. The degree of accuracy of the gravimetric procedure, the amount of iron coprecipitated, and the effect of the presence of iron on the solubility of the metastannic acid precipitate were determined. Coprecipitation of iron is shown to follow Freundlich's adsorption equation.

TIN in brass and bronze can be determined (*7, 11*) by digesting with nitric acid, filtering, igniting the resultant metastannic acid, and weighing as stannic oxide. Two significant sources of error are inherent in this method: Iron, copper, and zinc coprecipitate, and iron increases the solubility of the metastannic acid (*7*). Detailed quantitative studies of these interferences have not been reported. The purpose of the present study is to provide more extensive data by utilizing the improved sensitivity of radioisotope tracer techniques.

Earlier investigators recognized the existence of these errors in the determination of tin. In 1922 Meyer (*8*) experienced difficulty in the quantitative recovery of tin in alloys containing iron in excess of 0.5%. Lundell and Hoffman (*7*) state, "The precipitation of tin as metastannic acid

$$3Sn + 4HNO_3 + xH_2O = 3SnO_2.xH_2O + 4NO$$

is not complete if the alloy contains much iron, say above 0.25%." They further maintain that the metastannic acid that separates from solution is always contaminated by compounds of iron, copper, and zinc. If the alloy contains antimony, phosphorus, silicon, or arsenic, these are also found in the precipitate, often quantitatively (*2*). Willard and Furman (*13*) state that the volumetric determination of tin is capable of greater accuracy than the gravimetric determination because the coprecipitation error is eliminated. The present paper applies radioisotope tracer techniques to investigate the gravimetric procedure, to evaluate quantitatively the effect of iron on the precipitation of tin as metastannic acid in brass and bronze, and to elucidate its mechanism.

Various investigators differ regarding the digestion temperature of the metastannic acid precipitate. Hillebrand and Lundell (*4*) recommend digestion at boiling temperatures to help minimize the effects of the colloidal character and solubility of the precipitate. Kolthoff and Sandell (*6*) state that the digestion should be carried out at 90° to 100° C. to ensure a more quantitative separation of tin. Norwitz, Boyd, and Bachtiger (*9*) suggest that the acid digestion of the alloy be maintained at a boil for low-tin samples and at 95° C. for high-tin samples. Goldberg (*3*) advocates a nitric acid digestion at a boiling temperature for manganese bronzes.

It was desirable to resolve this problem of digestion temperature with respect to the two sources of error by investigating two digestion temperatures with samples of varied iron contents. This approach is in line with the research of Kolthoff and Moltzan

Table I. NBS Standards and Composites

Sample	Wt., Gram	Tin, %
Bronze 52c	1.0	7.85 ± 0.01
Bronze 52c plus brass 37d	0.5 each	4.410 ± 0.007
Brass 37d	1.0	0.970 ± 0.004

Composition of Standard Alloys, %

Sample	Cu	Zn	Sn	Pb	Ni	Fe
Bronze 52c	82.25	2.12	7.85	0.011	0.76	0.004
Brass 37d	70.78	26.65	0.97	0.94	0.58	0.076

Table II. Data for Below-Boiling Digestion

Fe, %	Sn[a], Uncorr., %	Coprecipitants, % Fe[b]	Cu[c]	Zn[c]	Sn, Corr., %	Recovery, Corr.
		Sn = 7.85 ± 0.01%				
0.10	7.81	0.068	0.05	0.006	7.68	97.8
0.30	7.92	0.163	0.05	0.004	7.70	98.1
0.55	7.93	0.229	0.04	0.006	7.65	97.5
1.05	7.96	0.346	0.03	0.001	7.58	96.6
2.05	7.90	0.442	0.03	0.001	7.43	94.6
4.05	7.33	0.506	0.01	0.004	6.81	86.8
		Sn = 4.41 ± 0.01%				
0.10	4.52	0.057	0.03	0.012	4.42	100.2
0.30	4.62	0.144	0.02	0.005	4.45	100.9
0.55	4.65	0.192	0.02	0.009	4.43	100.4
1.05	4.60	0.198	0.03	0.014	4.36	98.9
2.05	4.46	0.288	0.02	0.010	4.14	93.9
4.05	3.82	0.323	0.01	0.003	3.48	78.9
		Sn = 0.970 ± 0.004%				
0.09	1.036	0.018	0.008	0.006	1.014	104.5
0.39	1.039	0.046	0.010	0.007	0.976	100.6
0.59	1.038	0.055	0.006	0.006	0.971	100.0
1.09	0.987	0.055	0.010	0.006	0.916	94.4
2.09	0.830	0.075	0.010	0.005	0.740	76.3
4.09	0.462	0.100	0.009	0.006	0.347	35.8

[a] Gravimetric assay. [b] Radiometric assay. [c] Polarographic assay.

(*5*), who have demonstrated that adsorption is not a simple process, but often the result of several variables, among which are temperature and concentration of the ions involved.

APPARATUS

The radioactive counting equipment consisted of a shielded scintillation counter (Model DS-3, Nuclear Instrument & Chemical Corp., Chicago, Ill.) with a Harshaw well-type, sodium iodide, thallium-activated crystal. The crystal was coupled to a DuMont 6292 (K1186) phototube. This is a ten-stage multiplier phototube of the end-window type with a spectral response predominantly in the visible region. Dow Corning DC 200 silicone fluid was used for optical contact between crystal and phototube. The pulses were fed through a preamplifier to a Model 182 Amplicount scaler (Nuclear Instrument & Chemical Corp.) operating at 1900 volts at a 100-mv. threshold. The samples were contained in 16 × 150 mm. glass test tubes and inserted in the circular well of the sodium iodide crystal.

REAGENTS AND RADIOISOTOPE STANDARDS

Standard inert ferric nitrate solution containing 5 mg. of iron per ml. was prepared by dissolving 1 gram of pure iron wire (99.85% iron) in 20 ml. of 70% nitric acid and 5 ml. of distilled water. The solution was boiled gently for 20 minutes, cooled, and diluted to exactly 200 ml. with 70% nitric acid.

Standard radioactive ferric nitrate solution was prepared by dissolving 1 gram of pure iron wire in 20 ml. of 1 to 1 nitric acid, boiling gently for 10 minutes, and cooling. To the resultant solution was added 0.2 ml. of radioactive ferric chloride solution containing an initial activity of 1 mc. per ml., with a specific activity 3.39 mc. per mg. of iron. The solution was made slightly alkaline with ammonium hydroxide, boiled gently, filtered while hot through a No. 40 Whatman filter paper, and washed with hot ammonium hydroxide (1%). The precipitate was dissolved with a minimum of 1 to 1 nitric acid. The solution was evaporated to approximately 10 ml., cooled, and adjusted to exactly 200 ml. with 70% nitric acid. The resultant solution contained 5 mg. of iron and 1 μc., initially, of radioactivity per ml.

Standard radioactive tin chloride solution was prepared in a 100-ml. quantity to contain 0.2 ml. of the high specific activity Atomic Energy Commission processed isotope, Sn-113P, as tin chloride in 6*N* hydrochloric acid. The resultant solution contained 0.39 mg. of tin and 2 μc. of initial radioactivity per ml.

In order to obviate the necessary corrections in radiation measurements caused by the decay of the radioisotopes, aliquots of standard solutions were measured concurrently with the analytical samples.

PROCEDURE

To each of seven 1-gram samples in a set (NBS standards and composites, Table I), contained in 300-ml. beakers, was added 0.10 ml. of standard radioactive ferric nitrate solution. Increments of inert ferric nitrate standard solution were added corresponding to a range from 0.05 to 4.05% iron in the sample. The acid content of the ferric nitrate solution was supplemented with 70% nitric acid so that each beaker contained a total of 12 ml. of the acid. Approximately 25 ml. of hot distilled water was added and the solutions were boiled gently for 10 minutes to effect complete solution and to expel nitric oxide fumes. This was followed by the addition of approximately 60 ml. of hot distilled water to each beaker, with subsequent digestion for 30 minutes at 80° ± 5° C. This is referred to as the below-boiling procedure. A similar series of seven samples was treated as described above, except that the solutions were diluted to approximately 150 ml. and boiled gently for 30 minutes at 102° C. This is referred to as the boiling procedure. The solutions were filtered while hot through a No. 40 Whatman filter paper containing a small quantity of paper pulp. The precipitates were washed thoroughly with hot 1% nitric acid and transferred to tared porcelain crucibles. The filter papers were carefully charred and the precipitates were ignited at 810° C. for 1 hour under oxidizing conditions. The resulting oxides were cooled and weighed.

The following procedures were used to determine the amount of oxide impurities of iron, copper, and zinc coprecipitated with the metastannic acid.

Iron. The ignited residues were quantitatively transferred

Table III. Data for Boiling Digestion

(102° C.)

Fe, %	Sn[a], Uncorr., %	Coprecipitants, % Fe[b]	Cu[c]	Zn[c]	Sn, Corr., %	Recovery, Corr.
		Sn = 7.85 ± 0.01%				
0.10	7.81	0.082	0.044	0.004	7.68	97.8
0.30	8.02	0.201	0.040	0.004	7.77	99.0
0.55	8.10	0.284	0.016	0.001	7.80	99.4
1.05	8.17	0.401	0.028	0.001	7.74	98.6
2.05	8.25	0.476	0.024	0.006	7.74	98.6
4.05	7.74	0.580	0.012	0.004	7.14	91.0
		Sn = 4.410 ± 0.007%				
0.10	4.51	0.071	0.026	0.012	4.40	99.8
0.30	4.59	0.154	0.018	0.009	4.41	100.0
0.55	4.56	0.196	0.018	0.007	4.34	98.4
1.05	4.51	0.255	0.020	0.012	4.22	95.7
2.05	4.25	0.307	0.018	0.006	3.92	94.9
4.05	3.69	0.379	0.015	0.008	3.29	74.6
		Sn = 0.970 ± 0.004%				
0.09	1.013	0.018	0.008	0.006	0.981	101.1
0.39	1.042	0.044	0.006	0.009	0.983	101.3
0.59	1.001	0.048	0.006	0.006	0.941	97.0
1.09	0.973	0.059	0.010	0.007	0.897	92.2
2.09	0.822	0.069	0.005	0.006	0.742	76.5
4.09	0.581	0.095	0.008	0.007	0.471	48.5

[a] Gravimetric assay. [b] Radiometric assay. [c] Polarographic assay.

Table IV. Data for Boiling Digestion Using Tin-113 as Tracer with Brass 37d

Fe, %	Sn, C.P.M. Ppt.	Filtrate	Total	Sn Recovery, %	Solubility Loss, %
0.09	27,550	310	27,860	95.9	1.1
0.39	26,890	360	27,250	95.7	1.3
0.59	26,480	980	27,460	93.0	3.6
1.09	24,410	3,200	27,610	85.8	11.5
2.32	21,830	5,880	27,710	76.4	21.1
4.09	14,770	12,440	27,210	52.7	45.7

to 16 × 150 mm. glass test tubes which were placed in a scintillation well counter for measurement of the gamma radiation. Radiation emitted by radioactive iron-59 consists of two beta rays of moderate energy and two gamma rays of high energy (1.3 and 1.1 m.e.v.) decaying with a half life of 46.3 days. In order to obviate the need for self-absorption and Geiger dead time corrections associated with moderate beta counting, the iron content of the samples was determined solely by the gamma emission with the beta rays effectively screened out. The per cent radioactive iron present was determined by comparing the radiation count of the oxide residue to the count obtained with 0.10 ml. of standard radioactive ferric nitrate. This eliminated corrections for decay.

Copper and Zinc. In another series of experiments the metastannic acid precipitates obtained were not ignited but were redissolved by fuming with 96% sulfuric acid, adding 70% nitric acid as necessary to destroy the filter paper. Tin was volatilized by repeated fumings with hydrobromic acid (48.5%), and the copper and zinc were determined by polarographic methods.

Corrections for the coprecipitated impurities in the ignited residue were made by applying the following formula:

$$\%\ \mathrm{Sn\ (corr.)} = \left[A - \left(\frac{B \times C \times 1.42}{D} + E + F\right)\right] 0.7877 \times 100$$

where

A = SnO_2 residue in grams
B = activity of residue (c.p.m.)
C = iron (iron content of standard bronze + added iron)
D = activity of iron-59 standard (c.p.m.)
E = CuO in residue
F = ZnO in residue
1.42 = Fe_2O_3/Fe ratio
0.7877 = Sn/SnO_2 ratio

In an analogous procedure employing boiling digestion, aliquots of a standard solution of radioactive tin chloride, equivalent to 0.08 mg. of radioactive tin, were added to each 1-gram sample of brass 37d. Radioactive tin containing 0.4 μc. gave a total activity of 27,000 counts per minute ±3%. The iron concentrations in this procedure were the same as above but were not radioactive.

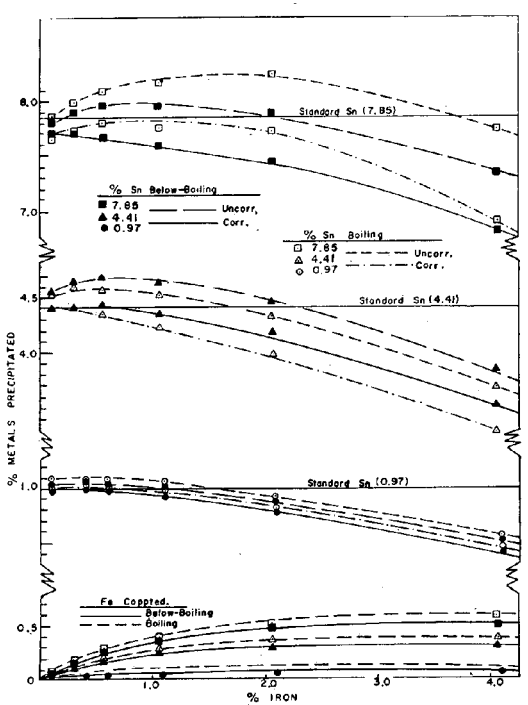

Figure 1. Effect of iron on precipitation of metastannic acid

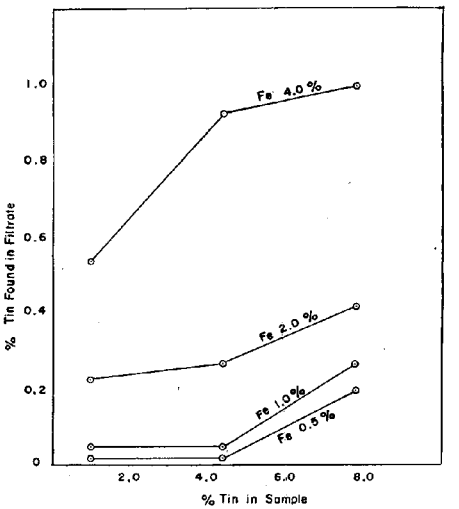

Figure 2. Relationship between tin present and tin found in filtrate for different iron contents

Below-boiling digestion

DISCUSSION

The quantitative data obtained confirmed earlier reports that the precipitation of tin as metastannic acid is subject to the iron-induced, partially compensating errors of coprecipitation and solubility. The detailed data are presented in Tables II and III, and Figure 1.

For purposes of routine gravimetric analysis with no corrections applied for adsorbed elements, the data reveal that the below-boiling digestion is superior to the boiling digestion for samples containing 7.85% tin and equivalent for those containing 4.41 and 0.97% tin.

Further confirmatory evidence of the solubility effect due to iron are the results obtained with the use of tin-113 as tracer. Data are presented in Table IV showing the amounts of metastannic acid contained in the filtrate when NBS standard brass 37d was used at boiling digestion.

The relationship between the tin present and tin found in the filtrate for different iron contents at below-boiling temperatures is illustrated in Figure 2. It is evident that the amount of tin dissolved increases or remains constant with the tin content of the sample for all iron and tin ranges investigated.

Figure 3 is an enlargement of the lower part of Figure 1, showing a sharp increase in adsorption up to 1% iron and a gradual increase from 1 to 4% iron.

The below-boiling digestion curves of Figure 1 are linear in the range from 0.5 to 2.0% iron. The deviation from 100% recovery is due to the solubility of the metastannic acid precipitate. It is, therefore, possible to derive a solubility correction equation of the form

$$y = ax + b \qquad (1)$$

where y is per cent tin (corrected for adsorbates), x is per cent iron in the alloy, and a and b are constants. Equations for the tin ranges studied, derived empirically, are listed as follows:

0.97% Sn: $y = -0.15x + 1.07$ (2)
4.41% Sn: $y = -0.15x + 4.47$ (3)
7.85% Sn: $y = -0.15x + 7.75$ (4)

If K is assumed to represent the NBS value of tin in the bronze, then the tin correction may be expressed as:

$$\text{Sn (corr.)} = K - (ax + b) \quad (5)$$

At each tin level the following equations are derived by substituting the respective constants for a and b of Equations 2 to 4 in Equation 5:

$$0.99\%\ \text{Sn (corr.)} = 0.15x - 0.10 \quad (6)$$
$$4.41\%\ \text{Sn (corr.)} = 0.15x - 0.06 \quad (7)$$
$$7.85\%\ \text{Sn (corr.)} = 0.15x + 1.00 \quad (8)$$

Equations 6 and 7 show similar values for a and b within experimental error and are averaged to produce a general equation useful in the analysis of tin with iron contents up to 4.4% as follows:

$$\text{Sn (corr.)} = 0.15x - 0.08 \quad (9)$$

Hence

$$K' = y + 0.15x - 0.08 \quad (10)$$

where K' is the calculated per cent tin in the sample.

In Table V NBS values for tin are compared with values obtained when applying Equation 10 to the experimental results.

Table V. Comparison of Calculated Tin with NBS Values

Tin, NBS Value, %	Iron Present (x)	Tin, % Corrected for adsorbates (y)	Tin, % Calcd.[a] (K')
4.41	0.10	4.42	4.36
	0.30	4.45	4.42
	0.55	4.43	4.43
	1.05	4.36	4.44
	2.05	4.14	4.38
0.970	0.09	1.014	0.94
	0.39	0.976	0.96
	0.59	0.971	0.98
	1.09	0.916	1.01
	2.09	0.740	0.98

[a] $K' = y + 0.15x - 0.08$.

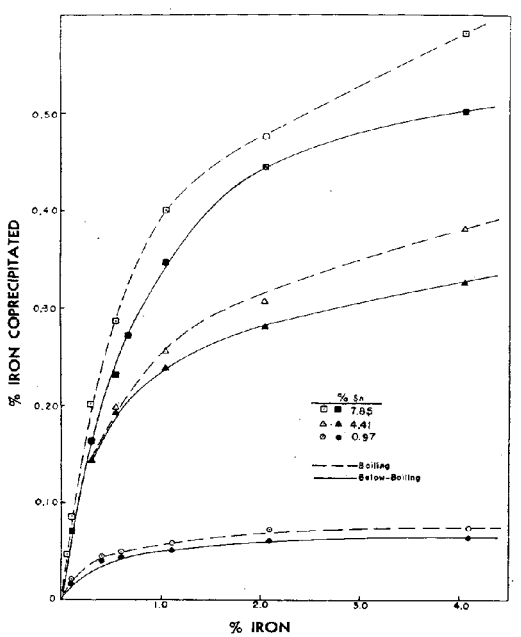

Figure 3. Relationship between iron content and amount of iron coprecipitated with metastannic acid

The average deviation is 0.025%, which is satisfactory for routine analysis.

Weiser (*12*) states that hydrous stannic oxide does not precipitate in the usual way from a nitric acid solution of tin containing iron. Hydrous stannic oxide is peptized by ferric nitrate or a mixture of ferric nitrate and nitric acid and coagulation does not take place at below-boiling or boiling temperatures. The colloid stabilizing action of the strongly adsorbed ferric ion contributes to the solubility of the metastannic acid precipitate. The present investigation has shown quantitatively that there is a definite solubility of the hydrous stannic oxide due to the presence of iron above 0.5%, both at boiling and below-boiling temperatures. Zsigmondy (*14*) has suggested that stannic oxide reacts with hydrogen ions to give positively charged stannic ions. In the analytical procedure for tin, stannic oxide peptized in the nitric acid medium may give the positively charged stannic ion, $(SnO_2 . H_2O)\ Sn^{+4}$. Ferric oxide sols have been obtained by Powis (*10*), and in the nitric acid medium ferric oxide behaves similarly. The structure of the colloidal unit may be written as $(Fe_2O_3 . H_2O)Fe^{+++}$. The similarity of these two structures makes more likely the entrainment of the ferric ion into the structure of the colloidal positively charged stannic ion, and contributes to the solubility of metastannic acid.

The present work shows that the adsorption of interfering ions is greater at boiling temperatures for the 8% tin and practically equivalent or slightly greater at the 4 and 1% tin levels. This phenomenon apparently does not conform to theoretical considerations, as indicated by the Gibbs equation:

$$a = -\frac{c}{RT}\frac{d}{dc}$$

in which a is moles absorbed per sq. cm. of interface, dc is change in concentration of solute, and d is change in interfacial tension. This equation indicates that the increase in temperature should result in decreased adsorption, due in part to the fact that the solubility of most impurities is increased by rise of temperature. Actually an increase in digestion temperature may permit an exchange and penetration of the interfering ions into the submicroscopic capillary structure of the colloidal metastannic acid, with development of an effective interface resulting in a greater degree of physical and chemical adsorption. The exchange of ions may be possible because of the similarity of the colloidal stannic oxide ion $(SnO_2 . H_2O)\ Sn^{+4}$ and ferric oxide ion $(Fe_2O_3 . H_2O)\ Fe^{+++}$.

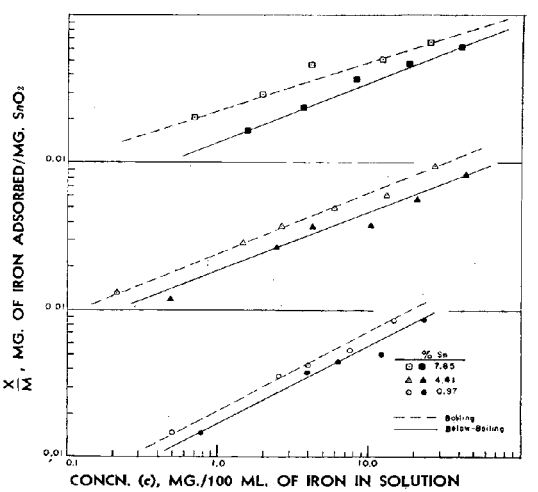

Figure 4. Freundlich's adsorption isotherm of effect of iron on metastannic acid

The data presented point to the possibility that the coprecipitation of iron by metastannic acid is governed by the Freundlich equation, $\frac{x}{m} = Kc^{1/n}$ (*1*), where x is milligrams of adsorbate (iron), m is milligrams of stannic oxide, and c is concentration of iron (mg. per ml.) remaining unadsorbed at equilibrium. The constants K and $1/n$ are particular to the system. A plot of $\frac{x}{m}$ against c on logarithmic paper resulted in a straight line, with a slope equal to $1/n$ as illustrated in Figure 4 for all tin levels studied. This indicated that the mechanism of coprecipitation of iron can be interpreted as a true Freundlich adsorption process.

Radioisotope techniques, utilizing iron-59 and tin-113 as tracer elements, provide a rapid, sensitive, and quantitative method for evaluating the interference of iron in the gravimetric determination of tin in brass and bronze alloys. The quantitative data obtained by this means confirm earlier reports of the dual phenomenon of solubility and coprecipitation exerted by iron on metastannic acid.

ACKNOWLEDGMENT

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Some Theoretical and Experimental Considerations of pH Gradient Elution Analysis

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In order to aid in the selection of optimum conditions for buffered pH gradient elution of chromatographic columns, some general equations relating pH change to effluent volume were derived. The equations are applicable to pH changes produced by the addition of one buffer to another or by the addition of acid (or base) to a buffer under a variety of different experimental conditions. With the proper choice of conditions nearly any gradient can be obtained. Superimposed concentration gradients were also considered.

MAINTENANCE of a sorption gradient on a chromatographic column by means of a continuous change in the composition of the eluting solution is referred to as gradient elution. Sorption gradients were apparently first considered by Tiselius as reported by Synge (*17*) and later by Strain (*16*). A theoretical treatment of concentration gradient elution was first developed by Alm, Williams, and Tiselius (*2*). The method was first used by Donaldson, Tulane, and Marshall (*8*), Alm (*1*), and Busch, Hurlbert, and Potter (*5*). A thorough theoretical discussion of sorption gradients and concentration gradient elution with particular reference to the effect on chromatographic behavior is the subject of a recent paper by Drake (*9*).

The experimental arrangement for gradient elution consists of a mixing chamber containing a solution of low eluting power and a reservoir of a solution of high eluting power. Solution is delivered continuously from the reservoir to the mixing chamber as the mixture passes to the column. The types of apparatus which have the greatest utility have been discussed by Bock and Ling (*3*). It is simplest and usually sufficient to employ either a constant volume or an equal level arrangement. In the former case the mixing chamber is a closed system, with the result that the flow rates into and out of the mixing chamber are equal. In the latter case the mixing chamber and reservoir are set at the same level and connected with a siphon or similar arrangement (*14*). During the progress of elution, the levels fall together. For vessels of identical size the rate of flow into the mixing chamber will be one half the flow out. For straight-sided but different sized vessels the ratio of the flow rates (f_1/f_2) is simply related to the cross sections (A) by the expression $A_1/(A_1 + A_2)$ where subscript 1 refers to the reservoir and subscript 2 to the mixing chamber. More complicated arrangements are possible (*3*, *9*) but these are sufficiently versatile for the great majority of applications.

The concentration gradients obtained from these simple devices are a function of the composition of the solutions, the volumes, and the relation between the flow rates. Equations relating these variables to concentration changes in the eluent were first derived for constant volume mixers (*2*, *6*, *8*) and later extended to systems with variable flow rates (*11*, *12*). Equations have also appeared describing multiple mixers (*9*) and arrangements where the ratio of the flow rates is continuously variable (*3*).

These equations are not directly applicable to chromatography employing buffered solutions where the sorption gradient is maintained by a continuous change in pH with, if desired, a constant eluent concentration. Because pH-gradient elution has been shown to have considerable utility (*4*, *7*, *10*, *13*, *15*, *18*), it is of interest to discuss some of the general considerations.

THEORY

Considering the volume relationships of an experimental arrangement consisting of a reservoir and mixing chamber where

the flow rates into and out of the mixing chamber can be varied at will, it is apparent that

$$dv' = -\frac{v'}{v}\,dV \tag{1}$$

where
v = total volume in the mixing chamber
v' = volume of original solution remaining in the mixing chamber
V = effluent volume

If the flow rates into and out of the mixing chamber are not equal (but have a constant ratio), v is not a constant but is related to the flow rates by the expression

$$v = v_o + \frac{f_1 - f_2}{f_2}\,V \tag{2}$$

where
f_1 = rate of flow into the mixing chamber
f_2 = rate of flow out of the mixing chamber
v_o = original volume of solution in the mixing chamber

Letting $(f_1 - f_2)/f_2 = a$, substituting Equation 2 into Equation 1, and integrating v' between the limits v_o to v' and V between 0 and V, there obtains

$$v' = v_o\left(1 + a\frac{V}{v_o}\right)^{-\frac{1}{a}} \tag{3}$$

This equation is equivalent to the one derived by Lakshmanan and Lieberman (*12*), except that it expresses a volume change rather than a concentration change.

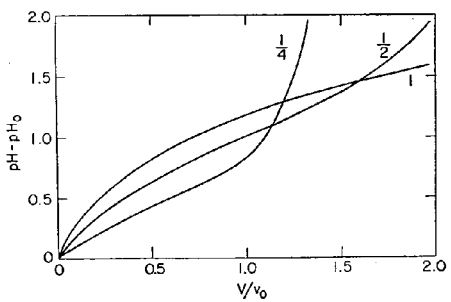

Figure 1. Effect of different ratios of flow rates on pH gradient obtained by adding one buffer to another of equal molarity

Plot of Equation 7

From this point several different ways by which a pH change might be produced will be considered: (I) the addition of one buffer to another; (II) the addition of the salt of a weak acid (or a weak acid) to a buffer; and (III) the addition of strong base (or strong acid) to a buffer.

Case I. The mixing chamber contains a buffer prepared from a weak monobasic acid and its conjugate base. The reservoir contains the same acid and salt but in different proportions. The pH may be either higher or lower. Within the buffering range and for buffers that are not extremely dilute, it is reasonable to assume that the changes in concentration of acid and salt are proportional to the corresponding volume changes and are additive. That is, the concentrations of acid (HA) and conjugate base (A^-) in the effluent can be expressed as

$$HA = \frac{v'}{v}\,HA_o + \frac{v - v'}{v}\,HA_r \tag{4}$$

$$A^- = \frac{v'}{v}\,A_o^- + \frac{v - v'}{v}\,A_r^- \tag{5}$$

where subscript o refers to original concentrations in the mixing chamber and subscript r to the concentrations in the reservoir. Substituting these equations into the equation for the dissociation constant of a weak acid and replacing v and v' with Equations 2 and 3, there obtains

$$pH - pH_o = \log\frac{1 + \left[\left(1 + a\frac{V}{v_o}\right)^{\frac{a+1}{a}} - 1\right]R_{A^-}}{1 + \left[\left(1 + a\frac{V}{v_o}\right)^{\frac{a+1}{a}} - 1\right]R_{HA}} \tag{6}$$

where $R_{A^-} = A_r^-/A_o^-$ and $R_{HA} = HA_r/HA_o$. It is apparent that the form of the gradient will depend only on the flow rates and the buffer concentrations; it is independent of the acid.

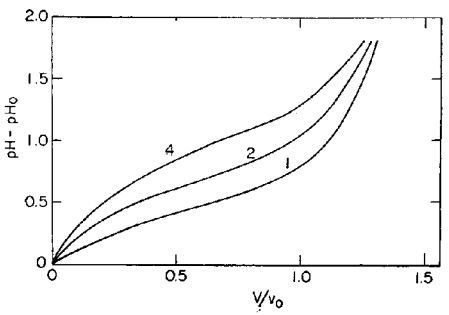

Figure 2. Effect of different ratios of buffer strengths on pH gradient obtained by adding one buffer to another with a ratio of flow rates of 1 to 4

Plot of Equation 8

Experimentally, the salt and acid concentrations are limited. To remain within the buffering range the ratio of salt to acid must lie between about 0.1 and 10. For an increasing gradient $A_r^-/HA_r = 10$ and $A_o^-/HA_o^- = 1/10$, if the widest possible pH change is to be obtained. If it is assumed that the buffers are of equal molarity, Equation 6 becomes

$$pH - pH_o = \log\frac{1 + \left[\left(1 + a\frac{V}{v_o}\right)^{\frac{a+1}{a}} - 1\right]10}{1 + \left[\left(1 + a\frac{V}{v_o}\right)^{\frac{a+1}{a}} - 1\right]\frac{1}{10}} \tag{7}$$

A plot of this equation for various values of the ratio of flow rates into and out of the mixing chamber (f_1/f_2) appears in Figure 1. At equal flow rates the gradient is convex upwards approaching the pH of the buffer in the reservoir asymptotically. As the ratio of flow rates decreases the first part of the gradient becomes nearly linear and the latter part concave upwards.

If the ratio of flow rates is held constant at 1 to 4, a reasonable experimental value, and if the ratios of the salt to acid concentration are 10 and 0.1 as before, Equation 6 becomes

$$pH - pH_o = \log\frac{1 + \left[\left(1 - \frac{3V}{4v_o}\right)^{-\frac{1}{3}} - 1\right]10\,R_M}{1 + \left[\left(1 - \frac{3V}{4v_o}\right)^{-\frac{1}{3}} - 1\right]\frac{R_M}{10}} \tag{8}$$

where $R_M = (A_r^- + HA_r)/(A_o^- + HA_o)$. The effect of different strengths of buffer, as expressed by the ratio of total molarities (R_M), is apparent from a plot of this equation (Figure 2) for several values of R_M. Increasing the strength of the buffer in

the reservoir ($R_M > 1$) has the same effect as increasing the ratio of the flow rates—that is, the pH gradient is more convex. Conversely, decreasing R_M produces a greater concavity.

If the salt to acid ratios other than 10 and 0.1 are employed, the gradients obtained will differ somewhat from those derived above, but they will have a similar form.

If the buffer in the reservoir has a lower pH than that in the mixing chamber, a decreasing gradient is obtained. Equations 7 and 8 still apply, except that the direction of pH change is reversed.

Case II, *A*. The mixing chamber contains a buffer as before. The reservoir contains a weak base in the form of the salt of the weak acid. This provides an increasing gradient. The situation is identical to Case I except that $HA_r = 0$. It follows that the denominator within the log term of Equation 6 becomes unity. That is,

$$pH - pH_o = \log \left\{ 1 + \left[\left(1 + a \frac{V}{v_o} \right)^{\frac{a+1}{a}} - 1 \right] R_{A^-} \right\} \quad (9)$$

To examine the effect of different values of the ratio of the salt concentration of the reservoir to the salt concentration in the buffer (R_{A^-}), let the flow rates be equal. Then $a = 0$ and Equation 9 becomes

$$pH - pH_o = \log \left[1 + \left(e^{\frac{V}{v_o}} - 1 \right) R_{A^-} \right] \quad (10)$$

A plot of this equation for various values of R_{A^-} appears in Figure 3. The gradient is linear if $R_{A^-} = 1$ with a slope equal to log e. The gradient is convex if $R_{A^-} > 1$ and concave if $R_{A^-} < 1$. However, in both cases it becomes essentially linear in a short time.

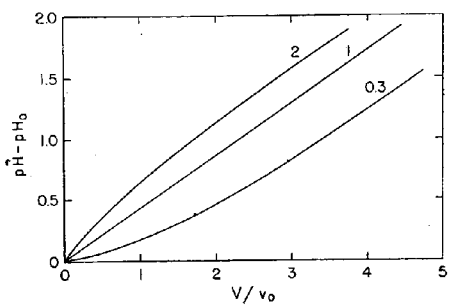

Figure 3. Effect of different ratios of salt concentrations on pH gradient obtained by addition of a salt of a weak acid to a buffer at equal flow rates

Plot of Equation 10

If $R_{A^-} = 1$, to study the effect of variations in flow rate, Equation 9 becomes

$$pH - pH_o = \frac{a+1}{a} \log \left(1 + a \frac{V}{v_o} \right) \quad (11)$$

A plot of this equation for various values of the ratio of the flow rates (f_1/f_2) appears in Figure 4. A convex gradient is produced if $f_1 > f_2$ and a concave gradient when $f_1 < f_2$.

Case II, *B*. The mixing chamber contains a buffer as before. The reservoir contains a solution of the weak acid which provides a decreasing gradient. Because $A_r^- = 0$, the second term in Equation 5 becomes 0 and the numerator within the log term of Equation 6 becomes 1. The situation is identical to Case II, *A* (Equation 9) except that the pH change is negative and the ratio of acid concentrations, rather than salt concentrations, becomes a controlling factor. The same considerations apply as in Case II, *A*.

Case III, *A*. The mixing chamber contains a buffer prepared from a weak monobasic acid and its salt. The reservoir contains a solution of a strong base. This results in an increasing gradient. In place of Equations 4 and 5 there obtains

$$A^- = \frac{v'}{v} A_o^- + \frac{v - v'}{v} OH^- \quad (12)$$

$$HA = \frac{v'}{v} HA_o - \frac{v - v'}{v} OH^- \quad (13)$$

where OH^- is the concentration of strong base in the reservoir. Substituting these equations into the equation for the dissociation constant of a weak acid and replacing v and v' with Equations 2 and 3, there obtains

$$pH - pH_o = \log \frac{1 + \left[\left(1 + a \frac{V}{v_o} \right)^{\frac{a+1}{a}} - 1 \right] \frac{OH^-}{A_o^-}}{1 - \left[\left(1 + a \frac{V}{v_o} \right)^{\frac{a+1}{a}} - 1 \right] \frac{OH^-}{HA_o}} \quad (14)$$

If the starting pH is at the lowest value ($A_o^-/HA_o = 0.1$) and $f_1 = f_2$, Equation 14 reduces to

$$pH - pH_o = \log \frac{1 + \left(e^{\frac{V}{v_o}} - 1 \right) \frac{OH^-}{A_o^-}}{1 - \left(e^{\frac{V}{v_o}} - 1 \right) \frac{OH^-}{10\, A_o^-}} \quad (15)$$

A plot of this equation for several values of the ratio of the concentration of base in the reservoir to the concentration of salt in the mixing chamber (OH^-/A_o) appears in Figure 5. In all cases the pH gradient is concave and the concavity increases with effluent volume. This is usually a desirable feature. The gradient effect decreases as the base to salt ratio increases.

If, again, $A_o^-/HA_o = 0.1$ and $OH^- = A_o^-$, Equation 15 becomes

$$pH - pH_o = \log \frac{\left(1 + a \frac{V}{v_o} \right)^{\frac{a+1}{a}}}{1.1 - \left(1 + a \frac{V}{v_o} \right)^{\frac{a+1}{a}}} \quad (16)$$

The effect of different flow rates is apparent from a plot of this equation (Figure 6) for several values of the ratio of the flow rates (f_1/f_2). As the ratio decreases the gradient becomes more concave.

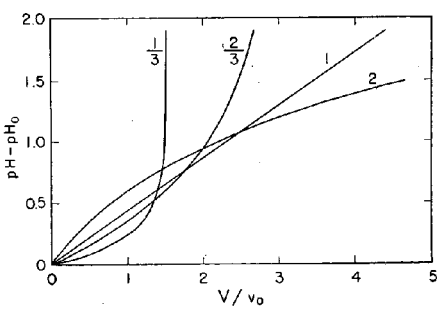

Figure 4. Effect of different ratios of flow rates on pH gradient obtained by addition of a salt of a weak acid to a buffer at equal salt concentrations

Plot of Equation 11

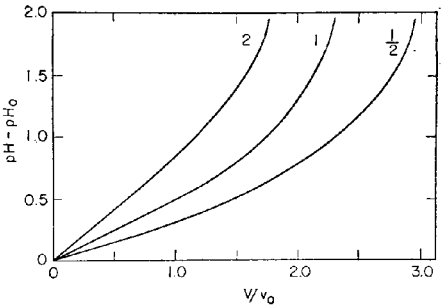

Figure 5. Effect of different ratios of base to buffer salt on pH gradient obtained by addition of strong base to a buffer at equal flow rates

Plot of Equation 15

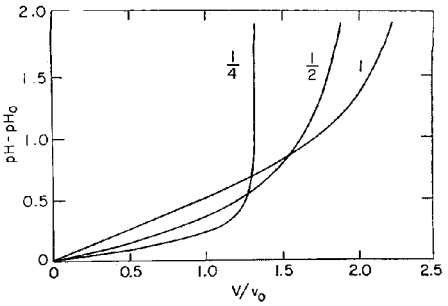

Figure 6. Effect of different ratios of flow rates on pH gradient obtained by addition of strong base to a buffer at equal concentrations of buffer salt and strong base

Plot of Equation 16

Case III, *B*. The mixing chamber contains a buffer as before. The reservoir contains a solution of a strong acid to provide a decreasing pH gradient. In a manner similar to Case III, *A*, an expression similar to Equation 14 is obtained. The pH change is negative and the concentration of acid in the reservoir, rather than base, becomes a controlling factor. The same considerations apply as in Case III, *A*.

DISCUSSION

Inasmuch as a buffer composed of a monobasic acid and its salt has a buffering range of only 2 pH units, it is often advantageous to use a polybasic acid and salt or a mixed buffer system. The equations derived above do not describe such situations quantitatively, but the same general considerations apply. For example, with a citrate buffer in the mixing chamber and sodium citrate in the reservoir the gradients predicted in Case II, *A*, are realized except that the rate of change of pH is approximately doubled. In any case, it is usually necessary to work out the final details by trial and error because it is seldom possible to predict accurately the behavior of the compounds to be chromatographed.

Lakshmanan and Lieberman (*11, 12*) have shown that a concentration gradient that is concave upwards is most desirable to minimize tailing. On the basis of the limited material now in the literature, particularly with regard to ion exchange chromatography, it seems likely that this is also true with buffered pH gradients. At the same time, it is often desirable to maintain a constant cation concentration in the eluent, when using a cation exchange resin, to minimize volume changes of the resin. This can be done in Cases II (Figure 4) or III (Figure 6) while the pH gradient is controlled by the flow rates. At other times, a superimposed concentration gradient is desirable to speed up the chromatographic process or to supplement a pH gradient. This can be attained most easily in Case III (Figure 5) while still maintaining a concave pH gradient. These same considerations apply to the anion concentration with anion exchange resins.

A constant volume mixer can conveniently produce a concave pH gradient only in Case III (Figure 5). If concentration differences exist the concentration gradient will be convex. An equal level arrangement employing identical vessels ($f_1/f_2 = 1/_0$) provides a concave pH gradient in either Case II (Figure 4) or Case III (Figures 5 and 6), and if a concentration gradient exists it is linear. It seems likely that this arrangement should be of considerable general use.

A convex pH gradient, particularly when used with a concentration gradient, may be useful for the separation of one group of compounds from another, or in cases where the stability of the compounds does not allow large pH changes. Case I (Figure 1) and Case II (Figures 3 and 4) provide gradients of the type.

In ion exchange chromatography, it is probably never desirable to have a negative concentration gradient, because this would work against the pH gradient. This situation would exist in Case II, for example, if a concave pH gradient was produced at equal flow rates by employing a low concentration of salt in the reservoir (Figure 3). Neutral salt could be added to the reservoir but a decrease in buffer strength would occur. This decrease in strength magnifies the retarding effect of the ion exchange resin on the pH gradient, a result of the buffering capacity of the resin.

Partly for the same reason, it is not always possible to realize very steep gradients. It is made more difficult by the fact that at values of f_1/f_2 of less than 1, which give the most rapidly changing gradients, the volume of the mixing chamber becomes very small. Technical difficulties, primarily the holdup volume of the column, prevent all of the volume from being used.

Although sorption gradients cannot increase the resolution of compounds that have a linear sorption isotherm (*2*), the technique is of more general value in that it speeds up the chromatographic process in an automatic manner (*13*) and sharpens the peaks. This allows smaller amounts to be chromatographed without loss of precision.

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Rapid Determination of Chlorine Content of Rubber Hydrochloride by Means of Density Gradients in Centrifugal Field

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The density of small particles can be determined by suspending them in a liquid of graduated density and centrifuging. Experimental improvements have been found which make the method suitable for accurate density measurements of rubber hydrochloride from latex. The density of amorphous rubber hydrochloride has been determined in the range from 24 to 33% chlorine. The density measurements provide a means of measuring the chlorine content of rubber hydrochloride in less than 10 minutes, thus enabling one to follow the hydrochlorination of latex closely. Differences as small as 0.1% in chlorine content can be determined. The method is applicable to other polymers as well.

PARTIAL mixing of liquids of different density produces a density gradient that is fairly linear and stable. The use of such liquid density gradients for determining the density of suspended particles has been demonstrated by Linderstrøm Lang (*6*, *7*). It is, of course, important that the liquid employed should not dissolve, dissolve in, or otherwise attack the suspended particles. The method is rapid and accurate, and has been applied previously in studies of high polymers. Boyer, Spencer, and Wiley (*1*) used density gradients for the measurements of the rate of crystallization of poly(vinylidene chloride), and Tessler, Woodberry, and Mark (*12*) for the analysis of fiber mixtures. Gordon (*3*) measured the degree of cyclization of cyclized rubber and Gordon and Macnab (*4*) studied the thermal expansion of polystyrene by means of density gradients.

However, it is impossible to measure the density of very small particles by this method under the influence of gravity alone, because they do not reach their equilibrium positions in reasonable times.

Brakke (*2*) demonstrated that this difficulty can be solved by placing the density gradient in a centrifugal field. He used this method for the purification of viruses. In this same way Kahler and Lloyd (*5*) determined the density of the particles of polystyrene latex and Low and Richards (*8*) determined the density of small crystals.

This principle has now been applied to derivatives of natural rubber prepared from latex (*9*). For the sake of brevity the method is discussed only in relation to rubber hydrochloride, but trivial variations of the method—e.g., the density ranges of the gradients—also make it applicable to chlorinated rubber and cyclized rubber.

The density of rubber hydrochloride from latex depends only on its chlorine content, if crystallization is avoided by keeping the emulsion at or below room temperature. When the emulsion is exposed to higher temperature, a rapid crystallization occurs (*10*, *11*) and the density depends both on chlorine content and degree of crystallization.

The preparation of an emulsion of completely saturated rubber hydrochloride takes approximately 50 hours at room temperature and atmospheric pressure. If a product of a certain chlorine content is needed, normal analytical methods are of only limited value, because sample preparation and chlorine determinations take at least several hours and the reaction has then proceeded further. However, a rapid method for determining chlorine content is provided by the measurement of the density, if the relation between density and chlorine content is known.

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EXPERIMENTAL

Gradient Tubes. The density gradient tubes should be as long as possible in order to obtain maximum accuracy. The centrifuge used in this laboratory (Major Model, Measuring and Scientific Equipment, Ltd., London, England) allowed a maximum length of 190 mm. An inside diameter of 10 mm. is best. If the diameter is considerably larger than this, the gradients are easily destroyed by turbulence caused by handling or centrifuging, whereas a much smaller diameter may give rise to wall effects.

Preparation of Gradients. The density gradients are made from sodium chloride solutions containing a few drops of hydrochloric acid. Low and Richards (*8*) found that the gradients cannot easily be calibrated with oil drops of known density, because small particles tend to adhere to these drops. This difficulty has also been encountered by the present authors, but it was solved by the addition of 1% Emulphor O (a condensation product of oleyl alcohol and ethylene oxide, General Dyestuff Corp., New York) to the sodium chloride solutions. This

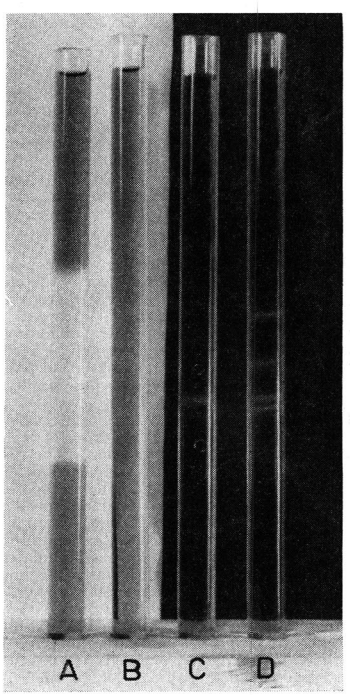

Figure 1. Gradient tubes

A, *B*. Gradients with several dyes before and after partial mixing, respectively
C. One sample and oil drops for calibration
D. One sample and several layers of rubber hydrochloride for calibration

Table I. Densities of Aroclor–Dibutyl Sebacate Mixtures

Aroclor 1221, Ml.	Dibutyl Sebacate, Ml.	Density, 20/20
100	30.1	1.1379
100	36.4	1.1285
100	50.2	1.1121
100	60	1.1026
100	79.8	1.0873
100	93	1.0660
100	113	1.0566

Table II. Chlorine Content and Density of Amorphous Rubber Hydrochloride

Sample	Chlorine Content		Density, 20/20	
		Av.		Av.
1	24.4, 24.4	24.4	1.057, 1.059	1.058
2	24.0, 24.4	24.2	1.060, 1.060	1.060
3	24.9, 24.3	24.6	1.066, 1.064	1.065
4	25.3, 25.7	25.5	1.070, 1.070	1.070
5	26.9, 26.7	26.8	1.079, 1.079	1.079
6	27.7, 28.1	27.9	1.090, 1.092	1.091
7	29.0, 29.6	29.3	1.096, 1.091	1.093
8	28.7, 29.1	28.9	1.101, 1.099	1.100
9	30.4, 30.1	30.2	1.107, 1.107	1.107
10	30.6, 30.8	30.7	1.118, 1.114	1.116
11	31.2, 31.2	31.2	1.118, 1.118	1.118
12	31.7, 31.3	31.5	1.120, 1.123	1.122
13	32.0, 32.2	32.1	1.126, 1.128	1.127
14	33.0, 32.6	32.8	1.128, 1.131	1.129

nonionic detergent prevents the adherence of small particles to each other or to the oil drops. To facilitate a reproducible and linear partial mixing of the solutions, small amounts of dyestuffs are added.

Figure 1, *A* and *B*, shows gradient tubes filled with 4.5 ml. of three different solutions both before and after partial mixing. The solutions are: 22% sodium chloride containing 1% Emulphor O and small quantities of hydrochloric acid and bromothymol blue (density, 20/20, 1.17); 16% sodium chloride with 1% Emulphor O and a trace of hydrochloric acid (density, 20/20, 1.12); and 10% sodium chloride with 1% Emulphor O and small quantities of hydrochloric acid and methyl red (density, 20/20, 1.07). The partial mixing is done in the usual way by passing a copper spiral slowly up and down until the colors pass into each other gradually.

Calibration of Gradients. Mixtures of nonvolatile oils are preferred in order to avoid alterations of the densities by differences in the rate of evaporation of the constituents. In these experiments mixtures of Aroclor 1221 (a chlorinated plasticizer of Monsanto Chemical Co., St. Louis, Mo.) and dibutyl sebacate were used. The densities of these mixtures are shown in Table I. Because the oil drops tend to adhere to the surface of the gradients, very small drops are added to the gradient just below the surface by means of a small pipet with a fine tip.

Preparation of Rubber Hydrochloride. The rubber hydrochloride is made from normal centrifuged latex by the method of van Veersen (*13*). The latex is stabilized by 1.75 grams of Emulphor O per 100 grams of rubber. It is then poured with stirring and cooling into half its volume of concentrated hydrochloric acid, and hydrogen chloride gas is passed through below 20° C. The reaction is completed in about 50 hours at atmospheric pressure. The rate of reaction can be increased considerably by a small increase in pressure.

Preparation of Curve. At certain time intervals samples are taken from the reaction vessel. A few drops of each sample are used for the determination of the density. The rest is precipitated in alcohol, filtered, washed, and dried at 50° C. The chlorine content is then determined by the method of Carius.

Determination of Density. The maximum speed of the Major centrifuge is only 3000 r.p.m. This is too slow to allow the particles of rubber hydrochloride to reach their equilibrium position rapidly. The particles are therefore first flocculated by adding a few drops of the sample to 1 ml. of ethyl alcohol. One or 2 drops of this flocculated dispersion are put into a gradient, which is then centrifuged for 2 minutes at 3000 r.p.m.

A sharp layer with a thickness of 0.5 to 1 mm. is obtained (Figure 1, *C*). The oil drops to calibrate the gradient are then added and the centrifuging is repeated. It is also possible to calibrate the gradient first and centrifuge the rubber hydrochloride particles afterward.

RESULTS AND DISCUSSION

The relation between chlorine content and density of amorphous rubber hydrochloride was established in the range from 24 to 33% chlorine. When the values were plotted a linear relationship was obtained, as shown in Figure 2, in which the points are the mean values of two chlorine determinations by the Carius method and two density measurements (Table II). The errors of the chlorine determinations are evidently substantially larger than those of the density measurements. This is partly due to the inaccuracy of the Carius method and partly to the fact that the washing and drying of products containing about 25 to 30% chlorine are rather difficult because of some tendency for decomposition. Products with a chlorine content of less than 24% are so unstable that changes of the chlorine content during coagulation, washing, and drying are likely to occur.

If the density of a rubber hydrochloride is determined, the corresponding chlorine content can now easily be found from the graph. The determination of the density takes less than 10 minutes, which enables one to follow the hydrochlorination process very closely. An additional advantage is that only minute quantities are needed.

It is possible to calibrate the gradients with rubber hydrochloride dispersions of known density. The various dispersions may be added and centrifuged simultaneously. Emulphor O prevents the flocculates from adhering to each other, and each sample gives a sharp layer as shown in Figure 1, *D*. It is immaterial whether the sample of unknown density or the samples used for calibration are centrifuged first. Particles with a greater density move right through existing layers with a lesser density.

Precision. The determination of the chlorine content by this method is, of course, not an independent one. Its precision depends on the accuracy of the plot shown in Figure 2; if all the chlorine determinations by the Carius method are too high or too low the density method will be in error, also. Relatively, however, the density measurements give more accurate results.

To demonstrate this fact a hydrochlorination was carried out under circumstances whereby the chlorine content increased from 28 to 30% in 3 hours. Samples taken at intervals of 10 minutes should differ by about 0.11% in chlorine content. This difference is too small to be detected by normal analytical procedures, but the samples clearly separated in individual layers. The differences in density corresponded with a difference

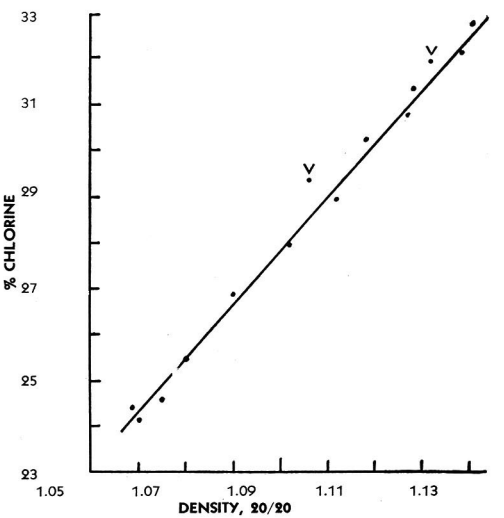

Figure 2. Chlorine content of amorphous rubber hydrochloride as function of its density

in chlorine content of about 0.1%. This means that the relative accuracy of this method is better than 0.1%, a substantial improvement over the Carius method. It may be possible to improve the sensitivity of the method still further by decreasing the steepness of the gradients, but this was unnecessary for the authors' purposes and, therefore, was not investigated.

This method has been applied for several years in this laboratory. Modified according to the exigencies of the case, it may be equally valuable for the investigation of other polymers.

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The authors wish to express their appreciation to the management of the Rubber-Stichting for permission to publish this paper.

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Fast Neutron Activation Analysis

Silicon and Aluminum

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A rapid method for the quantitative estimation of silicon and aluminum uses the deuterium-tritium reaction as an intense source of 14-m.e.v. neutrons. These neutrons are sufficiently energetic to produce the *n,p*; *n,α*; and *n, 2n* reactions in usable yields at the neutron fluxes available from relatively low voltage positive ion accelerators. The *n,p* reaction on silicon, producing the 2.4-minute aluminum-28 radioisotope, is used for silicon determination by comparison with yields from known samples. Similarly, aluminum determination uses the 15-hour sodium-24 activity produced by the *n,α* reaction. The method is accurate to within 5% for samples containing 0.4 gram or more of silicon dioxide and 0.5 gram or more of aluminum oxide. Phosphorus, praseodymium, and chromium interfere with the determination of silicon, while magnesium and iron interfere with the determination of aluminum.

THE general concept of activation analysis has been well established by a large number of papers (*2–6*), and summarized by Boyd (*1*). Previous studies have been primarily concerned with activation by thermal neutrons. With an increasing number of particle accelerators capable of producing high energy neutrons, the field of analysis by fast neutron activation deserves study. With relatively low voltage accelerators (100 to 250 kv.) good yields of 14-m.e.v. neutrons are readily obtainable, by using the deuterium-tritium reaction. These neutrons have sufficient energy to produce *n, 2n; n, α*; and *n, p* reactions, although the cross sections are usually smaller than for thermal neutron (n, γ) reactions.

In general, when an element to be detected is bombarded, the resulting intial intensity of activation, I_o, is given by:

$$I_o = \frac{W}{A} N \sigma f e m (1 - e^{-\lambda t}) \qquad (1)$$

where e is the counter efficiency for the radiation from the element concerned, W is the weight of the element, A is its atomic weight, N is Avogadro's number, f is the effective flux, σ is the elemental cross section for the particular reaction involved, λ is the decay constant of the produced radioactivity, m is the isotopic fraction of the element, and t is the bombardment time.

For practical purposes, A, N, σ, λ, and m may be combined as a single constant, F, characteristic of each element, and independent of the bombardment time.

Equation 1 then becomes

$$I_o = Wfe F \frac{(1 - e^{-\lambda t})}{\lambda} \qquad (2)$$

where the quantity F, called the "figure of merit," is

$$F = \frac{N \lambda \sigma m}{A} \qquad (3)$$

Because for short bombardment times, the quantity $(1 - e^{-\lambda t})$ may be approximated by λt, Equation 2 becomes

$$I_o = Wfe F t \qquad (4)$$

The figure of merit, F, for a particular element is a measure of the relative sensitivity of the method for bombardment times, which are short compared to the half life of the activity. Table

Table I. Computed Figure of Merit Values

(14-m.e.v. neutron bombardment)

Element	Half Life, Min.	F	Gamma Energy, M.E.V.	Isotope Produced
O	7 sec.	1.05×10^{-2}	6.13, 7.1	N-16
Pr	3.4	1.80×10^{-3}	1	Pr-140
Si	2.4	1.31×10^{-3}	1.8	Al-28
P	2.4	8.5×10^{-4}	1.8	Al-28
Cu	10.1	2.20×10^{-4}	0.6	Cu-62
Cr	3.7	1.41×10^{-4}	1.46	V-52
Al	9.6	8.56×10^{-5}	0.84, 1.01	Mg-27
Mg	1	7.54×10^{-5}	0.5	Na-25
Zr	4.5	4.24×10^{-5}	1.5	Zr-89
V	6	3.82×10^{-5}	0.32	Ti-51
Si	6.7	1.02×10^{-5}	1.2	Al-29
Fe	2.6 hr.	4.58×10^{-6}	0.8, 1.8, 2.1, 2.7	Mn-56
Mg	14.7 hr.	3.02×10^{-6}	1.38, 2.76	Na-24
Si	9.6	2.09×10^{-6}	0.84, 1.01	Mg-27
Al	14.7 hr.	1.41×10^{-6}	1.38, 2.76	Na-24
Cl	33	9.5×10^{-7}	1.16, 2.1	Cl-34
K	38	6.15×10^{-7}	1.6, 2.1	Cl-38
K	110	5.25×10^{-7}	1.3	A-41

I is a list of figure of merit values for several elements computed from cross-section values reported by Paul and Clarke (*8*) and data from the National Bureau of Standards (*7*). If, in a single series of analyses, the neutron flux and bombardment time are held constant, the induced radioactivity is directly proportional to the weight of the element present.

If the flux of the neutron source is not known or is not constant, a flux monitor or standard must be used. The flux monitor consists of a known amount of the element for which the analysis is desired. The weight of material in the unknown can then be determined by comparison with standard samples bombarded and counted under the same conditions.

APPARATUS

A Van de Graaff accelerator, manufactured by the High Voltage Engineering Corp., Cambridge, Mass., was used as the source of accelerated deuterons. Deuterons of 200- to 300-kv. energy were allowed to strike a target of zirconium-tritium 3/4 inch in diameter (1 mg. per sq. cm.), manufactured by the Oak Ridge National Laboratory.

The samples for analysis were mounted in plastic vials 9/16 inch in diameter, which were supported directly in front of the target. After bombardment the samples were placed in a well-type sodium iodide (thallium) scintillation crystal counter. Pulses from the RCA 6342 photomultiplier tube were amplified and fed into an integral-type discriminator, and thence to a scaler and register.

REAGENTS

National Bureau of Standards samples 97 (flint clay), 98 (plastic clay), and 99 (soda feldspar) were used as standards without further dilution or drying.

Table II. Analysis of Silica Samples

Sample	SiO_2, Grams	Specific Activity, Counts/Min./G. SiO_2			Av.
NBS 97	1.40	67,400	67,400	66,800	67,200
Sand	3.82	67,700	65,000		66,350
Quartz	2.72	68,250	(av. of 14 detns.)		68,250
Si metal	5.75	68,800			68,800
	1.20	65,400			65,400
	0.90	67,400	68,600		68,800
	0.60	68,800	68,300		68,550
	0.394	67,500			67,500
		Av. 67,600 ± 1160 or ±1.7%			
Si metal	0.100	84,600			84,600
	0.050	100,000			100,000
Shale	1.16	73,400	73,400		73,400

Silicon metal (Mallinckrodt Chemical Co.) analyzed at >99% silicon was used in preparation of some of the samples. Weighed amounts of the metal were mixed with an amount of neutron inert material (CaO) to give a constant final volume of sample. Although the neutron activation of calcium is low, its presence interfered with the analysis of samples of very low silicon content.

Aluminum oxide (Baker's C.P.) was used in the preparation of aluminum reference samples. In the case of the aluminum samples, potassium dichromate was used as the diluting agent to maintain a constant volume of sample.

Two samples, sand and quartz, were analyzed in these laboratories for silicon and aluminum content by ordinary wet chemical methods.

RESULTS

Silicon. Silicon, bombarded with 14-m.e.v. neutrons, yields the 2.4-minute half-life aluminum-28 activity (*7*) with a gamma-decay energy of 1.78 m.e.v. Silicon concentrations were determined with the discriminator set to accept only those pulses due to gamma rays of energy greater than 1 m.e.v., in order to bias out the effect of the magnesium activity produced from the aluminum in the sample. Bombardment times of approximately 30 seconds at a target current of about 30 μa. were used. As the neutron source was almost a point source, it was necessary to maintain a constant volume of sample in the plastic vial in order to hold the bombardment geometry constant. A monitor sample containing a known amount of silicon and bombarded at the same time as the unknown sample was used to correct the samples for variations in neutron flux.

Both the weighed sample (~ 3 grams) and monitor were bombarded in fixed geometries for approximately 30 seconds. After at least a 1-minute delay (to allow for 7.3-second nitrogen-16 decay), the sample was counted for a period of 2.5 minutes. Thirty seconds after the end of the sample count, the monitor was counted for 2 minutes. The sample count was then normalized to a given monitor count.

As the volumes of all the samples were maintained nearly constant, a correction for self-absorption was neglected. The total neutron attenuation through the thickness of samples used was determined by thin copper foil activation to be of the order of 10 to 12%, so that variations in self-absorption between samples was considered negligible for the purposes of this method. Similarly, the self-absorption correction for attenuation of the gamma radiation in the sample was also neglected.

Table II shows the results of the analyses of 11 samples, the silicon content of which ranged from 0.05 to 5.75 grams (reported as SiO_2). The observed standard deviation of the values for the first eight samples is 1.7%, with a maximum deviation from average of 3.7%. The last three samples of low silica content show a higher value for counts per gram. In the first two samples this is probably due to the effect of activity induced in the matrix material. One gram of calcium oxide yields an effect approximately equal to 0.01 gram of silicon dioxide. The shale sample is known qualitatively to contain phosphorus, which interferes with the analysis for silicon. From the list of computed figure of merit values the principal interfering elements, at the bias level used, are praseodymium, phosphorus, and chromium. The degree of interference is approximately equal to the ratio between their respective figure of merit values, with the exception of praseodymium. The bias level selected is such that the response to praseodymium is greatly decreased—i.e., 1 gram of praseodymium oxide yields the same count as 2.6 grams of silica.

Table III. Analysis of Alumina Samples

Sample	Al_2O_3, Grams	Specific Activity, Counts/Min./G. Al_2O_3				Av.
NBS 97	1.268	45,400	46,000	46,400		45,900
NBS 99	0.570	44,500	42,700	42,400		43,200
NBS 98	0.766	48,200	49,400	48,000		48,500
Al_2O_3	2.000	44,100	44,100	45,280		44,500
	1.000	46,000	44,000	44,450	43,600	44,450
	0.500	47,500	45,900	45,300	47,500	46,500
		Av. 45,500 ± 1700 or 3.8%				
Al_2O_3	0.250	50,500	50,500	49,000	49,200	49,800
	0.125	50,800	54,200	52,250		52,400

The results of 14 repeat runs on the same sample (quartz, 2.72 grams) yielded 185,500 ± 2200 counts, or an observed standard deviation of 1.2% with a maximum deviation from average of 2.2%.

Aluminum. Bombardment of aluminum by 14-m.e.v. neutrons yields two activities (*7*). Magnesium-27, produced by the $n - p$ reaction on aluminum-27, has a half life of 9.6 minutes and gamma-decay energies of 0.84 and 1.01 m.e.v. Sodium-24, produced by the $n - \alpha$ reaction, has a half life of 14.7 hours and gamma-decay energies of 1.38 and 2.75 m.e.v. Aluminum was determined by using the 14.7-hour sodium-24 activity and counting on the integral bias plateau of the crystal counter at a fixed discriminator setting. In order to enhance the effect of the 14.7-hour activity, the samples were bombarded for 1.5 hours at a neutron flux of the order of 5×10^7 n. sq. cm./second. A rotating sample holder, with 10 samples mounted around the periphery, was used to enable a number of samples to be bombarded simultaneously and to distribute the flux equally among all the samples.

As in the silicon determinations, a constant volume of sample was used in order to maintain a constant bombardment geometry. In each case, a flux monitor sample (1.00 gram of aluminum oxide) was included among the samples during bombardment. At least a 2-hour delay after the end of bombardment was required to allow for the decay of extraneous activities, such as that due to aluminum-28 (2.4 minutes) and to magnesium-27 (9.6 minutes). Under these conditions, the principal interfering elements are magnesium and iron. The observed relative specific activities produced by these elements are 1.37 for magnesium (as MgO), 1.00 for aluminum (as Al_2O_3), and 0.575 for iron (as Fe_2O_3). Although this paper is concerned with aluminum analysis, the method should be equally valid for any one of the three elements in the absence of the other two.

All samples were counted for 10 minutes and corrected for decay to the time of the start of the first sample count. The resulting value, when compared with the monitor count, was proportional to the aluminum content.

The results of 27 analyses of eight samples containing aluminum ranging from 0.125 to 2.00 grams (as aluminum oxide) are shown in Table III. The observed standard deviation of the values for the first six samples is 3.8%, with a maximum deviation from average of 7.6%. These errors are somewhat larger than would be anticipated and could conceivably be attributed to unequal neutron flux variations on the sample and the monitor, or to some unrecognized source of error. The last two samples of low alumina content show appreciably higher values for counts per gram presumably due to scattering or self-absorption effects. For these low concentrations, it may be possible to establish a calibration curve for the specific activity.

The results of 10 analyses of a single concentration of aluminum oxide (1.00 gram) yielded 45,500 ± 790 counts or an observed standard deviation of 1.8% with a maximum deviation from average of 2.8%.

CONCLUSIONS

A rapid method for the quantitative estimation of silicon and aluminum has been established. Although its accuracy is less than conventional wet chemical means, the considerable saving in time and effort justifies its use. At present the method is accurate to within 5% for samples containing 0.40 gram or more of silicon dioxide and 0.50 gram or more of aluminum oxide, with the flux of fast neutrons readily available from a low energy positive ion accelerator. With a suitable calibration curve the range of concentrations for useful application of the method may be extended.

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Rapid Analytical Electrophoresis Employing Prismatic Cell

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An optical arrangement for moving-boundary electrophoresis is described, which uses a cell of prismatic cross section and directly plots the refractive index gradient *vs.* the refractive index increment. Experimental results and diagrams for serum analyses, as well as the analysis of optical sensitivity and systematic errors, indicate that this arrangement is at least equal in accuracy to schlieren optical methods. The arrangement permits rapid analytical electrophoresis work of high accuracy, because resolved components are recognized as separate peaks just as with schlieren optical arrangements, while relative concentrations are read directly as lengths with a reticle eyepiece.

PRISMATIC arrangements have been employed for the study of inhomogeneous liquid columns occurring in sedimentation equilibrium (*5*) and electrophoresis (*8, 18*). These arrangements have the advantage of depicting the distribution of refractive index (or concentration) directly. Without some arrangement for also recording refractive index gradient (*5, 18*), however, a prismatic system does not offer satisfactory visual resolution of partially separated moving boundaries, the refractive index gradient function being superior in this respect (*13*).

The present arrangement takes advantage of a very simple projection optical system using a point source of light and a prismatic cell to plot directly the refractive index against the gradient of refractive index (*6*). In the diagrams obtained, visual resolution of partially separated boundaries is as satisfactory as that obtained with schlieren refractive index gradient recording techniques (*10, 11, 16*). However, diagrams from this prismatic arrangement also contain the relative refractive index increments in the form of distances between successive gradient dips in the diagram. Consequently, concentration measurement proceeds very rapidly, and can be made with a precision intermediate between that of schlieren and interferometric (*1, 3, 8, 9, 12, 14*) methods. No cell position coordinates are defined in these diagrams, and mobilities are obtained, when required, with auxiliary optical devices.

OPTICAL ARRANGEMENT

The schematic arrangement of the optics is shown in Figure 1. Light from the point source, P, is received by the astronomical telescope achromatic objective, L, placed at its focal length, F, away from the source. Parallel light is projected into the electrophoresis cell, C, whose rear window is externally silvered to return the light through lens L. Source P is slightly off the axis of lens L, so that the returning light beam impinges on mirror M and comes to focus, in the presence of uniform liquid throughout the cell, C, and its surrounding water bath, as a sharp image of the point source on the screen, S.

Figure 2 shows a schematic arrangement of the top view of one column of the cell, which aids in following the lateral light deflec-

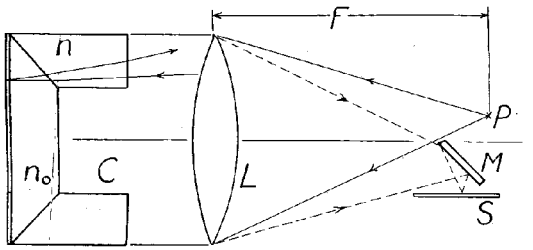


Figure 1. Schematic arrangement of optical system

P. Point source of light
F. Focal length of objective
C. Electrophoresis cell
L. Lens
M. Mirror
S. Screen
n. Refractive index of protein column in cell
n_0. Refractive index of buffer in cell

tem of moving boundaries, the lateral deflections at *S* will reproduce a continuous record of this variation in refractive index.

At the same time, vertical deflections of light are produced within inhomogeneous regions of refractive index in the cell, and the resulting deflections Z at *S* are proportional to the gradient of refractive index according to the principles of schlieren optics, $Z = 2F(dn/dx)\bar{a}$. Here $\bar{a}$ is the mean path of a ray passing once through the electrophoresis prism. The same ray which suffers a vertical deflection Z also suffers a simultaneous lateral deflection y, so that the distorted image on *S* of the source *P* constitutes a plot of refractive index gradient, $Z = (2F\bar{a})(dn/dx)$, against refractive index increment, $y = (2F \tan \alpha)(n - n_0)$.

This is illustrated in Figure 3, in which a hypothetical electrophoretic separation is being made of human plasma. The formed boundaries are descending into unaltered protein solution. In this figure, ray *b* represents light which has passed through buffer in both chambers of the cell, and has returned undeflected to form an image of source *P* at point *b* on the screen. Ray *c* represents light which has passed through homogeneous protein solution of the original concentration below all the moving boundaries, and through buffer solution in the reference chamber of the cell. Ray *c* consequently suffers no vertical deflection, but is deflected horizontally at *S* by the distance *bc* which is proportional to the total protein concentration of the original solution.

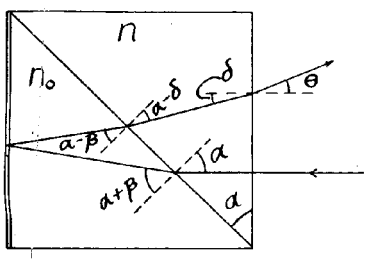


Figure 2. Prismatic light deflections

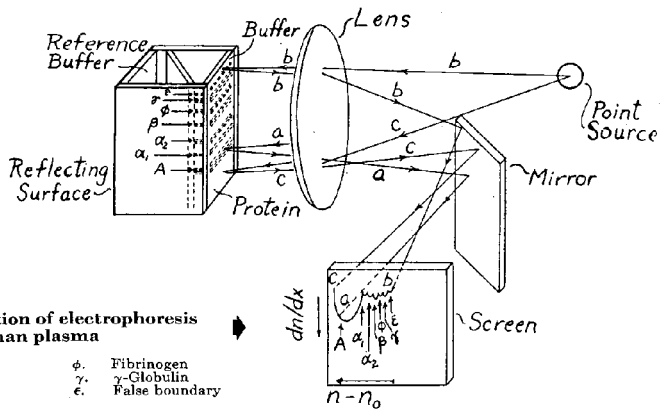


Figure 3. Production of electrophoresis patterns from human plasma

A. Albumin
α_1. α_1-Globulin
α_2. α_2-Globulin
β. β-Globulin
ϕ. Fibrinogen
γ. γ-Globulin
ϵ. False boundary

tions resulting from the prismatic cross section of the electrolysis chamber. The cell contains a reference chamber which is filled with buffer solvent of refractive index n_0 and sealed during the preparation of an experiment. The refractive index n of the protein column varies from that of original unseparated protein at the bottom of the column to that of the buffer, n_0, at the top of the column. Figure 2 may be taken as representative of a horizontal section through the cell at some level part way up the column. The ray of light entering the cell from the right, normal to its right-hand window, is deflected at the prism interface (the glass being omitted for simplicity of the diagram). The deflection follows Snell's law

$$n \sin \alpha = n_0 \sin (\alpha + \beta) \quad (1)$$

at the first passage, and

$$n_0 \sin (\alpha - \beta) = n \sin (\alpha - \delta) \quad (2)$$

at the second passage. When the returning beam enters air, the deflection angle becomes θ, where

$$\sin \theta = n \sin \delta \quad (3)$$

When the angles of deflection, β, δ, and θ are sufficiently small, these equations may be simplified to

$$\theta \backsimeq (2 \tan \alpha)(n - n_0) \quad (4)$$

According to Equation 4, when light passes through cell *C* at a height where the refractive index difference between the electrophoresis column and reference buffer is $n - n_0$, the light will be deflected at screen *S*, Figure 1, a lateral distance from the undeviated point source image equal to $y = 2F(n - n_0) \tan \alpha$. If the refractive index n varies vertically in the column (perpendicular to the page in the diagram) because of the separation of a system of moving boundaries, the lateral deflections at *S* will reproduce a continuous record of this variation in refractive index.

Ray *a* represents light which passes through the albumin boundary in the electrophoresis chamber and through buffer in the reference chamber. This ray, consequently, is deflected vertically according to the gradient of refractive index dn/dx and horizontally according to the refractive index increment $n - n_0$, in the level where it penetrates the cell. As a result it comes to position *a* on the screen, deflected both vertically and horizontally from reference point *b*. Continuing this reasoning, it is apparent that the resultant diagram constitutes a continuous plot of refractive index gradient *vs.* refractive index increment. The horizontal distances between successive cusps in this diagram (successive minima of dn/dx) represent protein component concentrations, which may then be read directly as lengths from the diagram.

In moving-boundary electrophoresis experiments it is a necessary consequence of the requirement for stability against convection that the density, concentration, and hence, in general, the refractive index, increase continuously as the boundaries are scanned from top to bottom of the cell. Consequently, the peaks in the present diagrams must appear in the same relative order as they appear in conventional schlieren (dn/dx *vs.* x) diagrams. The diagrams may be converted to Toepler schlieren diagrams (*20*, *21*) by removing the screen and interposing a horizontal blade and a telescope focused on the cell. This procedure has no advantage for analytical work, but permits the measurement of mobilities.

In this regard, if it is desired to remove samples from a region in the cell corresponding to a given part of the diagram, this is performed with a syringe in a perfectly straightforward manner, with the aid of the simple projection optics. The needle of the syringe used for sampling has the effect of obscuring the pattern, so that the location of the tip of the needle corresponds to the first visible part of the pattern closest to the undeflected reference point. In this optical arrangement, it is also practical and con-

venient, during formation and positioning of boundaries, to focus the unaided eye on the cell after removal of the screen.

EXPERIMENTAL

Electrophoresis experiments on a number of pooled human sera at several dilutions were performed in sodium diethylbarbiturate buffer (pH, 8.6, ionic strength, 0.1) after overnight dialysis in the cold. A number of experimental cells were tried, the optimum being one with a prism angle tangent of 1.33, and a mean $\bar{a}$ of approximately 13 mm. The cross-sectional arrangement of this particular cell is shown true to scale in Figure 4. The focal length of the lens was 146 cm. The cell cross section was 0.35 sq. cm., and optimal serum patterns were obtained from this cell filled with either one volume of serum plus two volumes of buffer or one volume of serum plus one volume of buffer. For an experiment in which one volume of serum was diluted with one volume of buffer for dialysis, only 2 ml. of serum was required. This represents the maximum requirement for this cell. The same cell still gave patterns which could be analyzed quantitatively at a concentration of one volume of serum plus four volumes of buffer.

In measurement of photographs, a Bausch & Lomb reticle eyepiece graduated for 20 mm. in 0.1 mm. was found adequate. In a given pattern it was usually possible to reproduce readings for a given component to the nearest 0.1 mm. A precision of 0.1 mm. corresponds, with these optical constants, to a refractive index increment of 0.000026, so that the sensitivity is about that of schlieren optical methods (*8, 14*). In practice, the need for integration of schlieren diagrams makes the comparison considerably more favorable to the present arrangement.

In the course of these serum analyses, results were compared for one serum sample which had been examined both with the Rayleigh interferometric method and with three separate experiments employing the present method. It was found uniformly true in all serum experiments using the prismatic arrangement that excellent resolution between γ-globulin and the δ-boundary occurred in the rising boundary patterns, whereas very poor resolution was exhibited between the γ-globulin and ϵ-boundary in the descending patterns. Resolution between albumin and α_1-globulin was also much better in rising than in descending patterns. Moreover, the β-globulin anomaly, after extensive electrolysis, showed up in the descending patterns as an apparent change of shape and refractive increment in the β-globulin region, clearly caused by local convections as density stability decreased with separation of the protein species. For analytical purposes in this particular protein system, therefore, only the rising patterns are suitable for precise work. Table I summarizes the results obtained from rising boundary patterns for this comparison.

Figure 5 shows direct photographic copies of the rising boundary patterns for one serum sample, using the cell with tan α = 1.33. (The instrument used for these experiments was arranged to rotate the image so that it was received at the screen exactly as shown here.) Figure 5, *a*, respesents electrolysis for 1 hour 18 minutes at approximately 6.8 volts per cm. in a solution containing one volume of serum *diluted* with one volume of buffer. Figure 5, *b*, represents electrolysis for 2 hours 15 minutes at approximately 6.5 volts per cm. in a solution containing one volume of serum diluted with two volumes of buffer. In this serum, the α_1-globulin component represented only 5% of the total protein, and was present during the experiment of Figure 5, *b*, at an absolute concentration of 0.17%. The optical sensitivity itself permits its determination to within 0.014% absolute concentration.

ERRORS OF MEASUREMENT

Some systematic errors may be anticipated in the use of this prismatic optical system for electrophoresis. Several authors (*2, 7, 15*) have computed the nonlinearity correction for relationships similar to Equation 4, the most detailed computations being those of Svensson. If Equations 1, 2, and 3 are reconsidered for normal entrance of light, without making any approximations, the sine of the angular prismatic deflection in air is given by

$$\sin\theta = \{n(1 + \cos\delta) - 2n_0 \cos\beta\}\tan\alpha \qquad (5)$$

If one takes as the error in Equation 4 the quantity $\epsilon = \sin\theta - \theta$, then by approximating $\beta \backsimeq (1/n_0)(n - n_0)\tan\alpha$ and $\delta \backsimeq (2/n)(n - n_0)\tan\alpha$ and expanding $\cos\beta$ and $\cos\delta$ in Taylor series and inserting in Equation 5, one obtains

$$\epsilon \backsimeq -(1/n_0)(n - n_0)^2 \tan^3\alpha \qquad (6)$$

as a first approximation to the error term. This error is such that it reduces the measured concentration below what would be expected from Equation 4 for high values of $n - n_0$ and tan α. With a single passage of light through a cell such as used here,

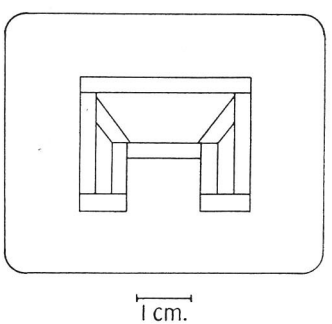
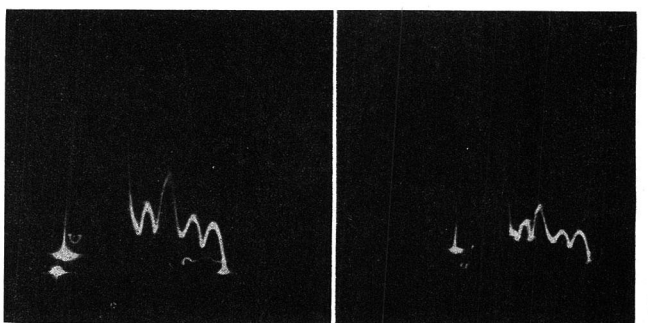


Figure 4. Scale drawing of cross section of electrophoresis prism cell



1 volume serum + 1 volume barbital buffer, pH 8.6; 1 hour 18 minutes at 6.8 volts per cm.

1 volume serum + 2 volumes barbital buffer, pH 8.6; 2 hours 15 minutes at 6.5 volts per cm.

Figure 5. Electrophoresis patterns obtained with human serum

Table I. Relative Per Cent Concentration of Proteins

Protein	Rayleigh[a]	Prism tan α = 3[a]	Prism tan α = 1.33[a]	Prism tan α = 1.33[b]
Albumin	50.9	51.5	50.0	..
α_1-globulin	6.5	6.6	7.2	..
Albumin + α_1-globulin	57.4	58.1	57.2	54.3
α_2-globulin	9.9	9.6	11.9	11.3
β-globulin	20.3	20.7	18.3	21.7
γ-globulin	12.4	11.6	12.4	12.7

[a] 1 volume serum + 2 volumes buffer.
[b] 1 volume serum + 4 volumes buffer.

giving the same angular deflection by doubling the prism angle tangent ($\tan \alpha' = 2 \tan \alpha$), the error becomes

$$\epsilon' \backsimeq (1/2n_0)(n - n_0)^2 \tan^3 \alpha' \quad (7)$$

which acts to increase the measured concentration above what would be expected from the relationship analogous to Equation 4 for this case, in agreement with previously published computations. For the same solution ($n - n_0$ constant), the ratio of errors for identical angular deflection becomes, in absolute magnitude

$$|\epsilon'/\epsilon| = \tan^3 \alpha'/2\tan^3 \alpha = 4 \quad (8)$$

Hence, the nonlinearity is reduced absolutely, and also percentagewise, by a factor of four by virtue of the autocollimating arrangement which passes light through the cell twice. According to Svensson (*15*), the present arrangement, which is equivalent to Figure 4, *d*, of Svensson, gives essentially the same results even though the light does not enter the cell normal to its first window. This leads to a great advantage in critical requirements for cell construction and alignment over a system in which the light passes the present type of cell only once.

For practical purposes, it is of considerable interest to calculate the highest protein concentration which may be measured to within 1%, without taking into account nonlinearity corrections given by Equation 6. Assuming a cell with a tangent of 1.33, and taking the specific refractive index increment of a protein as 0.00185 and the solvent refractive index as 4/3, it is required that $\epsilon/\theta = (1/2n_0)(n - n_0) \tan^2 \alpha < 0.01$, or translated in terms of concentration, the requirement becomes $(2/3)(0.00185\ c) < 0.01$, or $c < 8.1\%$. This represents somewhat more than twice the highest protein concentration at which this cell has been used; excellent patterns for analyses of human sera are still obtained at about one third of this critical concentration. It might then be stated that errors of this type would not be expected to exceed about 0.5% under the worst conditions, with this choice of optical constants.

It might also be asked how high a protein concentration can be examined with the same optics, without making a nonlinearity error greater than the stated reproducibility of 0.1-mm. deflection at the screen. In this case $\epsilon = (4/3)^2(0.00185)^2c^2$, and $\epsilon < 0.01/146$, which leads to $c < 3.4\%$. As this protein concentration is again near the upper limit used with this cell to obtain optimal patterns, it may be safely concluded that, with the optical constants chosen, a linear dependence of deflection on refractive index increment holds true.

Other systematic errors may be produced by the presence of refractive index gradients. These involve the fact, already recognized by Wiener (*22*), and recently more correctly recomputed (*17*) and experimentally evaluated (*4*), that light bent by a gradient of refractive index does not behave as if all the bending takes place in the middle of the path through the gradient. Ordinarily this may result in the assignment of a refractive index gradient value to a particular height in the cell to which it does not quite belong. In the present optical arrangement a given refractive index gradient may be assigned, in the diagram obtained, to a refractive index increment value to which it does not quite belong, because of such effects stemming from the curvature of the light path. This would tend to give a lateral shift to regions of the diagram containing high refractive index gradients. The calculation of such effects becomes extremely complicated, however, because of the interaction between the two types of deflection, and no satisfactory calculations have yet been made. A similar effect is produced because the lateral deflection occurs at the prism window rather than inside the protein solution. To do justice to the present optical arrangement, however, it must be pointed out that in practice the regions of interest for analytical determinations are just those where the refractive index gradients and the errors just discussed reach successive minima, and these errors must disappear entirely when the refractive index gradient becomes zero. In comparison with schlieren optical methods, where an error at high values of dn/dx results in an error in area and concentration, the present method is at least superior in principle, in so far as light-bending errors are concerned. In the regions of maximum protein concentration the lateral deflections may also cause the effective mean cell thickness for vertical deflections to differ appreciably from the cell thickness at low protein concentrations. While this effect has a tendency to distort the upper parts of the peaks, again the effect on measurements taken at successive minima of dn/dx in the diagram is not significant. To reduce the spread of the refractive index gradient deflections at high values of dn/dx, it has been found satisfactory to mask the cell aperture down to 2 mm.; the diagrams shown in Figure 5 were obtained with such masking. For very sharp initial boundaries, the intensity in the diagrams has been found to be inconveniently low. However, there is no need to observe these early diagrams for analytical work, as all manipulations can be performed by focusing the eye on the cell. As diffusion and separation proceed, the diagrams continually increase in brightness, becoming adequate for visual inspection on the screen, for visual measurement with an eyepiece, and for photography by the time sufficient physical separation has been accomplished in the electrophoresis cell.

From the standpoint of accuracy in analytical work, the present method is in no way inferior to schlieren optical arrangements.

DISCUSSION

Certain practical advantages have been found for this arrangement in analytical work. During physical separation of components by the electric current, the peaks in the diagram neither spread nor move laterally. Improvement of physical separation in the cell merely improves optical resolution in the diagram. As a result it is practicable to read component concentrations with an eyepiece in place of the screen during electrolysis. It is not necessary to stop the current for readings, and the diagrams serve to indicate when the physical separation is optimal. A further result is that photography is necessary only for the keeping of permanent records. Visual analysis while electrophoresis is still going on has been found satisfactory. Consequently, essentially no time is required to obtain the final analyses beyond that required for electrolysis, and the numerical calculations can be performed within minutes while electrolysis proceeds. This reduces the time for each experiment by a considerable factor without loss of accuracy.

In the case of routine analyses of samples such as sera, Svensson and Olhagen (*19*) have described how moving boundary work can be performed without dialysis. They dilute the serum with one buffer, and layer above it a second buffer solution containing buffer- and serum-ion components at proper concentration to produce only very small ion gradients across the protein boundary. When advantage is taken of this method and of the present optical arrangement, a complete quantitative analysis for the major protein components can be made available within about 3 hours after receipt of the serum sample. This point may be of considerable interest in clinical work wherever a serum protein analysis is useful as an aid in medical examinations.

ACKNOWLEDGMENT

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staff of the Memorial Hospital, Worcester, Mass., made available pooled human sera for these experiments.

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Colorimetric Determination of Phenolic Resins

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A modification of the nitrous acid test for free phenols is presented for phenol-aldehyde resins in varnishes and other coatings. An intense yellow color is developed with phenolic resins; the color is specific and can be used for quantitative measurement if the nature of the raw materials is known. The phenolic content of resins of unknown origin can be estimated.

RESINS obtained by phenol-aldehyde condensation are widely used, usually modified by dispersion in other resins or reacted with fatty acids. They impart desirable properties such as chemical and water resistance, exterior durability, and rapid drying to coating materials. These resins appear in such a large variety of chemical compositions that significant chemical analysis has been considered improbable. At the present time, tests for these resins are practically limited to qualitative identification and measurement of free phenol. No quantitative measurement of phenolic resins has been proposed, and no completely satisfactory qualitative test is available.

The most widely used qualitative test, the indophenol test of Gibbs (*2*), is very sensitive but indicates free phenols only. Most phenolic resins contain some free phenol and give a positive test with Gibbs reagent, although a few are completely free of uncombined phenol. The recent extensive use of free phenols such as *p-tert*-amylphenol as antiskinning agents in enamels has caused considerable difficulty in distinguishing qualitatively between agents of this type and phenolic resin modifications. To prevent such interference, the test is conducted on the dried vehicle film. Because the resulting film is insoluble, it is pyrolytically decomposed and the fumes are dissolved in water. The qualitative test with Gibbs reagent is then applied to this water solution.

Although this test appears to eliminate interference from the volatile antiskinning agents, it gives positive results with resins of nonphenolic origin or with resins not intended for the scope of the test. For example, polystyrene, epoxy resins, and coatings plasticized with tricresyl phosphate show positive results by this technique. As a result, this qualitative test is significant only when negative.

A variety of free phenols, cresols, xylenols, and aldehydes is used in making phenolic-type plastics, adhesives, and resins. Each phenolic raw material may appear in a variety of substituted forms, including many isomeric forms. There are so many potential different resins of this type that significant chemical analysis may well be considered impossible; however, oil-soluble phenol condensate resins for use in paints and varnishes are obtained from alkyl or aryl derivatives of phenol, the former possessing three or more carbon atoms. *p-tert*-Amylphenol, *p-tert*-butylphenol, and *p*-phenylphenol are widely used; resins made from the latter excel in desirable properties and have the distinction of responding to specific qualitative tests. Therefore, they can be distinguished from other phenolic resins and analyzed by the procedure described with a high degree of accuracy in any coating solution in which they are used.

The procedure is based on the nitrous acid test (*3*) for phenols. This test, usually conducted in aqueous medium for free phenols, produces an intense yellow color when applied to phenolic resins dissolved in a water-insoluble solvent such as butyl acetate or 1-hexanol. It is highly reproducible and specific as no other resin interferes. Epoxy resins, which use bisphenols as starting materials, do not develop color in this test.

PROCEDURE

Quantitative Determination. A small sample of resin, varnish, or enamel vehicle is carefully weighed, dissolved in *n*-butyl acetate and diluted to definite volume. For some heat-hardening resins which are not soluble 1-hexanol is substituted throughout the test, but butyl acetate is preferred for its cleaner and faster separations. From the nonvolatile composition of the sample, an aliquot (not exceeding 40 ml.) estimated to contain not more than 6 mg. of phenolic resin is transferred to a 250-ml. Erlenmeyer flask. Butyl acetate is then added to bring the total volume to 40 ml. Ten milliliters of 10 to 1 sulfuric acid (about 3.6*N*) is added, followed by 2 ml. of a freshly prepared 10% aqueous solution of sodium nitrite. All of these volumes may be approximate. The flask is covered with a vented stopper and placed in a water bath at 70° C. for 1 hour. Gentle agitation is applied several times during this period. The sample is then cooled to room temperature and transferred to a separatory funnel with water. The lower aqueous layer is removed and the solvent layer washed twice with water using gentle agitation. The solvent layer is then filtered through paper dampened with solvent into a 50-ml. volumetric flask and diluted to volume. Colorimetric comparison is made at 425 mμ. In most colorimeters, water may be used in the blank cell. Graphs of known samples are prepared in like manner, developing color individually on each known fraction. The phenolic resin content of the sample aliquot can be computed as desired.

Qualitative Identification of *p*-Phenylphenol–Formaldehyde Resins. Because this colorimetric method can be used with high accuracy on resins of known phenolic origin, and because *p*-phenyl-phenolformaldehyde resins can be readily distinguished from other types, the specific qualitative tests are given in detail.

FERRIC CHLORIDE. To 1 ml. of the resin solution in a test tube is added 10 ml. of methyl isobutyl ketone, followed by 10

drops of ferric chloride reagent (2% solution in pyridine). Alcoholic potassium hydroxide is then added dropwise and the sample examined after each drop. If no green color forms by the addition of 10 drops of alkali, this resin is absent.

CONCENTRATED SULFURIC ACID. Dried films of *p*-phenylphenol–formaldehyde resins develop an intense green color when dissolved in cold concentrated sulfuric acid, but this may be obscured by dark colors formed if large quantities of other resinous materials are present.

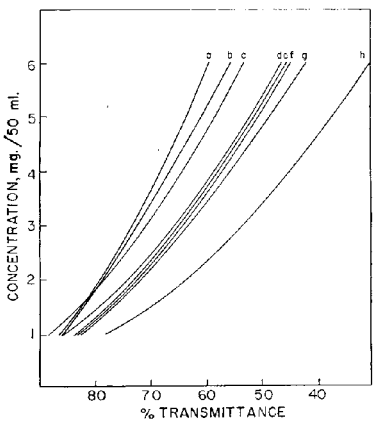

Figure 1. Color developed by phenol-formaldehyde resins

a, e. Heat-hardening
b, c, d, f, g. Non-heat-hardening
h. *p*-Phenylphenol–formaldehyde

MILLON'S REAGENT (*1*). The reagent is prepared by dissolving a small amount of mercury in an equal weight of concentrated nitric acid and diluting with an equal volume of water. When heated to boiling in the presence of *p*-phenylphenol–formaldehyde resin, or preferably the fumes from its pyrolysis, a purple color is formed. Other phenolic resins may form red colors that should not be confused.

RESULTS

To test the analytical possibilities of the procedure, color was developed and measured with a large variety of phenolic resins, mostly of unknown origin. Among this group were heat-hardening and non-heat-hardening resins, as well as heat-reactive and non-heat-reactive types. A variety of substituted phenols, many of which are used as raw materials for resin manufacture, was similarly tested. The color developed with resins is shown in Figure 1, and with phenols in Figure 2. The curves were obtained by plotting known weights (1 to 6 mg.) of each against the per cent transmittance at 425 mμ.

Because the origin of a few of the resins was known, comparison of the color produced by resins and their corresponding phenol was of particular interest. This comparison revealed that the color developed was similar but not identical, and each showed an individual spectrum in the visible wave-length region. At approximately 422.5 mμ each resin and its corresponding phenol absorbed identically. At longer wave lengths the color produced by resins absorbs more strongly, while at shorter wave lengths the phenols show stronger absorption. This is illustrated in the graph of Figure 3.

Most colorimeters do not provide for the use of the exact wave length of 422.5 mμ; some are graduated at 5-mμ intervals so that 420 or 425 mμ could be used. The 425-mμ wave length is available on practically all types of colorimetric instruments. The resins and phenols absorb closely at 425 mμ; therefore, this wave length was used throughout the investigation. Absorbances were measured on the Bausch & Lomb Spectronic 20 colorimeter with 0.5-inch cells. The choice of a wave length at which the resins and their corresponding phenols have similar absorbances is important to prevent error due to varying amounts of free phenols in the resins.

The usefulness of the method would appear at first to be limited to the analysis of resins of known origin or to resins that can be identified qualitatively—i.e., *p*-phenylphenol–formaldehyde. For high accuracy this is probably the case. But consideration of the relatively few phenols which produce oil-soluble resins for coatings and the relative similarity of color developed with the resins shown in Figure 1 indicates that the method appears to have possibilities for estimating resin content based on average color development of a variety of resins. One phenolic modified alkyd, stated by the manufacturer to contain 3.5 to 4.0% phenol modification, analyzed 3.9% by this method based on the average described.

Phenolic antiskinning agents produce color by this method, but these materials are used in paints in small quantities (0.2 to 0.6% on the nonvolatile basis) and their interference is not serious in quantitative estimations.

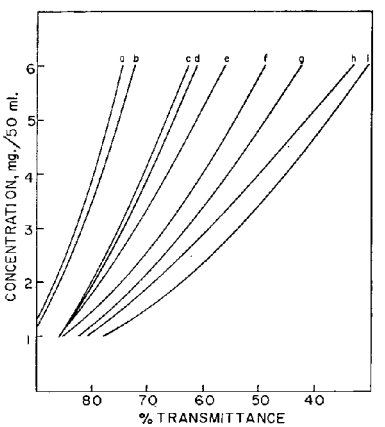

Figure 2. Color developed by some substituted phenols

a. *m*-Cresol
b. *o*-Cresol
c. *p-tert*-Amylphenol
d. *p-tert*-Butylphenol
e. Bisphenol A
f. *p*-Cresol
g. *tert*-Butylcresol
h. 2,4-Diamylpheno
i. *p*-Phenylphenol

Considerable interest and discussion have been centered around the chemistry of the reaction between oxidizing oils, such as tung or linseed, and *p*-phenylphenol–formaldehyde resins (*3, 5*). When oil-reactive phenolic resins are heated with drying oils which contain some conjugated unsaturation, there is elimination of water and an abnormal viscosity increase. It is generally believed that a form of diene synthesis takes place. It is also believed that the reaction is greatest with tung oil and less with linseed. When known varnishes of both types were prepared on a laboratory scale and analyzed for *p*-phenylphenol–formaldehyde, low results were obtained with both types of varnishes, but the residual phenolic resin content was much lower in the linseed varnish than in the tung oil varnish. The usual techniques, which involve higher reaction temperature for the linseed varnish, were used for the preparations. To demonstrate that

Table I. Analysis of Varnishes Made from Drying Oils and *p*-Phenylphenol–Formaldehyde Resins

Sample No.	Oil	Resin, % Present	Found
1	Tung	33.3	29.2
2	Tung	33.4	29.0
3	Linseed	32.8	22.5
4	Linseed (same conditions as for tung oil)	36.5	36.5
5	Paraffin oil (nonreactive)	33.3	34.0
6	Glyptal 2585 (phenol-modified alkyd)	3.5 to 4.0	3.9 (resin *c*, Figure 1)

heat degradation of the phenolic resin was not the reason for this difference, an inert oil such as paraffin oil was substituted for the drying oil and the resulting product analyzed for phenolic resin content, in which case 100% recovery was obtained. Some analytical results are shown in Table I.

Saponification has been known to cause reversal of some Diels-Alder type additions and products of diene synthesis, but saponification of the linseed varnish (sample 3, Table I), followed by solvent extraction and analysis for *p*-phenylphenol–formaldehyde resin, gave results identical to those shown.

The color developed with phenols by the action of nitrous acid is believed to be due to the formation of *p*-nitrosophenol, which condenses to form other complex colored compounds (*4*).

DISCUSSION

To analyze pigmented coatings for phenolic resin content, a clear pigment-free vehicle is necessary. Black and olive-drab coatings are not usually clean enough after ordinary centrifuging to permit satisfactory analysis. For these materials it is necessary to dilute about 50% with toluene and add 5 to 10 grams of dry powdered calcium hydroxide to each 100 ml. of paint before centrifuging. While this technique may affect some analytical results, such as acid number determinations it has not shown any undesirable effect on the phenolic resin analysis.

Prior to the development of the colorimetric method the only indication of the extent of phenolic modification of alkyd resins, for example, was from the determination of unsaponifiable content. This test is obviously affected by a number of other resinous modifications, and varies with different types of phenolic resins. Some of these saponify readily under ordinary saponification conditions while others are completely unsaponifiable even under drastic conditions.

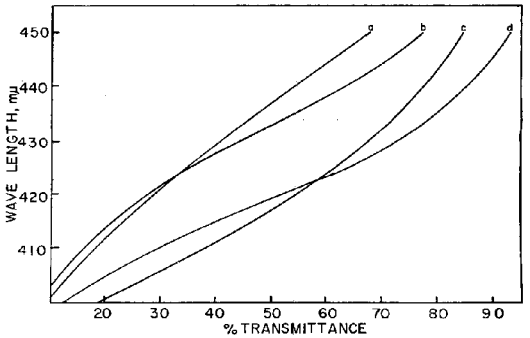

Figure 3. Spectra of substituted phenols and their formaldehyde condensates

a. *p*-Phenylphenol–formaldehyde resin
b. *p*-Phenylphenol
c. *p-tert*-Butylphenol–formaldehyde resin
d. *p-tert*-Butylphenol

To analyze materials of unknown phenolic resins origin such as phenol modified alkyds, it is recommended that the estimation be made by comparing the color developed by the unknown to a representative resin such as *c* of Figure 1. In choosing this resin it should be noted that curve *h* for *p*-phenylphenol–formaldehyde resin is not considered because this resin produces more color than any of the others and can be identified as such when present. The phenol-modified alkyd shown in Table I was the only resin solution of this type available with known composition.

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Radioassay of Tagged Sulfate Impurity in Cellulose Nitrate

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Two methods of sample preparation and counting are discussed for the application of radiotracer techniques, using radiosulfur, to the quantitative assay of sulfate impurity in samples of cellulose nitrate. One method entails the preparation of uniform films and the determination of the counting rate with a windowless flow counter. The other method involves solution of a fixed weight of cellulose nitrate in a liquid phosphor medium and measurement of beta activity with a coincidence liquid scintillation counter.

IN THE course of a study on the purification and stabilization of cellulose nitrate, it was necessary to analyze a large number of samples for total sulfate content. The sulfate, considered an undesirable impurity, is imparted to the nitrated polymer from the sulfuric acid used in the mixed nitrating acid to esterify the cellulose; it is believed to exist as both strongly occluded free acid and chemically combined sulfate ester.

The usual methods for total sulfate determination in cellulose nitrate and other cellulose esters have been surveyed by Hoffpauir and Guthrie (*1*). Essentially all of these techniques involve the wet combustion or decomposition of samples in a strong oxidizing or hydrolytic medium and subsequent gravimetric determination of sulfate as the barium salt. Hoffpauir and Guthrie concluded that "when the sulfate content of cellulose nitrate and other esters is very low, these methods require the use of large samples to provide sufficient amounts of the barium sulfate precipitate for convenient manipulation and the decomposition becomes lengthy and tedious."

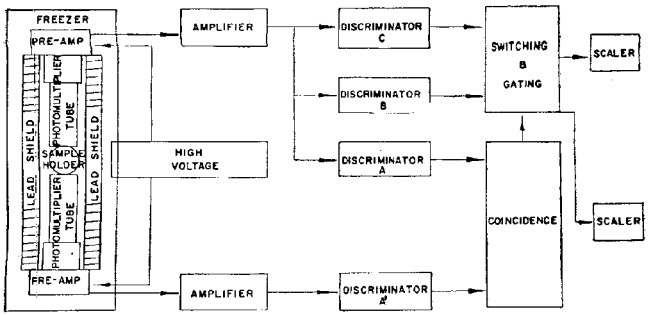

Figure 1. Simplified diagram of liquid scintillation spectrometer

In the same paper these authors reported a method whereby the residue from a nitric-perchloric acid wet oxidation is treated with barium chromate, precipitating barium sulfate and leaving an equivalent amount of free chromic acid in solution. The chromic acid is then determined iodometrically and the results are calculated as sulfate.

However, even with this improved volumetric modification, samples of lower sulfate content require proportionately larger sample weights and greater volumes of oxidizing acid; the method is thus still tedious and time-consuming. In addition, Hoffpauir and Guthrie reported that, when the sulfate content is of the order of a few hundredths of 1%, their method is not reliable. Experience in this laboratory has confirmed this, in that reproducible results could not be obtained by the same method with cellulose nitrate samples of less than 0.05% sulfate content.

Because this particular research application required determination of sulfate content of the order of 0.005% in cellulose nitrate samples, a method other than one involving wet oxidation had to be devised. Recent adaptations of radiotracer techniques to problems in analytical chemistry suggested an application of radioactive sulfur for this assay. Sulfuric acid tagged with sulfur-35 is conveniently available in high specific activity from the Oak Ridge National Laboratory. If a quantity of this tagged acid is added to the sulfuric acid in the mixed nitrating acid, any sulfate in the cellulose nitrate product will contain the same relative proportion of radioactive sulfur. The sulfate content of the product, initially or after any subsequent treatment, can then be determined by measuring the intensity of the beta emission (E = 0.167 m.e.v.). To eliminate the necessity for absolute beta counting and to avoid corrections for radioactive decay of sulfur-35 (half-life, 87.1 days) the activity of an unknown sample can be compared to a reference standard of known specific activity derived from the same tagged sulfuric acid used in the nitration.

Two methods are described here for the quantitative determination of sulfate in cellulose nitrate with sulfur-35 as a tracer. One involves the measurement of sulfur-35 activity in an infinitely thick film of fixed area using a windowless gas flow proportional counter; the other consists of dissolving a fixed sample weight of cellulose nitrate in a liquid phosphor solution and determining the activity with a liquid scintillation counter.

INSTRUMENTATION

A windowless gas flow counter (Model SC-16, Tracerlab, Inc., Boston, Mass.) was used for the radioassay of the cellulose nitrate films. The signals from the counter were fed into a pulse amplifier (Model SC-15, Tracerlab, Inc.) and registered on a conventional decade scaler. Helium–2-methylpropane (96 to 4 by volume) was used as the proportional counting gas.

The radioactive solutions were counted with a Tri-Carb liquid scintillation spectrometer (Model 314, Packard Instrument Co., La Grange, Ill.). This model is one of the several commercial instruments recently developed for the radioassay of low-energy beta emitters in solution. Figure 1 shows a simplified block diagram of the electronic circuitry characteristic of this type of equipment. Light produced by the combination of radioactive material and organic liquid phosphor is viewed by two photomultiplier tubes arranged in coincidence in a lighttight, low-temperature environment. The coincidence arrangement allows a registered pulse only when both tubes view a light-producing event simultaneously. Low temperature reduces thermionic events occurring in the photomultiplier tubes. The concerted action of the coincidence arrangement and low temperature reduces registered pulses other than those due to radioactive decay; as a result, background readings are kept low. The discriminator and gating sections of the circuitry are designed to sort pulses according to their heights; these sorted pulses are fed to scalers which yield integrated counts that can be correlated with amounts of radioactive material.

EXPERIMENTAL

Film Method. Preparation of Cellulose Nitrate Containing Sulfur-35. The cotton linters used for the preparation were supplied by the Hercules Powder Co., Wilmington, Del., and were of military nitration grade. The mixed acid used for the preparations was composed of sulfuric acid, nitric acid, and water, 66%, 21.6%, and 12.4%, respectively. In a typical nitration, 20 grams of cotton linters, dried in an oven at 100° C. for 2 hours, were added rapidly to 800 grams of the mixed acid containing 20 mc. of sulfur-35 as $H_2S^{35}O_4$. The temperature of the mixed acid at the start of the nitration was 34° C. Nitration was continued for 24 minutes with occasional mixing. The cellulose nitrate was then filtered from the spent acid for 3 minutes prior to drowning in 3 liters of ice water. After 20 minutes of standing with occasional stirring the nitrocellulose was filtered. The washing and filtering were repeated with 3 liters of distilled water and the product was finally washed three times on a Büchner funnel. In this manner the crude material was washed essentially free of surface acids.

Determination of Infinite Film Thickness. Varying weights of dry cellulose nitrate of the same specific activity were dissolved in a fixed volume of redistilled acetone and cast as smooth, flat films on a glass plate in the manner described below. This yielded films of different thickness measured as milligrams per square centimeter. Circular disks of identical area were prepared from these films and counted in the windowless gas flow counter. Figure 2 shows that the infinite thickness of cellulose nitrate films containing sulfur-35 is approximately 15 mg. per sq. cm. This value is in agreement with that found by Libby (*2*) for barium sulfate layers tagged with sulfur-35.

Preparation of Films of Unknown Activity. A 750-mg. sample containing an unknown amount of radioactive sulfate impurity was dissolved in 23 ml. of redistilled acetone. The resulting viscous solution was then poured into a fixed collimated area made by placing a glass cylinder (5.5 cm. in inside diameter and 11 cm. high) on a leveled, polished glass plate. The viscosity of the solution was such as to prevent any loss through the surface of contact between the glass plate and the cylinder. The height of the tube reduced the rate of solvent evaporation; smooth, flat films resulted. The solvent was allowed to evaporate into the atmosphere; approximately 18 hours was required to obtain dry films. From the dried films so prepared, two circular disks, each having an area of 4.92 sq. cm., were cut from a prepared die and mounted concentrically, with a polyisobutylene adhesive and use of pressure, on a copper disk of the same area. Each film weighed approximately 100 mg., which is well above

the critical infinite thickness value. The samples were then ready for radioassay with the windowless flow counter. The beta activities were determined at 1750 volts with a discriminator setting of 1 mv. Each film was counted long enough to yield a probable error of 1% or less. The background counting rate of a "cold" film was 30 counts per minute.

Liquid Method. PREPARATION OF UNKNOWN SOLUTIONS. For each unknown radioactive sample, 454.5 mg. of the sample was transferred to a 100-ml. volumetric flask and dissolved in redistilled tetrahydrofuran. The solution was diluted to the mark at 20° C. with the solvent. This resulted in a concentration of 100 mg. of nitrocellulose per 22 ml. of solution. Duplicate samples from this solution were then prepared for counting as follows. A 22-ml. portion was measured into a borosilicate weighing bottle (70-ml. capacity) and to it were added 6 ml. of absolute alcohol and 36 ml. of liquid phosphor consisting of a toluene solution containing 4 grams of 2,5-diphenyloxazole per liter. One milliliter of distilled water was also added to each unknown sample preparation, because the reference standards for these determinations (described later) contained 1 ml. of aqueous radioactive sulfuric acid.

SAMPLE COUNTING PROCEDURE. Samples containing the radioactive material in phosphor solution were placed in the sample holder between the photomultiplier tubes of the liquid scintillation counter. The sample bottle was optically coupled to the phototube windows by a special low viscosity fluid (Dow-Corning 200; viscosity = 1 cs.). The lead shield surrounding the phototubes and sample system was housed in a deep-freeze unit maintained at 20° F. The samples were exposed to the dark-adapted phototubes by manipulating the special shutter arrangement on the instrument. All samples were counted with 1050 volts applied to the photomultiplier tubes and the discriminators set between 10 and 100 volts. The background of a blank scintillator solution was 460 c.p.m. at these settings. Each sample was counted for a time sufficient to yield a probable error of less than 1% in the total number of counts.

RADIOACTIVE REFERENCE STANDARDS

Because it was not possible to prepare a synthetic cellulose nitrate film containing a known amount of radioactive sulfate, the activities of unknown samples within a series (where the ratio of sulfur-35 to sulfur-32 is constant) were compared with the activity of a reference film whose sulfate content had been determined by a wet oxidation method. A correlation was thus obtained between sulfate content and radioactivity; the sulfate content of any subsequent sample derived from the same nitration was readily calculated by a comparison of beta activities.

The reference sample was taken from a cellulose nitrate preparation directly after nitration, when the total sulfate content is of the order of 1%. This relatively high concentration of sulfate can be determined accurately by a wet oxidation method. In addition, two films of this reference sample were prepared for windowless flow gas counting in the same manner as the unknown. For example, using the same procedure as described by Hoffpauir and Guthrie, four 125- to 150-mg. samples of the same cellulose nitrate yielded the following values for per cent sulfate: 0.907, 0.881, 0.877, 0.888; average 0.888. Duplicate films of this same material gave activities of 7595 and 7578 c.p.m.

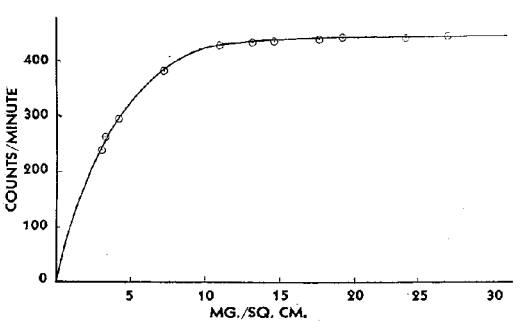

Figure 2. Determination of infinite thickness of sulfur-35-tagged cellulose nitrate films

The preparation of reference standards for the liquid phosphor samples presented less of a problem. Here it was possible to prepare solutions of known sulfate content using aqueous radioactive sulfuric acid.

This was accomplished by removing a small aliquot of the concentrated radioactive sulfuric acid before it was mixed with nitric acid. This aliquot was diluted to approximately 0.2*N* with distilled water and the normality accurately determined by titration with standardized 0.01*N* sodium hydroxide. From this solution, a stock reference solution was prepared containing 1 mg. of radioactive sulfate per ml. For the reference counting sample, 1 ml. of the stock solution was added to a system containing 100 mg. of "cold" cellulose nitrate dissolved in 22 ml. of tetrahydrofuran, 6 ml. of absolute alcohol, and 36 ml. of a toluene solution containing 4 grams of 2,5-diphenyloxazole per liter. This sample served as the reference liquid phosphor standard for the determination of sulfate content of unknown solutions containing cellulose nitrate derived from the same sulfuric acid used in the mixed nitrating acid.

To test the reliability of the preparation of these solution reference standards, as well as the precision of the liquid phosphor counting technique in general, a series of 10 samples containing known added amounts of radioactive sulfuric acid was prepared and their counting rates were determined with the liquid scintillation counter. The results obtained are listed in Table I. From the counting rate obtained with each sample the specific activity was calculated as counts per minute/per cent sulfate; the average value of all 10 samples was then used to calculate the sulfate content for each.

Table I. Liquid Scintillation Radioassays of Cellulose Nitrate Samples of Known Sulfate Content

Sample No.	Sulfate Added, %	Net Counts per Minute	Specific Activity $\times 10^{-4}$ [a]	Sulfate Found, %
1	1.000	103836	1.038	1.007
2	0.800	83656	1.046	0.811
3	0.600	61376	1.023	0.595
4	0.400	40816	1.020	0.396
5	0.200	21116	1.056	0.205
6	0.100	10346	1.035	0.100
7	0.080	8316	1.040	0.081
8	0.060	6236	1.039	0.060
9	0.040	4053	1.013	0.039
10	0.020	2000	1.000	0.019
			Av. 1.031	

[a] Counts per minute/% sulfate.

Table II. Sulfate Determinations in Cellulose Nitrate by Liquid Scintillation and Windowless Flow Counter Methods

Sample No.	Net Counts per Minute		Sulfate, %	
	Liquid scintillation	Windowless flow	Liquid scintillation[a]	Windowless flow[b]
1	72,120	7595	0.877	0.898
	72,280	7578	0.879	0.896
2	43,259	4457	0.522	0.527
	43,209	4474	0.522	0.529
3	35,154	3527	0.427	0.417
	34,624	3543	0.423	0.419
4	28,684	2859	0.349	0.338
	28,964	2893	0.352	0.342
5	27,190	2850	0.331	0.337
	26,910	2852	0.327	0.337
6	4,682	499	0.057	0.059
	4,846	496	0.059	0.059
7	3,285	355	0.040	0.042
	3,204	338	0.039	0.040
8	345	31	0.0042	0.0037
	353	30	0.0043	0.0036
9	255	29	0.0031	0.0034
	263	30	0.0032	0.0035

[a] Based on reference solution of specific activity 82,240 c.p.m./% sulfate.
[b] Based on reference film of specific activity 8458 c.p.m./% sulfate.

Table III. Radiotracer and Wet Oxidation Assays of Sulfate Content in Cellulose Nitrate

Sample No.	Sulfate, % Liquid scintillation	Windowless flow	Wet oxidation
1	0.308	0.323	0.316
	0.308	0.321	0.320
2	0.173	0.190	0.184
	0.174	0.186	0.182
3	0.154	0.150	0.149
	0.154	0.150	0.145
4	0.084	0.095	0.084
	0.086	0.095	0.090
5	0.080	0.078	0.074
	0.082	0.079	0.079

RESULTS WITH UNKNOWN SAMPLES

In the course of the study on the purification of cellulose nitrate, a batch of freshly nitrated cellulose (derived from the same sulfur-35–tagged sulfuric acid) was treated by various chemical methods to reduce the sulfate content to trace quantities. Samples were taken at various stages of the treatment and counted by both the liquid scintillation and windowless gas flow counter methods. The results for duplicate samples are listed in Table II showing agreement between the found sulfate content obtained by each method. The data for the last two samples demonstrate that, for the specific activity of the sulfuric acid used in these experiments, the film counting method is not sensitive enough below 0.004% sulfate content. A comparison of the counting rates obtained also shows that the liquid scintillation technique is ten times more sensitive than the film preparation method. The sensitivity of each method can, of course, be increased by using a nitrating acid having a higher specific activity for sulfur-35.

Table III lists duplicate analyses for five unknown samples obtained by each radiotracer method as well as by the Hoffpauir and Guthrie wet oxidation procedure. In this series each sample is derived from a different nitrating acid.

CONCLUSIONS

The results reported here show that radiotracer methods can be conveniently applied as a research analytical tool for the determination of sulfate impurity in samples of cellulose nitrate. Accuracies obtained are comparable to those obtained with a conventional wet oxidation procedure in the range where this latter method is applicable—i.e., above 0.05% sulfate content. Below this value the sensitivity of the radiotracer methods is practically limitless, depending only on the specific activity of the radiosulfur used.

Of the two techniques discussed, the liquid scintillation counting method is preferred over the film preparation method because of (1) the greater sensitivity obtained for a particular specific activity of radiosulfur in nitrocellulose, (2) the greater ease and speed of sample preparation, and (3) the greater convenience involved in the preparation of a reference standard.

ACKNOWLEDGMENT

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Determination of Phenolic Groups in Lignin Preparations

Titration with Potassium Methoxide Using Dimethylformamide as a Solvent

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Recent interest in lignin chemistry has led to refinement of the analytical methods available for characterization of the functional groups in this complex entity. The phenolic hydroxyl content of lignin preparations is of importance in this regard, and in the present paper a rapid and precise method is given for this determination. The method is based on titration with potassium methoxide in benzene-methanol using dimethylformamide as a solvent. Using an antimony-calomel electrode system it is possible to differentiate between phenols (or enols) and more strongly acidic groups. A number of model compounds were studied in the present work, and results were obtained for six lignin preparations.

ISOLATED lignins are known to contain free hydroxyl groups, some of which show weakly acidic reactions characteristic of phenols and enols. Brauns (*1*) has discussed the determination of hydroxyl groups in lignin preparations via acetylation, methylation, and esterification with *p*-toluenesulfonic acid. Bromination and coupling reactions have also been used for this determination (*7*).

Goldschmid (*6*) determined the phenolic content of various lignin preparations by spectrophotometric measurement of the absorbance of neutral and basic lignin solutions; the bathochromic shift of the 280-mμ absorption peak was attributed to the ionization of phenolic hydroxyl groups. The results obtained by this method do not include phenolic groups conjugated with carboxyl groups, or side-chain enolic groups conjugated with the aromatic nucleus. Eugenol and conidendrin were used as standards of reference.

In a recent paper Sarkanen and Schuerch (*8*) described the conductometric titration of isolated lignins in an aqueous mixture containing methanol and acetone, using lithium hydroxide as the titrant. Their results are at least minimum values for the amount of weakly acidic functional groups in isolated lignins.

Fritz and Keen (*5*) developed a method for determining substituted phenols by titration in dimethylformamide (DMF). These authors found that phenols with electrophilic groups in the

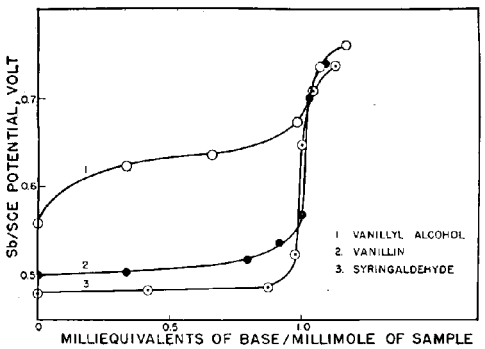


Figure 1. Titration of substituted phenols with potassium methoxide

ortho and para positions could be titrated in dimethylformamide using potassium methoxide in benzene-methanol as the titrant. Azo violet was recommended as a visual indicator, although the titration could be followed potentiometrically.

According to Fritz (*4*), such electron-withdrawing groups as —CHO, —CH_2—CH=CH_2, —CH=CH—CH_2, —C(=O)—R, —C(=O)—OR, —CH=CH—C(=O)—R, and others in the ortho or para positions increase the acid strength of phenols to such an extent as to enable titration in dimethylformamide. Ortho or para halogenation enhances the acidity of phenols, but carboxyl groups do not exhibit an activating effect. In the case of halogen-substituted phenols the increased acidity is due to inductive effects rather than resonance stabilization of the phenolate ion, which is the major consideration in the case of aldehyde, keto, and ester groups. Substitution in the meta position has a greatly decreased effect on the acidity of phenols, as would be predicted from a study of the possible resonance configurations.

Certain enols and imides which have adjacent or conjugated electron-withdrawing groups can also be titrated with potassium methoxide in dimethylformamide. Carboxyl groups react with potassium methoxide under the conditions of the titration, and it is often possible to determine carboxyl and phenolic groups by a single titration, owing to the difference in pK_a values. Some dihydric phenols can be titrated as dibasic acids, whereas a single break in the potentiometric titration curve is observed with other dibasic compounds. Simple phenols are not titrated with potassium methoxide in dimethylformamide.

Titration in dimethylformamide seemed to lend itself to the determination of the acidic groups in lignin preparations and wood extractives. Dimethylformamide is an excellent solvent for many organic compounds, and samples of isolated "native lignins" (or Braun's lignin) and commercial products are readily soluble. And, as discussed above, the nature of the solvent is such as to enable differentiation between substituted phenolic compounds and less acidic hydroxyl-containing materials.

EXPERIMENTAL

Reagents and Materials. Potassium methoxide solution, 0.05*M*. Mix 20 ml. of absolute methanol and 50 ml. of dry benzene in a 1-liter Erlenmeyer flask, and cautiously add 2 grams of freshly cut potassium metal in small increments. When the reaction subsides, add 55 ml. of methanol and dilute the solution to 1 liter with benzene. Store the solution in a glass-stoppered bottle and standardize against pure benzoic acid prior to use.

Dimethylformamide, Du Pont technical grade.

Azo violet indicator solution. Prepare a saturated solution of 4-(*p*-nitrophenylazo)resorcinol in benzene.

Model compounds. These were obtained in purified form, either from commercial sources or in the form of research samples prepared at The Institute of Paper Chemistry by I. A. Pearl and his associates.

Lignin preparations. Samples of various "native lignin" preparations were procured from F. E. Brauns. Indulin was obtained from the West Virginia Pulp and Paper Co. and Meadol from the Mead Corp., Chillicothe, Ohio.

Apparatus. A Beckman Model G pH meter was used as a potentiometer in this work. An antimony-saturated calomel electrode system was used. The calomel was a Beckman No. 1170 and the antimony electrode was prepared by drawing molten antimony up into a length of 5-mm. glass tubing and chipping away the glass when the melt had cooled. Contact with the antimony was effected by means of a silver wire.

Because of solvent attack on stopcock lubricants, an Ultramax buret (The Emil Greiner Co.) was used for all titrations. A needle valve buret would serve as well.

Titrations were performed in a 40 × 120 mm. tall-form weighing bottle fitted with a three-hole rubber stopper.

Stirring was provided by a magnetic stirrer and a glass-sealed stirring bar. The stirrer was grounded to minimize spurious potential readings.

Procedure. Pipet 15 ml. of dimethylformamide into a clean, dry weighing bottle, add several drops of indicator solution, and position the stopper, electrodes, and buret. Start the magnetic stirrer and neutralize the acidic impurities in the dimethylformamide by adding potassium methoxide solution until the indicator changes from red to blue.

Accurately weigh a sample, previously dried at 60° C. in vacuo, of such size as to contain 1 to 3 meq. of replaceable hydrogen, and transfer the sample to the titration vessel. Titrate the sample with 0.05*M* potassium methoxide solution and follow the titration potentiometrically.

An alternative procedure is to add 15 ml. of dimethylformamide to a titration vessel containing the weighed sample, titrate, and then correct the volume of titrant for the acidity of the solvent as measured in a blank determination.

The calomel electrode should bleed potassium chloride through the fiber capillary when functioning properly. Plugging of the capillary leads to insensitive electrode behavior. Grinding of the electrode tip with fine emery paper will alleviate this difficulty.

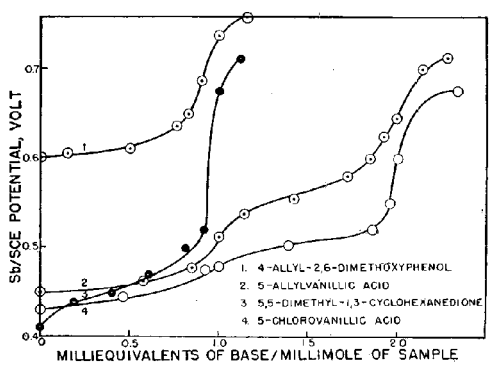


Figure 2. Titration of phenols, enols, and acids with potassium methoxide

Exposure to carbon dioxide should be avoided during the titration.

A gelatinous precipitate was observed during the titration of certain of the lignin preparations, but this did not seem to affect the sensitivity of the titration or the precision.

DISCUSSION OF RESULTS

Model Compounds. The titration curves for a number of model compounds with structures similar to skeletal portions of lignin are shown in Figures 1 to 5. In Figure 1 are shown the titration curves of vanillyl alcohol, vanillin, and syringaldehyde. The curves for vanillin and syringaldehyde are very similar,

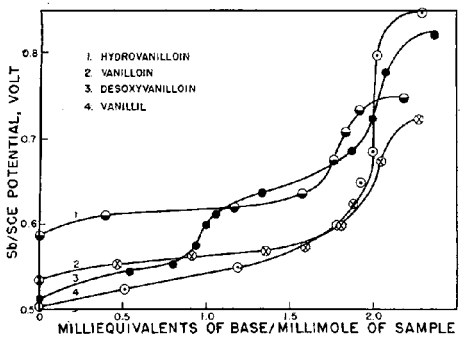


Figure 3. Titration of bisvanillyl derivatives with potassium methoxide

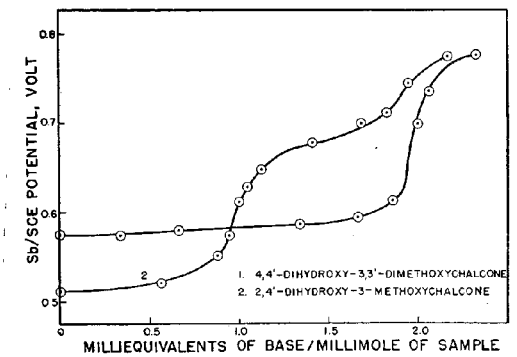


Figure 4. Titration of chalcone derivatives with potassium methoxide

first. 4,4'-Dihydroxy-3,3'-dimethoxychalcone shows only one break in the curve, and this at two equivalents.

Vanillalacetone, ethyl vanillate, eugenol, and isoeugenol gave fairly straightforward titration curves, as are shown in Figure 5. Vanillalacetone is the vinylog of 3-methoxy-4-hydroxyacetophenone, which contains an activating acetyl group. Ethyl vanillate behaves as a fairly strong acid, at least in comparison to the other compounds tested. The comparison between the acid strengths of eugenol and isoeugenol is interesting. Both are weakly acidic, but the propenyl group in isoeugenol appears to activate the phenolic group to a greater extent than does the allyl group. Actually, resonance stabilization of the phenolate anion facilitates loss of a proton in the case of isoeugenol.

A list of the structures of the various model compounds studied is given in Figure 6.

Lignin Preparations. The determination of the acidic content of lignin preparations by titration with potassium methoxide in dimethylformamide gave results which compared well with those

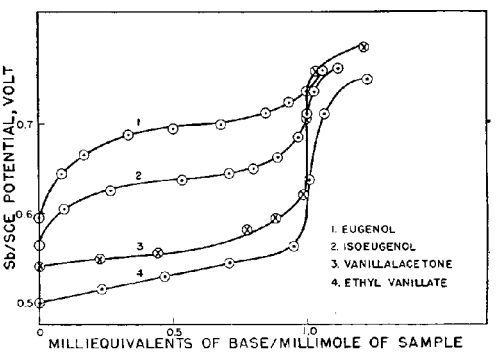


Figure 5. Titration of substituted phenols with potassium methoxide

and apparently the introduction of a second methoxyl group has little effect on the acidity of the phenol.

5,5-Dimethyl-1,3-cyclohexanedione behaves as a surprisingly strong acid, as shown in Figure 2, and this behavior can be explained on the basis of enolization. 5-Allylvanillic acid shows the typical titration curve for a dibasic acid and the phenolic and carboxylic groups can be readily differentiated. In the case of 5-chlorovanillic acid, however, only one break in the titration curve was observed. Here the inductive effect of the chlorine atom ortho to the hydroxyl group is such as to increase the acidity of the phenol to the point where it is indistinguishable from the acid entity.

The titration curves for four bisvanillyl derivatives are shown in Figure 3. As would be expected, hydrovanilloin is the most weakly acidic of the series, and both phenolic hydroxyl groups are titrated simultaneously. Vanilloin exhibits the same type of titration curve. Deoxyvanilloin behaves as a dibasic acid, and two sharp breaks are seen in the titration curve. Here the first break is probably attributable to removal of a proton from the enol and the second inflection to formation of a phenolate anion; the second phenolic group does not give an inflection in the curve. The curve for vanillyl shows a sharp break after the addition of two equivalents of base; the acidity of both phenols is enhanced by carbonyl substituents in the para position.

In Figure 4 are shown the titration curves for two chalcone derivatives. The 2,4-dihydroxy-3-methoxy compound behaves as a dibasic acid, with pK_{a_1} considerably smaller than pK_{a_2}. The second equivalence point is less sharply defined than the

obtained by other methods. Titration in dimethylformamide does not necessarily differentiate among carboxyl, enol, imido, or substituted phenol groups. However, in the case of native lignins, carboxyl groups are entirely absent (*2*) and the occurrence of imido groups is unlikely. Therefore, under the conditions of the determination one would expect to titrate only enols and substituted phenols.

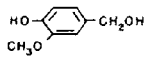
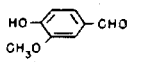
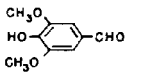
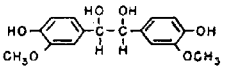
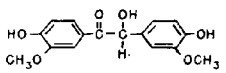
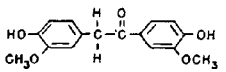
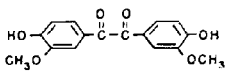
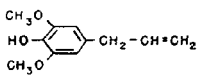
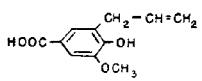
Representative titration curves for several lignin preparations are shown in Figures 7 and 8. A summary of the results obtained is given in Table I.

Shown in Figure 7 are the titration curves for four native lignins. Isolated spruce and aspen lignins were found to contain about 2.4 meq. of replaceable hydrogen atoms, presumably phenolic or enolic, per gram, while hemlock native lignin contained approximately 3.7 meq. per gram of such groups. The value obtained for *Eucalyptus regnans* was somewhat lower than the others.

Methylated, acetylated, and toluenesulfonated spruce native lignins were titrated and found to exhibit no acidic characteristics in dimethylformamide. This behavior was anticipated, as the reactive hydroxyl groups are no longer available for neutralization. A titration curve typical of these materials is given in Figure 7; this curve parallels the blank almost exactly.

Titrations of spruce native lignin were equally successful using dimethylsulfoxide instead of dimethylformamide as the solvent; the resultant curves were not significantly different from those obtained in dimethylformamide.

Two commercial lignin preparations were titrated in the course

COMPOUND	STRUCTURE
VANILLYL ALCOHOL	
VANILLIN	
SYRINGALDEHYDE	
HYDROVANILLOIN	
VANILLOIN	
DESOXYVANILLOIN	
VANILLIL	
4-ALLYL-2,6-DIMETHOXYPHENOL	
5-ALLYLVANILLIC ACID	

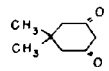
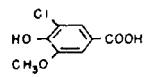
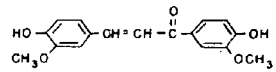
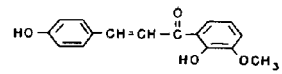
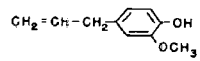
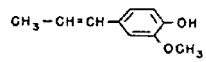
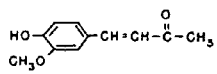
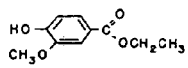
COMPOUND	STRUCTURE
5,5-DIMETHYL-1,3-CYCLOHEXANEDIONE	
5-CHLOROVANILLIC ACID	
4,4'-DIHYDROXY-3,3'-DIMETHOXYCHALCONE	
2,4'-DIHYDROXY-3-METHOXYCHALCONE	
EUGENOL	
ISOEUGENOL	
VANILLALACETONE	
ETHYL VANILLATE	

Figure 6. Structure of model compounds

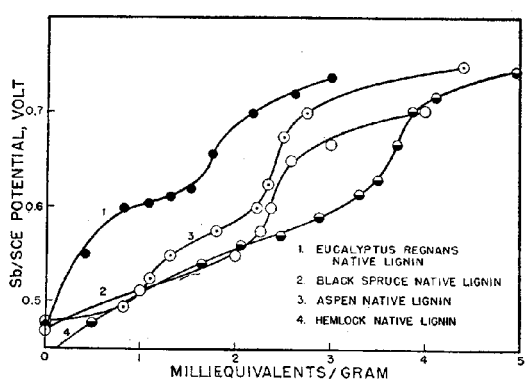


Figure 7. Titration of isolated "native lignins" with potassium methoxide

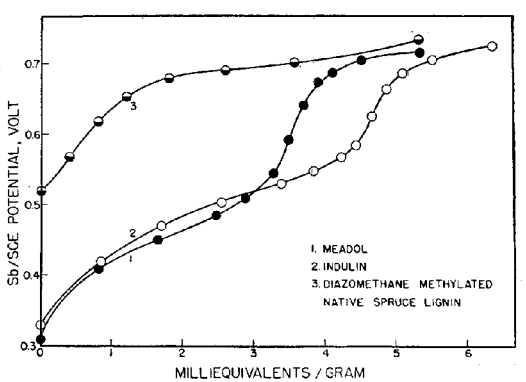


Figure 8. Titration of lignin preparations with potassium methoxide

Table I. Determination of Acidic Hydroxyl Groups in Lignin Preparations

Sample	OH, %	Meq. per Gram	No. of Determinations	Average Deviation, % $\left[\frac{\Sigma(\bar{x}-x)}{n\bar{x}} \times 100\right]$
Native lignins[a]				
Eucalpytus regnans	2.80	1.65		
Aspen				
First break	1.88	1.11		
Total	4.17	2.45	5	1.5
Black spruce	3.97	2.34	10	1.9
Hemlock	6.31	3.71	4	1.3
Commercial lignins				
Meadol[b]	5.94	3.49	6	1.8
Indulin[c]	7.91	4.65	5	0.5

[a] Prepared by F. E. Brauns.
[b] Hardwood soda lignin prepared commercially by the Mead Corp.
[c] Kraft lignin prepared commercially from pine wood by West Virginia Pulp and Paper Co.

of the present work. Meadol, an alkali hardwood lignin recovered from the spent liquor of the soda process, yielded an excellent titration curve, as shown in Figure 8, and 3.49 meq. of replaceable hydrogen atoms were found per gram of material. The titration curve for Indulin, a mixture of thio and alkali pine lignins, prepared by the Kraft process, is also seen in Figure 8; in this case there were 4.65 meq. of acidic groups per gram of material.

The speed and simplicity of this method for determining the acidic content of complex materials seem to offer a number of advantages over other available methods. The remarkable solvent properties of dimethylformamide make the method well suited to the analysis of isolated lignins, their degradation products, and wood extractives. Dimethylsulfoxide can be used as a solvent for materials that are difficultly soluble in dimethylformamide.

ACKNOWLEDGMENT

The authors are indebted to F. E. Brauns and I.-A. Pearl for supplying many of the samples used in this work, and to B. L. Browning for constructive criticism.

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Absolute Alpha Counting of Astatine-211

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A method has been developed for the absolute standardization of the α-emitter astatine-211. The isotope is dissolved in the liquid scintillator of a scintillating counter, thereby avoiding the usual difficulties due to absorption and scattering of the alpha particles. When a solution of 50 grams per liter of diphenyloxazole in *p*-dioxane was used, more than 1 ml. of water could be added before the efficiency of the counter was impaired.

THE absolute standardization of an α-emitting isotope is complicated by the short range of the α-particles emitted. The absorption that takes place in the source mounting, as well as the self-absorption in the source itself, is extremely difficult to determine, and can be done only by extrapolation technique (*4*), which is a rather complicated and lengthy procedure when compared to the ease with which $4\pi\ \beta$ counting is done. Chambers with a standard low geometry have indeterminable errors due to scattering, and this technique has been discarded, in the case of β-emitters, in favor of the 4π counter with 100% efficiency.

As reported by Basson and Steyn (*2*) these difficulties of absorption and scattering can be surmounted by dissolving the α-emitter in a liquid phosphor. This technique was further developed for the absolute counting of astatine-211.

All the isotopes of astatine (element 85), the heaviest of the halogen group, are radioactive. However, only astatine-211, which has a half life of 7.3 hours and emits α-particles, possesses nuclear properties that make it desirable for use either as a tracer or for studies of radiation effects. This isotope has been proved to be a valuable tool in thyroid physiology (*5*, *12*) and in studies of radiation damage, (*1*) and an increased interest is being shown by biological and medical researchers.

The disintegration scheme of astatine-211 is shown in Figure 1, reproduced from Mihelich, Schardt, and Segrè (*9*). It is clear that in every disintegration, one, and only one, α-particle—of at least 5.9-m.e.v. energy—is emitted. The only radiations of importance are the α-particles of 5.89 m.e.v. (40.9%) and 7.43 m.e.v. (59.1%) (*10*), with 80-k.e.v. x-rays accompanying the latter. The half life of 7.3 hours (*9*) was confirmed in these experiments.

Astatine-211 is produced by the reaction Bi^{209} (α, 2*n*) At^{211} with a threshold energy of 20 m.e.v. (*8*). The energy of the bombarding α-particles in the cyclotron should be kept below 29 m.e.v., as that is the threshold for the reaction Bi^{209} (α, 3*n*) At^{210}, producing the undesirable astatine-210, which decays by orbital electron capture to the radiotoxic polinium-210. The astatine can be easily separated from the bismuth target by simple volatilization techniques, if the separation does not need to be efficient (*11*).

APPARATUS

The simple equipment shown diagrammatically in Figure 2 was used. A cylindrical glass cell about 2.5 cm. in diameter and 2.5 cm. long, with a flat bottom, contained the liquid scintillator. Good optical coupling to the RCA 6342 photomultiplier (with a

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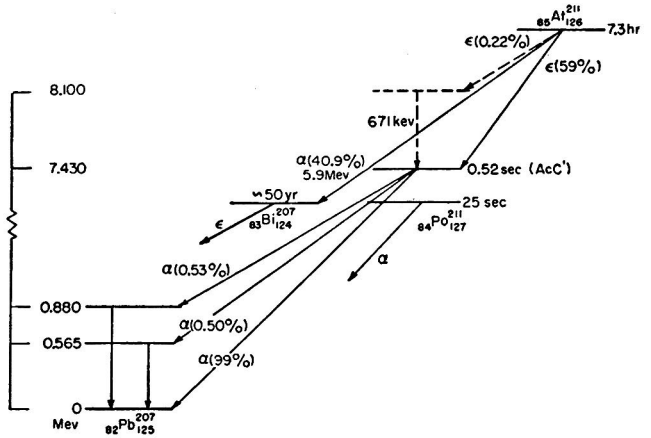

Figure 1. Disintegration scheme of astatine-211

flat cathode) was obtained with a thin layer of mineral oil. An aluminum-coated, semispherical glass reflector gave increased light collection efficiency. The whole system was enclosed in a light-tight metal tube with an easily removable top, and shielded with 1 inch of lead.

The photomultiplier tube was connected to a conventional scaling unit equipped with a nonoverloading amplifier with 5-microsecond resolving time. The gain of the amplifier could be switched to give three different input sensitivities of 0.3, 10, or 300 mv.

RESULTS

The first experiments were done with the commonly used liquid scintillator system of terphenyl dissolved in toluene. A two-thirds saturated solution was used for counting the astatine, which dissolves well in most organic solvents. With 6 ml. of this solution in the cell, the results plotted in Figure 3 were obtained. The astatine activity was arranged to give a suitably high counting rate for good statistical accuracy.

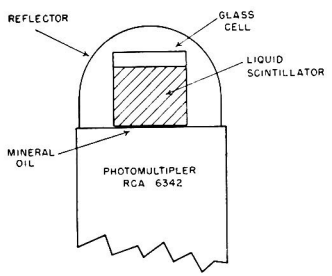

Figure 2. Diagrammatic sketch of apparatus

Coupling of liquid scintillator in glass cell to photocathode, with reflector

The plateau of counting rate *vs.* photomultiplier voltage, for constant amplifier input sensitivity of 10 mv., extended from 1150 to 1550 volts with a slope too small to measure. Above 1550 volts a rise in the counting rate took place due to the 80-k.e.v. x-radiation, and at 1600 volts the background of the photomultiplier tube became high and rather erratic. At 1550 volts the background (with scintillator) was about 250 counts per minute, decreasing to about 40 counts per minute at 1500 volts and less than 20 counts per minute below 1450 volts. No fluorescence effects were observed after the photomultiplier or scintillator had been exposed to light. This plateau could be repeated at a lower photomultiplier voltage with the 0.3-mv. input sensitivity. This was, however, considered to be unnecessarily prone to external noise.

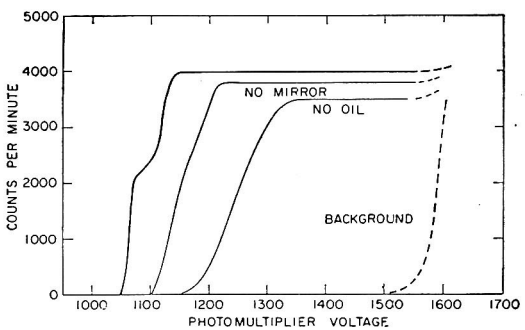

Figure 3. Counting rate of astatine-211 sample dissolved in liquid scintillator (terphenyl in toluene) for different voltages across photomultiplier tube

Effect of using no mirror, or no mirror and oil coupling, on plateau shown as well as background counting rate with no activity in liquid scintillator

The hump in the rise of the curve is due to counting of the 7.43-m.e.v. alphas before the 5.89-m.e.v. That this extremely good resolution is really obtained was substantiated by photographing a gray wedge spectrum (*3*) of the pulses (Figure 4). The two alpha groups as well as the 80-k.e.v. x-radiation are clearly resolved, and no pulses are apparent between the x-ray peak and the first alpha peak.

Figure 3 also demonstrates the effect on the plateau of the removal of the semispherical reflector and the coupling oil.

As astatine is usually worked with in aqueous solution, the author now tried to find a liquid scintillator which would give a good plateau even with an appreciable amount of water added. The only solvent miscible with water, that has been reported suitable for scintillation counting, is *p*-dioxane. The effect of varying the concentration of terphenyl dissolved in dioxane on the

plateau length obtained with astatine-211 was determined and it was observed that the plateau length is still increasing when the dioxane becomes saturated with terphenyl. When water is added to a terphenyl solution in dioxane, the resulting dioxane-water mixture cannot dissolve as much terphenyl as before and some of the terphenyl is precipitated—e.g., with a two-thirds saturated solution, 2 drops of water added to 6 ml. of solution in the cell is enough to cause some precipitation with resultant deterioration of the plateau.

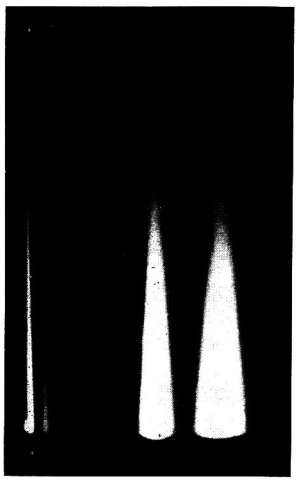

Figure 4. Gray wedge spectrum of astatine-211 dissolved in liquid scintillator (terphenyl in toluene)

Showing resolution of two alpha peaks, and absence of pulses between 80-k.e.v. x-ray and 5 .9-m.e.v. alpha peak

The compound, 2,5-diphenyloxazole, reported by Hayes, Hiebert, and Schuch (*6*) to be an efficient scintillator, is highly soluble in *p*-dioxane. The astatine plateau length was determined for different concentrations of this substance, and no decrease in plateau length was found until a concentration of less than 3% of saturation was reached, corresponding to about 15 grams per liter. This offers the possibility of adding a considerable amount of water to the liquid scintillator. Using a solution of 50 grams per liter, the effect of adding water on the plateau length was determined; up to 25% water could be added before the counter became unsuitable for accurate determinations. Changing the concentration of 2,5-diphenyloxazole from 20 to 200 grams per liter had very little effect on this result.

The addition of diphenylhexatriene as "spectrum shifter" (*7*), or using a mixture of solvents, such as phenylcyclohexane, did not result in any improvement.

The last experiment was repeated using a large cell, which covered the whole of the photomultiplier cathode and contained 50 ml. of 20 grams per liter solution. Although a reasonable plateau was obtained without any water, the length decreased rapidly with the addition of water and it was not found profitable to use a large cell for counting a greater volume of aqueous solution.

Determination of the specific activity of an astatine solution gave the same result regardless of the scintillation solution used if a reasonable plateau had been found to exist.

DISCUSSION

The decay of astatine-211 results in the emission of one α-particle per disintegration with an energy of 5.89 or 7.43 m.e.v. (Figure 1). The gray wedge spectrum (Figure 4) of the pulses obtained from the scintillation system used shows two clearly defined peaks—corresponding to the two α-particles—well separated from the peak corresponding to the 80-k.e.v. x-rays, and with no signs of any pulses of intermediary size being produced. It is therefore most likely that every α-particle emitted gives rise to a pulse within these two peaks. The counting rate obtained from the resulting plateau should, therefore, correspond to one count per disintegration—i.e., 100% efficiency.

The standardization was found to be consistent with that of a conventional counting chamber with 2π geometry. This has also been confirmed by chemical analysis in the case of natural uranium as α-emitter (*2*). The marked improvement in results is due to the smaller weight of the active material dissolved, which causes less quenching, as well as the improvements in photomultiplier tube and electronic circuitry.

ACKNOWLEDGMENT

The authors wish to thank W. L. Hughes for his help in the preparation of astatine and the interest shown in this work.

It is, furthermore, a pleasure to express sincere gratitude to J. S. Robertson and the Medical Physics Division for the cordial hospitality extended to the author during his stay at the Brookhaven National Laboratory. He is also indebted to the South African Council for Scientific and Industrial Research for enabling him to do this research.

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Determination of Microgram Quantities of Free Iodine Using *o*-Tolidine Reagent

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o-Tolidine in acid solution does not react with free iodine in the presence of iodide, but the addition of mercuric chloride to form a complex with the iodide ion carries the reaction shown by the equation $I_2 + H_2O \rightarrow H_2^+OI + I^-$ well over to the right-hand side. The hydrated iodinium ion reacts rapidly and completely with o-tolidine. This reaction permits the rapid and quantitative development of a yellow color in the presence of free iodine, which may be determined spectrophotometrically at a wave length of 425 mμ. The procedure is subject to interference from other oxidizing agents, with the exception of iodate, which does not react with o-tolidine under these conditions. The method is very sensitive; with 4-cm. absorption cells 0.1 p.p.m. of iodine may be determined with a sensitivity within ±5%.

TRACE quantities of free iodine in aqueous solution have, for a long time, been determined by measuring the blue color formed by the triiodide ion with starch. The method was improved by Lambert (*6*), who used a purified starch, which he termed a "linear starch reagent." Palin (*9*) used *p,p*-dimethylaminoaniline, which gives a blue color with iodine, but used the method to provide standards for chlorine estimations rather than to determine iodine, as iodine solutions of the order of 10^{-5} *M* are readily prepared and are much more stable than chlorine solutions of the same strength. Kramer, Moore, and Ballinger (*5*) found that tetramethylbenzidine gave good color development with a sensitivity of 0.05 p.p.m. in the range 0.05 to 0.2 p.p.m., and that fluorescein was unsatisfactory. Other workers have used the color of elementary iodine in organic solvents or as the triiodide ion in the presence of excess iodide. Sendroy and his coworkers (*10–12*) developed methods for the determination of the triiodide ion, both visually and using a filter photometer. More recently, Custer and Natelson (*3*) measured this ion by using its strong absorption in the near ultraviolet at 352 mμ, obtaining a high sensitivity. Kramer, Moore, and Ballinger (*5*) applied the amperometric titration method of Marks and Glass (*8*) for free chlorine to the determination of iodine, obtaining excellent results with an accuracy within ±0.01 in the range 0.2 to 2.0 p.p.m., when phenylarsene oxide was the titrating reagent.

Lange and Ward (*7*) used an alcoholic solution of *o*-tolidine as a neutral reagent, comparing the blue color formed with that from standards. They found a lower limit of 0.01 mg. in 15 ml. (0.66 p.p.m.).

As the customary reagent for free chlorine is an acid solution of *o*-tolidine (*1*), which gives a very intense yellow color in the presence of the former substance and is readily available, it was thought that it might be applied to the determination of free iodine.

An acid solution of *o*-tolidine reacts instantly with free chlorine or bromine, but a solution of iodine in potassium iodide did not react. A dilute solution of elementary iodine in distilled water, containing no added iodide, gave a slow development of color, addition of iodide reducing both the rate of color formation and the maximum intensity attained before fading commenced.

It was shown by Derbyshire and Waters (*4*) that iodination is due to the iodinium ion, which Bell and Gellis (*2*) have shown to exist in the hydrated form according to the equation

$$I_2 + H_2O \rightarrow H_2^+OI + I^- \qquad (1)$$

The above experiments suggest that the iodinium ion is the active entity in the reaction with *o*-tolidine, a quinone-iodoimine possibly being formed as a transition compound.

Consideration of the above facts suggested that the addition of a substance to remove iodide ions would facilitate Reaction 1 and hence the succeeding reactions. A mercuric salt seemed the best choice, as silver salts would form insoluble halides with a resultant turbidity. Mercuric chloride was found satisfactory, effecting a rapid and quantitative development of color. The amount of iodide present must not be such that the solubility product of mercuric iodide is exceeded.

PROCEDURE

To 25 ml. of test solution containing up to 3.0 p.p.m. of iodine and not more than 50 p.p.m. of iodide, add 1 drop of saturated mercuric chloride solution, immediately followed by 0.5 ml. of acid *o*-tolidine solution prepared according to directions of the American Public Health Association (*1*). Measure the absorbance at 425 mμ within 10 minutes. Standards may be prepared by diluting 0.1*N* iodine to 0.001*N*; 1.0 ml. of the latter diluted to 100 ml. will give a solution containing 1.27 p.p.m. Water of zero iodine demand should be used. The laboratory distilled water supply had an iodine demand of approximately 0.2 p.p.m. and before standard solutions could be prepared this demand had to be removed by adding 2 or 3 p.p.m. of iodine to a quantity of water and vigorously boiling off about a quarter of the volume.

Table I. Absorbance of Standard Iodine Solutions

10^{-4} *N* × Iodine Soln. Added, Ml./50 Ml.	Iodine, P.P.M.	Absorbance, 4-Cm. Cell	Mean Absorbance	Deviation from Mean
5.0	1.27	1.028		−0.008
		1.039		+0.003
		1.040	1.036	+0.004
2.0	0.508	0.404		+0.005
		0.398		−0.001
		0.394	0.399	−0.005
1.0	0.254	0.181		+0.004
		0.176		−0.001
		0.175	0.177	−0.002
0.50	0.127	0.074		0.0
		0.074		0.0
		0.074	0.074	0.0
0.25	0.064	0.024		0.0
		0.024		0.0
		0.024	0.024	0.0
0.10	0.025	0.008		0.0
		0.007		−0.001
		0.008	0.008	0.0
Nil (blank)	Nil	0.002		

The 0.1*N* iodine should be standardized by a suitable conventional method.

Table I shows the absorbance values obtained over the range 0 to 1.27 p.p.m. of iodine, three independent estimations being made at each concentration. At values below 0.1 p.p.m. the

relationship between concentration and absorbance is not linear, but above this value the relationship is linear. At all concentrations the results are highly reproducible.

INTERFERENCES

Oxidizing agents such as free and combined chlorine, bromine, ferric ion, or nitrites will react with the *o*-tolidine reagent. Iodate and bromate, however, do not react, as, in the presence of excess mercuric ion, the iodide-halate reaction cannot proceed.

The interferences due to some of the above substances may be dealt with as follows:

Chlorine or Bromine. To 25 ml. of test solution add 1.0 ml. of 1 to 4 hydrochloric acid, followed by 1.0 ml. of 1% freshly prepared aqueous phenol, and continue as directed in the procedure.

Chloramine. Follow the directions for chlorine or bromine, but add 0.5 ml. of 0.10% potassium bromide as well as the acid and phenol.

Ferric Iron in Trace Quantities. Add 20 to 25 mg. of sodium fluoride to 25 ml. of test solution.

Nitrite. To 25 ml. of test solution add 1.0 ml. of 1 to 4 hydrochloric acid, then approximately 20 to 25 mg. of sulfamic acid, mix, and continue as in the procedure.

This latter use of sulfamic acid destroys nitrite rapidly at trace level and has no effect on monochloramine. It appears to be the answer to the problem of nitrite interference in the determination of combined chlorine.

CONCLUSIONS

In the presence of mercuric chloride, iodine reacts rapidly and quantitatively with an acid solution of *o*-tolidine, providing a very sensitive method for determining free iodine.

The blue color of iodine with starch is due to an I_3^--starch complex and is bleached by mercuric salts, removing iodide from the system. Some semiquantitative experiments indicate the possibility of titrating iodide with mercuric salts, using, as an indicator, starch with a trace of elementary iodine.

A Photovolt filter photometer and later a Unicam S.P. 500 spectrophotometer were used in this work.

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Concentration of Microgram Amounts of Rare Earths in Thorium

Precipitation and Ion Exchange Procedures

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Two methods have been studied for the concentration of trace rare earths (<than 100 p.p.m.) present in relatively pure thorium. Lanthanum-140 and yttrium-90 were used to study the efficiency of separation. A precipitation procedure was devised in which more than 98% of the lanthanum present in 1.5 grams of thorium oxide is coprecipitated with less than 1% of the latter; however, this procedure was not effective for concentrating yttrium. An ion exchange procedure was also developed which was capable of concentrating both yttrium and lanthanum, and should be applicable to the concentration of rare earths up through terbium.

MANY methods have been proposed for the separation of thorium and the rare earths (*4–7*) but, in general, they are not applicable where the rare earths are present in microgram amounts.

Studies are described here of methods for concentrating small quantities of rare earths by procedures in which nitrilotriacetic acid and (ethylenedinitrilo)tetraacetic acid are employed to complex preferentially the major fraction of the thorium. The stability of the rare earth complexes (*13*) of these chelating agents increases with atomic number; thorium is more strongly complexed (*1*) than any of the rare earths. As the pH of a solution containing thorium, rare earths, and complexing agent is lowered, preferential dissociation of the complexes occurs. This dissociation is dependent on the magnitude of the stability constants, with the weaker complexes dissociating at a higher pH. As the pH is lowered, the lanthanum complex dissociates, then the cerium complex, then the other rare earths in order of increasing atomic number, with the very stable thorium complex being the last to dissociate. Thus, control of pH provides a means of keeping thorium in solution as a complex while the rare earths are present as uncomplexed ions.

When oxalate ions are added to a solution of thorium, a rare earth, and the complexing agent, it should be possible to utilize the preferential dissociation of the rare earth complex to achieve a separation from thorium. If the rare earth is present only in microgram amounts, it will not be possible to precipitate the rare earths directly; a carrier must then be employed. A small part of the thorium itself can be used as carrier. This results in a concentration of the rare earth rather than a complete separation.

REAGENTS AND APPARATUS

Thorium Solution. Thorium metal was used which contained less than 100 p.p.m. of rare earths. The relative abundance of

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these in descending order was: cerium, lanthanum, neodymium, praseodymium, yttrium, samarium, gadolinium, and dysprosium. The metal was dissolved with warm nitric and hydrofluoric acids, after which perchloric acid was added and the temperature elevated to remove fluoride. Suitable dilutions were then made with 10% nitric acid; the resulting solutions were gravimetrically standardized by precipitation of the oxalate from homogeneous solution, using essentially the method described by Gordon and coworkers (*3*).

Table I. Precipitation Method for Concentration of Rare Earths Using Nitrilotriacetic Acid

ThO_2 Present, Mg.	Rare Earth		ThO_2 Precipitated, %	Rare Earth Coprecipitated, %
	Substance present	Mg.		
100	CeO_2	0.10	2 to 4	93
100	CeO_2	0.50	2 to 4	98
500	CeO_2	0.41	1 to 2	96
1000	CeO_2	0.28	<1	90
1000	Y_2O_3	2.0	<1	25

Yttrium Solution. Yttrium oxalate (Lindsay Chemical Co., West Chicago, Ill.), consisting of 70 to 75% yttrium, 10 to 15% yttrium earths, and 10 to 15% terbium earths, was dissolved in warm nitric acid. Suitable dilutions were then made with 10% nitric acid, and the resulting solutions were gravimetrically standardized in the same manner as that used for thorium.

Disodium, Dihydrogen Ethylenedinitrilo Tetraacetate (EDTA). Analytical reagent grade material was obtained from the Bersworth Chemical Co., Framingham, Mass.

Nitrilotriacetic Acid and Hydroxy(ethylenedinitrilo)triacetic Acid. These were obtained from Alrose Chemical Co., Providence, R. I.

Dowex 50. A cation exchange resin (Dow Chemical Co., Midland, Mich.), spherically shaped, 20 to 50 mesh, with 8% divinylbenzene cross linking.

Yttrium-90 and Lanthanum-140. These radioisotopes were obtained from Oak Ridge National Laboratory as mixtures, respectively, of strontium-90–yttrium-90 and barium-140–lanthanum-140. The yttrium-90 and lanthanum-140 were separated from the parent isotopes by the methods of Salutsky and Kirby (*8, 9*). This technique of "milking" the radioactive rare earths from the radioactive alkaline earths results in a supply of carrier-free rare earths of better than 99% radioactive purity.

All other reagents used were of C.P. or reagent grade quality.

Radioactivity Measurements. A Tracerlab TGC-1 tube, 2.5 mg. of mica per square cm., was used in conjunction with a Tracerlab utility scaler. Samples containing activity were evaporated in glass specimen vials of uniform dimensions and counted in the usual manner in the experiments in which the precipitation procedure was employed. Liquid counting was utilized in the ion exchange procedure.

pH Measurement. A Beckman Model H-2 pH meter was used.

PRELIMINARY STUDIES

Nitrilotriacetic Acid Precipitation Method. Ammonium oxalate was used as the precipitant and nitrilotriacetic acid as the complexing agent. If the pH was adjusted to approximately 4, thorium (added as nitrate) remained in solution in the presence of severalfold excesses of ammonium oxalate and nitrilotriacetic acid. A fraction of a milligram of cerium was then added. The solution was heated to boiling and acidified by the dropwise addition of hydrochloric acid until a slight turbidity appeared, which occurred at a pH of about 2.5. The small amount of precipitate formed at the boiling point was easily filtered; if acidification was terminated at the first appearance of turbidity the extent of precipitation of thorium was slight. A semiquantitative spectrophotometric determination (*10*) of the cerium remaining in solution indicated that most of it had been carried with the small fraction of thorium precipitated. When yttrium was added rather than cerium, the extent of precipitation of yttrium was much less. The yttrium was determined by a flame photometric procedure (*10*); data are given in Table I.

Other Complexing Agents. Some exploratory work with hydroxy(ethylenedinitrilo)triacetic acid indicated that it was not as efficient as nitrilotriacetic acid for this separation.

The use of the stronger complexing agent, EDTA, in the same manner as nitrilotriacetic acid was not possible because excess EDTA precipitated on acidification, simultaneously with the dissociation of the thorium complex. However, a modified procedure was finally devised, using EDTA but avoiding the use of the severalfold excess of the reagent. This modification permitted precipitation of the small thorium fraction at a higher pH where EDTA is soluble. In addition, a more efficient separation of thorium and a rare earth could be obtained than with nitrilotriacetic acid. A similar procedure using EDTA was also devised for the removal of uncomplexed rare earths by ion exchange.

Attempts to use the EDTA procedure with carriers other than thorium oxalate were not successful; neither thorium iodate nor thorium fluoride would carry yttrium. Further attempts to utilize calcium, barium, or strontium fluoride as carriers for yttrium also failed.

RADIOCHEMICAL ANALYSIS

Neither the spectrophotometric method for cerium nor the flame photometric method for yttrium was satisfactory for small quantities of these rare earths. Thus, it became necessary to utilize a radiochemical procedure to determine the efficiency of the separation. Lanthanum-140 and yttrium-90 were selected as tracers to ascertain the efficiency of each concentration operation because they were the most suitable isotopes available to represent the two rare earth subgroupings. They had an easily measurable radiation, a reasonable half-life, and could be obtained in radiochemical purity.

Identical aliquots of a thorium solution containing the rare earth tracer were taken before and after each separation experiment. These aliquots were then counted in close sequence to avoid errors due to the short half-lives of the tracers. A comparison of the counts in the two samples provided a measure of the efficiency of the separation.

EDTA PRECIPITATION METHOD

Procedure. Mix 101 ml. of 0.00375*M* thorium solution (as perchlorate or nitrate) and 100 ml. of 0.00375*M* EDTA solution. If nitric acid is not present in the thorium solution, add 1.5 ml. of concentrated nitric acid. Adjust the pH of the mixture to between 5.0 and 5.5 with ammonium hydroxide.

Add 25 ml. of saturated ammonium oxalate solution which has been previously adjusted to pH 3.3 to 3.5 with hydrochloric acid. Then adjust the final pH to 3.2 to 3.3 with small portions of the ammonium oxalate solution. If the pH should fall below 3.2, which surprisingly enough it may do, add ammonium hydroxide again to attain the proper pH range. The pH must be adjusted in the manner described. If hydrochloric acid is added after pH 3.3 to 3.5 is reached, the precipitate of thorium oxalate may form prematurely and in an excessive amount. The lowering of the pH below this value without danger of precipitate formation can easily be accomplished by addition of the ammonium oxalate solution.

Add 1 drop of 1 to 50 nitric acid, stir, and let stand overnight or longer to permit a small amount of thorium oxalate to appear as a turbidity. Filter through a double Whatman No. 42 paper and refilter through another double thickness. The slight amount of precipitate or turbidity contains the major portion of the rare earths.

Results and Discussion. Thorium reacts stoichiometrically with EDTA at low pH values (*2*). This reaction provides the basis for controlling the relative quantities of thorium in solution as a complex or as the ion. Thus, it is possible to mix thorium and EDTA in such amounts that only about 1% of the thorium precipitates on the addition of oxalate. Thorium oxalate precipitated in this manner serves as a carrier for the rare earths.

Table II shows the results obtained with the precipitation procedure described. The data show that more than 98% of the

lanthanum can be concentrated with the thorium precipitate. The weights of thorium oxide precipitated in six of the experiments ranged between 2.6 and 12.5 mg. Thus, the average, 6.1 mg., indicates that less than 1% of the original 1.5 grams of thorium oxide present is used to carry lanthanum.

Table II. Precipitation Method for Concentration of Rare Earths Using EDTA

pH	Stable La_2O_3 Added, Mg.[a]	La Removal, %
3.5	0.5	99
3.5	0.5	99
3.5	0.5	99
3.3	0.3	99
3.2	0.3	100
3.2	0.1	98
3.2	0.1	99
3.2	0.05	100
3.2	0.05	98
3.3	0.01	98
3.2	0.01	99
3.2	0.001	99
3.2	0.001	99
...	0.000	100
...	0.000	100
...	0.000	100
pH	**Stable Y_2O_3 Added, Mg.[b]**	**Y Removal, %**
3.2	0.0	15
3.2	0.0	1
3.2	0.1	0
3.2	0.1	0

[a] Lanthanum-140 added as tracer.
[b] Yttrium-90 added as tracer.

A pH of about 3.2 to 3.3 seems to be optimum for the separation. Above pH 3.3 the slight amount of thorium oxalate begins to dissolve. Above pH 3.7 the turbidity does not persist in the solution, whereas below pH 3.1 additional thorium precipitates.

The precipitation procedure was not effective in concentrating yttrium.

ION EXCHANGE METHOD

Procedure. Use a thorium sample containing the equivalent of 1.5 grams of thorium oxide as nitrate or perchlorate. Add slightly less than an equivalent amount of EDTA. Adjust the pH to 2.0 to 2.3 and the volume to 70 to 80 ml.

Add 5 drops of 0.1% Naphtharson [*o*-(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid] (Smith-New York Co., Inc., Freeport, Long Island, N. Y.). Titrate the sample with 0.2- to 0.3*M* EDTA (*2*) until the reddish-orange color changes to yellowish orange. For comparison purposes, prepare a blank of equal volume containing 5 drops of Naphtharson and some thorium.

Next add to the titrated sample a quantity of formic acid equal to 10% of its final volume. Adjust the pH to 2.1 with ammonium hydroxide.

Prepare a Dowex 50 ion exchange column with 30 cm. of resin in a column 22 mm. in inside diameter. Pass a 10% ammonium formate buffer, pH 2.1, through the column until the pH of the effluent buffer is also 2.1.

Now pass the previously treated thorium solution (2 to 3 ml. per minute) through the column. After this, wash the column with approximately 50 ml. of the formate buffer. Next, wash with distilled water until about 350 to 400 ml. have passed through.

The major portion of the rare earths remains on the column along with a small fraction of the thorium.

Results and Discussion. The principle employed in the ion exchange method is similar to that used in the precipitation process. The preferential dissociation of the EDTA complexes with pH makes possible the retention on a cation exchange column of the traces of rare earths along with a small portion of the thorium which is uncomplexed.

A study of the effect of pH is shown in Figure 1. Solutions containing the EDTA complexes of thorium and yttrium (1 to 1 molar ratio of metal ion to EDTA) were adjusted to various pH values and passed through a Dowex 50 ion exchange column. Aliquots of 25 ml. were used to obtain each point on the curves shown in Figure 1; 25 ml. of the thorium-EDTA complex solution contained 1.369 grams of thorium oxide, and 25 ml. of the yttrium-EDTA complex contained 0.4417 gram of yttrium oxide. Below about pH 2.2 thorium begins to collect on the resin, whereas above this value it essentially passes through. However, the retention of yttrium is quite sensitive to pH in this range. It was not necessary to study lanthanum in a similar way because the difference between lanthanum and thorium could be qualitatively predicted once the thorium-yttrium relationship was established.

Figure 1 was used as a guide in arriving at the procedure described for the ion exchange separation of the trace rare earths.

Table III shows the results obtained with this procedure. At pH 2.1 more than 98% of the trace yttrium present in the thorium is collected on the resin. However, pH is critical, as shown by the erratic results obtained at slightly higher pH values. The stability constant of the lanthanum-EDTA complex is such that lanthanum is even more efficiently collected by the resin than is the case with yttrium. Table III shows that lanthanum is effectively collected at pH values up to 2.5.

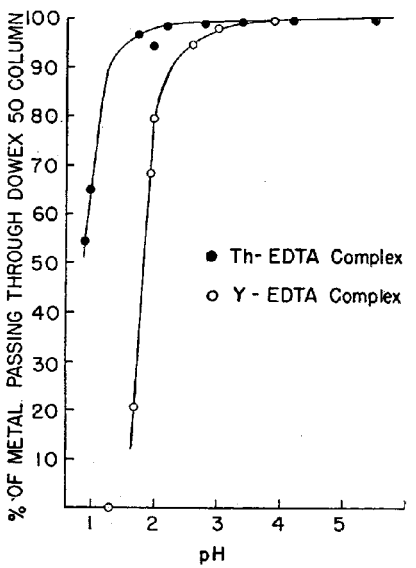

Figure 1. Effect of pH on elution of thorium and yttrium complexes

Some additional concentration of the rare earth traces can be realized by eluting the column with 2 liters of 1 to 1 hydrochloric acid. This operation removes from the column about 98% of the yttrium, 80% of the lanthanum, and 30% of the thorium. It is then possible to repeat the entire ion exchange procedure and attain further concentration. This was demonstrated in some experiments in which 14.3 and 1.43 mg. of thorium oxide was used in the starting mixtures instead of 1.5 grams, along with yttrium-90 and lanthanum-140. More than 99.5% of the tracers were retained by the column with about 20 to 30% of the thorium taken, indicating that the method as described is more efficient for the concentration of rare earths when the initial amount of thorium is relatively large—i.e., of the order of 1.5 grams. While

the use of a shorter column would undoubtedly result in less retention of thorium it would at the same time allow a greater rare earth loss.

The ion exchange method described is best applicable for concentrating microgram quantities of rare earths present in a relatively large amount of thorium. The initial weight ratio of thorium to rare earths in the present case is of the order of 10,000 to 1. This is reduced in a single step to about 100 to 1. Some further concentration can be achieved by repeated application of the method, although the efficiency is not so good when the quantity of thorium becomes smaller. Further concentration or separation could also be achieved by using some other method which is capable of handling the reduced quantity of the thorium–rare earth mixture obtained after a single pass through the ion exchange column. One such possibility is the modification of the Stine and Gordon iodate method (*12*) proposed by Shaver (*11*), whose work indicates that only 0.1 to 0.5% of trace rare earths is coprecipitated with the thorium precipitate.

The stability constant (*13*) of the EDTA complex of yttrium is 17.56 and that of terbium is 17.38. The stability constants of the rare earths heavier than terbium are greater than that of yttrium. Because of this, the concentration of the heavier rare earths—i.e., heavier than terbium—would not be as efficient as is the case with yttrium. However, the stability constant for lutecium is 19.65, whereas that of thorium (*1*) is 22. Thus, some concentration of these rare earths can be expected.

Table III. Ion Exchange Method for Concentration of Rare Earths[a]

pH	Rare Earth Remaining in Solution, %	Thorium Passing through Column, %[b]
	Yttrium-90[c]	
2.1	0.11	92.8
2.1	1.38	96.4
2.1	1.02	96.6
2.2	15	...
2.3	0.14	93.6
2.3	1.43	98.0
2.3	22	...
2.3	36	...
2.4	51	...
2.5	45	98.6
	Lanthanum-140[c]	
2.3	0.71	98.1
2.3	0.20	99.1
2.5	0.47	98.2

[a] Stable rare earths were not added as was done in precipitation procedure.
[b] Thorium analyzed gravimetrically by oxalate precipitation.
[c] Rare earth added as tracer.

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Quantitative Determination of Amino Acids by Fluorescence of Derivatives on Paper

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A method is presented for the semiquantitative estimation of amino acids, which depends on treating paper chromatograms with a xylose spray, heating, and measuring the fluorescence of the spots. Various tests and applications of the method are presented, and its advantages and disadvantages are discussed.

WITH the development of paper chromatography for separation of micro quantities of amino acids, a number of methods for the quantitative estimation of the separated amino acids have been developed (*3*). Considerable variations in rapidity, convenience, and precision occur among the methods, with rapidity usually being achieved at the expense of precision.

The method presented here evolved from the work of Wadman, Thomas, and Pardee (*18*), who determined reducing oligosaccharides by measuring directly on the filter paper the fluorescence intensity of derivatives formed when the sugars were made to react with a fluorescent amine. The measurements of the fluorescence were made with a Beckman Model DU spectrophotometer. The only modification required was that a filter which transmitted only the fluorescent light be placed in front of the photocell. Such a filter (Corning No. 3389) was supplied in the fluorescence accessory set for the Beckman spectrophotometer.

The amine-sugar reaction was used for the determination of amino acids. The fluorescence in this case is developed on paper by the interaction of xylose and amino acids in the presence of sodium bisulfite. This type of reaction has long been known as the browning or Maillard reaction. Under suitable conditions the reaction does not produce the familiar brown products, but rather intermediates that are highly fluorescent substances (*11*).

EXPERIMENTAL

Separation of Amino Acids. For the separation of amino acids in a mixture prior to development of fluorescence, the chromatographic procedures of Levy and Chung (*9*) and of Redfield (*18*) were tried.

In the method of Levy and Chung, the first dimension was run with *n*-butyl alcohol–acetic acid–water as the solvent, and

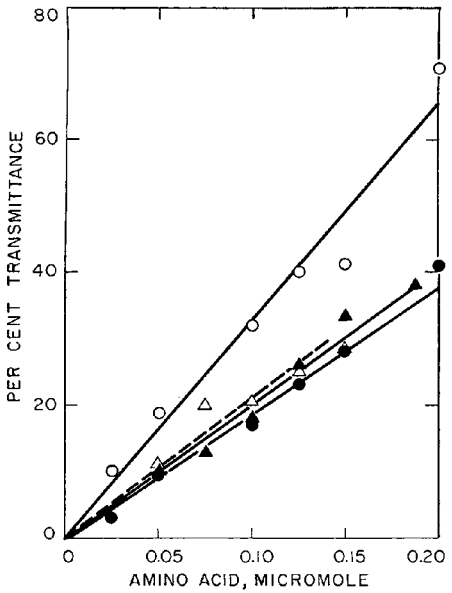

Figure 1. Fluorescence intensities of xylose derivatives of various amino acids as function of quantity of amino acid

Mixtures of four amino acids each were run at several concentrations by method of Levy and Chung (*9*).
○ Aspartic acid △ Serine
● Arginine ▲ Tyrosine

the second with phenol–*m*-cresol. Before the second dimension was run, the papers were sprayed with a buffer solution of pH 9.3 as in the Levy and Chung procedure. However, because the borate buffer they and McFarren (*10*) used reacts at this pH with sugars (and interferes with development of fluorescence) pyrophosphate buffer was substituted. Carbonate buffer was unsatisfactory for the production of fluorescence.

Whatman No. 52 was found to be the filter paper of choice for the chromatographic separation when the above solvents were used. The spots of amino acids were compact, the fluorescence developed well on it, and the background was very low provided purified solvents were used. Purification by distillation of the phenol and cresol seemed essential to the development of maximum fluorescence.

As an alternative separation procedure, the chromatographic method of Redfield was used. This is a two-dimensional separation using very small squares (about 14 cm. square) of Schleicher and Schuell No. 507 filter paper. Smaller amounts of amino acids (about one fifth as much) are used in this process, but the fluorescence developed on these chromatograms is not so intense as that obtained by using the previously described separation procedure.

In both methods of separation the fluorescence could be developed satisfactorily after the chromatogram was run in the first dimension only.

Development of Fluorescent Amino Acid Derivatives. Various solutions for spraying the amino acid chromatograms were tried to test the effect on production of fluorescence of sugar, pH, buffer, and agents which prevent browning.

Xylose and glucose were used at concentrations from 2 to 8%. Glucose reacted with amino acids to give fluorescence, but the intensity was less than one half as much as with xylose. Also, longer heating periods at higher temperatures (4 hours at 110° C.) were required for development of fluorescence. Furfural was also tested, because it has been reported that amino acids catalyze the formation of hydroxymethylfurfural and furfural (*15*), which may be intermediates in the formation of amino acid–sugar compounds. It gave no fluorescent derivatives.

The pH of the spray was varied between 5 and 10. Unbuffered solutions and solutions which were 0.02-, 0.05-, and 0.1*M* in phosphate or bicarbonate were tried. As others have found (*6*), the interaction of sugar and amino groups is dependent upon pH, as are the production of fluorescence and browning. Above pH 8, browning was considerably increased; below pH 6 the fluorescence was much weaker. Phosphate (or pyrophosphate) buffer is suitable for the control of pH, because it is a good buffer in this region and increases the production of fluorescence as well.

To prevent the formation of nonfluorescent brown pigments, sodium bisulfite and ascorbic acid were tried. It has been reported that bisulfite may prevent color development but not the loss of amino acids (*7*), depending on pH and moisture, and that a combination of ascorbic acid and bisulfite may be better than bisulfite alone to prevent browning (*8*). Bisulfite prevented browning and promoted the accumulation of the fluorescent intermediates. Addition of ascorbic acid to the spray caused browning, even of the background.

The most satisfactory spray contained 4% xylose and 1.5% sodium bisulfite in 0.05*M* phosphate buffer at pH 6.5 to 7. The papers were sprayed, dried, and then heated in an oven at 80° to 90° C. for 1.5 to 2 hours. The intensity of fluorescence developed is dependent upon the heating. Thus, it is desirable to have an oven in which the temperature is fairly uniform throughout and constant with time.

Measurement of Fluorescence Intensities of Amino Acid–Sugar Derivatives. The fluorescent spots produced by spraying and heating of the amino acid spots are easily located by observing the paper under an ultraviolet lamp. The spots are outlined and cut out later for reading in the Beckman spectrophotometer as follows.

The spots were fastened with rubber bands in front of a hole in a wooden block which was placed in the cell holder compartment. The paper was placed as near the phototube as possible, so that the latter could receive the maximum fluorescent light. A filter (Corning No. 3389) to absorb the exciting light (365 mμ) and to transmit light above 400 mμ was placed between the paper and the phototube. With the slit at 2.0 mm. and the sensitivity near the maximum, the fluorescence was read on the per cent transmittance scale. Because variations in the sensitivity occur, the per cent transmittance without any paper in the cell was checked occasionally. The selector switch was usually set at 1.0, but for readings of less than 10%, it may be set at 0.1.

The variation of fluorescence intensity with amount of amino acid is shown in Figure 1 and Table I. For other amino acids, except proline which fluoresced feebly, the results were similar. Results obtained with amino acids separated by the Redfield method were less satisfactory owing to the lower fluorescence, but were otherwise similar.

APPLICATIONS

The method was applied to the determination of some of the amino acids in insulin and β-lactoglobulin (Table II). The proteins were hydrolyzed in constant boiling hydrochloric acid at 150° C. for 5 hours. The amino acids were separated by the method of Levy and Chung (*9*), and were compared with known amounts of amino acids.

The method proved to be satisfactory for the comparison of the protein moieties of various lipoproteins of human plasma (*16*). Determination of the amino acid compositions of these proteins by the dinitrophenylation technique confirmed the results obtained with the fluorescence method.

The amount of azatryptophan incorporated into bacterial proteins was also estimated by this method (*12*).

DISCUSSION

The determination of fluorescence intensity has the advantage of being simple and fairly rapid by comparison with other methods for the determination of the concentration of amino acids directly on the chromatograms. In the total color density methods (*2, 5, 14*), the average color densities of a number of ninhydrin-sprayed strips of chromatographically separated amino acids are plotted on graph paper and the areas representative of each amino acid are determined. The absorption method requires the use of a base line (dependent on the colorless area) and certain assumptions with respect to the position of the dividing line between overlapping bands. These difficulties are avoided by the measurement of the fluorescence, which is proportional to the amount of material present. The maximum color density method of Block (*1*) also avoids this problem and, like the fluorescence method, is applicable to two-dimensional paper chromatography. The maximum color density and the total color density methods require more expensive reagents and a piece of apparatus, the densitometer, which is not as commonly found in the laboratory as the Beckman spectrophotometer.

Good accuracy (5 to 10% error) may often be obtained, although errors considerably larger than the mean are occasionally encountered (Table II). For qualitative location of spots, the method is similar in sensitivity to the ninhydrin method.

When the amino acids in the mixture to be analyzed can be separated in one dimension, the quantitative analysis can be carried out easily and rapidly. However, when two dimensions are required, the determination may become tedious because of the number of chromatograms necessary for mixtures containing a large number of amino acids in widely varying amounts. While rather time consuming for an absolute, complete protein analysis, the fluorescence method seems to be a rapid means of comparing relative amounts of amino acids in a series of samples.

Table I. Fluorescence Intensities of Xylose Derivatives of Various Amino Acids as Function of Quantity of Amino Acid

	Amino Acid, μmole						
	0.025	0.05	0.075	0.10	0.125	0.15	0.20
Amino Acid	Fluorescence Intensity						
Alanine	8	17	24	34	40	48	68
Glutamic acid	10	16	26	41	43	52	72
Leucine		12	17	20	25	30	42
Valine		8	10	14	16	22	
Glycine	9	18	24	33	38	46	
Lysine	17	30	42	53	58	65	

Table II. Amino Acid Composition of Insulin and β-Lactoglobulin by Measurement of Fluorescence Intensities

Amino Acid	Moles per 10^5 Grams of Protein		
	Found	Reported values	Diff., %
	Insulin		
		Brand (*4*)	
Glutamic acid	137	150	9
Aspartic acid	55	50	10
Glycine	90	60	50
Serine	60	55	9
Leucine + isoleucine	120	125	4
Phenylalanine	60	50	20
Valine	75	67	12
	β-Lactoglobulin		
		Stein, Moore (*17*)	
Alanine	85	80	6
Lysine	86	77	12
Aspartic acid	84	86	2
Leucine + isoleucine	170	163	4
Glutamic acid	140	130	8
Valine	60	49	22

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Determination of Acetylenic Compounds via Hydration

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The hydration reaction of the triple bond, when a mercuric ion catalyst is used in an acidic medium, converts the acetylenic compound to a ketone, which is then determined using hydroxylamine hydrochloride. This approach makes possible the determination of acetylenic compounds, for which earlier methods are unsatisfactory. The precision and accuracy are, in general, within ±2%, depending on the type of compound determined.

MANY methods have been developed for determining monosubstituted acetylenes of the type HC≡CR (*1–3, 5, 8, 10, 12–14*). All these methods involve replacing the acetylenic hydrogen atom by a metallic ion and titrating either the acid formed or the excess of metallic cation used. These methods cannot be used for determining disubstituted acetylenic compounds (RC≡CR). Also, in the case of monosubstituted acetylenes, these methods sometimes cannot be applied because of interfering substances, which react with the metallic ions used in the analysis.

Wagner, Goldstein, and Peters (*16*) describe a method for determining mono- and dialkyl acetylenes of four or five carbon atoms by reaction with methanol, using mercuric oxide and boron trifluoride as catalyst. The acetylenic compound is thus converted to the ketal. The ketal is distilled into hydroxylamine hydrochloride reagent, which hydrolyzes the ketals to the ketones and then forms the oxime of the ketone. The reactions used in this method are as follows:

$$RC{\equiv}CR' + 2MeOH \xrightarrow[BF_3]{HgO} RC(OMe)_2CH_2R'$$

$$RC(OMe)_2CH_2R' + H_2O \rightarrow R\overset{\overset{O}{\|}}{C}CH_2R' + 2MeOH$$

$$R\overset{\overset{O}{\|}}{C}CH_2R' + NH_2OH.HCl \longrightarrow R\overset{\overset{NOH}{\|}}{C}CH_2R' + H_2O + HCl$$

The hydrochloric acid formed in the last reaction is titrated, and the amount of acetylenic compound present in the sample is calculated from this value. The results obtained by this method are about 92% of the theoretical values.

The above procedure could not be used for the acetylenic compounds under investigation because of the high boiling points of the acetals formed, which made distillation impossible, the instability of the acetals, which resulted in decomposition at the distillation temperatures, or the low accuracy inherent in the method.

Koulkes (*8*) used the reaction between the acetylenic triple bond and mercuric acetate to determine several disubstituted acetylenic compounds. The mercuric acetate presumably adds onto the triple bond, and the excess of the acetate is determined by addition of sodium chloride and titration of the acetic acid liberated. This approach is fast, but impurities which react with mercuric acetate interfere: ethylenic compounds, some of which also add mercuric acetate, and inorganic and some organic halides, which complex with the mercuric ion. Some organic compounds such as carboxylates and sulfonates, form precipitates, and others are readily oxidized by the mercuric ion.

In the procedure described, the hydration reaction is used, with a mercuric catalyst in a strongly acidic medium, and the ketone

$$RC{\equiv}CR' + H_2O \xrightarrow[H_2SO_4]{HgSO_4} R\overset{\overset{O}{\|}}{C}CH_2R'$$

formed is a measure of the acetylenic material. This approach is subject to less interference than the mercuric acetate addition method, as the hydration will still proceed if a portion of the catalyst is consumed by other components. The accuracy of this system is, in general, 100 ± 2%, and a variety of acetylenic compounds are determinable (Table I).

Acidic or alkaline impurities in the sample do not interfere, as the system is neutralized prior to oximation. Ethylenic unsaturated compounds do not interfere, as they do not form carbonyl compounds under the conditions of the reaction. The only interferences which can be envisioned are from carbonyl compounds or carbonyl-forming compounds such as acetals or vinyl ethers. However, these can be determined by running the oximation analysis alone on a sample without first running the hydration; this should yield just the carbonyl compound. The hydration analysis should then yield the total of carbonyl compound and acetylenic compound; the acetylenic component can then be determined by subtraction. Some aldehydes are not stable (oxidize) with mercuric ion and samples containing large amounts of aldehydes or acetals should be examined thoroughly to make sure that the corrections applied are valid.

A potentiometric titration is used to measure the hydrochloric acid liberated in the oximation step. The end points are not sharp, but, in general, the precision is within ± 2% and sometimes within ± 1%.

Table I. Determination of Acetylenic Compounds

Compound	Reflux Time, Min. Hydration	Reflux Time, Min. Oximation	Mole Used	Mole Found	% Recovery	% Purity	Method
Butynediol[a]	15	15	0.01398	0.01339	95.8	98.9 ± 1	A
	30	30	0.01398	0.01391	99.5	..	..
	30	30	0.01398	0.01381	98.8	..	..
	30	30	0.00702	0.00682	97.2	..	..
	30	45	0.01404	0.01415	100.8	..	..
	30	45	0.01404	0.01422	101.3	..	..
	30	45	0.00702	0.00700	99.7	..	..
	45	60	0.01271	0.01277	100.5	..	..
	60	60	0.01398	0.01395	99.8	..	..
	120	60	0.01398	0.01388	99.3	..	..
2-Propyne-1-ol[b]	30	18 hr. at room temp.	0.01551	0.01473	95.0	99.0 ± 1	A
	30	30	0.01551	0.01387	89.4	..	..
	30	30-min. reflux + 18 hr. at room temp.	0.01551	0.01526	98.4	..	..
	30	60	0.01551	0.01517	97.8	..	..
	30	60	0.01551	0.01512	97.5	..	..
1-Butyne-3-ol[b]	60	60	0.01321	0.01125	85.2	84.1 ± 1	B
	60	60	0.00881	0.00732	83.1	..	..
3-Butyne-1-ol[b]	30	30	0.01477	0.01317	89.2	93.8 ± 1	B
	60	60	0.01507	0.01376	91.3	..	..
	60	60	0.01507	0.01414	93.8	..	..
	60	60	0.01484	0.01355	91.3	..	..
	60	60	0.01507	0.01364	90.5	..	..
	60	60	0.01507	0.01402	93.0	..	..
Ethynylcyclohexanol[c]	30	30	0.01381	0.01280	92.7	..[d]	..
	60	60	0.00810	0.00781	96.4	..	..
	60	60	0.00810	0.00793	97.9	..	..
Phenylacetylene[e]	60	..[f]	0.0001985	0.0001915	96.5	97.2 ± 1	B
	60	..[f]	0.0005958	0.0005744	96.4	..	..
1-Hexyne[c]	75	60	0.01223	0.01088	89.0	89.1 ± 1	C
	90	60	..	0.01083	88.6	..	..
3-Hexyne[c]	75[g]	60	0.01384	0.01235	89.2	91.5 ± 1	C
	75	60	..	0.01263	91.3	..	..
3-Octyne[c]	90[g]	60	0.00936	0.00875	93.5	93.0 ± 1	D
	150	60	..	0.00868	92.7	..	..
3-Octyne-1-ol[c]	90[g]	60	0.00806	0.00678	84.1	87.5 ± 1	C
	150	60	..	0.00675	83.7	..	..

A. Acetylation method (for alcohols) (*11*).
B. Acetylenic hydrogen method (*15*).
C. Bromination method (*9*).
D. Hydrogenation method (*4*).

[a] Recrystallized from ethyl acetate.
[b] Laboratory samples distilled once through helix-packed column.
[c] Analyzed as purchased from Farchan Research Laboratory.
[d] Could not be determined by method A, B, C, or D.
[e] Analyzed as purchased from Eastman Kodak.
[f] Dinitrophenylhydrazine method used because acetophenone could not be measured by oximation method.
[g] 20 ml. extra methanol used in hydration step because of insolubility of compounds.

Acetylenic compounds with substitutents on the carbons adjacent to the acetylenic linkage ($R_1CHC{\equiv}CCHR_2$, with R_3 and R_4) do not hydrate rapidly, owing to the hindrance caused by substituents. An analysis of $C_2H_5C(OH)(CH_3)-C{\equiv}C-C(OH)(CH_3)C_2H_5$ was attempted by this method, but conversions of only about 50% were obtained under the conditions described; 70% conversions were obtained when the hydration time was doubled. Unfortunately, not enough compounds could be obtained with substituents adjacent to the acetylenic linkage to compare the rates of hydration with the type of substituent.

Attempts were made to analyze acetylenic bromine compounds of the type $RC{\equiv}CCH_2Br$. However, the bromine atoms on these compounds are so labile that they are removed by the mercuric catalyst to form the mercuric bromine complex, and the catalytic action of the mercuric ion is much decreased; this type of compound cannot be determined by this method. The chloride compounds of the same structure will probably behave in the same manner, but this has not been tested.

In the case of phenylacetylene, the ketone formed on hydration is acetophenone. This ketone is one of the very few that cannot be determined by oximation in an aqueous or partially aqueous medium, because of the equilibrium which is present in the oximation system. Water is a product of the oximation reaction, and an aqueous system keeps the reaction from going to completion. In the case of phenylacetylene, after hydration any excess mercuric ion is removed by bubbling hydrogen sulfide through the solution and filtering off the mercuric sulfide. Then the acetophenone in the solution is determined by the 2,4-dinitrophenylhydrazine method of Iddles and Jackson (7). This same technique should be applicable to other acetylenic compounds which yield ketones that are not readily determined by oximation. This technique is applicable to samples containing small amounts of acetylenic compounds, as the 2,4-dinitrophenylhydrazine method requires only 4×10^{-4} mole of ketone for optimum operating conditions. This precipitation approach is less applicable to the hydroxyacetylenic compounds because of the solubilizing effect of the hydroxyl groups.

Before the 2,4-dinitrophenylhydrazine approach was tried on the acetylenic compounds, blanks were run, in which all the steps were included. This was to make sure that no extraneous precipitate formed on addition of the hydrazine, which would affect the results. Known samples of acetophenone were run through the entire procedure to establish the conditions for complete recovery of the ketone. Then the acetylenic compounds were used.

REAGENTS AND PROCEDURE

Reagents. Hydroxylamine hydrochloride (0.5*N*) in 1 to 1 methanol-water.

The catalyst is made from 0.5 gram of mercuric sulfate, 2 ml. of sulfuric acid, and 63.4 ml. of water.

Alcoholic sodium hydroxide (1.0*N*). Sodium hydroxide is dissolved in as little water as possible. The sodium carbonate is filtered off, and the solution is diluted with methanol to the desired volume. This solution need not be standardized.

Aqueous sodium hydroxide, 0.5*N* (standard), is used.

Apparatus. Glass and calomel electrodes, with a Model H-2 Beckman pH meter.

Procedure. A sample containing 0.05 to 0.20 mole of acetylenic compound is dissolved in methanol and diluted to 100 ml. in a volumetric flask; 10-ml. aliquots are used for the determinations.

Ten milliliters of sample solution are added to 20 ml. of catalyst in a 200-ml. three-necked flask connected to a reflux condenser. Glass stoppers are inserted in the two unused necks of the flask. The mixture is refluxed for 1 hour and then cooled in ice with the condenser still attached. After cooling, the condenser is washed with 10 ml. of 1 to 1 methanol-water and allowed to drain. The flask is disconnected from the condenser and glass-calomel electrodes are inserted into the flask through the two side necks. The acid is just neutralized (pH 7) with 1.0*N* alcoholic sodium hydroxide.

Fifty milliliters of hydroxylamine hydrochloride are added, the mixture is again refluxed for 1 hour and cooled in ice, and the condenser is washed with 1 to 1 methanol-water. The mixture is transferred to a 400-ml. beaker, using 50 ml. of 1 to 1 methanol-water to wash the flask. As much of the solid residue as possible is left in the flask during transfer.

The liberated hydrochloric acid is titrated potentiometrically with standard 0.5*N* sodium hydroxide, using the glass and calomel electrodes. The end point is determined from a plot of milliliters of reagent *vs.* pH.

If carbonyl compounds are present in the sample, they should be determined using the hydroxylamine hydrochloride analysis (see discussion above).

2,4-DINITROPHENYLHYDRAZONE METHOD

Reagents. Catalyst as described above.

A saturated solution of 2,4-dinitrophenylhydrazine at 0° C. in 2*N* hydrochloric acid.

Procedure. A sample is dissolved in methanol and diluted to 100 ml., so that a 10-ml. aliquot contains approximately 4×10^{-4} mole. Ten milliliters of sample are added to 20 ml. of mercuric sulfate–sulfuric acid catalyst and refluxed for 1 hour in a three-necked flask with glass stoppers in the two unused necks. After the hydration reaction period, the flask is cooled in ice with the condenser attached, and the condenser is washed with 10 ml. of 1 to 1 methanol-water. At this point there is a white precipitate in the flask, which does not appear to affect the results.

With the condenser still in position, hydrogen sulfide is passed into the solution to precipitate mercury as the sulfide. When this reaction is complete (5 to 10 minutes), the sulfide is filtered off through a No. 30 Whatman filter paper and the flask and paper are washed with a 1 to 1 solution of methanol-water.

To the filtrate are added 50 ml. of 2,4-dinitrophenylhydrazine solution, and the mixture is allowed to stand 0.5 to 1 hour. The resulting solution is warmed on a hot plate with constant stirring to coagulate the precipitate. When the supernatant liquid is clear, the precipitate is filtered off through a Gooch crucible with an asbestos mat, washed with water, dried at 100° C., and weighed. If the resultant hydrazone exhibits a significant solubility with the alcohol present (this must be predetermined), the solution is boiled for a few minutes to remove as much alcohol as possible before filtration. Acetylenic compounds containing hydroxyl groups cannot usually be determined by this method, because of the solubilizing effects of these groups.

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Determination of Partition Coefficients of Carotenoids as a Tool in Pigment Analysis

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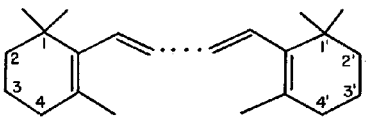
The partition of a carotenoid between two immiscible solvents such as hexane and 95% methanol is a characteristic that is determined by the presence or absence of certain functional groups. A photometric method for the estimation of partition coefficients is described to facilitate the identification of carotenoid pigments. Its combination with some other methods is briefly discussed.



β-Carotene, $C_{40}H_{56}$

FOR convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation." This statement was made 92 years ago by Stokes (*5*), who succeeded in "disentangling" the green leaf pigments. Subsequently, several authors, especially Willstätter and Stoll (*6*) and Kuhn and Brockmann (*2*), described the differentiation of some naturally occurring carotenoids by partitioning between petroleum ether and slightly dilute methanol or ethanol. Some data can also be found in pertinent monographs (*1, 7*). Thus, it was observed, after equilibration by shaking, that polyene hydrocarbons such as carotenes were found in the upper phase ("epiphasic" behavior), in contrast to dihydroxycarotenoids, the xanthophylls, that are "hypophasic." In the presence of a single —OH group an intermediate behavior was observed.

It is proposed that such rather qualitative descriptions be replaced by precise photometric determinations of partition coefficients of chromatographically homogeneous samples, as such data represent characteristic physical constants and are highly dependent on the structure. Table I contains partition coefficients of some naturally occurring carotenoids and in vitro conversion products (*4*). The numbering system used in the table is based on the following abbreviated formula.

The procedure described below may also be used for testing the homogeneity of samples. If, after equilibration and determination of the partition coefficient, one phase is removed and shaken with the pure solvent that was initially used as the other phase, an unchanged partition coefficient is an indication, although not a final proof, of purity.

This operation is being used in this laboratory in conjunction

Table I. Partition Coefficients of Some Carotenoid Pigments

(Determined in a two-phase system, hexane-methanol)

Type of Polyene	Compound	Functional Groups	Wave Length Used for Readings,[a,b] Mμ	Hexane-Methanol Ratio
	1. Hexane–95% Methano			
Hydrocarbon	α-Carotene	None	444 H	100:0
	β-Carotene	None	451 H	100:0
	γ-Carotene	None	460 H	100:0
	Lycopene	None	472 H	100:0
	Prolycopene (poly-*cis*)	None	440 H	100:0
	Phytofluene	None	348 H	100:0
	retro-Dehydrocarotene	None	474 H	99:1
	retro-Bisdehydrocarotene	None	490 H	100:0
Alcoho	4-Hydroxy-α-carotene	One —OH	444 H	84:16
	4-Hydroxy-β-carotene (isocryptoxanthin)	One —OH	451 H	86:14
	3-Hydroxy-β-carotene (cryptoxanthin)	One —OH	451 H	82:18
	3-Hydroxy-γ-carotene (?) (gazaniaxanthin)	One —OH	480 H	80:20
	3-Hydroxy-3′,4′-dehydro-α-carotene (desoxylutein I)	One —OH	460 H	78:22
	4-Hydroxy-3′,4′-dehydro-β-carotene	One —OH	460 H	80:20
	2-Hydroxy-3,4-dehydro-β-carotene	One —OH	460 H	90:10
	3,3′-Dihydroxy-α-carotene (lutein)	Two —OH	444 M	12:88
	3,3′-Dihydroxy-β-carotene (zeaxanthin)	Two —OH	451 M	11:89
	4,4′-Dihydroxy-β-carotene	Two —OH	451 M	22:78
	Vitamin A_1 ($C_{20}H_{28}O$)	One —OH	325 H	36:64
Ether	4-Ethoxy-α-carotene	One —OC_2H_5	444 H	99:1
	4-Methoxy-3′,4′-dehydro-β-carotene	One —OCH_3	460 H	99:1
Ester	Zeaxanthin dipalmitate (physaliene)	Two —$OOCC_{15}H_{31}$	451 H	100:0
	4,4′-Dihydroxy-β-carotene diacetate	Two —$OOCCH_3$	451 H	86:14
	4-Hydroxy-3′,4′-dehydro-β-carotene acetate	One —$OOCCH_3$	460 H	96:4
Ketone	4-Keto-α-carotene	One C=O	453 H	95:5
	4-Keto-β-carotene	One C=O	458 H	93:7
	4-Keto-3′,4′-dehydro-β-carotene	One C=O	468 H	92:8
	4,4′-Diketo-β-carotene	Two C=O	465 H	50:50
	β-Carotenone	Four C=O	484 M	8:92
Keto alcohol (and deriv.)	4-Keto-4′-hydroxy-β-carotene	One C=O, one —OH	458 M	34:66
	4-Keto-4′-ethoxy-β-carotene	One C=O, one —OC_2H_5	458 H	86:14
	4-Keto-4′-ethoxy-3′,4′-dehydro-β-carotene	One C=O, one —OC_2H_5 (allylic)	468 H	44:56
	Capsanthin	One C=O, two —OH	475 M	4:96
	Capsanthin diacetate	Two —$OOCCH_3$, one C=O	470 H	65:35
	Capsorubin	Two C=O, two —OH	450 M	1:99
Carboxylic acid and ester	Crocetin ($C_{20}H_{24}O_4$)	Two —COOH	450 M	4:96
	Methylbixin ($C_{26}H_{32}O_4$)	Two —$COOCH_3$	450 M	26:74
	2. Hexane–85% Methanol			
Alcohol	Lutein	Two —OH	445 H	43:57
	Zeaxanthin	Two —OH	450 H	40:60
	Neozeaxanthin A (*cis*)	Two —OH	444 H	40:60

[a] As a rule these wave lengths represent those at maximum extinction taken in the Beckman spectrophotometer. Any other maximum can be used—e.g., because of limitation of apparatus available.
[b] Extinction measured: H, in hexane; M, in methanol.

with chromatographic analysis. The latter method would reveal the presence of even small amounts of cis contaminants, whose influence on the partition coefficient is mostly negligible. A third current method of carotenoid identification is spectroscopy in the ultraviolet and visible regions. While the extinction curve alone will not differentiate between β-carotene and its hydroxyl derivatives, for example, identification of the pigment will be aided by the determination of the partition coefficient as suggested in the present paper. In favorable instances a further powerful tool can be used—infrared spectroscopy—by means of which not only functional groups but also some typical stereochemical features of the molecule may be detected (*3*).

PROCEDURE

The sample is dissolved in about 15 ml. of either hexane (previously shaken with 95% methanol) or 95% methanol (previously shaken with hexane). Usually, the more effective solvent is chosen for the dissolution. The concentration should be low enough to allow direct reading at $\lambda_{max.}$ of the absorbance, A_1, in a Beckman spectrophotometer. After this has been accomplished, 5.0 ml. of the pigment solution is pipetted into a stoppered 10-ml. graduate cylinder, followed by an equal volume of the other solvent. The two phases are equilibrated by slowly inverting the cylinder twenty times. The absorbance, A_2, is then taken again in the phase used for the dissolution of the sample. The per cent epiphasic character (when hexane was the solvent) or hypophasic (when the substance was dissolved in methanol) is given by $A_2/A_1 \times 100$. (The extinction values are usually measured in the hexane solution.)

The experiment described requires 15 minutes, and the accuracy limit is ±1%. The most favorable conditions for the analysis prevail when a two-phase system is chosen that causes the presence of 40 to 60% of the total pigment in either phase. Thus, in the analysis of xanthophylls, hydroxyketones, or polyketones, the photometric estimations may be carried out with advantage in the system hexane–85% methanol (Table I).

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Determination of Phosphorus by Precipitation as Oxine Molybdophosphate

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A study of the gravimetric determination of phosphorus as the oxine salt of molybdophosphoric acid removed several uncertainties about the method. The composition of the compound that is weighed corresponds to the formula $3C_9H_7ON.H_3(PMo_{12}O_{40})$. The method was checked against a variety of standard samples of alloys and rocks; it was found to be faster than the magnesium pyrophosphate method and of comparable accuracy. The high molecular weight of the molybdophosphate is an advantage in the analysis of low-phosphorus materials.

A GRAVIMETRIC method for the determination of phosphorus as the oxine salt of molybdophosphoric acid was described by Scharrer (*9*). He applied the method to plant ash, soils, and fertilizers (0.001 to 60% phosphoric oxide).

The high molecular weight and low phosphorus content of the precipitated oxine salt suggest a particular suitability of the method for the determination of small quantities of phosphorus—a major reason, perhaps, for appearance of the method in various reference books on analytical methods. The prospective user of the method is faced, however, with uncertainties about the composition of the precipitate and about the conditions under which the precipitate should be handled up to the time it is weighed.

Scharrer found an average of 3.063% phosphoric oxide (P_2O_5) in the dried oxine molybdophosphate precipitate (105° C.) by the Lorenz method (*8*). Berg (*2*) concluded that the dried precipitate is a dihydrate, $3C_9H_7ON.H_7[P(Mo_2O_7)_6].2H_2O$, with a theoretical phosphoric oxide content of 3.05%. The same composition of precipitate was reported as recently as 1953, when Duval (*5*) presented a pyrolysis curve from which he concluded that the dihydrate is the stable compound over the temperature interval from 176° to 225° C. Brabson and coworkers (*3*), on the other hand, presented evidence that the compound that remains when the precipitate is dried at 140° C. is anhydrous oxine molybdophosphate, $3C_9H_7ON.H_3(PMo_{12}O_{40})$, which contains 3.1393% phosphoric oxide.

In the analysis of reagent grade phosphate salts, Shik (*10*) did not confirm Scharrer's results. He found that extended drying at 105° C. was necessary to bring the precipitate to constant weight. This finding was confirmed in tests (*3*) which showed also that the precipitate could be dried at 140° C. without change in the x-ray pattern. Experience with the silicon analog of the phosphorus compound (*3*) indicates that Scharrer's directions for washing the precipitate are inadequate.

An interest in the oxine molybdophosphate method arose in connection with the analysis of products from a study of the synthesis of phosphorus nitrides (*6*). Some of the products were merely stains on the glass synthesis apparatus, and the determination of their phosphorus content was complicated by the large amount of sulfuric acid that was required for their dissolution.

Several improvements in the method and strong evidence regarding the composition of the precipitate resulted from the study described here.

REAGENTS

Oxine–Hydrochloric Acid Solution, 5%. Triturate 75 grams of 8-quinolinol with 75 ml. of concentrated hydrochloric acid. Dilute to 1500 ml.

Ammonium Molybdate Solution, 10%. Dissolve 400 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$ (large crystals), in 4000 ml. of water. Filter through a retentive paper.

Precipitating Solution. To 1680 ml. of concentrated hydrochloric acid add 1680 ml. of the ammonium molybdate solution and 640 ml. of the oxine–hydrochloric acid solution. Filter through a sintered-glass funnel of fine porosity.

Saturated Wash Solution. The precipitate for saturation of the wash solution is prepared in small batches to ensure its correct composition and to facilitate washing. Add reagent grade phosphoric acid equivalent to about 10 mg. of phosphoric oxide to each of nine 400-ml. beakers and dilute to 100 ml. Heat the solutions to 70° C. in a water bath and to each add 30 ml. of the precipitating solution from a buret while stirring. Continue the heating with occasional stirring for 15 minutes. Cool for 1 hour at 20° C. Filter through sintered-glass crucibles of fine porosity and wash 10 times with a hydrochloric acid solution made by diluting 850 ml. of the acid to 10 liters. Transfer the bulk of the precipitates to a 2-liter flask. Add 1500 ml. of the dilute hydrochloric acid to the flask and digest with occasional shaking at 70° C. in the water bath for 1 hour. Pour the supernatant liquid into a stock bottle (about 9 liters). Similarly digest the solid with successive charges of dilute acid until about 90% of it has dissolved, then transfer it to the stock bottle along with the last charge of acid. Filter a portion of the wash solution through a fine sintered-glass funnel immediately before use.

PROCEDURE

The optimum amount of phosphoric oxide for precipitation is 5 to 10 mg., and 0.1 mg. is the practical lower limit. Samples may be decomposed by various means, provided (1) the phosphorus is converted to orthophosphate; (2) silicon, germanium, arsenic, and nitrates either are absent or are removed; (3) the concentration of sulfate is under 35 mg. per ml. (otherwise a double precipitation is required); and (4) excess acid is neutralized with sodium hydroxide, not ammonium hydroxide.

Separation of Phosphorus. Transfer an aliquot containing no more than 15 mg. of phosphoric oxide to a 400-ml. beaker. Dilute to about 50 ml., add 2 drops of methyl red indicator, and adjust the pH to the intermediate shade of the indicator with sodium hydroxide solution and 1 to 9 hydrochloric acid. Adjust the volume to 100 ml. and heat the solution to 70° C. in a water bath. Add 30 ml. of precipitating solution from a buret while stirring. Hold the solution at 70° C. for 15 minutes and stir it occasionally. Then cool it for 1 hour at 20° C.

Under gentle suction, filter all the liquid through a weighed sintered-glass crucible of fine porosity. Wash the solid onto the filter with a jet of the saturated wash solution and the aid of a policeman. Wash the precipitate 10 times with the wash solution and finally once with a jet of water. About 85 ml. of wash solution is required for a determination. Dry the precipitate at 140° C. for 1 hour, cool to room temperature in an evacuated desiccator over anhydrous magnesium perchlorate, and weigh. Repeat the drying, cooling, and weighing to constant weight. Since the precipitate is somewhat hygroscopic, cool no more than three crucibles in one desiccator. Make the weighings quickly. Subtract the blank correction described below to obtain the net weight of precipitate.

Blank Determination. Prepare a standard phosphate solution by digesting 1 gram of NBS phosphate rock 56b with perchloric acid. Dilute to 1 liter and transfer 25-ml. aliquots to each of six 400-ml. beakers. Precipitate the phosphorus. Filter and dry the precipitates to constant weight. If the weights of the precipitates exceed the theoretical value by more than a few tenths of a milligram, apply the difference as a blank correction. Establish a new blank for each lot of reagents.

Calculations.

$$\frac{\text{Net weight of precipitate} \times 0.013701}{\text{weight of sample}} \times 100 = \% \text{ P}$$

$$\frac{\text{Net weight of precipitate} \times 0.031393}{\text{weight of sample}} \times 100 = \% \text{ } P_2O_5$$

EXPERIMENTAL WORK

In Scharrer's method (*9*) a neutral or weakly acidic solution containing about 10 mg. of phosphoric oxide is heated to 70° C. and an oxine–ammonium molybdate solution is added. An orange-yellow precipitate separates immediately. After at least 12 hours it is filtered on a Gooch crucible, washed with an ammonium nitrate solution, and dried to constant weight (4 hours or more) at 105° C.

A study of silica determination with oxine (*3*) suggested several changes in the older procedures. Parallel changes were made in Scharrer's method. Thus, the precipitated complex was allowed to settle for 1 hour instead of 12 hours and was washed with a saturated solution of the complex in dilute hydrochloric acid instead of a solution of ammonium nitrate. Also, the precipitate was dried at 140° C. for 1 hour.

Gravimetric Factor. Determinations of the phosphoric oxide content of the oxine molybdophosphate by analysis of the compound and by weighing precipitates from known amounts of phosphoric oxide yielded respective averages of 3.1378 and 3.1331% phosphoric oxide. Because these results agreed, within the limits of experimental error, with the formula of oxine molybdophosphate, $3C_9H_7ON.H_3(PMo_{12}O_{40})$, the theoretical factor 0.031393 was used.

Interfering Substances. Arsenic (*9*), germanium (*1*), and silicon (*3*) interfere with precipitations of oxine molybdophosphate, because these elements precipitate analogous heteropoly compounds. Nitrate interferes by decomposing oxine to a dark mass.

Ammonium salts in large amount interfere (*2*), presumably by precipitation of ammonium molybdophosphate. Sodium hydroxide, therefore, was used instead of ammonium hydroxide for neutralization of excess acid.

Table I. Accuracy of Oxine Molybdophosphate Method in Analysis of Rocks and Alloys

Standard Sample	No. Detns.	Oxine Method Av.	Oxine Method Max.	Oxine Method Min.	NBS value
		P_2O_5, %			
Rocks					
Argillaceous limestone, 1a	9	0.148	0.150	0.146	0.15
Phosphate rock, 56a	10	32.84	32.93	32.78	32.90[a]
Phosphate rock, 120	15	35.18	35.32	35.06	35.20[a]
Alloys					
		Phosphorus %			
Ferromanganese, 68a	9	0.290	0.291	0.288	0.294[b]
Ferrophosphorus, 90	5	26.12[c]	26.16	26.04	26.2

[a] Most probable values assigned by National Bureau of Standards.
[b] Average of 7 gravimetric results obtained after removal of arsenic, 0.290.
[c] Acid-insoluble residues were found to contain an additional 0.02% phosphorus.

Many phosphatic materials are easily decomposed by means of perchloric acid without loss of phosphorus. The effect of perchloric acid was studied by analyzing aliquots of a standard solution of diammonium phosphate to which had been added various amounts of the 72% reagent acid. Perchloric acid had no effect on the method in quantities as great as 20 ml. in the 100-ml. volume to which the precipitant was added.

The effect of sulfates was tested in the 100-ml. volume to which the precipitant was added by analyzing aliquots containing equal quantities of phosphorus and amounts of concentrated sulfuric acid ranging from 1 through 20 ml. The precipitates from solutions containing more than 2 ml. of sulfuric acid were contaminated by an extraneous compound. Even in the absence of phosphate, an orange-yellow compound was precipitated. From its composition and its x-ray diffraction pattern the precipitate was identified as molybdenum hydroxyquinolate, $MoO_2(C_9H_6ON)_2$.

Interference by sulfate was obviated by a double precipitation of the oxine molybdophosphate. The mixture first precipitated was collected on filter paper and wet-ashed with nitric and perchloric acids (*4*). The precipitated molybdic oxide was dissolved in excess sodium hydroxide solution. The second precipitation was done in the same manner as single precipitations in the absence of sulfate. The results showed that interference by sulfuric acid in the range from 1 to 20 ml. of the concentrated reagent per 100 ml. of solution had been eliminated.

Application to Standard Samples. The accuracy of the method was checked against NBS samples of rocks and alloys that varied widely in phosphorus content. Phosphate rocks were decomposed by digestion with perchloric acid. Argillaceous limestone was digested with perchloric acid after strong ignition. Ferromanganese was dissolved in boiling nitric acid, which then was re-

Table II. Analysis of Phosphorus Nitride by Single- and Double-Precipitation Methods

Phosphorus Taken, Mg.	Phosphorus Found, Mg.	
	Single pptn.	Double pptn.
0.25	0.29	0.30
0.50	0.51	0.50
1.33	1.34	1.33
2.86	2.99	2.86
4.19	4.19	4.15
5.58	5.57	5.50

moved in several evaporations with hydrochloric acid, and the sample finally was boiled with hydrochloric acid and ammonium bromide to remove arsenic. Ferrophosphorus was fumed with perchloric acid. The results obtained with the rocks and alloys are shown in Table I.

Application to Phosphorus Nitride. The oxine method was applied to a sample of phosphorus nitride (P_3N_5) that assayed 55.0% phosphorus by the magnesium pyrophosphate method. Portions of the nitride weighing about 0.35 gram were digested to complete solution in 15-ml. charges of concentrated sulfuric acid, and the solutions were fumed an additional 15 minutes. The aliquots contained approximately 5 mg. of phosphorus. Their sulfuric acid content was not enough to interfere in a single precipitation. Six determinations yielded an average of 55.22% phosphorus with a range of variation of 0.47%. When one result was rejected statistically, the average was 55.15% phosphorus with a maximum variation of 0.18%. The oxine method thus gave results comparable to those obtained by the magnesia method in the analysis of macro samples.

Micro samples of the phosphorus nitride were weighed into tared microbeakers and transferred to 250-ml. beakers for decomposition. Phosphorus was determined by single- and double-precipitation methods on aliquots representing 50% of each sample. The results are shown in Table II.

The last two entries in the double-precipitation column of Table II indicate minor losses of phosphorus, presumably through volatilization (7). The losses were offset by the effect of the sulfate in the single precipitations. Although the sample must be decomposed completely, the total time of fuming with sulfuric acid should not exceed 2 hours.

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Precipitation of Lead Chromate from Homogeneous Solution

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Homogeneous generation of chromate, by bromate, from chromium(III) is used to precipitate lead chromate. The procedure is relatively free from interferences and can be used for the quantitative recovery of lead or chromium. The crystals recovered are large and easily handled, and exhibit maximum purity. The volume of the precipitate is half that obtained by standard procedures. Purity is the same as of lead chromate recovered by the careful use of standard procedures, but lead chromate can be more easily precipitated and handled by this procedure.

A RECOMMENDED method for the separation and determination of lead employs the precipitation of lead chromate (*1*, *3*). Chromium may be determined by oxidation to chromate (*3*) with subsequent precipitation, but in the latter case chromate is usually titrated. The present paper describes a modification of these procedures, in which chromium(III) is slowly oxidized to chromate by bromate. This procedure results in the recovery of large, readily filtered, and easily washed crystals of lead chromate, which exhibit maximum purity.

MATERIALS

Baker and Adamson test lead was washed in nitric acid to free it of surface impurities, and was then dissolved in nitric acid. Lead nitrate was recovered by recrystallization. The lead nitrate so obtained was dissolved and recrystallized twice from 0.1% nitric acid. The resultant material was dried at 120° C. and weighed, as needed, as a primary standard, $Pb(NO_3)_2$. Lead content was checked by recommended procedures (*1*, *3*) as the sulfate, chromate, and electrolytically deposited dioxide.

Table I. Analysis of Precipitate from Hydrolysis of Urea

	Calculated $PbCrO_4$, %	Found, %		Average, %
		1	2	
Pb	64.11	65.26	65.52	65.4
CrO_4	35.89	27.83	27.98	27.9
Ratio (Pb-CrO_4)	1.000			1.313

Mallinckrodt analytical reagent grade potassium dichromate, with an experimentally determined oxidative purity factor of 1.000, was weighed as a primary standard. Reduction, to prepare individual stock chromium samples, was effected by a Jones reductor.

Other reagent grade chemicals were used as needed.

EXPERIMENTAL

The mechanism proposed for the generation of chromate (*6*, *7*), which employs the hydrolysis of urea, led to erratic and incomplete recoveries of lead. Furthermore, the material recovered was not a simple lead chromate (Table I).

The chromium content was too low to correspond to either

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simple lead chromate or a lead polychromate. Carbonate was shown to be absent from the precipitate. The analysis corresponded to a multiple salt of the form: $x\mathrm{PbO}.y\mathrm{PbCrO_4}.z\mathrm{K_2CrO_4}$. However, in view of the quantitative limitations of incomplete recovery and with no apparent enhancement of the precipitate form, further investigations of this problem seemed of limited utility. The difficulties presented by this precipitation approach have not been fully resolved.

The oxidative generation of chromate from chromium(III) by bromate proceeded slowly at 90° to 95° C. and yielded a quantitative recovery of lead in the form of large, easily washed and filtered crystals of high purity (Figure 1).

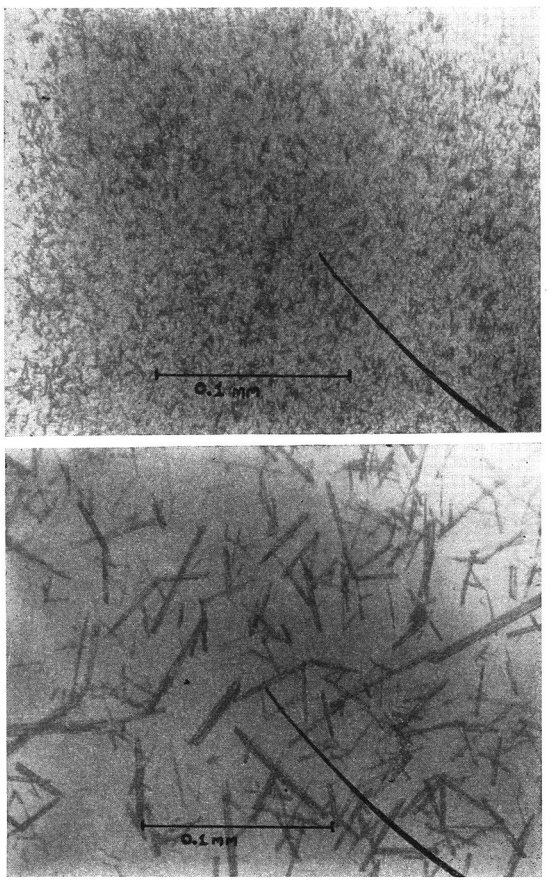

Figure 1. Form of lead precipitate

Upper. **Heterogeneous**
Lower. **Homogeneous**

The sequence of reactions for the generation can be represented as

$$2Cr^{+++} + BrO_3^- + 5H_2O \rightarrow CrO_4^{--} + Br^- + 10H^+$$
$$5Br^- + BrO_3^- + 6H^+ \rightarrow Br_2 + 3H_2O$$

Bromine boils from the solution, but bromide is the initial reduction product, as was demonstrated by adding silver in the absence of lead. An insoluble white residue that is sparingly soluble in ammonia is recovered after generation is complete. Consequently, silver ion should be absent.

INTERFERENCES

Complexants of chromium(III) inhibit the formation of chromate. Of these, only acetate and ammonia are commonly encountered. Acetate hinders appreciably only if present in concentrations approaching saturation in terms of sodium acetate. As chromate is formed in acidic solution, the concentration of free ammonia is low. The presence of ammonium salts, however, was also observed to inhibit generation of chromate.

Inhibition by ammonium salts was probably due to competitive oxidation of the ammonium ion by bromate. The reaction involved is represented by the expression:

$$2NH_4^+ + BrO_3^- \rightarrow N_2 + Br^- + 2H^+ + 3H_2O$$

Ammonium salts heated in aqueous solution with bromate slowly evolved a colorless gaseous product. The gas was collected over water; its inert character strongly suggested nitrogen. Bromide was the initial reduction product, causing a turbidity to develop in the presence of silver nitrate that in time became a heavy precipitate. In the absence of silver nitrate the reaction of bromide with bromate was not appreciable, but acidification with dilute sulfuric acid produced an unmistakable evolution of bromine.

If solutions of the sample are acidic after dissolution, they should be neutralized with sodium hydroxide to avoid the unnecessary use of large quantities of bromate and the attendant increase in the time necessary to complete precipitation.

The anions of the common mineral acids do not interfere.

Cations forming insoluble chromates, such as silver(I), barium(II), mercury(I,II), and bismuth(III), do not interfere in the recovery of lead chromate because the acidity is sufficiently high during the generation of chromate to prevent their precipitation. Bromide from the generation can be expected to form insoluble salts with silver(I) and mercury(I). Mercury(I) does not interfere, presumably because of oxidation to mercury(II), the bromide of which is more soluble. Silver (in small quantities) may be removed from the precipitate by repeated washings with ammonia, but is preferably separated by standard methods prior to precipitation (*4*).

PRECIPITATE FORM

Analysis of the precipitate made by the following procedure showed it to be lead chromate (Table II).

Procedure. Dissolve the precipitate in concentrated nitric acid, add sulfuric acid, and evaporate the solution to fumes of sulfur trioxide. Upon cooling, filter off the lead sulfate, dry, and weigh it. Determine chromate content by adding excess standard ferrous sulfate solution to the filtrate and back-titrating with dichromate.

Table II. Analysis of Precipitate

	Calculated $PbCrO_4$, %	Found, %		Average, %
		1	2	
Pb	64.11	64.22	64.14	64.18
CrO_4	35.89	35.80	35.84	35.82
Ratio	1.000	1.005	1.001	1.003

The purity of the precipitate was ascertained spectrographically. When barium and copper were added, impurities on the

order of 10^{-10} gram were found. No differentiation could be made in this respect between the precipitates obtained homogeneously and those obtained by standard procedures (*1*, *3*).

Aging had no effect on the amount of precipitate recovered or upon its composition.

The precipitate was dried at 120° C. in all cases. When heated to 450° C. it showed a negligible loss of weight (*2*).

Table III. Recovery of Lead from Stock Solution

Taken, G.	Found, G.	Difference, Mg.	Additions (10 Mmoles Each)
0.9036	0.9034	−0.2	None
	0.9037	+0.1	
	0.9038	+0.2	
0.9442	0.9440	−0.2	None
	0.9444	+0.2	
	0.9442	0.0	
	0.9437	−0.5	Al, Fe, Ni
	0.9442	0.0	
	0.9443	+0.1	
0.9218	0.9225	+0.7	Cu, Zn, Ba
	0.9220	+0.2	
	0.9222	+0.4	
0.7880	0.7885	+0.5	Cd, Bi, Hg
	0.7882	+0.2	
	0.7883	+0.3	
0.7880	0.7882	+0.2	Ag[a]
	0.7888	+0.8	
	0.7887	+0.7	
0.8332	0.8335	+0.3	Ag[b]
	0.8330	−0.2	
	0.8325	−0.7	

[a] Ag removed by washing with ammonia.
[b] Ag removed prior to precipitation as chloride.

The volume of the precipitate, compared to that obtained by standard procedures (*1*, *3*), was halved. The volume did not decrease to the extent sometimes observed with use of the homogeneous technique, notably in comparison to the hydrous oxides (*5*). The decrease is of the order obtained with crystalline precipitates such as barium chromate, barium sulfate, and rare earth oxalates, however.

RECOMMENDED PROCEDURE

Lead. Select a sample containing 0.1 to 0.2 gram of lead. After dissolution, neutralize the solution by adding sodium hydroxide until a precipitate just begins to form. Add 10 ml. of acetate buffer solution (6*M* in acetic acid, 0.6*M* in sodium acetate), 10 ml. of chromic nitrate solution (24 grams per liter), and 10 ml. of potassium bromate solution (20 grams per liter). Heat to 90° to 95° C. After generation and precipitation are complete (about 0.5 to 0.75 hour) as evidenced by a clear supernatant liquid, cool, wash with 0.1% nitric acid, and collect the crystals in a weighed Gooch crucible. Very little washing is required. Dry at 120° C. and weigh.

Chromium. Select a sample containing approximately 0.05 gram of chromium. After dissolution neutralize the solution by the addition of sodium hydroxide until a precipitate just begins to form. Add 10 ml. of acetate buffer solution, 10 ml. of lead nitrate solution (35 grams per liter), and 10 ml. of potassium bromate solution (20 grams per liter). Heat to 90° to 95° C., and proceed as with lead.

RESULTS

Determination of Lead. The results obtained with the recommended procedure are given in Table III.

Recovery of lead in multicomponent samples is reported in Table IV. Tin was separated as metastannic acid in the case of the brasses and bronze.

The Thorn Smith samples contained only copper, tin, and zinc in addition to lead. The National Bureau of Standards samples contained the same components, in addition to small amounts of iron and nickel. The bronze sample contained traces of sulfur and phosphorus.

Only the lead content of the ore was known.

Determination of Chromium. The recovery of chromium by the recommended procedure is shown in Table V. No additions were made to the samples.

Table VI reports recovery of chromium from steel samples containing iron, carbon, manganese, phosphorus, sulfur, silicon, chromium, and vanadium; less than 1% of all components, except iron, was present.

Table IV. Recovery of Lead in Multicomponent Samples

Sample	Pb Reported, %	Pb Found, %
Thorn Smith		
Brass	4.01	3.95, 3.99
Brass	4.75	4.75, 4.77
Brass	3.76	3.70, 3.71
National Bureau of Standards		
Cast bronze (52)	1.52	1.54, 1.53
Sheet brass (37B)	0.90	0.91, 0.91
Diack and Smith		
Lead ore	83.88	83.70, 83.82

Table V. Recovery of Chromium from Stock Solution

(0.1023 gram taken)

Found, G.	Difference, Mg.	Found, G.	Difference, Mg.
0.1020	−0.3	0.1025	+0.2
0.1023	0.0	0.1018	−0.5
0.1021	−0.2	0.1022	−0.1
0.1020	−0.3	0.1024	+0.1
0.1022	−0.1		

Table VI. Recovery of Chromium in Multicomponent Samples

Sample	Cr Reported, %	Cr Found, %
Thorn Smith steel	0.96	0.91, 0.96
	0.64	0.65, 0.75
	0.54	0.57, 58

The analysis of the low-chromium steel samples indicates that the procedure would be successful for the determination of chromium in chromite ores. Other procedures are probably better suited for determining chromium in steel.

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Determination of Dissolved Oxygen in Hydrocarbons

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A method is described for the determination of dissolved oxygen in hydrocarbons by a modified Winkler procedure. The procedure is easily run using simple equipment. Mercaptans do not appreciably affect the results, but peroxides interfere; a correction factor is proposed to compensate for the effects of peroxides. The repeatability of the method is good and the accuracy appears comparable to that obtained by more laborious techniques.

THE concentration of dissolved oxygen in petroleum fractions is of interest in connection with their storage and thermal stability, sweetening processes, and probably other applications. Most procedures for the determination of dissolved oxygen in organic solvents involve the removal of dissolved gases from the solvent and the subsequent determination of oxygen in these gases (*1, 2, 4, 8, 11*). These procedures generally require special or elaborate apparatus and most of them are time-consuming. The polarograph has been used by Hall (*7*) to determine dissolved oxygen in fuels; this method is fairly rapid and does not require the separation of dissolved gases.

A simpler method than those mentioned is the modified Winkler procedure proposed by Schulze, Lyon, and Morris (*15*). In this method the fuel is shaken with an alkaline suspension of manganous hydroxide, the hydrocarbon and aqueous phases are separated, and the aqueous phase is acidified in the presence of iodide. The resultant iodine is titrated with thiosulfate. This method, which has been proposed for the determination of dissolved oxygen in sweetened gasolines, presents considerable difficulty in the separation of hydrocarbon from caustic–manganese oxide suspensions, particularly when applied to fairly viscous fuels, such as kerosine and jet fuels.

The method proposed herein is a further modification of the Winkler method, which eliminates the problems of phase separation that are encountered in the Schulze procedure and is not significantly influenced by the mercaptan concentrations found in most fuels. Fuels are shaken with an alkaline suspension of manganous and ferrous hydroxides and the system is acidified prior to separating the fuel and aqueous phases. The net effect is to form ferric ion in proportion to the amount of oxygen dissolved in the sample. The amount of ferric ion is determined iodometrically. Both the classical Winkler method and the Schulze procedure use the iodide ion rather than the ferrous ion as a reducing agent. A reducing agent is required when the system is acidified; otherwise the manganic ion would go through a disproportionation reaction to yield the manganous ion and manganese dioxide. The dioxide is not soluble in acids of the strength used in this work and would not be as easily determined as either iodine or the ferric ion. The ferrous-ferric system is used in the proposed method, because it permits acidification in the presence of the fuel. If iodide were used, the resulting iodine would both dissolve in and possibly react with the fuel. For this reason the fuel and aqueous phases must be separated prior to acidification in the Schulze procedure (*15*).

Organic peroxides interfere in the proposed procedure and presumably a similar interference would be found in the Schulze method (*15*). However, an empirical correction is proposed to minimize errors due to peroxides. The method has been tested on a variety of fuels, solvents, and pure hydrocarbons and is believed to be generally applicable to liquids that are immiscible with water. It should also be applicable to partially or fully miscible liquids, provided these do not react with iodine and there is no interference with the starch indicator; however, the method has not been tested on such compounds. The method uses simple apparatus and common reagents. It requires 1.5 to 2 hours per determination, but several analyses can be run simultaneously.

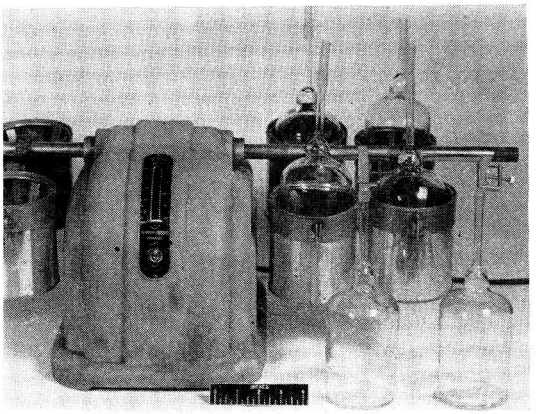

Figure 1. Apparatus used in determination of dissolved oxygen

APPARATUS

The fuels were shaken with the reagents in 500-ml. sealing ampoules similar in shape to the bottles in which bromine is sold. These were cylindrical glass bottles about 8 cm. in outside diameter and 12 cm. long, with a tubulature 1.0 cm. in outside diameter. Short lengths of Tygon tubing $^5/_{16}$ inch in inside diameter were fitted over the tubulature to leave a 5-cm. free length of tubing. The ampoules were closed by using pinchclamps on this free length. The ampoules were shaken with a rocking motion by a Burrell Model C12 shaking machine which had been fitted with cups to hold the ampoules. The ampoules and modified shaking machine are shown in Figure 1. It is probable that other containers and shaking methods would be equally effective.

REAGENTS

Manganous-ferrous solution, containing approximately 0.2*N* manganous sulfate, 0.2*N* ferrous ammonium sulfate, and 0.2*N* hydrochloric acid.
Sodium hydroxide, approximately 2.5*N*.
Hydrochloric acid, approximately 5.0*N*.
Potassium iodide crystals.
Sodium thiosulfate, approximately 0.1*N*, standardized.
Starch indicator.

PROCEDURE

Place 50 ml. of the manganous-ferrous solution in an ampoule and connect via the Tygon tubing to one leg of a three-way stopcock. Connect the other two legs to vacuum and to a source of oxygen-free gas. A vacuum of ca. 5 mm. absolute is adequate and any convenient inert gas may be used; propane was used in this work. Draw a vacuum on the ampoule until the solution boils for a few seconds under the reduced pressure and then admit the inert gas to near atmospheric pressure. Repeat the evacuation and repressurization three times to flush out atmospheric oxygen. Finally evacuate for 1 to 2 minutes and close off the

ampoule with a pinchclamp. Disconnect from the stopcock and, using a small funnel, add 20 ml. of sodium hydroxide without breaking the vacuum. Squeeze out any air bubbles trapped in the tubing before opening the pinchclamp. Leave the last few drops of caustic above the pinchclamp as a precaution against air's leaking into the ampoule. Add 250 to 300 ml. of fuel in the same manner.

Place the ampoule in a shaking machine and shake for 30 minutes. Then add 25 ml. of 5*N* hydrochloric acid and shake manually for a few seconds to neutralize the caustic. Open the pinchclamp and drain the contents of the ampoule into a separatory funnel. Wash the ampoule with 20 ml. of water and add this water to the separatory funnel. Drain the aqueous phase from the separatory funnel into an Erlenmeyer flask, add 5 grams of potassium iodide crystals, allow the solution to stand for 10 minutes, and titrate with 0.1*N* sodium thiosulfate, using starch solution indicator. Record the volume of thiosulfate required and then set the flask aside for approximately 30 minutes. If, at this time, color has returned to the starch indicator, titrate again with thiosulfate and add this volume to the first reading.

As the ferrous-manganous–hydrochloric acid solution is slowly oxidized by air, run a blank upon this solution each time it is used, repeating the above procedure in all its steps except the addition of the fuel sample.

Measure the volume of sample left in the separatory funnel, and calculate the amount of dissolved oxygen by the following equation:

$$\text{Dissolved oxygen, ml. (STP) per liter} = \frac{\text{net ml. of } Na_2S_2O_3 \text{ (sample} - \text{blank)} \times \text{normality} \times 5603}{\text{ml. of sample}}$$

RESULTS AND DISCUSSION

The repeatability of this procedure is shown in Table I by the results from quadruplicate tests run on seven fuels. One result (for kerosine) was omitted from the average, because the Dean and Dixon rejection coefficient (*3*) showed that there was a greater than 90% probability of experimental error. A standard deviation, σ, of 0.67 ml. per liter was found for the method.

Table I. Repeatability of Dissolved Oxygen Determinations with Air-Saturated Fuels

Fuel	Dissolved Oxygen, Ml.(STP)/Liter	Av.
Benzene	30.8, 33.4, 34.0, 33.0	32.8
Toluene	40.9, 41.0, 41.0, 40.8	40.9
n-Heptane	62.0, 62.4, 61.4, 61.2	61.8
Cyclohexane	46.6, 46.7, 46.4, 46.5	46.6
Kerosine	38.8, 39.0, 39.3, (37.1)[a]	39.0
Mineral oil, light	28.7, 28.3, 28.1, 27.9	28.3
Clear gasoline	41.4, 42.3, 42.7, 42.7	42.3

[a] Value in parentheses omitted from average. Standard deviation, σ, = $\sqrt{\frac{\Sigma x^2}{n-7-1}} = 0.67$.

Table II. Dissolved Oxygen Content of Air-Saturated Petroleum Fractions and Pure Hydrocarbons

Substance	Gravity, °API	Boiling Range, ° F.	Reid Vapor Pressure[a]	Saturation Temp., ° C.	Saturation Pressure, Mm. Hg	Dissolved Oxygen, Ml.(STP)/Liter	Ostwald Coefficients
Benzene	..	...	..	26	748	32.8	0.200
Toluene	..	...	..	26	751	40.9	0.224
p-Xylene	..	...	..	27	750	38.4	0.206
n-Hexane	..	...	..	23	749	58.0	0.372
n-Heptane	..	...	..	24	752	61.8	0.344
Iso-octane	..	...	..	25	745	64.7	0.368
n-Dodecane	..	...	..	25	748	38.3	0.202
n-Hexadecane	..	...	..	26	750	36.3	0.192
Kerosine	44.2	340–530	0	26	749	39.0	0.205
Varsol	49.2	350–388	0	26	744	44.7	0.238
Light mineral oil	30.4	...	0	28	748	28.3	0.151
Motor gasoline	60.0	...	9.0	26	743	30.4	0.305
Furnace oil No. 2	35.6	356–622	0	24	742	33.2	0.176
100/130 gasoline	71.4	110–328	6.4	22	747	48.8	0.353
Clear gasoline	58.7	112–351	5.3	26	744	42.3	0.306
JP-4	52.9	160–422	2.2	24	753	42.8	0.248
JP-5	42.2	360–502	0	23	745	39.3	0.206

[a] Measured on another sample after air saturation to eliminate effect of loss of light ends.

No experiments were run which directly show the sensitivity of the method. However, data were obtained on two jet fuels which contained relatively small amounts of dissolved oxygen. These were prepared by saturating the fuels with a gas containing 2.0 volume % of oxygen; this gas was made by taking a tank of air at 1.0 atm. and pressurizing it to 10.5 atm. with cylinder nitrogen. Dissolved oxygen determinations were made on these fuels and also on the same fuels when saturated with air. The experimental values are shown below. Also shown are the solubilities calculated for the 2% oxygen-saturated solutions using the data on air-saturated fuels and Henry's law.

Sample	Dissolved Oxygen, Ml. (STP)/Liter: Found, Air Saturated	2% O_2 Saturated: Found	2% O_2 Saturated: Calcd.	Difference
A	46.8	4.3	4.5	0.2
B	50.1	4.4	4.8	0.4

These data show that accuracy is preserved down to relatively low concentrations of dissolved oxygen. The differences are well below the standard deviation for air-saturated fuels.

Table II lists the dissolved oxygen concentration found for various air-saturated hydrocarbons. Because saturation was accomplished by bubbling large excesses of air through the solvent, the dissolved oxygen should be 0.21 that obtained with pure oxygen. Therefore Ostwald coefficients—i.e., the volume of gas at the pressure and temperature of the experiment dissolved in one volume of solvent—for pure oxygen were calculated and are also listed in Table II. The calculation of these coefficients requires the vapor pressures of the hydrocarbons at the temperature of the air-saturation experiment and these were taken from the data of Rossini (*14*) for the pure hydrocarbons and estimated (*12*) from the Reid vapor pressure for the commercial fuels.

As an example, benzene, when air-saturated at 26° C. and 748 mm. total pressure, had a dissolved oxygen content of 32.8 ml. per liter (Table II) or 0.0328 ml. per ml. of solvent. For pure oxygen the solubility would be $0.0328 \times \frac{100}{21} = 0.156$ ml. of oxygen per ml. As the vapor pressure of benzene is 100 mm. at 26° C. (*14*), the partial pressure of air was 748 − 100 = 648 mm. Converting the 0.156 ml. of oxygen from standard temperature and pressure to the conditions of air saturation (26° C. and 648 mm.) gives 0.200 as the Ostwald coefficient for benzene. The vapor pressures of dodecane, hexadecane, and fuels with zero Reid vapor pressure are so low that they need not be considered.

An attempt was made to estimate the accuracy of the proposed method by comparing data for air-saturated hydrocarbons with literature values for the solubility of oxygen in the same solvents. Comparison was made on the basis of Ostwald coefficients (Table III). The repeatabilities of both this method and the several methods proposed in the literature are good, well below 0.01 unit in Ostwald coefficients. For reasons unknown, the differences among the literature Ostwald coefficients for benzene and toluene are considerably greater than the repeatabilities of the several methods. Perfect agreement is shown between the literature values for *n*-hexane and iso-octane (*5, 6*); however, both references are by the same authors. Only single literature values could be found for *n*-heptane and cyclohexane. Because of spread in some of the literature Ostwald coefficients, the accuracy of the method cannot be stated, but the comparisons of Table III suggest that the accuracy of the method is probably within ±10% of the actual amount.

As peroxides readily oxidize the ferrous ion and are often determined by means of this reaction (*18*), the results of the dissolved oxygen con-

centrations are certain to be high in the presence of these compounds. If peroxides in fuels reacted completely with the ferrous-manganous reagent, one peroxide number—i.e., 1 meq. per liter—would be equal to 5.6 ml. of oxygen per liter of fuel. It has been shown (*18*), however, that many peroxides have efficiencies well below 100% in the conversion of ferrous to ferric ion; therefore a study was made of the effect of peroxides on this dissolved oxygen determination. The fuels used for this study were peroxidized by the action of pure oxygen in the presence of sunlight or by the addition of measured quantities of organic peroxides to the fuels.

The results are shown in Table IV, along with data taken from Wagner, Smith, and Peters (*18*). These data show that oxidation of both ferrous and ferrous-manganous systems by peroxides was far less than theoretical; the average oxidation efficiency of the peroxides was 21% for the ferrous-manganous system and 40% for the ferrous system. A correction, therefore, of 0.3 of theoretical is proposed for this method. This correction is about midway between the above mentioned average oxidation efficiencies. The correction factor amounts to 5.6 × 0.3 = 1.7 ml. of oxygen per liter of fuel per peroxide number of the fuel; it should be subtracted from the determined dissolved oxygen content. This correction, even though it is empirical and based on limited data, should rarely be in error by more than 2.0 ml. of oxygen per liter of fuel per peroxide number for commercial fuels. The errors might be slightly larger for pure compounds.

The method of Schulze, Lyon, and Morris (*15*) is recommended only for sweetened gasolines, presumably because the mercaptans in unsweetened gasolines are extracted by the alkaline manganous-manganic oxide suspension and subsequently oxidized by iodine. This gives low results for dissolved oxygen. A similar effect of mercaptans on the results of the proposed procedure would be expected only if the mercaptans remained in the aqueous phase after acidification, as the iodide ion is not added until after acidification and phase separation. Two fuels, iso-octane and kerosine, each containing approximately 0.01 weight % mercaptan sulfur as added *n*-butyl mercaptan, were studied in order to evaluate the effect of mercaptans on the results of this method (Table V).

Table III. Experimental and Literature Ostwald Coefficients

Hydrocarbon	Temp., ° C.	This Work	Literature[a] (*5*)	(*6*)	(*9*)	(*10*)	(*11*)	(*13*)
Benzene	26	0.200	...	0.228	0.224	0.174	...	0.209
Toluene	26	0.224	0.212	0.232	...	0.179	...	...
n-Hexane	23	0.372	0.359	0.359	...	...	...	...
n-Heptane	24	0.344	...	0.353	...	...	...	...
Iso-octane	25	0.368	0.368	0.368	...	...	0.373	...
Cyclohexane	25	0.282	...	0.222[b]	...	...	...	...

[a] Literature values interpolated to experimental saturation temperatures.
[b] Value questioned by authors (*6*).

Table IV. Oxidation Efficiencies of Peroxides

Substance	This Work: Dissolved oxygen found, ml./liter	This Work: Peroxide[a] No.	This Work: Oxidation efficiency based on dissolved O_2 analysis	(*18*) Table I: Peroxide No.[b]	(*18*) Table I: Oxidation efficiency based on peroxide analysis
Cumene	37.4	1.6[c]	..	...	...
Cumene + cumene peroxide	45.9	9.1	20	13.86	73.5
Tetralin	17.5	1.8[c]	..	...	...
Tetralin + Tetralin peroxide	46.0	17.8	32	7.92	25.5, 27.0
Iso-octane	65.1	0	..	...	...
Iso-octane + di-*tert*-butyl peroxide	70.5	5.5	18	...	...
JP-4	42.5	1.2[c]	..	...	...
JP-4, peroxidized	43.8	3.8	8	...	...
JP-5	39.5	0.3[c]	..	...	...
JP-5, peroxidized	47.8	6.4	25	...	...
tert-Butyl hydroperoxide	..	...	..	6.59	78.7, 79.1
Hydrogen peroxide	..	...	..	11.59	12.2
Benzoyl peroxide	..	...	..	10.10	13.6, 13.9
Ascaridole	..	...	..	8.40	31.7
			Av. 21		40

[a] Anlysis by method of (*17*).
[b] Calculated from data originally presented in terms of grams per liter.
[c] As received from commercial source.

Table V. Effect of Mercaptans on Dissolved Oxygen Determination

Sample	Dissolved Oxygen, Ml./Liter	Av.
Iso-octane	64.8, 65.4, 64.4, 64.2	64.7
Iso-octane + 0.01 mercaptan S	63.2, 63.6, 63.5	63.4
Kerosine	38.8, 39.0, 39.3	39.0
Kerosine + 0.01 mercaptan S	35.8, 35.7, 37.8	36.4

The data indicate that mercaptan sulfur concentrations of the order of 0.01 weight %, which are abnormally high for ordinary fuels, have but a minor effect on the dissolved oxygen results. There was no significant change in the mercaptan concentration of these fuels during the dissolved oxygen determination. It is believed that no significant inaccuracies due to mercaptans will result when the method is applied to normal petroleum products; for these products the mercaptan concentration is usually well below 0.005%.

Considerable difficulty was experienced during the development of this method, in obtaining permanent end points in the iodometric determination of the ferric ion. Swift (*16*) has shown that permanent end points can be obtained for this titration if at least 3 grams of potassium iodide and acid strengths of 0.25 to 12 meq. of hydrochloric acid in 30 ml. of solution are used for the iodide reaction. His conditions may not be applicable to the reactions in this procedure, as his solutions did not contain manganese. It was found in the development of this procedure that end points are nearly permanent if the acid strength used for the iodide reaction is about 0.5 to 1.0 meq. of hydrochloric acid per milliliter of solution. The amounts of reagents specified in this procedure give final acid concentrations within these limits.

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Colorimetric Determination of Nitromethane in the Presence of Other Nitroparaffins

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In a specific method for determining nitromethane in the presence of other nitroparaffins sodium 1,2-naphthoquinone-4-sulfonate reacts with nitromethane in an alkaline solution to yield a violet complex. This complex, when extracted with isoamyl alcohol (3-methyl-1-butanol) can be quantitatively measured at 585 mμ. The complex follows Beer's law in the range of 5 to 30 γ of nitromethane. The method has an accuracy within ±2% and a precision within ±1%. A specific method for separating nitromethane from other nitroparaffins depends upon an azeotropic distillation with methanol. This separation step effectively extends the lower limits of the colorimetric procedure so that as little as 1 part of nitromethane in 10,000 parts of other nitroparaffin may be quantitatively determined.

THE quantitative determination of any one member of the nitroparaffin series in the presence of the others is difficult because of closely related chemical properties. However, nitromethane, the simplest member, exhibits a few unique and characteristic color reactions.

Desvergenes (*3*) measured the yellow color formed with alkalies, Bose (*1*) used the Griess-Ilosvay reactants to determine the nitrous acid split off by alkaline degradation, and Meyer and Locher (*8*) formed nitrolic acids with nitrous acid. These tests apply to nitromethane as well as all other primary nitroparaffins. Manzoff (*6*) described a specific test for nitromethane using vanillin and ammonia as reagents, but Machle, Scott, and Treon (*5*) found that the test did not follow Beer's law and had a limited use. Meyer and Ambuhl (*7*) coupled diazonium salts with the alkali salts of the nitroparaffins to obtain highly colored phenylhydrazones of nitroaldehydes. Turba, Haul, and Uhlen (*10*) developed the diazonium reaction into a quantitative method for all primary nitroparaffins. This reaction was more specific for nitromethane when a buffer was used, but the other primary nitroparaffins interfered appreciably when present in high concentrations.

Recently, Turba, Haul, and Uhlen (*10*) presented a qualitative test for the trace detection of nitromethane. They found that nitromethane condenses with sodium 1,2-naphthoquinone-4-sulfonate in an alkaline solution to give a violet colored complex. In the present work this complex was found to be suitable for quantitative measurement at 585 mμ when extracted from the reaction mixture with isoamyl alcohol. The color develops rapidly, is stable for 1 hour in the solvent, and follows Beer's law in the range of 5 to 30 γ of nitromethane. The reaction is specific for nitromethane.

BASIC PROCEDURE

Apparatus. Spectrophotometer, Beckman Model DU with 1-cm. Corex cells.

Centrifuge, any laboratory type.

Flowmeter, Fisher Laboratory Model 11-163 or equivalent.

Aeration apparatus (*4*).

Distillation column, Penn State-type column 16 mm. in inside diameter and 150 cm. long, packed with $^3/_{16}$-inch single-turn glass helices. Wound with 182 turns of No. 22 Nichrome wire.

Reagents. Nitromethane standard, 99.99% mole, by freezing curve (*9*).

Methanol, anhydrous.

Phosphate Buffer, K_2HPO_4, 20 ± 0.5% aqueous solution of dibasic potassium phosphate adjusted to pH 9.50 with a saturated solution of tripotassium phosphate.

Isoamyl alcohol, purified by fractional distillation and saturated with phosphate buffer, pH 9.50.

Sodium 1,2-naphthoquinone-4-sulfonate, Eastman Kodak No. 1372, 0.10% aqueous solution. This solution is not stable, and is prepared fresh daily.

Preparation of Calibration Curve. Prepare a solution of nitromethane standard in water to contain 1.00 mg. per ml. Transfer 0.0-, 0.50-, 1.00-, 2.00-, and 3.00-ml. portions to separate 100-ml. volumetric flasks and dilute each to volume with water. Transfer 1.00 ml. of each dilution into a test tube. Add 10.0 ml. of the buffer and 1.0 ml. of the color reagent and mix. Allow the solution to stand at room temperature for 15 minutes. Add 5.0 ml. of the isoamyl alcohol and mix. Separate the phases by centrifuging. Transfer a portion of the upper isoamyl layer to a 1-cm. Corex cell and read the absorbance at 585 mμ, using the solution containing 0.0 ml. of standard as the blank.

Plot concentration against absorbance on linear graph paper. The above standards equal 0, 5, 10, 20, and 30 γ of nitromethane, respectively.

Determination. The determination is the same as the calibration, except that the sample is prepared so that a 1.00-ml. aliquot contains no more than 30 γ of nitromethane. A blank is prepared from 1.00 ml. of water treated with the same reagents as the sample.

Interference. Several additional aliphatic nitroparaffins were tested to determine the specificity of the color reaction: nitroethane, 1-nitropropane, 2-nitropropane, 1-nitrobutane, 2-nitrobutane, 1-nitro-2-methylpropane, 2-nitro-2-methylpropane, and 2,2-dinitropropane. Only nitromethane gave a positive reaction.

Applications. Although other nitrocompounds do not yield a colored complex, excessive amounts of primary nitroparaffin (nitroethane, 1-nitropropane, 1-nitrobutane, and 1-nitro-2-

Table I. Effect of Nitroparaffins on Determination of Nitromethane

Composition of Mixtures, Wt. %						Nitromethane Found, Wt. %	Difference, Wt. %
Nitroethane	1-Nitropropane	1-Nitrobutane	2-Nitropropane	2-Nitrobutane	Nitromethane		
19.50	13.40	14.55	22.35	20.15	10.05	10.00	−0.05
21.30	20.65	16.10	19.00	16.60	6.35	6.32	−0.03
11.65	9.82	15.20	21.05	18.63	23.65	23.67	+0.02
24.90	25.02	...	23.95	...	26.10	26.08	−0.02
98.90	...	...	...	...	1.10	0.34	−0.76
...	99.05	...	...	...	0.95	0.41	−0.54
...	...	98.60	...	...	1.40	0.69	−0.71
...	...	...	98.94	...	1.06	1.05	−0.01
...	...	...	...	99.30	0.70	0.70	0.00

Table II. Determination of Nitromethane in Air

Calcd., γ	Found, γ
1000	995
500	509
100	99
50	51

Table III. Stability of Colored Complex in Isoamyl Alcohol

Time, Min.	Absorbance of Nitromethane 10 γ	20 γ	30 γ
0	0.340	0.680	1.020
15	0.340	0.680	1.020
30	0.340	0.679	1.020
60	0.339	0.680	1.020
75	0.335	0.676	1.115

Table IV. Effect of pH on Color Development

pH	Absorbance, 20 γ NM
8.50	0.350
9.00	0.615
9.50	0.680
10.00	0.670
10.50	0.640

methylpropane) inhibit the color formation between nitromethane and sodium 1,2-naphthoquinone-4-sulfonate (Table I). When the nitromethane content is below 5% weight, it must be separated before analysis.

Determination of Nitromethane. IN PROCESS SAMPLES. In the preparation of derivatives from nitromethane where relatively pure (95% or better) nitromethane is the starting material, no interference is encountered due to the presence of other primary nitroparaffins.

Weigh the sample into a volumetric flask containing water, neutralize, and dilute to volume with water. From this solution, prepare a diluted sample such that a 1.00-ml. aliquot will contain 30 γ or less of nitromethane, and analyze.

IN AIR SAMPLES. Pass the air containing nitromethane vapor through the aeration train with each tube containing 20 ml. of phosphate buffer. Use a flow rate of 0.2 liter per minute. At the end of the sampling period, combine the buffer solutions from the three tubes into a volumetric flask, dilute to volume with water, and analyze a 1.0-ml. aliquot containing 30 γ or less of nitromethane.

Nitromethane determinations in known concentrations of air samples are presented in Table II.

Effect of Variables. The influence of several variables on the color formation and quantitative applications of the reaction was investigated: absorption curve, Beer's law, stability and intensity of color, time and temperature of reaction, concentration of reagents, pH of reaction, and azeotropic distillation.

ABSORPTION SPECTRUM. The absorbance curve of the violet complex of nitromethane and sodium 1,2-naphthoquinone-4-sulfonate shows maximum absorbances at 387 and 585 mμ. The maximum at 585 mμ was the stronger and was chosen for use.

TRANSMITTANCY AND CONCENTRATION. Calibration curves were determined for nitromethane by plotting absorbance against concentration. A straight-line calibration curve, originating at zero concentration and absorbance, is obtained for the range of 5 to 30 γ of nitromethane.

TIME AND TEMPERATURE OF REACTION. The colored complex was prepared and spectrophotometer readings were made intermittently to determine the time required for complete color development. A time of 15 minutes at room temperature was chosen as optimum.

COLOR STABILITY. The absorbance of the colored complex was determined at various time intervals (Table III). The color was stable for only 6 minutes in the buffer solution after the 15-minute development period. However, it was stable for 1 hour after extraction into isoamyl alcohol.

pH OF REACTION. The color development was determined in several phosphate buffer solutions of varying pH. The buffers were prepared from a stock 20% dipotassium phosphate solution and adjusted to the desired pH with either 85% phosphoric acid or a saturated aqueous solution of tripotassium phosphate. Ten milliliters of buffer solution were used and the colors extracted with isoamyl alcohol. The optimum buffer solution, as indicated in Table IV, had a pH of 9.50

STABILITY AND CONCENTRATION OF REAGENT. The stability and concentration of the sodium 1,2-naphthoquinone-4-sulfonate are critical. Aqueous solutions of the reagent are not stable and must be prepared fresh daily to obtain reproducible results.

The color development between nitromethane and the reagent was determined at different levels of reagent concentration (Table V). Excess reagent inhibits the color development. A 1.0-ml. aliquot of a 0.10% aqueous solution of sodium 1,2-naphthoquinone-4-sulfonate was chosen as optimum.

AZEOTROPIC DISTILLATION

The determination of small amounts of nitromethane in mixtures of other primary nitroparaffins is subject to interference (Table I).

Table V. Effect of Reagent Concentration

Concentration, G./100 Ml.	Absorbance, 20 γ NM
0.05	0.680
0.10	0.680
0.20	0.672
0.50	0.515
1.00	0.385

Table VI. Azeotropic Separation of Nitromethane

Added to Distillation Flask, Mg.	Found, 50-Ml. Distillate, Mg.
1.0	1.00; 1.01
3.0	3.00; 3.00
10.0	10.00; 10.03
100.0	99.00; 99.80

Table VII. Determination of Nitromethane Using Azeotropic Separation

Composition of Mixtures, Weight %							Nitromethane Found, Wt. %	Difference, Wt. %
Nitroethane	1-Nitropropane	1-Nitrobutane	2-Nitropropane	2-Nitrobutane	1-Nitro-2-methylpropane	Nitromethane		
33.67	20.00	6.46	20.06	10.02	10.08	0.010	0.010	0.000
41.42	12.63	10.18	17.02	7.80	10.92	0.030	0.030	0.000
37.81	26.60	32.89	...	...	2.69	0.010	0.010	0.000
99.50	...	...	...	...	...	0.500	0.490	−0.010
96.40	1.00	...	2.12	...	...	0.480	0.480	0.000
93.00	1.60	...	4.40	...	...	1.000	0.990	−0.010

Desseigne and Belliot (2) found that nitromethane formed an azeotrope with methanol containing 12.5 weight % nitromethane and boiling at 64.55° C. This provides a convenient method for separating nitromethane, as the higher nitroparaffins do not form azeotropes with methanol.

The separation of nitromethane from other nitroparaffins, by azeotropic distillation with methanol, was studied. Tests were made to establish the type and length of column needed, the most suitable rate of distillate removal, and the volume of distillate necessary to remove nitromethane quantitatively in the range of 1 to 100 mg. Removing 50 ml. of distillate with the described column at a 30 to 1 reflux ratio gave good results. Typical data on the recovery of nitromethane in 50 ml. of distillate are presented in Table VI.

Columns with shorter packed sections or increased take-off rates were not satisfactory.

Determination of Nitromethane Using Azeotropic Distillation. Weigh 1 to 2 grams of the sample into a 500-ml. round-bottomed distillation flask, add 200 ml. of dry methanol, and attach to the fractionating column. Operate the column at total reflux for 1 hour. Remove 50 ml. of distillate at a reflux ratio of 30 to 1, transfer the distillate to a 100-ml. volumetric flask, and dilute to volume with water. Analyze a 1.00-ml. aliquot for nitromethane. Repeat the analysis, using a larger dilution of the distillate if the nitromethane content is high.

Results of the determination of nitromethane in mixtures using azeotropic separation and the described color test are given in Table VII.

ACKNOWLEDGMENT

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Determination of Chromium and Vanadium in Silica-Alumina Cracking Catalyst

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A rapid and accurate wet chemical method for the determination of small amounts of chromium and vanadium in silica-alumina cracking catalyst and similar materials involves amperometric titration with ferrous iron to determine chromium plus vanadium. Vanadium is titrated directly after reoxidation with potassium permanganate, followed by selective reduction of chromium with sodium azide. Chromium is calculated by difference. The precision varies from within ±0.0003% for less than 0.005 weight % to ±1% of the mean for greater than 0.10 weight %.

CHROMIUM and vanadium are two contaminant metals that are known to produce adverse effects on the performance of silica-alumina catalysts in fluid cracking operations. Many methods are reported in the literature for estimation of these metals. Chromium has been determined colorimetrically in many materials with *s*-diphenylcarbazide (*1*); however, large amounts of vanadium interfere. Vanadium is frequently determined colorimetrically with phosphotungstate (*2*). Here again, interfering ions, such as chromium and iron, must be removed.

Willard and Young (*7*) have reported a volumetric method for determination of chromium and vanadium in steel. They used ferrous iron and potassium permanganate with *o*-phenanthroline as indicator. Ducret (*4*) employed ferrous iron as a titrant, with diphenylamine as indicator, to determine total chromium plus vanadium after permanganate oxidation. He then selectively oxidized vanadium with permanganate, destroyed the oxidant with sodium azide, and titrated the vanadium directly. Parks and Agazzi (*6*) employed Ducret's technique, but substituted amperometric titration for the visual indicator titration. They digested the sample briefly with sulfuric and perchloric acids before addition of permanganate. Johnson, Weaver, and Lykken (*5*) used electrodeposition to remove chromium and similar ions from solutions to prevent their interference with the determination of other ions. The methods that appeared most promising for the authors' purpose [*5–7*] were evaluated.

In the method presented here, a modification of the Parks and Agazzi procedure (*6*), samples are digested with hydrofluoric and sulfuric acids to remove silica and with perchloric acid to oxidize chromium and vanadium. Total chromium plus vanadium content is determined by amperometric titration with ferrous solution. Then, after reoxidation, chromium is selectively reduced with sodium azide, and the vanadium is titrated directly. Chromium is calculated by difference.

EQUIPMENT

The equipment consists of a Sargent Model XXI polarograph equipped with a saturated calomel reference electrode, salt bridge, constant-speed stirring motor, and rotating platinum indicator electrode.

REAGENTS

A 0.1*N* stock solution of ferrous ammonium sulfate in 1 to 9 sulfuric acid is made 0.01*N* and 0.001*N* by dilution.

Potassium permanganate is approximately 0.1*N*.

Sodium azide, 10 weight % aqueous, is prepared fresh daily.

RECOMMENDED PROCEDURE

Weigh 1 to 2 grams of silica-alumina catalyst into a 100-ml. platinum dish. Heat slowly to 650° C. in a furnace and ignite about 30 minutes at this temperature. (This ignition step may be omitted if carbonaceous matter is known to be absent.) Wet the catalyst thoroughly with water and add 5 ml. of C.P. concentrated sulfuric acid. Add 15 ml. of 50% hydrofluoric acid in

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Table I. Recovery of Known Amounts of Chromium and Vanadium

	Amperometric with Electrodeposition (5)					Willard and Young with 0.01N Fe and 0.005N $KMnO_4$ (7)				
Chromium, mg.										
Added	0.500	0.040	0.020	0.020	0.012	0.020	0.020	0.020	0.000	0.060
Found	0.510	0.040	0.023	0.019	0.008	0.025	0.024	0.034	0.010	0.020
Vanadium, mg.										
Added	0.500	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.240	0.300
Found	0.460	0.200	0.200	0.214	0.201	0.200	0.208	0.198	0.245	0.254
	Amperometric with Selective Oxidation (6)					Amperometric with Selective Reduction				
Chromium, mg.										
Added		0.040	0.020	0.000		0.040	0.026	0.020	0.14[a]	
Found		...	...	...		...	0.032	0.019	0.125	
Vanadium, mg.										
Added		0.200	0.200	0.200		0.200	0.500	0.400	0.14 ± 0.01[a]	
Found		0.206	0.200	0.200		0.206	0.505	0.402	0.151	

[a] Calculated from NBS values for NBS sample 98.

small increments (caution) and evaporate to sulfur trioxide fumes. Repeat the hydrofluoric acid addition and evaporation. Wash down the walls of the dish with about 15 ml. of water and evaporate to sulfur trioxide fumes.

Add 13 ml. of 72% perchloric acid and transfer the solution to a 150-ml. beaker containing a few boiling stones. Cover, heat to fumes, and allow to simmer 10 minutes after development of a full yellow or orange color. Cool rapidly by dipping and swirling in ice water for 6 to 8 seconds. Add 15 ml. of water, wash down the cover and sides, and boil the solution for 3 minutes. Cool, and titrate amperometrically with ferrous iron solution.

Make the solution up to approximately 75 ml., boil, and add potassium permanganate dropwise until a pink color remains 1 minute. Add sodium azide solution dropwise to the boiling solution to destroy excess oxidant and 1 ml. in excess (add a correspondingly larger excess when more than 0.05 meq. of total titrant is expected). After 30 seconds, remove the solution from the heat and cool in ice water. Titrate the vanadium as above and obtain chromium by subtracting the milliequivalents of vanadium (second titration) from the total number of milliequivalents (first titration).

APPLICATION TO KNOWN MIXTURES

Solutions of known chromium and vanadium contents were analyzed by the method presented and by three other methods for comparison (Table I).

Amperometric titration with electrodeposition permits good recovery of chromium and vanadium; however, this procedure requires extra equipment (electrodeposition unit) and about 1 hour more time per analysis than the proposed method.

The method of Willard and Young showed the poorest accuracy. End point determination was difficult with the visual indicator in the presence of large amounts of buffering salts, especially for the lower concentrations.

The method of Parks and Agazzi, which utilizes selective oxidation of vanadium, gives good recovery of vanadium. However, oxidation and reduction in the cold are slow.

While working with the Parks and Agazzi method, it was found possible to oxidize the boiling solution with permanganate and, then, selectively reduce the chromium with sodium azide. This method of selective reduction of chromium is new. The data show no difference in accuracy between the selective oxidation and reduction methods; however, the latter requires about 10 minutes less time per analysis. One National Bureau of Standards sample (No. 98) similar in composition to the fluid cracking catalyst was analyzed by the new procedure (Table I).

ANALYSIS OF CATALYST SAMPLES

Two silica-alumina cracking catalysts were analyzed by the four procedures (Table II).

The Willard and Young method is untenable for the catalyst samples studied because of difficulties with buffering in the presence of large amounts of alumina, and indicator difficulties with the low concentrations involved.

Electrodeposition removes the chromium satisfactorily; however, it requires too much time.

Values obtained by the selective oxidation and reduction methods are of the same magnitude; however, those by the selective reduction method show better repeatability.

The deviation, at 95% confidence level (3), for duplicate determinations has been calculated for the new procedure using approximately 100 analyses for various concentration ranges. The deviation was ± 0.0003 for less than 0.005 weight %, ±

Table II. Determination of Chromium and Vanadium in Catalysts

	Amperometric Titration			
	Selective reduction of chromium	Selective oxidation of vanadium	Electrodeposition	Willard and Young Procedure
Catalyst 1				
Cr, wt. %	0.0041	0.0058	0.0052	0.0056
	0.0051	0.0049	0.0052	0.0043
	0.0043	0.0036		
	0.0046	0.0038		
V, wt. %	0.041	0.044	0.043	0.043
	0.041	0.041	0.043	0.050
	0.041	0.040		
	0.041	0.042		
Catalyst 2				
Cr, wt. %	0.0010	0.0012	0.0011	0.0011
	0.0012	0.0011	0.0007	
	0.0009	0.0011		
	0.0010	0.0015		
V, wt. %	0.0030	0.0029	0.0030	0.0029
	0.0029	0.0029		
	0.0028	0.0026		
	0.0028	0.0020		

0.0005 for 0.005 to 0.01 weight %, ± 0.001 for 0.01 to 0.10 weight %, and ± 1% of the mean for greater than 0.10 weight %.

CONCLUSION

Amperometric titration with ferrous iron combined with either selective reduction of chromium or oxidation of vanadium affords a satisfactory method for determination of chromium and vanadium in silica-alumina cracking catalyst. Selective reduction gives better repeatability and can be handled in about 10 minutes less per analysis than selective oxidation. It is therefore recommended.

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Gradient Elution Chromatography of Phosphates

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This investigation was undertaken to improve the ion exchange chromatographic method for phosphate mixtures by employing a gradient, rather than a discontinuous, elution technique. Resolution of five phosphates from mixtures is demonstrated. Several advantages, including a shorter procedure, are obtained.

A KNOWLEDGE of the distribution of phosphate polymers is of considerable interest to the producers of synthetic detergent compositions from the point of view of phosphate stability, purity of raw materials, analysis of final products, and the kinetics of hydrolytic degradations. The present method, which is employed routinely in the authors' laboratory, is based upon previously published data and techniques (*2–4*). The published techniques call for a discontinuous (stepwise) increase in the eluent concentration by siphoning from a series of reservoirs.

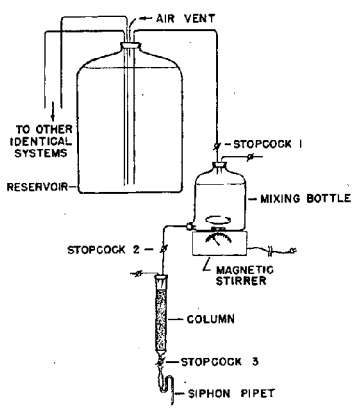

Figure 1. Diagram of apparatus

The present method employs the modification, commonly called gradient elution, in which the eluent concentration is increased continuously. Several advantages are realized with the gradient-elution technique: Only one standard potassium chloride solution, instead of a series, is needed; a higher flow rate (and consequent 35 to 40% reduction in elution time) is permissible; and the frequently observed "tailing" of triphosphate is eliminated.

APPARATUS

The apparatus is shown in Figure 1. A reservoir (16-liter carboy) is equipped with rubber tubing to feed solution to as many systems as is convenient. Solution feeds into an air-tight mixing bottle of 1-liter capacity, which in turn rests on a magnetic stirrer. Solution from the mixing bottle can then flow (or is siphoned) into the ion exchange column. Inlet tubing with stopcocks at the top of the mixing bottle and at the top of the column permits slight pressure to be applied. Stopcocks are fitted between the reservoir and the mixing bottle, between the mixing bottle and the column, and at the bottom of the column.

The resin employed is AG-1X8 anion exchange resin, 100–200 mesh (capacity = 3.2 meq. per dry gram), purchased from BIO-RAD Laboratories, 800 Delaware St., Berkeley, Calif. The prewashed resin fills the glass column (equipped with a medium-porosity fritted-glass disk) of 3.80-sq. cm. cross-sectional area to a height of 19.0 cm. A siphon pipet (*5*) is used to deliver equal volumes (approximately 11 ml.) of eluate from the column. The siphon pipet employed was purchased from the Ace Glass Co., Vineland, N. J., as a Rieman pipet.

REAGENTS

The eluent solution for the reservoir was 1.00*M* potassium chloride containing 100 ml. of stock solution of acetate buffer for each 16 liters of total eluent. A pinch of phenyl mercuric acetate was added to prevent mold growth.

The stock solution of acetate buffer was 0.80*M* potassium acetate containing enough glacial acetic acid to give pH 5.0.

To prepare nitric acid for hydrolyzing polymers, concentrated nitric acid was mixed with an equal volume of water.

PROCEDURE

Before the elution is started, the resin in the column must be in the chloride form and the interstices of the column bed filled with water. Polymeric phosphates remaining from previous experiments should be removed by allowing the resin to remain in contact with 1*M* hydrochloric acid overnight, followed by passage of 0.5 to 0.6 liter of acid, to ensure that the resin is in the chloride form. The acid is washed out of the column before use with about 0.2 liter of water.

The phosphate solution to be studied is diluted so that an aliquot of convenient volume (5 to 20 ml.) will contain 2.5 to 4.5 mg. of phosphorus. Such an aliquot is then pipetted onto the top of the column. The column is drained through stopcock 3, so that the level of liquid coincides with the level of the resin bed.

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One liter of water is introduced into the mixing bottle and the stirrer turned on. Sufficient pressure is applied in the mixing bottle so that eluent does not flow when stopcock 1 is opened. Then, sufficient pressure is applied at the top of the column to prevent liquid from entering the column when stopcock 2 is opened. The elution is then started by opening stopcock 3. With the aid of stopcock 3, the flow rate is adjusted to 5.7 to 6.1 ml. per minute. (The volume of solution in the mixing bottle thus remains constant at 1 liter.)

Fractions of approximately 22 ml. (two siphon pipetfuls) are collected in 100-ml. volumetric flasks. The number of fractions collected will depend on the number of phosphate species in the sample, on the capacity of the siphon pipet, and to a lesser degree, on the particular apparatus and batch of resin employed.

To each fraction is added 10.0 ml. of the nitric acid solution and each flask is allowed to stand in boiling water for 30 to 40 minutes, for the purpose of hydrolyzing the polymeric phosphates to orthophosphate. The flasks are then cooled to room temperature and orthophosphate is determined colorimetrically as the yellow phosphovanadomolybdate complex (*1*). A blank for the colorimetric measurements is prepared by treating 10 ml. of 1.0*M* potassium chloride in the above manner.

Those quantities of phosphorus found in the various fractions which represent one given phosphate species are added together to yield the total weight of phosphorus derived from that species. Another aliquot of the original sample, which is equivalent to about one twentieth of the total quantity taken on the column, is hydrolyzed as above and analyzed (in duplicate) for the total phosphorus taken. This permits the calculation of the percentage of the total phosphorus found in each phosphate and the percentage of the total phosphorus which was recovered from the column.

DISCUSSION

Shown in Figure 2 is a typical elution of a mixture of five phosphates—the monomer, dimer, trimer, cyclic trimer, and cyclic tetramer. The number of siphon pipetfuls collected and the time are also shown. The separation of the last three phosphates from each other is not quite complete, but a longer column should improve this.

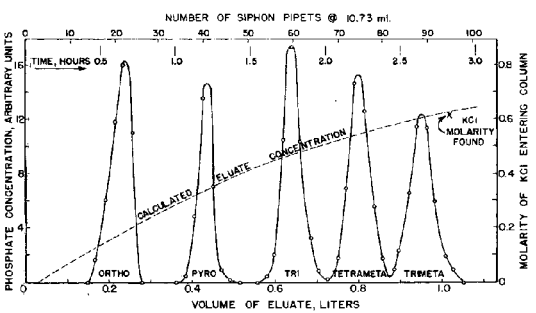

Figure 2. Elution of phosphates, with water initially in mixing bottle

Figure 3 shows an elution of a different mixture of the same phosphates, in which case the mixing bottle contained initially 0.10*M* potassium chloride buffered at pH 5.0 and the column interstices were filled with this same solution before the elution was started. All other conditions were the same as previously described. Although the separation in Figure 3 is superior and the elution required a smaller volume, one of the advantages—the use of only one standard eluent solution—has been lost.

It is the practice in the authors' laboratory to employ the conditions which gave Figure 2 for mixtures known to contain only the first three phosphates, and to use the conditions that gave Figure 3 when higher phosphates are present.

The dashed curves in Figure 2 and 3 were calculated from the following equation:

$$\log \frac{(M_1 - M_2 - M)}{M_1} = -\frac{rt}{2.303V} = -\frac{v}{2.303V}$$

where M_1 is the molarity of the eluent in the reservoir, M_2 is the molarity of eluent initially in the mixing bottle, and M is the molarity of the eluent entering the column at time t(minutes) and rate r(ml. per minute); V is the volume of solution (ml.) in the mixing bottle, and v is the total volume of eluate (ml.) that has entered the column up to time t.

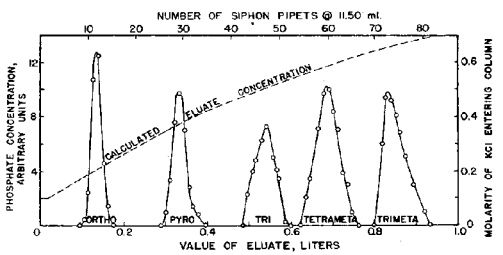

Figure 3. Elution of phosphates, with 0.10*M* potassium chloride initially in mixing bottle

Table I. Analysis of a Known Phosphate Mixture

(% Total Phosphorus)

Phosphate	Taken	Found 1	2	3	Mean
Ortho	20.4	20.6	20.5	21.1	20.7
Pyro	19.8	19.5	19.1	19.6	19.4
Tri	20.2	20.1	19.1	18.9	19.3
Tetrameta	20.0	20.3	20.8	21.0	20.7
Trimeta	19.6	19.5	20.5	19.4	19.8

Because allowance was made for the volume of rubber tubing between the mixing bottle and the column—i.e., 22 ml.—these dashed curves do not start at the origin.

One experimental determination of the concentration of eluate was made and is shown in Figure 2.

The discontinuous-elution method has been shown (*3*) to be subject to a mean error of 0.2%, which was believed to be due largely to the limited accuracy of the colorimetric method, and not to the chromatographic technique.

The results obtained with three continuous elutions of a synthetic mixture are given in Table I. The individual components of the mixture, each at least 95% pure, had previously been analyzed with this same technique.

In each case, the samples taken for elution contained a total of 4.8 mg. of phosphorus in 10 ml. The mean absolute error is 0.5%.

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Paper Disk Electrophoresis

New Device for Separation of Serum Proteins

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A simple paper disk electrophoretic apparatus has been developed and used successfully for the separation of the proteins present in blood serum. The method was standardized by studying the inherent variables, such as design of apparatus, concentration of dye, washing solution, tailing effect, wick size, and current applied. It has several advantages—namely, simplicity and rapidity of performance, the large number of samples that can be analyzed, and the uniformity of conditions that can be attained during analysis of different samples.

WITH the development of electrophoresis on filter paper, almost every worker has constructed apparatus differing only in some operational details from previous models. All can be classed under three types: (1) Durrum (*2*), with free-hanging paper from a vertical apex; (2) Wieland (*7*), with free-hanging horizontal paper; and (3) Cremer and Tiselius (*1*), with horizontal paper between glass plates. Cremer and Tiselius (*1*) and Kunkel and Tiselius (*5*) have been responsible for developments concerning the theoretical basis of paper electrophoresis.

The present paper describes a new, simple, and convenient apparatus for paper electrophoresis on circular filter paper, and includes a study of factors affecting the migration of serum proteins during electrophoresis.

APPARATUS

The apparatus (Figure 1) consists of a Petri dish, *A*, 20.0 cm. in diameter, in which are placed two Petri dishes, *B*, both 16 cm. in diameter, one inverted over the other. The top Petri dish has a hole, *C*, at the center, 1 cm. in diameter. Between these two dishes is another Petri dish, *D*, 9 cm. in diameter. Petri dishes *A* and *D* contain 100 and 40 ml. of buffer, respectively. The circular filter paper, *E*, shown in Figure 2, is kept between the two Petri dishes, *B*, as in circular paper chromatography, so that the V-shaped wedges are in the buffer in dish *A*. A filter paper wick, *F*, 3 × 2 cm., is folded round to a diameter of approximately 0.8 cm. and introduced through the center of the paper. The paper is then covered with the top dish. A buffer-agar bridge, *G*, is introduced through the top hole of dish *B* and finally through the paper wick, so that it reaches the buffer in *D*. The other end of the bridge is connected to electrode vessel *H* (a beaker), filled with buffer and containing a platinum electrode. Another buffer-agar bridge, *I*, is placed between the buffer in the large dish and the other electrode vessel, *J*.

Because the serum proteins are negatively charged under alkaline conditions, they are spotted near the center of the paper, which is made the cathode so that the proteins will move toward the circumference of the paper (anode). The electrodes can be reversed according to the charge of the sample to be analyzed, in order to ensure movement from the center of the paper toward the circumference.

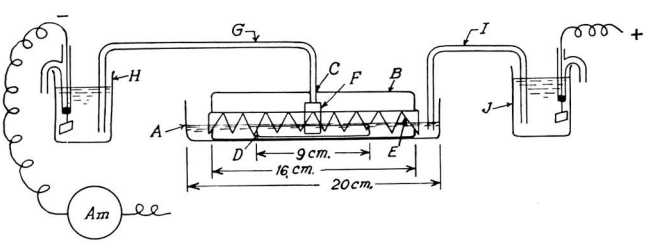

Figure 1. Apparatus for paper disk electrophoresis

A. Petri dish, 20.0 cm. in diameter
B. Petri dishes, 16 cm. in diameter
C. 1-cm. hole in top dish B
D. Petri dish, 9 cm. in diameter
E. Circular filter paper
F. Filter paper wick
G, I. Buffer-agar bridges
H, J. Electrode vessels
Am. Ammeter

MATERIALS

Filter Paper. A circle 16.0 cm. in diameter was drawn on Whatman No. 1 filter paper, which was 21.0 cm. in diameter (Figure 2). The circumference was then divided equally into 16 parts, and 16 equal V-shaped wedges were cut out. One paper was set aside as a master sheet to mark the others.

Buffer-Agar Bridge. This was prepared to give a 3% agar concentration in buffer. The agar was allowed to dissolve in the buffer for 3 to 4 hours at room temperature, and then heated in a water bath at 100° C. When the agar was dissolved, it was poured into glass tubes, 5 mm. in diameter, the ends of which were bent at right angles with a taper at one end. Care was taken to exclude air bubbles. The taper was included because it was observed during experimental operations that the gradual flow of current seems to force the buffer-agar mixture out the end of the tube; the taper minimizes this tendency. The bridges were allowed to set and were kept in a solution of the same buffer when not in use.

Electrodes. The electrodes consisted of a platinum wire fused into one end of a glass tube containing mercury for electrical contact. A piece of platinum foil, 1.5 × 1 cm., was attached to the platinum wire in order to expose a larger area for electrolysis. The electrodes rest on the rim of the beakers as shown in Figure 1.

Buffer. Barbital buffer, pH 8.6, was prepared according to Hardwicke (*3*). It had an ionic strength of $I/2 = 0.05$, and was $0.02M$ in diethyl barbituric acid and $0.1M$ in sodium diethyl barbiturate.

Staining Solution. A solution of 100 mg. of bromophenol blue in 100 ml. of ethyl alcohol saturated with mercuric chloride (33 grams per 100 ml.) was used.

Washing Solution. A solution of acetic acid, 0.5% in water, was used for washing the excess dye from the paper after staining.

PROCEDURE

A current of 8 ma. and a potential of 220 volts direct current were used for all the experiments.

Sample Preparation. A trace of bromophenol blue was added to the serum samples to combine with the albumin present and act as a marker for the length of the run. Spots of 0.01 ml. of this protein solution, total concentration not more than 1 gram per 100 ml., were applied to the paper with a micropipet.

Determination. A circle, 4 cm. in diameter, was drawn with pencil at the center of the filter paper. Twelve small circles, 5 mm. in diameter and equidistant from each other, were drawn on the circumference of this 4-cm. circle. Another circle, 12 cm. in diameter, was drawn on the paper to mark the distance through which the samples were to move. The paper was then soaked in barbital buffer and laid on another filter paper to remove the excess buffer. Then the paper was allowed to rest on the rim of the lower 16-cm. Petri dish, *B*, placed inside dish *A* containing buffer. All the V-shaped

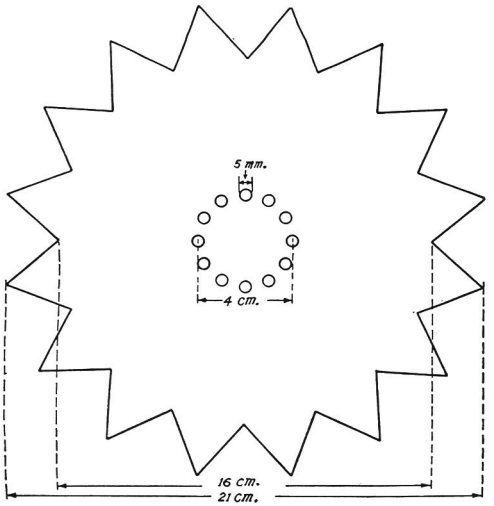

Figure 2. Filter paper disk with V-shaped wedges

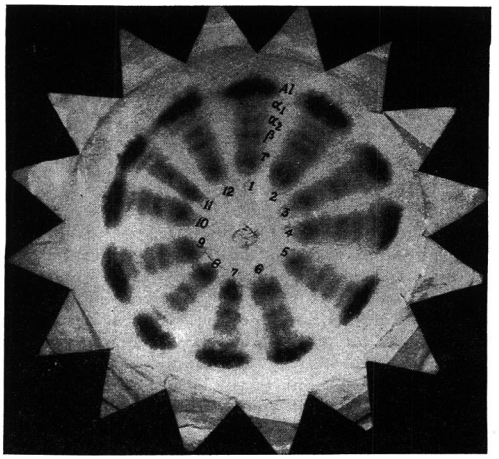

Figure 3. Separated pattern of 12 samples of normal human serum

4-hour run at 8 ma.
Al. Albumin
α_1, α_2, β, γ. Globulin fractions

wedges were then dipped one by one into the buffer in *A* so that they adhered to the side wall of *B* and all the tips of the wedges dipped into the buffer.

The sample solutions were immediately spotted on the wet paper on the 5-mm. circles. The paper wick was introduced at the center and the whole pipe was covered with the top cover of *B* to prevent evaporation of buffer during the passage of current. The buffer-agar bridges were positioned as described previously.

Current was then applied and noted from time to time on the milliammeter. With the passage of current, all the protein samples began to move, forming an arc (as in circular paper chromatography) which was revealed by the blue-stained albumin fraction.

The operation was continued for 4 hours, by which time the marked spots had reached the outer pencil-marked boundary. After separation the whole paper was carefully taken out and placed on a glass plate to prevent further spreading of the separated pattern during subsequent heating. The paper was then heated for 15 minutes at 100° C. in an air oven. After that the paper was dipped in bromophenol blue staining solution for 30 minutes. The paper was given five 10-minute washes with acetic acid solution until the background was colorless, then dried again in the oven at 100° C.

RESULTS AND DISCUSSION

The results of a typical experiment on 12 samples of normal human sera are shown in Figure 3. Because the quantity of bromophenol blue bound to denatured proteins on filter paper is dependent on the time of heat denaturation of the protein, straining time, and amount of rinsing, these conditions were kept constant.

Two types of apparatus were tried. The type described here is one of them; in another type the individual V-shaped wedges were dipped in separate buffer containers and all the buffer receivers were connected to a common buffer tank. Separation of the protein fraction in both cases was radial and uniform. The present device was more simple and convenient, and was used throughout the experiments.

Only bromophenol blue dye was used because this dye is readily available and gives good staining of proteins on paper (*3*). Dye concentrations of 0.1, 1.0, and 2.0% were tried; the 0.1% solution gave better results for the 30-minute staining period used. Similar findings have also been reported by Hardwicke (*3*).

A 0.5% solution of acetic acid gave better removal of excess dye from the paper than did tap water. This solution has also been recommended by others (*3–5*) because it does not remove appreciable amounts of dye from the protein samples. Five 10-minute washes were sufficient to remove excess unreacted dye.

The procedure adopted by Hardwicke (*3*) was followed in order to observe the tailing effect in the method reported here. No tailing effect was observed when egg albumin marked with bromophenol blue was run on a paper; the blue arc was cut out and eluted from the paper with buffer and again run on another paper.

Although wick size is important in circular paper chromatography (*6*), its role in this method was found to be negligible.

To ascertain the effect of time and potential gradient on mobility, experiments were carried out with a pure solution of egg albumin in barbital buffer. The albumin samples were colored with a trace of bromophenol blue. Table I shows that there is a gradual decrease in voltage across the paper with simultaneous increase of current. The mobility unit resulting from these experiments was about 0.006 and relatively constant.

Table I. Rate of Mobility with Time

Time, Minutes (t)	Current, Ma.	Voltage across Paper (V)	Distance Moved, Mm.	Mobility (Mm./V)	Mobility Unit (Mm./V/t)
0	8.0	70.0	...	...	...
20	8.2	68.0	11.0	0.16	0.008
40	8.5	61.0	16.0	0.26	0.006
60	8.8	51.0	18.0	0.35	0.005
80	9.0	46.0	23.0	0.50	0.006
100	9.2	40.0	25.0	0.62	0.006
120	9.4	37.0	28.0	0.74	0.006
140	9.6	36.0	31.0	0.84	0.006
160	9.7	32.0	32.0	0.98	0.006
180	9.8	28.0	34.0	1.20	0.006
200	10.0	30.0	36.0	1.20	0.006
220	10.2	28.0	38.0	1.33	0.006
240	10.3	28.0	40.0	1.40	0.005

When the current was varied between 3.0 and 15.0 ma., it was found that a current of 8 to 10 ma. gave the most distinctly separated pattern of serum protein fractions. At this amperage, water condensed on the top Petri dish, which was replaced by a dry dish when necessary. The momentary break of current flow for this replacement did not affect the separated pattern.

From one to 12 samples can be analyzed with this apparatus. When one sample is to be analyzed, it can be spotted on the

whole circumference of a circle 2 cm. in diameter which is placed at the center of the paper. This gives continuous ring bands of the separated proteins.

ACKNOWLEDGMENT

The author is deeply indebted to S. C. Roy, B. C. Guha, and S. K. Mukherjee of the University College of Science & Technology, Calcutta, India, for their helpful suggestions and kind interest during the work.

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CRYSTALLOGRAPHIC DATA

135. Tripotassium Diuranyl Heptafluoride Dihydrate, $K_3(UO_2)_2F_7.2H_2O$

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TRIPOTASSIUM diuranyl heptafluoride dihydrate was prepared by adding a solution of potassium hydrogen fluoride to an aqueous solution of uranyl nitrate in sufficient volume to prevent immediate precipitation and allowing the solution to evaporate at room temperature. Uranium by chemical analysis was 57.3%, calculated 57.61%.

CRYSTAL MORPHOLOGY

System and Class. Monoclinic, prismatic.

Axial Elements. $a:b:c = 0.7955:1:0.537$; $\beta = 94° 40'$. Baker (*1*) gives "approximate elements" $a:b:c = 0.918:1:0.978$; $\beta = 114°$ or, after transformation by matrix $(\bar{1}01/020/\bar{1}01)$ to conform to the orientation of the unit cell, $a:b:c = 0.800:1:0.510$; $\beta = 93°$.

Habit. Rectangular plates, flattened {101}, elongated [010] with {100}, {010}, {001}, and {121}.

Polar Angles. $(100) \wedge (101) = 52° 48'$; $(001) \wedge (101) = 32° 32'$; $(010) \wedge (121) = 49° 27'$.

[1] Present address, Department of Chemistry, University of Colorado, Boulder, Colo.

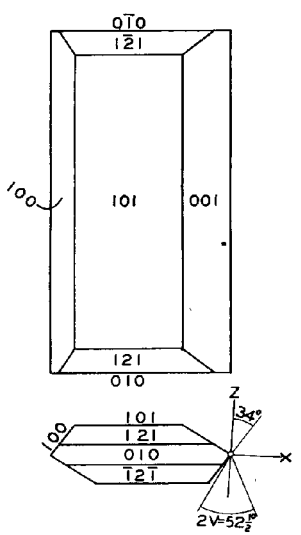

Figure 1. Orthographic projections on (101) and (010) of crystal of tripotassium diuranyl heptafluoride dihydrate

Partial Powder X-Ray Diffraction Pattern of $K_3(UO_2)_2F_7.2H_2O$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	*I*/*I*$_1$[b]
100	9.26	9.19	30
110	7.24	7.21	15
001	6.23	6.22	80
020	5.80	5.79	40
011	5.49	..	..
$\bar{1}$01	5.37	5.36	10
101	4.99	4.97	100
120	4.92	..	..
$\bar{1}$11	4.87	4.84	15
200	4.63	4.61	35
111	4.58	..	..
210	4.30	..	..
021	4.25	4.22	35
$\bar{1}$21	3.94	..	..
201	3.86	3.85	60
121	3.78	3.77	65
211	3.67	..	..
220	3.62	3.60	25
201	3.58	3.56	75
130	3.57	..	..
211	3.42	3.41	40
031	3.29	3.28	35
221	3.22	3.21	55
131	3.14	3.13	45

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

X-RAY DIFFRACTION DATA

Diffraction Symbol. $12/m1P\text{-}2_1\text{-}$. Observed development of crystal forms and failure to observe a piezoelectric effect makes it probable that the space group is $P2_1/m$ (C_{2h}^2).

Cell Dimensions. $a_0 = 9.29$ A.; $b_0 = 11.60$ A.; $c_0 = 6.25$ A.; $\beta = 94.5°$; $a_0:b_0:c_0 = 0.801:1:0.539$. Cell volume 671 A.^{3}

Formula Weights per Cell. 2.

Formula Weight. 826.46.

Density. 4.09 grams per cc. (x-ray); 4.108 (measured) reported by Baker (*1*).

OPTICAL PROPERTIES

Refractive Indices (5893 A.). $n_X = 1.448$; $n_Y = 1.459$; $n_Z = 1.502$; geometric mean 1.4695. Molecular refraction 56.1 cc. (based on measured density).

Optic Orientation. $Y = b$; $Z \wedge c = 34°$.

Optic Axial Angle (5893 A.). $2Vz = 52.5°$.

Color. X and Y yellow; Z nearly colorless.

Fluorescence. Strong, excited by mercury vapor lamp.

THERMAL DATA. Decomposition is evidenced by the crystals becoming cloudy at 148° C.

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WORK done under the auspices of the Atomic Energy Commission.

Convenient Alkoxyl Apparatus

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THE modified Viebock and Schwappach alkoxyl determination (Clarke, E. P., "Semimicro Quantitative Organic Analyses," p. 68, Academic Press, New York, 1943) uses either an adjustable microburner or an oil bath for heating the alkoxyl flask. These heating methods have the disadvantages of poor temperature control and large space requirement, especially when several determinations are run simultaneously.

To circumvent these difficulties the vapor bath and alkoxyl flasks shown in Figure 1 were constructed. The heater flask was made from a standard 1-liter three-necked flask by adding two side necks (not illustrated in Figure 1) to allow four samples to be run simultaneously. The constant boiling liquid used was mixed xylenes (b. p. 138° C.). Alkoxyl flasks constructed as shown were inserted in the side necks, and a reflux condenser was inserted in the center neck of the heating flask. The absorbers were of the type described by Gran [*Svensk. Papperstidn.* **57**, 702 (1954)], and were attached by means of springs or rubber bands.

The advantage of a common carbon dioxide supply soon became apparent and it was constructed as shown in Figure 2. Constant positive pressure was maintained by allowing the carbon dioxide to bubble slowly through the mercury trap. The glass wool plug prevented splashing of the mercury. The gas supply to each alkoxyl flask was adjusted by screw-type pinch-clamps.

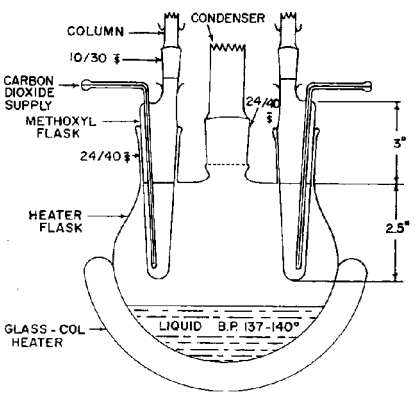

Figure 1. Modified alkoxyl flasks and vapor bath heater

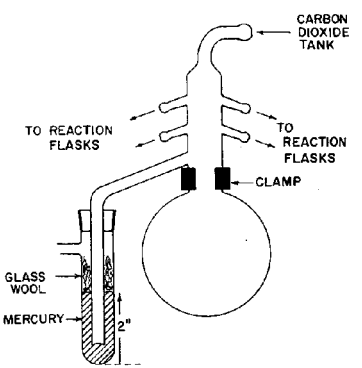

Figure 2. Carbon dioxide supply

This easily constructed apparatus is more compact than the usual alkoxyl apparatus. The vapor bath and carbon dioxide supply obviate continual checking. Attendance of the operator during the heating period is unnecessary.

The error of a series of seven total alkoxyl determinations on pure 4-ethoxy-3-methoxybenzoic acid ranged from 0 to 1.26%. The average of this series was 0.1% less than the calculated total alkoxyl.

ACKNOWLEDGMENT

The authors gratefully acknowledge the fellowship received from the Research Corp. for one of them (K. S.).

Field Kit for Estimating Moisture in Acrylic Molding Powder by a Small Sealed Vessel Technique

C. F. Roth and John Mitchell, Jr., Polychemicals Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

THE small sealed vessel (SSV) technique is finding an increasing number of applications in the authors' laboratory. Some of these were discussed by Smith, Mitchell, and Billmeyer (*7*), who described procedures for acids, bases, and esters and mentioned applications of similar equipment by other workers (*1–4*). The authors have used small sealed vessels extensively for sampling process streams to simplify otherwise complex problems of obtaining truly representative samples and preserving them for analysis.

The advantages of using small sealed vessels are numerous.

The basic tools required are simple and inexpensive. They consist primarily of pharmaceutical-type serum bottles with pressure seal stoppers and hypodermic syringes and needles.

Small sealed vessels offer a system protected from contamination by, or reaction with, constituents of the atmosphere, such as carbon dioxide, moisture, and oxygen.

Changes in sample composition due to loss of volatile components are avoided.

Changes in a sample because of its reactivity can be overcome by sampling into a small sealed vessel containing a chemical reagent (or solvent). The chemical reagent is chosen so that it will react instantaneously with the active component of the sample.

Only small amounts of samples and reagents are required. The technique is readily scaled down to the semimicro or micro level.

The present paper describes a compact field kit for estimating moisture in acrylic molding powder. Its development was prompted by the frequent need for a rapid and simple means of checking the moisture content of the polymer at locations where adequate laboratory facilities are not available. In addition, it was required that the method have an accuracy within ±10% relative in the range 0 to 0.5% moisture. Small sealed vessels seemed ideally suited for this purpose. The method described was adapted from the standard laboratory procedure, based on titration of a chloroform solution of the polymer with standardized Karl Fischer reagent.

MOISTURE TEST KIT

The compact kit contains everything required for making eight moisture determinations. It is designed so that persons not specially trained in analytical techniques can obtain accurate

results. The sample to be analyzed is measured volumetrically rather than gravimetrically, in order to obviate the need for an analytical balance. The Karl Fischer reagent employed is prepared from a stabilized reagent marketed by the Fisher Scientific Co. This reagent is characterized by good shelf-life and a constant water equivalence factor, so that it may be used with confidence months after it has been standardized.

Components of Kit. Narrow-mouthed serum bottles, 20-ml. (A. H. Thomas Co., Philadelphia, Pa., Catalog No. 2319A), filled with standardized Karl Fischer reagent, and sealed with red-rubber pressure-seal stoppers (Catalog No. 2319B). The stoppers are coated with beeswax to protect them from oxidative attack.

Narrow-mouthed screw-cap bottles (1-ounce) containing 15 ml. of chloroform pretitrated to the end point. Red-rubber stoppers are not used here because they are attacked by chloroform. The bottles are sealed by means of phenol-formaldehyde screw caps fitted with polyethylene liners, which are unaffected by chloroform. By drilling a 1/8-inch hole through the screw cap but not through the liner, a tight seal is maintained and insertion of a syringe needle is still possible. The caps are sealed with beeswax to protect against atmospheric moisture and to prevent the plastic caps from loosening.

Narrow-mouthed screw-capped bottles (1-ounce) containing colorimetric standards. These permanent standards are prepared from solutions of iodine in methanol and are included in order to simplify recognition of the end point of the titration. One bottle contains a solution whose color is to be matched. The other two standards represent the colors of slightly undertitrated and overtitrated solutions, respectively.

A vial containing six hypodermic needles (Yale Luer-Lok 20-gage, 1 inch long) in protective plastic sheaths.

Two 5-ml. hypodermic syringes (Yale Luer-Lok B-D 5 YL), a stainless steel scoopula (Fisher Scientific Co., Catalog No. 14-357), and a dissecting needle. The scoopula and dissecting needle are cut down to fit into the boxes containing the syringes.

Test tubes, calibrated to contain 2 grams of polymer. Two rimmed test tubes (A. H. Thomas Co., Catalog No. 9488, 12 × 75 mm.) are marked for measuring cube-cut molding powder such as Du Pont Lucite 140 acrylic resin. Two rimless test tubes (A. H. Thomas Co., Catalog No. 9450, 10 × 75 mm.) are marked for measuring granular polymer such as Du Pont Lucite 40 acrylic resin.

Table I. Moisture in Acrylic Molding Powders

Sample No.	Form	Water Found, Wt. %: Laboratory method	Water Found, Wt. %: Moisture kit
1	Cubes[a]	0.08	0.08
		0.08	0.09
		0.08	0.09
2	Cubes[a]	0.35	0.34
		0.34	0.34
3	Granules[b]	0.45	0.48
		0.47	0.49
			0.49
4	Granules[b]	0.48	0.49
		0.48	0.50
			0.50

[a] Du Pont Lucite 140 acrylic resin.
[b] Du Pont Lucite 40 acrylic resin.

The box itself is constructed from a solid piece of mahogany wood, into which holes are drilled to various depths, so that the different components are held tightly in place when the lid is closed. Over-all dimensions of the kit are 7 × 9 1/4 × 4 1/4 inches.

PROCEDURE

A calibrated test tube is filled to the mark with the sample by adding successive small portions of polymer to the tube by means of the scoopula and gently tapping the bottom of the tube on a wooden surface after each addition. The particles settle uniformly of their own weight. Any agglomerations of particles must be broken up into individual particles in order to avoid large voids in packing. The cap is unscrewed from a bottle containing solvent, the sample is quickly poured in, and the cap is screwed back on tightly. The mixture is shaken periodically until the sample is dissolved. This requires from 5 to 30 minutes, depending upon the physical form of the polymer.

After a clean, dry, hypodermic syringe has been rinsed with a small portion of reagent, exactly 5 ml. of Karl Fischer reagent are drawn from the storage vial into the syringe.

The dissecting needle is used to punch two holes through the polyethylene liner in the cap on the bottle containing the dissolved sample. The syringe needle is inserted through one hole, while the other serves as a vent. Then reagent is added until the color of the solution matches the colorimetric standard. The amount of reagent added is read to the nearest 0.1 ml. on the calibrated barrel of the syringe. The test is designed to cover the range 0.0 to 0.5% water. With a 2-gram sample and Karl Fischer reagent having a water equivalence factor of 2 mg. of water per ml., each 0.1 ml. of reagent represents 0.01% water. The factor for direct conversion of titer in milliliters to per cent water is typed on the label of each vial along with the date of preparation of the reagent. By suitable adjustment of the strength of the reagent, the range of moisture content may be extended as desired.

ANALYTICAL RESULTS

In order to demonstrate the degree of accuracy obtainable by use of the moisture kit, results were compared with those obtained by the standard laboratory method. In the laboratory method 10 grams of polymer are dissolved in 100 ml. of pretitrated chloroform contained in a 300-ml. glass-stoppered Erlenmeyer flask. The resulting solution is titrated with freshly standardized Karl Fischer reagent equivalent to about 3.5 mg. of water per ml. The excellent agreement obtained between the two methods is shown in Table I. Moisture was determined in Du Pont Lucite 140 acrylic resin, a cube-cut molding powder, and Du Pont Lucite 40, a granular formulation. In view of the simplicity of the moisture kit method, the method is remarkably accurate, more than meeting the initial requirement of an accuracy within ±10% relative.

Table II. Precision of Measuring 2 Grams of Sample

Sample	Analyst	No. of Measurements	Average Weight, G.	Standard Deviation, G.
Cubes	1	30	2.000	0.067
	2	30	2.008	0.032
		60	Av. 2.004	0.054
Granules	1	30	2.014	0.015
	2	30	2.010	0.013
		60	Av. 2.012	0.014

The accuracy with which 2 grams of polymer can be measured volumetrically in these small test tubes is shown in Table II. Sixty tubes were calibrated, 30 with cube-cut Lucite 140 and 30 with granular Lucite 40. Each calibrated tube was checked by two analysts, who weighed the amount of sample required to fill the tube to the mark. The average weight was very close to 2 grams in each case. The larger standard deviation obtained for cube-cut molding powder is not surprising, as cubes ($^1/_{16}$ to $^1/_8$ inch on edge) would not be expected to pack as uniformly and reproducibly as fine granules. Accurate measurement of samples requires tubes with a favorable ratio of inside diameter to length, with consideration for the particle size range and distribution.

STABILIZED KARL FISCHER REAGENT

The key to accurate results using the moisture kit described lies in the use of stable Karl Fischer reagent. This reagent is a powerful desiccant. Contact with moisture from any source results in instantaneous reaction and an attendant decrease in strength. In small sealed vessels the reagent is adequately protected. However, the usual Fischer reagent, which is a mixture of methanol, pyridine, sulfur dioxide, and iodine, undergoes an unavoidable deterioration even when protected from contact with water (*5*); daily standardization is necessary to obtain accurate results. Consequently, it cannot be used for an application such as the moisture kit, where daily standardization is not practical.

In the fall of 1953 the Fisher Scientific Co. marketed a stabilized Fischer reagent (Catalog No. SO-K-3). Shelf-life tests showed that this reagent had good stability. However, as sold it is much too concentrated for use in the moisture kit, having an equivalence factor of more than 6 mg. of water per ml. of reagent and a density of about 1.28. It was necessary, therefore, to reduce its strength without impairing its stability. Three methods were investigated: (1) dilution with methanol, (2) addition of water, and (3) dilution with methyl Cellosolve. Peters and Jungnickel (*6*) have demonstrated that relatively stable Karl Fischer reagent can be prepared by use of methyl Cellosolve in place of methanol.

Dilution with methanol was unsuccessful. In 145 days the strength of SO-K-3 Karl Fischer reagent, which was diluted with approximately 2 parts by volume of methanol, decreased almost linearly from 2.11 to 1.20 mg. of water per ml. This was not surprising, because it is known (*5*) that ordinary Fischer reagent prepared with methanol is unstable. Reducing the strength of SO-K-3 Fischer reagent by adding water resulted in a reagent of fairly good stability. In 138 days water-weakened SO-K-3 decreased in strength from 2.28 to 2.09 mg. of water per ml. Methyl Cellosolve–diluted SO-K-3, however, had excellent stability. Over a period of 110 days SO-K-3 reagent diluted with 2 volumes of methyl Cellosolve maintained its water equivalence factor unchanged at 2.16 mg. of water per ml.

The shelf-life tests of the three modifications of SO-K-3 discussed were all run in the same manner. A number of dried 20-ml. serum bottles were filled with the formulation to be tested and stoppered with pressure-seal rubber stoppers. The vials were stored at room temperature with no special protection against light. The stoppers were not coated with beeswax. One bottle of each formulation was standardized at approximately weekly intervals. This was a severe test of the reagent. It demonstrated not only that the methyl Cellosolve–diluted reagent was stable but also that the small serum bottles capped with pressure-seal rubber stoppers adequately protect the reagent from atmospheric moisture. This method of testing shelf-life was chosen because the conditions simulated storage of the reagent in the moisture kit. Although a tight seal was maintained by the stoppers during the course of the tests, the outer surface of the stoppers deteriorated badly, owing to oxidative degradation of the stretched rubber. In order to prolong their life, the stoppers of all serum bottles containing reagent were subsequently coated with beeswax, which effectively prevented such degradation.

The moisture kit principle should be applicable to the determination of water in other resins. In many cases the primary practical problem to be overcome is that of finding solvents which will dissolve the polymers quickly to form solutions of not inconveniently high viscosity.

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Cover Glass for Erlenmeyer Flasks

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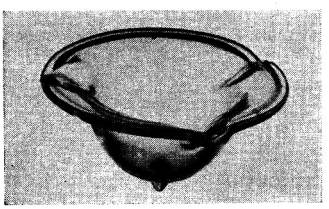
A NEED arose in this laboratory for a cover glass that would permit rapid evaporation of liquids, particularly organic solvents, from Erlenmeyer flasks. Neither a small plain watch glass nor a chemical funnel permits very rapid evaporation. No suitable watch glass was found in any supply catalog, so the cover glass shown in the photograph was designed and made for the purpose. It will fit 125-, 200-, and 300-ml. conical flasks. In addition to the advantage of rapid evaporation, the flasks may be swirled, or tipped to about 45° without loss of the cover.



Seal or blow a round (test tube) bottom at one end of a 15-cm. (6-inch) length of glass tubing 25 mm. in outside diameter. To the center of the bottom seal about 15 cm. of 4- or 5-mm. glass rod to serve as a handle for further working. When the glass has cooled, saw off the tube about 10 to 12 mm. above the point where the bottom curvature begins.

Secure the glass rod in the chuck of a glass lathe and heat the open end of the tube until it is soft and glowing. With a carbon rod (7 to 8 mm. in diameter) flare the end back to the point of curvature until it is at right angle to the axis of the tube and about 4 cm. in diameter. It may be necessary to reheat and rotate against a carbon paddle to flatten the flare evenly.

Cool and remove from the lathe. In a small flame heat a portion of the flare at a time and make the three indentations in the top surface with a tungsten rod (2 mm.) or a carpenter's pencil. If a pencil is used, whittle the wood from about 15 mm. of the lead, which is then shaved to a wedge shape. Finally, heat and remove the glass rod from the bottom of the cover.

The cover glass may be made entirely by hand working, but if the flaring is done by hand the cover may not fit the three sizes mentioned above. It may be necessary to start with a different size of tubing for each size of flask, depending on the skill of the glass blower. For hand working, a glass tube is chosen with an outside diameter within 1 mm. of the inside diameter of the neck of the flask. If desired, covers for 500-ml. flasks may be made from tubing 35 mm. in outside diameter and for liter flasks from 38-mm. tubing. The latter also serve as cover glasses for 50-ml. beakers. Covers for flasks with 24/40 standard-taper outer joints can be made from 20-mm. tubing.