ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

Selecting the Right Analytical Method

THERE are two subjects concerning analysis about which there is little concern until a gross error is introduced, and a careful study is made to determine the cause. One is sampling; the other is selecting the right analytical method. In both cases the analyst usually has little or nothing to say about either, because all too often he is handed the sample and told what determination is required. In many cases, especially in routine control, the procedures are so standardized that these two problems do not become important.

We will limit the present discussion to selecting the right analytical method because, with the complexity and versatility of modern analysis, this now presents a very real problem in many laboratories. The factors that make this a problem stem from several sources.

The chemist who seeks the services of the analytical department has in the course of his training had some courses in analysis, and he therefore feels qualified to request along with the sample the specific determination which he expects will give the information about the composition or structure of the material on which he is working. The analytical laboratory is geared to turning out requested analyses and has not the training or interest in discussing the reasons behind the requested work. It is not uncommon to find the chemical approach to analysis separated from the instrumental, so that where the problem is submitted depends somewhat on what group is the best salesman. The instrumental specialist is often weak in chemistry, but because he can usually run a curve in the matter of minutes, his customers at least have had action and something to talk about, even though they may not have an adequate answer. The chemical approach to analytical problems is too often time-consuming, and, with the usual heavy backlog of work, the analyst is often bypassed when he could perhaps give a better answer.

The answer, which is gradually being recognized, is to organize the analytical department so that it has both the chemical and instrumental to offer as service and so obviates the wasteful process of customers' shopping around to get their analytical problems solved.

The development of instruments is a slow process involving mechanical and electrical problems which the experts in these fields are best able to handle. Unfortunately, these specialists are not the best qualified to apply their methods to analytical problems. The development stage has passed, and well-engineered and excellent instruments can now be purchased. It is for this reason that analytical laboratories should think in terms of offering service in both physical and chemical methods. They should staff their laboratories with men qualified to work in all branches of analysis.

Management is becoming concerned about the lack of coordination of analytical effort and its continued mounting cost. In some large organizations it is possible to submit the same samples to two or more independent groups, which can lead to costly and ineffective analytical service.

To select the best and most economical analytical method requires an analytical department equipped and staffed with qualified personnel in all phases of analysis. When this is done, those requiring analytical service need only outline the information required and leave to the analytical department the task of deciding what method or combination of methods will give the required information. If required, the analytical data should be interpreted in consultation with those submitting work, and the too common practice of expecting the research chemist to draw his own conclusions should be discontinued.

To accomplish this rather simple task of selecting the right method is rather difficult under the usual organizational setup. With a better and more complete approach to analysis and a staff that commands the respect of those they serve, we believe that cost of analysis can be reduced, and certainly a faster and more complete answer can be given to the analytical problems submitted.

Gas-Liquid Partition Chromatography

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The technique of gas-liquid partition chromatography was investigated as a method for analyzing complex organic mixtures. This technique was found to be very rapid and versatile for the qualitative and quantitative analysis of complex hydrocarbon mixtures in the C_5 to C_8 range. The separations obtained by this method in a few minutes are similar to those obtained on precision distillation columns requiring several hours of operation. The separated components of a mixture are detected with a thermal conductivity cell as they emerge from the column. A complete description of the apparatus and the analytical results for several typical samples are presented to show the accuracy and versatility of this technique. This technique is now in use, both as an independent analytical tool and as a powerful adjunct to molecular spectroscopic procedures.

DURING recent years, numerous papers have appeared in the literature describing the scope and application of chromatographic methods of analysis. Recently chromatographic methods have been developed for the separation and analysis of gases and volatile liquids using the adsorption method (*2, 3, 10–13*) and the gas-liquid partition method (*1, 3–10, 13, 14*).

In the adsorption method, the mixture to be separated is adsorbed in a narrow band at the one end of a column filled with an adsorbent such as activated charcoal. Different authors have described techniques in which the sample is eluted from the adsorbent by a carrier gas. Recently a commercial apparatus (*2*) was made available in which the adsorbed material is displaced from the adsorbent by passage of a more strongly adsorbed vapor through the column.

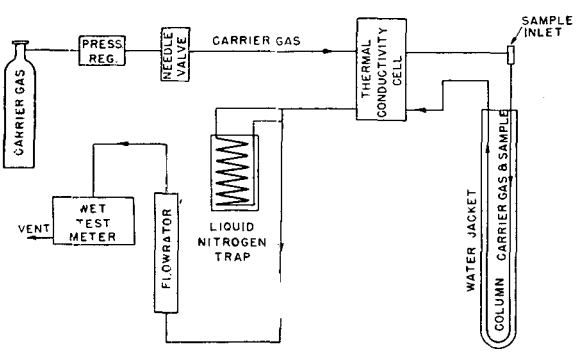

Figure 1. Schematic diagram of apparatus

The techniques of gas-liquid partition chromatography are similar in many ways to the liquid-liquid chromatographic columns except that the mobile phase is a gas instead of a liquid. Briefly the basis of this method is as follows:

A sample of the mixture to be analyzed is injected into the end of a narrow column packed with an inert granular material (Celite 545, Johns Manville Corp., Pittsburgh, Pa.) on which has been deposited a coating of a very high boiling organic liquid such as dioctyl phthalate. The column is prepared in a manner similar to that described by James and Martin (*7*). When a lower boiling mixture is charged to the end of the column, the individual components will partition between a gas phase in the pore space and a liquid phase absorbed in the high boiling organic coating. The column is then eluted with an inert gas which causes the components to move forward with individual velocities, which are less than that of the carrier gas. The velocity with which a particular component moves is dependent upon its partition coefficient. Since the partition coefficient varies for different compounds, a separation into zones results within the column. The separated components are detected with a thermal conductivity cell (Gow-Mac Instrument Co., Madison, N. J.) as they emerge from the column.

Gas-liquid chromatographic techniques have been shown to be a powerful and versatile tool for the analysis of organic compounds. For those interested in rapid and accurate means for analyzing hydrocarbons, this method has several advantages—namely, good separation is obtained readily; small samples are used, since analyses are run on only a few milligrams of sample; azeotrope formation causes no trouble; and only simple apparatus, easy to operate, is required.

The separations obtained are similar to those obtained on precision distillation columns. Hence the method can be used as a powerful adjunct to molecular spectroscopic techniques. The gas-liquid partition columns have two main advantages over the liquid-liquid partition columns. The low viscosity of the gas phase allows relatively longer columns to be used with a corresponding gain in efficiency. Also the methods for detecting a change in composition of a gas stream are generally simpler than those for a liquid stream.

APPARATUS

The schematic diagram of the apparatus is shown in Figure 1. In operation, a stream of carrier gas is continually passed through the system in the direction indicated by the arrows. The carrier gas is drawn from a cylinder through a series of reducing valves and finally a needle valve in order to maintain a constant flow of gas. The flow of carrier gas is indicated by a rotameter and measured with a wet test meter. The liquid nitrogen trap is used when fractions are collected for further analysis by the mass spectrometer. The electrical bridge of the thermal conductivity cell is balanced with carrier gas passing through both the reference channel and the sample channel. The top of the column is equipped with a rubber serum bottle cap for injecting the sample to be analyzed by means of a hypodermic syringe. During elution of a component from the hydrocarbon mixture, the sample channel of the thermal conductivity cell is exposed to a mixture of carrier gas plus the component leaving the column. This results

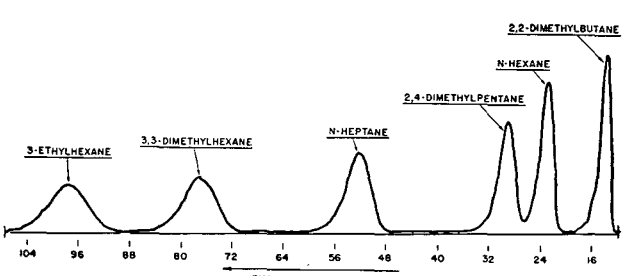

Figure 2. Chromatogram of synthetic mixture of saturated hydrocarbons

in an unbalanced voltage in the thermal conductivity cell bridge. This unbalanced voltage is fed to a recording potentiometer which automatically plots detector response versus time.

An example of this type of plot is illustrated in Figure 2, which is the chromatogram of a synthetic mixture of saturated hydrocarbons. This sample was run in a column 10 feet long with an inside diameter of 4.5 mm. at a temperature of 65° C. and a gas flow rate of 29 ml. per minute, using dioctyl phthalate as the liquid phase. This curve shows a continuous plot of the signal from the thermal conductivity detector sampling the gases leaving the column versus time of elution. The qualitative identification of each hydrocarbon is based on its retention volume. Because a constant flow rate of carrier gas was used for each run, it is more convenient to plot retention time instead of retention volume. The concentration of each component is determined from the areas under the peaks, as the response of the thermal conductivity cell for the hydrocarbons in this molecular weight range is essentially the same. It is seen from this chromatogram that in mixtures containing components with a wide range of boiling points, the higher boiling components tend to spread themselves out to give a long band of low concentration. For qualitative work, this difficulty can be easily overcome by increasing the column temperature during a run. For quantitative work, this procedure is undesirable, as it requires exact reproduction of temperature rise and flow rate, as well as calibrated peak areas for components at different temperatures.

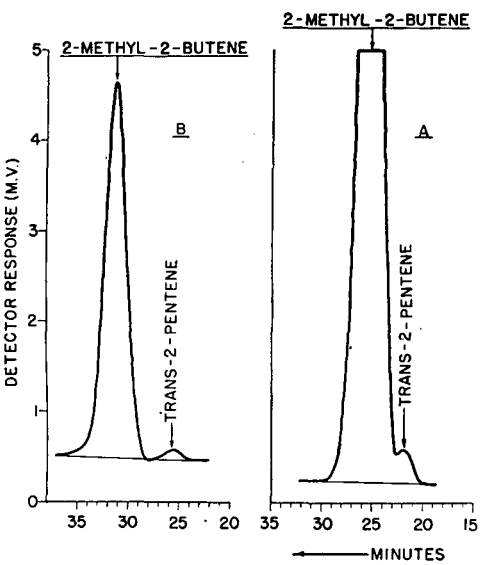

Figure 3. Special separations using different liquid coatings

Table I gives the order of elution for several of the saturated hydrocarbons in the light gasoline range. These data apply to a column 10 feet long with an inside diameter of 4.5 mm. operated at 65° C. and a carrier gas (hydrogen) flow rate of 28 ml. per minute, using dioctyl phthalate as the liquid phase. The measured retention time for each compound is the number of minutes between the air peak resulting from the injection of the component and the detection of maximum concentration of that component in the effluent gas. The retention time of a compound depends on the boiling point and relative solubility between the compound and the organic coating in which it is absorbed in the column.

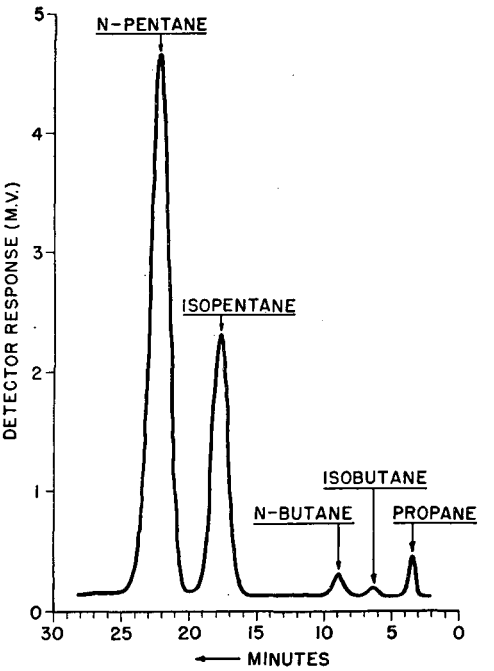

Figure 4. Chromatogram of typical C_5 and lighter hydrocarbon sample

Table I shows a smooth relationship between boiling points and retention time within a homologous series. It also shows that the order of arrival does not follow increasing boiling points when different classes of compounds are analyzed by this column. This difference makes possible the separation of close boiling components such as 2,4-dimethylpentane and cyclohexane. However, this column will not separate compounds with the same retention times such as cyclopentane and 3-methylpentane. The naphthenes and aromatics are more soluble in the dioctyl phthalate than the paraffins with comparable boiling points; thus their retention times are longer.

The versatility of this method can be greatly increased by varying the properties of the liquid phase. A column in which the granular coating was a paraffin wax was used for separating cyclopentane and 3-methylpentane, which could not be separated with the dioctyl phthalate column. A vacuum pump oil (Octoil-S, Consolidated Vacuum Corp., Rochester, N. Y.) was found to be an effective coating for separating several hydrocarbons that could not be separated on the dioctyl phthalate column. This is illustrated in Figure 3 showing the separation of a small amount of *trans*-2-pentene from 2-methyl-2-butene. This sample was

Table I. Order of Elution for Several Hydrocarbons

Component	B.P., ° C.	Retention Time, Min.	Component	B.P., ° C.	Retention Time, Min.
Isopentane	27.9	10	3-Methylpentane	63.2	24
n-Pentane	36.1	12	*n*-Hexane	68.7	28
2,2-Dimethylbutane	49.7	17	2,4-Dimethylpentane	80.6	36
2,3-Dimethylbutane	58.0	22	Cyclohexane	80.8	55
2-Methylpentane	60.3	22	*n*-Heptane	98.4	67
Cyclopentane	49.2	24	Benzene	80.1	87

Table II. Analysis of Synthetic C_5 and C_6 Hydrocarbon Mixture, Mole %

Component	Blended, %	Observed, % Run 1	% Diff.	Run 2	% Diff.
Isopentane	6.6	6.7	+0.1	6.7	+0.1
n-Pentane	16.7	16.0	−0.7	17.3	+0.6
2,2-Dimethylbutane	5.8	5.9	+0.1	5.8	0.0
2,3-Dimethylbutane	5.9	11.8	0.0	11.2	−0.6
2-Methypentane	5.9				
3-Methylpentane	29.8	29.4	−0.4	28.6	−1.2
n-Hexane	29.3	30.2	+0.9	30.4	+1.1
Av. % diff.			0.4		0.6

run in a column 14 feet long with an inside diameter of 4.5 mm. operated at 65° C. with a carrier gas (hydrogen) flow rate of 18 ml. per minute. For the chromatogram (A) using dioctyl phthalate as the liquid phase, incomplete separation was obtained. However, for the chromatogram (B) using Octoil-S at the same conditions, complete resolution of the *trans*-2-pentene and 2-methyl-2-butene was possible. Using a larger sample charge and calibrated peak areas for the minor components, this technique is useful to determine small percentages of impurities in pure grade hydrocarbons.

Figure 4 is the chromatogram of a sample which is typical of many encountered in hydrocarbon research. This sample was run in a column 14 feet long with an inside diameter of 4.5 mm. operated at 65° C., and a carrier gas (helium) flow rate of 18 ml. per minute, using Octoil-S as the liquid phase. The different components in the mixture are marked by the individual peaks. This chromatogram shows complete separation of all components in this particular mixture. Figure 5 is the chromatogram of a synthetic mixture of C_5 and C_6 hydrocarbons. The same column and conditions were used for this sample as in Figure 4. This mixture is typical of many samples now analyzed by this technique for control purposes. As 2,3-dimethylbutane and 2-methylpentane have the same retention time on this column, they could not be separated. Table II is a comparison of the blended percentages and observed percentages for the synthetic mixture shown in Figure 5. The average deviation in terms of total sample for run 1 is 0.4% and run 2 is 0.6%.

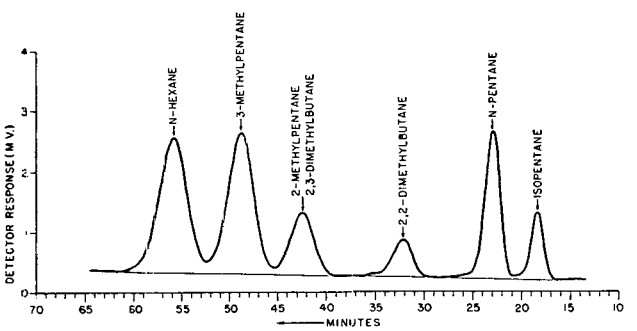

Figure 5. Chromatogram of synthetic mixture of C_5 and C_6 hydrocarbons

Figure 6 is the chromatogram of a more complex mixture of C_5 through C_7 hydrocarbons. This sample was run in the same column as in Figure 4 at a temperature of 85° C. and a gas flow rate of 29 ml. per minute. This chromatogram shows several cases wherein two components have similar retention time; thus they come off together as a single peak. However, this curve shows the difference in retention time for benzene and cyclo compounds compared to the paraffins of similar boiling points. Development work is now concentrated on the application of this technique as a separation tool to use in conjunction with the mass spectrometer. This technique would eliminate the time-consuming precision distillation now in use to separate these cuts. A charge of approximately 0.02 to 0.03 ml. of a light gasoline is sufficient to provide several cuts in the C_5 to C_8 hydrocarbon range for further analysis by the mass spectrometer.

Table III shows a comparison between the blended concentration and the observed concentration for the 18-component mixture shown in Figure 6. Although some peak areas are in error by more than 1%, the average error in terms of total sample is only 0.6%.

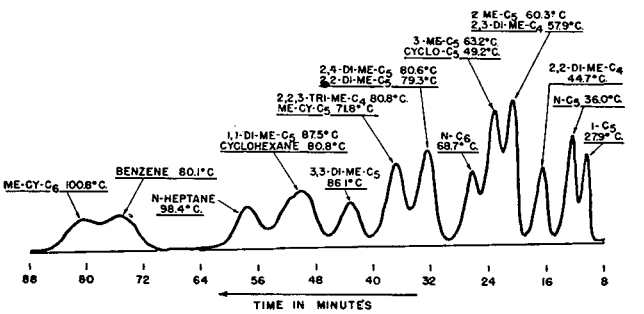

Figure 6. Chromatogram of an 18-component hydrocarbon mixture

An example of the technique applied to a very difficult separation is shown in Figure 7, which is the chromatogram for a complex heptene mixture. This sample was run in a column 14 feet long with an inside diameter of 4.5 mm. operated at 65° to 85° C. with a carrier gas (hydrogen) flow rate of 29 ml. per minute, using dioctyl phthalate as the liquid phase. By inspection of this chromatogram, it is obvious that the mixture contained several compounds as indicated by the breadth of some of the peaks and the fact that the curve did not return to the base line between peaks. The tentative identification of the peaks is made by correlating boiling points and retention times for several of the pure compounds that are available for calibration purposes. Since all of these components were not used in the calibration, this is only a tentative identification of the peak areas.

Figure 8 is a photograph of the apparatus used in the gas analysis laboratory for the routine analysis of liquid samples. This unit is assembled on a metal frame which is mounted on a dolly. The chromatographic column, which is assembled in the glass water jacket, is mounted on the left side. The insulation from this jacket is removed so the arrangement of the column would be visible for this picture. Water from the constant temperature bath is continuously circulated through the jacket to maintain the desired column temperature. The flow of carrier gas is indicated by the rotameter on the side of the apparatus and is measured by the wet test meter located beneath the constant temperature water bath. The thermal conductivity cell is enclosed in a constant-temperature air bath located directly

Table III. Comparison between Blended and Observed Concentrations for 18-Component Blend

Component	Blended %	Observed %	% Difference
Isopentane	4.5	4.7	+0.2
n-Pentane	5.8	6.3	+0.5
2,2-Dimethylbutane	5.8	5.4	−0.4
2-Methylpentane 2,3-Dimethylbutane	11.8	10.2	−1.6
3-Methylpentane Cyclopentane	10.6	10.9	+0.3
n-Hexane	5.9	6.4	+0.5
2,4-Dimethylpentane 2,2-Dimethylpentane	11.8	10.1	−1.7
2,2,3-Trimethylbutane Methylcyclopentane	9.4	9.5	+0.1
3,3-Dimethylpentane	5.9	5.6	−0.3
1,1-Dimethylpentane Cyclohexane	10.8	11.5	+0.7
n-Heptane	5.9	6.7	+0.8
Benzene	5.9	6.8	+0.9
Methylcyclohexane	5.9	5.9	0.0
		Av. % diff.	0.6

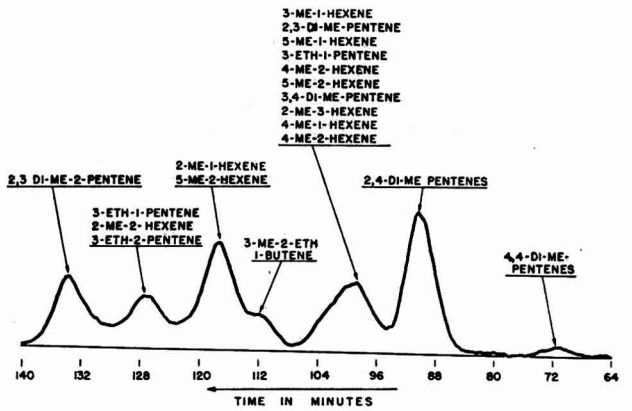

Figure 7. Chromatogram of a complex heptene sample

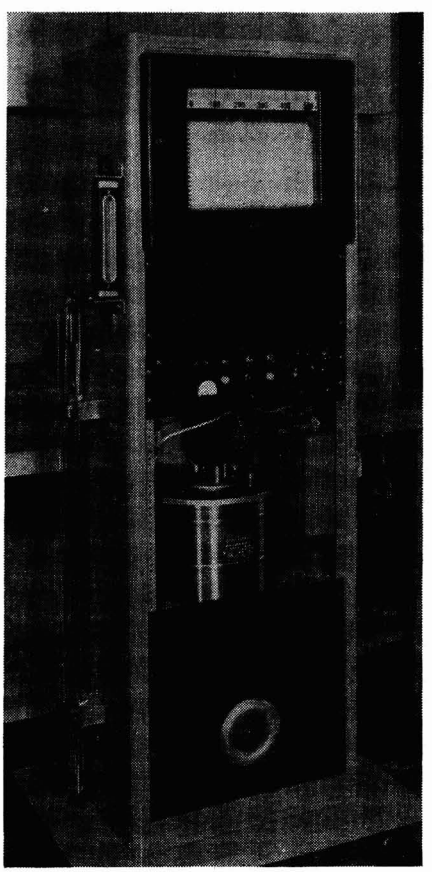

Figure 8. Photograph of routine apparatus

under the recorder. This bath is equipped with a mercury type thermoregulator, electronic relay, circulating fan, and heater for maintaining the thermal conductivity cell at a constant temperature. A modified Brown electronic recorder having a 0- to 5-mv. range and a chart travel speed of 1.3 cm. per minute is used to measure the signal from the thermal conductivity cell. This instrument range and chart travel speed provide sufficient area under the peaks for accurate measurement with a planimeter.

This unit is operated by the high school graduate technicians and used for operating the low temperature distillation apparatus. One of the features of this model is the ease and simplicity of operation. As soon as the sample is charged to the column, the apparatus is completely automatic. Less than 5 minutes of operator time is required to charge the sample and 10 to 20 minutes for calculating the peak areas. As soon as the last component in one sample is eluted, the column is immediately ready to be charged with another sample. Several hundred routine samples have been analyzed in the same dioctyl phthalate column with no apparent change in accuracy or reproducibility.

In conclusion the scope and accuracy obtained by this simple procedure should be emphasized. This technique is certain to supplement and in some cases supplant the use of infrared spectrometers, mass spectrometers, and distillation apparatus for the analysis of complex organic mixtures.

ACKNOWLEDGMENT

The authors wish to thank N. D. Coggeshall for his interest and help in carrying out this work, and J. R. Tomlinson, Lionel Domash, and G. F. Crable for their help in the initial stages of this project. The authors are also indebted to the management of Gulf Research & Development Co. for permission to publish this paper.

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RECEIVED for review April 8, 1955. Accepted June 30, 1955.

Determination of Some Components in Corn Sirups by Quantitative Paper Chromatography

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Paper chromatography has been demonstrated as a practical procedure for the quantitative separation of nine corn sirup components. These components, in order of decreasing chromatographic mobility, have been tentatively identified as: glucose, maltose, isomaltose, maltotriose, isomaltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose. The nine components, separated chromatographically, were quantitatively determined by absorptiometric techniques. A number of corn sirups differing in degree of hydrolysis were analyzed.

PRODUCTION control in corn sirup manufacturing has been based primarily on determinations of moisture, ash, pH, color, and reducing sugars (*8*). Little has been known about the composition of the sugar mixture except its D-glucose content, determinable from Sichert-Bleyer reducing values (*29*), by various fermentation procedures (*24*, *30*), or recently by the specific action of the enzyme glucose dehydrogenase (*33*).

For the determination of the individual oligosaccharides there have been no such direct methods. Maltose, maltotriose, and maltotetraose have been estimated in several sirups by fractional distillation of the methyl (*15*) or propionyl (*16*) derivatives, but the procedures were not adaptable to routine analysis.

In the present work, paper chromatographic methods (*2*, *7*, *18*, *23*, *28*, *36*) for the separation of sugar mixtures have been applied with absorptiometric methods of determination to provide methodology for the analysis of corn sirups.

Table I. Comparison of Irrigating Solvents in Chromatography of Corn Sirup Components

Irrigating Solvent	Volume Ratio	Time, Hours	Glucose Movement, Mm.	$R_{glucose}$ Maltose	$R_{glucose}$ Maltotriose
n-BuOH, pyridine, H_2O	6:4:3 (*4*)	33	228	0.60	0.38
EtOAc, pyridine, H_2O	8:2:1 (*36*)	31	105	0.28	...
	8:2:1 (*36*)	40	170	0.28	0.08
	8:2:1 (*36*)	56	273	0.32	0.14
	8:2:1 (*36*)	78	354	0.32	0.10
(Epiphase)	5:2:5 (*19*)	23	293	0.65	0.43
(One phase)	10:4:3	..	..	0.65	0.38
(One phase)	5:2:2	23	256	0.68	0.46
(One phase)	5:5:2	23	Trisacch. moved 330 mm.		
n-BuOH, EtOH, H_2O	10:1:2 (*36*)	31	75	0.24	...
	10:1:2 (*36*)	40	109	0.33	0.12
	10:1:2 (*36*)	78	188	0.26	0.08
	2:1:1	36	321	0.64	0.41
n-BuOH, EtOH, 27% NH_4OH	4:1:5	54	199	0.60	0.36
	4:1:5	81	268	0.56	0.31

SELECTION OF CONDITIONS FOR CHROMATOGRAPHIC SEPARATIONS

Irrigating Solvent. Several solvents were compared for separating corn sirup components on Whatman No. 1 filter paper (chromatographic grade) at 25° to 30° by the descending technique (*26*) (Table I).

Solvent selection was based on rate of movement of glucose and efficiency of separation of oligosaccharides. The epiphase of a mixture of ethyl acetate, pyridine, and water (5:2:5 volume per volume) seemed useful by these criteria. In practice it was found that equivalent results were obtained with a monophase of solvent made up of 10 volumes of ethyl acetate, 4 volumes of pyridine, and 3 volumes of water. Also used with equal success was a mixture of *n*-butyl alcohol, ethyl alcohol, and water (2:1:1 volume per volume). This solvent was used for most of the analyses reported here.

Indicator Spray Reagent. Aniline hydrogen phthalate (*26*), aniline hydrogen oxalate (*25*), and ammoniacal silver nitrate were compared as chromatographic spray reagents.

Aliquots of glucose or maltose solutions (0.1 γ per μl.) on filter paper were dried, sprayed with reagent, and heated at 115° to 120° for 10 to 15 minutes. From observations recorded in Table II, aniline hydrogen phthalate was chosen as the indicator spray reagent.

Table II. Comparison of Developers on Corn Sirup Components

(Spotted on paper)

Developer	Component Developed: Glucose, γ 0.5	Glucose, γ 1	Maltose, γ 1	Maltose, γ 10
Ammoniacal silver nitrate	−	+	+	+
Aniline hydrogen oxalate	+	+	−	−
Aniline hydrogen phthalate	+	+	+	+

SELECTION OF CONDITIONS FOR DETERMINATIONS

Recovery of Chromatographed Sugars. To recover sugars the paper was extracted with condensate from a refluxing solvent (*3*). This technique gave rapid, complete recoveries with minimum volumes and many units could be operated concurrently to provide efficiency. In the later phases of this work it was found that complete extraction of sugar could be accomplished by simply stirring the paper in a small beaker with about 5 ml. of distilled water, filtering the liquid through a glass wool plug in a funnel, and rinsing the paper with a second 5-ml. portion of water.

To test the completeness of the extraction, 42 D.E. corn sirup was depleted of glucose and maltose by absorption on a carbon column (*32*) and elution with water and 3.5% ethyl alcohol. On analysis, 1463 γ of this sirup were found to contain 32 γ of residual glucose and 31 γ of maltose. When 400 γ of D-glucose were added to the same amount of sirup and the mixture was separated on paper chromatograms, 436 γ of D-glucose (an average of 14 determinations) were recovered from the chromatogram. When 190 γ of maltose were added, 224 γ of maltose were recovered (an average of 11 determinations).

Color Production. Among the reagents compared in the generation of a colored complex with the sugar were anthrone-sulfuric acid (*7*), 1-naphthol–sulfuric acid, phenol–sulfuric acid (*21*), and alkaline 3,5-dinitrosalicylic acid–phenol (*3*). The latter two were found to be satisfactory and essentially equivalent. Both methods were employed in the analyses.

The alkaline method, operating in the range of 100 to 600 γ of D-glucose, was somewhat superior in regard to color stability but suffered from the disadvantage of a colored blank. At 543 mμ the method was more sensitive than at the suggested (*3*) 500 mμ.

The phenol–sulfuric acid method, operating in the range 10 to 125 γ of D-glucose, had the advantage of low reagent requirement

but required strongly caustic reagents and had poor reproducibility. Even blank determinations on carefully prepared paper were large and poorly reproducible.

To avoid the necessity of using individual standard curves for the oligosaccharides, each oligosaccharide was hydrolyzed by refluxing 0.01*N* hydrochloric acid in the extraction.

ANALYTICAL METHOD

Separation. For the determination of components *A* through *D* (see section on identification of corn sirup components), prepare chromatographic papers (18 × 57 cm.) by tracing along the heavily outlined edges of a template cut from thin aluminum (Figure 1). Serrate (*13*) the bottom edges—e.g., with pinking shears. Deposit replicate 10-μl. samples of sirup (diluted to about 25% solids) containing about 2.5 mg. of total sugars from a micropipet (Research Equipment Corp., Oakland, Calif.) along lines *B*. Put 5-μl. spots of the same solution at points *A*. Dry the papers, fold along line *C*, suspend in descending chromatographic equipment, and irrigate for 36 hours.

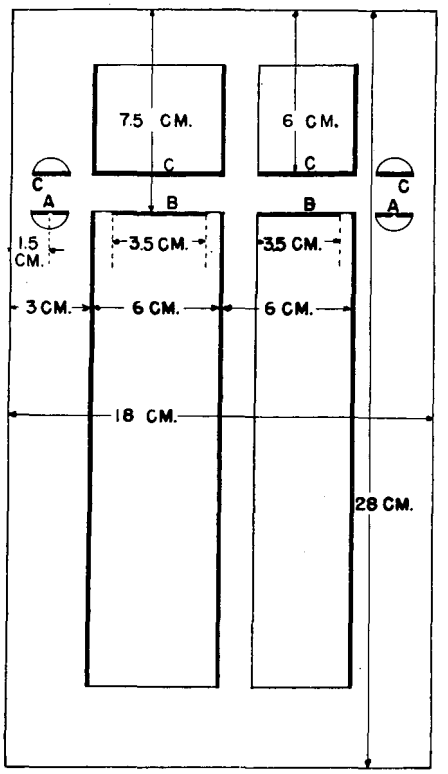

Figure 1. Template for preparing paper chromatograms

Template length (28 cm.)—about half that of paper (57 cm.)—is arbitrary

For the determination of the remainder of the oligosaccharides, *E* through *I*, prepare chromatographic papers as explained above, but add a vertical line bisecting one line *B*. Put 5-μl. spots of sirup at points *A* and at either end of the bisected line, *B*. Deposit one 10-μl. aliquot of sirup along the remaining line, *B*. Dry, fold along line *C*, and sever the three adjacent locator strips except for a 1-cm. band along the top edge. Suspend in descending chromatographic equipment and irrigate for 96 hours. Remove a locator strip each 24 hours to follow the progression of the sugars.

Dry the resulting chromatogram. Cut into strips along the longitudinal lines and develop the locator strips with aniline hydrogen phthalate reagent.

Locate the respective sugars and excise appropriate sections of the chromatogram. Roll these sections into cylinders about 3 cm. in length by 5 mm. in diameter and suspend them from the hooks on the extractor condensers by Nichrome wire clips.

To 2.0-ml. aliquots of 0.01*N* hydrochloric acid in test tubes, add small boiling chips. Fit the condensers with papers attached into the tubes and reflux in an oil bath at 120° for 1 hour. All values are corrected for filter paper blanks.

Table III. Determination of Components in Corn Sirup

Sirup	Dry Solids[a] %	Sirup D.E.[b]	*A*[c]	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*
1	94.10	18.0	5.1	4.8	1.1	4.8	1.2	5.1	5.0	4.1	3.6
2	83.92	26.3	8.3	7.8	1.0	6.2	1.1	7.0	6.3	5.9	5.2
3	79.00	32.6	11.4	9.2	1.3	10.5	1.3	8.6	7.8	5.8	5.1
4	81.80	43.3	19.4	14.4	1.1	10.6	1.2	9.7	8.6	6.2	5.1
5	81.7	49.7	26.1	15.1	2.9	11.2	2.3	9.4	8.4	..	..
6	82.50	55.6	30.9	15.2	3.5	10.0	3.5	9.4	7.2	7.0	5.0
7	82.60	59.9	34.6	15.6	3.7	10.6	3.2	8.7	6.4	..	..
8	70.29	63.0	38.9	22.0	3.8	9.4	3.4	8.2	5.5	3.7	2.4

[a] Dry solids determined by Filtercel method (*5*).
[b] D.E. determined by modified Lane-Eynon method (*22*).
[c] Glucose values *A* corroborated by glucose dehydrogenase and Sichert-Breyer determination (*33*).

DETERMINATION

Alkaline 3,5-Dinitrosalicylic Acid–Phenol Method (*3*). To enough of the extract to contain 100 to 600 γ of D-glucose, add 3.00 ml. of phenol–3,5-dinitrosalicylic acid reagent (*3*), 2 ml. of 6*N* sodium hydroxide, and 1 ml. of 50% potassium sodium tartrate. Dilute to 10.0 ml. with water and mix thoroughly. Heat the tube in boiling water for 10 minutes, cool to room temperature under tap, dilute with water to 10.0 ml., and measure the transmittance at 543 mμ. The amount of sugar present is determined by reference to a previously prepared standard curve.

Phenol–Sulfuric Acid Method (*21*). To enough of the extract to contain 10 to 125 γ of D-glucose, add 0.15 ml. of 80% aqueous phenol and 5.0 ml. of 96% sulfuric acid. Mix vigorously and let stand for 30 minutes, then measure the transmittance at 490 mμ.

Determine the D-glucose by reference to the corresponding standard curve and convert to appropriate oligosaccharide values by applying the respective hydrolysis factors.

Other Methods. In the later phases of this work it was found that excellent quantitative results could be obtained with anthrone or the microferricyanide method of Hagedorn and Jensen (*12*). The ferricyanide method in particular gives excellent reproducibility and is not affected by the presence of filter paper fibers. For this reason it is now routinely used in the authors' laboratories.

ANALYTICAL RESULTS

Eight corn sirups of various degrees of hydrolysis, as expressed by the dextrose equivalent (D.E.) were analyzed by these methods. The first six components (*A* through *F*) were determined by the alkaline 3,5-dinitrosalicylic acid–phenol procedure in all of the sirups. Values for components *C* through *F* in some of these sirups were determined either by the phenol–sulfuric acid method or the ferricyanide method and the analyses extended through spot *I*. Each value of the analytical results recorded in Table III is the average of four to eight replications the mean deviations of which were no more than ±1.3%.

IDENTIFICATION OF CORN SIRUP COMPONENTS

By chromatographic comparisons with authentic samples spots *A*, *B*, and *C* from representative sirups were identified as glucose, maltose, and isomaltose, respectively. From relative chromatographic positions and spot intensities, components *D*, *F*, *G*, *H*, and *I* were inferred to be maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltoheptaose, respectively. It is very likely that at least components *G*, *H*, and *I* are not constituted wholly of pure malto homologs but are admixed with their isomers. Spot *E* was believed to be an isomaltotriose, but it did not correspond chromatographically to *O*-α-D-glucopyranosyl- (1 → 6) -*O*-α-D-glucopyranosyl- (1 → 4)-D-glucopyranose (panose).

To establish their identities, samples of components *B*, *D*, *F*, and *G* were isolated from representative sirups by cellulose column chromatography (*14*) using ethyl acetate–pyridine-water solvent (10:4:3). Concentrates, so prepared, were rechromatographed by the same method, decolorized in 25% aqueous ethyl alcohol with activated carbon and dried for 16 to 30 hours at 78° under reduced pressure over phosphorus pentoxide. These

Table IV. Degree of Polymerization by Hypoiodite Oxidations

Component	Degree of Polymerization	
	Inferred	Found
B	2	1.98
D	3	2.83
F	4	3.74
G	5	5.08

Table V. Analysis of Sirup Components by Periodate Oxidation

Component	D.P. Inferred	Mole-Equivalents of Substance					
		Periodate (*17*)		Formic Acid (*17*)		Formaldehyde (*27*)	
		Calculated	Found	Calculated	Found	Calculated	Found
B	2	5.0	5.1	3.0	3.3	1.0	1.2
D	3	6.0	6.1	3.0	3.1	1.0	1.2
F	4	7.0	7.1	3.0	3.1	1.0	1.3
G	5	8.0	8.2	3.0	3.2	1.0	1.3

fractions were subjected to periodate (*17*, *27*) and hypoiodite (*34*) oxidations; relative chromatographic mobilities (*10*) and specific optical rotations were compared with calculated values.

Hypoiodite Oxidations. The degree of polymerization of an oligosaccharide is equivalent to the increase in reducing power (*34*) on hydrolysis. This information (Table IV) confirms the deduced degrees of polymerization.

Periodate Oxidation. The analytical information obtained by periodate oxidation (Table V) confirms the inferred degrees of polymerization.

Molecular Rotations. Specific optical rotations of the maltopolymer homologous series follow a regular order as expected (*11*) (Table IV).

The information gained in the foregoing investigation demonstrates that components *B*, *D*, *F*, and *G* are maltose, maltotriose, maltotetraose, and maltopentaose, respectively.

Table VI. Molecular Rotations of Corn Sirup Components

Component	Oligosaccharide	Mol. Wt.	[*M*]	Observed [α]
B	Maltose	342	+ 46,512	+136
D	Maltotriose	504	+ 80,640	+159°, +160 (*31*)
F	Maltotetraose	666	+117,432	+176.4
G	Maltopentaose	828	+148,543	+179.4
H	Maltohexaose	989	+179,998	+182.0
I	Maltoheptaose	1152	+204,300	+179, +176 (*9*)
	Maltodocosanose	3582	+691,300	+193 (*20*)

Chromatographic Mobilities. The isolation of components *H* and *I* by column chromatography was not attempted; hence there was no evidence obtained by chemical reactions that they belonged to the maltose homologous series. It has been observed (*1*, *10*, *35*) that, for members of a homologous series, there is a linear relationship between a function of the relative chromatographic mobility and the degree of polymerization. In the solvent employed, the components were found to exhibit the relative mobilities expressed in Table VII.

These data can be correlated by the α-function (*6*), a relationship between the areas of the mobile and stationary phases. French (*10*) has simplified the estimation by employing the relationship $\alpha' - R_f/(1-R_f)$. This relationship, for the nine corn sirup components determined in this work, is demonstrated in Figure 2. Hence, components *H* and *I* appear to be maltohexaose and maltoheptaose, respectively. Component *E* is shown not to be a maltose homolog, and appears to belong to the "iso" series.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the Corn Industries Research Foundation for funds in support of this investigation; to the Corn Products Refining Co. and the American Maize Co. for corn sirup samples; and to Edna Montgomery, Northern Utilization Branch, for a sample of isomaltose. They also wish to acknowledge the assistance of Wan Chen L. Ming, J. W. Armstrong, Ila G. Carroll, and C. K. Kolstadt in the course of the work.

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Table VII. Relative Mobilities of Corn Sirup Components

Component	Sugar	R_f[a]	*R* glucose
A	Glucose	0.270	1.00
B	Maltose	0.176	0.65
C	Isomaltose	0.122	0.45
D	Maltotriose	0.103	0.38
E	Isomaltotriose	0.078	0.29
F	Maltotetraose	0.057	0.21
G	Maltopentaose	0.041	0.15
H	Maltohexaose	0.030	0.11
I	Maltoheptaose	0.022	0.08

[a] Solvent. Ethyl acetate, pyridine, water (10:4:3 v./v.). Temperature. 28–30° C. Whatman No. 1 filter paper, descending technique.

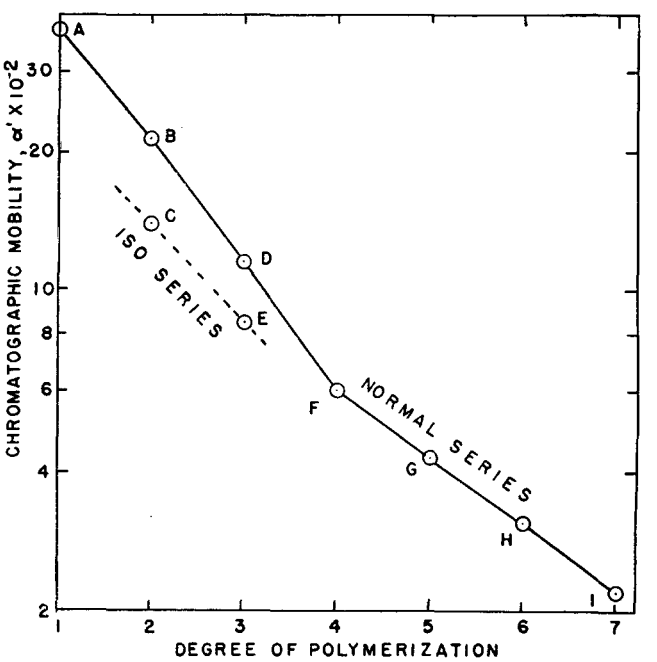

Figure 2. Relationship of chromatographic mobility to degree of polymerization

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RECEIVED for review October 12, 1953. Accepted June 9, 1955. Journal Paper No. 758, Purdue University Agricultural Experiment Station, Lafayette, Ind.

Determination of Alpha-Ketolic Substances in Urinary Extracts and Paper Chromatograms

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The procedure described was developed to fill a need for a reproducible method for determination of α-ketolic steroids in urinary extracts. The procedure has been extended to include determinations of α-ketolic substances on paper chromatograms. Paper strips containing the samples were sprayed with the blue tetrazolium color reagent. The color was eluted with a 7 to 3 mixture of ethyl acetate–methanol and the density determined. Recoveries of added α-ketolic steroids averaged 97%, and the duplicates showed an average deviation from the mean of 6%. The blanks have not been more than 2% of the absorbance because of the α-ketolic content of the sample. A study has been made of normal, pregnancy, and cortical carcinoma urines.

SEVERAL procedures have been reported for the determination of corticosteroids by the use of tetrazolium reagents (*1, 2, 4, 6, 10*). The results obtained with these methods when applied to urinary extracts are inconsistent because of the high blanks in some samples and the instability of the color. Hoffmann and Standinger (*5*) have mentioned a procedure for eluting formazan spots from paper chromatograms and quantitation in a colorimeter, but gave no details and stated that the method was not successful with urine extracts. In experiments with paper chromatography, it was found that certain solvents used for elution of steroids from paper did not remove urinary pigments. Investigation was then instituted in an attempt to find a solvent mixture which would elute the color formed by various color reagents while leaving behind extraneous material, which would interfere with color density determinations. It was found that the blue colored formazan formed by reaction of α-ketols with blue tetrazolium (dianisole bisdiphenyltetrazolium chloride) was water-insoluble and could be washed on the paper with water. The blue formazan was eluted quantitatively from paper strips by a 7 to 3 mixture of ethyl acetate and methanol, after prior washing on the strip with water, and was quantitated colorimetrically. The procedure has been extended also to include α-ketolic steroids on paper chromatograms.

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EXPERIMENTAL

Extraction of Urine. The urines were extracted with chloroform after incubation with glucuronidase (*9*). The values reported here are not maximum, as ideal conditions for extraction of urine have not been attained (*8*). It is probable that the values, in general, are low because 100 units per cc. of glucuronidase were used in hydrolysis of the urine specimens, and more recent experiments show slightly increased amounts when more glucuronidase is used.

Procedure for Determination. For the determination of α-ketolic material, $^1/_{100}$ to $^1/_{150}$ of the extract of a 24-hour sample, extracted as above, is sufficient. Using a minimum amount of 1 to 1 chloroform-methanol, the extract is placed directly on 0.5-inch strips of Whatman No. 1 filter paper and marked into sections of 3.5 inches; care is taken to keep the extract at least $^1/_4$ inch within the dividing line to allow for seepage when the strip is sprayed. The drying is aided by using a jet of dry nitrogen, or the strip is allowed to dry at room temperature. A strip for the standard is set up in the same manner using 25 and 50 γ of desoxycorticosterone. Handling is facilitated when no more than three or four samples are placed on any one strip. The strips are sprayed with blue tetrazolium (two parts of 0.2% aqueous blue tetrazolium and one part of 10% sodium hydroxide solution, freshly prepared) until soaking wet (about 1 cc. of solution is required for each section) and allowed to dry at room temperature. The strips are then washed 3 minutes by immersion in water and again allowed to dry partially at room temperature. While still damp, each section is cut into small pieces, and the formazan eluted in a separate test tube containing 5 ml. of 7 to 3 (by volume) ethyl acetate–methanol mixture. The strips must not dry completely, or all the color will not be eluted. After 10 to 15 minutes with occasional shaking, the solvent is decanted into Evelyn tubes, another 5 ml. of elution mixture is added to the strips, and elution continued an additional 10 to 15 minutes. The second 5 ml. of solvent is decanted from the strips into the initial eluate, and the color density is read in an Evelyn photoelectric colorimeter using a 565 mμ filter. Results are obtained by intrapolation against the curve obtained with the reference standards.

For blanks, the following are used: a solvent blank consisting of the 7 to 3 ethyl acetate–methanol mixture, the reagent blank which is the eluate of a blank strip sprayed with the blue tetrazolium color reagent and eluted as described, and a urine color

blank represented by a urine sample sprayed on the strip with 3.3% aqueous sodium hydroxide and eluted.

It is recommended that no more than 25 to 75 γ of α-ketols be placed on any 3.5-inch section of strip. Too concentrated amounts on the strips led to inaccurate results.

Specificity of Blue Tetrazolium Color Reaction. The specificity of the blue tetrazolium color reaction is not limited to primary α-ketols and other structures under different conditions will give a positive reaction. It has been found that 16-keto-17-hydroxy steroids also exhibit color formation with blue tetrazolium. However, 17-α-hydroxyprogesterone did not. Both androstane-17β-ol-3,16-dione (kindly supplied by M. N. Huffman, Oklahoma Medical Research Foundation) and 16-ketoestradiol-17-β showed pronounced blue color formation with the blue tetrazolium reagent. Meyer and Lindberg (*7*) have reported that Δ^4-androstene-17-β-ol-3,16-dione gave a pink color with the triphenyl tetrazolium reagent.

Many non-α-ketolic Δ^4-3-keto steroids under certain conditions give a positive test with the tetrazolium reagents. In spot plate type tests, alcoholic solutions of several Δ^4-3-keto steroids, notably progesterone, testosterone, 3-keto-Δ^4-etiocholenic acid (courtesy of Leland Chinn, G. D. Searle & Co., Chicago), and Δ^4-pregnene-17α-20-diol-3-one gave a positive reaction with alkaline blue tetrazolium (made as used in the present method). However, the blue tetrazolium reaction appears to be more specific for α-ketols. When non-α-ketolic Δ^4-3-ketones were placed on paper strips and sprayed with the reagent, there was no color formation. Thus, it appears that solubility may be important in this reaction

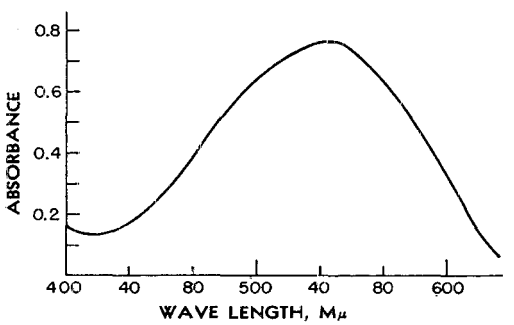

Figure 1. Absorption spectrum of blue formazan formed by α-ketols with blue tetrazolium

(15 γ Compd. F per cc.)

Properties of Color. The color produced by varying amounts of α-ketolic steroids followed Beer's law over the range (5 to 100 γ) studied. The color was stable over a 12-hour period. The absorption spectrum of the color formed by the steroidal α-ketols studied after elution from strips with 7 to 3 ethyl acetate-methanol is shown in Figure 1. The maximum of 550 to 560 mμ was found in all cases; thus only one curve is shown. Cortisone, hydrocortisone, corticosterone, 11-dehydrocorticosterone, 17-α-hydroxy-desoxycorticosterone, and desoxycorticosterone all gave essentially the same absorptivity as did the "tetrahydro" (3α-OH-5β-H) derivatives. The dihydro (5β-H) derivative of cortisone also gave similar results. These results are in agreement with those of Zaffaroni (*11*), who found that equimolar quantities of various free and esterified α-ketolic steroids produced practically identical amounts of color in the Mader-Buck method (*6*).

Precision and Accuracy. In studies on urinary extracts, the urine color blanks have not had an absorbance more than 0.0132, and the reagent blank averaged 0.0269 for each section of strip; both read against the elution solvent. Nonspecific material apparently is adsorbed by the paper, or is removed by the water washing. The laboratory procedure is simple, accurate, and reproducible. In 20 duplicate determinations, the average deviation from the mean was 6%. As shown in Table I, the average recovery of standards added to urinary extracts was 97%.

RESULTS

Table II gives the results obtained in studies with normal and pregnancy urines and that from a patient with an adrenal cortical tumor. For comparison, the neutral reducing lipid values determined by the method of Heard and Sobel (*3*) using phosphomolybdic acid are included. The ratio between these values and the blue tetrazolium value varies more widely. Further studies on pathological urines will be reported elsewhere.

Application to Paper Chromatograms. To test the applicability of the method to determination of α-ketols on paper chromatograms, reference compounds were chromatographed in two

Table I. Recovery Experiments

Subject	Initial BT Value, γ	Added Standard, γ	Found, γ	% Recovery of Standard
1	14	10	24	100
2	40	10	48	80
3	35	10	43	80
4	15	20	34	95
5	1	20	22	105
6	2	20	20	90
7	12	20	33	105
8	9	20	29	100
9	30	30	60	100
10	21	40	70	120
11	10	40	48	95
12	17	40	56	98
13	11	50	62	102
14	11	50	61	100
15	24	50	68	90
16	10	50	55	90
17	27	50	71	90
18	11	50	63	104
19	11	50	61	100
20	19	60	83	106
21	30	80	106	96

Table II. Results of Blue Tetrazolium Determinations on Urinary Extracts

Subj.	Date of Collection	Age	Sex	BT[a], Mg. per 24 Hr.	NRL[b], Mg. per 24 Hr.	NRL/BT
			NORMALS			
1		25	M	2.9	9.9	3.4
2		36	M	2.6	12.6	4.8
3		28	M	1.6	8.6	5.2
4		41	M	2.4	5.8	2.4
5		42	F	1.2	8.6	7.1
6		23	F	1.6	7.1	4.4
7		28	F	2.3	13.4	5.8
8		32	F	1.3	8.5	6.5
9	Aug. 27	29	F	1.8	12.9	7.2
	Sept. 18			1.9	7.8	4.1
	Nov. 17			2.8	14.7	5.3
			PREGNANT			
1	Feb. 11	26		1.4	8.8	6.3
	March 4			1.2	8.3	6.9
2		32		1.1	12.6	11.4
3		28		2.2	18.8	8.5
4		31		1.9	24.5	12.9
5		34		2.4	18.3	7.6
6		36		1.6	12.6	7.8
7		34		1.9	24.0	12.6
8		30		1.2	8.3	7.0
9	Oct. 4	24		2.2	15.7	7.1
	Oct. 23			2.4	21.5	9.0
10		25		2.5	8.4	3.4
11		27		2.1	13.2	6.3
			ADRENAL CORTICAL TUMOR			
1	April 7	42	F	12.1	83.7	6.9
	8			14.0	73.4	5.3
	9			14.2	71.9	5.9
	10			14.1	58.5	4.2
	13[c]			13.4	76.2	5.7
	15[c]			15.9	75.2	4.7
	16[c]			18.8	95.6	5.1

[a] Blue tetrazolium values for α-ketols by present method.
[b] Neutral reducing lipide determinations using phosphomolybdic method of Heard.
[c] Intravenous corticotropin was administered to subject during this period.

different solvent systems. Desoxycorticosterone and dehydrocorticosterone in 25-, 50-, and 75-γ amounts were chromatographed in the methylcyclohexane–propylene glycol system until the steroids had migrated approximately two thirds of the distance down the strip. After drying in air, the strips were sprayed, washed, and dried according to the above procedure. The colored zones were then cut out and eluted, and the color density was determined. The same procedure was followed using cortisone and hydrocortisone in the toluene–propylene glycol system. In all cases, the color density of the sprayed and eluted chromatograms gave values identical with that indicated by the standard curve prepared according to the method.

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Received for review November 11, 1954. Accepted June 20, 1955. Investigation supported in part by a research grant H-922 from the National Heart Institute, National Institute of Health, Public Health Service.

Identification of Imidazole Compounds by Paper Chromatography

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The locations of 20 imidazole compounds are described following paper chromatography. Three solvent systems were used, singly or in combination. The identity of spots was further established by their appearance with ultraviolet light, a *p*-phenyldiazonium sulfonate spray, or an iodine spray. The iodine spray revealed the location of 1-substituted imidazole compounds which other sprays for imidazole compounds did not reveal.

A PAPER chromatographic method for the separation and identification of imidazole compounds is required in connection with biochemical studies in this laboratory. Current methods described in the literature (*1*, *4*, *5*) for the chromatographic separation of imidazole compounds either do not encompass the compounds of interest in these studies, or are not applicable to them.

The range of imidazole compounds for which current paper chromatographic methods are applicable is limited by the nearly exclusive use of *p*-phenyldiazonium sulfonate or a similar diazonium compound as a spray to reveal the spot. Exceptions to this are sprays which react with certain substituents on the imidazole ring—for example, the ninhydrin spray for the α-amino group of histidine. The *p*-phenyldiazonium sulfonate spray (diazonium spray) is a sensitive test for many imidazoles. Unfortunately, it fails to produce colored spots with all such compounds. An important group which does not react with the diazonium spray consists of the 1-substituted imidazoles. This group includes the biologically important 1-methyl histidine which occurs in muscle as the dipeptide anserine. Use of iodine to reveal a variety of organic bases following paper chromatography has been described by several workers (*2*, *6*). Such an iodine spray also reveals spots of these imidazoles refractory with the diazonium spray, as well as all other imidazole compounds tested. The combination of the iodine spray, diazonium spray, and the ultraviolet lamp is capable of revealing spots and in many cases of distinguishing between spots of a large variety of imidazole compounds (Table II).

Further, solvent mixtures described in the literature do not effect a distinct separation of several of the compounds listed in Table I. Two new solvent mixtures (solvent I and II) that separate most of these compounds were devised. The solvent mixture of Inoue (*5*) was found to separate the compounds of Table I which are not separated by solvent mixtures I and II; also the former solvent leads to higher R_f values for imidazoles that bear carboxyl groups. Since the solvent mixture of Inoue complements solvent mixtures I and II, the R_{Im} values are reported for all three (Table I).

EXPERIMENTAL DETAILS

Conventional paper chromatographic techniques are used. Imidazole compounds are spotted on 11.4 × 40 cm. sheets of Whatman No. 52 paper. The sheets are hung lengthwise in jars for development by solvent flow in descent. Jars are equilibrated with the solvent mixture at least 1 hour before the papers are introduced.

Three single-phase solvent mixtures are used for development of the chromatogram. Ethyl alcohol, diethyl ether, water, and 28% ammonia in a 4 to 5 to 1 to 0.1 ratio (solvent I) and acetone, chloroform, water, and 28% ammonia in a 30 to 5 to 4 to 0.2 ratio (solvent II) were formulated in this laboratory. The third, acetic acid, *n*-butyl alcohol, ethyl acetate, water, in a 1 to 1 to 1 to 1 ratio (solvent III) was described by Inoue (*5*). All solvents are distilled before use, except 28% ammonia and glacial acetic acid. The rates of solvent flow over a 30-cm. path are 5 cm. per hour for solvent I, 9 cm. per hour for solvent II, and 3.5 cm. per hour for solvent III.

Table I. Movement of Compounds Relative to Movement of Imidazole

(Whatman No. 52 paper with solvent flow in descent)

Compound	R_{Im} Values		
	Solvent I	Solvent II	Solvent III
Imidazole	1.00	1.00	1.00
4(or 5)-COOH Imidazole	0.10	0.02	0.63
4,5-$(COOH)_2$ Imidazole	0.02	0.02	0.60
4(or 5)-$COOCH_3$ Imidazole	0.98	1.14	1.37
4(or 5)-CHO Imidazole	0.81	0.89	1.06
4(or 5)-CH_2OH Imidazole	0.72	0.48	0.85
1-CH_3 Imidazole	1.05	1.20	1.03
2,4,5-$(Me)_3$ Imidazole	1.26	1.35	1.34
4(or 5)-Br Imidazole	1.16	1.48	1.56
2,4,5-$(Br)_3$ Imidazole	1.00	0.98	1.83
Benzimidazole	1.15	1.42	1.37
L-Histidine	0.08	0.02	0.37
L-Histidine anhydride	..	0.02	0.41
Phthalyl-DL-histidine	0.39	0.08	1.26
Methyl ester of L-histidine	0.68	0.97	0.81
Methyl ester of phthalyl-L-histidine	1.14	1.45	1.50
Pilocarpine	1.03	1.38	1.17
Histamine	0.53	0.80	0.28
NH_4Cl	0.25	0.07	0.38

Table II. Appearance of Spots of Imidazole Compounds after Paper Chromatography

Compound	Appearance with Light: Ultraviolet, initial	Appearance with Light: After I_2 spray: Ordinary	Appearance with Light: After I_2 spray: Ultraviolet	Appearance after Diazonium Spray: Color	Appearance after Diazonium Spray: Relative intensity
Imidazole	None	Brown	Brown	Yellow	Medium
4(or 5)-COOH Imidazole	Blue	Brown	Dark brown	Yellow	High
4,5-$(COOH)_2$ Imidazole	Blue	Dark brown	Dark brown	Yellow	Medium
4(or 5)-$COOCH_3$ Imidazole	Blue	None or ext. weak	Blue	Yellow	Low
4(or 5)-CHO Imidazole	Blue	Faint brown	Blue	Brown	Low
4(or 5)-CH_2OH Imidazole	None	Grayish brown	Brown	Orange	High
1-CH_3 Imidazole	None	Reddish brown	Brown	No color	...
2,4,5-$(CH_3)_3$ Imidazole	Faint blue[a]	Reddish brown	Dark brown	No color	...
4(or 5)-Br Imidazole	None	Faint brown	Brown	Red-orange	High
2,4,5-$(Br)_3$ Imidazole	Blue	Grayish brown	Dark blue	Yellow	High
Benzimidazole	Light blue	Brown	Light blue	No color	...
L-Histidine	None	Dark brown	Brown	Red-orange	High
1-Me-L-histidine[b]	None	Reddish brown	Brown	No color	...
1,3-$(Me)_2$-L-histidine[b]	None	Reddish brown	Brown	No color	...
L-Histidine anhydride	None	Reddish brown	Brown	Red-orange	High
Phthalyl-DL-histidine	None	Brown	Brown	Red-orange	Medium
Methyl ester of L-histidine	None	Brown	Brown	Yellow	Medium
Methyl ester of phthalyl L-histidine[b]	Blue	Brown	Brown	Orange	Medium
Histamine	None	Brown	Brown	Red-orange	High
Philocarpine	None	Reddish brown	Brown	No color	...
NH_4Cl	None	Yellow[a]	Blue	Yellow	Low

[a] No color with solvent III.
[b] Syntheses of these compounds described in a subsequent paper.

The iodine spray is a solution of 1.0 gram of iodine in 100 ml. of 95% ethyl alcohol. The spray is stable for several days, but can not be kept for more than 1 week for best results with all imidazoles. Papers to be sprayed are dried overnight in the open, or 30 minutes in an oven at 100° C. to expell all solvent vapors. Both ammonia and acetic acid interfere with this spray; ammonia by production of highly colored background and acetic acid by inhibition of color formation of the spot. The iodine solution is sprayed lightly over the paper. As the iodine vaporizes from the surface of the paper, the imidazole spots appear. For most compounds these spots rapidly fade and must be marked within a few minutes. However, the paper may be sprayed again, and the spots revived repeatedly if necessary.

Spots may be visualized by their absorption of ultraviolet light. Certain compounds in Table II can be detected on the developed chromatogram without further treatment; others require the iodine spray for appearance. Whereas the spots brought out by the iodine spray rapidly fade when viewed in ordinary light, the spots remain visible permanently with the ultraviolet lamp. Spots are best seen by passage of rays from the lamp through the paper. The lamp used in this laboratory is equipped with a General Electric germicidal bulb and a 4 × 15 cm. window. The window serves as a filter to absorb most rays except those of longer wave lengths of the ultraviolet region (black light). Although less convenient, a Mineralight (Ultra-Violet Products, Inc., South Pasadena, Calif.) with similar characteristics may be used.

The diazonium spray was prepared and applied as directed by Ames and Mitchell (*1*).

The diazonium spray, iodine spray, and ultraviolet light all may be applied to the same chromatogram. The best sequence is as follows: initial view of spots with ultraviolet light, application of the iodine spray, and location of transient spots; a second view with ultraviolet light for spots brought out by the iodine spray; and finally the application of the diazonium spray.

Paper chromatography in two dimensions may be carried out. For this purpose the procedure described by Redfield (*7*) is suitable. The author used 20 × 20 cm. squares of Whatman No. 52 paper rather than the smaller sheets of Schleicher and Schuell No. 507 specified by Redfield. Cylinders formed from these larger sheets can be accommodated in inexpensive, 1-gallon jars. The best sequence of solvent development is the use of solvent III, then I or II, or the use of solvent I, then solvent II.

DISCUSSION

All compounds listed in Table I may be separated by one or more of the three solvent mixtures. [4(or *5*)-Chloromethylimidazole decomposed in all three solvent mixtures.] Further distinction between compounds may be secured by the characteristic response to the combination of sprays and ultraviolet light described in Table II. Movement of spots is reported in terms of R_{Im} in preference to R_f. Movement of spots relative to readily obtainable imidazole (R_{Im}) has been found more consistent in this laboratory than movement relative to the solvent front (R_f). If desired, R_f values may be calculated from the R_{Im} values in Table I and from the R_f values for imidazole. The R_f values for imidazole are 0.73 in solvent I, 0.57 in solvent II, and 0.51 in solvent III.

The compounds histidine, 1-methyl histidine and 1,3-dimethyl histidine, are of special interest in connection with studies in this laboratory. The brief time of solvent flow used for the chromatograms described in Table I was not sufficient to differentiate the above three compounds from each other. The three could be distinguished readily by longer periods of solvent flow. The solvent was allowed to flow down the sheet and drip off the bottom for periods of 24 to 48 hours. Results are shown in Table III; spot location is expressed relative to movement of histidine.

Samples of imidazoles applied to the paper as their hydrochloride salts, yielded a spot which apparently was due to ammonium ion retention at the position of migration of the chloride ion. This effect was due to the ammonium cation rather than the chloride or other anion. In solvent I (which contains ammonia) potassium chloride, magnesium chloride, sodium chloride, and ammonium chloride gave spots with the diazonium spray at identical positions; sodium sulfate and ammonium sulfate gave spots at identical positions, but distinct from those for chloride salts; and silver nitrate and ammonium nitrate gave a third set of identical spots, but distinct from those for chloride or sulfate salts. Conversely, in solvent III (which does not contain ammonia) ammonium sulfate, ammonium nitrate, and ammonium chloride, all gave yellow spots with the diazonium spray, but at different positions on the paper, whereas sodium sulfate, silver nitrate and sodium, potassium, or magnesium chlorides did not give spots. These facts should be remembered in the study of chromatograms of mixtures which contain anions, because the ammonium spot reacts with all three spot tests of Table II.

Table III. Movement of Methyl Histidine Compounds Relative to Movement of Histidine (R_H)

(Whatman No. 52 paper with solvent flow in descent)

Compound	R_H: Solvent I	R_H: Solvent III
L-Histidine	1.00	1.00
1-Me-L-Histidine	1.26	0.87
1,3-$(Me)_2$-L-Histidine	0.63	1.05

When possible, solvents I or II were used in preference to solvent III. Spots were more compact, and background with the iodine spray was less with solvents I and II. With solvent III the chromatogram was overloaded more readily with the result that spots were streaked. Spots of not more than about 0.05 micromole should be developed with solvent III, but tenfold or even fiftyfold more may be developed with solvents I and II. After application of spots, the formation of streaks with solvent III was reduced by exposure of the paper to ammonia vapors for 15 minutes. The paper was aerated well to expel all ammonia before it was placed in the jar for development with solvent III. A second exposure to ammonia vapors was of advantage after

development with solvent III in the event that spot formation with the iodine spray was poor.

Sensitivity of the diazonium spray and iodine spray vary with the nature of the imidazole compound, but an order of magnitude may be indicated. The diazonium spray is capable of detecting 10^{-3} micromoles of imidazole and the iodine spray is capable of detecting 10^{-2} micromoles. Whether maximum sensitivity with the iodine spray occurs with the transient color in ordinary light, or with the ultraviolet light, depends on the nature of the compound. For example, the transient spot in ordinary light was the most sensitive indicator of 1-methylimidazole whereas the corresponding spot with 4(or 5)-bromoimidazole was weak. The latter compound gave a strong spot when viewed with ultraviolet light.

The nature of the reaction with the iodine spray is not known (*2*). Substitution on the imidazole ring is unlikely since imidazoles which bear substituents on the ring nitrogen are reported not to undergo iodination (*3*). Addition of iodine to the unsaturated imidazole ring is unlikely also in view of the known resistance of the imidazole ring to this type of reaction. Possibly a reversible physical combination of iodine with the imidazole compound occurs. This is indicated by the fact that the colored spots formed with most imidazole compounds rapidly faded, but could be revived repeatedly by further application of the iodine spray. Highly-colored unstable products were observed by Brunings (*3*). He has proposed that these products are iodinated on the ring nitrogen at position No. 3.

ACKNOWLEDGMENT

The author takes this opportunity to thank Reuben Jones for a sample of the methyl ester of 4(or 5)-carboxyimidazole and Eva Wormser for the preparation of several other compounds used in these studies.

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RECEIVED for review March 12, 1955. Accepted May 23, 1955.

Colorimetric Method for Analysis of Histidine and Certain Related Imidazole Compounds

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A colorimetric method is described for the quantitative analysis of histidine and certain of its derivatives, histamine and imidazole. Phenols and aromatic bases which interfere with other colorimetric procedures for histidine did not interfere with this method unless present in relatively large amounts.

THE Pauly (*6*) diazo reaction has long been used for the colorimetric determination of imidazole compounds. This reaction between imidazoles and diazotized aromatic amines in alkaline solution leads to the formation of azo dyes. This is the basis of the classic Koessler-Hanke procedure (*5*) for histidine. A more specific method for histidine is the Kapeller-Adler method (*3*). This method is based on the observation of Knoop (*4*) that a heated solution of histidine in bromine water yields a reddish-colored product. Both of these methods suffer from lack of specificity, and many compounds other than imidazoles contribute color. These include phenols, aromatic amines, pyrroles, and indoles.

A more specific method was desired by the author in order to analyze for certain imidazole compounds in the presence of other imidazoles and other compounds known to interfere with the above methods. A number of reactions that might lead to colored products were investigated. Of these, the most promising reaction was based on the Bamberger degradation (*1*) of imidazoles with benzoyl chloride in alkali. In order to obtain a colored product, *p*-nitrobenzoyl chloride was substituted for benzoyl chloride (Equation 1). Conditions were devised so that the amount of light absorbed at the wave length of maximum absorption by (I) was proportional to the concentration of the imidazole compound. (See structural Reaction I.)

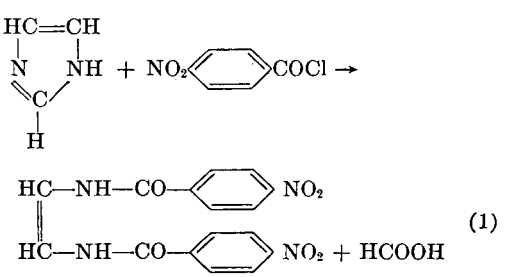
The identity of the colored product of the reaction with imidazole was established to be 1,2-di(*p*-nitrobenzamido)ethylene-(I). Properties of the colored product from the procedure for the colorimetric analysis proved to be identical with those of a purified preparation of 1,2-di(*p*-nitrobenzamido)ethylene.

The specificity of the colorimetric method was tested with 16 imidazole compounds. Of these, only imidazole, histamine, histidine, and derivatives of histidine were reactive. Other compounds such as tryptophane, tryosine, arginine, or phenol did not interfere with this procedure unless present in relatively large amounts.

EXPERIMENTAL DETAILS

Procedure for Colorimetric Analysis. STOCK REAGENTS Aqueous sodium bicarbonate, 1.0*M*, and 1.0*N* aqueous sodium hydroxide.

p-Nitrobenzoyl chloride, 0.06*M*, in acetone. The *p*-nitrobenzoyl chloride from Eastman Kodak Co., was used without purification. The *p*-nitrobenzoyl chloride from Matheson Co. was crystallized from petroleum ether (boiling point 60° to 70° C.) before use. The acetone solution of *p*-nitrobenzoyl chloride



(1)

(I)

is unstable and should be prepared no more than a few minutes before use.

PROCEDURE. An amount of sample up to 1.0 ml. is placed in a colorimeter tube calibrated for 10-ml. volume. The volume of the sample is adjusted to 1.0 ml. by addition of water, to which 0.1 ml. of 1*M* sodium bicarbonate and then 5.0 ml. of 0.06*M* *p*-nitrobenzoyl chloride are added. The mixture is vigorously swirled and kept at room temperature for 10 minutes.

Two milliliters of 1*N* sodium hydroxide is added, the mixture is vigorously swirled again, and is allowed to stand for 5 minutes.

The mixture is diluted to 10 ml. with water. After 30 minutes the color intensity is measured in a colorimeter with a filter of maximum transmittance at 420 mμ.

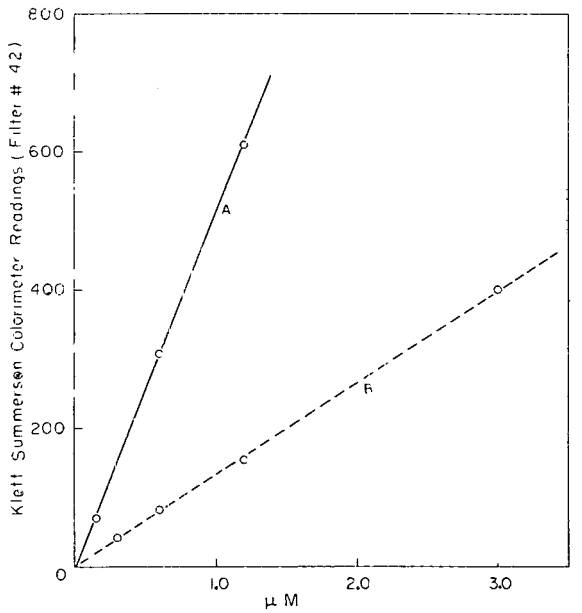


Figure 1. Standard curves for analysis of imidazole and histidine

A. Imidazole
B. Histidine

A standard curve should be made from aliquots of a standard solution of the same imidazole compound that is present in the sample for analysis. This standard curve was found to be linear for all compounds tested (Figure 1).

Nature of Colored Product of Reaction. SYNTHESIS OF 1,2-DI(*p*-NITROBENZAMIDO)ETHYLENE (I). Two grams (0.03*M*) of imidazole was dissolved in 40 ml. of 2*N* sodium hydroxide, and 5.5 grams (0.03*M*) of *p*-nitrobenzoyl chloride was added with stirring at 0° C. The mixture was maintained at 0° C. for 6 hours and stored overnight at 20° C. The mixture then was extracted with 100 ml. of benzene. The benzene extract was discarded. The pH of the aqueous mixture was adjusted to 2 to 3 with concentrated hydrochloric acid, and 50 ml. of water was added. The slurry of yellow crystals was extracted with 50 ml. of ether and 50 ml. of benzene. Both extracts were discarded. The yellow crystals were filtered and washed with water. Weight of the dried product was 3.6 grams; this was a 68% yield, based on the *p*-nitrobenzoyl chloride used.

Table I. Compounds Not Yielding Colored Products with *p*-Nitrobenzoyl Chloride Reagent

1-Methylimidazole
4(or 5)-Bromoimidazole
4(or 5)-Chloromethylimidazole
4(or 5)-Hydroxymethylimidazole
4(or 5)-Imidazolecarboxyaldehyde
4(or 5)-Imidazolecarboxylic acid
Methyl ester of 4(or 5)-imidazolecarboxylic acid
2,4,5-Tribromoimidazole
2,4,5-Trimethylimidazole
Pilocarpine
Ammonia
Phenol
Pyridine

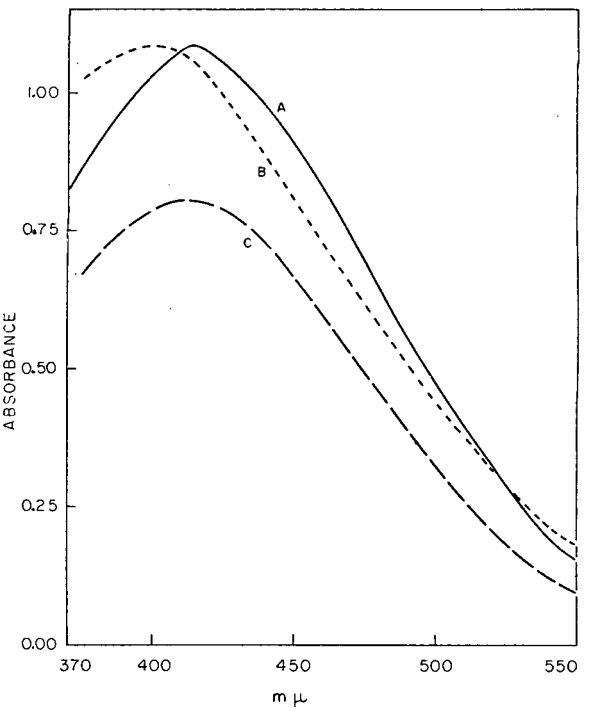


Figure 2. Absorption spectra of products of modified *p*-nitrobenzoyl chloride procedure

Histidine and imidazole were treated with 1.0 ml. of *p*-nitrobenzoyl chloride reagent. All other conditions were in accordance with the prescribed colorimetric procedure. The spectrophotometer was adjusted to 100% transmittance with a reagent blank.
A. $1 \times 10^{-4}M$ 1,2-di(*p*-nitrobenzamido)ethylene in aqueous alkali (instrument adjusted to 100% transmittance with water)
B. 5.5 Micromoles of histidine
C. 1.5 Micromoles of imidazole

Table II. Compounds Yielding Colored Products with *p*-Nitrobenzoyl Chloride Reagent

Compound	Micromoles of Compd. for Equiv. Color Intensity at 420 mμ
Imidazole	1.0
Histidine	3.4
Phthalyl histidine	3.4
Methyl ester of histidine	7
Histamine	8
Benzimidazole	>100
Aniline	25
Arginine	>100
Hydrazine	1.3
Hydroxylamine	18
Phenylalanine	50
Tryptophane	45
Tyrosine	100

The product was recrystallized from ethyl alcohol and again from acetone. Decomposition point was 256° C.

Element Analysis, %	Calcd. for $C_{16}H_{12}O_6N_4$	Found
Carbon	53.93	53.81
Hydrogen	3.40	3.56

The absorption spectrum of the compound in aqueous alkali had a single peak at 417 mμ; the molar absorption coefficient was 1.104×10^4 at 417 mμ (Figure 2).

ISOLATION OF COLORED PRODUCT FROM PROCEDURE FOR COLORIMETRIC ANALYSIS OF IMIDAZOLE. In 18 ml. of water 0.12 gram of imidazole was dissolved. Then 1.8 ml. of 1*M* sodium bicarbonate followed by 90 ml. of 0.06*M* *p*-nitrobenzoyl chloride in acetone were added. The mixture was kept at room temperature for 30 minutes. To this 36 ml. of 2.0*N* sodium hydroxide and 34 ml. of water were added. The clear red solution was extracted with ether. The ether extract was dried over calcium sulfate and evaporated to leave 0.2 gram of yellow powder. The yield as 1,2-di(*p*-nitrobenzamido)ethylene was 32%. The

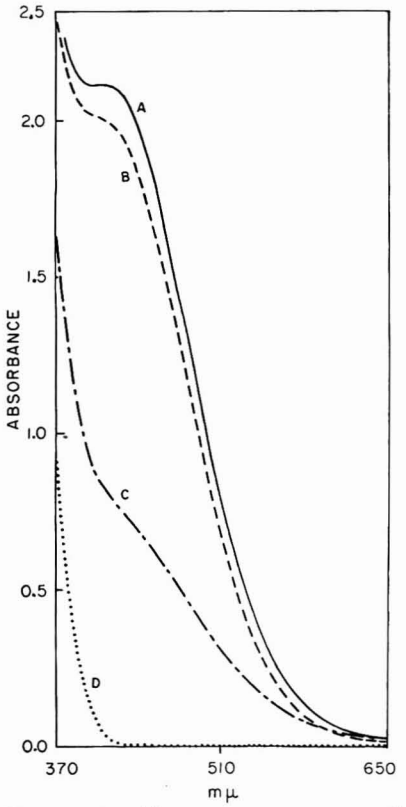


Figure 3. Absorption spectra of products of *p*-nitrobenzoyl chloride procedure

All samples were subjected to prescribed colorimetric procedure.
A. 2 Micromoles of 1,2-di(*p*-nitrobenzamido) ethylene
B. 2 Micromoles of imidazole
C. 5 Micromoles of histidine
D. Reagent blank

product was recrystallized from ethyl alcohol. Decomposition point was 254° C. This point was not lowered when an intimate mixture of this product and 1,2-di(*p*-nitrobenzamido)ethylene prepared above were heated together. The absorption spectrum of this product coincided with that of the 1,2-di(*p*-nitrobenzamido)ethylene.

DISCUSSION

Histidine and its derivatives, histamine and imidazole, gave colored products with the *p*-nitrobenzoyl chloride reagent. Eight other imidazole compounds tested (Table I) failed to give colored products. Several compounds listed in Table II other than imidazoles gave colored products, but the concentrations of these compounds required for equal color intensity were at least tenfold greater than those for the reactive imidazole compounds. Exceptions were hydrazine and hydroxylamine. The sensitivity of the method to 1×10^{-6} gram of hydrazine may make the method of some value for the analysis of hydrazine. However, this sensitivity for hydrazine is no greater, and in the case of hydroxylamine is less, than methods now in the literature (*2*, *7*).

From Figure 1, the method is capable of detecting 0.1 micromole of histidine when a colorimeter is used. By use of a more sensitive spectrophotometer at 417 mμ the sensitivity of the method is increased to 0.025 micromole of histidine or 4 γ.

Routine analyses of imidazole and histidine over a period of several months gave a standard deviation (σ) of ±5.0%. Accuracy of the method will be considered in subsequent papers on the applications of the method to the analysis of histidine in urine, and of carnosine and anserine in muscle tissues.

Comparison of absorption spectra of purified 1,2-di(*p*-nitrobenzamido)ethylene and of colorimetric analysis mixtures was difficult because of the high absorption of the latter mixtures below 400 mμ (Figure 3). This absorption below 400 mμ was due to *p*-nitrobenzoic acid from the hydrolysis of excess *p*-nitrobenzoyl chloride reagent. A better comparison of spectra could be obtained by development of the color with one fifth of the prescribed amount of *p*-nitrobenzoyl chloride reagent, and by adjustment of the spectrophotometer to 100% transmittance with a reagent blank. This comparison is made in Figure 2. The spectral peaks of 1,2-di(*p*-nitrobenzamido)ethylene and of imidazole color product occur at 417 mμ. The peak for the histidine color product appears to be at 400 mμ.

A few comments on details of the procedure should be made. The method gives erratic results if sodium bicarbonate is omitted or replaced by sodium acetate. The amount of sodium bicarbonate added is not sufficient to neutralize all hydrochloric acid liberated by reaction of the *p*-nitrobenzoyl chloride present. However, addition of greater amounts of sodium bicarbonate than are called for in the procedure was unsatisfactory. The ratio of acetone to water during the reaction of sample and *p*-nitrobenzoyl chloride is critical. Greater or lesser amounts of acetone led to erratic results. Neither *m*-nitrobenzoyl chloride nor 3,4-dinitrobenzoyl chloride could replace *p*-nitrobenzoyl chloride. The time of reaction with *p*-nitrobenzoyl chloride prior to addition of alkali is not critical, and a longer period than 10 minutes is without harm. The color produced after addition of alkali is stable for several hours.

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RECEIVED for review March 12, 1955. Accepted May 23, 1955.

Separation of Mixture of Nine Monosaccharides by Two-Dimensional Ascending Paper Chromatography

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Two-dimensional ascending chromatography has been employed to separate a mixture of monosaccharides not resolved by the use of a single solvent system. A mixture of rhamnose, fucose, ribose, xylose, arabinose, fructose, mannose, glucose, and galactose was clearly resolved and separated from two hexuronic acids. Two initial runs were made in 1-butanol, pyridine, and water (3 to 2 to 1.5) followed by a single run in a second dimension in phenol saturated with water. Characteristically colored spots, which tended to enhance the degree of separation, were obtained by the use of a β-naphthylamine spray. Fructose may be selectively demonstrated by the use of an orcinol spray. The methods described are simple and applicable to the separation and identification of mixtures of monosaccharides in the acid hydrolyzate of material of biological origin.

MANY solvents are available for paper chromatographic separation of mixtures of simple sugars. A solvent often used for this purpose is 1-butanol, pyridine, and water in the volume proportions of 3 to 2 to 1.5 (*3*). Glegg and Eidinger (*6*) and Sophian and Connolly (*14*) separated mixtures of a number of sugars by unidimensional ascending paper chromatography using this solvent. However, certain sugars have identical R_f values in this solvent and repeated unidimensional runs fail to separate them. Jeanes, Wise, and Dimler (*8*) showed that mannose, fructose, and arabinose migrate together in the above solvent, as well as in other combinations of 1-butanol or fusel oil, pyridine, and water.

Partridge (*11*) used phenol saturated with water, in an atmosphere of ammonia, to separate mannose and fructose in unidimensional descending chromatography. Evans and Mehl (*5*) had similar results with ascending chromatography. However, the phenol solvent alone fails to separate most of the hexoses.

Unidimensional chromatography is therefore of limited value in the separation of certain sugars. Single spots on the chromatograms may actually represent two or more sugars that have migrated together. The identity of these spots, though they may correspond in location to those of the standard sugars employed, cannot be known with certainty. To provide for more rigorous identification, Partridge (*11, 12*) employed two-dimensional descending chromatography using widely differing solvent systems to separate a mixture of certain sugars.

This paper describes a method for completely resolving a mixture of nine monosaccharides and separating these from two hexuronic acids by the use of two-dimensional ascending chromatography.

MATERIAL AND METHODS

Paper. Whatman No. 1 filter paper was used in these studies.

Apparatus. Large glass cylinders with flat bottoms were lined with filter paper, which was saturated with the solvent when this was a one-phase system, or with the aqueous layer when the solutions were not miscible. A large Petri dish filled with solvent was placed at the bottom of the cylinder. The top of the cylinder was lined with stopcock grease to ensure a tight fit of the glass cover.

Sugar and Hexuronic Acid Solutions. Twenty milligrams of the sugar was dissolved in 1 ml. of distilled water in small glass vials. The sugars used were from commercial sources and consisted of rhamnose, fucose, xylose, arabinose, ribose, mannose, glucose, galactose, and fructose. A solution of reductic acid and galacturonic acid in distilled water was similarly prepared. Glucuronic acid solutions 2 weight by volume % were prepared from the lactone by neutralization.

Preparation of Chromatograms. A sheet of filter paper 31 × 31 cm. was used. For unidimensional ascending runs, 40 γ of the sugar or hexuronic acid solution was applied with a 2 λ micropipet along a line, 2 cm. from the bottom edge of the paper. Each spot was 2.5 cm. apart. For two-dimensional runs, the sugars and acids were superimposed on one spot in the lower right hand corner of the paper. The paper was formed into a cylinder and the approximated edges were stapled through masking tape.

All chromatograms were run in a room with the temperature controlled at 23° to 24° C. Two-unidimensional ascending runs were made each time for 17 hours. The paper was dried and replaced in fresh solvent for each run.

When a second dimensional run was carried out, the staples were cut; the paper was opened and then turned 90°. A smooth edge was provided for the base of the paper, the edges restapled, the paper placed in the phenol solvent, and the solvent front allowed to advance for 15 hours.

Solvents. Table I lists the volume proportions of the solvents used, in order of decreasing resolving ability, for the initial ascending runs. In all cases, the alcohol was redistilled before use.

Phenol saturated with water (*11*) was the solvent used for chromatography in the second dimension. An atmosphere of ammonia was provided in the cylinder. A small amount of 8-quinolinol was added to the solvent to inhibit darkening of the paper (*7*).

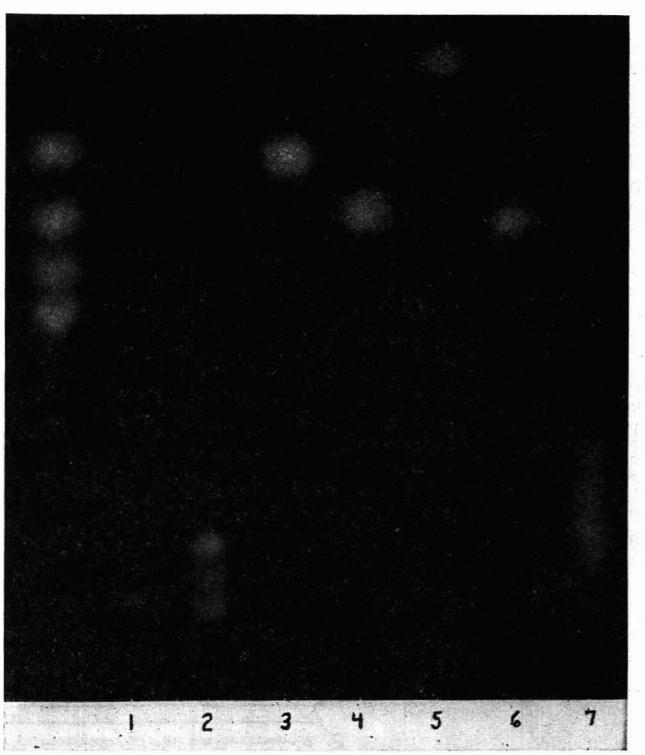

Figure 1. Undimensional ascending chromatogram in 1-butanol, pyridine, and water

The mixture (far left) consists of (from the top down) fucose, mannose, glucose, and galactose. Continuing to the right are:

1. Glucuronic acid
2. Galacturonic acid
3. Xylose
4. Arabinose
5. Rhamnose
6. Fructose
7. Reductic acid

Table I. Solvents for Initial Unidimensional Ascending Chromatography of Mixture of Monosaccharides

(In order of decreasing resolving ability)

Alcohol		Pyridine	Water	Acid	Reference
1-Butanol	3	2	1.5	.....	(*3*)
1-Butanol + ethanol	40 11	..	19	.....	(*1*)
3-Methyl-1-butanol	35	35	30	.....	(*15*)
2-Propanol	70	..	10	Glacial acetic-20	(*13*)

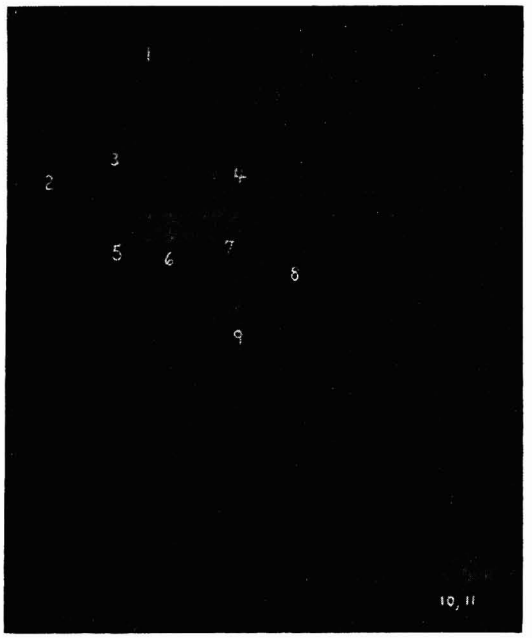

Figure 2. Two-dimensional chromatogram

Run first in butanol, pyridine, and water, and then in phenol, showing positions of methylpentoses (yellow), aldohexoses (brown), pentoses (orange-pink), and hexuronic acids (pink). Point of origin, lower right hand corner.

1. Rhamnose
2. Fucose
3. Ribose
4. Xylose
5. Arabinose
6. Fructose
7. Mannose
8. Glucose
9. Galactose
10. Galacturonic acid
11. Glucuronic acid

Detection of Sugars. A β-naphthylamine spray (*10*) was used for the detection of sugars and hexuronic acids.

For the specific detection of fructose, orcinol (*2*) recrystallized from benzene was used. The paper was sprayed lightly with this reagent in acidified alcohol, and then heated at 100° C. for 1 minute. The paper was next sprayed with the β-naphthylamine reagent and heated at 140° C. for 3 to 5 minutes to detect the nonketose spots.

RESULTS

Chromatographic Separation of Sugars. The most satisfactory solvent for initial, unidimensional ascending chromatography of a mixture of sugars was 1-butanol, pyridine, and water (3 to 2 to 1.5). Other suitable solvents are listed in Table I. Hexoses were clearly resolved (Figure 1), and hexuronic acids were separated from the sugars. However, xylose and fucose, and arabinose, mannose, and fructose moved together on the chromatograms in all the solvents listed in Table I.

Complete separation of the sugars was accomplished by a second dimensional run in the phenol solvent (Figure 2). Color characteristics imparted by the β-naphthylamine spray served to enhance the degree of resolution above that noted in the black and white reproduction. The hexuronic acids were separated from the sugars, but were not themselves resolved in these solvents.

Specific Detection of Fructose. Fructose appeared as a bright orange spot on chromatograms sprayed with orcinol reagent. Other sugars present, as well as the hexuronic acids, did not appear provided the paper was heated for only 1 minute at 100° C. Subsequent spraying with β-naphthylamine and further heat brought out the spots of the nonketose substances, and fructose turned from orange to dark green.

DISCUSSION

Mixtures of arabinose, fructose and mannose, and xylose and fucose could not be separated by means of two unidimensional runs in a number of solvent systems listed in Table I; and phenol alone, which could resolve these particular groups of sugars, was not able to satisfactorily separate most of the hexoses. The separate use of both solvents clearly achieved the desired result.

In addition to chromatographic separation, fructose may be differentiated from other sugars, particularly mannose and arabinose, by use of an orcinol reagent (*2*, *9*). Nonketoses on the same paper may then be detected with the β-naphthylamine spray. Similar results have been reported using urea as the first spray to detect fructose followed by aniline phthalate to demonstrate the other sugars (*4*).

The methods described have proved useful in identifying monosaccharides in the acid hydrolyzate of material of biological origin.

ACKNOWLEDGMENT

The authors are grateful to the staff of the Department of Medical Illustration, Subsection, Fitzsimons Army Hospital, for photographs of the chromatograms. Theodore E. Friedemann, Medical Nutrition Laboratory, generously supplied the galacturonic and reductic acids.

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RECEIVED for review February 14, 1955. Accepted May 31, 1955. Opinions expressed in this paper are those of the authors and do not necessarily represent those of any governmental agency.

Automatic Coulometric Titrations Involving an Amperometric End Point

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An instrument has been developed for automatic control of coulometric titrations employing the dual indicator electrode amperometric end point. The instrument is capable of detecting the end of the titration and stopping the generation, freeing the analyst of this task. The titration is stopped at a preset indicator current, either as a dead-stop end point or as a preliminary operation to determining the exact end point by extrapolation of the post-end-point current to zero. The operation of the instrument on three different types of coulometric titrations is described.

VARIOUS coulometric titrations and the end points used in this laboratory have been described (*2*, *4*, *7–11*). This paper is concerned with titrations that make use of dual indicator electrode amperometric end points which have been used in this laboratory (see Figures 1 and 2).

The dual indicator electrode amperometric end-point system forms a basis for a versatile titrator. The simplicity of the amperometric system, both functionally as to the physical arrangement and in its direct application to a number of different titrations, is essential in a system where different types of analyses are to be performed. The fact that the response of the indicator circuit is almost instantaneous simplifies circuit design. The dual indicator electrode system has the additional advantage that in most instances the indicator current does not change the concentration of the system being measured.

The three types of amperometric end points which have been encountered and toward which the instrument has been designed are shown in Figure 1 (*A*, *B*, and *C*). The most common type of end point is that represented by curve *A*, where the indicator current has a steady or very slowly rising value until the vicinity of the end point. After the end point is passed it increases rapidly in a linear manner (when plotted against generation time as shown in Figure 1). A representative end point of this type occurs in the titration of arsenic with electrolytically generated bromine (*6*). Several authors describe automatic coulometric titrators which use this type of end point (*1*, *5*, *6*). An automatic coulometric titrator that employs potentiometric detection of the end of the titration has been described (*3*).

Curve *B* represents the case where the end point is preceded by a sudden reversal of indicator current, as has occasionally been experienced in titrations involving electrolytically generated bromine and chlorine (*6*). This reversal is believed to be due to a shift at an electrode from one controlling half cell to another.

Curve *C* represents the indicator current in a third type of titration represented by the titration of iodide with electrolytically generated bromine (*9*). In this type of titration, the end point is preceded by a high maximum of indicator current.

The usual method of locating the end point has been described in detail by several authors (*2*, *6*, *9*), but essentially consists in all cases of extrapolating the linearly rising post-end-point current to zero current and then applying a correction obtained from a blank titration. Thus, when used with this amperometric technique, this instrument is not required to stop the titration exactly at the end point, for the exact end point of a titration is obtained mathematically, as indicated. The instrument which has been developed will stop a titration at any preset level of indicator current within its range. The analyst then records the value of this indicator current and the elapsed generation time from the timer, generates manually for 1 second more, and again records the necessary data, from which he can calculate the slope of the indicator current (against generation time) for the purpose of extrapolating to zero end point current. He then applies corrections obtained from a blank determination to find the corrected end point.

Although a dead-stop type of instrument is not necessary for this technique, it would be possible to use the instrument here for routine analyses as a dead-stop instrument with accuracy on the order of 1%. If a number of similar titrations were being made, it would be feasible to calculate a factor (on the basis of a blank titration) to enable the analyst quickly to compute the results of the determination on the basis of the elapsed time as indicated directly by the timer.

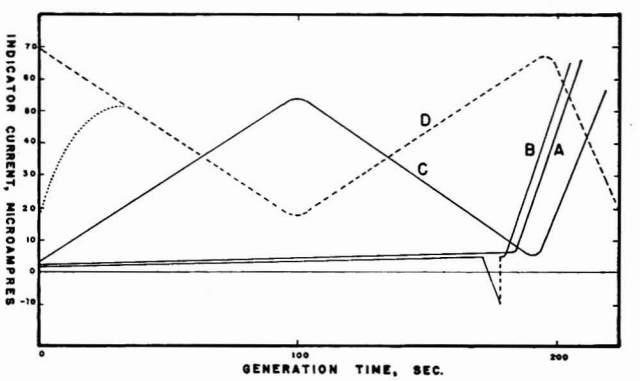

Figure 1. Indicator current during three types of coulometric titrations

One other means of locating the end point would be to record the indicator current and use the automatic controller to stop the titration after the end point has been passed. The end point could be obtained from the recorded indicator current in the manner described above.

PRINCIPLE OF OPERATION

The instrument described was constructed as an accessory unit which can easily be attached to existing coulometric titrators. The basic titrator (*5*) consists of a titration cell, an indicating circuit which employs two platinum electrodes in the titration cell, and the generation circuit. The latter consists of an electronically controlled constant-current power supply with an elapsed time indicator, in connection with two other platinum electrodes in the titration cell. For automatic operation, the progress of the titration is followed by measuring the current between the indicator electrodes and the potential drop across the electrodes (the latter having an inverse relationship to the magnitude of the indicator current). The indicator current is actually measured in the form of a potential created by the flow of the indicator current through a series resistor, *R* (Figure 2).

Because of the magnitude of the indicator current used in the dual indicator electrode amperometric method of indication described here (0 to 50 μa.) and the amounts of resistance that can be placed in series with the indicator electrodes (generally 1000

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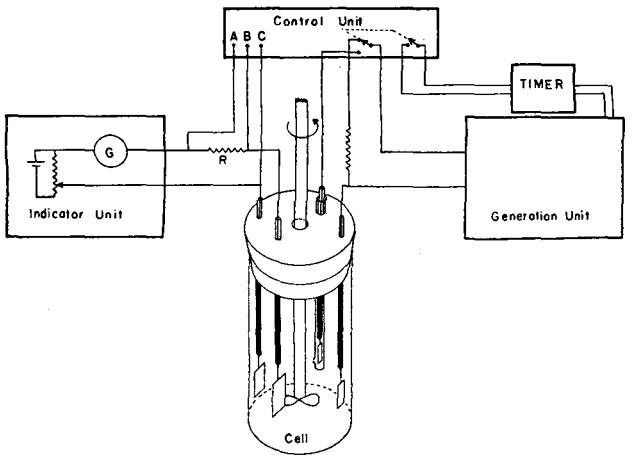

Figure 2. Complete coulometric titrator

ohms), the potential available for control purposes is on the order of 0 to 50 mv. The instrument therefore must contain some sort of amplifier to produce power sufficient to actuate control relays. The amplifier must be of the direct-current type, as the indicator current is of this classification, and the direction of flow of this current is of importance. The amplifier selected for this application is a so-called "chopper amplifier" which converts a high-gain, stable alternating current amplifier into a direct current amplifier.

Block diagrams of the apparatus are shown in Figure 3.

V_1 and V_2 constitute the direct current amplifier. V_3 and V_4 are electronic relay circuits. V_4 is a cathode-coupled amplifier intended to give inversion of polarity, which operates on a positive input (normal flow of indicator current). Vacuum tubes V_3 and V_4 actuate relays when the output of the amplifier reaches a certain preset value—that is, when the indicator current or potential drop between the indicator electrodes reaches a certain value.

The operation of the instrument on the various end points is discussed with the aid of the block diagrams of Figure 3.

Titration curve A (Figure 1), the simplest of the three, is described first in a simple way, and then after titrations B and C are discussed, titration curve A is described fully. Thus, first, circuit V_3 will be ignored. In all three types of end points shown, the termination of the titration occurs on the post-end-point linear rise of indicator current.

End point A consists only of this linear current rise. Relay tube V_4 can be preset to stop the titration at any desired value of indicator current—let us say 10 μa. (Figure 3,*a*). The potential developed across the sensing resistor, R, is amplified by V_1 and V_2 and applied to V_4. When it reaches the preset value, the relay in the plate circuit of V_4 closes and the titration is terminated. In actual operation with a titration made on a fast generation rate there may be a lag in the operation of the device and the final indicator current may be 15 μa. when shutoff was set for 10.

Titration B is similar to A, but contains a sudden reversal of indicator current. When and if this occurs, it is necessary to stop the titration and give the titration system a few seconds to attain equilibrium conditions again. This reversal does not always occur. The appropriate block diagram is Figure 3,*b*.

Relay tubes V_3 and V_4 are both connected to the amplifier output. V_4 again responds to a positive flow of indicator current as was described for end point A. V_3 responds to a negative flow of indicator current and is set to a low value. If a value of indicator current more negative than this is encountered, this circuit stops the titration. This can be of the form of a permanent termination of the titration (which is customary, as this occurs near the end point) or the machine can be set to resume the titration when the indicator current becomes more positive than the preset negative value for V_3, and V_4 then terminates the titration as for end point A.

End point C of Figure 1 requires the use of the potential drop across the indicator electrodes. The appropriate block diagram is Figure 3,*c*. Consider the circuit of Figure 2, which shows a resistance in series with the indicator electrodes. The combined resistance of the sensing resistor, R, and the resistance of the indicator current meter is of such a value that it is small compared to the resistance of the cell when very little indicator current is flowing and large compared to the cell resistance when 10 or more microamperes of indicator current are flowing. The

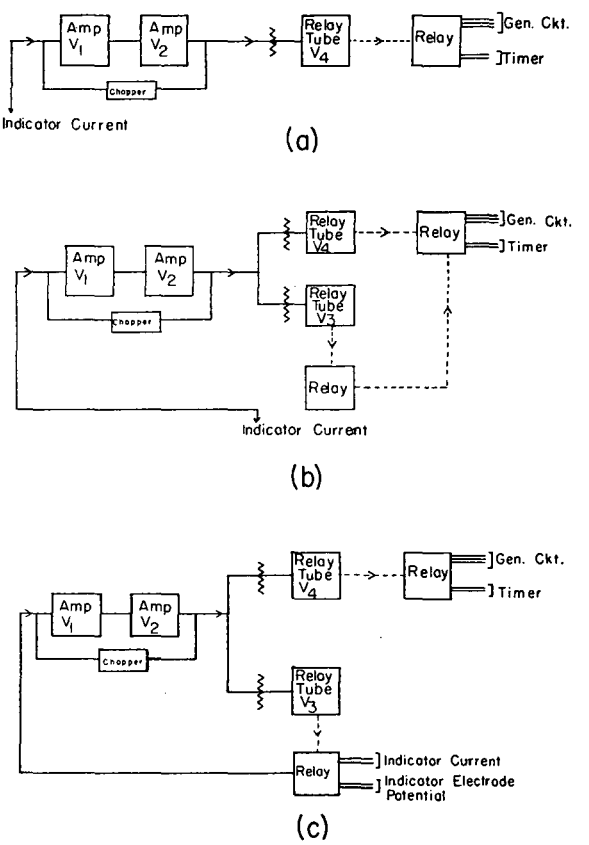

Figure 3. Block diagrams of operation of automatic controller

potential drop across the indicator electrodes will not be constant, but, because of the external resistance, will be strongly dependent on the indicator current. This potential drop is shown qualitatively by the dashed curve, D, of Figure 1. When very little indicator current is flowing, this potential is almost the applied indicator electrode potential. When indicator current flows during the titration, because of the potential drop across the external resistance in the indicator circuit, the potential across the indicator electrodes decreases. If the same system were used for end point C as for A, relay tube V_4 would respond at the first rise of indicator current and the titrations would be stopped prematurely. To avoid this, V_3 is used in such a way as to prevent application of the amplifier output to V_4 until just before the end point.

The relay associated with V_3 is used to switch the amplifier input from across the indicator electrodes to across the sensing resistor, R. A titration involving end point C begins with the amplifier input connected to the indicator electrodes, the potential

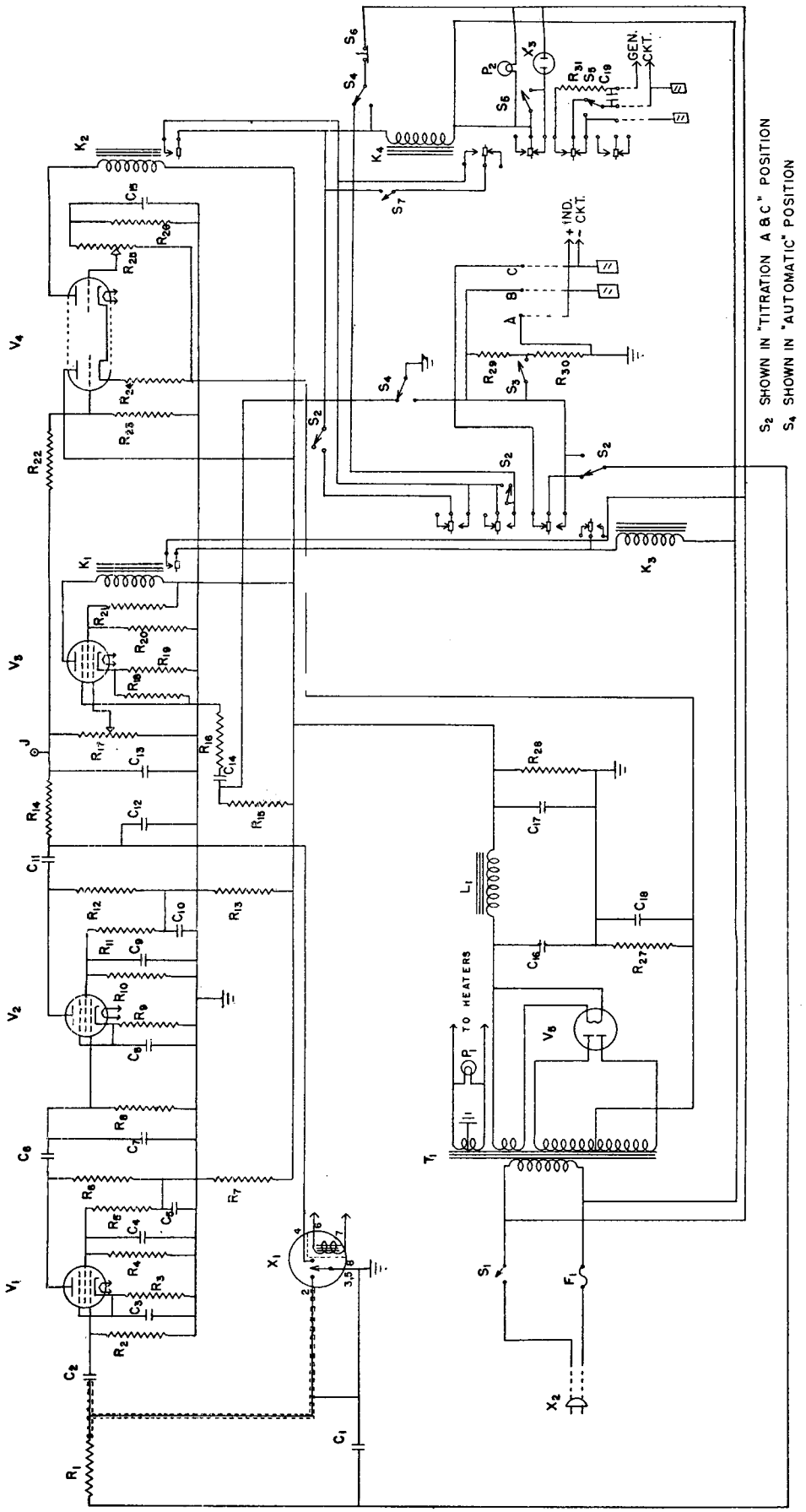

Figure 4. Circuit diagram of automatic controller

C_1. 0.5-μfd. 600-volt paper
C_2. 0.02-μfd. 600-volt paper
C_3. 25-μfd. 25-volt electrolytic
C_4. 0.1-μfd. 400-volt paper
C_5. 0.1-μfd. 400-volt paper
C_6. 0.05-μfd. 600-volt paper
C_7. 100-μμfd. disk ceramic
C_8. 25-μfd. 25-volt electrolytic
C_9. 0.1-μfd. 400-volt paper

R_{11}. 100-K 0.5-watt
R_{12}. 250-K 0.5-watt
R_{13}. 5-K 0.5-watt
R_{14}. 1.8-M 0.5-watt
R_{15}. 68-K 1-watt
R_{16}. 100-K 0.5-watt
R_{17}. 100-K potentiometer (control B)
R_{18}. 47-K 0.5-watt
R_{19}. 1-K 1-watt

C_{10}. 0.1-μfd. 400-volt paper
C_{11}. 0.5-μfd. 600-volt paper
C_{12}. 100-μμfd. disk ceramic
C_{13}. 0.5-μfd. 400-volt paper
C_{14}. 8-μfd. 450-volt electrolytic
C_{15}. 0.1-μfd. 200-volt paper
C_{16}. 8-μfd. 450-volt electrolytic
C_{17}. 8-μfd. 450-volt electrolytic
C_{18}. 50-μfd. 50-volt electrolytic
C_{19}. 1-μfd. 400-volt paper

F_1. Fuse, 3 amperes

J. Test prod jack for meter

K_1. Sensitive relay; Leach No. 1037 (1750 ohms d.c. resistance)
K_2. Sensitive relay; Leach No. 1037 (1750 ohms d.c. resistance)
K_3. Control relay; Leach No. 2127 (110-volt a.c.)
K_4. Control relay; Leach No. 2127 (110-volt a.c.)
L_1. 8-henry 80-ma. choke
P_1. 6.3-volt pilot light
P_2. 110-volt pilot light

R_1. 1.5-M 0.5-watt
R_2. 2-M 0.5-watt
R_3. 2.2-K 1-watt
R_4. 100-K 0.5-watt
R_5. 100-K 0.5-watt
R_6. 250-K 0.5-watt
R_7. 5-K 0.5-watt
R_8. 1-M 0.5-watt
R_9. 2.2-K 1-watt
R_{10}. 100-K 0.5-watt
R_{20}. 25-K 0.5-watt
R_{21}. 33-K 1-watt
R_{22}. 100-K 0.5-watt
R_{23}. 380-K 0.5-watt
R_{24}. 560-K 1-watt
R_{25}. 100-K potentiometer (control A)
R_{26}. 33-K 0.5-watt
R_{27}. 470-K 10-watt
R_{28}. 40-K 5-watt
R_{29}. 9-K 0.5-watt
R_{30}. 500-K 0.5-watt
R_{31}. Dummy load (300 ~ 1-watt)

S_1. SPST toggle switch (power on-off)
S_2. Four PDT telephone switch (titration A, C-B)
S_3. SPST toggle switch (sensitivity low-high)
S_4. DPDT toggle switch (automatic-manual)
S_5. DPDT push button (manual generation)
S_6. SPST push button (N.C.) (clearing button)
S_7. SPST toggle switch (permanent stop)

T_1. Power transformer; Thordarson No. T22R31

V_1. 5879
V_2. 5879
V_3. 6AS6
V_4. 6J6
V_5. 5Y3GT

X_1. Stevens-Arnold d.c.-a.c. chopper Type 243
X_2. Plug for 110-volt a.c. mains
X_3. Socket for timer power

K = 10^3 ohms. M = 10^6 ohms.

of which is chosen to be opposite in polarity to the potential across R. The amplifier output is always connected to both V_3 and V_4 and, because the output of the amplifier will be negative during the first part of the titration, V_4 will not respond to the signal. V_3 does respond to a negative output and is preset to respond at a potential near to the applied indicator potential. The desired point of response is just before the maximum on curve D of Figure 1, which occurs just before the end point. When this happens, the relay associated with V_3 changes the input to the potential drop across R and the titration completes as was described for titration A. By the process just described, the sampling of the high intermediate indicator current by the amplifier is avoided. As V_5 would respond to the potential across the indicator electrodes at the start of the titration when it is high (Figure 1, D), a gate tube is used for V_3, and is disabled during the first part of the titration, effectively producing a condition replacing the initial dashed portion of curve D with that part indicated by dots.

Titrations involving end point A can now be accurately described, as the same arrangement is used as for end point C. The indicator electrode potential (when plotted against generation time) bears an inverse relationship to the indicator current (compare Figure 1, C and D). During a titration which culminates in an A type of end point, the indicator electrode potential differences will be high until the linear rise of current denoting the end point. Thus, after the disabling of V_3 at the start of the titration, V_3 will respond at once, owing to the high negative input, transferring the input to the amplifier to the potential drop across R. The titration will then terminate in the manner described for end point A at the beginning of this discussion.

No changes in switching need to be made to differentiate between end points A and C. End point B requires a different arrangement, the possibility of a current reversal is known beforehand and necessary switching (from c, Figure 3, to b) can be done. In the titrations where indicator current reversals are sometimes experienced, intermediate indicator current maxima have not been found.

ELECTRONIC CIRCUITS

With the instrument set for end point B, the input to the amplifier is always the potential drop across the sensing resistor, R, and the response of either V_3 or V_4 can terminate the titration. With the instrument set for titration C (or A) the input to the amplifier is switched by the relay associated with V_3 and only V_4 can terminate the titration. Provision has been made for more than one value of sensing resistor to be available. The sensing resistor may be either 500 or 10,000 ohms, depending on the conditions of the titration. If the indicator current and its pre-end-point maximum are small, R_{29} (10,000 ohms) is used. The sensing resistance is selected by a switch, S_3.

The schematic circuit for the instrument is presented as Figure 4. With the titrations that have been discussed above, two types of end-point control are available. In the case of a titration involving a slow rate of reaction, it may be desirable to have the instrument stop the generation when the preset value of indicator current is reached, but to resume the titration if the indicator current falls below the preset value of indicator current when the excess intermediate has reacted with the substance being titrated. On the other hand, it may be desirable to have the instrument permanently stop the titration when the preset value of indicator current has been reached. This control is accomplished by a switch, S_7, which connects holding contacts on the generation control relay, V_4, representing the "permanent stop" condition and when open is the "slow rate" position.

V_1 and V_2 of Figure 4 and associated components comprise the direct current amplifier. V_1 and V_2 are connected in the form of a high-gain audio-frequency amplifier and this is converted to a direct-current amplifier by means of the chopper indicated. The operation of this unit is not discussed here. The direct

Table I. Titrations of Iodide by Bromine

Run	A	i_0, μa.	t_{end}, Sec.	i_t, μa.	i_{t+1}, μa.	T, Sec.	W, γ
1	60	1	87.6_5	29	34	81.8_5	546
2	60	0	87.0_2	31	37	81.8_6	546
3	51	1	85.5	22	27	81.1_0	541
4	51	1	85.0	22	30	82.2_5	549
5	51	1	85.6	22	29.5	82.6_7	551
6	60	1	86.0	24.5	30.5	81.8_8	547
7	60	1	85.9_2	28	35	81.9_0	547
8	60	1	86.2	26	32	71.8_7	547
9	62	0	86.1_8	29.5	36.5	81.9_7	548
10	50	0	84.7_8	16	21	81.5_8	544
11	62	1	85.8_8	28	35	81.8_8	547
						Av. found	546
	548 γ of iodide taken for each run					S.d.	2.5

current amplifier was found to be the most critical part of the assembly as far as construction was concerned, careful isolation and shielding are necessary to avoid pickup. Instabilities were encountered and traced to two sources. The adjustment of the chopper is important, it is a make-before-break switch and coupling between the input and output of the alternating current amplifier occurs if the adjustment is otherwise. It was also found necessary to bypass the plate circuits of V_1 and V_2 with small condensers to avoid oscillation (C_7 and C_{12}).

V_3 is used as a power amplifier. The sensitive relay in the plate circuit actuates a larger relay which does the necessary switching. Desensitization of V_3 at the start of the titration is accomplished by the network in the suppressor grid circuit. When switch S_4 is thrown from "manual" to "automatic," condenser C_{14} (which was charged to the supply voltage) discharges through R_{13}, with a time constant such that V_3 is gated for about 10 seconds. During this time the plate current is zero and response of this stage to the output of the amplifier is avoided. The amplifier output is applied to the tube by means of R_{21}, which in effect determines the indicator electrode potential, or negative indicator current at which relay K_1 will close.

V_4 is a cathode-coupled current amplifier. The value of the cathode resistor is somewhat critical and may have to be determined by experiment to put the operation of the tube in the proper region of plate current. The maximum output of the chopper amplifier is several volts and this is fed to the grid of V_4. The cutoff level is set by potentiometer R_{25} in the grid of the second half of the tube, which applies a negative bias. This bias is obtained from a resistor, R_{27}, in series with the negative supply connection in order to get a fairly constant voltage. A suitable filter (C_{18}, R_{26}, C_{15}) is provided to remove any ripple components that might be present.

Ordinary constructional practice for wiring has been followed. Pains as to proper shielding on the high-gain and high-signal parts of the chopper amplifier must be taken. A shield across the chopper socket, to isolate the input and output leads of the high-gain alternating current amplifier, is recommended. Most component values are not critical. Although the sensitive relays in the plate circuits of V_3 and V_4 were placed under the chassis in this instrument, an arrangement which would permit them to be accessible for adjustment in the normal operating position would be very desirable, as their operation is greatly influenced by gravity.

The amplifier used has more than ample gain, and some sort of feedback could be profitably included for the purpose of stabilization and prevention of amplifier saturation. It has not been attempted here. In the interest of reducing transients which may be produced by switching, all relay contacts carrying 110-volt current have been appropriately bypassed by capacitors, which are not shown in Figure 4.

A lever-type telephone switch, S_2, changes the circuitry from end point B to A and C (Figure 1), switching the input of the amplifier and the sequencing of V_3 and V_4. A push-button switch, S_6, is used as a "clearing" switch to open all relays at the start of a titration.

EXPERIMENTAL

Several different types of titrations have been successfully performed with the automatic instrument. The prime function of the instrument is to stop the titration in the vicinity of the end point. Although experimental results are presented, the accuracy of coulometric titrations involving dual indicator electrode amperometric end points determined as described above is not dependent upon whether the titration is performed by manual or automatic control of the generation. The crucial test was that the titration was automatically stopped at or near the proper post-end-point current.

Titrations of tripositive antimony by means of electrolytically generated bromine of the type described by Brown and Swift (*2*) have been carried out with the instrument described above by E. A. Butler of this laboratory. In every case the titration was stopped at the preset indicator current level, and the results of the titrations agreed within experimental error (0.2%) with the calculated amount of tripositive antimony taken.

Titrations of thiosulfate with electrolytically generated iodine (*9*) were made by J. K. Rowley of this laboratory with both manual and automatic control of the generation. The instrument worked satisfactorily in this application, both methods of control giving similar results.

To test this instrument on an end point such as represented by C of Figure 1, iodide was titrated by electrolytically generated bromine using both manual and automatic control. The titrations were done so as to duplicate the method of Wooster, Farrington, and Swift (*11*), except that by necessity here the indicator current was allowed to flow throughout the titration. The results of one series of titrations are summarized in Table I. Here A is the potentiometer setting which adjusts the shutoff level on V_4, i_0 is the indicator current at the start of the titration, t_{end} is the reading on the timer when the titration stops, i_t is the indicator current at the time of stopping, and i_{t+1} is the indicator current after 1 second of manual generation after stopping. The calculated time of the end point is T, and W is the calculated weight of iodide found. The correction from a blank determination was negligible. The accuracy and reproducibility of the results are comparable to those of Wooster, Farrington, and Swift (*11*) and independent of the setting of the potentiometer A.

Before making titrations with the automatic controller, as with any piece of electronic apparatus, it is advisable to allow about a 20-minute warm-up period. It has been found necessary here to perform a couple of initial titrations at the beginning of each new series of runs in order to establish the two potentiometer settings required for correct shut-off points. The use of the instrument for dead-stop titrations has not been explored. For a series of titrations carried out under similar conditions, it should be possible to calibrate the machine for dead-stop usage when maximum accuracy of the method is not required.

ACKNOWLEDGMENT

The advice and interest of Ernest H. Swift, under whom this problem was started as undergraduate research, have been appreciated. The author would like to thank Bart Locanthi, now of Computer Engineering Associates, for advice on chopper amplifiers.

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RECEIVED for review April 1, 1955. Accepted June 29, 1955. Presented in part before the Division of Analytical Chemistry at the 123rd Meeting of the AMERICAN CHEMICAL SOCIETY, Los Angeles, Calif., March 1953. Contribution 1981, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, Calif.

Precise Assay of Trichloroacetic Acid by Coulometry at Controlled Potential

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The reduction of trichloroacetate ion from an ammoniacal medium proceeds quantitatively to dichloroacetate ion at a mercury cathode whose potential is maintained at a constant suitable value. Integrating the current which flows during such an electrolysis permits the determination of trichloroacetate in pure samples or in the presence of at least 8 times as much dichloroacetate, with an accuracy and precision of within ±0.2%.

MONO- and dichloroacetic acids are universal contaminants of trichloroacetic acid, and no method of assaying trichloroacetic acid (*4*, *9*) has yet been proposed, which is free from interference by the lower chlorinated acids. Such a method is presented in this paper. It is based on the quantitative electroreduction of trichloroacetate ion to dichloroacetate ion in an ammoniacal medium, which proceeds with 100% current efficiency at a mercury cathode at a suitably chosen potential. An integration of the current flowing during this reduction gives the amount of trichloroacetate present in the sample to ±0.2% or better.

The reduction of the chloroacetic acids at a mercury cathode has been studied several times by polarographic techniques. Elving and Tang (*2*, *3*) showed that dichloroacetate ion in ammonical media gives a single wave at a fairly negative potential, corresponding to the reaction

$$Cl_2CHCOO^- + H_2O + 2e \rightarrow ClCH_2COO^- + Cl^- + OH^-$$

Trichloroacetate ion under the same conditions gives a double wave: The second wave is identical with the single dichloroacetate wave, while the first wave represents the reduction of trichloroacetate to dichloroacetate. This first wave, which is well defined, was used by Elving and Tang (*2*) for the polarographic determination of trichloroacetate in the presence of dichloroacetate; of course this is insufficiently accurate for assay purposes. Elving and Tang (*3*) found that monochloroacetate ion was not reducible from an ammoniacal solution.

Neiman, Ryabov, and Sheyanova (*8*), on the other hand, asserted that monochloroacetate does give a single wave in 0.1*M* sodium hydroxide, and that dichloroacetate and trichloroacetate correspondingly give two and three waves, respectively. This the present authors were unable to confirm; the polarographic characteristics of the chloroacetic acids in sodium hydroxide media do not differ in any significant respect from those found in ammoniacal media by Elving and Tang (*3*).

From this information it appeared possible to select conditions under which trichloroacetate ion alone could be reduced, and quantitatively, and to apply this to the coulometric determination of trichloroacetate, either alone or in mixtures with dichloroacetate.

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EXPERIMENTAL

The double diaphragm cell, the potentiostat, and the current integrator, which are manufactured by Analytical Instruments, Inc., Bristol, Conn., have been described (*6*), as has the recording polarograph used (*7*). All weights and volumetric apparatus were carefully calibrated by conventional techniques.

The chloroacetic acids were secured from a commercial supplier. The trichloroacetic acid was dried for a week over anhydrous magnesium perchlorate. Titration of a sample of the dried acid with sodium hydroxide standardized against potassium hydrogen phthalate gave (on the assumption that no lower chlorinated acid was present) an "assay" of 99.98%. Stock solutions of the acid appeared to be stable indefinitely when ammonium and potassium chlorides were present alone, but when ammonia was also present the values secured by coulometric analysis decreased at the rate of about 1% per week. The kinetics of this reaction have been studied by Verhoek (*10*).

Some of the mixtures of di- and trichloroacetic acids were prepared from the commercial dichloroacetic acid. Analysis of this material, either polarographically (*2*) or by the coulometric procedure described below, indicated the presence of roughly 0.3% trichloroacetic acid. The necessity of correcting for this amount of impurity would have severely limited the accuracy attainable with the mixtures containing much dichloroacetic acid. Consequently, a pure dichloroacetate solution was prepared by the following procedure. A solution of 4.6 grams of trichloroacetic acid in the ammoniacal supporting electrolyte used throughout the work was electrolyzed at a mercury cathode at −0.8 volt *vs.* S.C.E. until the current had fallen to zero, and the resulting solution was transferred to a 250-ml. volumetric flask and diluted to the mark with the stock supporting electrolyte. Whereas ammoniacal solutions of the commercial dichloroacetic acid rapidly yellowed on standing, the electrolytically prepared solution appeared stable for many months.

RECOMMENDED PROCEDURE

Prepare a stock solution containing 2.5*M* ammonia, 1*M* ammonium chloride, and 2*M* potassium chloride. (The potassium chloride serves primarily to decrease the cell resistance; and none of the concentrations is in any way critical.) Dissolve a sample of trichloroacetic acid weighing 0.03 to 5 grams in a little water and add about 40 ml. of the supporting electrolyte solution. If the weight of the sample exceeds 1 gram, enough additional ammonia should be added to restore that lost by neutralization.

Fill the auxiliary electrode and central compartments of a double diaphragm cell for controlled potential electrolysis in the manner described previously (*6*), and transfer the trichloroacetate solution to the working electrode compartment. Since the time required to complete the electrolysis is proportional to the volume of the solution (*5*), not more than an additional 50 ml. of the supporting electrolyte should be used in completing the transfer.

Pass a stream of tank nitrogen or hydrogen through an efficient gas washing bottle filled with the supporting electrolyte and containing 1 to 2 grams of hydrazine dihydrochloride to facilitate the removal of oxygen, and thence into the solution in the working electrode compartment of the cell. Since oxygen is reducible at a mercury cathode under the conditions used in this procedure, it is essential to remove all dissolved air before the electrolysis is begun. This normally requires about 5 minutes, provided that a sufficiently rapid stream of gas is used and the solution is well stirred.

Add about 30 ml. of pure mercury, read the coulometer register,

and adjust the potentiostat to maintain the potential of the mercury electrode at −0.9 volt *vs.* S.C.E. Do not disconnect the gas stream until the electrolysis is completed.

The electrolysis may now be allowed to proceed unattended. After 60 to 90 minutes the current will fall to zero and the integrator will stop. Subtract the initial from the final register reading to give directly the number of milliequivalents of trichloroacetic acid. (The integrator's 1-ohm resistor is always used unless the weight of trichloroacetic acid present is less than about 0.05 gram.) Since two faradays are consumed in the reduction of each mole of trichloroacetic acid, the equivalent weight of the acid is 81.70 grams.

After the completion of the electrolysis, discard the solution and rinse the cell thoroughly. The mercury may be re-used many times without purification.

RESULTS AND DISCUSSION

The half-wave potentials corresponding to the reactions

$$Cl_3CCOO^- + H_2O + 2e \rightarrow Cl_2CHCOO^- + OH^- + Cl^- \quad (1)$$

and

$$Cl_2CHCOO^- + H_2O + 2e \rightarrow ClCH_2COO^- + OH^- + Cl^- \quad (2)$$

are −0.73 and −1.65 volts, respectively, in the supporting electrolyte recommended. (In the absence of the potassium chloride they are −0.93 and −1.62 volts; thus the potassium chloride also serves the unexpected purpose of providing a better separation of the waves.) Since both of the waves are irreversible, and therefore cover a considerable range of potentials, the selection of the optimum potential at which to conduct the electrolysis is more difficult than it would be if the waves were reversible.

Normally one would anticipate that a potential of about −1.2 volts *vs.* S.C.E., midway between the two half-wave potentials, would effect complete reduction of trichloroacetate without initiating Reaction 2. This is not the case, however, for some dichloroacetate is reduced at this potential during the latter stages of the electrolysis, and the value found is a few tenths of a per cent too high (Table I). At −1.3 volts the reduction of dichloroacetate is even more extensive, and the error becomes about +2%. Even at −1.1 volts one may be on dangerous ground in dealing with solutions containing much dichloroacetate for, as is well known, the potential at which a wave begins becomes more positive as the concentration of the reducible substance increases.

Table I. Assay of Trichloroacetic Acid

Cathode Potential, Volts *vs.* S.C.E.	Trichloroacetic Acid Taken, Grams	Trichloroacetic Acid Found, Grams	Assay, %
−0.80	4.5894	4.5855	99.91
−0.90	0.03762	0.03763	100.03
		0.03760	99.95
		0.03760	99.95
	0.07369	0.07364	99.93
		0.07320	99.33
		0.07337	99.56
		0.07315	99.26
	0.15096	0.1505	99.70
		0.1503	99.56
		0.1504	99.63
		0.1503	99.56
		0.1503	99.56
	0.3649	0.3638	99.70
		0.3635	99.62
		0.3655	100.16
	0.3773	0.3757	99.58
		0.3770	99.92
	0.7575	0.7565	99.87
		0.7583	100.11
		0.7550	99.67
	1.5150	1.5080	99.54
	1.5183	1.5132	99.66
	5.6928	3.6823	99.72
	3.9976	3.9870	99.74
	Average of 24 values at −0.90 v.		99.72 ± 0.22 (standard deviation)
−1.00	0.2993	0.2993	100.00
		0.2990	99.90
		0.2993	100.00
		0.2991	99.93
		0.2992	99.97
	0.3649	0.3628	99.42
		0.3638	99.70
		0.3629	99.45
		0.3638	99.70
		0.3633	99.56
		0.3635	99.62
		0.3634	99.59
	Average of 12 values at −1.00 v.		99.74 ± 0.21 (standard deviation)
−1.10	0.2993	0.2993	100.00
−1.20	0.2993	0.3002	100.30
−1.30	0.2993	0.3056	102.1
		0.3050	101.9

Table II. Determination of Trichloroacetic Acid in Presence of Dichloroacetic Acid

Cathode Potential, Volts *vs.* S.C.E.	Trichloroacetic Acid Taken, Grams	Dichloroacetic Acid Added, Grams	Trichloroacetic Acid Found, Grams	Assay, %
−1.00	0.2994	0.0125	0.3005	100.37
−0.90	0.3773	0.0286	0.3770	99.92
−1.00	0.2994	0.0250	0.2986	99.73
	0.2995	0 0376	0 2992	99 90
−0 90	0 3773	0.0718	0.3767	99.85
−1.00	0.2996	0.0627	0.2988	99.73
−0.90	0.3773	0.108	0.3766	99.81
	0.3773	0.145	0.3766	99.81
−1.00	0.2991	0.125	0.2976	99.50
−0.90	0.3773	0.217	0.3764	99.76
	0.3773	0.362	0.3767	99.84
	0.3773	0.579	0.3763	99.73
	0.3773	0.868	0.3770	99.92
	0.1501	1.16	0.1500	99.93
			0.1500	99.93
			Average	99.85 ± 0.18 (standard deviation)

As the averages of the 24 values secured at −0.90 volt and of the 12 values secured at −1.00 volt differed by only 0.02%, it seems evident that electrolysis at either potential suffices for the complete reduction of trichloroacetate without any danger of reducing dichloroacetate. Table II shows this to be true even when the original solution contains 8 times as much dichloroacetate as trichloroacetate. Though samples poorer in trichloroacetate than this would probably be most efficiently analyzed by the procedure of Elving and Tang (*2*), there is no reason to believe that a coulometric analysis conducted at, say, −0.90 volt, would not provide a more accurate result if the need arose. Since a considerable excess of dichloroacetate does not measurably affect the results, it is equally clear that the presence of substantial amounts of monochloroacetate would also be without effect.

The average of the 36 values at these two potentials, 99.73 ± 0.22% (standard deviation), together with the result of the alkalimetric "assay" previously described, indicates that the dried trichloroacetic acid contained approximately 0.25% lower chlorinated acids. Though this seems to be entirely credible, its confirmation by any other method would be extremely difficult.

Of special interest from a theoretical standpoint is the result of the electrolysis at −0.80 volt shown in Table I. This is only 0.07 volt more negative than the half-wave potential of trichloroacetate, and the current measured at this potential with a dropping electrode is barely two thirds of the diffusion current, yet the reduction at the large electrode proceeded to completion. Of course an electrolysis at so low a potential requires considerably more time than does one at −0.9 or −1.0 volt. The fact that quantitative reduction is eventually secured merely reflects the chemical irreversibility of the electrode reaction. At −0.8 volt one is dealing, not with an equilibrium mixture of di- and trichloroacetate at the surface of the electrode, but with a

reduction which simply proceeds at a lower rate than it would at a higher potential. Though these considerations were outlined by Delahay (*1*), this is believed to be their first practical application.

ACKNOWLEDGMENT

It is a pleasure to thank Analytical Instruments, Inc., of Bristol, Conn., for the grant-in-aid which made this work possible.

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RECEIVED for review March 29, 1955. Accepted May 3, 1955. Contribution No. 1330 from the Department of Chemistry of Yale University.

Application of Organic Solvent Extraction to Flame Spectrophotometry

Determination of Iron in Nonferrous Alloys

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This investigation describes an application of organic solvent extraction to flame spectrophotometry. Iron was selectively extracted from aqueous solutions with acetylacetone, which served as chelating agent and solvent. The extract was aspirated directly into an oxy-acetylene flame. This procedure circumvents many spectral and radiation interferences encountered in iron determinations by flame spectrophotometry and provides a superior aspirating medium compared to an aqueous solution containing variable concentrations of diverse elements. Acetylacetone contributes greatly to the size of the flame and increases sixfold the luminescence of the 372-mμ iron line. No interferences were found when this method was applied to aluminum-, copper-, and nickel-base alloys and limestone. Replicate samples show a standard deviation of 3%.

THE present investigation resulted from the need for a faster method for the flame spectrophotometric determination of iron. In a preceding study a considerable number of elements were found either to interfere directly or to affect the luminescence from the 372.0-, 373.7-, 374.7-, or 386.0-mμ flame emission lines of iron (*5*). Manganese emits a series of weak bands in the region 363 to 400 mμ, and magnesium possesses three intense band systems whose heads appear at 372, 375, and 385 mμ. In addition to these coincidences, interference with the excitation process has been observed for aluminum, zinc, the alkaline earths, lithium, and potassium. Through the addition of cobalt, as an internal standard, interference from aluminum, zinc, and lithium was circumvented. However, difficulties due to magnesium, manganese, and the alkaline earths could be circumvented only if the iron were first separated as the hydrous oxide or by some other chemical method.

Although solvent extraction is recognized as a powerful method for accomplishing analytical separations, it has not yet been exploited in flame spectrophotometry. The solvent extraction of iron as the iron acetylacetonate chelate is a convenient and rapid method for isolating iron from other elements encountered in nonferrous alloys and limestone. Iron is 50% extracted at a pH of zero (*8*). Thus several extractions with acetylacetone (2,4-pentanedione) quantitatively remove iron from a 1*N* acid solution. As a result, the extraction of iron can be accomplished in the presence of metals which hydrolyze at higher pH values.

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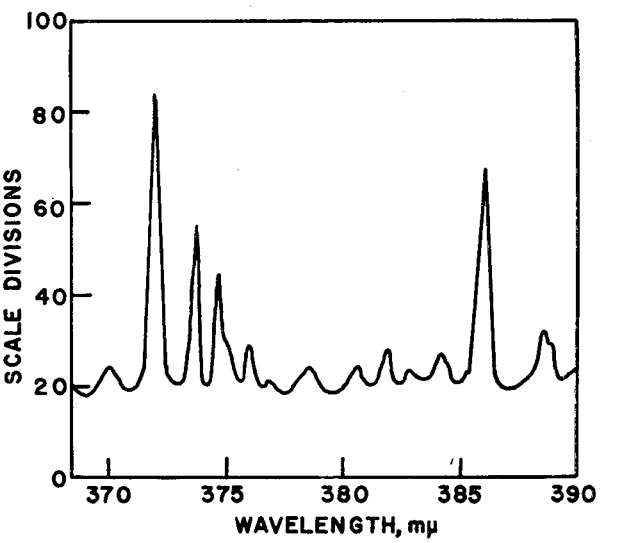

Figure 1. Flame emission spectra of iron

Present 65 p.p.m. iron. Slit width 0.030 mm.

The use of acetylacetone, as both solvent and reagent for iron, offers several other advantages. Iron acetylacetonate is very soluble in acetylacetone, soluble to an extent that macroseparations are possible. The solvent is readily available at reasonable cost and is combustible itself, thus contributing to the degree of excitation of the iron lines.

EXPERIMENTAL WORK

Apparatus. A Beckman Model DU spectrophotometer with Model 9220 flame attachment and photomultiplier unit was used. A metal atomizer-burner unit, supplied with the flame attachment, was used as the excitation source. Oxygen and acetylene were the gases used.

The wave-length knob on the spectrophotometer was replaced by a 4 to 1 gear-reduction knob to facilitate positioning the wave length dial. The iron lines are sharp, arc-emission lines. One must move slowly back and forth over the peak of each line to ascertain

the maximum reading. It is inadvisable to set the instrument on the apparent peak of one particular solution and make a series of readings. The sensitivity knob on the spectrophotometer also was replaced with a ten-turn dial arrangement to permit the sensitivity knob settings to be reproduced accurately.

A Beckman Model H-2 pH meter was used for pH adjustments.

Reagents. Acetylacetone, technical grade, was distilled to remove iron impurities.

A standard aqueous solution of iron, 1.00 ml. equivalent to 1.00 mg. of iron, was prepared by dissolving 7.022 grams of ferrous ammonium sulfate hexahydrate in demineralized water, and then adding 45 ml. of 12*N* hydrochloric acid and sufficient 30% hydrogen peroxide (or other suitable oxidant) to oxidize the iron. The excess peroxide was removed by boiling the solution, after which the solution was diluted to 1 liter with demineralized water. The final pH should be about 0.5.

A standard acetylacetone solution of iron, 1.00 ml. equivalent to 1.00 mg. of iron, was prepared by extracting an aqueous solution containing 250 mg. of iron with successive 25.0-ml. portions of acetylacetone until the aqueous phase was free of the iron complexes, and then diluting with acetylacetone to a final volume of 250 ml. The completion of the extraction can easily be seen as the iron complex is a deep red color. Weaker standard solutions are made by appropriate dilution with additional acetylacetone.

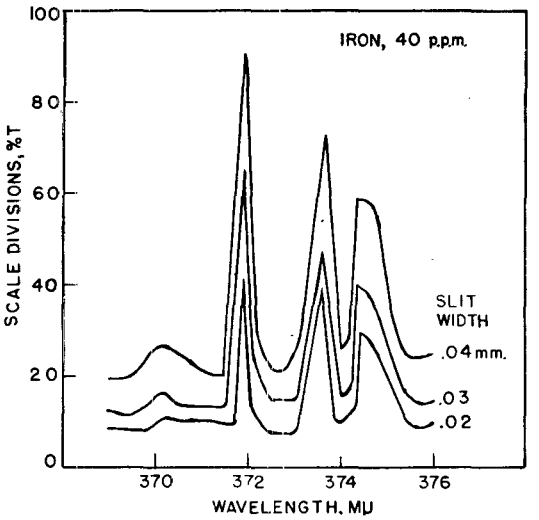

Figure 2. Effect of slit width on iron spectral lines

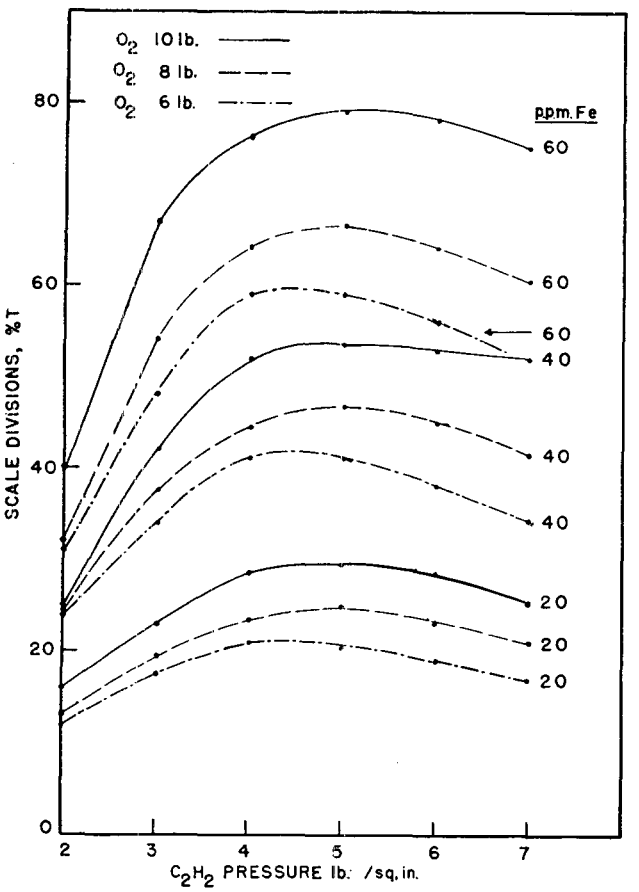

Figure 3. Iron luminescence as function of acetylene pressure at various oxygen pressures

Flame Spectrophotometer Settings. The instrument settings used to measure the iron luminosity were as follows:

Sensitivity control, turns from clockwise limit	6
Selector switch, position	0.1
Phototube resistor, megohms	22
Phototube, volts per dynode	60
Slit, mm.	0.030
Acetylene pressure, pounds per square inch	4.5
Oxygen pressure, pounds per square inch	10

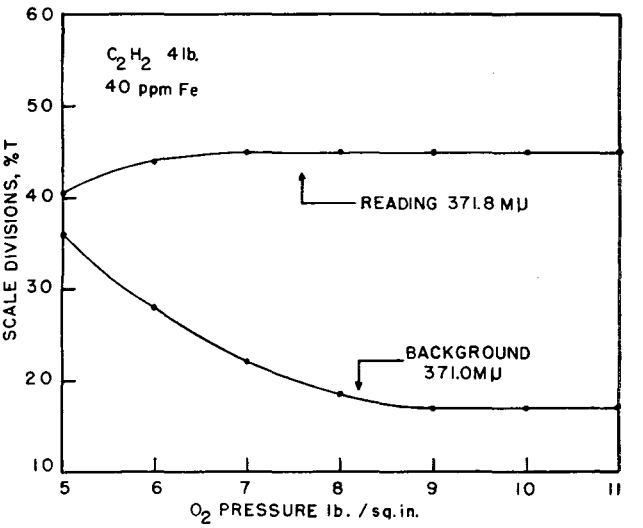

Figure 4. Intensity of iron emission and flame background as function of oxygen pressure

Flame Spectra of Iron. The major flame emission lines of iron are shown in Figure 1, taken from the work of Dean and Burger (*5*). Of these iron lines, the 372.0- and 386.0-mμ lines are the stronger with the 372.0-mμ line being the most intense. It was therefore chosen for this study. However, the 386.0-mμ line can be used, if desired. The background radiation in the vicinity of the iron lines is essentially continuous and is attributable to the continuous spectra of the carbon monoxide molecules in the flame and the superposition of innumerable feeble iron lines. Correction was made for the background radiation by measuring the luminosity at 371.0 mμ when using the iron 372.0 line. The background may be measured at either 385.0 or 387.0 mμ for the 386.0 iron line.

The luminosity of the iron lines in acetylacetone solution is approximately sixfold greater than those from aqueous solutions. Other authors have pointed out that the use of organic solvents may enhance the emission of certain elements (*1*, *2*, *4*, *6*, *7*). The use of acetylacetone in place of water as the aspirating medium results in a much larger flame in terms of the area, particularly the width, of the flame structure immediately above the inner cone. The flame may be more energetic, although this point

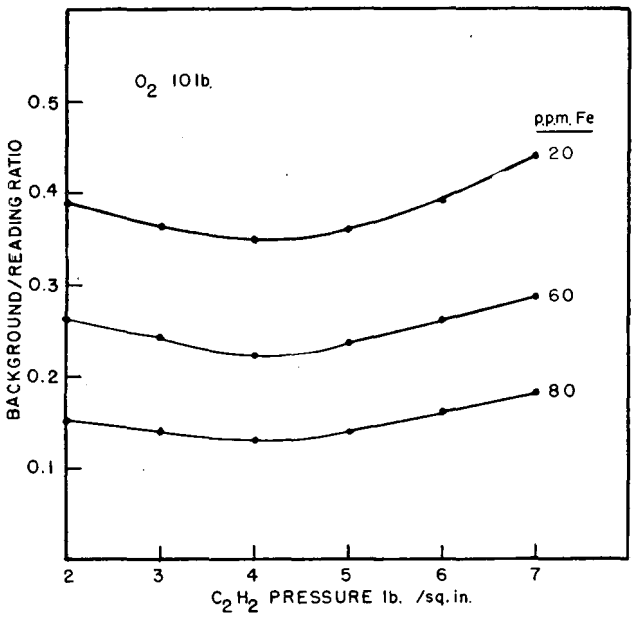

Figure 5. Ratio of flame luminescence to iron luminescence as function of acetylene pressure

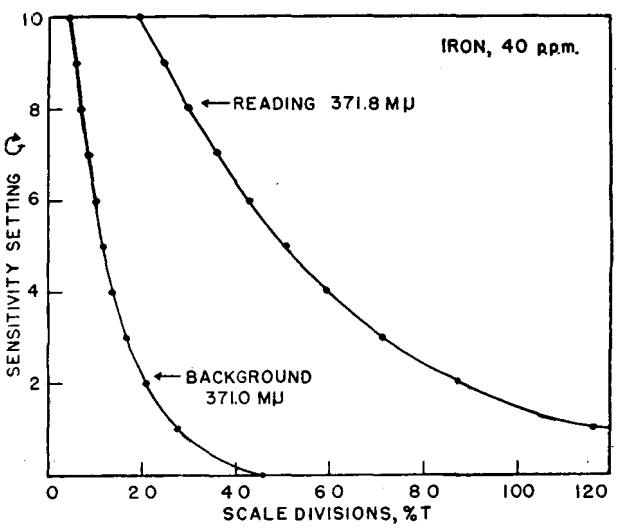

Figure 6. Influence of sensitivity control upon flame and iron luminescence

Ordinate is number of turns from counterclockwise limit.

has not been investigated. There is little difference in aspiration rates of an aqueous and acetylacetone solution.

OPTIMUM INSTRUMENT SETTINGS

Slit Width. The slit width used throughout was 0.030 mm. Figure 2 shows that the individual iron lines at 372.0 and 373.7 mμ are not resolved completely from each other at larger slit widths. Cowan and Dieke (*3*) have found that this slit width is sufficiently wide to integrate the total line intensity, particularly where there is a possibility of self-absorption.

Fuel and Oxygen Pressures. Optimum acetylene and oxygen pressures are those which give the greatest iron luminescence, and at the same time provide a low flame background. Figure 3 is a plot of the iron luminescence as scale reading above the flame background versus the acetylene pressure for various oxygen pressures at different iron concentrations. The maximum iron emission is achieved for acetylene pressures varying between 4 to 6 pounds per square inch. Within this range of acetylene pressures little change in iron luminescence results from slight fluctuations in pressure of acetylene.

The optimum oxygen pressure was obtained as shown in Figure 4, a plot of the iron luminescence and flame background versus oxygen pressure. The acetylene pressure was held constant at 4 pounds per square inch. The flame background gradually decreases to a constant value for oxygen pressures exceeding 9 pounds per square inch. The resultant iron emission attains its maximum value at 8 pounds per square inch and remains constant at higher oxygen pressures. There is a tendency for the burner to blow out at oxygen pressures exceeding 10 pounds per square inch.

As a further check on operating pressures, a plot of the ratio of flame background to iron luminescence (corrected for background radiation) for various acetylene pressures is shown as Figure 5. The ratio passes through a broad minimum at about 4 pounds per square inch acetylene pressure, confirming the data shown in Figure 3. For the remainder of the work the pressure was maintained invariant at 4.5 pounds per square inch of acetylene and 10 pounds per square inch of oxygen. Individual operators should be aware that different aspirator-burners, even though of similar construction, do not necessarily reproduce the luminosities on the graphs. The information on which the choice of optimum pressures was made is included merely to illustrate the nature of the variables involved, and to indicate approximately the range of fuel and oxygen pressures which were found satisfactory.

Sensitivity Control. The choice of the sensitivity control setting on the monochromator depends upon the concentration range employed. As shown in Figure 6, the luminescence of the iron line is a function of the sensitivity setting. Both the iron luminescence and the flame background increase as the sensitivity control is turned toward the counterclockwise limit. However, settings nearer the counterclockwise limit do not permit more accurate luminosity readings. Even though the emission sensitivity (defined as the number of transmittance dial divisions observed per 1 p.p.m. of iron present) is much greater, there is a corresponding decrease in galvanometer sensitivity, which offsets any gain in accuracy. This factor is brought out in Figure 7, which is a plot of emission sensitivity and of galvanometer sensitivity (defined as the deflection of the galvanometer needle in scale divisions for one-division change of the transmittance dial) versus the setting of the sensitivity control. Working at

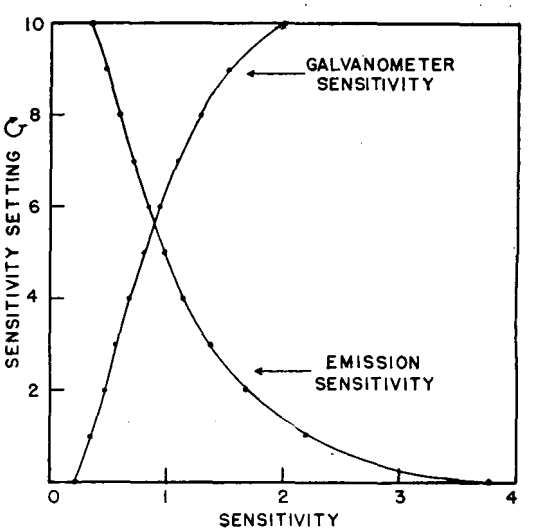

Figure 7. Emission sensitivity of iron and galvanometer sensitivity as function of sensitivity control setting

Ordinate is number of turns from counterclockwise limit.

a position of high emission sensitivity requires working at a low galvanometer sensitivity, and vice versa. From Figure 7 the per cent "relative error"—i.e., the uncertainty involved for an error of one galvanometer division—may be calculated for any given sensitivity setting and iron concentration by dividing into 100 the product of the galvanometer sensitivity and the luminescence above background. For 40 p.p.m. of iron, a relative error of approximately 3.3% is calculated from all sensitivity control settings. Consequently, the precision of a reading is independent of the position of the sensitivity control. The choice of this setting should be governed chiefly by the range of concentrations employed. If very small concentrations of iron are being determined, instrument settings corresponding to higher emission sensitivity may be desirable even though the background readings are increased. On the other hand, for higher concentrations of iron, instrument settings corresponding to higher galvanometer sensitivities may be more desirable. In the present investigation the sensitivity control was positioned six turns from the clockwise limit, which gave an emission sensitivity of approximately 1 p.p.m. of iron per one transmittance scale division. Even though the choice of the sensitivity control setting is arbitrary, it is necessary to maintain a fixed setting for any particular series of unknowns and standards.

PROCEDURE

Weigh samples containing 5 to 35 mg. of iron. Dissolve the samples by conventional methods and transfer to 100-ml. volumetric flasks. Dilute to the mark with demineralized water and mix well.

Transfer a 10.0-ml. aliquot portion of each sample to a small beaker and adjust the pH between 0.5 and 1.0. Transfer the solution to a 60-ml. cylindrical separatory funnel. Rinse the beaker several times with demineralized water and add these rinsings to the separatory funnel. Add 10 ml. of acetylacetone and shake the mixture for 1 minute. When the emulsion has settled, transfer the upper acetylacetone layer to a 50-ml. volumetric flask. Repeat the extraction two more times with fresh 10-ml. portions of acetylacetone, and three times with 5-ml. portions. The completion of the extraction is indicated by the disappearance of the red color of the iron complex from the aqueous layer. Dilute the contents of the volumetric flask to the mark with acetylacetone, which has been saturated with water.

Aspirate the samples and measure the luminosities of the iron line at 372.0 mμ and the flame background at 371.0 mμ. Bracket the unknowns with a series of standards containing iron acetylacetonate dissolved in acetylacetone. The appropriate background readings are subtracted from the unknown and standard readings to obtain net relative luminosities. Read the amount of iron present from the calibration curve.

DISCUSSION

Table I summarizes the results obtained on National Bureau of Standards samples of limestone and aluminum-, copper-, and nickel-base alloys. On two types of aluminum-base alloys a series of replicate samples were analyzed. The values are shown in Table I with the mean of the replicate samples and the associated standard deviation. For the other samples only the values found are enumerated.

Only copper, among all the elements encountered in the sample types investigated, gave any interference, and then only if the final concentration of copper in the aliquot portion taken for extraction exceeded 0.1 gram per 10-ml. aliquot volume. When larger amounts of copper were handled, precipitation of copper acetylacetonate occurred in the aqueous phase. Some of the precipitate passes into the acetylacetone phase. If allowed to enter the capillary of the aspirator-burner, it quickly plugs the capillary, otherwise it is not harmful.

Table I. Analysis of Bureau of Standards Samples

Sample	Certified Fe Value, %	Value Found, %
Aluminum-Base Alloys		
Aluminum alloy 85a 94 Al, 2 Cu, 2 Mg	0.208	0.205, 0.207
Aluminum alloy 86c 91 Al, 6 Si, 8 Cu	0.90	0.94, 0.88, 0.87, 0.90, 0.89, 0.88, 0.81, 0.88 Average value 0.88% Associated std. dev. 0.03%
Aluminum alloy 87 89 Al, 2 Zn	0.46	0.468, 0.500, 0.506, 0.477, 0.462, 0.491, 0.497, 0.506, 0.468 Av. value 0.486% Associated std. dev. 0.016%
Copper-Base Alloys		
Manganese bronze 62b 58 Cu, 38 Zn	0.82	0.75, 0.78
Phosphorus bronze 63b 78 Cu	0.47	0.46, 0.47
Ounce metal 124b 84 Cu, 5 Zn	0.26	0.25, 0.26
Silicon bronze 158 91 Cu, 2 Zn	1.48	1.46, 1.47
Aluminum brass 164 64 Cu, 22 Zn	2.52	2.50, 2.40
Nickel-Base Alloys		
Monel 162 66 Ni, 29 Cu	0.34	0.335 0.312
Nickel-base casting 161 64 Ni, 17 Cr	15.0	14.8, 14.8
Limestones		
Argillaceous 1a 41 CaO, 2 MgO	1.14	1.10, 1.10
Dolomite 88	0.059	0.057, 0.058
Burnt magnesite 104 86 MgO, 3 CaO	4.94	4.64, 4.64

The use of acetylacetone to isolate the iron by means of a preliminary extraction offers several advantages in addition to isolating iron from serious spectral interferences. The organic solvent is a fuel and apparently adds to the energy of the flame. The introduction of acetylacetone in place of water produces a flame considerably larger in size and increases the luminosity of the iron sixfold. A selective extraction also avoids the introduction of high concentrations of diverse ions into the flame, thus eliminating any error which might arise from their presence.

ACKNOWLEDGMENT

J. H. Lady is indebted to the Tennessee Eastman Corp. for a fellowship under which this work was carried out.

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RECEIVED for review April 4, 1955. Accepted June 22, 1955. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1955. Material taken from a portion of a dissertation submitted by J. Harold Lady to the Graduate School of the University of Tennessee in partial fulfillment of the requirements for the degree of doctor of philosophy. Contribution 144 from the Department of Chemistry.

Quantitative Application of Potassium Bromide Disk Technique in Infrared Spectroscopy

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In a study of the potassium bromide disk technique for the quantitative infrared spectrophotometric analysis of solids, several procedures for dispersing solids in potassium bromide have been compared. A vibrator-grinding technique was found to be advantageous especially for the preparation of sample mixtures from difficultly ground materials. This simple procedure is rapid, requires no elaborate equipment, and is generally applicable to a wide variety of substances not soluble in useful infrared solvents. The reproducibility of the various methods of sample preparation was determined, and the optimum procedure tested by analyzing model organic and inorganic systems. The precision of data obtained by the disk method compares favorably with conventional liquid-phase measurements. Although the techniques described have been applied to only a limited number of quantitative studies, these experiments suggest the wide applicability of the disk method to the solution of complex analytical problems, which previously were not subject to infrared spectrophotometric treatment because of solubility limitations.

STIMSON and O'Donnell (*8*) and Schiedt and Rheinwein (*6, 7*) have demonstrated the advantages of preparing samples for solid phase infrared spectroscopy by mixing the finely ground specimen with potassium bromide and pressing the mixtures into clear transparent wafers under high pressure. This method, known as the "potassium bromide disk" techinque, is particularly applicable to the study of minute quantities of insoluble solids. Anderson and Woodall (*1*) have obtained infrared absorption curves on as little as 10 γ of sample. Infrared spectra taken by the disk technique show little or no scattered energy above 2 microns and are generally of better quality than those obtained by other methods of solid sampling. In addition, potassium bromide does not exhibit any absorption maxima up to about 28 microns whereas mineral oil or other suspending media have bands which mask portions of the spectrum.

Although investigators (*2, 4*) have used disk methods for the solution of specific problems, they have not discussed a sample preparation procedure which is suited for general quantitative purposes. During the investigation reported, several methods of dispersing finely divided solids in potassium bromide were compared critically, and the reproducibility of these procedures was determined. The most reproducible method was then tested by analyzing model organic and inorganic systems. With this procedure, the precision of solid-phase measurements compared favorably with that of conventional liquid-phase methods, thus permitting the application of precise infrared spectrophotometry to a much wider variety of analytical problems.

EQUIPMENT AND MATERIALS

A cylindrically ground, divided-casing die of the type described by Schiedt (*6*) was found to produce disks which could be used satisfactorily for both qualitative and quantitative purposes (Figure 1). This evacuable die was constructed of Carpenter Vega tool steel hardened to Rockwell C-60 and was designed to press transparent disks 12 mm. in diameter in thicknesses varying from 0.5 to 2.0 mm., depending on the weight of potassium bromide mixtures used. Since the two halves of the die casing can be easily separated, the fused disks are readily removed without breakage. An evacuable, solid-casing die for preparing disks 12 mm. in diameter was found to be generally inferior to that of the divided-casing design for removing disks without fracture or breakage.

A Perkin-Elmer Model 21 infrared spectrophotometer was used to obtain all of the data recorded in this paper. The 12-mm. diameter potassium bromide disks were mounted in a simple stainless steel holder (Figure 2), which was designed to fit in the microcell adapter of the Model 21 instrument without interfering with the sample beam. This holder was designed to accommodate disks ranging from 0.5 to 2.0 mm. in thickness.

A hydraulic press of 10-ton capacity was employed in the pressing operations.

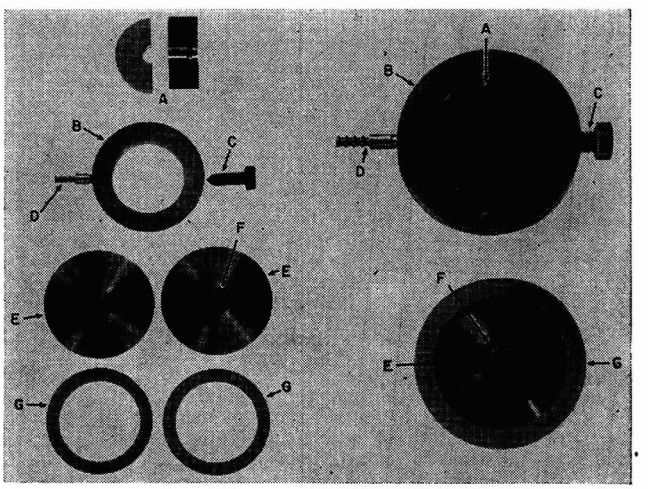

Figure 1. Die for pressing potassium bromide disks

A. **Divided casing**
B. **Casing collar**
C. **Positioning set screw**
D. **Air evacuation connection**
E. **Upper and lower pressing pins**
F. **O rings**
G. **Gum rubber gaskets**
Left, **disassembled view;** ***right,*** **assembled view**

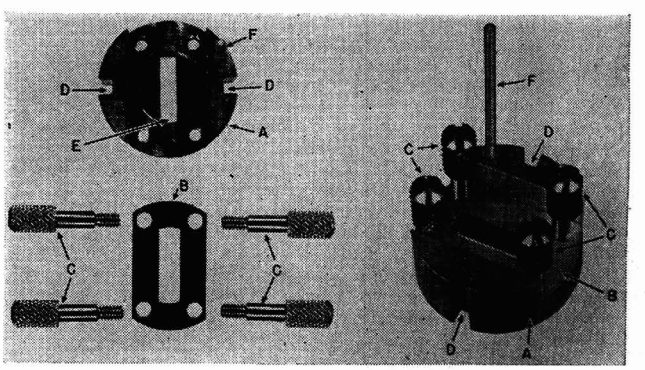

Figure 2. Disk holder

A. **Holder body**
B. **Keeper**
C. **Keeper screws**
D. **Holder positioning slots**
E. **Disk cavity**
F. **Mounting pin**
Left, **disassembled view;** ***right,*** **assembled view**

Harshaw Chemical Co. optical grade potassium bromide was found to be the most suitable suspending material. Fused disks 1 mm. thick made from this highly purified material show a transmittance of 85% or better in the 2- to 15-micron range. This pure salt exhibits none of the 7- and 9-micron impurity bands sometimes seen in the spectra of disks prepared from reagent grade or chemically pure materials. These less expensive materials may be used satisfactorily for quantitative studies in cases where the bands chosen for analysis do not occur in wave-length regions in which these minor impurities interfere. The potassium bromide powder used to prepare sample mixtures was obtained by first grinding the salt in a motor-driven mortar until it was thoroughly powdered. The material was then screened through a 230-mesh stainless steel cloth and dried at 135° C. for 48 hours at atmospheric pressure. Disks 1 mm. thick pressed from this powder show no bands except very weak peaks at 2.9 and 6.1 microns, which cannot be conveniently removed by continued drying. The 2.9-micron band of a 1-mm. disk made from potassium bromide usually has a base-line intensity of less than 0.04 absorbance unit, whereas the 6.1-micron band appears as only a slight depression in the "background" line. It is most desirable to grind and screen a relatively large amount of salt (25 to 50 grams) and then to separate the powder into 3- to 5-gram lots, which are dried separately just prior to use.

Table I. Lambert's Law Check

3-(*p*-chlorophenyl)-1,1-dimethylurea concn. −0.150%		
Disk Thickness, Mm.	k^a 9.19 μ	k^a 11.99 μ
0.50	...	3.38
0.74	1.31	3.35
1.20	1.31	3.39
1.45	1.35	3.34
1.70	1.32	...
1.96	1.35	3.38
Av.	1.33	3.37
Av. dev.	1.4%	0.9%

[a] $k = \frac{\text{baseline absorbance}}{\text{weight per cent} \times \text{disk thickness, mm.}}$

EXPERIMENTAL

Disk Pressing Technique. During the present investigation, all disks were pressed by a standard procedure. First, the desired quantity of potassium bromide–sample mixture was placed in the partially assembled die (Figure 1). The mound of salt mixture was then smoothed to a uniform surface with a stirring rod, the end of which had been flattened. (All operations involving exposure of potassium bromide to the atmosphere were conducted as rapidly as possible to minimize the adsorption of water.) Next the plunger was inserted and the assembled die placed in the hydraulic press. A slight pressure was exerted on the plunger to seal the gaskets to the casing, and evacuation of the die was begun. After a minimum of about 2 minutes of evacuation at less than 15 mm. of mercury, the force on the plunger was slowly increased to 8.5 tons and allowed to remain for 5 minutes (or 10 tons for 2 minutes) while the system was still under vacuum. The pressure was released slowly and air vented into the evacuation chamber. Next the die was disassembled, and the disk removed. For quantitative purposes, the disks were weighed or their thicknesses measured in order to relate the intensity of the absorption maxima to the quantity of sample placed in the beam. Thickness measurements used in all of the quantitative studies reported were made with a No. 522M dial micrometer with 1/4-inch contact point and anvil manufactured by the B. C. Ames Co., Waltham 54, Mass. The surfaces of disks 1 mm. thick are essentially uniform and parallel when prepared by the procedure described. Measurements taken on various sectors of the disks have shown variations of no more than 0.05 mm., and usually are in the range of 0.01 to 0.02 mm. The average of several measurements made on the face of the disk was taken as the apparent disk thickness. The density of a potassium bromide disk prepared by the procedure described was measured as 2.65 grams per cc., as compared to 2.75 grams per cc. reported for crystalline potassium bromide.

Lambert's Law Check. Adherence to Lambert's law by the solid-phase mixtures in disks was tested using 3-(*p*-chlorophenyl)-1,1-dimethylurea as the sample compound. The pure organic material was preground in a motor-driven mortar for 30 minutes. A portion of this finely pulverized material was then ground with dry, powdered potassium bromide in this manner for 45 minutes. This procedure was used in order to prepare a sufficiently large sample (about 3.5 grams) so that several disks could be pressed from the same mixture.

The data in Table I show that the absorptivity of the analytical band was constant when the disk thickness was varied (constant sample concentration); therefore, it must be assumed that disk thickness is an accurate measure of the number of absorbing units in the sample beam when the disks are prepared in the manner described. Close adherence to Lambert's law has also been found for all materials tested to date using the vibrator-grinding procedure.

Study of Grinding Methods. To obtain sharp, distinct infrared spectra of solids, energy losses resulting from scattering must be reduced to a minimum. Scattering may be decreased by reducing the absorbing particles to dimensions significantly smaller than the wave lengths of energy which are being used in the spectrophotometric study. If the average sample particle size is maintained below about 0.1 micron, energy losses by scattering are usually negligible, even in the 2- to 3-micron region. Additional reductions are realized by fusing the finely ground sample with potassium bromide, thereby effecting an excellent matching of the refractive indices of absorbing materials with that of the suspending medium.

The particle size effect is of particular importance in quantitative solid phase spectroscopy. Lejeune and Duyckaerts (*5*) have shown that in the case of powdered calcite, the absorptivity of the maxima depends very largely on the particle size of the dispersed substance. Harp, Stone, and Otvos (*3*) have reported that not only do particle size variations cause changes in the observed absorptivities but also that the dependence of apparent absorptivity with particle size is different for absorption bands of different intensity. These investigators concluded that sample particle size must be controlled carefully when quantitative results are desired.

Since uniformly small particle size appeared to be a principal requirement in quantitative potassium bromide disk spectroscopy, a study of this important variable was undertaken. Organic materials which had been found particularly resistant to particle size reduction were selected for the investigation, as it was felt that if quantitative techniques could be developed with substances which were difficult to grind, they could also be applied to samples which could be ground more easily. 3-(*p*-Chlorophenyl)-1,1-dimethylurea and related substituted ureas meet these requirements and were selected for study. These compounds give highly aggregated particles when ground in a mortar and develop a static charge which greatly increases handling difficulties. In addition, they are virtually insoluble in useful infrared solvents.

Three modes of particle size reduction for solid-phase infrared sampling were studied: ball milling the sample with potassium bromide, grinding the material in a mortar with powdered potassium bromide, and grinding the substance with potassium bromide in a mechanical vibrator-grinder with the help of small steel balls. The use of a vibrator was suggested by Schiedt (*6*). The study of the efficiency of the vibrator-grinding technique was carried out using a Wig-L-Bug amalgamator, manufactured by the Crescent Dental Manufacturing Co., Chicago, Ill. This amalgamator mechanically vibrates a stainless steel cylinder 3/4 inch long and 3/8 inch in diameter at about 3200 cycles per minute in a back-and-forth motion. The most efficient grinding of mixtures takes place when two 1/8-inch steel balls are placed in the cylinder which is about one third filled with the potassium bromide–sample mixture. If larger quantities are loaded into the cylinder, less efficient particle size reduction takes place. Approximately 0.8 to 1.5 mg. of sample, depending on the nature of the substance, and 0.35 ± 0.03 gram of powdered potassium bromide are usually employed in the grinding-mixing process. Most mixtures are easily removed from the cylinder if the potassium bromide has been properly dried. Since the mixture is homogeneous after the grinding operations, it is not necessary to recover it quantitatively. About 0.30 gram of the preparation is required for a 12-mm. diameter disk approximately 1 mm. in thickness.

Table II. Comparison of Grinding Techniques for 3-(*p*-Chlorophenyl)-1,1-dimethylurea

(Concn., 0.2% in KBr)

Grinding and Mixing Technique	Av. Absorptivity (k^a) (Min. of 6 Disks) 9.19 μ	11.99 μ	Av. Dev., % 9.19 μ	11.99 μ	Max. Dev., % 9.19 μ	11.99 μ
A. Preground sample[b] mixed with KBr by hand-grinding for 5 minutes.	1.12	2.48	10.2	10.5	17.0	16.9
B. Preground sample[b] mixed with KBr by motor-mortar grinding for 15 minutes.	1.27	2.81	5.1	5.4	10.2	7.5
C. Same as B, except ground with KBr for 30 minutes.	1.45	3.35	1.8	2.3	4.1	5.4
D. Large crystals of sample, mixed and ground with KBr for 15 minutes by vibrator method described	1.57	3.54	1.2	0.8	3.2	2.5

[a] $k = \frac{As}{Cl}$ where k = absorptivity
As = absorbance, baseline
C = concentration, weight %
l = thickness of disk, mm.
[b] Ground in a motor-driven mortar for 30 minutes before dilution with KBr.

To test the effectiveness of the vibrator technique as compared with other methods of particle size reduction, a series of mixtures containing 3-(*p*-chlorophenyl)-1,1-dimethylurea were prepared by various grinding procedures. Disks were pressed from these mixtures and selected absorption bands scanned. The data obtained during this study are summarized in Table II.

Since the vibrator-grinding method produces disks showing the highest and most reproducible band absorptivities, it may be concluded that this technique achieves the smallest average uniform particle sizes of any tested.

The advantages of the vibrator-grinding technique are most pronounced with difficultly ground materials such as 3-(*p*-chlorophenyl)-1,1-dimethylurea. Many easily ground compounds give satisfactorily reproducible disks by simple hand grinding with potassium bromide in a mortar.

The grinding time required to disperse a sample for quantitative investigation should be determined for each material to be analyzed by the disk technique. This interval may vary with the nature of the compound and may be determined empirically by observing the absorptivities of the bands to be used for the quantitative measurement as a function of grinding time. The results of a vibrator-grinding study using 3-(*p*-chlorophenyl)-1,1-dimethylurea are shown in Table III.

Table III. Vibrator-Grinding Time Versus Absorptivity for 3-(*p*-Chlorophenyl)-1,1-dimethylurea

(Concn., 0.200% in KBr)

Minutes Vibrated	k^a 9.19μ Large Crystals	k^a 9.19μ Preground Sample[b]
1	1.80	1.71
3	1.78	1.65
5	1.69	1.66
10	1.65	1.59
15	1.70	1.57
20	...	1.55
25	...	1.61

[a] $k = \frac{\text{base-line absorbance}}{\text{weight per cent} \times \text{disk thickness, mm.}}$
[b] 30-minute grinding in a motor-driven mortar before dilution with KBr.

The apparent absorptivities of the 9.19-micron band in disks prepared from both large crystals and preground material show a slight decrease at the start of the grinding process. This may have been caused by the slight shift in a base-line tangent angle, which was observed and is presumably a function of sample particle size. The apparent absorptivity of the 9.19-micron band appears to be fairly constant after 5-minute vibration of mixtures prepared with large crystals of sample, and after 3 minutes using preground material. The variation in the band absorptivity data obtained using the two starting materials is unexplained, but may give some indication of the expected long-term precision of the over-all method on samples of varying starting particle size, because the two sets of data were taken a week apart.

Other methods for obtaining reproducible particle size reduction were also attempted, but were found to be generally less satisfactory or more tedious than the vibrator technique. Preparation of mixtures by ball milling the sample with potassium bromide in a small stainless steel cylinder with steel balls is not feasible, because of serious caking of the mixture which takes place. Some studies were also carried out using liquids such as acetone and ethyl alcohol to assist in the mortar grinding. This necessitates a solvent removal step and results in only a slight increase in the efficiency of mortar grinding. The technique of dissolving the compound in an organic solvent and dropping or spraying the solution on the finely pulverized salt, followed by evaporation of the solvent under vacuum, has been used in this laboratory for obtaining qualitative spectra of extremely small amounts of sample; however, this procedure is more difficult to use quantitatively than the vibrator method. Preparation of potassium bromide–sample mixtures by freeze-drying techniques is time-consuming and is not generally applicable, because of solubility limitations.

The vibrator-grinding technique has an additional important advantage over most of the other methods of sample preparation in that contamination of the potassium bromide mixture by water is maintained at a low level. This is possible as the entire grinding and mixing process is carried out in an enclosed cylinder; hence, the powdered dry potassium bromide in the resulting mixtures is exposed to the air only during the weighing operations. Serious interference by water bands was found in the spectra of disks prepared by the hand- or motor-driven mortar grinding methods even when the operations were carried out in an enclosed box through which dry nitrogen was flowing rapidly. Quantitative analyses in the 3- and 6-micron regions are difficult when these water impurity bands are significantly intense.

In order to evaluate the techniques described for the solution of practical quantitative problems, model organic and inorganic quantitative analyses were devised and tested.

Quantitative Applications to Organic Systems. ANALYSIS FOR MINOR CONSTITUENT. 3-(*p*-Chlorophenyl)-1,1-dimethylurea and 3-(3,4-dichlorophenyl)-1,1-dimethylurea were selected for demonstrating a typical solid-phase quantitative analysis in which the determination of the minor constituent in a two-component system is desired. Calibration mixtures were prepared by weighing the necessary quantities of the two pure organic materials into the vibrator cylinder. Approximately 0.35 gram of pure, powdered potassium bromide was then accurately weighed into the same vessel so that the total organic content was 0.800% of the mixture. The total weight was maintained at about 0.35 gram in order to obtain maximum reproducibility in particle size reduction. These preparations were then vibrator-ground for 15 minutes as described. Approximately 0.30 gram was then pressed into disks about 1 mm. in thickness, mounted in the metal holder and scanned. The calibration data plotted in Figure 3 show that Beer's law follows throughout the concentration range investigated. Several synthetic mixtures containing known

Table IV. Determination of Minor Component

Synthetic	3-(*p*-Chlorophenyl)-1,1-dimethylurea Added, %	3-(*p*-Chlorophenyl)-1,1-dimethylurea Found, %
1	2.5	2.3
2	8.0	7.5
3	9.6	9.7
4	14.9	14.1
5	16.9	16.6

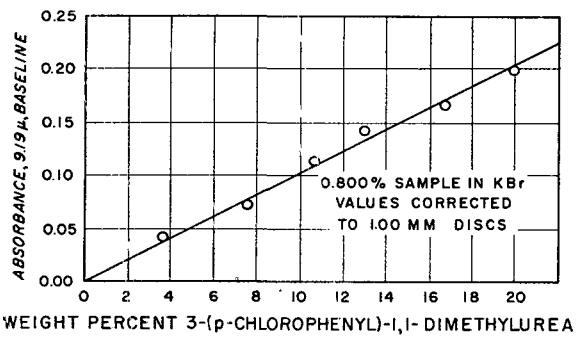

Figure 3. Calibration curve for 3-(*p*-chlorophenyl)-1,1-dimethylurea in 3-(3,4-dichlorophenyl)-1,1-dimethylurea

amounts of pure 3-(*p*-chlorophenyl)-1,1-dimethylurea in technical grade 3-(3,4-dichlorophenyl)-1,1-dimethylurea were prepared and analyzed as a means of testing the accuracy of the method. The results of these analyses are shown in Table IV.

Table V. Analyses of Synthetic Two-Component Samples

Added, %	Found, %
51.7 pure 3-(*p*-chlorophenyl)-1,1-dimethylurea (I)	52
48.3 technical 3-(3,4-dichlorophenyl)-1,1-dimethylurea (II)[a]	44[a]
51.7 pure (II)	52
48.3 technical (I)[a]	50[b]
28.9 pure (II)	32
71.1 technical (I)[a]	73[b]

[a] Technical samples about 97% pure.
[b] No corrections applied for known interfering impurities.

Analysis of Two-Component Mixtures. Binary mixtures of 3-(*p*-chlorophenyl)-1,1-dimethylurea and 3-(3,4-dichlorophenyl)-1,1-dimethylurea in the concentration range 15 to 70% were prepared with potassium bromide by the vibrator technique. Absorption bands characteristic of each component were chosen so that these peaks were in region of maximum transmittance for the other compound. Self-calibrating base-line intensity measurements were used to obtain the calibration data shown in Figure 4. The base-line procedure used to obtain the data in Figure 4 was necessarily different from that in Figure 3 and also different from that employed in obtaining the more fundamental data in Tables II and III; therefore, no consistency in 9.19-micron band absorptivity could be expected. Adherence to Beer's law was found for both compounds in the concentration ranges shown; however, additional points in the 90 to 100% range for 3-(*p*-chlorophenyl)-1,1-dimethylurea indicated a noticeable downward trend in the curve. The apparent deviation from Beer's law in this concentration range probably is a function of instrumental limitation since the intensity measurements were taken in the high absorbance region.

The results of analysis of several synthetic samples containing the two test compounds are given in Table V.

Quantitative Application to Inorganic Systems. Several of the

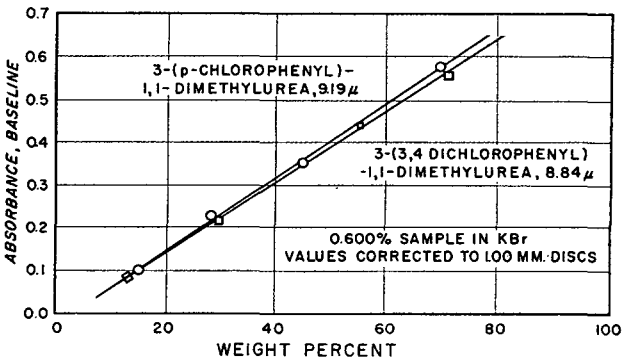

Figure 4. Calibration curves for 3-(*p*-chlorophenyl)-1,1-dimethylurea and 3-(3,4-dichlorophenyl)-1,1-dimethylurea mixtures

different crystalline forms of silica were used to demonstrate the applicability of the disk technique to the solution of quantitative inorganic problems. The study of silica seemed particularly interesting, because the usual methods of x-ray and electron diffraction, and differential thermal analysis do not possess sufficient sensitivity to permit the determination of small amounts of one form in the presence of another.

Quartz and α-cristobalite display characteristic absorption bands at 14.41 and 16.13 microns, respectively, which are not shown by any of the other modifications of silica. These bands were utilized for the quantitative analysis of mixtures of these materials by the disk technique. Independent Beer's law curves were constructed for each of these two substances by preparing a series of disks containing known concentrations of the test substance using the general vibrator procedure described. Both silica modifications were preground by hand in a mullite mortar and ground for 15 minutes with potassium bromide by the vibrator technique. Band intensity measurements were again made by the base-line method. A potassium bromide prism was employed in the spectrophotometer in order that the 16.13-micron α-cristobalite band could be studied.

Beer's law is obeyed for both quartz and α-cristobalite throughout the concentration ranges studied (Figure 5). A slight deviation in the α-cristobalite curve was found when the concentration exceeds about 0.25% in potassium bromide. In order to test the applicability of these curves for practical quantitative analyses, several synthetic samples containing small amounts of α-cristobalite in quartz were prepared and analyzed. Disks incorporating this mixture of silica modifications were made by the same technique employed for the Beer's law curves except that the total silica content was maintained at 0.800%. The results of the analyses of the synthetic samples are given in Table VI.

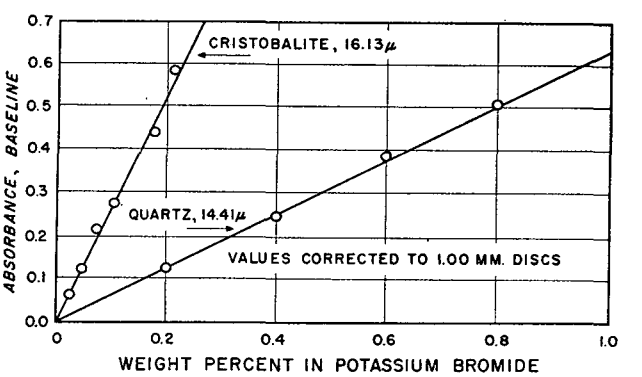

Figure 5. Beer's law plots for quartz and cristobalite

α-Cristobalite also may be determined quantitatively in amorphous silica by the disk technique. The calibration data shown in Figure 6 were obtained from disks containing known mixtures of the two silica modifications using the same experimental technique described for the earlier silica studies. The infrared method appears to be capable of detecting about 1% α-cristobalite in amorphous silica.

DISCUSSION

No quantitative studies were attempted with gummy or tarry materials, or with high-boiling liquids; however, several satisfactory qualitative spectra have been obtained on samples of this nature. Dispersion of materials of this type is probably the result of the coating of the sample fragments with potassium bromide, which prevents reaggregation and facilitates particle size reduction.

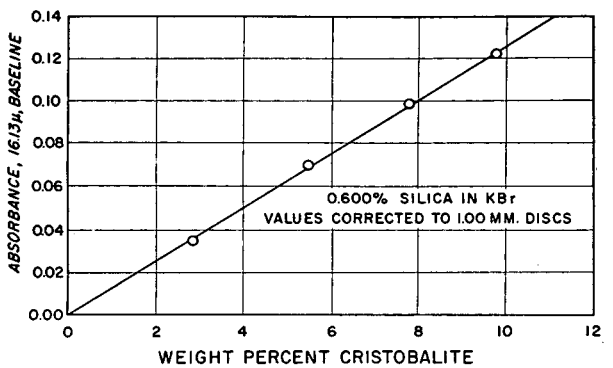

Figure 6. Calibration curve for cristobalite in amorphous silica

Absorption bands to be used for a particular quantitative analysis are selected in the same manner as for solution work. A study of band absorptivity as a function of grinding time usually indicates the feasibility of utilizing a particular peak for quantitative purposes. A further test is to prepare a series of replicate disks containing the sample and determine if the reproducibility of band absorptivities is within acceptable precision limits. To ensure maximum reproducibility in the preparation of disks by the vibrator method (and presumably by any other method employing a grinding process), the ratio of sample weight to potassium bromide and the total weight of potassium bromide–sample mixture should be kept uniform. The weight of the mixture to be vibrator-ground should also be held at a minimum.

An important advantage of the disk technique is that it permits the precise quantitative study of variables associated with the crystallinity of materials, which otherwise would be impossible by solution techniques. The solid-state analysis of two very similar species, such as diasterioisomers, can often be carried out on substances which show little or no spectral differences in the liquid phase. Absorption bands associated with crystallinity of a substance must be used with caution, however, since occasionally they give anomalous results. The absorptivities of these bands are sometimes more susceptible to particle size changes than is the case for those originating from functional group vibrations. In several cases it was found that the wave length of the maximum and the shape of this type of absorption band were also dependent on the particle size of the sample.

Table VI. Analysis of α-Cristobalite in Quartz

Sample No.	Cristobalite Added, %	Cristobalite Found, %
1	2.4	2.3
2	4.7	4.4
3	10.0	10.0
4	15.2	15.1

The illustrative quantitative examples given in this paper were all simple mechanical mixtures of solids. Caution must be used when attempting to apply the disk technique to systems containing solid solutions or mixed crystals. Obviously, calibration data must be obtained on the same solid systems as are to be encountered in the desired analysis. In instances where it is not known whether the samples to be analyzed are mechanical mixtures or solid solutions, mixed crystals, etc., it might be necessary to dissolve the calibration components in a common solvent (preferably the solvent from which the unknown sample is isolated), then evaporate the solvent before preparing the disk in the usual manner.

No detailed study of the effect of storage of disks on the absorption spectrum of an imbedded material has been made. Although the majority of disks prepared remain clear when kept in a desiccator, some do become turbid on standing; therefore, it is recommended that disks be scanned as soon as possible after preparation. No significant changes have been noted in the spectra of the clear disks which have been rescanned in this laboratory.

ACKNOWLEDGMENT

The author is indebted to Warren K. Lowen for his helpful suggestions and encouragement offered during this investigation and to George S. Walser, who obtained much of the data reported.

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RECEIVED for review February 7, 1955. Accepted June 27, 1955.

Preparation of Spectrographic Standards of Low Boron Content for Determination of Boron in Iron

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Spectrographic determination of boron in steel has been hindered by the lack of reliable standards in the low boron range. By using vacuum melting techniques and careful ingot processing, precise boron standards can be made which contain as little as 1 p.p.m. of boron.

BORON, when present in steel in minute amounts, produces an important improvement in hardening characteristics. Five parts per million is the usual minimum boron content specified for commercial boron-treated steels. Lately, studies of the effects of boron in steel have been concerned with boron present in even smaller amounts. With increased interest in very low boron contents, improved spectrographic methods for boron determination have been developed (*5*). Unfortunately, the accuracy of boron determination in the range below 6 p.p.m. has been questionable, because of the lack of reliable spectrographic standards for boron contents of that level.

This paper describes a method by which spectrographic standards have been prepared containing as little as 1 p.p.m. of boron. The method of preparation involved the addition of known amounts of boron to iron melted in a vacuum. By the use of careful vacuum melting practice and ingot processing it is believed that 100% recovery of the boron additions was realized.

MELTING TECHNIQUE

Vacuum melting was used for the preparation of these alloys, because of the degree of control of the oxygen and nitrogen contamination afforded by the vacuum melting process. Experience has shown that careful melting in air results in only a slight loss of boron (*3*); however, even the best air melting techniques were judged unreliable for the preparation of low boron alloys of precisely known boron contents. Melting in air would risk the formation of boron oxide and possibly boron nitride and their subsequent loss by interaction with the slag or with the refractory crucible.

Fifteen-pound heats were made using electrolytic iron with 0.10% carbon added. Upon melting in vacuum (1 to 5 microns of mercury) the carbon combined with the initial oxygen impurity and was drawn off as carbon monoxide. Oxygen contents were reduced in this way from an initial value of 0.02%, to 0.002%, whereas nitrogen, which is readily removed in vacuum, was reduced to about 0.00005%. These remarkably low residual oxygen and nitrogen contents could not have caused any loss of boron from the melts.

It has been observed that boron pickup from refractory materials during melting may be as important as the loss of boron, especially at low boron levels (*4*). Magnesia crucibles are especially troublesome in this respect; as much as 0.001% boron has been absorbed by pure iron during melting in magnesia. High purity stabilized zirconia crucibles were used in the present work to prevent boron pickup. Detectable amounts of boron were not picked up by pure iron melted in such crucibles if the melts were held in the crucible less than 30 minutes.

Each alloy was held molten for 10 minutes to allow the removal of oxygen and nitrogen before adding boron. The boron additions were in the form of Bureau of Standards spectrographic standard No. 830, containing 0.019% boron. This addition technique was used, because it was the most reliable way of adding a precisely known amount of boron. The heats were held molten for an additional 5 minutes after adding the boron to ensure complete solution and mixing of the boron into the liquid iron. Ingots 2.5 inches in diameter were then cast in a water-cooled copper mold in the vacuum chamber.

Spectrographic analysis showed that the very rapid solidification in the mold eliminated any segregation of boron from the top to the bottom of the ingots. Some slight radial segregation was noted, but subsequent steps in the processing eliminated any compositional errors which could have been caused by this condition.

It is not possible to give any quantitative data concerning the radial segregation. Radial segregation was never examined in the actual standards, as this would have required the destruction of large portions of the ingots. The radial segregation of a similar ingot containing a nominal 0.0006% boron was examined. It was found in this 2.5-inch diameter ingot that a surface layer, about 0.25 inch deep, was low in boron, whereas the rest of the ingot cross section was uniform in boron concentration. Segregation from top to bottom was nil. Likewise, tests on the actual standards showed no measurable longitudinal segregation.

PROCESSING OF INGOTS TO SPECTROGRAPHIC PINS

The ingots were not given the prolonged high temperature anneal which is the common practice. This type of treatment was avoided because it has been shown that at high temperatures boron steels are very susceptible to progressive boron loss by oxidation at the surface in much the same way that steels are decarburized. Deboronization has been shown to occur much more readily than decarburization; indeed, it is impossible to heat-treat boron steels at a high temperature and avoid deboronization in any reasonably attainable atmosphere other than a vacuum (*6*).

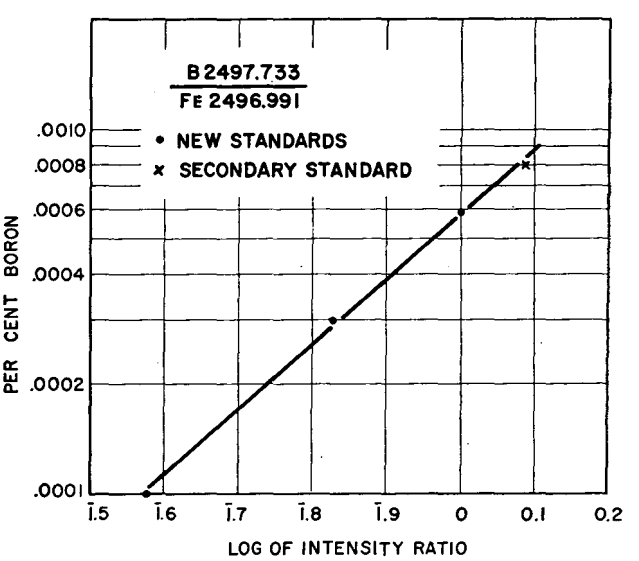

Figure 1. Analytical curve showing ratio of intensities of 2497.733 A. boron line to 2496.991 A. iron line

Boron content of secondary standard established by National Bureau of Standards boron steels (*5*)

The problem of eliminating radial segregation by means of high temperature annealing was avoided by rolling the entire ingot down to the final size. Thus, the average boron content of the final product was the same as that of the ingot and any radial segregation pattern in the ingot was simply retained in the proces-

sed pins. Such radial segregation does not affect analytical result if a spectrographic method is used in which a representative cross section of the sample material is consumed during excitation.

The ingots were reduced by hot rolling to 0.25-inch diameter rod. In this step the problem of deboronization again had to be reckoned with. It is known that boron diffuses much more rapidly in the high temperature face-centered-cubic modification of iron than it does with body-centered-cubic form (*1*, *2*). In pure iron the body-centered-cubic phase is stable below 910° C. Therefore, all rolling and processing were limited to temperatures below 865° C.

After hot rolling to 0.25-inch rods, the surfaces of the standards were cleaned by centerless grinding to $^{7}/_{32}$ inch in diameter.

Figure 1 shows an analytical curve made using the three new low boron standards and one other established standard. The spectrographic data obtained were by the Runge, Brooks, and Bryan (*5*) improved method for determination of low boron content. The agreement between these data is another indication of the reliability of standards produced by the described method. Although the analytical curve appears linear, it is not feasible to extrapolate data from high boron standards to low boron levels. Different exposure conditions are required for the accurate determination of high and low boron contents, making reliable low boron standards necessary.

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RECEIVED for review March 24, 1955. Accepted August 10, 1955.

Spectrographic Determination of Trace Quantities of Boron in Steel

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A quantitative spectrographic procedure has been devised for the determination of boron in steel within the range 0.0001 to 0.0006%. Precision of the method is ±10% at 0.0003% boron concentration. Accuracy is estimated to be within 0.00005% boron. The improved procedure combines the following features: measurement of the 2497.73 A. boron line by means of a Littrow spectrograph crossed with an echelle; recording of only the initial portion of the arcing period in order to improve line-to-background ratio; superimposition of several spectra to obtain adequate over-all spectral intensity; and quantitative calibration based on materials prepared by diluting a high concentration standard with electrolytic iron.

COMMERCIAL boron steels have no rigid specifications in respect to boron content since production and inspection control is difficult, and since absolute minimum and maximum effective quantities are not, as yet, well determined. Apparently, boron can be helpful in promoting hardenability of steels when present in concentrations roughly between 0.0005 and 0.02%. And for this concentration range, considerable successful work has been done in this country with both chemical and spectrographic (*4*, *6*, *9*, *10*) analytical procedures. Since 1946, the National Bureau of Standards has provided reliable standards (*7*) together with spectrographic procedures (*1*) to cover essentially this range.

Current metallurgical research in this laboratory on the mechanism of boron in the hardenability of steel has required extension of analytical procedures to include minimum effective quantities with a fair degree of accuracy. As a result, emphasis has been placed on the concentration range from 0.0006 to 0.0001% boron.

In extending the detectability of spectrographic procedures, several serious difficulties needed to be surmounted. First, it was considered desirable to use the most sensitive boron line at 2497.73 A. which is within 0.09 A. of an iron line of comparable intensity. Corliss and Scribner (*1*) have suggested that a twofold increase in sensitivity might be realized by using this line, which is the stronger of the two boron lines available in this region of the spectrum. The instrument used to resolve the preferred line is a Bausch & Lomb echelle attachment crossed with a Littrow quartz prism spectrograph (*6*). This arrangement provides sufficient dispersion and resolving power to separate adequately the desired boron line from its neighboring iron line.

A second difficulty lay in the inherently poor line-to-background intensity ratio for boron when conventional excitation and exposures are used (*9*). The extreme volatility of boron relative to iron, however, will allow the boron line to be recorded before an appreciable background accumulates. Thus the superimposition of spectra from short exposures of separate samples provides a more favorable line-to-background ratio than a spectrum from a single long exposure.

The third problem was that of calibrating for a concentration range where chemical standardization is impractical. Rather than attempt analyses on a few grams of sample where the absolute error is likely to equal the concentration, it was decided to synthesize calibration materials on a large enough scale to make weighing errors and contamination negligible quantities. This latter procedure is practical for boron steels, provided that high purity electrolytic iron and vacuum melting equipment are employed.

APPARATUS

Commercially available spectrographic equipment is employed throughout. A direct current arc source is used with an open-circuit voltage of 250 volts. Two mercury-vapor rectifier tubes produce a fully rectified direct current arc discharge.

A Bausch & Lomb echelle grating attachment crossed with a

Table I. Quantities of NBS No. 830 Steel Added to Electrolytic Iron to Produce Standards of Lower Boron Concentration

Amount of NBS Steel Added, Grams	Resulting Standard, % Boron
225.6	0.0006
109.0	0.0003
34.9	0.0001

large Littrow quartz spectrograph is employed. This optical arrangement results in a reciprocal linear dispersion of 0.31 A. per mm. at 2500 A. The echelle-Littrow system is illuminated by forming an image of the electrode gap on the echelle slit by means of a spherical crystalline quartz lens.

The spectrum is recorded photographically on Eastman Spectrum Analysis No. I plates which are developed in a thermostatically controlled processing machine. Transmittances of the analytical lines are measured on a recording-type projection microphotometer.

PROCEDURE

Preparation of Standards. Three standard steels were synthesized in the following manner. Fifteen pounds of electrolytic iron were melted in a vacuum furnace for each of the standards. The iron selected was examined spectrographically and was found to be free of boron within the limits of sensitivity of this method. To the molten electrolytic iron, accurately weighed amounts of National Bureau of Standards steel containing 0.019% boron were added. Quantities used are shown in Table I. The vacuum melted ingots were then rolled to 1/4-inch rod at a temperature below 1600° F., a range wherein the diffusivity of boron is low enough to prevent significant loss by oxidation at the surface. From the rod, slugs of 7/32 inch in diameter and 1/4 inch in length were machined to serve for the calibration. The diameter was selected to conform with conventional rod-type steel samples obtained for production control by either the glass tube sampling method (*8*) or by the cast pin technique (*5*).

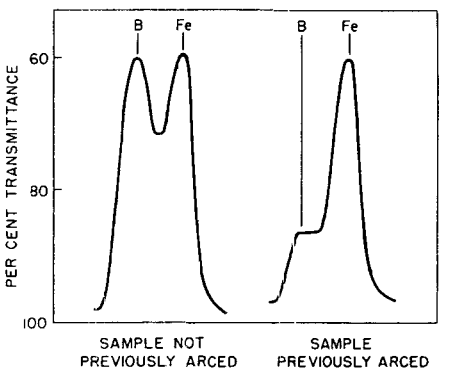

Figure 1. Effect of arcing on boron line intensity

Analysis of sections of the cast cylindrical ingot indicated some radial segregation of boron. Therefore the rolling operation, in reducing this cross section, minimizes the effect of segregation on analytical results. Precision data are noticeably better on segments of rod than on segments of the corresponding ingot.

Preparation of Samples. A representative sample of steel is machined into four cylindrical slugs, each 1/4 inch long and having a diameter of 7/32 inch. Extreme care must be exercised to prevent the sample from coming into contact with boron-containing materials such as borax cleaners. Precaution should also be taken to avoid the use of metal which has been previously arced or heat treated to the extent of having lost boron by oxidation. Slugs taken from the ends of rods previously arced provide analytical values much lower than slugs taken from the opposite unburned ends of the same rods. The extent of discrepancy between boron line intensities obtained from a sample not previously arced and one which has been previously arced is illustrated in Figure 1.

ELECTRODE SYSTEM

The lower, sample-containing, electrode (anode) is formed from a purified boron-free graphite rod 1 1/2 inch long and 5/16 inch in diameter, by drilling a hole 1/4 inch in diameter and 1/8 inch deep in one end to receive the metal slug. In order to concentrate heat in the slug, the graphite electrode is undercut to a diameter of 1/8 inch over a length of 1/4 inch at a distance of 5/16 inch from the top. Figure 2 illustrates this electrode shape.

The counterelectrode (cathode) is also made from purified boron-free graphite 1 1/2 inch long and, 5/16 inch in diameter by reducing the diameter to 5/32 inch over a distance of 3/8 inch from the top. The restricted diameter of the counter electrode confines the arc position and thus maintains the necessary optical alignment.

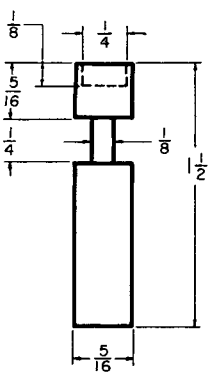

Figure 2. Preformed graphite anode

Analytical gap width is monitored at 2 mm. by manual adjustment of the electrode holders. Gap spacing can be maintained to within ±0.1 mm. by means of a projected image of the gap on a reference target.

EXCITATION

Standards and samples are excited in a direct current arc obtained from a 250-volt regulated power line. A variable transformer provides a current of 10 amperes when the electrodes are short circuited. Presumably, an interrupted direct current arc (*9*) or an overdamped condenser discharge (*3*) could have been used with equivalent success, had such units been available in this laboratory.

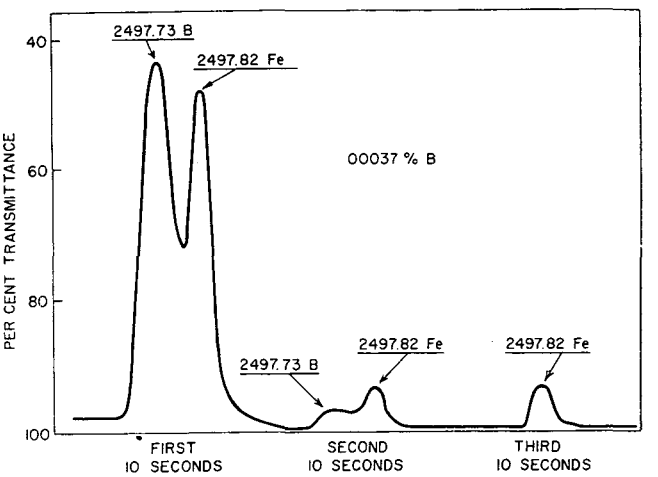

Figure 3. Time-intensity characteristics of boron and adjacent iron line

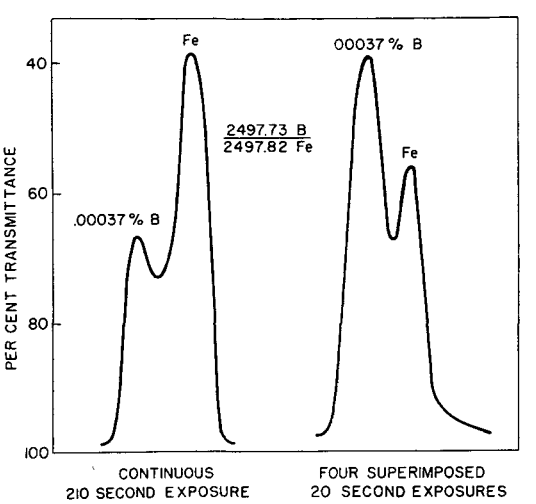

Figure 4. Comparison of a single long exposure and superimposed short exposures

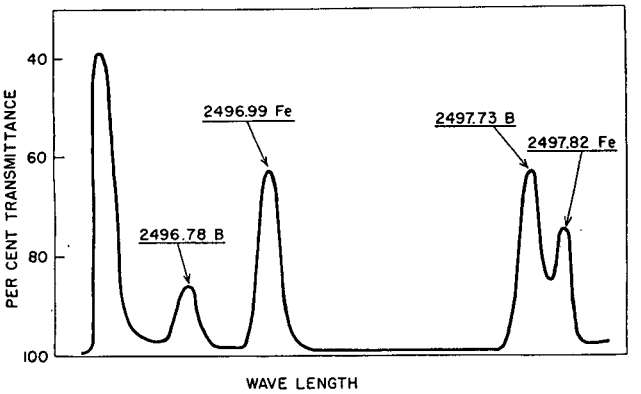

Figure 5. Typical microphotometer recording

EXPOSURE CONDITIONS

Instrument settings and exposure periods are as follows:

Spectral region	2200–2850 A.
Echelle slit width	0.050 mm.
Littrow slit width	0.4 mm.
Pre-exposure period	None
Exposure period	20 seconds (4 superimposed)

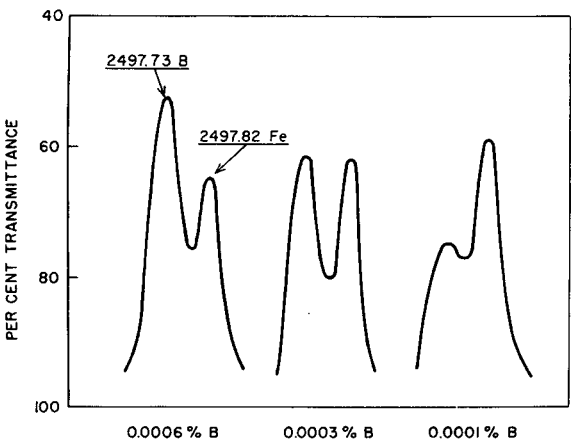

Figure 6. Recorded spectra from synthesized low boron standards

The selection of the exposure period is based on a study of line intensity versus time relationships of the boron and iron lines. The results of this study indicate that boron tends to vaporize and produce intense lines during the first 10-second period, after which there is a sharp diminishing of the boron spectrum. Figure 3 illustrates the time-intensity characteristics of the boron and adjacent iron line as photographed during the first, second, and third 10-second intervals after striking the arc. It was necessary to superimpose four separate spectra from each period to obtain ample line density. A comparison of boron and iron line intensities for a single long exposure and for a series of superimposed 20-second exposures is shown in Figure 4. It is apparent that a series of short exposures, superimposed, provides more favorable relative line intensities than a single long exposure.

PHOTOGRAPHIC PROCESSING

Emulsion	Eastman SA No. 1 plates
Development	Eastman D-19, agitated for 3.5 minutes at 68° F.
Stop bath	Dilute acetic acid for 30 seconds
Fixing	Eastman rapid liquid fixer for 1 minute
Washing	Running tap water 3 to 5 minutes, distilled water rinse
Drying	Warm air blast for 3.5 minutes

EMULSION CALIBRATION AND PHOTOMETRY

The emulsion is calibrated, normally only once for each emulsion lot number, from a separate iron arc spectrum. This spectrum provides a group of lines of known relative intensities (*2*) which are plotted against their corresponding transmittance values to produce the characteristic emulsion response curve.

Transmittance measurements are made with the microphotometer for the analytical line boron 2497.73 A. and the internal standard line iron 2496.99 A. (Figure 5). The iron reference line is chosen to match the boron line as nearly as possible in transmittance as well as in wave length and exitation potential. The exposure conditions provide measurements within the 20 to 80% transmittance range corresponding to the linear portion of the emulsion response curve. Spectral recordings of the boron line obtained from standards containing 0.0006, 0.0003, and 0.0001% boron are shown in Figure 6. Transmittances of the analytical lines and the internal standard lines are converted to log intensity ratios by means of the emulsion calibration curve. This procedure applied to standards serves to establish an analytical curve relating log intensity ratio to concentration for the pair of lines (Figure 7). Log intensity ratios for analytical determinations are converted to concentrations by referring to the analytical curve. Since 20-second exposures of four separate pieces of sample are superimposed on the plate to produce one echellegram, the log intensity ratio obtained from the reading of sample lines is, in effect, an average of four runs.

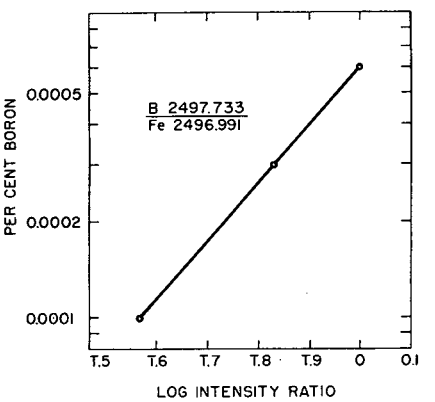

Figure 7. Analytical curve based on synthesized standards

PRECISION AND ACCURACY

Midpoint in the concentration range, the 0.0003% boron standard has shown an average deviation of ±10%. The 0.0001% boron standard repeats to within approximately the same absolute value of ±0.00003% boron, representing a deviation of ±30% of the amount present. The 0.0006% boron standard has not yet been tested for precision, but would be expected to repeat within ±10% of the amount present.

Accuracy of the results depends largely on the precision of the spectrographic determinations, the care used in sampling, and the reliability of the reference standards. Of these, reliability of the reference standards is thought to be the limiting factor. The amount of boron that may be present in the electrolytic iron is unknown, and, although undetectable by this same analytical

procedure, may exist to the extent of perhaps 0.00005% without being observed above background.

ACKNOWLEDGMENT

The authors wish to express their appreciation to E. R. Morgan and J. C. Shyne, Scientific Laboratory, Metallurgy Department, Ford Motor Co., for directing the preparation of the standard steels.

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RECEIVED for review December 27, 1954. Accepted March 3, 1955.

Indirect Spectrophotometry

A Study of Precision

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This paper presents the theory of the precision of indirect spectrophotometry, which is concerned with the measurement of the decolorization of a colored system. It is shown that the lowest relative error is achieved at low absorbance readings. The precision is increased by taking the readings against a partly decolorized standard. The optimum concentration range can be selected by the use of known functions which give close approximations to the usual calibration curves. A function of suitable form is proposed. When the calibration curve flattens out, errors in reading the concentration from the calibration curve may become more important than the uncertainty in reading the transmittance scale of the instrument. Means of calculating these errors are outlined, and the advantage of using a transmittance-concentration plot is pointed out for a specific case.

THE lack of precise methods for determination of fluoride was strongly felt during an analytical investigation of fluorine compounds. An indirect spectrophotometric procedure, based on measuring the bleaching of the thorium–alizarin Red S complex (*9*), was originally used, but the repeatability turned out to be very low, only about 5%. This led to an investigation of the maximum attainable precision of indirect spectrophotometry. Indirect spectrophotometry is in this paper defined as those methods by which the decolorization of a solution of a colored system is measured, in contrast to direct spectrophotometry, by which the unknown forms a colored product, the absorbance of a solution of which is a direct measure of concentration. Methods of obtaining high precision in indirect spectrophotometry are presented in this paper. In a later paper a spectrophotometric procedure of high precision for the determination of fluoride will be described.

The precision of ordinary direct spectrophotometry has previously been studied by a number of authors (*1*, *4–6*, *12*), who showed that the minimum error occurs at an absorbance of 0.434 when the measurements are done in the usual way. Means of increasing the precision by "differential spectrophotometry," using standard solutions in place of the pure solvent in the reference beam, have been pointed out by Bastian (*2*, *3*) and Hiskey (*5*).

A survey of the available literature on the spectrophotometric determination of fluoride indicated that several previous workers (*7*, *9–11*) have assumed implicitly that the precision is poor at low absorbance readings, where the bleaching is nearly complete, and as a result have worked with solutions in which the absorbance was rather high, above 0.25.

In this paper it is shown that the lowest absolute, as well as the lowest relative, error in indirect spectrophotometry is obtained at rather low absorbance values, usually outside the linear region of the calibration curve.

The discussion is mainly concerned with errors arising from the uncertainty of reading the transmittance scale of the spectrophotometer. These errors are for convenience called the photometric errors. Errors arising from the uncertainty of reading the concentration from the calibration curve, or the graphical errors, are discussed briefly.

PRECISION IN SIMPLIFIED CASE OF INDIRECT SPECTROPHOTOMETRY

Let us consider, as a typical case of indirect spectrophotometry, a system where the metal ion, M, forms a colored complex with the reagent, R.

$$M + R = MR \text{ with } K_R = \frac{(MR)}{(M)(R)} \qquad (1)$$

The introduction of the unknown element, X, into a solution of the complex, MR, causes a bleaching of the color by the formation of a stable complex, MX, between M and X:

$$M + X = MX \text{ with } K_X = \frac{(MX)}{(M)(X)} \qquad (2)$$

For the sake of clarity only 1:1 complexes are considered in the following material. The results are equally valid for systems of higher complexes. In this paragraph it is furthermore assumed that the stability constant, K_X, is sufficiently large even to cause all of the X introduced to form the complex MX. Later it is shown how the basic principles may be applied to cases where K_X is finite.

If now, in addition, an excess of R is present, the following relation holds:

$$c_x = c_1 - x_0 \tag{3}$$

where

c_1 = original concentration of complex MR in unbleached solution
x_0 = amount of X added, expressed as concentration of final solution
c_x = concentration of complex MR remaining in partly bleached sample solution
c designates the concentrations of the colored complex, MR, and x indicates the concentration of X

For the readings obtained on the unbleached reagent solution and partly bleached sample solution against pure solvent, or against a completely bleached reference solution, the following relations hold:

$$\frac{I_1}{I_0} = 10^{-abc_1} = 10^{-A_1} \tag{4}$$

$$\frac{I_x}{I_0} = 10^{-abc_x} = 10^{-ab(c_1 - x_0)} = 10^{-A_x} \tag{5}$$

where a and b are absorptivity and cell length, respectively, and A is absorbance.

All the terms used in this paper are in conformity with the recommendations made by the Joint Committee on Nomenclature in Applied Spectroscopy (*8*).

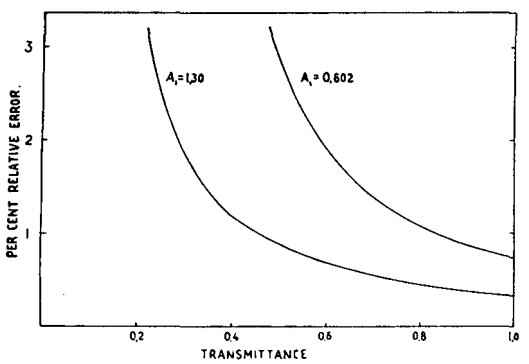

Figure 1. Relative analysis error for 1% error in transmittance

Solution of Equations 4 and 5 for the concentration x_0 of the unknown element X gives:

$$x_0 = \frac{1}{ab} \times \log \frac{I_x}{I_1} = \frac{1}{ab}(A_1 - A_x) = \frac{1}{ab} \times \Delta A \tag{6}$$

The relative error, given by $\Delta x_0/x_0$, is found by taking the logarithm and differentiating:

$$\frac{dx_0}{x_0} = \frac{0.434(dI_x/I_0)}{\frac{I_x}{I_0}(\log I_x/I_0 - \log (I_1/I_0)} = \frac{0.434 \times (dI_x/I_0)}{10^{-A_x}(A_1 - A_x)} \tag{7}$$

In the following only errors arising from the uncertainty of reading the transmittance scale of the spectrophotometer are considered. The discussion is furthermore limited to errors in measuring A_x, the absorbance of the sample solution. Errors in measuring A_1, the absorbance of the unbleached reagent solution, can be disregarded as the absorbance difference $\Delta A = A_1 - A_x$ is not involved in the measurements. Assuming that the error in transmittance, or the photometric error, is a constant, say 1%, independent of I_x, the relative error in concentration, expressed as a per cent, is plotted in Figure 1 for two different values of A_1.

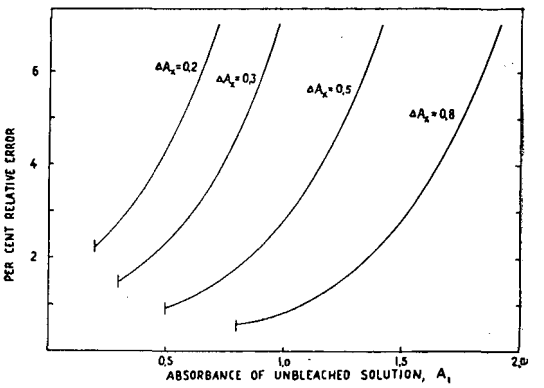

Figure 2. Relative error *vs.* absorbance of unbleached reagent solution

A comparison of the relative errors in direct and indirect spectrophotometry is highly instructive. In direct spectrophotometry the error curve is U-shaped (*5*, *12*), and the minimum error is achieved at a transmittance of 36.8%, at which a 1% error in transmittance produces a relative error of 2.72% in concentration.

In this simplified case of indirect spectrophotometry the relative error decreases steadily with increasing transmittance of the sample solution (Figure 1), and attains its minimum value at 100% transmittance. If the absorbance of the unbleached solution is $A_1 = 0.602$, the relative error for a 1% error in transmittance is 8.5% at a transmittance of 35% ($A_x = 0.456$), and only 0.72% at a transmittance of 100%. Working with higher concentrations of the reagent solution ($A_1 = 1.30$) and of the element X, the relative error is further reduced to only 0.33% at 100% transmittance.

A word of caution is necessary in interpreting these results. Using a reagent solution of high absorbance is in itself not a guarantee of high precision. What is of primary importance is to bring the readings into the low-absorbance region where the absorbance scale is greatly expanded. The relationship between error and the absorbances of the unbleached reagent and the partly bleached sample solutions is further illustrated in Figure 2, where the relative error is plotted against the absorbance, A_1, of the unbleached reagent solution for four different values of $\Delta A = A_1 - A_x$. ΔA is, according to the assumptions for this simplified case, proportional to the amount x_0 of X added. These curves show that the relative error decreases as the absorbance of the sample solution approaches zero, and that the error is decreased on going to higher concentrations of the reagent solution and the unknown element X.

DIFFERENTIAL INDIRECT SPECTROPHOTOMETRY

Instead of making the readings against the pure solvent or against a completely bleached reference solution, much may be gained in actual practice by using a partly bleached reference standard. As in the previous paragraph the simplified case of a nearly infinite value of the stability constant of the MX complex is first considered. The discussion of cases with finite stability constant is left to a later paragraph.

Consider four solutions containing, respectively, pure solvent (or completely bleached reference solution), partly bleached

reference standard of concentration c_2, partly bleached sample solution of concentration c_x, and unbleached reagent solution of concentration c_1 of the colored complex MR. According to the assumptions made above Equation 3 holds.

In addition to Equations 4 and 5 the following equation describes the absorbance of the reference standard relative to the pure solvent:

$$\frac{I_2}{I_0} = 10^{-abc_2} = 10^{-A_2} \tag{8}$$

If the readings are made against the partly bleached reference standard of concentration c_2, the following transmittance ratio is obtained from Equations 5 and 8:

$$\frac{I_x}{I_2} = 10^{-ab(c_1 - x_0 - c_2)} = 10^{-A} \tag{9}$$

Primes are used for absorbance readings obtained against the partly bleached reference standard solution of concentration c_2.

Solving Equation 9 with respect to x_0, taking the logarithm and differentiating, the following expression for the relative error is obtained:

$$\frac{dx_0}{x_0} = \frac{0.434 \times (dI_x/I_2)}{\frac{I_x}{I_2}\left[\log (I_x/I_2) - \log (I_1/I_2)\right]} = \frac{0.434 \times (dI_x/I_2)}{10^{-A_x'}(A_1' - A_x')} \tag{10}$$

This equation is of the same form as Equation 7, which was derived for the ordinary case of indirect spectrophotometry. A set of curves, showing the relative error as a function of the absorbance, A_2, of the partly bleached reference standard, can be constructed from Equation 10. They are not reproduced here, as their form is easily visualized from the curves of Figure 2.

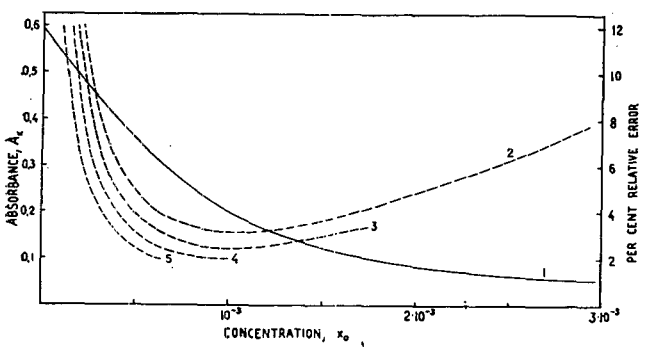

Figure 3. Calibration curve and per cent relative error

$K_X = 10^6$ $K_R = 10^5$
$M_0 = 10^{-3}$ $R_0 = 2 \times 10^{-3}$
$a = 600$ $b = 1$

1. Calibration curve, left ordinate scale
2–5. Error curves, right ordinate scale
2. $A_2 = 0$ 4. $A_2 = 0.2$
3. $A_2 = 0.1$ 5. $A_2 = 0.3$

The conclusion is that, working with a given concentration of X, the minimum error appears as the absorbance of the sample solution approaches zero. This may be achieved in the ordinary indirect method by an appropriate choice of the concentration of the reagent solution, or in the differential technique by taking the readings against a partly bleached reference standard of the same absorbance as the sample solution or slightly lower. Obviously, it is much more convenient to keep the concentration of the reagent solution constant, and choose from a set of partly bleached reference standards. Nothing is, however, gained in precision by using the differential technique in this case.

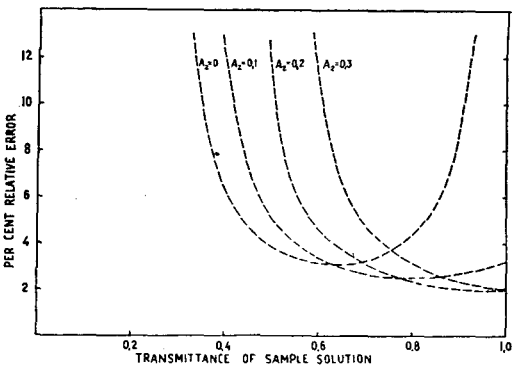

Figure 4. Per cent relative error for different absorbances of reference standard

$K_X = 10^6$ $K_R = 10^5$
$M_0 = 10^{-3}$ $R_0 = 2 \times 10^{-3}$
$a = 600$ $b = 1$

The real importance of differential indirect spectrophotometry occurs in those cases in which the stability constant of the MX complex has a finite value. Here readings obtained against a partly bleached reference standard may be advantageously used to bring desired portions of the calibration curve into the low absorbance region.

PRECISION OF INDIRECT SPECTROPHOTOMETRY IN ACTUAL CASES

In the previous paragraphs a simplified case of indirect spectrophotometry was considered, in which the decrease in concentration of the colored complex, MR, was assumed to be equal to the amount of X added. When going to cases of finite values of the stability constant K_X the following set of equations, in addition to Equations 1 and 2, describes the system:

$$\begin{aligned} (R_0) &= (R) + (MR) \\ (M_0) &= (M) + (MR) + (MX) \\ x_0 &= x + (MX) \end{aligned} \tag{11}$$

The subscripts refer to the total amount added of the respective components.

Solution of Equations 1, 2, and 11 for x_0 gives:

$$x_0 = \left[(M_0) - (MR) - \frac{(MR)}{K_R\left[(R_0) - (MR)\right]}\right] \times \left[1 + \frac{K_R}{K_X} \times \frac{(R_0) - (MR)}{(MR)}\right] \tag{12}$$

In Equation 12 one finds an expression that permits the construction of spectrophotometric calibration curves for the element X. The investigation starts with the usual case of indirect spectrophotometry for which the readings are made against pure solvent, or completely bleached reference standard. In this case the concentration of the colored complex, MR, is proportional to the absorbance of the sample solution.

With a set of assumed values for the constants, curve 1 in Figure 3 represents the calibration curve for the spectrophotometric determination of X. A curve of this type is the common one in spectrophotometric determinations of fluoride. The calibration curve becomes flatter with increasing concentrations of X. At the same time the absorbance readings move into the greatly expanded low absorbance region. It follows then that a point is finally reached where the relative error in concentration for a given spectrophotometric error is at its minimum.

It is possible also to calculate the relative error in concentration as a function of the transmittance of the sample solution.

Substituting c_x for the concentration (MR) in Equation 12, differentiating this equation with respect to c_x, and dividing by x_0, one obtains:

$$\frac{dx_0}{x_0} = -\frac{B}{x_0} \times \frac{dc_x}{c_x} \tag{13}$$

where

$$B = c_x + \frac{(R_0) \times c_x}{K_R\,[(R_0) + c_x]^2} - \frac{K_R}{K_X} \times c_x + \frac{K_R}{K_X} \times \frac{(R_0)(M_0)}{c_x} \tag{14}$$

Introducing in Equation 13 the following relations between the concentration, c_x, and the transmittance, I_x, of the sample solution:

$$\frac{I_x}{I_0} = 10^{-abcx} = 10^{-A_x} \tag{15}$$

$$dc_x = -\frac{0.434}{ab} \times \frac{(dI_x/I_0)}{(I_x/I_0)} \tag{16}$$

the expression for the relative error in concentration as a function of the transmittance of the sample solution is obtained:

$$\frac{dx_0}{x_0} = -\frac{B}{x_0} \times \frac{0.434 \times (dI_x/I_0)}{(I_x/I_0) \times \log (I_x/I_0)} = \frac{B}{x_0} \times \frac{0.434(dI_x/I_0)}{10^{-A_x} \times A_x} \tag{17}$$

A plot of the relative error, assuming a 1% error in transmittance, for the system represented by the calibration curve of Figure 3, is shown by the curve for $A_2 = 0$ in Figure 4. In this particular case the minimum relative error occurs at a transmittance of 63% ($A_x = 0.20$) at which a 1% error in transmittance causes a relative error of analysis of 3.08%. If the experimental data are confined to between 48 and 78% transmittance, the error is kept below 4% per 1% error in transmittance.

Inspection of the calibration curve of Figure 3 indicates that it should be possible to increase the precision by using a differential technique. When the readings are made against a partly bleached reference standard of absorbance $A_2 = 0.20$ for instance, a more linear portion of the calibration curve is brought into the expanded low-absorbance region, and higher precision would be expected.

An algebraic expression of the relative error in differential indirect spectrophotometry may also be obtained from Equation 13. From Equations 5 and 8 it follows that

$$\frac{I_x}{I_2} = 10^{-ab(c_x - c_2)} \tag{18}$$

Solution of this equation for c_x and differentiation with respect to I_x gives:

$$dc_x = -\frac{0.434}{ab} \times (dI_x/I_2)/(I_x/I_2) \tag{19}$$

Introducing Equation 19 into Equation 13 leads to the expression of the relative error in differential indirect spectrophotometry where the readings are made against the partly bleached reference standard of concentration c_2:

$$\frac{dx_0}{x_0} = -\frac{B}{x_0} \times \frac{0.434 \times (dI_x/I_2)}{\frac{I_x}{I_2}(\log (I_x/I_2) + \log (I_2/I_0))} = \frac{B}{x_0} \times \frac{0.434 \times (dI_x/I_2)}{10^{-A_x'}(A_x' + A_2)} \tag{20}$$

The relative error is plotted in Figure 4, using the same set of constants as previously, for the following values of the reference standard: $A_2 = 0.10$, 0.20, and 0.30. It is evident that a great improvement may be achieved by the differential technique. When the absorbance of the reference standard is $A_2 = 0.20$, the minimum relative error is only 1.95% per 1% photometric error, and is achieved at 100% transmittance.

Another important advantage of the differential technique is that sometimes a large concentration range may be analyzed within the limits of a given error. This is shown by the error curves of Figure 3. The error curves, calculated for different values of the absorbance of the reference standard, A_2, are here plotted against the concentration x_0. The permissible concentration range corresponding to a relative error of less than 3.5% per 1% photometric error is $0.75 - 1.45 \times 10^{-3}$ when the readings are made against the pure solvent, and $0.55 - 1.75 \times 10^{-3}$ when using a reference standard of absorbance $A_2 = 0.1$. With reference standards of higher absorbance, the permissible concentration range becomes less, however.

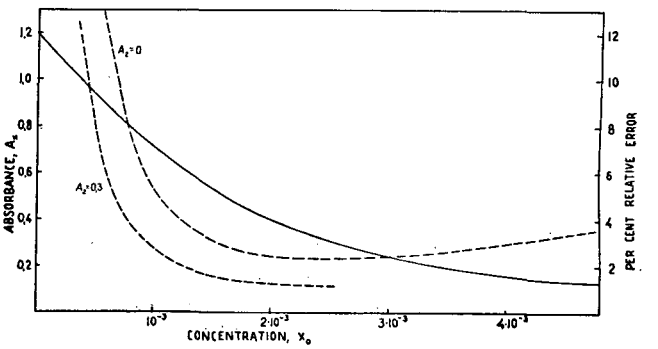

Figure 5. Calibration curve and per cent relative error

$K_X = 10^6$ $K_R = 10^5$
$M_0 = 2 \times 10^{-3}$ $R_0 = 4 \times 10^{-3}$
$a = 600$ $b = 1$

It is also interesting to note from Figure 3 that following the usual procedure of measuring the absorbance against the pure solvent ($A_2 = 0$), the best readings are obtained on the curved part of the calibration curve. In fact, if the experimental data were limited to the linear portion of the calibration curve the relative error would be well above 5%.

A substantial increase in precision is obviously possible by using higher concentrations of reagent and sample solutions. This is illustrated by the curves in Figure 5 which apply to the same system as Figure 3, except that the concentrations of the reactants have been increased by a factor of 2. Taking the readings against a reference standard of absorbance $A_2 = 0.3$, the minimum relative error per 1% photometric error is only 1.1%. It should be kept in mind, however, that in some systems interferences from foreign elements may become more pronounced at the higher concentration levels.

The conclusion from the discussion in this paragraph is that the use of reference standards in indirect spectrophotometry is highly desirable. An increase in precision may be expected, and a somewhat larger concentration range becomes available. The differential technique offers an important increase in precision, especially when one is interested in the low concentration range. Here the difference in absorbance between the sample solution and the unbleached reagent solution is rather small, and it is obviously necessary to bring the readings on the sample solution into the expanded low-absorbance region of the instrument.

TREATMENT OF EXPERIMENTAL DATA

After construction of a preliminary calibration curve from the absorbancies, measured against pure solvent, of a series of standard solutions, the question about the optimal absorbance value and the corresponding relative analysis error per unit of spectro-

photometric error arises. Once this question is answered, one needs to consider how much the error may be decreased by measuring against a reference standard in place of the solvent, and what absorbance the reference standard should have.

The optimal absorbance value is most easily obtained from a Ringbom plot of per cent transmittance versus the logarithm of concentration (*12*). The absorbance value corresponding to the inflection point of the curve will be the one that gives the lowest error of analysis. The per cent relative error per 1% spectrophotometric error can be evaluated by dividing 230 by the slope of the curve at its point of inflection (*1, 12*).

The curve of per cent transmittance versus the logarithm of concentration is not very convenient, however, for determining the best concentration range and the corresponding errors, and for evaluating the optimal absorbance of the reference standard. A curve showing per cent relative error as a function of concentration (or transmittance of sample solution) would be more instructive, but is not easily calculated from the experimental data. It is possible, however, to arrive at the error curve by using known functions that are similar to the usual calibration curves of indirect spectrophotometry. A function that is convenient to handle and that usually follows closely the experimental calibration curve is:

$$x_0 = \alpha A_x + \beta + \frac{\gamma}{A_x} \qquad (21)$$

where x_0 is the concentration of the sample, and A_x is the absorbance of the sample solution. Having determined the constants that fit best to the experimental data, it is easy, by differentiating with respect to the transmittance and dividing by x_0, to obtain the error function

$$\frac{dx_0}{x_0} = \frac{0.434\left(\frac{\gamma}{A_x^2} - \alpha\right)}{x_0 \times 10^{-A_x}} \times \frac{dI_x}{I_0} \qquad (22)$$

that permits the construction of curves of the relative error versus concentration or absorbance. The absorbance value that gives the minimum relative error is easily obtained from the minimum of this curve.

Having obtained the absorbance value, A_{min}, that gives the minimum relative error, E_{min}, the answer to the question about the absorbance of the reference standard solution depends on whether one is primarily interested in the maximum attainable precision or whether one is satisfied with a certain increase in precision without impairing the permissible concentration range for the procedure.

A comparison of Equations 17 and 20 shows that the lowest relative error, when using a reference standard of absorbance A_2, is obtained at an absorbance reading of:

$$A_x' = A_{min} - A_2 \qquad (23)$$

and that the corresponding error is

$$E'_{min} = \frac{I_2}{I_0} \times E_{min} \qquad (24)$$

It follows now that the absolute lowest value of the error is obtained with a reference standard of absorbance slightly above A_{min}. Usually, however, the permissible concentration range is then somewhat reduced.

If a further increase in precision is desired, one might consider the use of higher concentrations of reagent and of sample. It should be borne in mind, however, that the interferences from foreign elements may sometimes be increased in indirect spectrophotometry when working with higher concentrations.

Up to now only errors arising from uncertainty in reading the transmittance scale of the spectrophotometer have been considered, and it has been shown in which regions these errors are at a minimum.

Another source of error is the uncertainty in reading the concentration from the calibration curve, which will be called the graphical error. A plot of absorbance versus concentration, which is usually linear in direct spectrophotometry, is difficult to read exactly at the concentrations that are high enough to cause curvature in indirect spectrophotometry. An alternative choice is to plot per cent transmittance versus concentration. Such a plot may sometimes become linear for a very large concentration range.

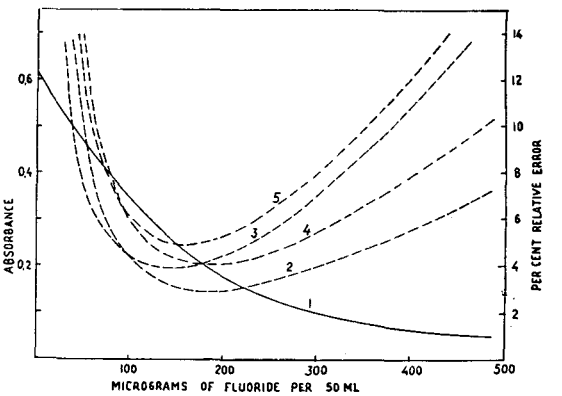

Figure 6. Calibration curve and error curves for the spectrophotometric determination of fluoride

1. **Calibration curve, $x_0 = -290A + 148 + \frac{18}{A}$**
2. **1% error in reading instrumental scale or reading calibration curve of transmittance vs. concentration**
3. **0.01 unit error in reading calibration curve of absorbance vs. concentration**
4. **Combined effect of 1% scale reading and 1% transmittance calibration curve errors**
5. **Combined effect of 1% scale reading and 0.01 absorbance unit errors**

The graphical error may sometimes become the main factor limiting the precision of an indirect spectrophotometric procedure. It is fortunately easy, once the constants of the function of Equation 21 are determined, to evaluate this error. For a plot of absorbance versus concentration an expression for the graphical error is obtained by differentiating Equation 21 with respect to the absorbance and dividing by x_0:

$$\frac{dx_0}{x_0} = -\frac{(\gamma/A_x^2) - \alpha}{x_0} \times dA_x \qquad (25)$$

When the calibration curve is a plot of transmittance versus concentration the expression for the graphical error is obviously identical with the photometric error given by Equation 22.

The photometric and graphical errors are further illustrated in Figure 6, which refer to data in use in this laboratory for the spectrophotometric determination of fluoride. Curve 2 is the usual photometric error curve, and gives at the same time the graphical error for a calibration curve of transmittance versus concentration. Curve 3 gives the relative error in concentration assuming a 0.01 unit error in reading the calibration curve of absorbance versus concentration. Curves 4 and 5 are total error curves, for transmittance and absorbance plots, respectively, when both photometric and graphical errors are considered. It is seen that in this specific case a transmittance plot is somewhat superior to the absorbance plot in the concentration region where the relative error is at a minimum.

It is not possible to make a general statement as to the relative merits of the transmittance and the absorbance plots. One

should in each case determine the best way of plotting the experimental data using the procedure outlined above.

It should be kept in mind that this treatment does not consider the chemical errors, which sometimes are more serious than both the photometric and graphical errors. The error curves that have been discussed in this paper should only be used to obtain an indication of the best concentration range for a spectrophotometric method. The actual error of analytical methods is dependent on many factors and must be determined experimentally.

ACKNOWLEDGMENT

The author desires to express sincerest thanks to David N. Hume for his valuable criticism of the manuscript.

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RECEIVED for review April 22, 1955. Accepted July 14, 1955.

Separation and Determination of Scandium

Spectrophotometric Method Using Alizarin Red S

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A colorimetric method for the determination of scandium has been developed. The scandium alizarin sulfonate lake is used as color complex in an ammonium acetate buffer medium. The color reaction conforms to Beer's law in the concentration range of 10 to 120 γ of scandium oxide per 100 ml. Scandium is isolated stepwise from all interferences by a cupferron-chloroform extraction; an iodate precipitation in nitric acid; a tributyl phosphate extraction in a hydrochloric acid system; an ammonium tartrate precipitation; and a tributyl phosphate extraction in a hydrochloric acid system. Extraction data for scandium in hydrochloric acid–tributyl phosphate systems are presented. Results are reported on the recovery of scandium in the presence of the major metal ions and the analyses of some waste residues and process liquors.

A RELIABLE method for the determination of scandium in uranium feed materials, waste products, ores, and minerals was required in connection with the recovery of scandium from these materials.

Existing qualitative tests for scandium were reviewed for possible quantitative development. The known color and fluorescence reactions of scandium have limited applicability and are not specific. Numerous color reactions of scandium have been reported (*1–5, 9, 13, 14*). Some of these reactions are applicable only for spot-test and microscopic techniques and are unsuitable for colorimetric determinations because of the formation of insoluble products and numerous interferences.

Miller (*11*) separated scandium from iron, titanium, zirconium, and vanadium by a chloroform extraction of the cupferrates in mineral acid solution. Lundell and Hoffman (*10*) have shown that the other elements which are completely removed by this extraction are hafnium, gallium, tin, molybdenum, antimony, and bismuth.

Fischer and Bock (*6*) separated scandium as the insoluble ammonium scandium tartrate in a hot ammoniacal medium, but found it difficult to effect a quantitative separation from thorium, iron, zirconium, and manganese.

Fischer and coworkers (*7*) found that small quantities of scandium were carried when yttrium was precipitated as ammonium yttrium tartrate from a hot ammoniacal medium. They then separated scandium from yttrium by extraction with ether from a hydrochloric acid solution containing a high concentration of ammonium thiocyanate.

Peppard and coworkers (*12*) showed that scandium could be separated completely from the rare earths, without loss of scandium, by extraction from a hydrochloric acid system with tributyl phosphate.

Singly, none of these methods will isolate scandium in a sufficiently pure state to permit a color development with existing organic reagents without interference from other metal ions. However, by combining these separation schemes, a procedure has been devised whereby scandium is isolated sufficiently pure to permit a spectrophotometric determination by color development with alizarin red S.

The method developed involves a cupferron-chloroform extraction to remove many metals, including zirconium, from a sulfuric or hydrochloric acid solution of the sample. After conversion to a nitric acid system, thorium is removed by coprecipitation with mercury (*8*) as the insoluble iodate. A tributyl phosphate extraction of the scandium then removes the bulk of the remaining elements with the exception of uranium. The scandium is separated from the uranium by means of a series of tartrate precipitations with yttrium as the carrier. Scandium is then separated from yttrium by extraction from a hydrochloric acid system with tributyl phosphate. The scandium is removed from the tributyl phosphate and the color developed with alizarin red S in a buffered medium. For materials that contain no zirconium and thorium, the method is considerably simplified by omission of the steps used for their removal.

The distribution of scandium between tributyl phosphate and hydrochloric acid was investigated for low and high concentrations of the acid, extending the existing data (*12*). The recovery of scandium in an extraction, washing, and stripping procedure was studied. The efficiency of yttrium as a carrier for scandium was studied for low concentrations of scandium in pure solution and in the presence of many common ions.

APPARATUS AND REAGENTS

Spectrophotometer. Absorbancy measurements were made with a Model DU Beckman spectrophotometer, using 5.00-cm. matched Corex cells.

Filtration Apparatus. An 85-ml. sintered-glass Büchner funnel of medium porosity and a Fisher filtrator were used for all filtrations.

Standard Scandium Solution. Dissolve 50 mg. of scandium oxide in 25 ml. of concentrated hydrochloridic acid and dilute to 500 ml. Dilute 10 ml. of the stock solution to a volume of 100 ml. to obtain a standard solution of 10 γ of scandium oxide per milliliter.

Yttrium Chloride Solution. Dissolve 1.0 gram of yttrium oxide in 10 ml. of concentrated hydrochloric acid and dilute to 200 ml. with water.

Alizarin Red S Solution. Prepare a 0.1% solution by dissolving 0.250 gram of alizarin red S in 250 ml. of water.

Ammonium Tartrate Solution. Prepare a 40% solution by dissolving 100 grams of reagent grade ammonium tartrate in 200 ml. of 10% ammonium hydroxide and diluting to 250 ml. For washing purposes dilute 1 part of this solution with 4 parts of water.

Ammonium Acetate Buffer. Dissolve 100 grams of reagent grade ammonium acetate in 300 ml. of water. Adjust the solution to pH 3.5 with concentrated hydrochloric acid and dilute to 500 ml.

Mercurous Nitrate Solution. Dissolve 5 grams of reagent grade mercurous nitrate monohydrate in 200 ml. of 20% nitric acid.

Potassium Iodate Solution. Saturate 500 ml. of 10% nitric acid with reagent grade potassium iodate.

Hydrogen Peroxide, 30% analytical reagent grade.

Cupferron. Prepare a 6% aqueous solution from reagent grade material.

Tributyl Phosphate.

Diethyl Ether, analytical reagent grade.

Table I. Extraction of Scandium into Tributyl Phosphate from Hydrochloric Acid[a]

(D_a° for scandium in HCl of indicated concentration)

2.3*M*	3.5*M*	4.7*M*	5.8*M*	7.0*M*	8.2*M*	9.4*M*	10.5*M*
0.02	0.07	0.26	1.4	7.6	36	110	>1000

[a] Tributyl phosphate was used as received. No pre-equilibrations were made with HCl. Tracer used was Sc^{46}.

EXPERIMENTAL

Extraction of Scandium from Hydrochloric Acid with Tributyl Phosphate. Peppard and coworkers (*12*) have reported distribution data for scandium and the rare earths in tributyl phosphate and 3 to 8*M* hydrochloric acid systems.

It was desired to know how completely scandium could be extracted with tributyl phosphate at hydrochloric acid concentrations greater than 8*M* and to have extraction data at hydrochloric acid concentrations less than 3*M* in connection with the subsequent removal of scandium from the organic phase. Extraction data were obtained over a wide range of hydrochloric acid concentrations with tracer scandium-46 (Table I).

Effectiveness of Yttrium as a Carrier for Traces of Scandium. A known quantity of scandium-46 tracer was added to 100 ml. of a 10% hydrochloric acid solution of 25 mg. of yttrium oxide. To the solution was added 25 ml. of 40% ammonium tartrate. The solution was made alkaline with ammonium hydroxide and heated just to the boiling point. After being stirred for a few minutes to flocculate the ammonium yttrium tartrate, the mixture was filtered.

The activity determinations made on the precipitate and filtrate showed that the yttrium carried the scandium completely.

To study the effect of diverse ions on the recovery of scandium, a known quantity of scandium-46 tracer was added to 100 ml. of a 10% hydrochloric acid solution in which were dissolved 0.5 gram of a sample and 25 mg. of yttrium oxide. The sample contained from 2 to 10% each of cobalt, copper, nickel, iron, aluminum, and magnesium, from 0.1 to 1% of uranium, molybdenum, vanadium, and manganese, and about 20% of calcium. The yttrium was precipitated as ammonium yttrium tartrate and filtered.

From the activity determinations it was found that at least 96% of the scandium-46 was present in the yttrium precipitate.

PROCEDURE

Preparation of Standard Curve. Pipet a suitable aliquot of the standard scandium chloride solution into a 100-ml. beaker to give from 0 to 120 γ of scandium oxide and add 5.0 ml. of the yttrium chloride solution. Dilute the solution to 30 ml. with water and add 25 ml. of the 40% ammonium tartrate solution and 10 ml. of concentrated ammonium hydroxide. Heat the solution just to the boiling point. Stir until the tartrate precipitate becomes flocculent and filter the precipitate on an 85-ml. sintered-glass Büchner funnel. Dissolve the tartrates from the funnel with 25 ml. of concentrated hydrochloric acid and collect the solution in the beaker used in the precipitation step. Wash the funnel with 25 ml. of concentrated hydrochloric acid and hold the washing in reserve.

Transfer the acid solution to a 125-ml. separatory funnel and equilibrate with 25 ml. of tributyl phosphate for about 30 seconds. Discard the aqueous phase. Backwash the organic phase containing the scandium with the reserved washing and then with two additional 25-ml. portions of concentrated hydrochloric acid. Discard the washings. Wash the scandium from the organic phase by shaking with 50 ml. of water, and transfer the water phase containing the scandium to a 125-ml. separatory funnel. Free the solution of residual tributyl phosphate by washing with 25 ml. of diethyl ether and transfer to a 150-ml. beaker.

Add 8.0 ml. of concentrated ammonium hydroxide and stir the solution until the ether, which is dissolved in the solution, stops evolving. Add 2.0 ml. of the alizarin red S solution and titrate carefully with concentrated ammonium hydroxide until the red end point is reached. If the end point is passed, add a few drops of hydrochloric acid and repeat the titration. To the solution add 5.0 ml. of the 20% ammonium acetate buffer, cool to room temperature, and dilute to 100 ml. with water.

Measure the absorbance of the solution with a Beckman Model DU spectrophotometer at a wave length of 520 mμ, slit width 0.04 mm., with 5.00-cm. Corex cells. As a reference solution use a standard containing no scandium which has been carried through the above procedure. Plot the calibration curve, which is a straight line indicating conformance to Beer's law.

Procedure in Absence of Zirconium and Thorium. Prepare a solution of the sample in concentrated hydrochloric acid. Transfer an aliquot containing from 10 to 120 γ of scandium oxide to a 125-ml. separatory funnel and adjust the volume to not less than 25 ml. with concentrated hydrochloric acid. Add 0.5 ml. of 30% hydrogen peroxide and 25 ml. of tributyl phosphate. Extract the scandium and discard the aqueous phase. Backwash the organic phase with three 25-ml. portions of concentrated hydrochloric acid. Discard all the washings. Add 70 ml. of water to the separatory funnel and wash out the scandium by shaking the contents for 30 seconds. Transfer the aqueous phase to another 125-ml. separatory funnel. Add 25 ml. of diethyl ether and extract the residual tributyl phosphate. Draw the aqueous solution into a 150-ml. beaker and add 5.0 ml. of the yttrium chloride solution. Add 25 ml. of the 40% ammonium tartrate solution. Slowly add concentrated ammonium hydroxide, in small portions and with constant stirring, until the solution is alkaline and then add a few milliliters in excess. When most of the ether has been expelled, heat the solution on a hot plate to a near boil. Discontinue heating and stir the mixture for a few minutes. Filter the insoluble tartrates on a sintered-glass Büchner funnel and wash the precipitate with 25 ml. of the ammonium tartrate wash solution. Dissolve the tartrates with 50 ml. of 20% hydrochloric acid and collect the solution in the 150-ml. beaker used in the precipitation step. Repeat the tartrate precipitation, filter, dissolve in hydrochloric acid, and repeat the precipitation. Treat the purified tartrates as in the preparation of the standard curve.

Procedure in Presence of Zirconium and Thorium. Prepare a solution of the sample in either sulfuric or hydrochloric acid, free of nitrate and fluoride ions. Transfer an aliquot containing from 10 to 120 γ of scandium oxide to a 250-ml. separatory funnel. Dilute the aliquot to 100 ml. with water and sulfuric or hydrochloric acid, so that a concentration of 10% acid is obtained. Add 10 ml. of the 6% cupferron solution to precipitate the cupferrates and extract with three 25-ml. portions of chloroform. Add 5 ml. of the 6% cupferron solution and extract with four 25-ml. portions of chloroform to remove the cupferrates and excess cupferron. (Additional cupferron extractions should be made if the initial amount of impurities is high and a heavy precipitate is obtained after the second addition of the cupferron.)

Transfer the solution to a 400-ml. beaker and dilute to about 300 ml. with water. Boil out any chloroform droplets and then make the solution slightly alkaline with ammonium hydroxide If no precipitate is observed, make the solution acid with nitric

Table II. Recovery of Scandium from Synthetic Solutions in the Presence of Foreign Ions

No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
Element	Mg.	Element	Mg.	Element	Mg.	Element	Mg.	Element	Mg.	Element	Mg.
Al	10	Al	10	Al	10	Al	10	Be	10	Ce	10
Be	10	Ba	10	Fe	10	Ca	10	Bi	10	Th	10
Bi	10	Ca	10	Mg	10	Ce	10	Cd	10	Ti	10
Cd	10	Co	10	Ni	10	Cu	10	La	10		
Co	10	Cu	10	Ti	10	Ga	10	Pb	10		
Cr	10	Fe	10	U	10	La	10	Sn	10		
Mn	10	Mg	10	Zn	10	Mo	10	Th	10		
Sb	10	Ni	10	Zr	10	Sn	10	Zn	10		
Th	1	Sr	10								
Ti	1	Th	2								
V	10	U	10								
Zr	1										
Sc_2O_3 added, γ	20		25		25		20		25		25
Sc_2O_3 found, γ	22		25		26		20		24		24

acid, add about 200 mg. of aluminum nitrate, and precipitate the aluminum with ammonium hydroxide. Macerate two or three Whatman filter accelerators (1 × 3/4 inch) in 10 ml. of water and add this pulp to the mixture. Filter the hydroxides on an 85-ml. sintered-glass Büchner funnel. Dissolve the hydroxides with 100 ml. of warm 20% nitric acid. If a hydrochloric acid solution was used in the cupferron precipitation, the hydroxides must be reprecipitated to free the solution of chloride. If sulfuric acid was used, the hydroxides need not be reprecipitated.

Add 2.0 ml. of the mercurous nitrate solution to the nitric acid solution of the hydroxides. Add 75 ml. of the saturated potassium iodate solution and stir frequently for 15 minutes. Filter the insoluble iodates on a sintered-glass Büchner funnel. Wash the beaker and the iodates with 25 ml. of the saturated potassium iodate solution. (The Büchner funnel containing the iodates can be cleaned in a hood with concentrated hydrochloric acid.)

Dilute the filtrate and washing containing the scandium to about 300 ml. with water. Make the solution alkaline with ammonium hydroxide and heat to about 80° C. Add two or three macerated Whatman filter accelerators and filter. Dissolve the hydroxides with 25 ml. of concentrated hydrochloric acid and collect the solution in a 100-ml. beaker. Wash the Büchner funnel with 25 ml. of concentrated hydrochloric acid and reserve the washing.

Extract, purify, and determine the scandium as described under the procedure in the absence of zirconium and thorium.

RESULTS

Recovery of Scandium in Presence of Foreign Ions. Six synthetic solutions were prepared to contain known quantities of scandium together with other metal ions. The solutions were analyzed by the appropriate procedure, with the results given in Table II.

In Table III the results of the analyses of scandium-bearing samples and residues and liquors obtained by processing these samples are presented.

DISCUSSION

The cupferron-chloroform extraction removes many metals which interfere with the color development with the alizarin red S. The chief need for this extraction, however, is the removal of zirconium (and probably hafnium). Zirconium, if not removed before the extraction of the scandium from hydrochloric acid with tributyl phosphate, prevents the complete washing out of the scandium from the organic phase with water. Qualitative tests with tracer scandium-46 showed that small quantities of zirconium prevented the complete removal of scandium from the organic phase even after repeated backwashings with equal volumes of water. No other metal ion tested exhibited this effect.

The iodate precipitation is required for the removal of thorium. Thorium is not removed in the cupferron-chloroform extraction, cannot be separated from scandium by the tributyl phosphate extraction from concentrated hydrochloric acid, and cannot be removed completely by repeated ammonium yttrium tartrate precipitations. Mercurous nitrate is added before the thorium is precipitated as the iodate, because microgram quantities of thorium are not removed quantitatively if the mercury carrier is absent.

After the removal of the zirconium and thorium, a preliminary tributyl phosphate extraction is made to separate scandium from the bulk of the remaining elements, which are essentially aluminum, beryllium, uranium, chromium, and the rare earths. Hydrogen peroxide retains cerium in the trivalent state and prevents its extraction. The peroxide also prevents the extraction of titanium if this element is present. Uranium and iron are extracted completely. On washing out the scandium with water, however, some of the uranium is retained in the organic phase. If the preliminary tributyl phosphate extraction is not carried out, the rare earths will interfere in the tartrate precipitations.

The multiple tartrate precipitations are made to free the scandium of any remaining traces of impurities. If thorium, zirconium, titanium, and rare earths other than yttrium—e.g., cerium—are present, the ammonium yttrium tartrate is slimy in nature and does not completely carry the scandium. Aluminum, iron, and uranium in quantities up to 10 mg. do not affect the tartrate precipitation.

Table III. Analysis of Initial and Process Samples

Material	Quantity	Sc_2O_3 Found	Total Sc_2O_3, Mg.
Initial sample A	2500 g.	0.0095%	237.5
Process liquor 1	14.5 l.	n.d.	...
Process liquor 2	2.37 l.	0.070 g./l.	165.9
Process liquor 3	2.20 l.	0.014 g./l.	30.8
Residue	546 g.	0.006%	32.8
Total found in processed samples			229.5
Initial sample B	1.0 l.	0.070 g./l.	70.0
Process residue 1	14.3 g.	0.030%	4.3
Process residue 2	3.5 g.	1.36%	47.6
Process residue 3	134.0 g.	0.011%	14.7
Filtrate	1.93 l.	0.0004 g./l.	0.8
Total found in processed samples			67.4

The final tributyl phosphate extraction is made to separate scandium from yttrium. Traces of yttrium may still be present after the hydrochloric acid washings of the organic extract. For this reason, the reference solution for the spectrophotometric determination is prepared by precipitating yttrium as the tartrate and extracting a hydrochloric acid solution of the tartrate with tributyl phosphate.

The data in Table II show that scandium can be determined in a great variety of process materials and minerals with an indicated accuracy within ±5%. Beryl and rutile samples have been analyzed readily.

The data in Table III are given to show the material balance

obtained. Those materials resulting from processing the initial samples contained very large quantities of some of the metals listed in Table II.

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RECEIVED for review May 17, 1955. Accepted July 21, 1955.

Spectrographic Determination of Rubidium in Plant and Animal Tissues

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Because wet chemical methods generally are unsatisfactory, rubidium usually is determined by spectrographic methods. Excitation of prepared samples of natural products by an electric arc or spark produce spectral lines of other elements that interfere with reading those of rubidium. This difficulty may be reduced by using flame excitation and a spectrograph of high dispersion. If the potassium concentration of the sample is high, as usually occurs in plant tissue, interference due to scattered radiation from intense potassium lines may be reduced by a mask, which prevents the energy from the potassum lines striking the spectrographic plate. Under proper conditions the method presented here is satisfactory for as low a concentration as 1 p.p.m. of rubidium in plant or animal tissue. The rubidium content of a number of human and animal foods was determined. Soybeans contained the largest concentrations of rubidium, 160 to 225 p.p.m., dry basis. In wheat flour, bread, and oleomargarine, less than 1 p.p.m. was found.

CHEMICAL methods for determination of rubidium are difficult, because of the tedious procedures required to effect separations from other alkali metals. In fact no published procedure has been found for quantitative separation of cesium and rubidium (*14*). Colorimetric methods also have not been found in the literature. Only spectrographic methods appear to have been used for determinations of the rubidium content of plant and animal tissues. Many difficulties, however, have arisen in development of spectrographic methods for the quantitative determination of this element, although it was one of the first to have been discovered by spectrographic means (*16*).

Two sets of spectral lines of rubidium are sufficiently intense for analytical purposes, the violet pair at 4201.9 and 4215.6 A. and the red pair at 7800.2 and 7947.6 A. Arc, spark, gas discharge, and flame techniques have been used for excitation of rubidium in spectrographic work. Sheldon and Ramage (*15*) rolled samples of plant tissues in ashless filter paper and burned them in an arc before the slit of a spectrograph, rubiduim being one of the elements determined. Strait (*17*) employed the condensed spark between an upper pointed electrode and a lower cylindrical electrode of copper upon which 0.04 ml. of plant sap had been deposited. The rubidium lines at 4215.6 and 4201.8 A. were used with indium line at 4101.8 A. serving as the internal standard.

Bertrand, Bertrand, and Courty (*4*) used a method of background correction to overcome the difficulties encountered in arc excitation. They used the 4201.9 A. line of rubidium. Halperin and Sambursky (*10*) also employing arc excitation, chose to read the 7800.2 and 7947.6 A. lines in attempting to overcome interference from other elements.

Cohn (*8*) excited rubidium by placing the powdered sample in a gas discharge tube. Vanstone and Philcox (*18*) pressed dried plant materials into a cellophane tube, which was fed into an oxyacetylene flame for excitation. Rusanov and Vasil'ev (*11*) introduced powdered materials into the oxyacetylene flame by means of an air stream. Borovik-Romanova (*6*) claimed both the acetone lamp and the direct current arc were suitable sources of excitation for spectrographic determination of rubidium using the lines at 7800.23 and 7947.68 A.

Flame rather than arc excitation has the advantage of producing spectral lines of the alkali metals along with relatively few lines of interfering elements (*9, 10*). Cyanogen bands also are eliminated by flame excitation methods.

Because the spectral lines of potassium (7664.9 and 7699.0 A.) and rubiduim (7800.2 and 7947.6 A.) are relatively close together, the Beckman DU flame photometer or similar instrument does not permit sufficient resolution for the determination of rubidium in the presence of potassium. Freytag (*9*) reported considerable interference of potassium and lithium in determination of rubidium using a Zeiss flame photometer. Instruments with interference or absorption filters likewise do not give sufficient resolution to permit the determination of rubidium in the presence of potassium.

Table I. Rubidium Content of Experimental Feed Samples[a]

Sample	Calcd. Rb Added, %	Rb Detd., %
1	0.000	0.000
2	0.000	0.000
3	0.01	0.009
4	0.01	0.011
5	0.10	0.14
6	0.10	0.14
7	0.20	0.21
8	0.20	0.21
9	0.30	0.30
10	0.30	0.30
11	0.40	0.37
12	0.40	0.40

[a] Feed compounded of casein, sucrose, oil, vitamins, and minerals.

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Table II. Replica of Determining Rubidium Content of Rat Liver Tissue[a]

Trial No.	Plate No.	Rb %, Dry Basis
1	98	1.00
2	99	1.22
3	100	1.06
4	101	0.96
5	103	1.10
6	104	1.13
7	122	1.09
8	123	0.91
9	124	1.09
10	125	1.20
11	126	1.18
12	127	1.20
13	129	1.24
14	130	1.16
15	133	1.15
	Av.	1.11
	Std. dev.	0.031

[a] Liver from rat receiving experimental rations containing Rb.

This laboratory has used the Beckman flame attachment (Model 10,300) as an excitation unit and a high dispersion spectrograph (Bausch & Lomb large Littrow) for the determination of sodium, potassium, and calcium (*12*, *13*). It appeared desirable to investigate the use of this equipment for the determination of rubidium. A special mask was devised for use with samples containing relatively large amounts of potassium to prevent scattered radiation from potassium interfering with reading the intensity of the rubidium line at 7800.2 A.; thus making this line of rubidium available for analyses under these conditions.

EXPERIMENTAL PROCEDURES

Preparation of Samples. Five-gram samples of plant or animal tissues were weighed into platinum dishes and dried at 105° C. The samples were dry-ashed at 550° C. They then were covered with 1 ml. of a solution of hydrochloric acid (1 to 1), and after approximately 1 hour were heated until only a dry residue remained. Distilled water was added, the solution filtered, and the filtrate made to 10 ml. This filtrate was the stock solution, which was diluted for spectrographic analyses.

Preparation of Standards. Several series of standards were used, the selection depending on the contents of rubidium, sodium, and potassium. As it was found that the intensity of the rubidium line of standards was increased by presence of sodium and potassium, these ions were added to standard solutions. Lithium served as the internal standard. All salts were added as chlorides. Typical of the standards used were solutions containing 0.4 mg. per ml. of potassium, 0.1 mg. per ml. of sodium, 0.0125 mg. per ml. of lithium with the rubidium concentration varying from 0.000 to 0.100 mg. per ml.

Effect of Extraneous Elements. The possible effects of extraneous elements on line intensities of test elements are well known in emission spectroscopy (*7*). Such effects were investigated with respect to the proposed method of analysis for rubidium. Since sodium, potassium, calcium, and magnesium are the elements most likely to be present in appreciable quantities in the ash of plant and animal tissues, the effects of these elements on line intensities or rubidium were investigated.

It was found that the intensities of the rubidium lines were enhanced by sodium and/or potassium. Calcium and magnesium however, had no apparent effect. For this reason, all standards were prepared containing quantities of sodium and potassium at approximately the concentrations present in the samples for analysis. Calcium and magnesium were not added to standards.

When analyzing samples having an exceptionally high rubidium content, the concentrations of rubidium, sodium, potassium, and lithium were increased to compensate for the shorter exposure required. The concentrations of the first three mentioned elements in the standards were selected to be in the approximate ranges as in samples analyzed

Instruments, Accessories, and Operational Data. SPECTROGRAPH. Bausch and Lomb large Littrow, quartz prism.

SETTING. Focus 8, tilt 310, wave-length range 3600 to 8500 A.

OPTICS. Cylindrical lens before slit, stainless steel concave mirror back of flame.

SLIT. Height 1.5 mm. width 65 to 90 microns, depending on concentrations.

EXPOSURE. 45 to 180 seconds, depending on concentrations.

EXCITATION. Beckman flame photometer excitation unit, Model 10,300. Natural gas fuel, pressure 5 cm. of a manometer fluid (specific gravity 1.04). Oxygen pressure, 40 inches of water. Air pressure on aspirator 16 pounds per square inch. Rate of feed, approximately 0.2 ml. per minute.

PHOTOGRAPHIC PLATES. Eastman 1-*N* plates, developed according to recommended procedures.

MASK. A black paper mask, fitted over the photographic plate in a position so that potassium lines 7664.9 and 7699.0 A. were masked, was used when analyzing samples of potassium content greater than 1 mg. per ml.

SPECTRAL LINES. Rubidium 7800.2 and 7947.6 A., lithium 6707.8 A.

DENSITOMETER, ARL-Dietert.

Analytical Curves. Spectra of a number of standards, the concentrations of which covered the range of those of the samples, were recorded on each plate which also contained spectra of samples. From densitometer readings and emulsion calibration curves, intensity ratios rubidium to lithium of standards were calculated. A calibration curve was plotted on a log-log scale, placing the ratio rubidium to lithium on one axis and concentration on the other. From this curve the concentrations of rubidium in the samples were determined. It also is possible to determine sodium and potassium on the same plate with rubidium if the concentration ranges of sodium and potassium give appropriate line intensities.

Effect of Masking. When the concentrations of rubidium and potassium are similar, no masking of potassium lines is necessary. However, samples containing small quantities of rubidium, as in many plant tissues, require extended exposure times. Because of the high potassium content in plants this may result in the

Table III. Comparison of Duplicate Determinations of Rubidium in Soybeans

Sample No.	Description	P.P.M. Rb, Dry Basis Trial 1	Trial 2	Mean	Dev. from mean
S-1	Aerial part	160	210	185	25
S-2	Aerial part	230	280	255	25
S-3	Aerial part	230	280	255	25
S-5	Seeds (commercial)	180	170	175	5
S-6	Seeds (commercial)	230	240	235	5
S-7	Meal (solvent extracted)	250	260	255	5
S-8	Seeds (commercial)	250	260	255	5
S-9	(612)	200	210	205	5
S-10	(Illini)	170	150	160	10
S-11	(Chief)	180	190	185	5
S-12	(Adams)	180	180	180	0
S-13	(Lincoln)	190	180	185	5
S-14	(Wabash)	200	170	185	15
S-15	(Wabash)	200	190	195	5
S-16	(Wabash)	200	210	205	5

Table IV. Rubidium Content of Some Human Foods and Animal Feeds, Dry Basis

Product	Rubidium Content, P.P.M.
Tomato, whole fruit	140
Beef, rib muscle	140
Tea, Orange Pekoe	110
Molasses	92
Liver, beef	90
Coffee, ground	89
Cocoa	81
Milk, dry skim	79
Pork, steak	78
Sweet potato	63
Coconut, shredded	58
Peanut butter	57
All-bran cereal	55
Ham	52
Beans, dry lima	51
Apple	50
Pecan, meat	41
Dates	33
Rice, white	27
Banana	18
Squash	17
Cabbage	12
Turnip	11
Raisins	7
Orange, peeled	6
Tapioca	4
Rye bread	4
Oats, rolled	2
Onion	1
Cheese	1
Flour, white wheat	<1
White bread	<1
Oleomargarine	<1
Soybean pellets	220
Atlas sorghum silage	130
Brome grass pellets	130
Silage, Tenn. Orange	65
Cottonseed meal	61
Prairie hay	60
Red clover	44
Alfalfa pellets	98

potassium line, 7699.0 A., being so dense and diffused on the spectrographic plate that the reading of the rubidium line, 7800.2 A., is inaccurate, because of background density. As the 7800.2 A. line is the denser of the rubidium pair it is the only rubidium line suitable for analytical purposes. It was found possible, however, to insert a black paper mask over the photographic plate prior to making exposures so that the potassium line was not recorded. Thus readings were possible on samples containing only a small quantity of rubidium and large quantities of potassium.

Accuracy and Precision. Several samples of experimental feeds prepared from casein, sucrose, oil, vitamins, and minerals were analyzed for rubidium content (Table I). Although some differences were found between the added and determined quantities of rubidium, not all differences should be attributed to the spectrographic analyses, for the samples were handled in several operations prior to analysis. As indicated in Table I, recovery of rubidium from the samples was satisfactory.

Data obtained on a single sample of rat liver tissue, which was analyzed 15 times for rubidium content, are presented in Table II. The results were obtained under various conditions of exposure and development, on separate spectrographic plates, each exposed on different days. The precision, average 1.11%, standard deviation 0.031, compares favorably with results on the same samples for potassium, average 0.66% standard deviation 0.051, and sodium, average 0.31%, standard deviation, 0.018, using the Beckman spectrophotometer.

The precision of the method also may be judged from data in Table III. Each of the soybean samples was analyzed in duplicate, one determination on each sample being read from one plate and the second determination from a second plate. In two thirds of the samples, deviations from the means were 5 p.p.m. or less.

Using standards of low rubidium content (0.001 to 0.020 mg. per ml.), exposures of 90 to 180 seconds, and a slit width of 90 microns, it was possible to detect as little as 1 p.p.m. of rubidium in solution, equivalent to 1 p.p.m. in the original dry sample. By proper concentration procedures it is possible to determine even smaller amounts of rubidium.

RUBIDIUM CONTENTS OF SOME PLANT AND ANIMAL TISSUES

Data on the rubidium content of soybeans are presented in Table III. In addition, a number of common human foods and animal feeds were examined for rubidium content (Table IV). Soybeans contained 160 to 225 p.p.m. of rubidium, more than any other food or feed examined. Other foods containing the larger quantities of rubidium were: tomato, 140 p.p.m.; beef muscle, 140 p.p.m.; brome grass and Atlas sorghum silage, 130 p.p.m.; tea, 110 p.p.m. At the other extreme, white flour, bread, and oleomargarine contained less than 1 p.p.m.

Few data have been found for comparing results using the proposed method for determinations of rubidium in plant and animal products with those of other authors. Borovik-Romanova (*5*) reported a range of 0.1 to 1.0 p.p.m. in plants. Bertrand and Bertrand (*3*) reported an average of 11.9 p.p.m. in 29 samples of monocotyledons and 20.2 p.p.m. for 29 dicotyledons. The same authors (*1*) report a range of 2.1 to 81 p.p.m. for phanerogams. By the present method 27 samples of human foods of plant origin averaged 35 p.p.m. dry basis, from a high of 140 p.p.m. in tomato to 1 p.p.m. in onion. Of the four meat foods listed, ham had the lowest rubidium content, 52 p.p.m. dry basis, beef muscle was highest, 140 p.p.m. Bertrand and Bertrand (*2*) investigated the rubidium content of animal tissues and found from 2 to 135 p.p.m. dry basis.

It is indicated by data of this study that the method of determining rubidium in plant and animal tissues using the high dispersion spectrograph and flame excitation is practical. The same plates from which concentrations of rubidium are read also may be used for determinations of sodium and potassium, provided the relative quantities of the latter are not markedly greater than that of rubidium and use of the mask is not required.

ACKNOWLEDGMENT

The authors wish to express appreciation to the Nutrition Foundation for financial assistance in this investigation.

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RECEIVED for review January 24, 1955. Accepted July 8, 1955. Fall Chemistry Conference, sponsored by Kansas City and University of Kansas Sections of ACS, December 4–5, 1953, Kansas City, Mo. Contribution No. 512, Department of Chemistry, Kansas Agricultural Experiment Station, Manhattan, Kan.

Polarographic Determination of Tin in Ores

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This work was undertaken as a part of a research program on the flotation concentration of tin ores. Existing methods of tin determination proved to be time-consuming, inaccurate, or insensitive for this purpose. The present method has been applied to many different types of tin ores, and is believed applicable to all ores, alloys, compounds, or mixtures containing tin if no better than the usual polarographic precision is to be expected at any given concentration. The procedure involves fusing with sodium peroxide, filtering off the cations insoluble in sodium hydroxide, and making the filtrate approximately 6*N* hydrochloric acid. The tin is then determined polarographically from a known volume of this solution. Few ions produce waves that interfere with the tin wave, and those that do interfere are easily removed. The time necessary to complete one analysis is less than 2 hours, but on a routine basis averages under half an hour.

ANALYTICAL methods for the determination of tin are usually specific, and depend to a great extent not only on the amount of tin in the ore, but also on the number and kind of possible interfering ions. An investigation has been undertaken to develop a polarographic procedure for the rapid determination of small amounts of tin in any ore. A great deal is known about the polarography of tin (*3*), and successful attempts have been made to determine tin in alloys (*2*) and in ores (*1*). However, these procedures are either too long, or not of general enough applicability.

Both stannous and stannic tin can be reduced at the dropping mercury electrode. The reduction of stannous tin to the metal produces waves suitable for analytical purposes in supporting electrolytes of 1*N* hydrochloric acid, 1*N* nitric acid, 1*N* sulfuric acid, 1*N* sodium hydroxide, tartrate, and alkaline citrate. The reduction of stannic tin produces waves suitable for analytical purposes only in hydrochloric acid solutions where the concentration of chloride ion is greater than about 4*N*. Higher order tin-chloro complexes are formed with increasing chloride concentrations until the chlorostannate complex predominates (*5*). This highest order chloro complex produces two waves upon reduction, both of which are diffusion controlled. The first wave occurring at a half-wave potential of −0.25 volt *vs.* S.C.E. results from the reduction of the chlorostannate ion to the chlorostannite ion, and the second wave at a half-wave potential of −0.52 volt *vs.* S.C.E. results in complete reduction to the metal. The second wave is best for analytical purposes.

APPARATUS AND REAGENTS

Polarographic data were obtained from a Leeds & Northrup Electro-Chemograph Type E, and an H-type polarographic cell with a saturated calomel electrode. The cell was kept at a temperature of 25.0° ± 0.1° C. in a constant temperature water bath. Dissolved oxygen was removed satisfactorily by bubbling nitrogen through the solution for 10 minutes before each polarographic determination. Oxygen was removed from the nitrogen stream by bubbling the stream through a vanadous sulfate solution (*6*). Characteristic polarographic properties of the capillary used were: drop time, 4.50 seconds per drop; m for the capillary, 1.59 mg. per second; and 1.75 $mg.^{2/3}$ $sec.^{-1/2}$ for $m^{2/3}t^{1/6}$ at h = 65.8 cm. Approximately 6*N* hydrochloric acid was the supporting electrolyte employed in all cases.

CONSTANT BOILING HYDROCHLORIC ACID. Add 1000 ml. of concentrated hydrochloric acid (specific gravity, 1.19) to 850 ml. of distilled water and boil for one half hour.

GELATIN SOLUTION, 2.0%. Dissolve 2.0 grams of gelatin in about 90 ml. of hot water. Cool and dilute to 100 ml.

The standard tin samples used were Mallinckrodt's granulated 20-mesh tin metal, Baker's Analyzed stannous chloride, National Bureau of Standards cast-bronze standard sample No. 52c containing 7.85% tin, 89.25% copper, and 0.76% nickel; and a cassiterite sample containing 77.9% tin.

PROCEDURE

Thoroughly mix 1 gram of −100-mesh tin ore or other tin sample with 8 grams of sodium peroxide in an iron crucible. If the tin is in solution, evaporate to dryness first, or if much organic matter is present, roast the ore at about 725° C. Fuse the contents as quickly as possible over a Tirrell burner, allowing it to be at a bright red heat for 1 minute. During this 1-minute period the crucible should be swirled with the aid of a pair of tongs to ensure complete mixing of the sample with the molten sodium peroxide. Cool the crucible and its contents and place them in a 250-ml. beaker containing 150 ml. of distilled water. Heat the solution to its boiling point to dissolve the melt out of the crucible, and then remove the iron crucible from the solution with a pair of platinum-tipped tongs using about 25 ml. of distilled water from a wash bottle to wash the small amount of adhering solution from the crucible to the rest of the solution. Cool to room temperature and transfer the contents of the beaker to a 200-ml. volumetric flask. Use about 25 ml. of distilled water to rinse the remaining contents of the beaker into the volumetric flask and make up to the mark.

Filter about 50 ml. of this solution through a dry filter paper into a small dry beaker. Pipet 25 ml. of the filtrate into a 50-ml. volumetric flask and make up to the mark with concentrated hydrochloric acid. If the sample contains more than 2% tin, add 0.1 ml. of the prepared 2% gelatin solution before making up to the mark. Make a polarogram of the solution over the range of −0.4 to −0.8 volt *vs.* S.C.E. Convert the wave height to microamperes and find the per cent tin from the following equation:

$$\% \text{ Sn} = 1.075\, I_d\ (\mu\text{a.})/\text{sample weight (grams)}$$

RESULTS AND DISCUSSION

The value of I_d/C for different tin samples shows excellent reproducibility considering the wide variety of samples used. For 14 determinations of the value of I_d/C on four different samples the range was 4.34 to 4.46, the average 4.40, and the coefficient of variation ±0.93%. It makes little difference in what form the tin occurs, because it is all oxidized to the plus four oxidation state by the procedure employed and determined polarographically as the complex chlorostannate ion.

Table I. Relationship of Diffusion Current to Concentration of Various Standard Tin Samples in Hydrochloric Acid and 0.004% Gelatin at 25° C.

Sample	Tin Concn., *C*, Millimoles/Liter	Diffusion Current I_d, μa.	K = I_d/C, Diffusion Current, μa./Millimole/Liter
Tin metal (100% Sn)	0.0256	0.114	4.46
	0.234	1.020	4.36
	1.162	5.16	4.44
	5.60	24.3	4.34
Cassiterite (77.9% Sn)	0.104	0.457	4.39
	0.852	3.74	4.39
	5.64	24.9	4.42
Stannous chloride assayed 99.2% $SnCl_2.2H_2O$ (52.4% Sn)	0.122	0.535	4.38
	0.585	2.57	4.40
	2.51	10.9	4.35
National Bureau of Standards cast-bronze standard sample No. 52c (7.85% Sn)	0.0147	0.0653	4.44
	0.174	0.777	4.46
	0.832	3.63	4.36
	2.31	10.2	4.43

The concentration range of the tin for which this procedure may be employed is given in Table I. As the values of concentration fall on a good straight line when plotted against the diffusion current, it is felt that higher and lower percentages of tin can be determined by extrapolation of this straight line. The concentration range can also be easily varied by the size of the aliquot taken after the filtration of the sodium hydroxide–insoluble cations.

The tin is not adsorbed or coprecipitated with the sodium hydroxide–insoluble cations. These precipitates consisting almost completely of the ferric hydroxide from the iron crucible are approximately of the same quantity for each determination. Since there is no trend in the constant of the ratio of the diffusion current to the concentration of the tin, no tin is lost in this step of the procedure.

If a supporting electrolyte containing approximately 6*N* hydrochloric acid is used, few ions interfere with the tin wave. Over 30 of the more common cations were tested for possible interfering effects by adding small amounts of the material to 100 ml. of 6*N* hydrochloric acid and observing whether a polarographic wave was obtained in the region of the tin wave. It was known that nickel, tungsten, vanadium, and lead would produce possible interfering waves.

Nickel is quantitatively separated from tin by precipitation as the hydroxide upon addition of water to the sodium peroxide melt. One tenth of a gram of nickel chloride was added to 0.0551 gram of granulated tin metal. A polarogram made according to the above procedure gave a diffusion current for tin of 5.20 μa. and a value of I_d/C equal to 4.47. The half-wave potential for nickel in this supporting electrolyte is −0.59 volt *vs.* S.C.E. The half-wave potential for tin under the same conditions is −0.54 volt *vs.* S.C.E.

Tungsten is not eliminated. It produces a wave that interferes with the tin wave. The tungsten may be removed easily, however, by filtering the tungstic acid in the presence of cinchinone from the sodium peroxide solution, which has been made slightly acid by the addition of a small amount of hydrochloric acid. The tungsten may then be dissolved in constant boiling hydrochloric acid and determined polarographically. Filter paper should not be used in this separation because the decomposition products formed by its reaction with acids produce a wave that interferes with the tungsten wave. One tenth of a gram of sodium tungstate was added to 0.0547 gram of granulated tin metal. A diffusion current of 5.12 μa. was obtained for the tin wave upon removal of tungsten, giving a value of I_d/C equal to 4.44. The half-wave potential for tungsten in this supporting electrolyte is −0.60 volt *vs.* S.C.E.

Vanadium can easily be separated from the tin, if present. A good indication of its presence is shown by the characteristic vanadium color of the solution produced upon the addition of water to the sodium peroxide melt, or from noticing that the tin and vanadium wave starts at a much more positive value and is much more drawn out than the wave produced by the tin alone. Starting the polarogram at −0.40 volt *vs.* S.C.E. a straight line is obtained always to the beginning of the reduction of the tin. With even small amounts of vanadium present this portion of the wave will be curved. As there is a large amount of ferric hydroxide produced from the decomposition of the iron crucibles, the vanadium may be precipitated quantitatively upon the addition of ammonium hydroxide to the sodium peroxide melt and water solution. Ammonium hydroxide alone does not precipitate vanadium; the ferric hydroxide must be present. One tenth of a gram of ammonium vanadate was added to 0.0550 gram of granulated tin metal. A diffusion current for the tin of 5.08 μa. was obtained giving a value of I_d/C equal to 4.38. The half-wave potential for vanadium in this supporting electrolyte is −0.46 volt *vs.* S.C.E.

Lead gives a half-wave potential of −0.55 volt *vs.* S.C.E. in the supporting electrolyte used here. Advantage is taken of the property of ferric hydroxide to occlude small amounts of lead so that it does not interfere with the tin wave. Wilkie (*7*) found that five parts of ferric hydroxide removed one part of lead from solution. One tenth of a gram of lead nitrate was added to 0.0548 gram of granulated tin metal. A diffusion current of 5.13 μa. was obtained for the tin wave giving a value of I_d/C equal to 4.44. Since large amounts of ferric hydroxide are formed from the fusion in the iron crucible, large amounts of lead can be tolerated. However, if very large amounts of lead are present, the lead wave may be subtracted from the tin wave by a method proposed by Lingane for the determination of tin in copper-base alloys (*4*).

It was believed that in addition to nickel, tungsten, vanadium, and lead, copper, arsenic, and molybdenum also interfere with the tin wave obtained. One tenth of a gram of each of these metals was added to a tin sample, and the tin determined. No change in the value of I_d/C for the tin wave was found for any of these additional metals. The National Bureau of Standards cast-bronze standard sample No. 52c contained 89.25% copper, and was used satisfactorily as a standard. Arsenic(V) is not reduced at the dropping mercury electrode. Molybdenum(VI) produces a wave far removed from the tin wave in this supporting electrolyte.

Aluminum, silver, gold, boron, barium, bismuth, calcium, cadmium, cobalt, chromium, iron, mercury, magnesium, manganese, phosphorus, platinum, rhodium, antimony, strontium, titanium, uranium, zinc, and zirconium were not expected to interfere in this tin analysis. However, a mixture of these elements in their various oxidation states was prepared and added to a tin sample. No effect on the shape of the tin wave or I_d/C was observed. A polarogram made with these possible interfering elements present gave a diffusion current of 5.23 μa. for 0.0553 gram of granulated tin metal and a value of I_d/C equal to 4.48.

If it is necessary to determine iron or some other element in the ore that may be precipitated from an alkaline solution, the following type of separation may be employed:

Fuse the sample in the usual manner in a nickel crucible. After the melt is dissolved in water, the iron and other substances insoluble in sodium hydroxide are filtered off and determined by any appropriate procedure. The tin in the filtrate may be determined by boiling down the filtrate to a volume less than 50 ml., during which time concentrated hydrochloric acid is added so that the final solution is constant boiling hydrochloric acid. This solution is placed in a 50-ml. volumetric flask, and made up to the mark with constant boiling hydrochloric acid.

Lingane (*5*) suggested the use of a supporting electrolyte of 4*N* ammonium chloride and 1*N* hydrochloric acid to provide a high chloride concentration and a low hydrogen ion concentration. This results not only in the formation of the highest order chloro complex, but also the creation of a small enough hydrogen ion concentration to allow the formation of a well-defined diffusion current plateau for the tin wave before the start of the hydrogen wave. In this procedure the concentration of chloride ion is high, about 6*N* from the hydrochloric acid added (only 2.5 ml. of concentrated hydrochloric acid is used for neutralization of the sodium hydroxide). Lingane's supporting electrolyte is not used, because the final solution is almost saturated with sodium chloride, which produces the same effect on the tin wave as the ammonium chloride. For samples containing more than 0.01% tin, the hydrogen wave is far removed from the tin wave. However, at very small tin concentrations the tin and hydrogen waves approach each other, because the potential of the hydrogen wave varies with the magnitude of the current. This difficulty may be overcome by increasing the weight of the sample, increasing the chloride ion concentration with ammonium chloride, and decreasing the normality of the hydrochloric acid, or increasing the size of the aliquot of alkaline solution containing the melt.

The half-wave potential of the tin in the supporting electrolyte

used is −0.54 volt *vs* S.C.E. This value does not vary with the tin concentration over the range of concentrations listed in Table I. In all cases the half-wave potentials are well within the range of −0.53 to −0.55 volt *vs.* S.C.E. The half-wave potential increases to more negative potentials as the hydrogen ion concentration decreases.

Although this procedure has been developed for the analysis of tin ores, there is no reason to suppose that it could not be applied to tin alloys and other tin-containing materials; especially when the tin concentration is small and there are a large number of interfering ions. Over 200 various tin ores containing 0.01 to 10% tin have been analyzed with a standard deviation always less than 2%. An average time for one analysis is less than half an hour on a routine basis and under 2 hours for one complete analysis.

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RECEIVED for review March 28, 1955. Accepted June 23, 1955

Polarography in Molten Ammonium Formate

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A wide variety of inorganic compounds has been investigated polarographically in molten ammonium formate at 125° C. Among the compounds studied were uranium, thorium, and plutonium, as well as typical fission products such as zirconium and the rare earths. Possible applications to qualitative and quantitative analyses of both water-soluble and water-insoluble inorganic compounds are suggested by the polarographic results obtained. Results indicate the relative ease of reduction of the various ionic species in the molten ammonium formate system under the conditions prevailing in this investigation.

NONAQUEOUS polarography can be applied to advantage in the analysis of many organic substances that are insoluble in water. Polarography in nonaqueous solvents performed at room temperature often results in decreased sensitivity due to the lower diffusion coefficients of the reducible species as well as greater difficulty in removing interfering dissolved oxygen. Both of these disadvantages can be minimized if the boiling point of the nonaqueous solvent permits the polarographic determination to be made at higher than room temperature.

Nachtrieb and Steinberg (*9*, *10*) report the use of ternary salt mixtures acting as both solvent and supporting electrolyte in qualitative and quantitative analysis of inorganic compounds by application of dropping mercury polarography in the temperature range 125° to 150° C. Lyalikov and Karmazin (*7*) report the use of solid microelectrodes in molten salt polarography at still higher temperatures. Apparently even under the latter conditions, concentration-dependent current-voltage curves are obtainable in certain cases. The present status of polarography in fused salt media has been reviewed by Lingane (*6*).

The main disadvantage of fused salt polarography is the tendency for reaction between the constituents of the system at high temperatures. In many cases the choice of salt and electrode system will help eliminate or control these side reactions, so that reproducible polarographic results can be obtained. Under these conditions, advantage can be taken of the excellent inorganic-solubility properties afforded by the use of molten salt media—for example, ordinarily insoluble oxides and carbonates are readily dissolved in many fused salt media.

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In the present investigation, molten ammonium formate was chosen as the solvent-supporting electrolyte system, as it afforded the advantage of an operating temperature of 125° C., which permitted use of a dropping mercury electrode without employing a ternary eutectic salt mixture. The heat stability and versatile solubility properties permitted a broad coverage of compounds investigated. In addition to demonstrating the possible application of fused salt polarography to the analysis of a variety of inorganic compounds, the results obtained indicate the relative ease of reduction under these conditions of the various component metal ions studied.

EXPERIMENTAL

Polarography. Polarographic measurements were made at 125° ± 1° C. with a Leeds & Northrup Electro-Chemograph, Type E. Temperature control was maintained using Arochlor (Monsanto Chemical Co.) fluid in a large stainless steel constant temperature bath (Precision Scientific Co., Catalog No. 10192). The solutions were deoxygenated by passing dry nitrogen gas through the molten ammonium formate for 10 minutes. A simple dropping mercury electrode assembly employing a mercury-pool anode-type cell (see Figure 1) with ground-glass connections was used. A capillary (E. H. Sargent Co., S-29351) was sealed into the end of the electrode assembly with Sauereisen No. 1 paste, (Sauereisen Cements Co., Pittsburgh, Pa.), followed by a coating of Fisher High-Pyseal (Eimer and Amend Co.) to seal the pore space within the rigid Sauereisen union. Capillary characteristics and polarographic properties employed at 125° ± 1° C. were: m = 3.04 mg. per second, drop time = 2.7 seconds at h = 37.5 cm., and $m^{2/3}t^{1/6}$ = 2.10 mg.$^{2/3}$ sec.$^{-1/2}$

Under the conditions employed, the molten ammonium formate yielded a useful reduction voltage range of +0.1 to −0.9 *vs.* a mercury pool. A similar voltage span of −0.1 to −1.1 *vs.* an external anode containing mercury, the ternary salt mixture, and potassium chloride was observed by Nachtrieb and Steinberg (*10*).

Freshly opened sample bottles of C.P. anhydrous ammonium formate (Baker & Adamson) were used in each polarographic run. Check polarograms were made from time to time on the ammonium formate alone to ensure its continued purity. Passing nitrogen gas through the solutions at the elevated temperature removed traces of water.

To ensure anhydrous conditions during sample preparation, the ammonium formate and the easily hydrolyzed materials such as uranium(III) chloride, uranium(IV) chloride, zirconium chloride, thorium chloride, aluminum chloride, and the anhydrous rare earth salts were all weighed out and melted within a dry box. Polarography on the plutonium compounds was performed inside an alpha-box.

Preparation of Compounds Studied. Unless otherwise stated, the compounds used were Baker & Adamson C.P. chemicals.

Uranium(VI) oxide was a purified grade from the Mallinckrodt Chemical Co. $V_2O_2Cl_4$ (vanadyl chloride) was from Eimer & Amend. Uranium tetrachloride was a specially prepared and purified anhydrous grade, custom made by the A. D. MacKay Chemical Co. The hydrated salts, zirconium sulfate tetrahydrate, zirconium nitrate tetrahydrate, and zirconyl chloride octahydrate, were prepared in this laboratory from purified zirconium oxide by ordinary metathetical procedures. Their purity was substantiated by ignition analyses (*1*). The purified zirconium oxide was obtained by ignition at about 350° C. of the zirconium hydroxide precipitate formed from a solution of purified zirconyl chloride. Anhydrous zirconium chloride was made from the purified zirconium oxide and C.P. carbon tetrachloride by a method similar to that described by Venable and Bell (*15*). Purity of the zirconium chloride sample was assured by gravimetric analysis after ignition at 850° C. (per cent ZrO_2: theoretical 52.9; found, 52.7).

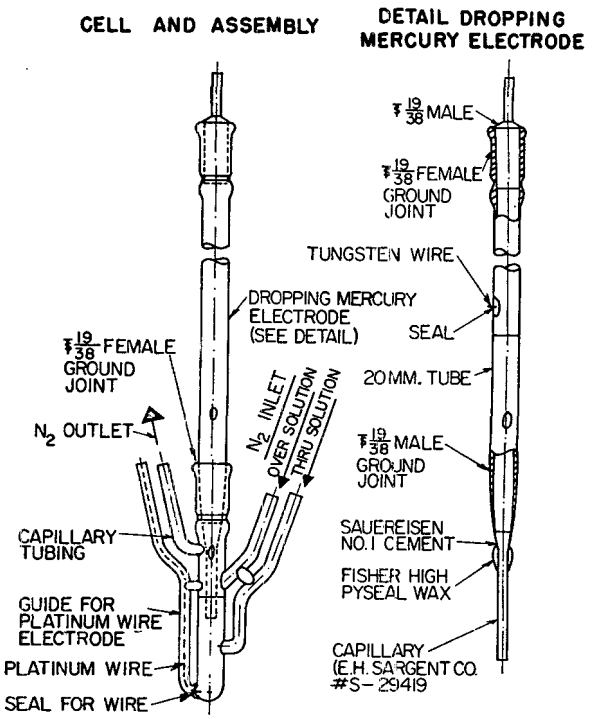

Figure 1. High temperature polarographic apparatus

Anhydrous lanthanum chloride, ytterbium chloride, and thorium chloride and the anhydrous rare earth chlorides, praseodymium, neodymium, gadolinium, and samarium, were prepared by dehydrating the corresponding hydrated salts in a stream of anhydrous hydrogen chloride at about 250° C. (*13*). These hydrated salts were the best grades available from Research Chemicals, Inc., Burbank, Calif. Purity in each case was reported to be at least 99.8%. Anhydrous uranium(III) chloride was prepared from uranium metal (Mallinckrodt) via conversion to uranium hydride and hydrochlorination, as described by Spedding and others (*11, 14*). The uranium(III) chloride was analyzed for uranium gravimetrically by ignition of a small sample, covered with oxalic acid, at 850° C., forming U_3O_8. Theory for UCl_3: 81.5% U_3O_8; found 81.8.

Sodium Uranyl Acetate. 0.5*M* uranyl nitrate (33 ml.), 4*M* sodium nitrate (33 ml.), and 0.1*M* nitric acid (33 ml.) were combined and the mixture was heated to 70° to 80° C. Six milliliters of glacial acetic acid was added. Upon the addition of 10 grams of sodium acetate, a precipitate was obtained. The precipitate was digested for a few minutes at 75° C. and then filtered, washed with small portions of ethyl alcohol, and dried in a vacuum desiccator over sulfuric acid.

Ammonium Uranyl Acetate. This salt was prepared by essentially the same method as the sodium uranyl acetate, except that ammonium nitrate and ammonium acetate were used instead of the corresponding sodium salts. It was found necessary to use three 10-gram portions of ammonium acetate and evaporate the mixture to about one half of its total volume in order to cause precipitation. Gravimetric ignition at 850° C. (per cent U_3O_8: theoretical, 60.4; found, 60.3 and 60.2) substantiated the formula as $NH_4UO_2(C_2H_3O_2)_3$.

Zirconium Mandelate (*5*). A 1.0-gram sample of zirconyl chloride octahydrate was dissolved in 20 ml. of 5% hydrochloric acid. On addition of 165 ml. of 1*M* mandelic acid, a white precipitate formed. The temperature of the solution was raised to 85° C. for 20 minutes, and the precipitate was filtered and washed with small portions of a hot solution containing 2% hydrochloric acid and 5% mandelic acid. After drying over sulfuric acid in a vacuum desiccator, a sample was ignited to 850° C. (per cent ZrO_2: theoretical 17.7; found 17.2).

Thorium Succinate (*3*). Approximately 25 grams of succinic acid was dissolved in 400 ml. of water. The pH was adjusted to 3.3 by adding 30% sodium hydroxide. About 2.5 grams of thorium nitrate (Baker & Adamson) was added and the mixture was heated to 85° C. The white precipitate that formed was filtered, washed with ethyl alcohol, and dried over sulfuric acid in a vacuum desiccator. The ignition analysis at 850° C. was (per cent ThO_2: theoretical 56.9; found 56.2), based on the formula $Th[(CH_2COO)_2]_2$.

Lanthanum Fluoride (*12*). Exactly 5.00 grams of lanthanum nitrate hexahydrate was dissolved in 50 ml. of water and then 15 ml. of 48% hydrofluoric acid was added. The precipitate was filtered and washed with 5% hydrofluoric acid and once with water. Polyethylene equipment was used. After thorough drying in a vacuum desiccator over sulfuric acid, the sample was weighed. The weight factor based on conversion of $La(NO_3)_3.6H_2O \rightarrow LaF_3.2H_2O$ is 53.6%. The weight conversion factor found was 53.8%; on this basis, material was designated as the dihydrate.

The loss in weight on heating accurately weighed samples of the dihydrate at 300° C. for about 2 hours under partial vacuum seemed to correspond to the formation of anhydrous lanthanum fluoride.

Cerium Fluoride. Starting with ammonium ceric sulfate dihydrate from G. F. Smith Chemical Co., a procedure similar to that described above gave $CeF_4.xH_2O$ where x is 1 to 2. Dehydration as above gave a compound which was considered as being anhydrous ceric fluoride. Polarographic reduction properties of the compound, designated as anhydrous ceric fluoride, indicated that material was different than the hydrate.

Uranium Dioxide. Exactly 5.00 grams of uranium(VI) oxide was heated at 330° C. for several hours while dry hydrogen gas was passed over the heated solid. A sand bath was used to maintain temperature control without overheating. The weight conversion factor, based on the reaction $UO_3 + H_2 \rightarrow UO_2 + H_2O$, is 94.4%. The weight conversion factor found was 94.2%. The uranium(IV) oxide formed was dark brown. The original uranium(VI) oxide was yellow. This controlled temperature reduction resulted in an acid-soluble product which apparently was not refractory.

Plutonium Compounds. A purified plutonium(IV) nitrate solution provided by Hanford Works, Richland, Wash., was standardized by a counting technique and then used in preparing all of the plutonium compounds investigated polarographically.

Accurately known quantities of plutonium(IV) oxide were obtained by evaporating aliquot portions of the standard plutonium(IV) nitrate solution to dryness and then heating at 350° to 400° C. for about 6 hours.

The plutonium(VI) used in preparing the plutonyl salt was obtained by oxidizing plutonium(IV) in 0.5*M* nitric acid by heating to approximately 90° C. for 10 hours (*13*). Solid sodium plutonyl acetate, $NaPuO_2(C_2H_3O_2)_3$, was precipitated from the plutonium(VI) solution by buffering with sodium acetate and acetic acid by a procedure similar to that described for sodium uranyl acetate. Purity of the salt was assured by counting technique—i.e., activity relative to original plutonium(IV) used in preparation.

RESULTS AND DISCUSSION

The polarographic results obtained on the various compounds investigated are given in Table I. The more reduction-resistant compounds—e.g., barium, magnesium, and zirconium—did not form half waves, presumably because of preferential or simultaneous reduction of the ammonium formate solvent. In these cases, decomposition potentials of these compounds in ammonium formate were evaluated and tabulated for comparison with the ammonium formate alone. Decomposition potentials in the range −0.55 to −0.65 volt, obtained with the annydrous barium

and magnesium compounds, possibly indicate that these cations are reduced, but simultaneous reduction of some ammonium formate solvent also occurs, thereby preventing the formation of clearly defined half waves. That hydration of a salt increases the reduction resistance is seen by noting that anhydrous lanthanum fluoride yields a $E_{1/2}$ value while lanthanum fluoride dihydrate does not. Further evidence in regard to the influence of hydration is found by comparing the polarographic results obtained from anhydrous chromium(III) fluoride with those from chromium(III) chloride hexahydrate and anhydrous thorium chloride and results from thorium succinate with those from thorium nitrate tetrahydrate.

Apparently neither hydrated nor anhydrous zirconium salts yield half-wave potentials. That the decomposition potentials ($E_{d.p.}$) of the hydrated zirconium salts are less than those for the anhydrous zirconium salts is probably due to the reduction of water and its influence on the $E_{d.p.}$ values. All ammonium formate solutions investigated polarographically were completely clear, indicating complete solubility.

A comparison of the polarographic results obtained with such compounds as cadmium chloride, cadmium carbonate, and cadmium oxide seems to indicate that counter ions within a compound exert little influence on half-wave potentials ($E_{1/2}$) as long as complexation does not occur. Apparently the situation is analogous to aqueous polarography in this respect.

Without knowing diffusion coefficients of the various ionic species investigated, it is not possible to apply the Ilković equation (*4*) and thereby evaluate precisely the number of electrons involved in the various reductions. Plausible or "possible electrode reactions" are indicated below for various reductions. The number of electrons postulated in these cases is based on half-wave height comparison—i.e., I_d/C values—between the reducible species and ionic species of known reductions—e.g.,

$$Pb^{++} + 2e \rightarrow Pb^{\circ} \text{ and } Cd^{++} + 2e \rightarrow Cd^{\circ}$$

Plots of $\log \frac{I}{I_d - I}$ *vs.* E usually were nonlinear and slope analysis values were considerably greater than those corresponding to reversible reductions.

Hydrated hexavalent uranium salts—e.g., uranyl nitrate hexahydrate and uranyl chloride dihydrate—showed well-defined reduction waves, thereby permitting evaluations of half-wave potential. A given concentration of uranium salt yields different half-wave potentials and varying diffusion currents (wave heights) depending upon the length of time the system is maintained at 125° C. For this reason these $E_{1/2}$ values are not reported. In all cases, these half-wave potential values were between −0.10 and −0.50 volt. Probably the uranyl ion is reduced slowly by molten ammonium formate. The mercury normally present during the polarographic runs apparently promotes the reaction, as evidenced by the deep yellow solution formed only when mercury is present during the aging of the melt. Data obtained on anhydrous uranium compounds under various aging conditions (Table II) show the influence of reaction between uranium(VI) and molten ammonium formate. When hexavalent uranium compounds were aged for a minimum period —i.e., only long enough to deoxygenate the solutions, approximately 5 minutes—half-wave potentials were not obtained. Only after the aging period allowed reduction of the uranium(VI) to some lower valence state, probably quadrivalent, was polarographic reduction evident. Quadrivalent uranium compounds, uranium(III) oxide and uranium(IV) chloride, at low concentrations—e.g., 0.001*M*—were reduced very slowly by the ammonium formate and then well defined polarographic properties were obtained (see Table II). Using the comparative wave height method discussed above, the two reduction waves observed for uranium(IV) oxide and the three waves found for uranium (IV) chloride can be explained as follows:

$$U^{++++} \xrightarrow{e} U^{+++} \xrightarrow{e} U^{++}$$

and

$$U^{++++} \xrightarrow{e} U^{+++} \xrightarrow{e} U^{++} \xrightarrow{2e} U^{\circ}$$

Table I. Half-Wave and Decomposition Potentials of 0.001*M* Solutions of Various Compounds in Molten Ammonium Formate at 125° C.

Compound	$-E_{1/2}$	I_d/C, $\frac{\mu a.}{mN}$	$-E_{d.p.}$
Ammonium formate alone			0 90
AgCl	[a]		
$AuCl_3 \cdot HCl \cdot 3H_2O$	[a]		
$AlCl_3$	0.82	19	
$Al(OH)_3$	0.83	20	
$NaAlO_2$			0.90
As_2O_3	I 0.31	I 7	
	II 0.60	II 14	
	I 0.60	I 14	
$NaAsO_2$	II 0.71	II 7	
BaF_2			0.55
$BaSO_4$			0.65
$BiCl_3$	[a]		
BiOCl	[a]		
Bi_2O_3	[a]		
CaF_2	0.76	10	
$CdCl_2$	0.17	8.2	
$CdCO_3$	0.19	8.3	
CdO	0.26	10	
$CoCO_3$	0.55	10.5	
$CoSO_4$	0.56	11	
Co_2O_3	0.70	4.0	
$CrCl_3.6H_2O$			0.55
CrF_3	0.27	5.1	0.55
CrO_3	I 0.25	I 5.0	
	II 0.56	II 9.7	
K_2CrO_4	0.17	5.2	0.55
$CuSO_4$	0.17	7	
$FeCl_2.4H_2O$			0.90
Fe_2O_3	0.68	17	
$FePO_4$	0.70	14	
$MgCO_3$			0.60
$MgSO_4$			0.60
MgO			0.60
$MnCO_3$			0.65
$MnSO_4$			0.70
			l.d.w.[b]
MnO_2			0.55
			0.90[c]
$(NH_4)_2MoO_4$	0.05	17	
$NiCO_3$	0.45	10	
PbO	0.12	9.8	
$PbSO_4$	0.21	10	
$SbCl_3$	0.05[c]	10	
Sb_2O_3	0.04[c]	4.5	
Na_2SiF_6			0.90
SnO	0.08	9	
SnO_2	I 0.31	I 9.2	
	II 0.72	II 10.3	
SrF_2			0.65
$SrSO_4$			0.90
$ThCl_4$	0.76	5.0	
$Th[(CH_2COO)_2]_2$	0.75	5.5	
Thorium succinate			
$Th(NO_3)_4 \cdot 4H_2O$			0.55
TiO_2			0.65
$(V_2O_2Cl_4)$	I 0.48	I 5.0	
Vanadyl chloride	II 0.76	II 4.8	
NH_4VO_3	0.61	7	
$NaWO_4 \cdot 2H_2O$			0.45
$ZnCO_3$	0.70	7	
$ZrOCl_2 \cdot 8H_2O$			0.60
$Zr(NO_3)_4 \cdot 4H_2O$			0.60
$Zr(SO_4)_2 \cdot 4H_2O$			0.58
$ZrCl_4$			0.82
$Zr(C_6H_5\text{-}CHOH\text{-}C\text{⊖}O)_4$			0.83
Zirconium mandelate			
Rare Earths and Related Compounds			
$CeF_4 \cdot x\ H_2O$			0.60
$(NH_4)_4Ce(SO_4)_4 \cdot 2H_2O$			0.60
CeF_4	0.85	5.5	0.90
$GdCl_3$	0.40	5.3	0.90
$LaCl_3$	0.78	16	
$LaF_3 \cdot 2H_2O$			0.60
LaF_3	0.75	16	0.82
$La(NO_3)_3 \cdot 6H_2O$			0.60
$NdCl_3$	0.85	15	0.65
$PrCl_3$	0.84	16	0.70
$SmCl_3$	0.76[c]	11	0.60
$YbCl_3$	0.30	5.0	0.90

Id/C. Final units $\left(\frac{\mu a.}{mN}\right.$ = microamperes per millinormality$\left.\right)$

[a] Formed black ppt. on dissolving in ammonium formate, presumably due to reduction to metal.
[b] l.d.w. Large decomposition wave initially.
[c] Potential after 1 hour in mercury pool cell.
$E_{1/2}$. Half-wave potential *vs.* mercury pool.
$E_{d.p.}$ Decomposition potential *vs.* mercury pool.

These results are in agreement with Driggs and Lilliendahl's observations (*2*) that hexavalent uranium compounds (uranyl radical) do not electrolyze to uranium metal, while quadrivalent uranium compounds such as UCl_4 and KUF_5 yield metallic uranium on ordinary electrolysis in fused salt media. It should not be inferred, however, that the polarographic results obtained actually prove the existence of specific intermediate valence states for uranium or the formation of uranium metal under the conditions prevailing in the system investigated here.

The plutonium analogs of the above uranium compounds were also investigated polarographically (Table II). In this case, plutonium(VI), plutonyl ion, showed no half-wave potential, nor any tendency to be reduced by the molten ammonium formate—mercury system. Plutonium(IV) as either the oxide or the hydrated nitrate showed a single reduction wave. The extremely small solubility of plutonium(III) oxide prevented a wave height analysis. The I_d/C values for quadrivalent plutonium nitrate reduction seem to indicate the following reaction;

$$Pu^{++++} \xrightarrow{e} Pu^{+++}$$

Table II. Polarographic Data on Uranium and Plutonium Compounds in Molten Ammonium Formate at 125° C.

Compound	M	Aging Time in Cell, Hr.	$-E_{1/2}$ I	$-E_{1/2}$ II	$-E_{1/2}$ III	I_d/C, $\frac{\mu a.}{mN}$ I	I_d/C, $\frac{\mu a.}{mN}$ II	I_d/C, $\frac{\mu a.}{mN}$ III
UO_3	0.001	[a]	...	...		0	...	
		0.5	0.10	...		2	...	
		1.5	0.15	...		5	...	
		2.5	0.19	...		6	...	
		4.5	0.22	...		6.6	...	
		6.0	0.25	...		7.0	...	
UO_3	0.001[b]	[a]	0.11	0.75		7.0	7.0	
		0.5	0.16	0.80		7.0	3.3	
		1.0	0.21	...		7.2	0	
		2.0	0.24	...		7.2	0	
		3.5	0.27	...		7.3	0	
$NH_4UO_2(OAc)_3$	0.001	[a]	...	...		...	...	
		0.5	0.12	0.83		4.0	5.0	
		2.0	0.32	...		8.0	...	
		3.0	0.37	...		10.0	...	
		4.0	0.37	...		10.2	...	
		5.0	0.38	...		10.3	...	
$NH_4UO_2(OAc)_3$	0.001[b]	[a]	0.18	...		6	...	
		0.5	0.22	...		7	...	
		2.0	0.32	...		9.6	...	
		3.0	0.36	...		10.2	...	
		4.0	0.38	...		10.5	...	
$NaUO_2(OAc)_3$	0.001[c]	[a]	0.37	...		10.0	...	
$UO_2(OAc)_2 \cdot 2H_2O$	0.001	[a]	...	...		...	...	
		0.5	...	...		...	...	
		1.5	0.14	...		4.5	...	
		3.0	0.20	...		6.0	...	
		4.0	0.27	...		7.5	...	
		6.0	0.35	...		10.0	...	
		7.0	0.36	...		10.1	...	
UO_2	0.001	[a]	0.12	0.80		4.0	3.9	
		1.0	0.15	0.82		4.0	4.0	
		2.0	0.17	0.83		4.0	4.0	
		3.0	0.19	0.85		4.1	3.9	
UCl_4	0.001	[a]	0.10	0.73	0.94	5.0	5.3	12[d]
		0.5	0.10	0.74	0.95	5.2	5.0	11[d]
		1.0	0.11	0.74	0.95	5.1	5.2	11[d]
UCl_4	0.0025[e]	[a]	0.12	n.r.	...	4.8	...	...
UCl_4	0.0050[e]	[a]	0.11	n.r.	...	4.7	...	...
UCl_4	0.0100[e]	[a]	0.13	n.r.	...	5.0	...	...
UCl_3	0.01[f]	[a]	0.76	...	...	16	...	...
$NaPuO_2(OAc)_3$	0.001	[a]	No waves $-E_{d.p.} = 0.90$				...	...
$NaPuO_2(OAc)_3$	0.001[c]	[a]	No waves $-E_{d.p.} = 0.90$			...	...	...
$Pu(NO_3)_4 \cdot XH_2O$	0.001	[a]	0.45	...		4.1	...	
		1.0	0.46	...		4.2	...	
		2.0	0.45	...		4.2	...	
PuO_2	0.001[g]	[a]	0.47	...		0.5	...	
		1.0	0.47	...		0.5	...	
		2.0	0.48	...		0.5	...	

[a] Run as rapidly as possible in cell after minimum deoxygenating period.
[b] Preaged before polarographic run for 3 hours at 125° C., no mercury present.
[c] Preaged before polarographic run for 6 hours at 125° C., mercury present.
[d] Diffusion current corrected for small solvent decomposition current at these voltages.
[e] Blackening on adding solution to mercury pool cell indicates some reduction of UCl_4 immediately at the higher concns.
[f] Concns. higher than 0.001 wm. (weight molar) formed black deposit too rapidly and could not be run polarographically.
[g] Only a small quantity of PuO_2 dissolved even after 1 hour at 125° C.; thus soln. is satd., not 0.001 wm.
n.r. Not reliable, reduction wave II merges with solvent-supporting electrolyte decomposition wave giving $(E_{1/2})_{II} \simeq 0.9$ volt.

Table III. Concentration Study on Cadmium Salts

Salt	M	$-E_{1/2}$	I_d/C, $\frac{\mu a.}{mN}$
$CdCl_2$	0.001	0.17	8.2
	0.005	0.23	8.2
	0.010	0.26	8.0
$CdCO_3$	0.001	0.19	8.3
	0.005	0.25	8.0
	0.010	0.29	8.4

The results in Tables I and II indicate the relative ease of reduction of the various ionic species investigated under the conditions prevailing in this investigation—namely, in molten ammonium formate containing mercury at 125° C.

That the polarographic system described here can be applied to quantitative analysis is seen by a consideration of results given in Table III. A given ionic species must be stable in the molten ammonium formate system if quantitative results are to be obtained. The excellent solubility properties exhibited by molten ammonium formate for both water-soluble and water-insoluble compounds make it a desirable system for the polarographic determination of a large variety of compounds.

ACKNOWLEDGMENT

Thanks are due J. M. Scarborough and B. J. Corts of this laboratory for preparing some of the compounds used in this investigation. T. J. Biel's help in recording many of the polarograms is greatly appreciated. Work was performed under Contract No. AT(11-1)-74 with the U. S. Atomic Energy Commission.

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RECEIVED for review March 3, 1955. Accepted July 21, 1955.

Polarographic Determination of Phthalic Anhydride in Alkyd Resins

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This work was carried out in order to find a simple and convenient method—not subject to interference from other dibasic acids or nitrocellulose—for the determination of total phthalate as phthalic anhydride in alkyd resins and resin solutions. The method consists of saponifying the sample, dissolving the precipitated potassium phthalate alcoholate in aqueous sulfuric acid, and measuring the diffusion current at a dropping mercury electrode. Phthalic acid yields a well-defined polarographic wave in aqueous sulfuric acid, when tetramethylammonium bromide is the inert electrolyte. The wave height is proportional to the concentration. The diffusion coefficient for phthalic acid in this solvent is 8.6×10^{-6} cm.^{2} sec.$^{-1}$ The method permits determination of phthalate more quickly than by simple precipitation of potassium phthalate alcoholate. Terephthalic, isophthalic, succinic, sebacic, and adipic acids do not interfere. Interference from maleic or fumaric acids or nitrocellulose may be eliminated by a controlled potential electrolysis.

PHTHALIC anhydride in alkyd resins and similar materials is often determined by simple saponification and precipitation of potassium phthalate alcoholate (*1*). The saponification and precipitation of the alcoholate are satisfactory in the absence of interfering materials but if other materials are present which precipitate during the saponification, a procedure based on a more specific determination of phthalate must be used. Because of such interferences—e.g., other dibasic acids or nitrocellulose—the procedure has been extended to include dissolving the potassium phthalate alcoholate in water and (*a*) polarographic determination of the phthalic acid in an acetate buffer (*4*); (*b*) reprecipitation of the phthalate as lead phthalate (*2*); or (*c*) determination of phthalic acid spectrophotometrically (*3*). Nitrocellulose not only precipitates or is carried down during the saponification but also interferes with the lead phthalate precipitation. Furthermore, this procedure takes over 30 hours after the saponification.

The spectrophotometric method yields good results in the absence of maleic, fumaric, and isophthalic acids. In the presence of any one of these acids, the quantity of phthalic acid may be determined by the use of a pair of simultaneous equations in which the two measured quantities are the total absorption at 276 mμ and the weight of the Kappelmeier precipitate. Obviously, if two or more dibasic acids are present, one of which interferes spectrophotometrically, independent determinations are required. Also, there is some doubt about the nature of the Kappelmeier precipitate in the case of maleates (*6*, *7*). When slightly soluble acids—e.g., sebacic—are present, an additional filtration step is required to clarify the solution.

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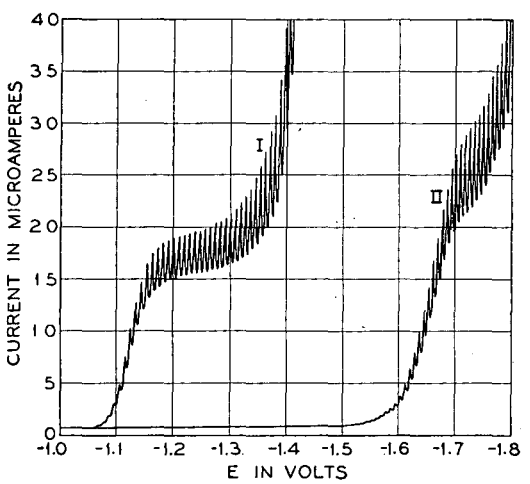

Figure 1. Polarographic waves of potassium acid phthalate

I. In aqueous sulfuric acid
II. In sodium acetate–acetic acid buffer (pH 4.2)

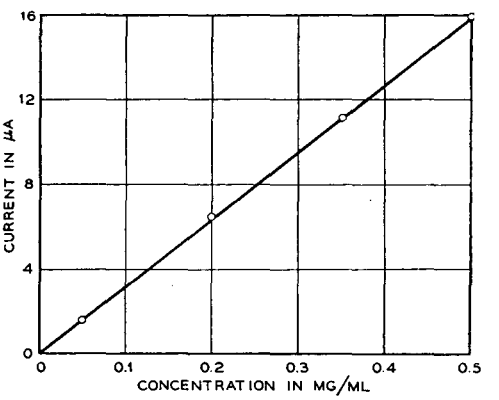

Figure 2. Relation between current and concentration of potassium acid phthalate

Phthalic acid is reducible at the dropping mercury electrode. Furman and Bricker (*4*) carried out a study of the dependence of the position and magnitude of the wave on the pH of buffered and unbuffered solutions. The diffusion current is not easily measurable because of the slope of the plot of *i vs. E*, and because the hydrogen wave follows closely.

Preliminary work showed that phthalic acid produces a simple well-defined wave in an aqueous solution of sulfuric acid with a pH of 1.5 to 1.6, tetramethylammonium bromide being the inert electrolyte. A comparison of the waves obtained in the acetate buffer and in sulfuric acid is given in Figure 1.

The wave height is proportional to the concentration of phthalic acid, as shown in Figure 2. Nitrocellulose does yield a wave at a less negative potential than phthalic acid, as shown in Figure 3, but the wave is often sufficiently well defined in the sulfuric acid system so that the diffusion current of the nitrocellulose may be subtracted from the total diffusion current.

After a method had been developed, certain resin materials containing nitrocellulose were found which yielded waves of comparatively irreproducible wave height. The deviations in the results were as high as about 8% (relative). No chemical reducing agent was found which would eliminate the wave resulting from the nitrate group without interfering in some manner with the phthalic acid wave. For this reason, a controlled potential separation was employed (*5*).

The controlled potential apparatus was assembled from a battery, a potentiometer, and a cell consisting of a beaker, the

necessary electrodes, and a magnetic stirrer. The reference electrode was a silver wire anodized in the electrolyte. The potential between the mercury pool and the silver–silver bromide electrode was measured with a Leeds & Northrup pH meter. The potentiometer was adjusted at frequent intervals so that the reference potential was held between 1.00 and 1.05 volts.

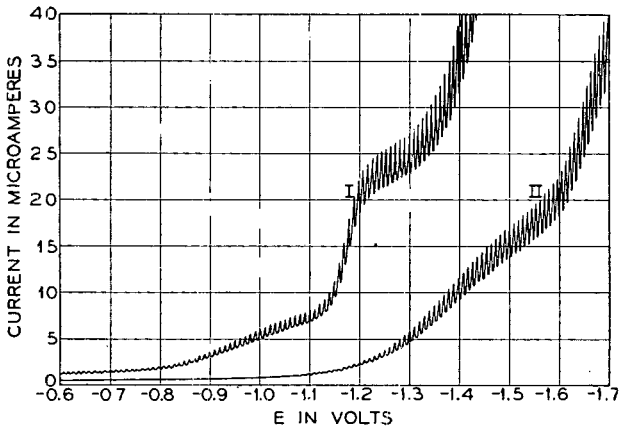

Figure 3. Polarographic waves of phthalic acid and nitrocellulose

I. In aqueous sulfuric acid
II. In sodium acetate–acetic acid buffer (pH 4.2)

For the Bell System needs, this analytical procedure must be adaptable to considerable variation in samples. The method of standard addition is naturally suggested. (The customary procedure using simple calibration should be satisfactory for routine control when samples are similar.)

Table I. Determination of Phthalic Anhydride by Polarographic and Gravimetric Methods

	Phthalic Anhydride, %			
		Found		
Sample	Present	Polarograph	Alcoholate	Lead phthalate
Phthalic acid (99.0% pure)	88.3	88.6	89.0	86.8
	...	88.8	88.8	86.7
General Electric Glyptal 2477	...	23.3	24.6	23.8
2570	...	25.5	26.0	24.3
	...	26.1	26.2	24.3
2462	...	18.6	19.8	18.9
	...	18.0	20.4	18.5
American Cyanamid Rezyl 869	...	24.6	24.8	23.9
	...	24.1	24.9	23.0
Lacquer compounded with nitrocellulose	...	7.0	9.7	7.0
	6.8	6.5	10.2	5.9
		6.8	15.1	7.5
		7.1	14.2	7.7

APPARATUS AND REAGENTS

The polarograph used is a Leeds & Northrup Electro-Chemograph, Type E, with the Leeds & Northrup dropping mercury electrode assembly.

The electrolytic solution is a 0.2*N* tetramethyl ammonium bromide solution in aqueous sulfuric acid with a pH of 1.3 (about 0.05*N*).

The standard solution is a water solution containing 4.00 grams per liter of potassium acid phthalate.

PROCEDURE

1. Weigh a sample, sufficient to contain 0.4 to 0.6 gram of phthalic anhydride, and saponify using the ASTM procedure (*1*). Collect the precipitate, potassium phthalate alcoholate, on a fritted-glass filter and wash as set forth in the procedure.

2. After washing, change the fritted-glass filter to a clean suction flask. Dissolve the precipitate in about 15 ml. of water and draw it through the filter with suction. Rinse the filter with 25 to 40 ml. of water and draw washings through the filter. Transfer the *filtrate to a 100-ml. volumetric* flask and dilute to volume.

3. To each of three 50-ml. volumetric flasks transfer by pipet 25.00 ml. of the sulfuric acid solution. To two of these (A and B), transfer by pipet 5.00 ml. of the sample solution. Sample C is the blank. To one of these (A) transfer by pipet 5.00 ml. of potassium acid phthalate solution. Dilute contents of all three flasks to volume with distilled water.

4. Transfer a portion of sample A to the polarograph cell and degas 10 minutes with nitrogen or hydrogen. Determine diffusion current at 0.02-volt intervals between −1.20 and −1.30 volts *vs.* the mercury pool electrode. Select the potential at which the change in current with voltage is a minimum. Degas samples B and C and determine the diffusion currents at −1.08 volts and the selected potential in each case.

5. Calculate the concentration of phthalic anhydride by the relation

$$\% \text{ phthalic anhydride} = \frac{i_B - i_C}{i_A - i_B} \times \frac{0.400}{\text{sample weight}} \times \frac{100 \times 50}{\times 5} \times \frac{C_8H_4O_3\ (148.1)}{KHC_8H_4O_4\ (204.2)} \times \frac{100}{1000}$$

where i_A, i_B, and i_C are the diffusion currents obtained with the indicated samples at the selected potential. If the diffusion current for sample B at −1.08 volts is significantly larger than that of C, the presence of other dibasic acids or nitrocellulose is indicated. This current is subtracted from the current due to the sample, the first expression in the calculations then becomes $\frac{i_B - i_C - (i_B' - i_C')}{i_A - i_B}$, where i_B' and i_C' are the diffusion currents measured at −1.08 volts *vs.* the mercury pool electrode.

If the results are not satisfactorily reproducible, owing to the presence of nitrocellulose or other dibasic acids, the procedure may be modified at step 3.

Transfer by pipet 10 ml. of the sample solution to a 100-ml. volumetric flask. Transfer to the flask 50.0 ml. of the electrolyte solution. Dilute to volume. Transfer this solution to the controlled potential electrolysis cell. Electrolyze using a stirred mercury cathode at a potential of −1.02 ± 0.02 volt *vs.* a silver–silver bromide electrode. Degas simultaneously using nitrogen or hydrogen previously saturated with water. Continue the electrolysis until the current is constant. Then to each of two 50-ml. volumetric flasks transfer, by pipet, 20 ml. of the electrolyzed solution. Transfer 10.0 ml. of electrolyte solution to each flask. To one of these (A) transfer by pipet 2.00 ml. of the standard solution. To a third 50-ml. volumetric flask (C) transfer by pipet 20.0 ml. of electrolyte solution. Dilute contents of all three flasks to volume with distilled water. Proceed with step 4. There will generally be a current at −1.08 volts sufficient to require the measurement of i_B' and i_C'.

RESULTS

Experimental determinations of phthalic anhydride by the polarographic method, the gravimetric alcoholate method, and the gravimetric lead method are compared in Table I. The purity of the phthalic acid was determined by alkimetric titration. The other samples, except for the lacquer containing nitrocellulose, are commercial resins.

The data show a fairly consistent relation. The results calculated from the alcoholate precipitate are invariably the highest in the absence of nitrocellulose, while the results calculated from the lead phthalate are generally the lowest. The results obtained using phthalic acid as the sample indicate that the polarographic method is the most accurate.

The time required for the determination of phthalic anhydride, after saponification, is about 30 to 40 minutes for the polarographic method, about 1.5 hours for the alcoholate method, and about 2 hours of operator time and several waiting periods (including two overnight periods) for the lead phthalate method. The time required for the spectrophotometric method is less than that for the polarographic determination unless interfering materials are present.

Terephthalic, isophthalic, succinic, sebacic, and adipic acids

show no polarographic reduction wave in the solvent system described. Addition of equal weights of these acids had no effect on the *o*-phthalic acid wave. Fumaric and maleic acids cause a diminution of the height of the phthalic acid wave when present in amounts equivalent to the phthalic acid. When present in smaller quantities—i.e., up to a mole ratio of about 0.5—these acids have no effect on the phthalic acid wave height. The reduction waves from these acids, of course, permit the determination of their concentration, providing only one is present and its identity is known. After such a determination, if the polarographic wave height is more than half that of the phthalic wave, the acid may be reduced by the controlled potential electrolysis and the phthalic acid determined. [If both maleic and fumaric acids are present, another portion of the sample solution from the Kappelmeier precipitate can be taken for simultaneous determination of maleate and fumarate ion concentrations (*8*).]

The reproducibility of the data obtained from the electrolyzed samples was essentially the same as that obtained from the samples containing no nitrocellulose. With a properly designed cell, there is no great loss of time resulting from the electrolysis, since the degassing of the sample is also carried out during the electrolysis.

Some work was carried out using potassium bisulfate–potassium sulfate buffers. There appears to be no change of wave height with pH in the range 0.8 to 1.7. This seems reasonable, as the phthalate is virtually all in the acid form. The half-wave potential and hydrogen wave shift with pH in the usual manner. Control of pH within a few tenths of a pH unit should be sufficient.

The half-wave potential of phthalic acid was determined by the standard method of plotting E *vs.* $\log i/_d - i$, using an H-cell supplied by Leeds & Northrup Co. The half-wave potential is -1.194 volts *vs.* the saturated colomel electrode. The slope of the plot is 0.033 volt, indicating that two electrons are required for the reduction. The flow of mercury from the capillary at a potential of -1.3 volts *vs.* S.C.E. was 2.46×10^{-3} gram per second and the drop time was 2.61 seconds. The diffusion coefficient was calculated as 8.6×10^{-6} sq. cm. per second using the Ilkovič equation. The pH of the sample was 1.61.

ACKNOWLEDGMENT

The authors are grateful to L. M. Towsley and S. M. Vincent for their assistance in this work.

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RECEIVED for review April 13, 1955. Accepted June 25, 1955. Presented before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March 1955.

Ultraviolet Absorption Determination of Nitrogen Dioxide

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The analysis of mixtures containing all the oxides of nitrogen is severely complicated by the high reactivity of nitrogen dioxide and the equilibrium between nitrogen dioxide and nitrogen tetroxide. Existing methods require a long time or extensive equipment. The present report covers the experimental and calculation details necessary to convert a proposal previously made by Frey and Moore into a practical method. In this procedure nitrogen dioxide and the associated nitrogen tetroxide are determined by ultraviolet absorption at 394 mμ, where the other oxides of nitrogen are known to be transparent. The other components are determined by mass spectrometry. As the latter procedure is rather straightforward, this report covers only the ultraviolet determination of nitrogen dioxide and nitrogen tetroxide, wherein the principal difficulties are encountered. The equilibrium relations of Verhoek and Daniels are applied to allow a complete accounting of nitrogen dioxide and nitrogen tetroxide at all times. Because of the reactive nature of the material, special gas-handling equipment and procedures must be used, in which these oxides of nitrogen do not come in contact with either mercury or organic stopcock grease.

THE determination of nitrogen dioxide in gas systems, such as arise from the oxidation of organic materials with nitric acid, is beset with peculiar difficulties, due to the high reactivity of nitrogen dioxide and to the fact that it exists in equilibrium with nitrogen tetroxide and, if nitric oxide is present, in equilibrium with nitrogen trioxide. Johnson (*4*) has described a chemical method whereby both nitric oxide and nitrogen dioxide are determined. This method is fairly time-consuming and does not detect the presence of organic constituents. Friedel and others (*2*) have described a mass spectrometer method for determining all of the constituents. As nitrogen dioxide apparently reacts with minute carbonaceous deposits in the ionization chamber, this method requires a very extensive pretreatment of the mass spectrometer and a frequent reconditioning or checking of instrument response to nitrogen dioxide. The results are good, but the program of conditioning involved makes the method impractical where the mass spectrometer is in constant use on other work.

Frey and Moore (*1*) have proposed a method for a complete determination of all of the oxides of nitrogen plus the other components normally encountered—i.e., hydrogen, carbon dioxide, carbon monoxide, and nitrogen. Their procedure requires four steps: (1) the determination of nitrogen dioxide by ultraviolet absorption techniques; (2) the addition of a few per cent of an inert monatomic gas as an internal standard, preferably neon or argon; (3) the obtaining of a mass spectrum of the sample from step 2; and (4) the obtaining of a mass spectrum of the sample from step 2 after scrubbing over potassium hydroxide. The information from these steps can then be assembled to yield a determination of all components present in the original sample.

The procedure described here, although simple in theory, is not simple in practice because of the high reactivity of nitrogen dioxide and the fact that it exists in equilibrium with nitrogen tetroxide. This report covers the details of technique and cal-

culations necessary to reduce the procedure to a practical and usable method. As the mass spectrometer portion of the method is relatively straightforward, this discussion deals only with the gas handling, ultraviolet absorption, and calculation procedure for the nitrogen dioxide.

EQUILIBRIA CONSIDERATIONS

First consider the equilibrium of the $N_2O_4 \rightleftharpoons 2NO_2$ system.

Let P = sum of the pressure of NO_2 and N_2O_4—i.e., $P = P_1 + P_2$
P_1 = pressure of nitrogen dioxide
P_2 = pressure of nitrogen tetroxide
P_0 = idealized pressure—i.e., all material in nitrogen tetroxide form
K_p = dissociation constant for nitrogen tetroxide
α = degree of dissociation

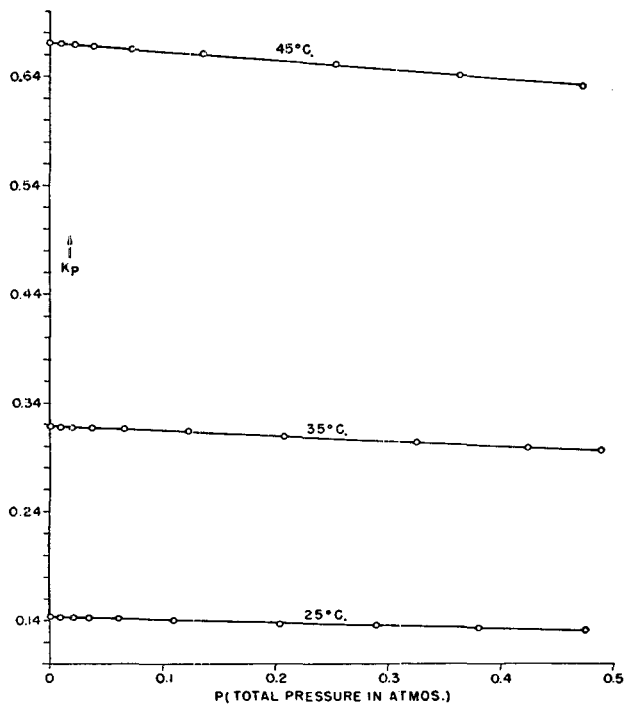

Figure 1. Plot of K_p as determined by relations of Verhoek and Daniels as a function of total pressure of nitrogen dioxide and nitrogen tetroxide

Then simple considerations show that:

$$K_p = 4\,\alpha^2\,P/(1 - \alpha^2) \tag{1}$$

$$\alpha = (P - P_0)/P_0 \tag{2}$$

Verhoek and Daniels (*5*) have shown that K_p varies not only with temperature but also with concentration. The dependence on concentration is relatively slight, whereas K_p varies rapidly with temperature. In the range of pressures employed in the gas analysis operations (generally less than 0.1 atm.) the variation of K_p is small enough to be safely ignored in some cases. However, Verhoek and Daniels have given general relations for K_p in the following equations, which can be used for best results.

$$\left.\begin{aligned} K_p(25^\circ\text{ C.}, C_0) &= 0.1426 - 0.7588\,C_0 \\ K_p(35^\circ\text{ C.}, C_0) &= 0.3183 - 1.591\,C_0 \\ K_p(45^\circ\text{ C.}, C_0) &= 0.6706 - 3.382\,C_0 \end{aligned}\right\} \tag{3}$$

where C_0 represents the concentration of nitrogen dioxide plus nitrogen tetroxide expressed as moles per liter of nitrogen tetroxide equivalent. By interpolation or extrapolation K_p may be derived for any temperature and concentration in the region of interest.

In the development and application of a spectral method for gas analysis there are three phases: calibration, determination of accuracy by the use of synthetically blended samples, and application to gas samples of interest. In this method for determination of nitrogen dioxide particular attention must be paid to the equilibrium changes occurring to the $N_2O_4 \rightleftharpoons 2NO_2$ system with changes in temperature and pressure, so that an accurate and unambiguous accounting is made at all times. In order to do this one must be able to calculate P_1, P_2, and P_0 at all times and evaluate the changes in each as a result of changes of pressure, volume, or temperature. To do this, the following additional equations which may be derived from the above relationships are necessary.

$$\left.\begin{aligned} P &= P_1 + P_2 \\ P_0 &= (P_1/2) + P_2 \end{aligned}\right\} \tag{4}$$

$$P_1 = [-K_p + (K_p^2 + 4K_pP)^{1/2}]/2 \tag{5}$$

$$P_1 = [-K_p + (K_p^2 + 16K_pP_0)^{1/2}]/4 \tag{6}$$

From these equations it is possible to relate K_p to total pressure, P, of nitrogen dioxide and nitrogen tetroxide in a manner practical for direct use. This is done by calculating that P that applies to a particular K_p for a series of values and plotting K_p as dependent on P. To do this for one point a value of C_0 is chosen which through use of Equations 3 evaluates the particular K_p. The same C_0 is converted to P_0 applicable at the particular temperature by the assumption of perfect gas behavior. This P_0 is then used in Equations 4 and 6 to evaluate the P that applies to the above K_p. When this is done it is found that for pressures up to 0.5 atm., which is beyond the pressures utilized in this method, K_p varies essentially linearly with P for each of the three temperatures of 25°, 35°, and 45° C. The results of these calculations are shown in Figure 1. The circles represent points determined by the calculation steps outlined above. These relations may alternatively be represented by the following equations:

$$\left.\begin{aligned} K_p(25^\circ\text{ C.}, P) &= 0.1426 - 0.0248P \\ K_p(35^\circ\text{ C.}, P) &= 0.3183 - 0.0462P \\ K_p(45^\circ\text{ C.}, P) &= 0.6706 - 0.0864P \end{aligned}\right\} \tag{7}$$

Interpolation or extrapolation may also be used with these relations to obtain K_p as a function of any desired temperature and total pressure in the region of interest.

In the range of partial pressures utilized in this procedure one can safely ignore the equilibrium

$$NO + NO_2 \rightleftharpoons N_2O_3$$

Verhoek and Daniels (*5*) showed that for temperatures near ambient, K_g would have a value of the order of 2 atm. K_g is defined by:

$$K_g = (P_1 \times P_3)/P_4$$

where P_3 and P_4 are the pressures in atmospheres of nitric oxide and nitrogen trioxide, respectively. With the values of P_1 and P_3 normally encountered in this method—i.e., substantially less than 0.1 atm.—P_4 can be neglected.

CALIBRATION

The absorption cell compartment is thermostated to maintain a constant temperature of 25° C. Unless a special gas-handling system is built to allow the absorption cell to be filled in place concomitant with a measurement of total pressure, it will be necessary to derive P_1 (25° C.) from total pressure of nitrogen dioxide and nitrogen tetroxide and temperature data representing the conditions of cell charging. From these K_p can be evaluated as applies to the charging conditions by use of Equations 7. This K_p can be used with the observed charging pressure to evaluate P_1 for the cell charging conditions by use of Equation 5. This value of P_1 then may be used with Equations 4 to evaluate

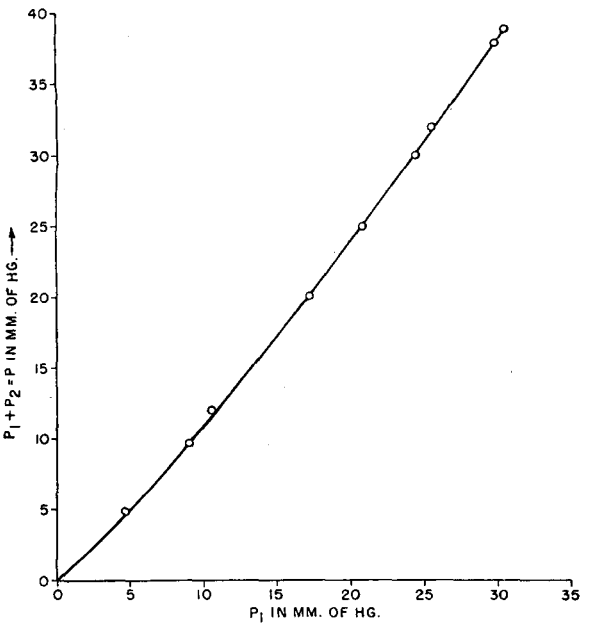

Figure 2. Total pressure, P, of nitrogen dioxide plus nitrogen tetroxide as a function of P_1

P_2 and P_0 as they apply to the charging conditions. From the latter of these C_0 can be determined, and as it will not change with temperature it may be applied in Equations 3 to determine K_p and P_0 applicable to the conditions at 25° C. in the instrument. Using the latter in Equation 6, P_1 may then be evaluated as it exists in the cell at the time of the absorbance measurement. This datum and the observed absorbance are, of course, the primary quantities used in establishing one point of an absorbance calibration.

CALCULATIONS FOR SYNTHETIC BLENDS AND SERVICE SAMPLES

In applying this method it is advisable for each laboratory to check the accuracy of the entire experimental and calculation procedures by the use of synthetic samples, blended to contain all components expected in service samples. In blending complex mixtures it is frequently necessary to blend the minor constituents in a preliminary stage at relatively high pressures in the mixing volume. This is followed by a reduction of pressure by removing a portion of the preliminary blend, after which the major components are added to the mixing volume. Needless to say, the accurate accounting of partial pressures and composition percentages is complicated by the $N_2O_4 \rightleftharpoons 2NO_2$ equilibria. For this reason it is convenient to express the component concentrations in each step as C_0—i.e., moles per liter of undissociated nitrogen tetroxide equivalent. This may be done by steps similar to those outlined above and it establishes an unambiguous concentration to which the optically determined value may be compared.

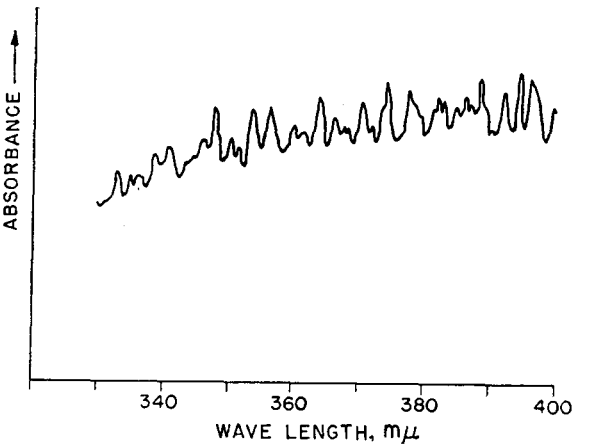

Figure 3. Absorption spectrum of nitrogen dioxide–nitrogen tetroxide mixture in 330 to 400 mμ region

In analyzing the synthetically blended sample the observed quantity is the absorbance, which can be translated into P_1 as exists in the absorption cell at 25° C. To relate this to concentration in the sample as blended requires that P_2 be calculated. As both K_p and P_2 are unknown, it requires laborious algebraic calculations using the relations above. It is far simpler to construct in advance a curve of total pressure, P, of nitrogen dioxide and nitrogen tetroxide versus P_1 for 25° C. This is easily done by choosing a series of P values from which the corresponding P_1 values may be calculated by the relations given above. This curve is shown in Figure 2. In use the P_1 value is optically determined and then used with the curve to determine P. With this information it is simple to calculate C_0 for comparison with the blended value. The same procedures for establishing C_0 apply to service samples.

EXPERIMENTAL DETAILS

Spectral. Hall and Blacet (*3*) have studied the ultraviolet and visible absorption spectra of nitrogen dioxide and nitrogen tetroxide. Any spectrum directly obtained is a superposition of the spectra for the two forms. By using the relations of Verhoek and Daniels (*5*) and by assuming the validity of Beer's law of absorption, Hall and Blacet were able to calculate from the spectral behavior of $N_2O_4 \rightleftharpoons 2NO_2$ system the separate spectra of nitrogen dioxide and nitrogen tetroxide. Their calculations showed that nitrogen tetroxide is transparent for wave lengths longer than about 390 mμ, whereas nitrogen dioxide absorbs heavily at 390 mμ and as far as 500 mμ in the visible. Thus, absorbances observed past about 390 mμ for any mixture may be assigned to the nitrogen dioxide component. The wave length chosen for the present method was 394 mμ. In conjunction with the determination of nitrogen dioxide at 394 mμ, the total pressure of nitrogen dioxide and nitrogen tetroxide may be estimated at the isobestic point around 353 mμ. Nitrogen tetroxide alone

may be estimated around 240 mμ. These additional data will not eliminate the previous calculations but will serve as a check on the calculation procedure.

Figure 3 shows the spectrum of a mixture between 330 and 400 mμ. As the spectrum contains a great deal of fine structure and the individual peaks are sharp, it is necessary to make accurate wave-length settings. Special tests were made to ensure that there were no pressure-broadening effects such as are experienced for the fine structure of infrared bands of the materials of low molecular weight. These tests comprised the examination of a series of blends, each containing the same amount of nitrogen dioxide and nitrogen tetroxide but containing varying partial pressures of the other nonabsorbing gases expected to be present in this type of sample. No pressure-broadening effects were observed.

The instrument employed in this work was a Cary recording spectrophotometer equipped with a constant temperature cell compartment, the temperature of which was controlled by circulating water from a temperature-controlled water bath. A 5-cm. quartz absorption cell was used (available from the American Instrument Co., Silver Spring, Md.). A stopcock with a male standard-taper joint was joined to the cell through a graded seal.

In Figure 4 may be seen the calibration curve of absorbance versus partial pressure of nitrogen dioxide—i.e., P_1 over the range of 0 to 35 mm. of mercury, which is the usable range for the analytical wave length. Beer's law is obeyed satisfactorily throughout the range.

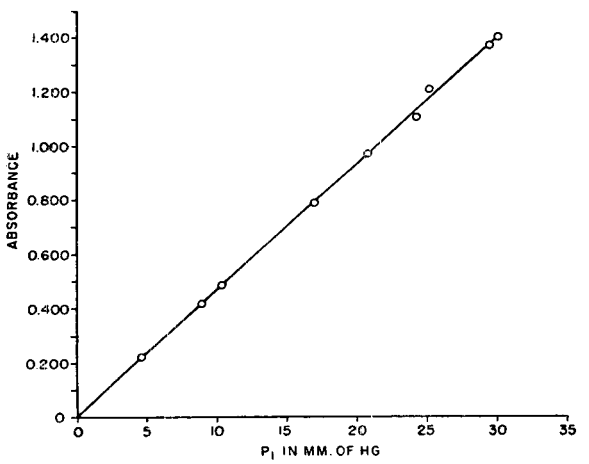

Figure 4. Absorbance of nitrogen dioxide at 394 mμ *vs.* P_1

Gas Handling. The $N_2O_4 \rightleftharpoons 2NO_2$ system is very reactive and mixtures containing it cannot be handled in ordinary gas-handling systems wherein contact is made with mercury and/or organic stopcock grease. High vacuum silicone stopcock grease was found to be satisfactory. The reaction with mercury complicates the gas handling, and the usual manometric and gas displacement techniques cannot be used.

It was decided to use a manometer wherein the nitrogen oxides would come in contact with a fluid having a low vapor pressure and in which the oxides would have a very low solubility. Four fluids were tried: Dow Corning silicone fluid, dibutyl phthalate, hexachlorobutadiene, and 1-bromonaphthalene. The first three were unsatisfactory because of the solubility of the nitrogen oxides. The 1-bromonaphthalene was found satisfactory and was used as a 0.5-inch layer over the mercury in an ordinary manometer. This works well if the 1-bromonaphthalene is not exposed to the nitrogen oxides for an excessive time. The manometer readings were corrected for the layer of the 1-bromonaphthalene. The vapor pressure of this material is low enough that vapors from it do not produce optical interference at the analytical wave length used in this analysis. The gas-handling system used no rubber or Tygon connections and was equipped with a female standard-taper joint to receive the standard-taper joint of the absorption cell directly.

The gas blends were mixed in a constant volume bulb. The nitrogen dioxide (in equilibrium with nitrogen tetroxide) was always admitted first and the concentration determined by a pressure determination. The other gases were then admitted by displacing them from a calibrated gas buret. The volume of the mixing bulb was known, which allowed the latter gas volumes to be converted to pressure readings. The mixtures were blended to contain nitrogen dioxide, nitric oxide, nitrous oxide, carbon dioxide, carbon monoxide. oxygen, hydrogen, nitrogen, and argon in varying amounts.

Table I. Nitrogen Tetroxide Equivalent of Synthetically Blended Samples

Sample No.	C_0, Mole per Liter Blended	C_0, Mole per Liter Determined	% Deviation	% NO_2 plus N_2O_4 in Original Sample
1	7.37×10^{-4}	6.46×10^{-4}	12.3	3.3
2	1.28×10^{-4}	1.39×10^{-4}	8.1	0.6
3	1.88×10^{-4}	2.04×10^{-4}	9.0	0.9
4	5.63×10^{-3}	6.00×10^{-3}	6.5	23.0
5	2.64×10^{-4}	2.74×10^{-4}	3.6	1.2
6	1.35×10^{-3}	1.48×10^{-3}	9.9	6.0
7	2.62×10^{-3}	2.73×10^{-3}	4.2	11.7

Standard deviation = 8.8%

The same gas-handling and manometer system was used for charging the absorption cell in service analyses as was used for the blending work.

Accuracy of Analysis. The method was evaluated by the analysis of a series of synthetically blended samples. Table I gives the blended concentrations and optically determined concentrations in terms of moles per liter of nitrogen tetroxide equivalent. The last column gives the percentage of nitrogen dioxide plus nitrogen tetroxide in the samples if calculated directly on the basis of total partial pressures. This information is included to demonstrate the range of concentration utilized in the synthetic blends.

The standard deviation for these analyses, based on total amount present, is approximately 9%. This is less accurate than many spectrophotometric gas analyses. This fairly large standard deviation is believed to be attributable to the highly reactive nature of the material and to the difficulty of accurately accounting for the exact degree of dissociation at all times. More refined techniques, both experimental and in the calculations, could improve the accuracy but at the cost of increased complexity of operations.

ACKNOWLEDGMENT

The authors are greatly indebted to N. D. Coggeshall for many helpful suggestions.

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RECEIVED for review April 2, 1955. Accepted June 21, 1955.

X-Ray Diffraction Powder Data for the Steroids

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X-ray diffraction powder data and powder diffraction photographs for 39 steroids are presented.

SINCE the publication of the previous paper (*3*) on x-ray diffraction data for steroids, a number of additional compounds have become available for study. The data and diffraction patterns obtained are here presented.

The procedure followed was identical with that outlined in the previous paper (*3*) with the exception of the method of determining relative intensities. The relative intensities, which are presented with these 39 patterns, were measured using a technique of graded intensity scale comparison, developed by Hanawalt, Rinn, and Frevel (*1*) and modified by Klug and Alexander (*2*).

Table I. Index to X-Ray Diffraction Powder Data on Steroids

Pattern No.	Name	M.P. (Uncor.), ° C.
	I. C_{19-29} Steroids	
	A. Monochloro	
A1	3(β)-Chloro-Δ⁵-cholestene (cholesteryl chloride)	92 –94
	B. Monohydric Alcohols	
B1	3(β)-Cholestane-3-acetate (dihydrocholesterol acetate)	108 –109
B2	22,23-Dihydrostigmasterol-3-acetate (β-sitosterol acetate)	125 –127
B3	Stigmasta-Δ⁵,²²-dien-3(β)-ol-3-acetate (stigmasterol acetate)	140 –141.5
B4	Δ⁵-Cholestene-3-ol-3-acetate (cholesterol acetate)	115 –116
	C. Dihydric Alcohols	
C1	Pregnane-3(α),20(α)-diol	237 –239
C2	Pregnane-3(α).20(β)-diol	232.5–235
C3	17(α)-Methyl-Δ⁵-androstene-3(β),17(β)-diol (mestenediol)	204 –205
	D. Monoketones	
D1	Cholestane-3-one (cholestanone)	128 –129
	E. Diketones	
E1	Pregnane-3,20-dione (pregnanedione)	117 –120
	F. Monohydroxymonoketones	
F1	Allopregnane-3(β)-ol-20-one (allopregnanolone)	192 –195
F2	Δ⁵-Androstene-3(β)-ol-17-one-3 methyl ether (dehydroepiandrosterone methyl ether)	137.5–140.5
F3	Δ⁴-Androstene-17(β)-ol-3-one-17-propionate (testosterone propionate)	117.5–120
F4	Δ⁵,¹⁶-Pregnadiene-3(β)-ol-20-one-3-acetate (16-dehydropregnenolone acetate)	173 –175
	G. Dihydroxymonoketones	
G1	Δ⁵-Pregnene-3(β),21-diol-20-one-3,21-diacetate (21-acetoxypregnenolone acetate)	163 –165
G2	16,17-Oxido-Δ⁵-pregnene-3(β),21-diol-20-one-21-acetate (16,17-epoxy-21-acetoxypregnenolone)	185 –189
G3	Δ⁵-Pregnene-3(β),17-diol-20-one (17-hydroxypregnenolone)	272 –276
	H. Trihydroxymonoketones	
H1	Δ⁴-Pregnene-17a,20b,21-triol-3-one-20b,21-diacetate (17a-pregnentriolone diacetate)	188 –190.5
	I. Tetrahydroxymonoketones	
I1	3(α),17(α),11(β),21-Tetrol-pregnane-20-one (tetrahydrocortisone)	216 –218
	J. Monohydroxydiketones	
J1	16,17-Oxido-Δ⁴-pregnene-3,20-dione-21-ol-21-acetate (16,17-epoxydeoxycorticosterone acetate)	166 –168
J2	Δ⁴-Pregnene-3,20-dione-21-ol-21-acetate (deoxycorticosterone acetate)	155 –157
J3	Allopregnane-21-ol-3,20-dione-21-acetate (21-acetoxyallopregnanedione)	194 –196
J4	Δ⁴-Pregnene-3,20-dione-21-ol (deoxycorticosterone)	138 –141
J5	Δ⁴-Pregnene-3,20-dione-17-ol (17-hydroxyprogesterone)	215 –217
	K. Dihydroxydiketones	
K1	Δ⁴-Pregnene-17(α),21-diol-3,20-dione [Reichstein's Substance S (alcohol)]	207 –210
K2	Δ⁴-Pregnene-17(α),21-diol-3,20-dione-21-acetate [Reichstein's Substance S (acetate)]	235 –237.5
K3	Pregnane-17(α),21-diol-3,20-dione-21-acetate	195.5–197.5
	L. Dihydroxytriketones	
L1	Δ⁴-Pregnene-3,11,20-trione-17(α),21-diol (cortisone)	222 –225
L2	Δ⁴-Pregnene-17(α),21-diol-3,11,20-trione-21-acetate (cortisone acetate)	236 –240
	II. Bile Acids	
M1	Hyodeoxycholic acid	
M2	Lithocholic acid (3α-hydroxycholanic acid)	185 –187
	III. Steroid Sapogenins and Derivatives	
N1	Sarsasapogenin	198 –201
N2	Diosgenin acetate	195 –197
N3	Cholesterol digitonide	...
N4	β-Sitosterol digitonide	...
N5	Δ⁵-Androstene benzoate digitonide	...
	IV. Digitalis Glycosides	
O1	Gitoxin	Dec. 282
O2	Digoxin	260 –265
O3	Digitoxin	245 –249

Table II. X-Ray Diffraction Powder Data

d, A.	I/I_1	*d*, A.	I/I_1	*d*, A.	I/I_1
		I. C_{19-29} Steroids			
		A. Monochloro			
		A1. 3(β)-Chloro-Δ⁵-cholestene			
15.4	0.13	4.32	0.20	2.78	0.03
10.5	0.53	3.95	0.30	2.58	0.02
7.90	0.13	3.78	0.07	2.49	0.07
6.89	0.20	3.54	0.30	2.27	0.13
6.13	0.40	3.21	0.03	2.16	0.07
5.49	1.00	3.07	0.03	2.05	0.13
4.66	0.53	2.94	0.03	1.93	0.02
		B. Monohydric Alcohols			
B1. 3(β)-Cholestane-3-acetate		B2. 22,23-Dihydrostigmasterol-3-acetate		B 3. *Contd.*	
16.7	0.20	12.8	0.13	5.10	0.13
10.0	0.15	6.40	0.07	4.87	1.00
9.02	0.07	6.07	1.00	4.66	0.20
7.47	0.15	5.74	0.20	4.30	0.13
6.81	0.07	5.42	0.02	4.12	0.13
6.09	1.00	5.10	0.53	3.98	0.13
5.72	0.50	4.86	0.40	3.87	0.13
5.31	0.27	4.16	0.02	3.78	0.13
4.89	0.27	3.75	0.20	3.74	0.27
4.68	0.37	3.51	0.02	3.59	0.13
4.47	0.27	3.10	0.20	3.38	0.13
4.18	0.37	3.02	0.13	3.26	0.13
3.81	0.20	2.94	0.07	3.10	0.13
3.55	0.20	2.60	0.02	3.02	0.01
3.28	0.15	2.43	0.03	2.94	0.01
3.07	0.20	2.14	0.07	2.78	0.01
2.89	0.20	2.02	0.07	2.57	0.13
2.79	0.10	1.94	0.02	2.50	0.13
2.68	0.15			2.39	0.13
2.51	0.15	B3. Stigmasta-Δ⁵,²²-dien-3(β)-ol-3-acetate		2.34	0.13
2.41	0.10			2.28	0.20
2.26	0.20	16.5	0.04	2.21	0.13
2.20	0.15	10.2	0.04	2.14	0.01
2.04	0.15	9.21	0.20	2.09	0.20
1.90	0.15	7.60	0.04	2.00	0.01
1.83	0.03	7.03	0.49	1.94	0.01
1.77	0.01	6.19	0.36	1.88	0.02
1.72	0.01	5.75	0.13	1.82	0.02
1.59	0.01	5.44	0.13	1.78	

(*Continued on page 1571*)

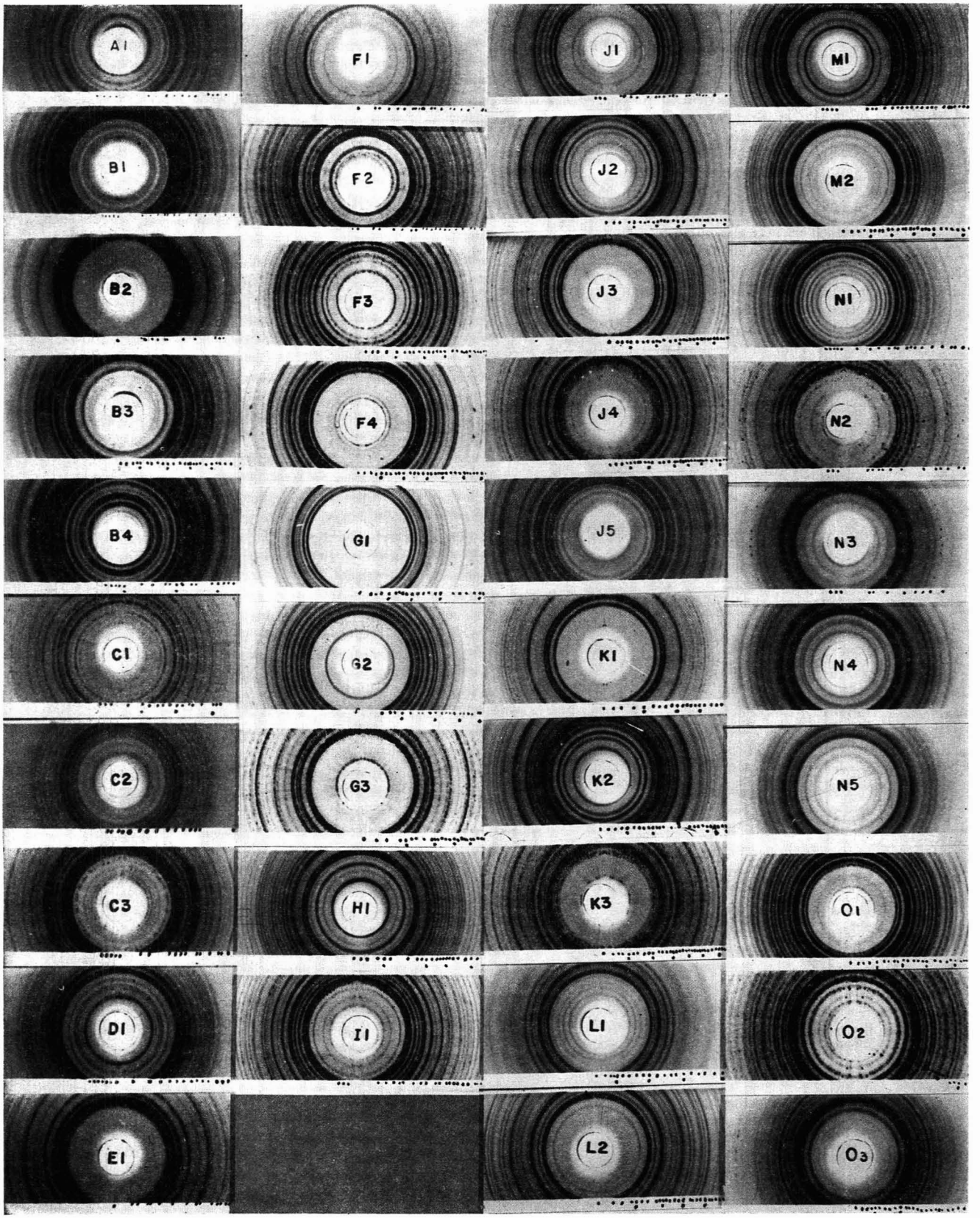

Figure 1. X-ray diffraction powder patterns of steroids

Key found in Table

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

B4. Δ^5-Cholestene-3-ol-3-acetate

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
16.36	0.10	4.13	0.27	2.30	0.15
13.81	0.10	3.70	0.37	2.25	0.15
10.40	0.50	3.53	0.27	2.20	0.15
8.23	0.50	3.12	0.20	2.14	0.15
7.06	0.15	2.97	0.15	2.06	0.15
6.17	0.27	2.88	0.10	2.00	0.01
5.42	1.00	2.75	0.10	1.97	0.01
4.77	0.75	2.65	0.10	1.89	0.20
4.56	0.15	2.47	0.20	1.80	0.07
4.33	0.27	2.39	0.20	1.74	0.07

C. Dihydric Alcohols

C1. Pregnane-3(α),20(α)-diol

d, A.	I/I_1
12.3	0.38
9.46	0.38
7.83	0.38
7.03	0.75
6.33	0.25
5.79	1.00
5.35	0.25
5.08	1.00
4.68	0.75
4.42	0.03
4.12	0.56
3.93	0.03
3.79	0.38
3.46	0.38
3.17	0.06
3.08	0.03
2.95	0.03
2.55	0.25
2.48	0.25
2.41	0.25
2.30	0.03
2.15	0.38
2.06	0.03
2.01	0.03
1.97	0.13

C2. Pregnane-3(α),-20(β)-diol

d, A.	I/I_1
11.6	0.55
10.5	0.05
9.07	0.27
8.39	0.55
7.17	0.55
6.44	0.55
5.83	0.73
5.32	1.00
4.87	0.41
4.49	0.41
4.14	0.41
3.92	0.41
3.69	0.41
3.68	0.05
2.71	0.02
2.52	0.02
2.35	0.02
2.21	0.02

C3. 17(α)-Methyl-Δ^5-androstene-3(β),17(β)-diol

d, A.	I/I_1
12.1	0.13
10.5	0.13
9.46	0.03
8.47	0.07
7.09	0.30
6.37	0.53
5.75	1.00
5.38	0.53
4.85	0.40
4.53	0.30
4.32	0.40
4.15	0.30
3.78	0.20
3.56	0.30
3.29	0.30
3.11	0.03
2.99	0.03
2.84	0.40
2.70	0.02
2.53	0.02
2.43	0.03
2.16	0.02
2.08	0.03

D. Monoketones

D1. Cholestane-3-one

d, A.	I/I_1
16.7	0.13
12.7	0.13
11.3	0.01
9.66	0.27
8.59	0.09
7.87	0.13
7.34	0.13
6.49	0.13
5.54	1.00
4.95	0.67
4.56	0.27
4.21	0.20
3.92	0.20
3.68	0.13
3.51	0.13
3.37	0.20
3.23	0.20
3.08	0.01
3.00	0.01
2.90	0.01
2.54	0.13
2.45	0.04
2.38	0.04
2.30	0.04
2.22	0.02
2.09	0.01
1.99	0.01

E. Diketones

E1. Pregnane-3,20-dione

d, A.	I/I_1
10.3	0.20
6.56	0.20
6.28	1.00
5.79	0.73
5.56	0.73
5.11	0.53
4.46	0.53
3.91	0.20
3.53	0.40
3.40	0.13
3.13	0.07
2.97	0.13
2.88	0.07
2.77	0.07
2.67	0.07
2.53	0.03
2.43	0.07
2.39	0.03
2.23	0.07
2.16	0.13
2.10	0.03
2.01	0.03
1.96	0.13

F. Monohydroxymonoketones

F1. Allopregnane-3(β)-ol-20-one

d, A.	I/I_1
8.59	0.30
6.66	0.30
6.33	0.40
5.70	1.00
5.49	0.30
5.15	0.02
4.89	0.53
4.65	0.02
4.44	0.02
4.21	0.53
4.10	0.30
3.80	0.02
3.68	0.02
3.49	0.40
3.29	0.13
3.19	0.03
2.99	0.03
2.77	0.03
2.44	0.30
2.38	0.30
2.25	0.07
2.18	0.30

F2. Δ^5-Androstene-3(β)-ol-17-one-3 methyl ether

d, A.	I/I_1
11.2	0.11
9.35	0.20
7.83	0.01—
6.76	0.53
6.26	1.00
5.74	0.11
5.20	0.20
4.66	0.20
4.42	0.53
4.23	0.11
4.08	0.11
3.88	0.08
3.70	0.08
3.58	0.11
3.39	0.15
3.28	0.11
3.09	0.15
2.92	0.11
2.78	0.01—
2.69	0.08
2.55	0.08
2.41	0.11
2.32	0.01—
2.22	0.15
2.10	0.01
2.07	0.01
2.01	0.08
1.91	0.15
1.81	0.01
1.79	0.01
1.76	0.01—
1.70	0.01—
1.65	0.01—
1.63	0.01
1.57	0.01—
1.50	0.01—

F3. Δ^4-Androstene-17(β)-ol-3-one-17-propionate

d, A.	I/I_1
10.8	0.36
7.94	0.27
7.14	0.36
6.09	1.00
5.49	0.67
5.08	0.13
4.84	0.04
4.71	0.67
4.40	0.27
4.22	0.04
3.97	0.27
3.87	0.27
3.73	0.01
3.60	0.27
3.45	0.27
3.24	0.09
3.11	0.09
2.95	0.27
2.87	0.13
2.80	0.13
2.73	0.09
2.68	0.09
2.60	0.09
2.55	0.09
2.50	0.02
2.45	0.04
2.41	0.04
2.37	0.04
2.28	0.27
2.22	0.02
2.15	0.27
2.07	0.04
2.01	0.02
1.98	0.02
1.94	0.02

F4. $\Delta^{5,16}$-Pregnadiene-3(β)-ol-20-one-3-acetate

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
19.0	0.55	3.58	0.09	2.23	0.18
11.6	0.41	3.49	0.09	2.17	0.18
9.36	0.05	3.41	0.55	2.13	0.02
6.89	0.09	3.34	0.41	2.07	0.09
6.33	0.73	3.16	0.09	2.01	0.09
6.17	1.00	3.07	0.09	1.96	0.02
5.77	0.73	3.01	0.09	1.93	0.18
5.50	0.73	2.88	0.09	1.89	0.09
5.35	0.73	2.77	0.09	1.85	0.09
5.05	0.73	2.73	0.09	1.77	0.09
4.67	0.55	2.66	0.18	1.74	0.02
4.52	0.09	2.59	0.02	1.71	0.02
4.33	0.73	2.50	0.02	1.66	0.02
4.13	0.18	2.43	0.02	1.62	0.02
3.94	0.55	2.36	0.09		
3.85	0.09	2.31	0.18		
3.69	0.18	2.28	0.18		

G. Dihydroxymonoketones

G1. Δ^5-Pregnene-3(β),21-diol-20-one 3,21-diacetate

d, A.	I/I_1
15.5	0.13
7.83	0.07
6.61	0.02
6.09	0.73
5.64	0.53
5.33	0.30
5.19	0.30
5.00	0.13
4.75	1.00
4.45	0.13
4.28	0.07
3.90	0.40
3.76	0.40
3.56	0.40
3.40	0.07
3.03	0.30
2.89	0.30
2.74	0.13
2.60	0.07
2.51	0.07
2.41	0.02
2.36	0.07
2.28	0.07
2.21	0.07
2.15	0.02
2.08	0.07
2.11	0.07
2.01	0.02
1.93	0.02
1.84	0.02

G2. 16,17-Oxido-Δ^5-pregnene-3(β),21-diol-20-one-21-acetate

d, A.	I/I_1
9.35	0.73
6.15	1.00

G2. (*Contd.*)

d, A.	I/I_1
5.67	0.30
5.37	0.53
5.04	0.53
4.65	0.40
4.29	0.53
4.10	0.53
3.92	0.40
3.56	0.02
3.37	0.53
3.27	0.02
3.19	0.02
3.08	0.30
2.92	0.02
2.83	0.07
2.75	0.13
2.70	0.13
2.58	0.02
2.54	0.02
2.39	0.07
2.33	0.07
2.18	0.13
2.04	0.07
1.96	0.02
1.82	0.02

G3. Δ^5-Pregnene-3(β),17-diol-20-one

d, A.	I/I_1
12.1	0.27
6.01	1.00
5.83	1.00
5.06	0.75
4.85	0.27
4.62	0.27
4.30	0.75
4.08	0.20
3.75	0.10

G3. (*Contd.*)

d, A.	I/I_1
3.62	0.20
3.52	0.10
3.40	0.15
3.31	0.15
3.17	0.15
3.08	0.15
3.00	0.27
2.92	0.15
2.86	0.15
2.82	0.15
2.74	0.10
2.68	0.15
2.57	0.03
2.47	0.03
2.42	0.03
2.38	0.07
2.36	0.03
2.30	0.07
2.28	0.03
2.21	0.03
2.13	0.01
2.08	0.20
2.04	0.20
2.01	0.01
1.99	0.20
1.95	0.20
1.92	0.01
1.90	0.01
1.88	0.01
1.85	0.03
1.83	0.03
1.74	0.03
1.68	0.03
1.64	0.02
1.57	0.01
1.54	0.01
1.52	0.03
1.47	0.01
1.45	0.01

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

H. Trihydroxymonoketones

H1. Δ⁴-Pregnene-17a,20b,21-triol-3-one-20b,21-diacetate

d, A.	I/I₁	d, A.	I/I₁
11.19	0.50	3.14	0.15
8.55	0.20	3.06	0.15
7.14	0.27	2.92	0.09
6.28	0.10	2.81	0.10
5.79	1.00	2.70	0.10
5.50	0.27	2.60	0.09
4.65	0.50	2.40	0.15
4.28	0.20	2.21	0.09
4.08	0.20	2.15	0.09
3.87	0.15	2.08	0.10
3.72	0.15	2.01	0.04
3.58	0.15	1.92	0.04
3.45	0.10	1.83	0.02
3.32	0.10	1.76	0.01
3.24	0.15		

I. Tetrahydroxymonoketones

I1. 3(α),17(α),11(β),21-Tetrol-pregnane-20-one

d, A.	I/I₁	d, A.	I/I₁
11.9	0.30	2.89	0.13
9.45	0.20	2.82	0.02
7.40	0.30	2.76	0.13
6.51	0.40	2.67	0.13
6.22	0.40	2.55	0.07
5.97	1.00	2.50	0.03
5.47	0.73	2.44	0.40
5.14	0.53	2.30	0.40
4.85	0.40	2.24	0.13
4.69	0.73	2.23	0.02
4.27	0.73	2.16	0.13
4.03	0.02	2.11	0.13
3.74	0.73	2.06	0.07
3.50	0.07	1.98	0.13
3.37	0.20	1.93	0.02
3.24	0.02	1.89	0.02
3.10	0.20	1.85	0.02
2.98	0.13	1.81	0.07

J. Monohydroxydiketones

J1. 16,17-Oxido-Δ⁴-pregnene-3,20-dione-21-ol-21-acetate

d, A.	I/I₁
13.6	0.13
11.4	0.13
9.12	0.20
7.28	0.03
6.66	0.03
6.07	1.00
5.63	0.73
5.15	0.53
4.72	0.53
4.55	0.03
4.33	0.53
4.21	0.07
4.06	0.03
3.92	0.02
3.77	0.03
3.63	0.13
3.49	0.20
3.43	0.07
3.33	0.07
3.27	0.07
3.14	0.02
2.95	0.02
2.76	0.40
2.57	0.13
2.46	0.07
2.41	0.03
2.37	0.03
2.25	0.13
2.18	0.13
2.13	0.13
1.98	0.02
1.91	0.13
1.76	0.02

J2. Δ⁴-Pregnene-3,20-dione-21-ol-21-acetate

d, A.	I/I₁
11.4	0.13
8.23	0.13
7.23	0.27
6.23	0.27
5.89	0.04
5.59	0.67
5.33	1.00
5.11	0.27
4.90	0.27
4.62	0.27
4.50	0.02
4.37	0.27
4.20	0.36
3.98	0.02
3.81	0.09
3.60	0.36
3.38	0.01
3.24	0.20
3.15	0.20
3.00	0.20
2.93	0.20
2.81	0.20
2.74	0.20
2.65	0.01
2.57	0.20
2.48	0.20
2.43	0.20
2.35	0.20
2.30	0.01
2.26	0.20
2.21	0.20
2.16	0.02
2.01	0.13
1.98	0.01
1.84	0.01

J3. Allopregnane-21-ol-3,20-dione-21-acetate

d, A.	I/I₁
7.00	0.07
6.68	0.07
6.56	1.00
6.33	0.40
6.19	0.40
5.79	0.30
5.22	1.00
5.10	0.30
4.94	0.02
4.68	1.00
4.59	0.02
4.39	0.13
4.13	0.02
3.87	0.30
3.75	0.20
3.63	0.20
3.54	0.13
3.43	0.30
3.33	0.40
3.24	0.07
3.09	0.03
3.04	0.03
2.98	0.03
2.94	0.03
2.82	0.30
2.72	0.30
2.65	0.30
2.61	0.02
2.56	0.20
2.52	0.20
2.43	0.02
2.38	0.02
2.25	0.02
2.20	0.02
2.18	0.30
2.15	0.13
2.12	0.07
2.08	0.07
2.04	0.07
2.00	0.13
1.96	0.02
1.87	0.13
1.77	0.03
1.66	0.13

J. Monyhydroxydiketone (*Contd.*)

J4. Δ⁴-Pregnene-3,20-dione-21-ol

d, A.	I/I₁
8.43	0.30
7.69	0.20
7.37	0.02
6.97	0.30
6.71	0.30
6.54	0.30
6.26	0.53
5.89	0.73
5.33	0.02
5.17	1.00
5.02	0.02
4.65	0.53
4.48	0.30
4.20	0.20
4.15	0.40
3.77	0.40
3.71	0.53
3.10	0.30
2.96	0.30
2.87	0.13
2.80	0.13
2.37	0.13
2.33	0.13
2.25	0.20
2.17	0.13
2.11	0.07
1.99	0.20
1.80	0.02

J5. Δ⁴-Pregnene-3,20-dione-17-ol

d, A.	I/I₁
11.7	0.06
11.4	0.08
6.54	0.08
5.91	1.00
5.45	0.20
4.90	0.30
4.54	0.20
4.19	0.06
4.10	0.20
3.92	0.06
3.63	0.20
3.48	0.04
3.36	0.04
3.26	0.06
3.12	0.11
3.04	0.01
2.84	0.06
2.66	0.11
2.52	0.11
2.46	0.04
2.34	0.11
2.31	0.11
2.22	0.03
2.10	0.08
2.01	0.03
1.96	0.08
1.91	0.01
1.86	0.08
1.81	0.01
1.75	0.01
1.64	0.06
1.54	0.06
1.39	0.03
1.25	0.01

K. Dihydroxydiketones

K1. Δ⁴-Pregnene-17(α),21-diol-3,20-dione

d, A.	I/I₁
10.9	0.05
8.35	0.09
6.33	0.73
5.59	1.00
5.29	0.73
4.58	0.02
4.33	0.02
4.17	0.73
3.66	0.05
3.71	0.02
3.69	0.27
3.40	0.18
3.29	0.18
3.18	0.41
3.08	0.27
2.96	0.09
2.84	0.09
2.67	0.18
2.59	0.27
2.40	0.27
2.23	0.02
2.18	0.18
2.09	0.09
2.04	0.18
2.00	0.02
1.96	0.02
1.89	0.02
1.84	0.09
1.79	0.02
1.69	0.09

K2. Δ⁴-Pregnene-17(α),21-diol-3,20-dione-21-acetate

d, A.	I/I₁
11.1	0.27
8.31	0.36
7.23	0.49
6.24	0.36
5.91	0.09
5.59	0.49
5.32	1.00
5.10	0.13
4.87	0.13
4.68	0.27
4.52	0.13
4.39	0.36
4.21	0.01
3.82	0.09
3.60	0.36
3.24	0.13
3.13	0.09
3.04	0.20
2.94	0.20
2.84	0.13
2.75	0.09
2.71	0.04
2.66	0.04
2.62	0.27
2.55	0.27
2.47	0.20
2.34	0.27
2.30	0.09
2.24	0.02
2.19	0.09
2.25	0.27
2.11	0.04
2.06	0.13
2.01	0.13
1.98	0.01
1.93	0.13
1.87	0.09
1.82	0.02
1.77	0.02
1.73	0.01

K3. Pregnane-17(α),21-diol-3,20-dione-21-acetate

d, A.	I/I₁
13.1	0.15
6.89	0.10
6.56	0.20
6.24	0.15
5.85	1.00
5.70	0.75
5.50	0.15
5.22	0.15
4.93	0.10
4.80	0.27
4.42	0.37
4.25	0.07
4.18	0.10
4.09	0.01
3.96	0.01
3.85	0.07
3.75	0.07
3.64	0.20
3.47	0.07
3.33	0.27
3.28	0.01
3.18	0.01
3.12	0.15
3.06	0.01
2.98	0.15
2.92	0.07
2.83	0.20
2.74	0.01
2.67	0.01
2.62	0.10
2.58	0.10
2.53	0.01
2.47	0.03
2.44	0.01
2.40	0.10
2.35	0.10
2.31	0.03
2.27	0.02
2.21	0.10
2.15	0.07
2.09	0.07
2.05	0.03
1.98	0.03
1.95	0.03
1.91	0.03
1.87	0.01
1.83	0.02
1.80	0.02
1.74	0.01
1.68	0.01
1.59	0.01
1.53	0.01

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

L. Dihydroxytriketones

L1. Δ^4-Pregnene-3,11,20-trione-17(α),21-diol

d, A.	I/I_1
13.0	0.03
10.6	0.25
8.89	0.25
7.94	0.13
6.63	0.13
6.22	1.00
5.83	0.25
5.31	0.75
4.97	0.25
4.71	0.56
4.34	0.56
4.00	0.13
3.72	0.13
3.64	0.13
3.38	0.03
3.16	0.03
2.94	0.25
2.85	0.13
2.65	0.03
2.50	0.03
2.34	0.03
2.26	0.03
2.19	0.13
2.11	0.13
2.07	0.13
1.98	0.13

L2. Δ^4-Pregnene-17(α),21-diol-3,11,20-trione-21-acetate

d, A.	I/I_1
7.34	0.15
6.19	0.50
5.93	0.37
5.49	0.15
5.10	0.03
4.94	1.00

L2. (*Contd.*)

d, A.	I/I_1
4.65	0.10
4.29	0.15
4.15	0.03
4.00	0.10
3.83	0.07
3.73	0.20
3.58	0.02
3.43	0.15
3.32	0.15
3.11	0.10
3.06	0.10
2.97	0.10
2.89	0.20
2.74	0.02
2.67	0.07
2.64	0.01
2.58	0.01
2.52	0.07
2.43	0.07
2.38	0.07
2.30	0.07
2.21	0.15
2.17	0.02
2.12	0.03
2.07	0.02
2.05	0.02
1.99	0.10
1.94	0.03
1.91	0.03
1.86	0.02
1.82	0.07
1.79	0.03
1.73	0.03
1.68	0.02
1.64	0.02

II. Bile Acids

M1. Hyodeoxycholic acid

d, A.	I/I_1
14.6	0.20
10.9	0.13
9.21	0.53
7.63	0.40
6.26	0.13
5.83	1.00
5.59	0.40
5.29	0.40
4.91	0.53
4.51	0.53
4.27	0.30
4.11	0.40
3.95	0.07
3.73	0.40
3.60	0.07
3.41	0.40
3.25	0.30
3.13	0.30
3.04	0.20
2.86	0.07
2.76	0.07
2.70	0.13
2.61	0.13
2.55	0.07
2.47	0.30
2.40	0.30
2.34	0.30
2.22	0.30
2.15	0.13
2.10	0.13
2.06	0.30
1.99	0.13
1.88	0.13

M2. Lithocholic acid

d, A.	I/I_1
13.1	0.20
9.07	0.02
7.23	0.20
6.13	0.20
6.15	0.53
5.81	1.00
5.61	0.73
5.39	0.02
5.05	0.53
4.76	0.40
4.48	0.73
4.32	0.53
4.20	0.40
4.03	0.40
3.88	0.02
3.74	0.40
3.57	0.30
3.46	0.40
3.36	0.30
3.23	0.30
3.10	0.02
2.90	0.02
2.80	0.07
2.65	0.20
2.56	0.13
2.48	0.20
2.35	0.20
2.26	0.30
2.23	0.20
2.16	0.02
2.09	0.30
2.02	0.02
1.98	0.20
1.90	0.02
1.87	0.07
1.82	0.02
1.74	0.02
1.66	0.02

III. Steroid Sapogenins and Derivatives

N1. Sarsasapogenin

d, A.	I/I_1
13.5	0.13
12.0	0.20
9.82	0.30
8.59	0.40
7.63	0.30
6.94	0.40
5.54	1.00
4.98	0.53
4.62	0.40
4.48	0.53
4.15	0.20
3.99	0.30
3.82	0.02
3.75	0.30
3.55	0.30
3.33	0.13
3.02	0.40

N2. Diosgenin acetate

d, A.	I/I_1
14.0	0.13
9.41	0.13
7.05	0.13
6.11	0.73
5.72	1.00
5.44	0.03
5.21	0.40
4.72	0.40
4.38	0.40
3.58	0.30
3.19	0.30
3.05	0.20
2.82	0.13
2.64	0.03
2.53	0.07
2.38	0.03
2.31	0.07

III. Steroid Sapogenins and Derivatives (*Contd.*)

N1. (*Contd.*)

d, A.	I/I_1
2.86	0.07
2.74	0.07
2.59	0.30
2.45	0.13
2.32	0.07
2.22	0.20
2.17	0.20
2.10	0.20
2.02	0.20

N2. (*Contd.*)

d, A.	I/I_1
2.14	0.03
2.05	0.07
1.97	0.03
1.86	0.03
1.71	0.03

N3. Cholesterol digitonide

d, A.	I/I_1
14.0	0.40
9.77	0.30
7.73	0.30
6.15	1.00
5.29	0.53
4.42	0.07
3.87	0.13
3.60	0.13
3.34	0.07
2.99	0.07

N4. β-Sitosterol digitonide

d, A.	I/I_1
14.0	0.20
11.7	0.01
7.73	0.15
8.63	0.02
7.66	0.15
7.14	0.10
6.15	1.00
5.72	0.27
5.04	0.27
4.21	0.15
3.82	0.20
3.60	0.15
3.35	0.10
3.05	0.10
2.56	0.03
2.45	0.02
2.11	0.01
2.00	0.01

N5. Δ^5-Androstene benzoate digitonide

d, A.	I/I_1
13.7	0.56
11.2	0.03
9.71	0.38
7.63	0.03
6.92	0.56
6.09	1.00
5.49	0.25
5.05	1.00
4.21	0.56
3.87	0.75
3.61	0.56
3.36	0.13
3.13	0.13

IV. Digitalis Glycosides

O1. Gitoxin

d, A.	I/I_1
13.7	0.20
9.41	0.09
7.06	0.36
6.49	1.00
5.79	0.67
5.47	0.49
5.26	0.01
5.05	1.00
4.81	0.01
4.63	0.36
4.34	0.36
4.16	0.36
3.96	0.36
3.78	0.36
3.63	0.36
3.40	0.36
3.26	0.36
3.19	0.36
3.08	0.20
2.95	0.13
2.87	0.13
2.75	0.36
2.69	0.04
2.65	0.04
2.54	0.09
2.48	0.01
2.41	0.09
2.32	0.09
2.27	0.01
2.22	0.01
2.18	0.27
2.10	0.01
2.03	0.27
2.02	0.01
1.97	0.01
1.90	0.01
1.86	0.09

O2. Digoxin

d, A.	I/I_1
12.0	0.13
10.8	0.20
8.98	0.13
7.17	0.36
6.09	0.67
5.63	0.09
5.29	1.00
5.11	1.00
4.66	0.13
4.43	0.36
4.17	0.27
3.98	0.27
3.78	0.13
3.59	0.09
3.47	0.20
3.32	0.02
3.21	0.27
3.08	0.20
2.96	0.13
2.91	0.13
2.74	0.04
2.59	0.20
2.46	0.20
2.31	0.13
2.23	0.13
2.15	0.13
2.09	0.13
2.05	0.13
1.99	0.09
1.94	0.09
1.90	0.13
1.83	0.09
1.78	0.02
1.74	0.09
1.70	0.01
1.64	0.01

O3. Digitoxin

d, A.	I/I_1
15.0	0.07
9.07	0.20
8.01	0.07
7.28	0.30
7.00	0.30
6.26	0.40
5.95	1.00
5.63	0.53
5.32	0.13
5.00	0.40
4.80	0.40
4.62	0.40
4.32	0.73
4.10	0.30
3.93	0.30
3.75	0.30
3.62	0.07
3.52	0.07
3.39	0.07
3.30	0.20
3.23	0.07
3.06	0.07
2.89	0.07
2.75	0.07
2.60	0.03
2.52	0.02
2.43	0.03
2.37	0.02
2.20	0.02
2.10	0.02
2.05	0.02
2.01	0.02
1.95	0.02

ACKNOWLEDGMENT

The authors would like to acknowledge the assistance of Marion Smale in the preparation of the x-ray data and photographs.

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Received for review May 9, 1955. Accepted June 1, 1955.

New Substituted 1,10-Phenanthrolines as Ferroine and Cuproine Reacting Ligands

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Substituted groups in the 4,7- and 2,9-positioned hydrogens of 1,10-phenanthroline provide marked alterations in property. The ferroine and cuproine reactions of chelation with 3,8- and 5,6-positioned hydrogens replaced by other functional groups exert significant, but less effective modulation in property. Eleven new mono- and di-substituted 1,10-phenanthrolines are described. The group substituents replacing hydrogen are compared as electron donors. The new alterations are evaluated with determination of their spectrophotometric constants of the wave length of maximum absorption, ($\lambda_{max.}$), and their molecular extinction coefficients, (ϵ). Suggested predictions which favor productive objectives in proposed preparation of new modified 1,10-phenanthrolines are thus implied.

PREVIOUS studies of modified 1,10-phenanthrolines have included a number of group substitutions for the eight replaceable hydrogens. Electron donor and acceptor groups of substitutions have been supplied. The methyl and phenyl, single and multiple substitutions, both symmetrical and unsymmetrical, have proved to be highly effective in the alteration of resultant physical and chemical properties. Methyl and phenyl groups are of the electron donor types. Nitro-, bromo-, and chloro-groups are the electron acceptor types. Their employment modifies the π electron concentrations at the (—C(=C)—) and (=N—) terminals which accounts for the alterations in property.

These donor and acceptor types of substitutions for replaceable hydrogens in 1,10-phenanthrolines alter the essential chelation properties listed as follows:

Both electron donor and acceptor substitutions produce significant alterations in the wave length of maximum absorption of their reactions of chelation involving iron(II) and copper(I) (*2*, *4*).

Both types of substitution produce major alterations in the molecular extinction coefficient in the same chelation relations (*2*, *4*).

The oxidation potential of the iron(II) complex cations to the corresponding iron(III) complexes is markedly modified owing to the same type of substitutions. Electron donor groups, (*4*), such as methyl, lower the oxidation potential. Electron acceptor types such as the NO_2^- and the Cl^- groups increase the oxidation potential. The same effect may be postulated in the corresponding chelations with copper(I), but experimental verification remains in substantiation of this premise.

Alkyl and aryl substitutions for the 2,9-hydrogens of 1,10-phenanthroline permit the cuproine reaction of chelation with copper(I), but prevent the ferroine reaction involving iron(II) chelation. Modified and increased specificity is thus provided. The effect is attributed to steric hindrance (*1*).

Alkyl and aryl substitutions markedly affect the solubility of the ligand and its chelation complexes in water and organic solvents immiscible with water. Such solvents include chloroform, benzene, iso-amyl alcohol, hexanol, and others. Application of immiscible solvent extraction markedly increases spectrophotometric sensitivity.

Substitutions in the 4,7- and 2,9-positioned hydrogens produce the greatest alteration in chemical and physical properties.

One hundred substituted 1,10-phenanthrolines, including the present examples, have been prepared and described. Literature references to most of these are included in a published review on this subject (*3*); the reader is referred to this source for cited references to this publication.

PREPARATION OF MATERIALS

The 11 newly prepared, substituted 1,10-phenanthrolines were synthesized by Francis H. Case and coworkers at Temple University, Philadelphia. The synthetic processes involved are to be published subsequently.

The new 1,10-phenanthrolines are shown in Table I together with the physical constants determined in each instance.

Either aqueous solutions of these reagents or their hydrochlorides were employed. A pH 4 is productive of rapid chelation and the stability of the resulting complex cations includes a pH range of 3 to 9 in most cases. The complexes are in most cases extracted using commonly employed immiscible solvents.

Table I. New Ferroine and Cuproine Reacting Substituted 1,10-Phenanthrolines

(Determined Physical Constants)

Type Substitution	Ferroine Reacting ϵ[a]	Ferroine Reacting λmax.	Cuproine Reacting ϵ[a]	Cuproine Reacting λmax.
(1,10-Phenanthroline)	11100	510	7250	435
2,9-Diphenyl-	...	...	3620	441
4-Ethyl-	13200	514	8060	437
5-Ethyl-	12420	518	7960	442
4,6-Diethyl-	13700	518	8410	443
4,7-Diethyl-	15020	515	8675	438
4,7-Diphenoxy-	14660	500	8020	425
5,6-Dimethoxy-	11960	518	7500	441
4,7-Dimethoxy-	12270	500	...	U.V.
5-Amino-	12060	524	...	U.V.
5,6-(2-Pyrido)-	12670	518	...	U.V.
5,6-(Cyclohexeno)-	12660	524	6970	446

[a] Accuracy not better than ±100 units, approx.

From an examination of the data of Table I, the comparison of the different group substitutions for the 4,7-hydrogens of 1,10-phenanthroline and their effect upon the ferroine and cuproine reactions (assuming negligible influence from steric hindrance), the following observations may be noted in connection with molecular extinction modulations:

The order in increasing magnitude of the substituent groups as electron donors is methoxy, < phenoxy, < ethyl, < phenyl.

The percentage increases in electron donor intensity (as approximations) are, respectively, methoxy, 10; phenoxy, 31; ethyl, 34; and phenyl, 100. The methyl group if included has the value 26%, falling between methoxy and phenoxy groups (*2*).

The effect of amino substitutions in the 5 position replacing hydrogen is minor in magnitude when compared with amino substitutions in the 4 and 4,7 positions (*5*). In the latter cases the electron donor intensities are comparable with the increases provided by the phenyl group substitutions in the same positions —namely, 80 to 90%.

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The magnitude of electron donation by the same group substitutions in the ferroine chelation, is greater than that for the cuproine reaction. In the latter case the percentage increase is phenoxy, 13.5; < ethyl, 20; < phenyl, 67.5. This decrease in effective modulation in the case of cuproine chelation is possibly due to steric hindrance. These conclusions have been substantiated (*1*).

The influence of substitutions by these newly described phenanthrolines does not promote alterations in the wave length of maximum absorption, ($\lambda_{max.}$), which are accurately additive, as was found in the case of corresponding methyl group substitutions (*2*).

Assuming that synthesis of 4,7-diethyl-1,10-phenanthroline may be provided with equal facility to the 4,7-dimethyl substitutions, the former is much to be preferred (ϵ = 15020 as compared to 14000), for the ferroine reaction. The preferred cuproine reacting ligand of those newly described is 4,7-diethyl-1,10-phenanthroline, (ϵ = 8675). This sensitivity does not approach the sensitivity of bathophenanthroline, [4,7-diphenyl-1,10-phenanthroline, (ϵ = 12140)], or that of bathocuproine, [2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, (ϵ = 14200)].

The results obtained in modulation of physical constants of the chelates of the modified 1,10-phenanthrolines are not in accord with the known electron donor properties of the modifying substituent groups employed. An explanation of these facts at present is not capable of interpretation.

SUMMARY

Eleven newly synthesized substituted 1,10-phenanthrolines have been studied with determination of the wave length of maximum absorption and molecular extinction coefficients of their iron(II) and copper(I) complex formulations. The new substitutions for replaceable hydrogens in the parent ligand are ethyl, methoxy, phenoxy, pyrido, and cyclohexeno groups. The hydrogen replacements are in the 2,9 position, the 4 and 5 positions, the 4,7 positions, and the 5,6 substitutions.

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RECEIVED for review January 17, 1955. Accepted June 20, 1955. Presented before the Division of Analytical Chemistry at the 125th meeting of the AMERICAN CHEMICAL SOCIETY, Kansas City, Kan., March 1954.

Identification of Accelerators and Antioxidants in Compounded Rubber Products

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A new, simple, and comprehensive approach to the identification of the accelerators and antioxidants used in rubber products is described. This procedure is unusual in that it utilizes the tendency of accelerators to decompose during mixing or vulcanization, or upon extraction from compounded stocks. The accelerator fragments are isolated using distillation and liquid-liquid extraction principles. The identification of the fragments is simple, rapid, and unambiguous. The accelerators used are then determined from a knowledge of the decomposition behavior of known compounds. The antioxidants are recovered unchanged and can be identified by their ultraviolet absorption characteristics and color tests. This method is applicable to all types of rubber products, including cements, and is particularly valuable in the identification of accelerators which decompose upon mixing, vulcanization, or extraction. Most of the commonly used accelerators and amine antioxidants can be classified or identified by the analytical scheme described. The procedure will be useful in identifying many accelerators and antioxidants which may be developed in the future.

THE identification of rubber compounding ingredients in the past has been accomplished mainly through the use of color reactions (*5, 7, 10, 14, 19, 21*). Recently these identifications have been made more positive through the use of chromatographic separation techniques (*2*) and characterization by ultraviolet (*8, 12, 13*) and infrared (*15*) spectrophotometry. Parker and Berriman (*17*) have recently published an excellent comprehensive study on the chromatographic determination of accelerators and antioxidants in vulcanized rubber, including data on many of the accelerators and antioxidants which are widely used today. A complete characterization of the composition of an unknown vulcanizate necessitates a detailed knowledge of the chromatographic behavior, not only of all the commercially used accelerators and antioxidants, but also of their decomposition products which are produced during vulcanization. Therefore, considerable background experience is necessary before the chromatographic technique can be used successfully.

The tendency of accelerators to decompose or react during mixing, vulcanization, or extraction has plagued many analysts in the past and has resulted in loss of accelerators, or their conversion to other chemical compounds. Examples of some of the difficulties which have been encountered are given below. Parker and Berriman (*17*) have shown that stearic acid interferes with the detection of bis(dimethylthiocarbamoyl) disulfide (Methyl Tuads) in vulcanized rubber. Similarly, bis(diethylthiocarbamoyl) disulfide (Ethyl Tuads) and bis(dimethylthiocarbamoyl) sulfide (Monex) could not be detected by these investigators after vulcanization. The conversion of bis(dimethylthiocarbamoyl) disulfide to zinc dimethyldithiocarbamate (Methyl Zimate) in the presence of zinc oxide during vulcanization has been observed by Du Fraisse and Jarrijon (*9*), Mann (*15*), and others (*17, 21*).

Mann (*15*) states that he was unable to detect either the piperidine salt of 1-piperidinecarbodithioic acid (Pip-Pip) or zinc pentamethylenedithiocarbamate (Pipazate) in vulcanizates where the former was used as an accelerator. Mann (*15*) has also observed that *N*-cyclohexyl-2-benzothiazolesulfenamide (Santocure) is converted to 2-mercaptobenzothiazole (MBT) during vulcanization. The cyclohexylamine fragment of the molecule could not be found after vulcanization.

Du Fraisse and Houpillart (*8*) identified both 2-mercaptobenzothiazole and 2,2′-dithiobisbenzothiazole (MBTS) in vulcanized rubber compounds which originally contained only one of these accelerators. They failed to detect diphenylguanidine (DPG) in

acetone extracts of vulcanized rubber compounds accelerated with diphenylguanidine (*8*).

The procedure proposed here simplifies these problems by using an extraction technique which promotes decomposition of the accelerators and collects the fragments obtained.

Table I. Accelerators Studied

Abbreviation or Trade Name	Chemical Name	Source
MBT	2-Mercaptobenzothiazole	Du Pont
MBTS	2,2′-Dithiobisbenzothiazole	Naugatuck
Zenite Special	2-Mercaptobenzothiazole, zinc derivative (zinc benzothiazolyl sulfide)	Du Pont
Santocure (CBS)	*N*-cyclohexyl-2-benzothiazolesulfenamide	Monsanto
NOBS Special	2-(Morpholinothio)benzothiazole (*N*-oxydiethylene-2-benzothiazolesulfenamide)	American Cyanamid
Vulkacit AZ	*N,N*-diethyl-2-benzothiazolesulfenamide	I. G. Farbenindustrie
IBS	*N*-isopropyl-2-benzothiazolesulfenamide	Firestone (*6*)
CPBS	*N*-cyclopentamethylene-2-benzothiazolesulfenamide	Firestone (*6*)
CDETS	*N*-cyclohexyl-*S*-(diethylthiocarbamoyl)-hydrosulfamine (*N′*-cyclohexyl-*N,N*-diethylthiocarbamylsulfenamide)	Firestone (*1*, *20*)
Naugatuck 124	Composition not given; identified in this study as *N,N*-dicyclohexyl-2-benzothiazolesulfenamide	Naugatuck
Monex	Bis(dimethylthiocarbamoyl) sulfide (tetramethylthiurammonosulfide)	Naugatuck
Methyl Tuads	Bis(dimethylthiocarbamoyl) disulfide (tetramethylthiuram disulfide)	R. T. Vanderbilt
Ethyl Tuads	Bis(diethylthiocarbamoyl) disulfide (tetraethylthiuram disulfide)	R. T. Vanderbilt
Tetrone A	Bis(piperidinothiocarbonyl)tetrasulfide (*N,N′*-pentamethylenethiuram tetrasulfide)	Du Pont
Methyl Zimate	Zinc dimethyldithiocarbamate	R. T. Vanderbilt
Ethyl Zimate	Zinc diethyldithiocarbamate	R. T. Vanderbilt
Pipazate	Zinc-*N*-pentamethylenedithiocarbamate	Naugatuck
Pip-Pip	1-Piperidinecarbodithioic acid, piperidine salt (*N*-pentamethyleneammoniumpentamethylenedithiocarbamate)	Monsanto
Philcure 113	*tert*-Butylsulfenyldimethyldithiocarbamate	Phillips Petroleum
DPG	Diphenylguanidine	Monsanto
DOTG	Di-*o*-tolylguanidine	Du Pont
TPG	Triphenylguanidine	Du Pont
Ethylac	*N,N*-diethylthiocarbamoyl-2-benzothiazolyl sulfide	Sharples
SRA No. 2	Zinc diphenylguanidinobenzothiazolylmercaptide	Du Pont

A perusal of the accelerators commonly used in rubber compounding shows that all contain one or more of the following components: a thiazole (or related compound), an amine, a guanidine, and carbon disulfide. The proposed procedure isolates and identifies each of these components along with the amine antioxidant used. The accelerator which was present in the rubber compound can then be determined, in most instances, from a knowledge of the fragments obtained from known accelerators.

Consequently, many of the difficulties encountered by previous investigators are eliminated. Easily decomposed accelerators can be classified or identified, as the analytical scheme is designed primarily to isolate and identify the decomposition products and not the original accelerators.

The extraction medium used must not only promote the accelerator decomposition but it must also be efficient in extracting the decomposed fragments or unchanged compounding ingredients. Humphrey (*11*) has shown that a mixture of benzene and aqueous hydrochloric acid facilitates the removal of the extraction-resistant guanidine-type accelerators. In the present work a mixture of ethyl alcohol and 1*N* aqueous hydrochloric acid was found to be an excellent extraction medium, in that it successfully decomposes unstable accelerators and recovers the guanidine and antioxidants unchanged.

The extracted components are subsequently separated using distillation and liquid-liquid extraction techniques. The separations involved are complete and little interference is encountered in the identification of the products.

Table II. Antioxidants Studied

Abbreviation or Trade Name	Chemical Name	Source
AgeRite Stalite	Mixture of mono- and diheptyldiphenylamines	R. T. Vanderbilt
BLE	Acetone-diphenylamine condensation product	Naugatuck
Flectol H	Acetone-aniline condensation product	Monsanto
J-Z-F (DPPD)	*N,N′*-diphenyl-*p*-phenylenediamine	Naugatuck
Neozone A	*N*-phenyl-1-naphthylamine	Du Pont
Neozone D (PBNA)	*N*-phenyl-2-naphthylamine	Du Pont
Santoflex AW	6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline	Monsanto
Santoflex B	6-Phenyl-1,2-dihydro-2,2,4-trimethylquinoline	Monsanto
Santoflex BX	Santoflex B plus DPPD	Monsanto
Stabilite	*N,N′*-diphenylethylenediamine	C. P. Hall
Thermoflex	Di-*p*-methoxydiphenylamine	Du Pont
Thermoflex A	Thermoflex plus PBNA and DPPD	Du Pont

APPARATUS

Norelco x-ray diffraction equipment was used to identify the crystalline products isolated. The photographic technique was employed, using copper K-alpha radiation.

A Beckman Model DU ultraviolet spectrophotometer was used in the identification of some of the products. Absolute ethyl alcohol was the solvent used.

The apparatus used for extracting rubber compounds under reflux is shown in Figure 1. It consists of a 1-liter, single-necked, round-bottomed flask, fitted with a Claisen-type adapter which connects it to an air inlet tube, and to a Hopkins type reflux condenser. The upper end of the air inlet tube is connected to a gas-washing tube containing 0.5*N* alcoholic alkali to remove any traces of carbon disulfide and hydrogen sulfide from the incoming air. The lower end of this tube dips below the liquid level in the flask. Connected to the outlet tube of the reflux condenser is another gas-washing tube which contains 50 ml. of 10% aqueous copper sulfate to remove any hydrogen sulfide generated during the refluxing period. A third gas-washing tube containing 0.2*N* alcoholic sodium hydroxide is used to trap the carbon disulfide that may be liberated from the rubber compound. This tube is connected to a vacuum line.

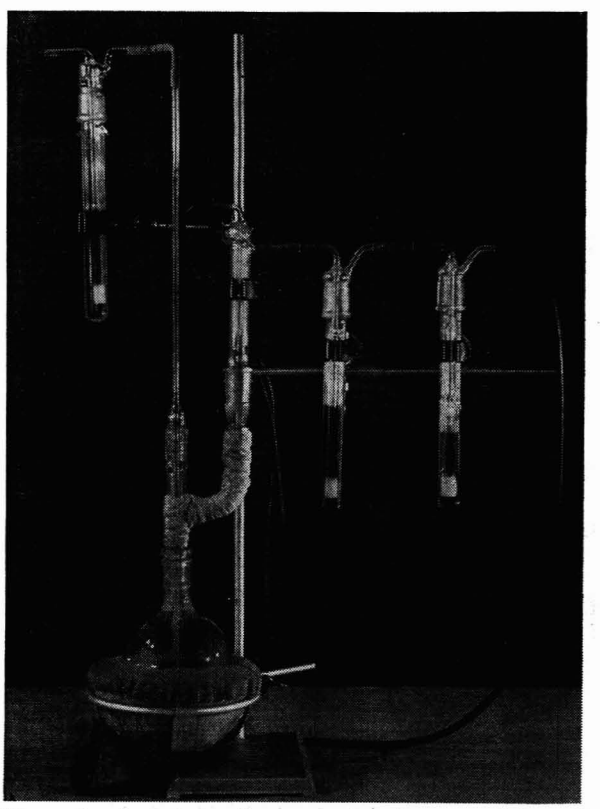

Figure 1. Apparatus for extractive decomposition of rubber compounding materials

Except for gum rubber tubing connecting the gas-washing tubes, ground-glass joints are used throughout this assembly and the distillation apparatus described below.

For distillation of the amines and their absorption in acid, a conventional type of apparatus may be used, such as that illustrated in Figure 2. A modified cylindrical Kjeldahl spray trap is inserted between the distillation flask and the condenser. Modification consists of fusing an insulating jacket around the bulb, to prevent excessive liquid holdup. The receiving adapter has an outlet tip consisting of a small perforated glass bulb.

MATERIALS

All reagents and solvents used were analytical grade.

The accelerators and antioxidants used in this study were all commercial grade. They are listed in Tables I and II, with the trade names, chemical names, and suppliers. These accelerators and antioxidants were compounded in the test formula given in Table III. *N*-phenyl-2-naphthylamine (PBNA) was used as an antioxidant in some of the stocks compounded for accelerator identification. *N*-cyclohexyl-2-benzothiazolesulfenamide was the accelerator used for the stocks containing different antioxidants.

Table III. Compounding Formula

Material	Parts
Natural rubber	100.0
Carbon black	50.0
Zinc oxide	3.0
Stearic acid	3.0
Paraflux (softener)[a]	4.0
Sulfur	2.3
Antioxidant	1.0 to 1.5
Accelerator[b]	0.5 to 1.0

All stocks, except TPG, cured 50 minutes at 280° F. TPG cured 60 minutes at 298° F.

[a] Supplied by C. P. Hall.
[b] Triphenylguanidine compounded at 1.5 parts.

PROCEDURE

The rubber vulcanizates containing the different antioxidants and accelerators listed in Tables I and II were analyzed by the procedure given below. The results of the accelerator analyses are shown in Table IV. All of the antioxidants studied were isolated in the neutral or basic fraction and successfully identified.

Extraction of Antioxidant and Accelerator Fragments from Rubber Products. The rubber products are prepared in the usual way by mixing and sheeting out on a rubber mill. Vulcanizates can also be ground in a Wiley mill. Cements are prepared by evaporation to the solids, preferably in a vacuum oven. The solids can then be mixed and sheeted out. Compounded latex and latex products may be prepared in the same way.

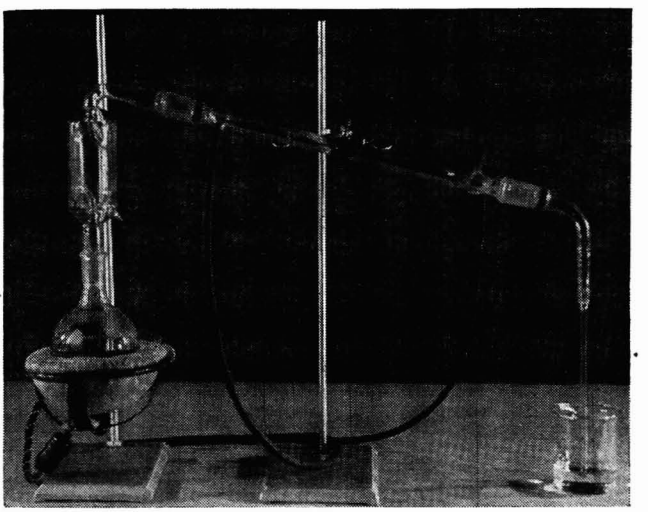

Figure 2. Apparatus for distilling volatile amines

Place 15 to 20 grams of the prepared rubber product in the 1-liter, single-necked, round-bottomed flask, and add 100 ml. of ethyl alcohol and 100 ml. of 1*N* aqueous hydrochloric acid. Connect the flask to the reflux setup, and attach the gas-washing tubes. Apply enough suction to the outlet of the carbon disulfide absorption tube to permit a slow bubbling of air through all the solutions.

Turn on the heating mantle and allow the alcoholic-acid solution to reflux for approximately 2 hours. After the heat is turned off, allow air to bubble through the solutions for about 5 minutes. Disconnect the apparatus and filter the alcoholic-acid solution into a 500-ml. single-necked, round-bottomed flask. Wash the rubber residue with 100 ml. of water and add the washings to the main solution.

Table IV. Results of Accelerator Analyses

Accelerator Used	Components Identified: Thiazole	Amine	Carbon disulfide	Guanidine	Accelerators Indicated
Thiazolesulfenamides					
Santocure (CBS)	MBT	Cyclohexylamine	Neg.	....	Santocure
NOBS Special	MBT	Morpholine	Neg.	....	NOBS Special
Vulkacit AZ	MBT	Diethylamine	Neg.	....	Vulkacit AZ
Naugatuck 124	MBT	Dicyclohexylamine	Neg.	....	*N,N*-Dicyclohexyl-2-benzothiazolesulfenamide
IBS	MBT	Isopropylamine	Neg.	....	IBS
CPBS	MBT	Piperidine	Neg.	....	CPBS
Thiocarbamylsulfenamide					
CDETS	...	Cyclohexylamine, Diethylamine	Pos.	....	CDETS
Thiazoles					
MBT	MBT	.....	Neg.	....	MBT, MBTS or Zenite Special
MBTS	MBT	.....	Neg.	....	MBT, MBTS or Zenite Special
Zenite Special	MBT	.....	Neg.	....	MBT, MBTS or Zenite Special
Thiuramsulfides and dithiocarbamates					
Monex	...	Dimethylamine	Pos.	....	Monex, Methyl Tuads, or Methyl Zimate
Methyl Tuads	...	Dimethylamine	Pos.	....	Monex, Methyl Tuads, or Methyl Zimate
Methyl Zimate	...	Dimethylamine	Pos.	....	Monex, Methyl Tuads, or Methyl Zimate
Ethyl Tuads	...	Diethylamine	Pos.	....	Ethyl Tuads or Ethyl Zimate
Ethyl Zimate	...	Diethylamine	Pos.	....	Ethyl Tuads or Ethyl Zimate
Pip-Pip	...	Piperidine	Pos.	....	Pip-Pip, Pipazate, or Tetrone A
Pipazate	...	Piperidine	Pos.	....	Pip-Pip, Pipazate, or Tetrone A
Tetrone A	...	Piperidine	Pos.	....	Pip-Pip, Pipazate, or Tetrone A
Guanidines					
DPG	...	.....	Neg.	DPG	DPG or DPG salt
TPG	...	.....	Neg.	TPG	TPG or TPG salt
DOTG	...	.....	Neg.	DOTG	DOTG or DOTG salt
Miscellaneous					
Ethylac	MBT	Diethylamine	Neg.	....	Ethylac or Vulkacit AZ
SRA No. 2	MBT	.....	Neg.	DPG	SRA No. 2 or benzothiazole-DPG mixture
Philcure 113[a]	...	Dimethylamine	Pos.	....	*tert*-Butylsulfenyldimethyldithiocarbamate

[a] Not compounded in rubber stock, *tert*-butyl mercaptan distilled over with amine and procedure modified to isolate and identify mercaptan as mercury salt.

Table V. Separation of Nonvolatile, Neutral, Basic, and Acidic Materials

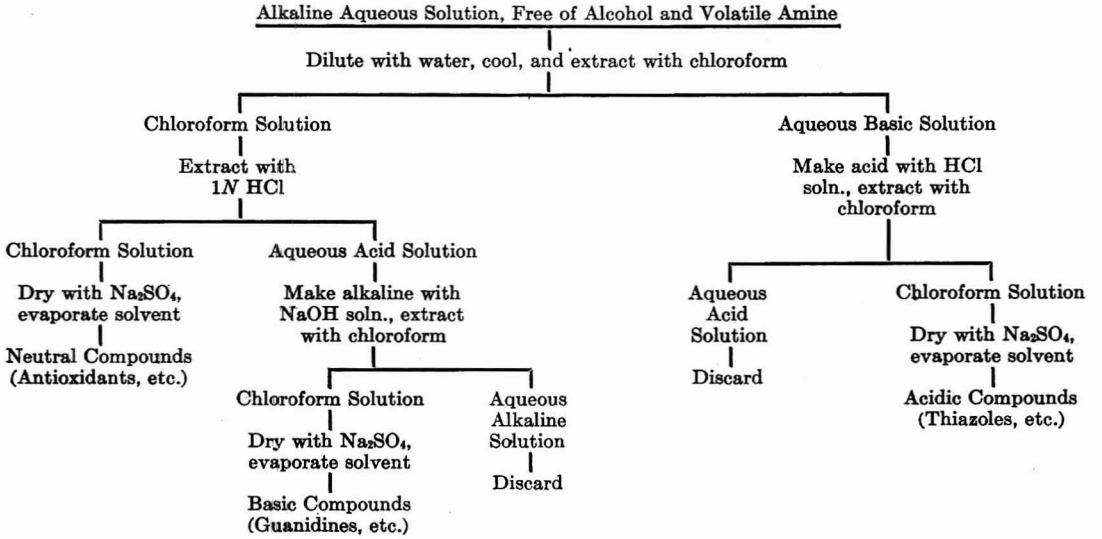

Table VI. Ultraviolet Absorption Characteristics of Amine Antioxidants and MBT

	Min., mμ	Maxima, 200–250 mμ	Min., mμ	Maxima, 250–300 mμ	Min., mμ	Maxima, 300–350 mμ
AgeRite Stalite	..	208(I)	253	288(II)	..	....
BJF (DPPD)	228	208(I) 240(IIIs)[a]	258	....	..	304(II)
BLE	..	208(I)	251	288(II)	..	....
Flectol H	225	212(I) 236(II)	285	....	..	310(IIIs)
Neozone A (PANA)	..	217(I)	236	253(II)	288	340(IIIs)
Neozone D (PBNA)	..	220(I)	232	272(II)	283	310(III)
Santoflex AW	..	230(I)	..	....	300	350(IIs)
Santoflex B	..	....	..	256(I)	278	310(II)
Santoflex BX	..	208(I)	219	256(II)	278	310(III)
Stabilite	218	212(I) 250(II)	272	294(IIIs)	..	....
Thermoflex	234	208(I) 245(IIIs)	253	288(II)	..	....
Thermoflex A	..	220(I)	232	272(III)	282	300(II) 310(II)
MBT	234	230(II) 238(III)	..	....	272	325 (I)

For each antioxidant maxima are designated I, II, and III in descending order of intensity.

[a] s denotes slight maximum.

Distillation, Recovery, and Identification of Amine. Make the cooled alcoholic-acid solution alkaline with approximately 25% aqueous sodium hydroxide solution, connect the flask to the distillation apparatus, turn on the heating mantle, and distill the amine into 35 ml. of 0.5*N* aqueous hydrochloric acid. Continue the distillation until about 150 ml. of distillate are collected or until most of the alcohol has been distilled from the mixture. The appearance of foam in the boiling, alkaline solution indicates that the bulk of the alcohol has been distilled. Concentrate the distillate by boiling, and finally evaporate to dryness in a 110° C. oven. Boil the dried residue gently with 2 or 3 ml. of chloroform and filter to separate the more soluble amine hydrochloride from the sodium and ammonium chlorides. Evaporate the chloroform at 70° C. and identify the dried amine hydrochloride by the x-ray diffraction method of Brock and Hannum (*4*).

Separation and Identification of Acid, Basic, and Neutral Materials. Dilute the alkaline residue from which the amine was distilled with 50 ml. of water, allow it to cool, and then subject it to an extraction procedure for separating the nonvolatile neutral, basic, and acidic materials.

The liquid-liquid extraction procedure used is similar to that employed by Braus, Middleton, and Ruchhoft (*3*) for separating the constituents of organic industrial wastes. A schematic diagram of the separation procedure is given in Table V.

The neutral fraction thus obtained normally contains the amine antioxidants, which can be identified by their ultraviolet absorption characteristics. Table VI lists the principal maxima and minima of the antioxidants included in this study. Color tests are also helpful in identifying these antioxidants. The tests of Burchfield and Judy (*5*) as given below have been extremely useful.

The basic fraction will contain the guanidines, which can be identified by their x-ray diffraction diagrams. The principal diffraction maxima together with the relative intensities of diphenylguanidine, triphenylguanidine (TPG), and di-*o*-tolyl-guanidine (DOTG) are listed in Table VII. In order to eliminate interferences due to polymorphism, all samples were recrystallized, by evaporation to dryness from chloroform, in a 110° C. oven before the x-ray data were obtained.

The acidic fraction will contain 2-mercaptobenzothiazole, if a benzothiazole-type accelerator is used. 2-Mercaptobenzothiazole is identified by its ultraviolet absorption characteristics given in Table VI. Other acidic thiazoles and related acidic materials will be isolated in this fraction. They can usually be identified by their ultraviolet absorption characteristics or x-ray diffraction diagrams.

Test for Carbon Disulfide. Transfer 10 ml. of the alcoholic sodium hydroxide solution from the carbon disulfide absorption tube to a test tube and make the solution weakly acid by the addition of glacial acetic acid. Add 5 ml. of 1% aqueous copper sulfate and shake the tube thoroughly. If carbon disulfide is absent, a blue solution or a blue precipitate results. The blue precipitate will dissolve upon the addition of several drops of concentrated nitric acid. When carbon disulfide is present, the precipitate is yellowish green in color, and the addition of nitric acid and reshaking leave a yellow precipitate of the copper xanthate.

Color Tests for Antioxidants (*5*). TEST SOLUTIONS. Stannic Chloride Solution. Dissolve 14.7 ml. of fuming stannic chloride in anhydrous analytical reagent grade benzene and make the volume up to 250 ml.

Amyl Nitrite Solution. Dilute 5 ml. of amyl nitrite to 100 ml. with benzene.

TEST I. Stannic Chloride–Amyl Nitrite Reaction. Dissolve approximately 1 mg. of the basic fraction in 5 ml. of benzene. Add 1 ml. of stannic chloride solution and 3 drops of amyl nitrite solution. A red precipitate indicates the presence of Stabilite.

TEST II. Stannic Chloride–Benzotrichloride Reaction. Dissolve approximately 1 mg. of the neutral fraction in 5 ml. of pure ethylene dichloride. Add 1 ml. of stannic chloride solution and 2 drops of benzotrichloride. BLE (an acetone-diphenylamine condensation product) gives a violet color with this test, differentiating it from AgeRite Stalite (a mixture of mono- and diheptyldiphenylamines), which gives an insignificant yellow color.

INTERPRETATION OF RESULTS

The accelerators used in the rubber product are determined by the fragments or combination of fragments identified. A knowledge of normal compounding practices will aid in reconstructing the accelerator system used. In general, the detection of a thiazole, a guanidine or carbon disulfide will classify the accelerator as to type—namely, a thiazole, usually 2-mercaptobenzothiazole or a derivative, a guanidine, or a thiuramsulfide or dithiocarbamate. The specific amine identified, if any, designates the particular amine activator, sulfenamide, thiuram sulfide, or dithiocarbamate used. Table IV lists the accelerators, which are indicated by the components identified.

Although the antioxidant is usually identified using ultraviolet absorption characteristics, some antioxidants are indicated by color reactions inherent in the procedure. Antioxidants containing *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) and di-*p*-methoxydiphenylamine (Thermoflex) are indicated by a green or blue-green color in the cooled extraction medium. When Thermoflex is present, the color turns red as the extraction medium is made alkaline with sodium hydroxide.* If DPPD is present, the alkaline solution is a yellow-brown color. Other color tests are used to distinguish between antioxidants having similar ultraviolet absorption characteristics. Consequently BLE can be distinguished from AgeRite Stalite using color test II (stannic chloride–benzotrichloride reaction).

N,N'-Diphenylethylenediamine (Stabilite) is the only one of the antioxidants studied which was recovered in the basic fraction. Stabilite can be identified in the presence of the guanidines using color test I (stannic chloride–amyl nitrite reaction).

The xanthate test was found to give a good positive test for carbon disulfide in the case of thiuram and dithiocarbamate type accelerators, and a negative test with other accelerators. If a more sensitive test is used, accelerators other than thiurams and dithiocarbamates may give a slight positive test for carbon disulfide and confuse the identification.

DISCUSSION

One disadvantage of this procedure is that some accelerators give identical decomposition products. As a result, it is impossible to differentiate between 2-mercaptobenzothiazole, 2,2'-dithiobisbenzothiazole, and 2-mercaptobenzothiazole, zinc derivative (Zenite Special). Similarly, no distinction can be made between thiuram sulfides and dithiocarbamates containing the same amine. This disadvantage is minimized in view of the fact that these accelerators undergo interconversion or decomposition during vulcanization, and identification of the original accelerator is always difficult.

The identification of accelerators and antioxidants in aged vulcanizates introduces the problem of decomposition due to aging. Burchfield and Judy (*5*) have recognized this difficulty in the identification of antioxidants. No detailed study of aged samples was made in the present investigation. It was observed, however, that 2-(morpholinothio)benzothiazole (NOBS Special) could not be identified in aged stocks and was difficult to identify in freshly cured vulcanizates. Santocure and DPG, on the other hand, could be identified easily in vulcanizates aged as long as 3 years. Antioxidants isolated from aged stocks gave ultraviolet absorption characteristics which were generally more difficult to identify.

Mixtures of antioxidants, as would be expected, are more difficult to identify, but they can usually be resolved by careful examination of the ultraviolet absorption data and by using color tests. Mixtures of accelerators generally do not present too much of a problem, as their decomposition products usually occur in different fractions.

To demonstrate the usefulness of the method in identifying mixed accelerators and antioxidants, a vulcanizate was prepared containing Santocure, Ethyl Tuads, TPG, BLE, and Santoflex BX (a mixture of 6-phenyl-1,2-dihydro-2,2,4-trimethylquinoline and DPPD). All five of the isolated fractions contained at least one product. The identification of cyclohexylamine and MBT indicated the presence of Santocure. Diethylamine and carbon disulfide indicated the use of Ethyl Tuads or zinc diethyldithiocarbamate (Ethyl Zimate). TPG was identified in the basic fraction. BLE, DPPD, and 6-phenyl-1,2-dihydro-2,2,4-trimethylquinoline (Santoflex B) were all indicated to be present in the neutral fraction. As Santoflex B and DPPD partially obscure the ultraviolet absorption characteristics of BLE, the presence of the latter is confirmed by color test II.

The extracted portion of the softeners used in compounding will usually be isolated in the neutral fraction. If these materials exhibit characteristic ultraviolet absorption, they could possibly interfere with the identification of the antioxidants. However, in the present study, no undue interference was encountered from either Paraflux or pine tar. This is primarily due to the fact that the aqueous alcohol extraction medium has little solvent power upon softeners that are insoluble in water.

The presence of large amounts of oils, in oil-extended polymers, interferes seriously with the identification of antioxidants. In such cases it may be necessary to resort to chromatographic techniques, before satisfactory results can be obtained.

APPLICATIONS

The procedure described here is offered as a new approach to the determination of accelerators and antioxidants in rubber products. It has been applied successfully to both vulcanized and unvulcanized rubber compounds. It is equally useful in the analysis of latex products and rubber cements. Data on 24 accelerators and 12 antioxidants have been obtained in this study.

The method has been used also in the identification of new accelerators. The analytical scheme is such that it will include many of the accelerators which are likely to be developed in the near future—for example, the recently reported accelerator, *tert*-butylsulfenyldimethyl dithiocarbamate (Philcure 113) (*18*), has been identified using the method. The procedure was modified slightly to include the identification of the mercaptan portion of the molecule (see Table IV).

The method will also have use in identifying some of the newer combinations of accelerators, such as the combination of the

Table VII. X-Ray Diffraction Data of Guanidines

Diphenylguanidine (DPG)		Triphenylguanidine (TPG)		Di-*o*-tolylguanidine (DOTG)	
d[a]	I/I_1[b]	*d*	I/I_1	*d*	I/I_1
10.36	0.82	9.15	1.00	10.33	0.46
6.96	0.07	8.24	0.38	6.79	0.25
6.43	0.12	7.05	0.06	6.18	1.00
6.07	0.07	6.50	0.03	5.22	0.25
5.06	0.17	5.37	0.46	4.63	1.00
4.68	1.00	5.00	0.03	4.04	0.46
4.51	0.24	4.72	0.25	3.88	0.17
4.19	0.32	4.52	0.31	3.76	0.46
3.95	0.24	4.34	0.46	3.37	0.05
3.80	0.17	4.07	0.38	3.18	0.11
3.63	0.40	3.79	0.19	3.03	0.05
3.49	0.24	3.56	0.38	2.50	0.05
3.26	0.04	3.42	0.03	2.43	0.05
3.09	0.07	3.08	0.25	2.09	0.03
2.99	0.07	2.70	0.19		
2.91	0.04				
2.64	0.02				
2.54	0.04				
2.44	0.02				
2.41	0.02				
2.31	0.04				
2.21	0.04				
2.14	0.04				
2.01	0.02				
1.93	0.02				
1.86	0.04				
1.69	0.04				

[a] *d*. Interplanar spacing in Angstrom units calculated from Bragg's law where $d = \frac{\lambda}{2 \sin \theta}$. λ is wave length of characteristic CuKα radiation, and θ is one half the angle of diffraction.
[b] I/I_1. Relative intensity.

thiuram and guanidine types which has been recommended for use in the vulcanization of neoprene rubber Type W (*16*).

ACKNOWLEDGMENT

The authors wish to acknowledge the help of Marjorie Jean Hannum in preparing this paper. Thanks are expressed to the Firestone Tire and Rubber Co. for permission to publish the results of this investigation.

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RECEIVED for review September 27, 1954. Accepted April 18, 1955. Presented before the Division of Rubber Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y., 1954.

4-Aminopyridine as Standard in Acidimetry

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4-Aminopyridine is a high melting (161° C.) nitrogen base with a dissociation constant of 1.3×10^{-5}. Methyl red indicator may be used with either 0.1*N* or 0.5*N* acid. The free base may be purified by recrystallization from toluene or benzene, or by sublimation at reduced pressure, and may be recovered easily after use. Standardization of acids with 4-aminopyridine yields normalities within 1 part per thousand of those found using sodium carbonate.

THE search for new substances which have the required properties of primary standards is never ending (*3*). This is particularly true with respect to bases suitable for the standardization of acids. The most recent suggestion, tris(hydroxymethyl)aminomethane (*4*), requires a mixed indicator which is unsatisfactory in the hands of students (*8*), because inexperienced people have trouble with the color change. 4-Aminopyridine satisfies most of the requirements for a primary standard.

4-Aminopyridine was first synthesized by Camps (*2*) in 1902. The most widely used synthesis was suggested by Koenigs and Greiner (*6*) and is based on the hydrolytic cleavage of 4-pyridylpyridinium dichloride to yield 4-aminopyridine and polymerized tarry material. Technical grade 4-aminopyridine is also available (Reilly Tar and Chemical Corp., Indianapolis, Ind.) 4-Aminopyridine is a weak, monoprotic base with an equivalent weight of 94.12. Tropsch (*9*) reported a dissociation constant of 1.3×10^{-5} at 25° C. from conductivity measurements, and Albert (*1*) cited data which lead to a value of 1.6×10^{-5}. 4-Aminopyridine is soluble in water and ethyl alcohol, and moderately soluble in benzene, toluene, and chloroform (*1*).

EXPERIMENTAL

4-Aminopyridine used in this work was prepared by the procedure of Koenigs and Greiner (*6*), and was recrystallized from benzene, ground to a powder, and dried for 2 hours at 105° C. before use. The melting point was 161° C. with a heating rate of 2° to 3° per minute.

A 6-liter carboy of approximately 0.1*N* hydrochloric acid solution was prepared by dilution of reagent grade concentrated acid and was protected against temperature changes. The exact normality was determined by the method of Kolthoff and Sandell (*7*), using Mallinckrodt primary standard grade sodium carbonate which was treated according to the directions of Kolthoff and Sandell (*7*) before use. The acid was also compared against carbonate-free sodium hydroxide solution, which had been standardized against potassium acid phthalate according to the method of Hillebrand, Lundell, Bright, and Hoffman (*5*).

All titrations in this work were made with one volumetric buret, which was calibrated at 25° C. in the normal way. All other volumetric equipment used was calibrated if necessary. The concentrations of indicator solutions were those normally used in analytical work. Indicator corrections were applied for phenolphthalein and bromocresol green used in the carbonate titration, but no correction was necessary with methyl red indicator. The pH titrations were made with a Beckman pH meter, Model H-2, equipped with a glass indicator electrode and a calomel reference electrode. All distilled water was boiled freshly before use.

All samples of 4-aminopyridine were dried at 105° C. for 2 hours, unless otherwise stated. All weighings were made with calibrated brass weights and were not corrected to vacuo.

STABILITY OF 4-AMINOPYRIDINE

Quantitative experiments to show the stability of 4-aminopyridine were carried out in the following manner. Samples of the original recrystallized material (the material after standing 96 days at room temperature, the material after 56 hours at 105° C., and some material kept in a closed bottle for 6 months) were dried 2 hours at 105° C. and titrated with approximately 0.1*N* hydrochloric acid solution. The normality of the acid was calculated assuming that all the samples of 4-aminopyridine were pure, and a value of 0.1020 was found from all samples. It, therefore, must be concluded that 4-aminopyridine is stable under the conditions described.

The hygroscopicity of 4-aminopyridine was observed by placing a weighed portion in a weighing bottle, storing the sample and another weighing bottle in a beaker (covered to keep out the dust) at room temperature, and weighing both the sample and the tare at frequent intervals. The tare was used to determine how much of the change was due to adsorption of moisture on the glass surface. The sample continuously lost weight over a period of 95 days, the total loss being 0.15%. During this time the temperature range was 22° to 31° C., and the relative hu-

midity range was 50 to 95%. It appears that 4-aminopyridine is not hygroscopic.

Since 4-aminopyridine has a definite vapor pressure as evidenced by its steam volatility, samples kept at 105° C. for drying would be expected to sublime. A sample of 4-aminopyridine was kept in an electric oven at 105° C. for 56 hours. A continuous loss of weight amounting to 0.2% per hour was observed. Therefore, drying times must be limited to conserve material.

The same experiments described above were carried out with 4-aminopyridine which had been sublimed at 55° to 60° C. and 10 mm. of mercury. Sublimation as a means of purification had been suggested by Wibaut, Herzberg, and Schlatmann (*10*). The same results were found using the sublimed material as the original material, except that the rates of loss were increased because the particle size was decreased.

DETECTION OF THE EQUIVALENCE POINT

A major requirement for a volumetric standard is that the equivalence point be easily detected. From previous work which reported a dissociation constant of 1.3×10^{-5} (*9*) for 4-aminopyridine, methyl red indicator appeared to be suitable. Weighed samples of 4-aminopyridine were dissolved in freshly boiled distilled water stirred with nitrogen, which had been passed through ascarite. Titrations were made with approximately 0.1*N* and 0.5*N* hydrochloric acid solutions. The pH of the solution was measured with a glass-calomel electrode pair after each addition of acid. Two drops of 0.1% methyl red indicator solution were also present. The results of these titrations are shown in Figure 1.

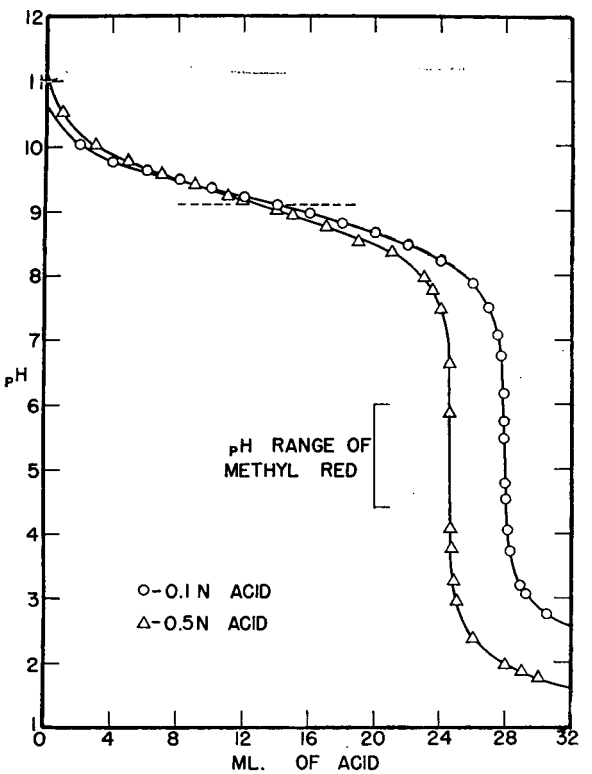

Figure 1. Titration of 4-aminopyridine with hydrochloric acid

From Figure 1 it is clear that methyl red is a satisfactory indicator with both 0.1*N* and 0.5*N* acids. The pH at the mid-point of both titration curves is 9.1 at 25° C. from which the dissociation constant is calculated to be 1.3×10^{-5} as previously reported.

The question of carbon dioxide interference had to be checked. Samples of 4-aminopyridine, which had been dried at 105° C. for 2 hours, were titrated to a methyl red end point under nitrogen and under air only. The normalities of the acid calculated from the titrations under both conditions were identical, and the sharpness of the indicator change was not affected by the presence of carbon dioxide from the air.

GENERAL RESULTS

A summary of the normalities found for a hydrochloric acid solution using a standard sodium hydroxide solution, sodium carbonate, and 4-aminopyridine is given in Table I. These results were obtained over a period of 6 months, thus do not contain a time bias. The results using sodium hydroxide solution and sodium carbonate check well, but the results using 4-aminopyridine are 1 part per thousand lower. However, the sum of the errors is probably 1 part per thousand, so that 4-aminopyridine appears to be worth while for further investigation in other laboratories.

Table I. Normality of Hydrochloric Acid Solution

			vs. 4-Aminopyridine	
	vs. NaOH[a]	*vs.* Na_2CO_3	Recrystallized	Sublimed
	0.10210	0.10209	0.10201	0.10201
	0.10210	0.10211	0.10202	0.10196
	0.10213	0.10214	0.10200	0.10196
	0.10210	0.10211	0.10201	0.10196
	0.10210	0.10213	0.10200	0.10196
	0.10210	0.10214	0.10194[b]	0.10193
	0.10216	0.10204	0.10195[b]	0.10198
	0.10210	0.10209	0.10195[b]	0.10191[b]
	0.10210	0.10210	0.10203[b]	0.10198[b]
	0.10216		0.10202[b]	0.10203[b]
			0.10203[b]	0.10196[c]
			0.10198[b]	0.10197[c]
Av.	0.10212	0.10211	0.10200	0.10197
Std. dev.	0.000026	0.000032	0.000033	0.000032

[a] 0.11437*N*, av. of 10 determinations *vs.* potassium acid phthalate std. dev. 0.000036.
[b] 4-Aminopyridine recovered from earlier titrations.
[c] 4-Aminopyridine sublimed twice.

AVAILABILITY OF 4-AMINOPYRIDINE

4-Aminopyridine is not available as a reagent grade chemical at this time. It is available in a technical grade (Reilly Tar and Chemical Corp.), and is not difficult to purify. Three recrystallizations from toluene or benzene, with activated carbon treatment the first time, yield long needles which after powdering and drying for 2 hours at 105° C. assay 100.0 ± 0.1% by titration with hydrochloric acid standardized with sodium carbonate.

RECOVERY OF 4-AMINOPYRIDINE

4-Aminopyridine may be recovered readily from titration residues. The solution is made alkaline to a pH of about 14 and evaporated to dryness. The dry residue is powdered, placed in a Soxhlet extractor, and exhaustively extracted with benzene. The extract is treated with activated carbon, filtered, and reduced to a small volume so that crystallization may occur. In a typical case, from 11.2 grams of 4-aminopyridine used in titrations 9.5 grams (85%) of material suitable for use were recovered. As seen in Table I, the recovered material was just as good as the original 4-aminopyridine.

RECOMMENDED PROCEDURE

The following procedure is designed for the standardization of 0.1*N* acid, but by increasing the amount of 4-aminopyridine may be used as well for 0.5*N* acid.

Weigh 0.4- ± 0.05-gram samples of 4-aminopyridine dried for 2 hours at 105° C. into 250-ml. flasks and dissolve in 100 ml. of freshly boiled distilled water. Add two drops of 0.1% methyl red indicator solution and titrate to the first change away from yellow. The milliequivalent weight of 4-aminopyridine is

0.09412. The indicator blank is normally negligible, but it should be checked.

SUMMARY

4-Aminopyridine is a solid, monoprotic base which is suitable as a standard for acidimetry when methyl red is used as the indicator in the titration.

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RECEIVED for review February 10, 1955. Accepted June 24, 1955. Abstracted from M.S. thesis submitted by Clayton E. Van Hall in June 1954 to Graduate Faculty of Michigan State University. Presented before the Division of Analytical Chemistry, 127th Meeting, ACS, Cincinnati, Ohio.

Complexometric Titration of Indium

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This investigation was undertaken to develop a rapid and accurate method for the titration of small amounts of indium. Indium may be titrated directly with (ethylenedinitrilo)tetraacetic acid using 1-(2-pyridylazo)-2-naphthol as an indicator at pH 2.3 to 2.5 or pH 7 to 8 with an accuracy within 0.5%. At pH 2.3 to 2.5, alkali metals, alkaline earth metals, aluminum, and manganese do not interfere. At pH 7 to 8, copper, zinc, cadmium, nickel, silver, mercury, and other metals which form very stable complexes with cyanide do not interfere if cyanide is added. Iron may be masked by the addition of fluoride. The common anions such as chloride, sulfate, nitrate, perchlorate, fluoride, tartrate, and citrate do not interfere. Bismuth, lead, gallium, and tin interfere.

A COMMON method for the determination of indium involves weighing the precipitate as indium sesquioxide after ignition of the hydroxide at 1100° to 1200° C. An amperometric method (*7*) and a flame photometric method (*6*) have been proposed. Recently, Flaschka and others (*2–4*) have reported the titration of indium by (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid) using eriochrome black T as the indicator and a lead solution for back-titration at pH 10. In the course of an investigation of the reaction of metals with 1-(2-pyridylazo)-2-naphthol (*1*) it appeared that this azo dye might be used as an indicator in the complexometric titration of indium.

This paper describes a simple and quick method for the direct titration of indium by (ethylenedinitrilo)tetraacetic acid using 1-(2-pyridylazo)-2-naphthol as the indicator. The titration is carried out at pH 2.3 to 2.5 using acetic acid as a buffer. Alkali metals, alkaline earth metals, aluminum, and manganese(II) do not interfere with the titration. The heavy metals which form strong complexes with cyanide do not interfere if the titration is made in the presence of cyanide at pH 7 to 8. Jentzsch and others (*5*) developed a simple scheme for the separation of indium. When this separation is followed by a complexometric titration, the result is a rapid and widely applicable analytical method for indium.

REAGENTS

(Ethylenedinitrilo)tetraacetic acid solution, 0.01*M*. Approximately 3.72 grams of the disodium salt of (ethylenedinitrilo)tetraacetic acid were dissolved in water and diluted to 1 liter. This solution was then standardized against the standard indium solution using the procedure recommended in this paper.

Standard indium solution, 0.01*M*. An accurate weight of 1.1 to 1.2 grams of indium metal (99.95%) in a small amount of hydrochloric acid was heated on a hot plate and diluted to 1 liter with water. The pure indium metal was obtained from the Indium Co. of America.

Indicator solution, 0.01%. Approximately 0.001 gram of 1-(2-pyridylazo)-2-naphthol was dissolved in 10 ml. of methanol. The indicator solution is very stable. The preparation of the azo dye has been described (*1*).

Sodium hydroxide, 1*N*.

All other chemicals used were of reagent grade.

PROCEDURE

Titration at pH 2.3 to 2.5. The solution containing 0.05 to 0.2 millimole of indium in a 200-ml. beaker was diluted to approximately 50 ml. and neutralized with 1*N* sodium hydroxide until a slight white precipitate was formed. Two milliliters of glacial acetic acid were added to dissolve the precipitate. The solution was titrated with (ethylenedinitrilo)tetraacetic acid solution after addition of 2 drops of the indicator solution. The end point from red to pure yellow was very sharp.

Titration at pH 7 to 8. In the presence of copper, zinc, nickel, and other metals which form strong complexes with cyanide, the titration of indium in alkaline medium using cyanide to mask the interference is recommended. The solution containing 0.05 to 0.2 millimole of indium in a 200-ml. beaker was diluted to approximately 50 ml. and adjusted to pH 7 to 8 using acetic acid and ammonium acetate after the addition of a suitable amount of potassium cyanide and approximately 1 gram of potassium sodium tartrate. The solution was titrated with (ethylenedinitrilo)tetraacetic acid solution after addition of 2 drops of the indicator solution. The end point was also from red to pure yellow. The calculation may readily be made according to a 1 to 1 ratio of the indium-(ethylenedinitrilo)tetraacetic acid complex.

DISCUSSION

Effect of pH. Flaschka and Amin (*3, 4*) used eriochrome black T as the indicator, which requires a pH of 10 for detecting the end point. It was found, by the author, that indium was still strongly complexed by (ethylenedinitrilo)tetraacetic acid at pH 2 and that indium could be titrated by (ethylenedinitrilo)-tetraacetic acid at a wide range of pH between 2 to 10 using 1-(2-pyridylazo)-2-naphthol as an indicator. No sharp end point was obtained when the solution was adjusted to pH 1.5 or below because the indium-indicator complex is not stable at that low pH. It was also impossible to titrate indium at very high pH because the formation of indium hydroxide caused a slow end point and because the indicator itself is an acid-base indicator; it changes

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Table I. Titration of Indium in Presence of Foreign Metals

Metal Added, Mg.	Indium, Millimole Taken	Found[a]	Error, %
None	0.0520	0.0522	+0.38
None	0.1040	0.1040	0.00
None	0.1560	0.1558	−0.13
None	0.2080	0.2070	−0.48
Al, 10	0.0520	0.0522	+0.38
Ca, Mg, Ba, Sr, 100 each	0.0520	0.0520	0.00
Mn(II), 10	0.0520	0.0521	+0.19
Fe(III), 1	0.0520	0.0523	+0.58
Cu(II), 10	0.0520	0.0520	0.00
Zn(II), 10	0.0520	0.0518	−0.38
Ni(II), 10	0.0520	0.0521	+0.19
Cd(II), 10	0.0520	0.0521	+0.19
Co(II), 10	0.0520	0.0523	+0.58

[a] Average of triplicates.

color from yellow to pinkish at pH above 11. The selection of pH for the titration depends upon the presence of interfering metals.

Interferences. When eriochrome black T is used as the indicator in the complexometric titration of indium at pH 10, interferences from aluminum, manganese, alkaline earth metals, and iron would be expected because they form colored complexes with the eriochrome black T indicator. Furthermore, the titration should be made in the boiling solution. It was found that these metals did not form colored complexes with 1-(2-pyridylazo)-2-naphthol and were not strongly complexed by (ethylenedinitrilo)-tetraacetic acid in the acid medium. Therefore, indium may be titrated with (ethylenedinitrilo)tetraacetic acid using 1-(2-pyridylazo)-2-naphthol as the indicator in the presence of these metals at pH 2.3 to 2.5.

When the titration is made at pH 7 to 8 and suitable amounts of cyanide are added, indium may be titrated in the presence of copper, zinc, nickel, cadmium, cobalt, silver, mercury, and other metals which form very stable complexes with cyanide. Some typical results are also shown in Table I. A slow end point was encountered if the titration was made in the absence of tartrate at high pH. Iron(III) interference could be eliminated if the titration was made at pH 7 to 8 and 1 gram of potassium fluoride, 1 gram of potassium sodium tartrate, and a small amount of potassium cyanide were added. The common anions such as chloride, sulfate, nitrate, perchlorate, fluoride, tartrate, and citrate did not interfere. Bismuth, lead, gallium, and tin interfered.

Accuracy. By using a microburet, an accuracy of 0.5% or better was obtained for 0.05 to 0.2 millimole of indium.

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RECEIVED for review May 5, 1955. Accepted July 13, 1955.

Flame Photometric Determination of Silver in Cadmium and Zinc Sulfide Phosphors

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Because silver is a common activator for sulfide-type phosphors, a rapid method for its determination was desired. This paper describes a flame photometric method for silver which is both rapid and accurate. No separations are required. The interferences from other ingredients in the sample have been studied and methods developed for overcoming these interferences.

NEARLY all the flame photometric methods reported in the literature have been for the determination of alkali and alkaline earth metals. Extensive coverage has been given to the determination of these elements in a wide variety of materials and to the interferences caused by other metals and anions in the samples. With the advent of photomultiplier tubes and improved burners, the sensitivity of the flame photometer has been greatly increased, making possible the determination of small amounts of many other elements. The rather low detection limit reported for silver (*4*) made such a determination feasible in the sulfide-type phosphors. Silver is commonly used as the activator in zinc sulfide and zinc cadmium sulfide phosphors, in a concentration on the order of approximately 0.01%. This paper presents a rapid method for accurately determining small amounts of silver in these phosphors without troublesome separations.

APPARATUS AND REAGENTS

Beckman Model DU spectrophotometer equipped with a Model 9200 flame photometry attachment, hydrogen-oxygen burner, and photomultiplier attachment.

Zinc sulfide, silver-free, may be made by precipitating zinc from a solution of zinc sulfate (conforming to ACS specifications) with hydrogen sulfide, washing with water, and drying at 105° C.

Cadmium sulfide, silver-free, may be made by precipitating the cadmium from a solution of ACS grade cadmium sulfate with hydrogen sulfide, followed by washing with water and drying at 105° C.

Standard silver solution, 50 γ of silver per ml. Dissolve 0.1575 gram of ACS grade silver nitrate in 2 liters of distilled water.

Sodium chloride solution, 100 γ of sodium per ml. Dissolve 0.2542 gram of ACS grade sodium chloride in 1 liter of distilled water.

Magnesium chloride solution, 1.0 mg. of magnesium per ml. Dissolve 8.36 grams of ACS grade magnesium chloride hexahydrate in 1 liter of distilled water.

EXPERIMENTAL

Silver exhibits two relatively strong flame emission lines, one occurring at 328.1 mμ and the other at 338.3 mμ. Of the two, the former is somewhat less intense and has a higher flame background; hence, the line at 338.3 mμ was chosen for all experimental work. Under the conditions employed the flame emission is directly proportional to the silver concentration in the range 0 to 500 γ of silver per 100 ml. of solution (Figure 1).

Use of Acetone to Increase Sensitivity. Previous workers have shown the effect of many organic solvents on flame emission (*1–3, 5*). In an attempt to increase the sensitivity for silver, a number of organic liquids miscible with water, including acetone, methyl ethyl ketone, methanol, ethyl alcohol, and ethylene gly-

Table I. Effect of Acetone Concentration on Flame Emission of Silver at 338 Mμ

Acetone, Ml./100 Ml. Soln.	Relative Luminosity at 338 Mμ
0	30[a]
15	45
25	50
40	60
50	71

[a] 250 γ of silver in each solution. Instrument control settings identical for all solutions.

col, were added to the sample solutions. Of these, the acetone and methyl ethyl ketone caused the greatest enhancement of silver emission. Although the latter was somewhat better in this respect, acetone was chosen because it is more readily available and less expensive. Table I shows the effect of varying the acetone concentration on the relative luminosity at 338 mμ.

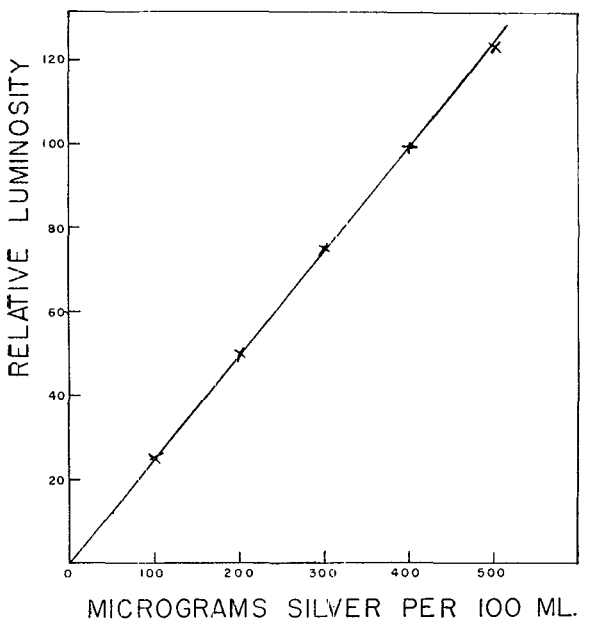

Figure 1. Flame intensity at 338 mμ *vs.* silver concentration

In each case, the relative luminosity reading is a net reading compared against a blank containing the same amount of acetone but no silver. This eliminates the effect of background radiation, which changes seriously with a change in the acetone-water ratio. The background radiation in each case is adjusted to zero by employing the zero suppression switch incorporated in the photomultiplier attachment. This allows the use of the entire transmittance scale for silver intensity readings.

The presence of acetone in the sample solutions causes slightly greater fluctuations in the flame intensity than normal with a water solution. The intensity readings change rather quickly once the solution is poured into the sample beaker, probably because of volatilization of acetone which causes a change in the water-acetone ratio. Hence it is very important that the volumetric flask containing the sample solution be tightly stoppered prior to the determination and that the luminosity reading be taken immediately after the solution is poured into the sample beaker. Because of this volatility interference, 30 ml. of acetone rather than 50 was added for each 100 ml. of solution, even though 50 ml. gave somewhat greater sensitivity.

Effect of Sodium and Magnesium. Sodium chloride and magnesium chloride are commonly used as fluxes in the firing of sulfide phosphors. Although the phosphors are washed after firing, it is difficult to remove residual flux completely by washing with water. In the samples analyzed, the sodium content was generally around 0.040% while the magnesium content approximated 0.12%. The effect of this sodium and magnesium on the luminosity readings at 338 mμ was studied. Both these elements increase the luminosity at 338 mμ, as shown in Tables II and III. An examination of the data shows that the increased readings at 338 mμ are due not to any enhancement of silver emission by these elements but rather to an increase in the background radiation. Magnesium has a weak emission band throughout the 338-mμ region and, hence, its presence tends to increase the intensity reading at the position of silver emission.

The effect of sodium on the background radiation is shown more clearly in Table IV. Although sodium exhibits a relatively weak emission line at 330 mμ, the radiation from this line falls off sharply and, at the slit width used (0.2 mm.) should be down to zero at about 332 mμ. Hence the increase in background radiation is due to some other cause, probably to continuum interference from the sodium. (The rather high readings at 330 mμ are due largely to a very strong flame background in the region of 300 to 330 mμ. This flame background tapers off rapidly at 330 mμ but is still moderately strong at 338 mμ, although the zero suppressor may be used to cancel out the background.)

This interference from sodium and magnesium can be compensated for in two ways. If the concentration of these two elements in the sample is known from previous analyses (also by a flame photometric procedure), the exact amount of each may be added to the blank and to the standard solution. If these concentrations are unknown, compensation may be made by measuring the light intensity close to the line on both sides and subtracting the interpolated intensity at 338 mμ. Such a procedure is justified in this case, as the background radiation in this region is more or less continuous or bandlike.

Table II. Effect of Sodium on Silver Emission

Silver Concn., P.P.M.	Sodium Concn., P.P.M.	Relative Luminosity at 338 Mμ
0	0	0[a]
5	0	92
5	5	94
5	10	96
5	15	96
5	20	97
0	20	5

[a] Adjusted to zero by means of zero suppressor switch and dark current adjustment.

Table III. Effect of Magnesium on Silver Emission

Silver Concn., P.P.M.	Magnesium Concn., P.P.M.	Relative Luminosity 335 mμ	338 mμ	341 mμ
0	0		0[a]	
5	0	0	112	0
5	30	7	119	7
5	60	13	124	12
5	120	22	132	20
5	180	31	141	30

[a] Adjusted to zero by means of zero suppressor switch and dark current adjustment.

Effect of Zinc and Cadmium. Small changes in the ratio of zinc to cadmium have a negligible effect on the silver emission. However, it is advisable to prepare standards having the approximate zinc and cadmium concentrations found in the sample.

Solution of Sample. Hydrochloric acid proved to be very effective in dissolving the samples, giving clear solutions requiring no filtration. The small amount of silver chloride formed is readily soluble in the large excess of hydrochloric acid employed, owing to the formation of a complex argentichloride. Nitric acid is less satisfactory, as free sulfur is always formed which must be filtered off and may tend to adsorb or occlude some of the silver.

The amount of hydrochloric acid used is not critical. Con-

Table IV. Effect of Sodium on Flame Background

Wave Length, Mμ	Relative Luminosity[a]		
	No sodium	20 p.p.m. sodium	Difference
330	82	125	43
331	44	51	7
332	33	40	7
335	5	10	5
338	5	10	5
341	0	5	5

[a] No silver present. Solutions contain only water, hydrochloric acid, acetone, and sodium chloride.

centrations from 10 to 30% by volume gave almost identical intensity readings for solutions containing 8 p.p.m. of silver.

PROCEDURE

Weigh into a 100-ml. borosilicate glass beaker an amount of the sulfide phosphor which will contain 50 to 500 γ of silver (generally 1 to 5 grams). Add 5 ml. of distilled water, then 30 ml. of concentrated hydrochloric acid, and cover immediately with a 9-cm. borosilicate glass cover glass. Add several small glass beads to prevent bumping later. Place the sample on a steam plate until completely decomposed, then place on a wire gauze over a low flame and boil gently for several minutes until the solution is perfectly clear. After cooling the beaker in a tray of cool water, quantitatively transfer the contents into a 100-ml. volumetric flask containing 30.0 ml. of acetone, dilute to the mark with water, and mix well.

Table V. Recovery of Silver by Flame Photometric Method

Sample[a]	Silver Added, γ	Silver Found, γ	% Error
ZnS	435	440	+1.1
	320	312	−2.5
	55	47	−14.5
	195	186	−4.6
	460	465	+1.1
	55	61	+11
ZnCdS	325	332	+2.2
	110	120	+9.1
	200	212	+6.0
	100	102	+2.0

[a] 5 grams of sample used in each case.

Measure the relative luminosity of the solution at 338 mμ. Control settings will vary with different instruments. However, the following conditions were found to be optimum for this investigation and may serve as a guide: oxygen pressure, 10 pounds; hydrogen pressure, 7 pounds; slit width, 0.20 mm.; sensitivity control at counterclockwise limit; selector switch at 0.1; photomultiplier at full sensitivity; zero suppression switch at No. 1 position. Allow the instrument to "warm up" for 10 to 15 minutes before taking measurements.

Keep the shutter open throughout all subsequent procedure and make all luminosity measurements immediately after placing the solutions in the sample beakers.

Place in atomizing position a "blank" solution carried through the entire procedure simultaneously with the unknown and containing approximately the same amount of zinc and/or cadmium sulfide, hydrochloric acid, and acetone. Set the transmittance control at 0% T and balance the needle by rotating the dark current control knob. Subsequently, determine the relative luminosity on a "standard" solution of the same composition but also containing 500 γ of silver. Finally, obtain the reading on the sample solution. Rinse the burner frequently with distilled water between readings. Repeat all readings on fresh samples to be sure they are reproducible.

Because the sodium and magnesium generally found in the samples affect the silver luminosity readings, a correction must be made for these impurities. This may be done in the following manner:

Obtain the luminosities for the sample and the standard at both 335 and 341 mμ and interpolate to obtain the luminosity at 338 mμ. The difference between the interpolated readings represents that part of the silver luminosity reading due to sodium or magnesium interference and should be subtracted from the reading obtained on the sample at 338 mμ to give the net silver luminosity.

If both the sodium and magnesium concentrations in the sample are known from previous analyses, the proper amount of each may be added to the blank and to the standard. Such a procedure would eliminate the need for a correction.

Determine the silver concentration of the sample by comparing the net relative luminosity of the sample with that of the standard. Within the range employed the silver luminosity is directly proportional to the silver concentration.

A typical set of data together with the method of calculation is shown below:

Wave Length, mμ	Luminosity Readings	
	Sample	Standard
338	92	100
335	11	3
341	5	1
Interpolated reading at 338 mμ	8	2

Increase in background due to sodium and magnesium 8 − 2 = 6
Sample reading corrected for sodium and magnesium background 92 − 6 = 86
Silver present, $\frac{86}{100} \times 500 = 430$

At first it might appear that a background correction should also be made for the standard—i.e., that the reading of 2 should be subtracted from the reading of 100 on the standard to give a net reading of 98. However, this would be incorrect. The background of the blank has already been adjusted to zero by means of the zero suppressor. Because the blank and the standard contain no sodium or magnesium and are alike in every respect except silver, the reading of 100 is due entirely to silver. The low readings on the standard at 335 and 341 mμ are not due to any additional background but rather to a "tailing off" of the silver emission line. This effect should be the same on the sample. Therefore, the increase in intensity on the sample at 335 and 341 mμ is due to sodium and magnesium. The increased background should be subtracted only from the sample reading at 338 mμ.

RESULTS

In order to test the accuracy of the method, recovery studies were made on both zinc sulfide and zinc cadmium sulfide phosphors. Known amounts of silver, ranging from 55 to 460 γ, were added to silver-free samples of these phosphors, which contained approximately 0.04% sodium and 0.12% magnesium. The samples were then carried through the entire procedure and the silver was determined. The results are listed in Table V.

For these analyses the largest deviation was only 12 γ, indicating that the accuracy is entirely satisfactory. The results are reproducible within 2 or 3 scale divisions corresponding to 10 to 15 γ of silver. The relative error is greatest for low silver concentrations, so that a practical limit of 5 to 10 p.p.m. in the phosphor samples should be set.

The chief advantage of this method is its speed. The time required to analyze five to six samples simultaneously, including weighing and solution of the samples, is only about 2 hours.

ACKNOWLEDGMENT

The author is deeply grateful to Gerrit Dragt for his helpful suggestions in preparing this manuscript.

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RECEIVED for review September 23, 1954. Accepted July 1, 1955.

Analytical Determination of Some Elastomeric Components in Aqueous Extracts

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The use of elastomers in the manufacture of specialty papers has stimulated the development of analytical methods for the determination of various elastomer components which may be extracted by contact with aqueous solutions. Analytical methods are described for the determination of trace quantities of zinc, phenothiazine, *p-tert*-butylcatechol, a disproportionated rosin (Resin 731 SA), and the sodium salts of condensed mononaphthalenesulfonic acid (Lomar PW), and 2-anthraquinonesulfonic acid (Silver Salt) in aqueous mixtures. The first four components are measured colorimetrically. The latter two components are determined by simultaneous ultraviolet spectrophotometry. The methods are capable of reliably detecting the above materials below 0.5 p.p.m. The synthesis and application of *m*-(*p*-dimethylaminophenylazo)benzyltrimethylammonium chloride as a new reagent for the disproportionated rosin are described.

THE use of elastomers such as GR-S, natural rubber, neoprene, and butadiene-acrylonitrile polymers in the manufacture of specialty papers has become of increasing importance in recent years. Although natural rubber had been of interest for impregnating fibrous materials as early as 1824, it was not until the work of Kaye (*29, 30*) that serious consideration was directed toward the incorporation of synthetic latices into paper pulp to produce products in which cellulose is the continuous phase. Owens (*41*) studied the effect of styrene-butadiene ratio of Buna S latices. Walsh, Abernathy, Pockman, Galloway, and Hartsfield (*64*) found the addition of neoprene (polychloroprene) latex in the beater gave paper sheets of superior wet strength and other physical properties. This work with polychloroprene latex was favorably extended by Wheeler, Borders, Swanson, and Sears (*65*). Oliner and O'Neil (*39*) and Yost and Aiken (*67, 68*) compared properties and studied the effects of variables on beater addition of butadiene-acrylonitrile polymers.

Since generally the addition of an appropriate elastomer or elastomer combination appreciably increases the tensile, burst, fold, and tear strength of paper, diversified applications have been found for these papers for many essential services such as food wrappers. This application required the development of analytical methods to obtain information on the extractability of elastomer components in various solvents, particularly for trace amounts of these components which are used in the manufacture of synthetic elastomers. This information would determine possible contamination of packaged products by the elastomer components and would aid in interpretation of changes in physical properties of treated papers or elastomer films on exposure to these solvents.

Paper sheets treated with one or more elastomers were individually extracted with six aqueous solvents. These extractants simulated components and conditions of acidity and basicity present in many food mixtures. The final extracts were analyzed for trace quantities of elastomer components by analytical methods described. The components of principal interest in this work included zinc, phenothiazine (thiodiphenylamine), *p-tert*-butylcatechol, a disproportionated rosin (Resin 731 SA), and the sodium salts of a condensed mononaphthalenesulfonic acid (Lomar PW) and 2-anthraquinonesulfonic acid (Silver Salt).

GENERAL CONSIDERATIONS

Extracts. The aqueous solvents employed for extraction included: water; Ringer's solution (8.6 grams of sodium chloride, 0.3 gram of potassium chloride, and 0.33 gram of calcium chloride per liter of final solution) (*61*); sodium carbonate, pH 10; acetic acid, pH 3; lactic acid, 5%; and sucrose solution, 5%. These extractants simulated components and conditions of acidity and basicity present in many food mixtures. Extractions were carried out also with a dill pickle juice to which the described procedures were applied successfully.

The aqueous solutions were prepared for analysis by extracting approximately 900 grams of elastomer-treated paper with 1800 grams of the particular solvent. The extractions were performed in large glass-stoppered borosilicate glass tubes ($2^3/_4 \times 55$ inches) charged with the paper-solvent mixture by rotating end to end for 7 days in a room conditioned to the temperature of extraction. At the end of the extraction, the solutions were filtered and analyzed for the various components mentioned.

Apparatus. CARY RECORDING SPECTROPHOTOMETER, Model 11, Applied Physics Corp., Pasadena, Calif., fitted with appropriate 5-cm. matched quartz cells.

PHOTOELECTRIC COLORIMETER. A Cenco-Sheard-Sanford photelometer was used. The 5-cm. rectangular cells were held in position by two blocks of plywood ($6 \times 5 \times 1.5$ cm.) so that they were transversed by light lengthwise in the conventional cell holder. During transmittance measurements, the usual diaphragm of the instrument was placed in position to transmit light through its standard rectangular aperture of 5×15 mm.

Any good photoelectric colorimeter or spectrophotometer that can accommodate 5-cm. cells may be used.

CELLS, absorption, rectangular, matched $55 \times 50 \times 10$ mm., Will Corp., New York 12, N. Y. These cells were used only with the Cenco instrument.

Reagents. All reagents used in this work were of analytical reagent grade. Solvents—e.g., chloroform and *n*-butyl alcohol—were carefully distilled prior to use to eliminate impurities.

Sample Calculations. The final concentrations of zinc, *p-tert*-butylcatechol, phenothiazine, and Resin 731 SA are calculated as follows:

$$\frac{A - B}{S} = \text{parts per million}$$

where A = component found in sample as read from standard curve, micrograms
B = component found in blank as read from standard curve, micrograms
S = sample weight, grams

As a corrective blank, the particular solvent used to extract the elastomer film or elastomer-impregnated paper should be analyzed simultaneously with the elastomer extract samples in order to calculate the actual quantity of the particular neoprene component extracted from the film or impregnated paper.

The calculations for condensed mononaphthalenesulfonic acid and 2-anthraquinonesulfonic acid are considered in their section.

Statistical Treatment of Data. An analysis of variance (*11*) was performed on each set of data in order to detect variability due to solvent effects. In this way a random standard deviation, s_r, and confidence interval, L, were calculated for each table at the particular concentration level at which the analytical methods were tested. The random standard deviation repre-

sents the precision to be expected if any food solvent taken from among those represented by the various types studied were to be analyzed.

ZINC

The determination of zinc in small quantities has been attempted by a number of techniques which include nephelometry (*16*), turbidimetry (*3*), polarography (*5, 8, 17, 36, 45, 58*), spectrography (*36, 49, 53, 55*), amperometry (*37*), coulometry (*20*), fluorometry (*47*), flame spectrophotometry (*2*), and colorimetry with reagents such as di-2-naphthylthiocarbazone (*6*), 5-nitroquinaldic acid (*32*) and dithizone (phenylazothionoformic acid phenylhydrazide) (*9, 18, 24, 26, 48, 50, 51, 62*). Colorimetric methods employing dithizone are extremely sensitive, and with appropriate control of pH and interfering ions very satisfactory results may be obtained.

In this investigation zinc was determined in aqueous extracts by the mixed-color dithizone method (*48, 50*). As the details of this procedure are extensively discussed in the literature only the sample preparation is presented here. The liquid sample or dissolved ash was extracted with a carbon tetrachloride solution of dithizone-complexing metals. The extract was shaken with dilute hydrochloric acid to separate zinc, small quantities of lead and cadmium into the aqueous phase from copper, cobalt, and nickel which remained in the carbon tetrachloride. The acid solution was finally extracted with a carbon tetrachloride solution of dithizone in the presence of diethyldithiocarbamate and ammonium citrate at pH 8.5 to 9. In this step, the red zinc dithizonate was extracted into the carbon tetrachloride layer away from the dithizonates of lead, cadmium, and other metals and determined colorimetrically.

Sample Preparation. A. Extracts of water, sodium chloride (5%) sodium carbonate (pH 10), acetic acid (pH 3).

Weigh a 5-gram sample into a platinum dish. Add 10 ml. of 1*N* hydrochloric acid (more if necessary) and heat on a steam bath until all substances soluble in hydrochloric acid are brought into solution. Continue as directed under B with "Filter off any insoluble matter, etc."

B. Extracts of sugar solution (5%), lactic acid (5%), and other solutions containing organic matter.

Ash a 5-gram sample in a platinum dish in an electric muffle furnace at 500° to 550° C. Dilute solutions should be carefully evaporated to prevent spattering until carbonization occurs. Wet the cooled ash with 1 to 3 ml. of distilled water, then add 10 ml. of 1*N* hydrochloric acid (more if necessary) and heat on a steam bath until all substances soluble in hydrochloric acid are brought into solution. Add 5 ml. of hot water. Filter off any insoluble matter on a 7-cm. filter paper (Whatman No. 42 or equivalent) which has been washed with two 5-ml. portions of hot 1*N* hydrochloric acid, then washed with hot water until free of hydrochloric acid. Collect the filtrate in a 100-ml. volumetric flask, and wash the filter with hot water until washings are no longer acid to methyl red. Add a drop of methyl red indicator to the filtrate in the 100-ml. flask, next add 1*N* ammonium hydroxide until neutral to methyl red, then add 4 ml. of 1*N* hydrochloric acid. Allow the contents of the flask to cool, then adjust the volume to the 100-ml. mark with water. Aliquots of this diluted sample are analyzed for zinc by a mixed-color (*9, 50*) or mono-color dithizone method (*51*).

Discussion. Upon analysis of four known samples in 5% lactic acid solution at the 10-p.p.m. level by the mixed-color dithizone method, the confidence interval for average of duplicate determinations was ±0.74 p.p.m. at a 95% probability. The accuracy was completely within the limits of the precision of the method. At the 50-p.p.m. level the confidence interval for duplicate determinations was ±0.43 p.p.m. zinc as indicated by five analyses. The accuracy at this level is approximately 98%.

For most accurate analyses, all glass apparatus used in the application of this method should be thoroughly cleaned. The apparatus should be rinsed with sulfuric acid followed by four or five rinses with distilled water, and finally be given a rinse with zinc-free water. If chromic acid is used for cleaning glassware, it must be completely removed by thoroughly rinsing with zinc-free distilled water. Also, all reagents should be carefully purified to be free of zinc contamination. Zinc-free distilled water and some organic reagents may be conveniently prepared by percolation through IR-120 or IR-410 resin columns (*62*).

One hundred micrograms of copper, lead, mercury, bismuth, cobalt, nickel, and tin ions do not interfere. When more than 15 to 25 γ of cadmium are present high results are obtained. Most elastomer extracts should not contain large concentrations of the above metals. When large concentrations of interfering metals are present a mono-color method (*51*) should be considered. Iron and aluminum do not interfere.

p-tert-BUTYLCATECHOL

Catechols may be added to elastomers to act as stabilizing agents. A number of methods are available for the determination of phenolic derviatives in both high and low concentrations. In low concentrations the methods of Gibbs (*21*) and Snell (*57*) using 2,6-dibromoquinone chloride have been widely applied. In alkaline media 2,6-dibromoindophenols are formed with phenolic compounds having substituted para positions. Emerson (*14*), Gottlieb (*23*), and Ettinger (*15*) used 4-aminoantipyrine. This method has the disadvantage of being sensitive to pH variables, giving a test color in the absence of test material. The sensitivity of this method is below 1 p.p.m.

Catechols in alkaline solution readily absorb oxygen with the formation of colored compounds. *p-tert*-Butylcatechol gives a stable pink to blood-red coloration that is proportional to catechol present at optimum concentrations. Color apparently is caused by the oxidation of the dihydroxy molecule to the quinoid. The quinoid structure readily reverts to the hydroxy structure in acid media. Colors obtained by quinoid formation in basic media were weak below 1 p.p.m. and lacked acceptable sensitivity.

A quantitative diazometric method for the determination of phenolic derivatives has been described by Theis (*60*) and Deichman and others (*12*). In this method the phenolic compound is coupled in basic media to diazotized *p*-nitroaniline to give a red color, which may be measured spectrophotometrically. Because of the ready availability of reagents and apparent simplicity of the coupling reaction, this technique was employed.

Reagents. Sodium acetate solution, 31%.
Sodium carbonate, 20%.
Hydrochloric acid 0.1*N*.
Acetic acid 0.1*N*.

p-Nitroaniline solution. Dissolve 1.5 grams (Eastman Kodak No. 179, melting point = 147–148° C.) in 40 ml. of concentrated hydrochloric acid and dilute to 500 ml. with water in a glass-stoppered volumetric flask.

Diazotized *p*-nitroaniline solution. Deliver 25 ml. of *p*-nitroaniline solution into a 50-ml. Erlenmeyer flask. Cool the solution in an ice bath for 10 to 15 minutes. Add 1 ml. of sodium nitrite solution, and agitate the solution. It is suggested that this solution be prepared just before use and held in the ice bath.

p-tert-Butylcatechol. Purify this material to white crystals by vacuum sublimation.

Procedure. Into a 125-ml. Squibb separatory funnel carefully weigh a sample to contain approximately 50 γ of *p-tert*-butylcatechol. Add 5 drops of 0.1*N* acetic acid and gently mix the solution. Extract the test solution with two 10-ml. portions of ethyl ether. Deliver the ether extract into a 25-ml. glass-stoppered volumetric flask. Add 1 ml. of 95% ethyl alcohol and 1 ml. of 0.1*N* hydrochloric acid. Immerse the bulb of the flask in a water bath at 50° to 55° C. After the ethyl ether has evaporated remove the flask from the water bath, and add 5 ml. of water and 1 ml. of sodium acetate solution. Cool the solution for 10 minutes in the ice bath. Add 1 ml. of diazotized *p*-nitroaniline solution. Mix the solution and add 2 ml. of 20% sodium carbonate. Agitate the solution for about 1 minute in the ice bath. Remove the flask from the bath, cool the flask contents to room temperature, and dilute to the 25-ml. mark with water. Determine the per cent light transmittance of the solution with a photoelectric colorimeter equipped with 5-cm. cells and a 525-mμ green filter. To standardize the instrument to 100% transmittance, prepare a reference blank solution by

performing all the steps of the analysis except the ether extraction. Read the micrograms of *p-tert*-butylcatechol corresponding to the per cent transmittance from a calibration curve.

CALIBRATION. Weigh 50.0 mg. of pure *p-tert*-butylcatechol into a 100-ml. glass-stoppered volumetric flask. Dissolve completely in 95% ethyl alcohol and dilute to the mark with the same alcohol. One milliliter of this solution contains 500 γ of *p-tert*-butylcatechol. Dilute 10 ml. of this solution to 100 ml. with 95% ethyl alcohol in a glass-stoppered volumetric flask. This diluted standard (50 γ of *p-tert*-butylcatechol per ml.) is to be used in preparing the standard curve. These solutions should be protected from light by using amber-glass volumetric flasks.

Employing a 1-ml. Mohr calibrated pipet, put 0, 0.10, 0.20, 0.40, 0.60, 0.80, and 1.00 ml. of the diluted standard into 25-ml. glass-stoppered flasks. Now add 1.00, 0.90, 0.80, 0.60, 0.40, 0.20, and 0.00 ml. of 95% ethyl alcohol, respectively. The flasks contain 0, 5, 10, 20, 30, 40, and 50 γ of *p-tert*-butylcatechol, respectively. Add 1.0 ml. of 0.1*N* hydrochloric acid, 5 ml. of water, and 1.0 ml. of sodium acetate solution. Continue as described in the regular procedure starting with "cool the solution for 10 minutes."

The standard curve (Figure 1) is constructed by plotting micrograms of *p-tert*-butylcatechol against per cent light transmittance on semilogarithmic graph paper.

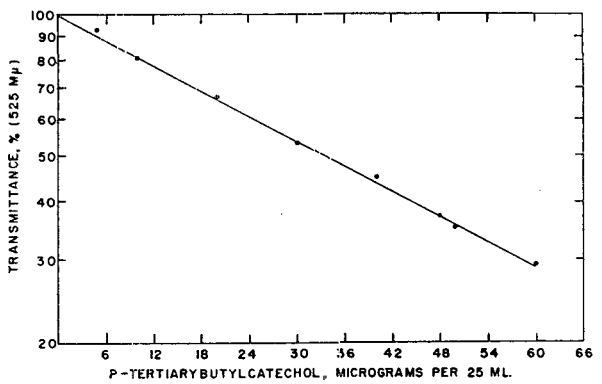


Figure 1. Calibration curve for *p-tert*-butylcatechol determination

Experimental. For accurate analyses the pH of the solution during extraction of the sample and the ethyl alcohol concentration present during the coupling reaction should be controlled. To obtain quantitative extraction of *p-tert*-butylcatechol the solution must be acidic (< pH 5). Above pH 7, the catechol recovery may be as much as 60% low. The presence of acidified aqueous ethyl alcohol was found necessary to prevent the loss of catechol after the ethyl ether had evaporated. If more than 5 ml. of 95% ethyl alcohol were present during the coupling reaction, a white precipitate formed together with an extraneous brownish color which erratically affected both sample and blank. Good results were obtained when 1 to 2 ml. of 95% ethyl alcohol were used. The addition of 5 ml. of water was necessary before adding diazotized *p*-nitroaniline to prevent insoluble salt formation after the addition of sodium acetate. More reproducible colors were obtained with sodium carbonate than with an alkali metal hydroxide. Figure 2 contains the spectrum of a solution resulting from the coupling of *p-tert*-butylcatechol with diazotized *p*-nitroaniline. It may be seen that the absorption maximum is at 466 mμ. Figure 1 indicates that a good standard curve may be obtained with the above procedure at 525 mμ for *p-tert*-butylcatechol when present in concentrations of 0 to 2 γ per ml. Even higher sensitivity would be attained at 466 $\pm$ 5 mμ.

Results. The method was evaluated at two levels of concentration. Analyses were run with each solvent after the addition of known quantities of *p-tert*-butylcatechol. The results are presented in Table I.

Discussion. Table I indicates that accurate recovery of *p-tert*-butylcatechol may be obtained at both 1- and 25-p.p.m. levels. Statistical variance analysis of the data presented in Table I indicated no systematic errors.

Table I. *p-tert*-Butylcatechol Determination in Various Solvents by Diazometry

Solvent	*p-tert*-Butylcatechol, P.P.M. Present	Found	Present	Found
Water	1.2	1.0	25.0	23.9
		1.2		27.5
Ringer's solution		1.2		29.0
		1.2		31.5
Sodium carbonate, pH 10		0.9		27.5
		1.2		26.0
Lactic acid, 5%		1.4		31.5
		1.4		33.9
Acetic acid, pH 3		1.2		27.5
		1.2		33.9
$\bar{X}$		1.2		29.2
s_r		0.16		3.45
L		$\pm$0.25		$\pm$5.38
P		95%		

$$\bar{X} = \frac{\sum_{i=1}^{N} Xi}{N} = \text{mean}$$

s_r = standard random deviation (*12*)

$L = \pm s_r \frac{t_{100-P}}{N}$ = confidence interval for average of duplicate determinations (N = 2) at a probability level P

P = probability level, % (95% used here)

t_{100-P} = "student's t" at a significance level 100 $-$ P (corresponding to a confidence interval of P = 95%)

N = number of determinations

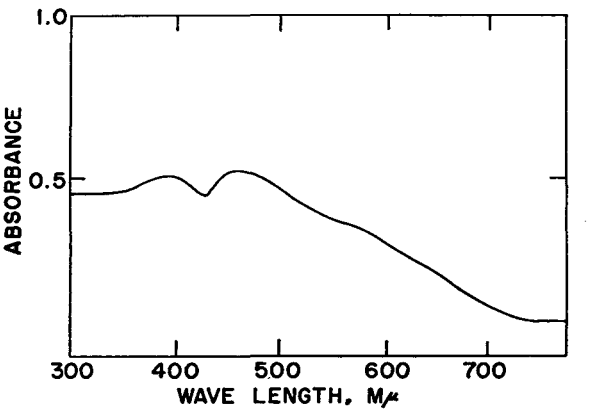


Figure 2. Absorption spectrum of *p-tert*-butylcatechol coupled to diazotized *p*-nitroaniline

1.9 p.p.m. *p-tert*-butylcatechol in sodium carbonate solution 5-cm. cells

If aromatic amines or phenols other than *p-tert*-butylcatechol are present which form colors with diazotized *p*-nitroaniline under the conditions of the analysis, the spectral characteristics of the final solution should be investigated. Thus, suspected aromatic amines or phenols may be detected and determined together with *p-tert*-butylcatechol after appropriate spectral standardization.

PHENOTHIAZINE

Phenothiazine (thiodiphenylamine) is usually utilized in compounding elastomers as a stabilizing agent. Methods available for the determination of phenothiazine include the work of Smith (*56*), Eddy (*13*), and Cupples (*10*), who employed the oxidation of phenothiazine to red 3,7-dihydroxyphenazathionium bromide (*63*). Kniaseff (*31*) suggested the use of cuprous chloride in alcoholic media to give a purple red color. Since white cuprous chloride on exposure to air changes its composition to green basic cupric chloride, $CuCl_3.3CuO.3H_2O$ (*35*), the exact composition of the active ingredient has not been determined. This method required approximately 52 hours for complete color formation.

Two papers had employed precious metal salts for pheno-

thiazine analysis. The gravimetric procedure of Payfer and Marshall (*42*) involving a precipitation of platinum phenothiazine tetrachloride $Pt(C_{12}H_9NS)_2Cl_4$, was not considered here. Overholser and Yoe (*40*) suggested the use of palladous chloride to form a dark blue complex with phenothiazine having the formula $Pd(C_{12}H_9NS)Cl_2$. Difficulties were encountered with this method because of the instability of the colored solutions.

The oxidation of phenothiazine to the red phenothiazine derivative initially described by Smith (*56*) formed readily in ethanolic media with bromine. This reaction was employed in this investigation.

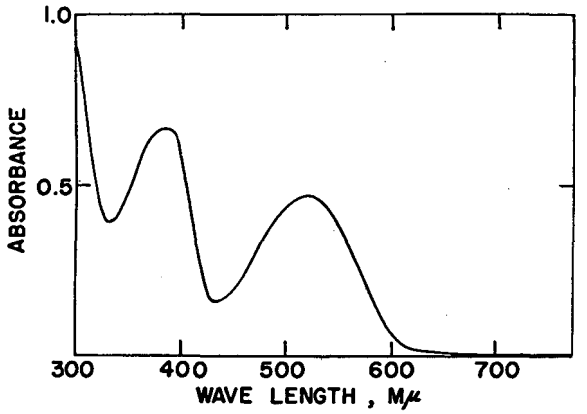


Figure 3. Absorption spectrum of 3,7-dihydroxyphenazathionium bromide

3.6 p.p.m. phenothiazine in ethyl alcohol 5-cm. cells

Reagents. Bromine, saturated aqueous solution.

Phenothiazine, recrystallized (*54*). Dissolve phenothiazine in 10 parts of C.P. toluene with the aid of heat. Add 0.2 gram of activated charcoal for each 4 grams of phenothiazine. Boil 10 minutes under reflux and filter while hot through a heated filter. Cool solution and collect phenothiazine crystals on a suction filter. Dry crystals in oven at 100° C. and then in vacuum desiccator containing paraffin chips. Repeat recrystallization process, if necessary, until product melts at 184–185° C.

Procedure. Into a 125-ml. separatory funnel weigh a sample to contain approximately 50 γ of phenothiazine. Extract with two 10-ml. portions of ethyl ether. Deliver the ether extracts into a 25-ml. glass-stoppered volumetric flask. Immerse the bulb of the volumetric flask in a water bath at 60° to 70° C. to evaporate ether. After the evaporation is complete, add 10 ml. of 95% ethyl alcohol and allow the solution to heat approximately 10 minutes. Add 5 ml. of saturated bromine water and gently agitate the solution. Allow the solution to stand in the water bath for 15 to 20 minutes. Add a second portion of bromine water, agitate, and allow the solution to stand another 15 to 20 minutes in the water bath until all traces of free bromine are eliminated. At this point the presence of phenothiazine is indicated by a red color. Remove the flask from the water bath, cool to room temperature, and adjust the volume to the 25-ml. mark with 95% ethyl alcohol. Agitate the solution well. Determine the per cent transmittance of the solution in a 5-cm. cell at 525 mμ. Ninety-five per cent ethyl alcohol should be used to adjust the instrument to 100% transmittance. Read the micrograms of phenothiazine corresponding to the per cent transmittance from a calibration curve. Run a blank on reagents and solvents used and correct the results accordingly.

Calibration. Standard phenothiazine solution A. Dissolve 100 mg. of pure phenothiazine in 95% ethyl alcohol and dilute to 100 ml. in a glass-stoppered volumetric flask.

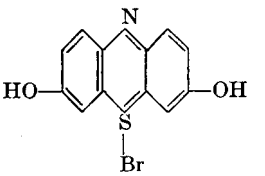
Standard phenothiazine solution B. Dilute 5.0 ml. of solution A to 500 ml. with 95% ethyl alcohol in a glass-stoppered volumetric flask. This solution contains 0.010 mg. (10 γ) of phenothiazine per ml.

Into glass-stoppered 25-ml. volumetric flasks put 0.50, 1.00. 2.50, 3.50, 5.00, 7.50, and 10.00 ml. of solution B, respectively. These solutions contain 5, 10, 25, 35, 50, 75, and 100 γ of phenothiazine. To each flask add 9.5, 9.0, 7.5, 6.5, 5.0, 2.5, and 0.0 ml. of 95% ethyl alcohol, respectively. Immerse the flasks in a water bath adjusted to 60° to 70° C., and allow the solutions to heat approximately 10 minutes. Now continue as described in the above procedure beginning with "Add 5 ml. of saturated bromine water." Construct a calibration curve to relate per cent transmittance at 525 mμ and micrograms of phenothiazine per 25 ml.

Experimental. A number of variables were investigated during method development. Each variable is discussed briefly.

Miscellaneous Oxidizing Agents. A number of oxidizing agents were investigated as possible color formers for phenothiazine. Potassium perborate, sodium vanadate, and lead peroxide had very weak color-forming tendencies in ethyl alcohol or ethyl alcohol–water mixtures with phenothiazine even when heated to 70° C. Ferric chloride readily converted phenothiazine to a colored compound in aqueous media acidified with hydrochloric acid. However, when a hydroxylic solvent such as ethyl alcohol was added, color development decreased by as much as 50%. When hydrogen peroxide is added to an acidified ethyl alcohol solution of phenothiazine and heated to about 80° C., the phenothiazine is converted to a highly colored compound which is believed to be 9-hydroxyphenothiazone, also known as thionol. For quantitative analysis, however, a close adjustment of the amounts of acid, ethyl alcohol, and hydrogen peroxide relative to the quantity of phenothiazine was found necessary. Therefore, this method of producing the highly colored thionol from an unknown quantity of phenothiazine cannot conveniently be made the basis of an accurate colorimetric method for estimating phenothiazine.

Bromine as an Oxidant. When phenothiazine is treated with saturated bromine water, under appropriate conditions, a red alcohol-soluble compound is formed. This compound is believed to be 3,7-dihydroxyphenazathionium bromide with the following possible formula (*66*).



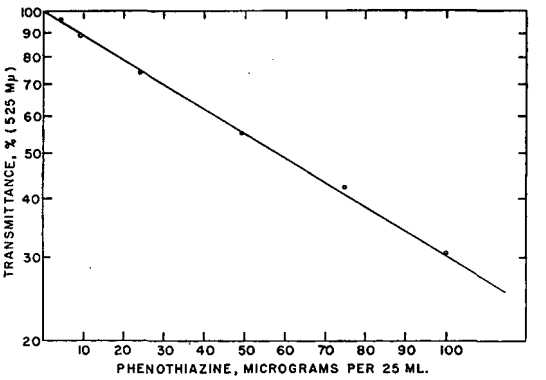


Figure 4. Calibration curve for phenothiazine determination

The absorption spectrum is given in Figure 3. Distinct absorption maxima are present at 384 and 520 mμ.

Reproducible Color Formation. It has been found that in alcoholic media dropwise additions of bromine (*10, 56*) may cause a greenish purple hue. Three experimentally controllable factors have been found to favor the formation of normal red colors: rapid addition of bromine water; two additions of excess bromine; and maintenance of the temperature of the solution during the addition of oxidant between 60° and 70° C. With the aid of these conditions it is possible to obtain consistent color formation. A standard curve is shown in Figure 4.

Table II. Phenothiazine Determination in Various Solvents by Oxidation with Bromine

Solvent	Phenothiazine, P.P.M. Present	Found	Present	Found
Water	2.0	1.9	50.0	49.2
		2.0		46.6
Ringer's solution		2.0		46.2
		1.9		46.7
Sodium carbonate, pH 10		1.8		45.2
		2.2		49.9
Lactic acid, 5%		1.8		49.8
		1.8		53.4
Acetic acid, pH 3		2.0		49.8
		2.0		47.3
Sucrose solution, 5%		2.1		...
		2.0		...
$\bar{X}$		2.0		49.2
s_r		0.12		2.49
L		±0.19		±3.88
P		95%		

Results. The reliability of the method was tested at the 2- and 50-p.p.m. levels. Known quantities of phenothiazine were added to the respective solvents and analyzed by the above procedure. The data are given in Table II.

Discussion. Table II shows that the method gives very good recovery of phenothiazine from the various solvents. Statistical variance analysis of the data presented in Table II showed no significant solvent effects. *p-tert*-Butylcatechol, Resin 731 SA (disproportionated rosin), Lomar PW, and 2-anthraquinonesulfonic acid in 50-p.p.m. level did not interfere. Generally, the method may be applied where aqueous bromine does not oxidize or brominate components to produce interfering side products. The method detects 0.2 p.p.m. of phenothiazine. In a single experiment with lactic acid a turbidity developed in the final solution after adjusting the volume to 25 ml. The solution was filtered through 12.5-cm. Whatman No. 12 folded filter and the per cent transmittance obtained. The filtration affected the results by less than 0.1 p.p.m.

RESIN 731 SA

Many colored reactions for the detection of various resins either in their natural state or in admixtures have been proposed from time to time. Some of these include the Liebermann-Storch reaction (*33*) and variations of the Halphen test (*19, 25*). These reactions are not specific since other compounds such as terpenoids give similar colors. Recently Swann (*59*) has suggested a colorimetric method for determining free rosin and rosin esters by a modified Liebermann reaction employing 18*N* sulfuric acid and acetic anhydride. The red to violet colors were measured colorimetrically. While this method gave fairly good colors with rosin in relatively concentrated solutions, it was not sufficiently sensitive for use with a disproportionated rosin. A modification by Sandermann (*52*) of the Liebermann-Storch reaction to allow a sulfonation of the resin for 12 hours followed by neutralization of the excess acid with concentrated sodium hydroxide was ineffective for quantitative application. Various other suggested reagents such as chlorosulfonic acid (*7*) or phloroglucinol (1,3,5-benzenetriol) (*46*) were useless for trace analysis. A disproportionated rosin does not have favorable fluorescent characteristics such as rosin (*66*) to make this property attractive for quantitative trace analysis.

As no adequate method was available in the literature for the determination of Resin 731 SA in trace quantities, the development of a sensitive method was undertaken. Paraffin chain cation-active compounds have been shown by Auerbach (*1*) to react with bromophenol blue to form colored complexes, which may be extracted with benzene and the active compound measured colorimetrically. A similar technique was utilized by others (*27, 28, 43, 44*) to determine relatively complex molecules. Following preliminary work by Campbell (*4*), a method was developed which is based on the formation of a colored compound by Resin 731 SA and a specially synthesized new cationic azo dye, *m*(*p*-dimethylaminophenylazo)benzyltrimethylammonium chloride, in an organic solvent, extraction of excess dye into aqueous alkali, followed by colorimetric or spectrophotometric measurement of the yellow resin-dye compound preferentially retained in the organic phase.

Reagents. Sodium hydroxide, 0.5*N*. Prepare this reagent from 50 weight % sodium hydroxide.

Hydrochloric acid, 1 to 1.

Resin 731 SA. This material is a commercial product purchased from Hercules Powder Co. Three samples obtained from different containers indicated excellent uniformity.

m-(*p*-Dimethylaminophenylazo)benzyltrimethylammonium chloride. The dye solution, 0.1% in water, is stable for at least 1 week.

The dry powder for this dye solution is prepared in three steps.

A. *m*-Nitrobenzyltrimethylammonium Chloride. Bubble trimethylamine through a solution of 200 grams of *m*-nitrobenzyl chloride in 2 liters of dry acetone for 2 hours. The temperature rises from room temperature to about 40° C. Filter the mixture and wash the product well on the filter with acetone. Dissolve the damp press cake in 700 ml. of warm absolute ethyl alcohol and add 1500 ml. of dry acetone. Heat to 50° C., then cool to 5 to 10° C. Filter and wash the product on the filter with a little dry acetone. Air dry the product, then dry at 90° C. for about 15 minutes. The dry weight is 146 grams.

B. *m*-Aminobenzyltrimethylammonium Chloride. Add 0.2 gram of platinum oxide (*22*) to a solution of 21.7 grams (0.1 mole) of the above nitro compound in 75 ml. of distilled water. Hydrogenate at 70° C. under 40 to 45 pounds of initial hydrogen pressure until the theoretical amount of hydrogen is taken up (about 2 hours). Prolonged hydrogenation results in excessive cleavage of the desired product. Filter to remove catalyst and extract the filtrate three times with 100-ml. portions of chloroform to remove side products. Discard the chloroform extracts. Heat the aqueous layer (boiling chips) on a steam bath to remove traces of chloroform. Cool and add 20 ml. of 37% hydrochloric acid. Bottle the solution until used in the following coupling reaction.

C. *m* - (*p*-Dimethylaminophenylazo)benzyltrimethylammonium Chloride. Diazotize the above amine hydrochloride solution with 5*N* sodium nitrite at 0° C. About 15 ml. of nitrite is required, indicating 0.075 mole of amine. Add the diazo solution in 1 portion to an ice cold solution of 0.078 mole of *N,N*-dimethylaniline in 10 ml. of 37% hydrochloric acid plus a little water. To the resulting solution add an ice-cold 30% aqueous solution of sodium acetate trihydrate in portions initially raising the pH to 3.0, then in small portions to maintain the pH in the range 2.8 to 3.0 as coupling progresses. A total of about 110 ml. of the sodium acetate solution is required. Allow the coupling to proceed for 7 hours below 5° C., then allow the mixture to stand overnight at room temperature. Raise the pH to 6.0 with 10*N* sodium hydroxide. Extract the mixture with four 200-ml. portions of chloroform; three layers appear upon separation. Discard the lower layer after each separation. Separate the dark center layer, discard the top layer. Heat the center layer on a steam bath to remove a considerable amount of chloroform. Cool the residual viscous oil and add 400 ml. of dry acetone. Agitate and boil down to a volume of 300 ml. on the steam bath. Cool and filter. Wash the orange crystalline product on the filter with cold dry acetone. Air dry the material, then dry at 90° C. for a few minutes to obtain 20.7 grams of dry product.

In order to test the purity of the dry benzyltrimethylammonium chloride derivative, develop a circular chromatogram of a dilute aqueous solution with Beckman pH 7.0 buffer solution. A single sharp orange band should result. If impurities are present, dissolve the benzyltrimethylammonium chloride derivative in water and adjust the solution to pH 6.0 with 10*N* sodium hydroxide. Repeat the extraction and isolation steps indicated.

Procedure. Into a 125-ml. Squibb separatory funnel weigh a sample to contain approximately 60 γ of resin. Add 3 ml. of hydrochloric acid (1 to 1) to adjust pH below 1. Extract this mixture with four 5-ml. portions of chloroform. Shake 2 minutes for each extraction. Transfer the chloroform extracts (20 ml.) quantitatively to a second 125-ml. Squibb separatory funnel. Add 10 ml. of 0.5*N* sodium hydroxide and 0.2 ml. of 0.1% dye solution. Thoroughly agitate the funnel contents for approximately 2 minutes. Allow the two liquid phases to separate. Quantitatively transfer the lower chloroform layer to a third 125-ml. Squibb separatory funnel. Discard the aqueous sodium

hydroxide layer. Add 10 ml. of 0.5*N* sodium hydroxide to the third funnel, stopper, and thoroughly agitate for approximately 2 minutes. Allow the phases to separate and then carefully transfer the lower chloroform layer to a glass-stoppered 25-ml. volumetric flask containing 0.5 gram of anhydrous sodium sulfate. Agitate the volumetric flask for about 15 seconds, dilute to the 25-ml. mark with distilled chloroform, stopper the flask, and thoroughly agitate. Determine the per cent transmittance of the solution in a 5-cm. cell with a 435 mμ filter against pure chloroform. As the azo dye is somewhat sensitive to light, the per cent transmittance must be obtained within 5 minutes after solution preparation. Also, the solution in the colorimeter cell must be exposed no more than 1 minute to the direct light beam in the colorimeter. Read the micrograms of Resin 731 SA corresponding to the per cent transmittance from a standard curve.

Calibration. Weigh 100 mg. of Resin 731 SA into a 100-ml. glass-stoppered volumetric flask, dissolve completely in distilled chloroform, and dilute to the mark with chloroform. One milliliter of this solution contains 1000 γ of Resin 731 SA. Dilute 10 ml. of this solution to 100 ml. with distilled chloroform in a glass-stoppered volumetric flask and agitate well. Use this diluted standard (100 γ of Resin 731 SA per ml.) in preparing the standard curve.

Employing a 1-ml. Mohr calibrated pipet, put 0, 0.20, 0.40, 0.60, 0.80, and 1.00 ml. of the diluted standard into 125-ml. Squibb separatory funnels containing 20 ml. of distilled chloroform. The separatory funnels contain, 0, 20, 40, 60, 80, and 100 γ of Resin 731 SA, respectively. Now continue as described in the above procedure beginning with "add 1.0 ml. of 0.5*N* sodium hydroxide and 0.2 ml. of dye solution."

Construct the standard curve by plotting micrograms of Resin 731 SA against per cent light transmittance on semilogarithmic paper. The standard is illustrated in Figure 5.

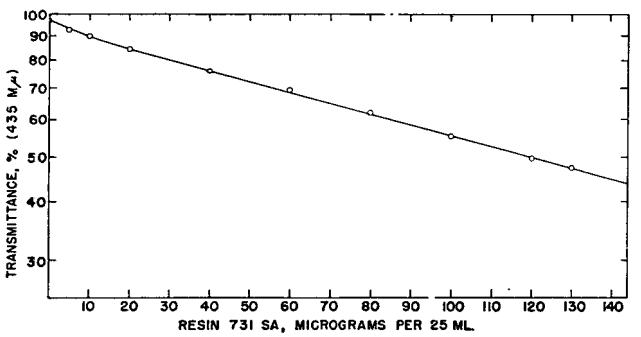


Figure 5. Calibration curve for Resin 731 SA determination

Experimental. The *m*-(*p*-dimethylaminophenylazo)benzyltrimethylammonium chloride reagent was specifically synthesized in this laboratory as a cationic-chromogenic reagent for the anion of relatively strong organic acids that may be present in materials such as Resin 731 SA. Preliminary investigation showed that this dye compound readily formed colored complexes with Resin 731 SA in a halogenated solvent such as chloroform, and the excess dye easily partitioned into aqueous sodium hydroxide while the dye-resin compound remained in the organic solvent. To achieve maximum accuracy and reproducibility, a number of variables were investigated.

Solvents. The following solvents proved inferior to chloroform as extracting media: carbon tetrachloride, cyclohexane, benzene, ethyl acetate, ethyl ether, iso-octane, petroleum ether, *n*-butyl alcohol, and methyl ethyl ketone. The resin-dye compound solubility was unsatisfactory in the first seven solvents, while the excess dye did not partition favorably into aqueous sodium hydroxide from the latter two.

Hydrogen Ion Concentration. The most complete extraction of Resin 731 SA from the sample into chloroform was obtained when the aqueous sample was adjusted to pH < 1. To achieve complete extraction of the excess dye, the pH of the aqueous wash solution should be approximately pH 12 to 12.5. Two 10-ml. portions of 0.5*N* sodium hydroxide were found to remove dye completely. Extractions performed with solutions containing known quantities of Resin 731 SA yielded results that could be reproduced to ±0.5% transmittance. Extracting with three 10-ml. portions of 0.5*N* sodium hydroxide or with aqueous solutions below pH 9.5 tended to remove about 2% of the resin-dye color compound.

Dye Solution. If more than 150 γ of Resin 731 SA is suspected in the samples, 0.4 to 0.5 ml. of the dye solution may be employed instead of 0.2 ml. It is suggested that a standard curve be prepared under similar conditions.

Spectral Characteristics of Yellow Complex. Figure 6 shows the spectra of the Resin 731 SA–dye compound in chloroform resulting from extracting 60 and 130 γ of Resin 731 SA from 25-gram samples. The absorption maximum of the yellow compound is at approximately 430 mμ.

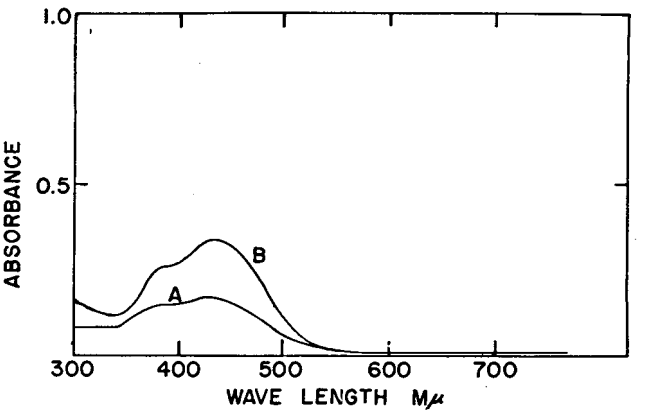


Figure 6. Absorption spectra of Resin 731 SA-dye complex in chloroform

A. 2.4 p.p.m. Resin 731 SA complexed
B. 5.2 p.p.m. Resin 731 SA complexed
5 cm. cells

Table III. Colorimetric Determination of Resin 731 SA with *m* - (*p* - Dimethylaminophenylazo)benzyltrimethylammonium Chloride

Solvent		Resin 731 SA, P.P.M. Present	Found	Present	Found
Water		2.4	2.1	20.0	17.8
			2.1		17.6
Ringer's solution			2.6		24.2
			2.9		21.3
Sodium carbonate, pH 10			2.4		19.7
			2.1		17.8
Acetic acid, pH 3			2.1		25.3
			2.2		17.6
Lactic acid, 5%			2.6		21.2
			2.4		19.7
Sucrose solution, 5%			2.6		24.2
			2.9		21.3
	$\bar{X}$		2.4		20.6
	s_r		0.31		2.79
	L		±0.49		±4.35
	P		95%		

Interfering Materials. Generally, organic materials such as long chain organic acids of anionic nature that are extractable into chloroform and can combine with the cationic dye to form species not readily extractable into aqueous 0.5*N* sodium hydroxide tend to give positive errors.

Individual experiments were performed by thoroughly agitating 300 ± 10 γ of the elastomeric component to be tested for interference with 25 ml. of aqueous sodium carbonate (pH 10), and the solution was then analyzed for Resin 731 SA by the above procedure. When 2-anthraquinonesulfonic acid, Lomar PW, phenothiazine *p-tert*-butylcatechol, and the sodium salt of dodecylsulfate were considered, the yellow color remaining in the chloroform layer after extracting with alkali was equivalent to 4.1, 9.5, 9.5, 10.0, and 25.8 γ, respectively. With a 25-gram sample, the contribution would be +0.2 to +1.0 p.p.m. Nancy

wood rosin behaves the same as Resin 731 SA. Inorganic salts present in the sample do not interfere.

Results. The accuracy of the method was determined by adding known quantities of Resin 731 SA at two levels to solvents of interest. The results are summarized in Table III.

Discussion. Table III shows that Resin 731 SA may be determined with good accuracy at both the 2- and 20-p.p.m. levels. Statistical variance analysis of the data presented in Table III indicated a slight solvent effect when determining resin at low concentrations.

The 2-minute extractions indicated in the procedure consisted of approximately 150 to 175 cycles and were performed at 25° ± 2° C. Although the extracted resin-dye compound is slightly sensitive to a direct light beam of 430 to 435 mμ in a colorimeter, transmittance readings may be easily obtained before noticeable decomposition occurs. The aqueous azo dye solutions were stable for at least a week. Decomposition is generally indicated by low rosinate recovery together with increasing blanks (low transmittance measurement). These blanks may be adversely affected by impurities in undistilled chloroform. Under proper conditions blanks had a transmittance of 98 to 99%.

LOMAR PW AND 2-ANTHRAQUINONESULFONIC ACID

Quantitative analytical methods are not readily available in the literature for determining trace quantities of sodium salt of a condensed mononaphthalenesulfonic acid (Lomar PW) and sodium salt of 2-anthraquinonesulfonic acid (Silver Salt) in relatively complex elastomer extracts. A simultaneous ultraviolet spectrophotometric determination was developed for these components in mixtures containing materials such as *p-tert*-butylcatechol, phenothiazine, disproportionated rosin derivatives, and xylene.

In principle, the method consists in removing interfering components from an acidified sample with chloroform or ethyl ether extraction. The Lomar PW and 2-anthraquinonesulfonic acid are then extracted into *n*-butyl alcohol from the aqueous solution, the *n*-butyl alcohol is evaporated, the residue is dissolved in acidified ethyl alcohol and the components are determined spectrophotometrically.

Reagents. Hydrochloric acid (1 to 1).

n-Butyl alcohol, distilled.

Lomar PW, sodium salt of a condensed mononaphthalenesulfonic acid. Jacques Wolf & Co., Passaic, N. J. Recrystallized from water.

Silver Salt, sodium salt of 2-anthraquinonesulfonic acid, Du Pont. Recrystallized from water.

Procedure. Into a 125-ml. separatory funnel carefully weigh a sample to contain approximately 50 to 60 γ of any single component. Add 3 ml. of hydrochloric acid (1 to 1). Extract this mixture with four 5-ml. portions of chloroform. Shake for 2 minutes for each extraction. Transfer the chloroform extracts (20 ml.) to a 50-ml. beaker. The chloroform extract may be discarded or examined.

Extract the aqueous solution with four 5-ml. portions of *n*-butyl alcohol. Shake for 1 minute for each extraction. Transfer the *n*-butyl alcohol extracts to a 50-ml. beaker. Gently evaporate the alcohol just to dryness on an asbestos-covered hot plate. Remove the beaker from the hot plate and add 1 ml. of (1 to 1) hydrochloric acid. Gradually swirl the aqueous acid to dissolve the residue. Add approximately 5 ml. of water, swirl the solution, and then carefully transfer the solution to a 25-ml. glass-stoppered volumetric flask. Employing three additional 5-ml. portions of water, thoroughly wash the 50-ml. beaker to quantitatively transfer Lomar PW and 2-anthraquinonesulfonic acid to the volumetric flask. Finally, adjust the volume with water to the 25-ml. mark, stopper, and thoroughly agitate the flask contents. Obtain the ultraviolet spectra (200 to 400 mμ) in a 5-cm. quartz cell employing a Cary automatic recording spectrophotometer with the following instrument adjustment:

Scanning speed	1.5 mμ per second
Silt control	15
Chart range	0 to 2.5

Balance the instrument initially with the 5-cm. quartz cells employing an aqueous solution containing 1 ml. (1 to 1) of hydrochloric acid per 25 ml. of solution. Calculate the base line absorbance A, at 228 and 256 mμ for Lomar PW and 2-anthraquinonesulfonic acid, respectively.

CALIBRATION. Weigh 100 mg. of Lomar PW into a 100-ml. glass-stoppered volumetric flask, dissolve completely in water, and dilute to the mark with water. Agitate thoroughly. One milliliter of this solution contains 1000 γ of Lomar PW. Dilute 10 ml. of this solution to 100 ml. with distilled water in a glass-stoppered volumetric flask, and agitate well. Use this diluted standard (100 γ of Lomar PW per ml.) in preparing a standard solution for spectrophotometric examination. Into a 25-ml. glass-stoppered volumetric flask add 0.60 ml. (60 γ) of the diluted standard. Add 1 ml. of hydrochloric acid (1 to 1) and dilute to the mark with distilled water. Stopper the flask and agitate the solution well. Examine the standard solution of Lomar PW as described in the above procedure starting with "Obtain the ultraviolet spectra (200 to 400 mμ) in a 5-cm. quartz cell . . . ." This standardization may be performed at more than one concentration. Calculate the base line absorbance A and absorbance index at 228 (principal absorption maximum for Lomar PW) and 256 mμ. Repeat the procedure and calculations for 2-anthraquinonesulfonic acid. The principal absorption maximum for it is at 256 mμ.

Experimental. ULTRAVIOLET ABSORPTION SPECTRUM OF LOMAR PW. Figure 7, A, shows the ultraviolet spectrum of Lomar PW. The curve is characterized by an absorption maximum at 228 mμ. Beer's law was found to apply at wave lengths 228 and 256 mμ for concentrations ranging from 0 to 80 γ per 25 ml. solution in 5-cm. cells. The reasons for selecting these wave lengths for testing for conformity to Beer's law are evident on examination of curve B, Figure 7. The absorbance index was calculated by the Bouguer-Beer law (*34*).

$$\log \frac{I_0}{I} = A = a_s bc \qquad (1)$$

where I_0 = energy incident on sample
I = energy transmitted by sample
A = sample absorbance
a_s = absorbance index
b = centimeters of path length of light in absorbing medium (5 cm.)
c = sample concentration, grams per liter

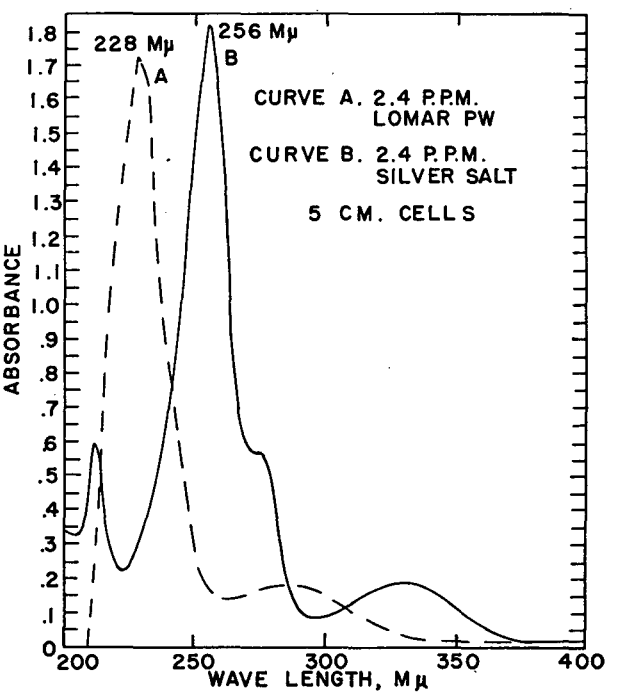


Figure 7. Absorption spectra of condensed mononaphthalenesulfonic acid (A) and 2-anthraquinonesulfonic acid (B) in ethyl alcohol

Table IV. Lomar PW Spectrophotometric Determination

(5-Cm. cells)

Solvent	Lomar PW, P.P.M. Present	Found	Present	Found
Water	2.4	2.2	30.0	27.5
		2.4		29.8
Ringer's solution		2.3		30.4
		2.4		29.0
Sodium carbonate, pH 10		2.1		26.2
		2.4		29.6
Acetic acid, pH 3		2.4		29.6
		2.4		29.4
Lactic acid, 5%		2.3		28.5
		2.4		30.0
Sucrose solution, 5%		2.4		30.0
		2.4		30.3
$\overline{X}$		2.3		29.2
s_r		0.10		1.24
L		±0.16		±1.93
P		95%		

Table V. 2-Anthraquinonesulfonic Acid Spectrophotometric Determination

(5-Cm. cells)

Solvent	2-Anthraquinonesulfonic Acid, P.P.M. Present	Found	Present	Found
Water	2.4	2.4	30.0	30.0
		2.4		30.2
Ringer's solution		2.4		30.3
		2.4		30.4
Sodium carbonate, pH 10		2.5		30.6
		2.4		30.1
Acetic Acid, pH 3		2.7		33.2
		2.6		32.3
Lactic acid, 5%		2.7		33.5
		2.7		33.3
Sucrose solution, 5%		2.6		34.5
		2.7		33.0
$\overline{X}$		2.6		31.9
s_r		0.14		1.73
L		±0.23		±2.70
P		95%		

The absorbance indices of Lomar PW at 228 and 256 mμ were 143.66 and 12.75, respectively.

Ultraviolet Absorption Spectrum of 2-Anthraquinonesulfonic Acid. Curve *B* in Figure 7 shows the absorption spectrum of this compound. The absorbance maximum occurred at 256 mμ. Conformity to Beer's law was found at 228 and 256 mμ for concentrations ranging from 0 to 80 γ per 25 ml. solution in 5-cm. cells. Higher concentrations were not measured in this long cell. The absorbance index of 2-anthraquinonesulfonic acid at 228 and 256 mμ were 22.08 and 151.08, respectively.

Simultaneous Ultraviolet Spectrophotometric Determination. As absorbances of a mixture of Lomar PW and 2-anthraquinonesulfonic acid were found to be additive, the following equations were used to calculate the concentration of both:

$$A_{228} = 718.5\ C_l + 110.5\ C_s \quad (2)$$

$$A_{256} = 64.0\ C_l + 755.5\ C_s \quad (3)$$

where $A\lambda$ = sample absorbance at the wave length (λ, mμ) indicated
C_l = concentration of Lomar PW, grams per liter
C_s = concentration of 2-anthraquinonesulfonic acid, grams per liter

To express results in micrograms per 25 ml., Equations 2 and 3 were modified as follows:

$$X = 35.25\ A_{228} - 5.16\ A_{256} \quad (4)$$

$$Y = 33.53\ A_{256} - 2.99\ A_{228} \quad (5)$$

where X = micrograms of Lomar PW per 25 ml.
Y = micrograms of 2-anthraquinonesulfonic acid per 25 ml.

or $\frac{X}{S}$ = Lomar PW, p.p.m.

$\frac{Y}{S}$ = 2-anthraquinonesulfonic acid, p.p.m.

where S = sample weight, grams

Sample Preparation. Components that absorb in the ultraviolet in the vicinity of 228 and 256 mμ would interfere. Some of these interferences such as *p-tert*-butylcatechol, phenothiazine, Resin 731 SA, xylene, and various aromatics may be removed conveniently by extracting an acidified solution of the aqueous extract with chloroform or ethyl ether. Neither Lomar PW nor 2-anthraquinonesulfonic acid is soluble in these solvents.

Results. The accuracy and precision of the method were evaluated by analyzing two synthetic samples for each solvent, which were prepared to contain both Lomar PW and 2-anthraquinonesulfonic acid in the 2- and 30-p.p.m. level. The data are presented in Tables IV and V.

Discussion. Tables IV and V indicate that good recovery may be obtained for synthetic samples in both the 2- and 30-p.p.m. levels for both Lomar PW and 2-anthraquinonesulfonic acid. Variance analysis of the data showed that the high results found for the latter with the last three solvents in Table V are due to distinct solvent effects. No systematic errors are indicated in the Lomar PW procedure.

Materials that are not extracted by ethyl ether or chloroform, but are soluble in *n*-butyl alcohol, are relatively nonvolatile, absorb near 228 and 256 mμ, and interfere with the Lomar PW and 2-anthraquinonesulfonic acid determination.

When employing ultraviolet spectrophotometry for trace analysis, it is important to use clean cells. The cells used in this work were cleaned thoroughly by rinsing three times with 95% ethyl alcohol and drying the cells with a stream of clean dry air.

If a Beckman quartz spectrophotometer is used for this work, a cell correction should be considered unless the cells are perfectly matched.

For maximum accuracy, it is suggested that the ultraviolet spectra be prepared at the temperature of instrument calibration. When a sample is to be analyzed whose temperature differs appreciably from the cell block of the spectrophotometer, the sample should remain in the cell block long enough for temperature equilibrium to be reached before preparing spectra.

The chloroform or ethyl ether extract may be discarded or the solvent carefully evaporated in a 60° to 70° C. water bath, the residue dissolved in absolute ethyl alcohol, diluted to 25 ml., and the solution examined in a 5-cm. cell with ultraviolet light in a Cary automatic recording spectrophotometer. The spectra obtained may indicate the possible presence of phenothiazine (252 mμ), *p-tert*-butylcatechol (280 mμ), and Resin 731 SA (258 mμ, 276 mμ).

ACKNOWLEDGMENT

The author wishes to acknowledge the valuable assistance in this work of many persons at the Du Pont Jackson Laboratory and Rubber Laboratory. Special credit is given to R. J. Barnhart for his wholehearted cooperation and aid with bulk extractions. Sincere appreciation also is expressed to J. W. Libby, Jr., A. M. Neal, G. H. Patterson, A. C. Stevenson, and R. H. Walsh for very helpful technical advice during the investigation.

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RECEIVED for review September 27, 1954. Accepted July 5, 1955. Contribution No. 172 from Jackson Laboratory, Division of Rubber Chemistry, 126th Meeting ACS, New York, September 1954.

Precipitation of Metals with Potassium Ferrocyanide in Presence of Complexing Agents

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The reactions of metals with potassium ferrocyanide in the presence of complexing agents have been studied. By utilizing (ethylenedinitrilo)tetraacetic acid, thiosulfate, and fluoride to sequester interfering ions, a qualitative test for zinc and manganese in the presence of other metals has been developed. The test is capable of detecting 1 γ of manganese at a limiting concentration of 1 to 1,000,000 and 50 γ of zinc at a limiting concentration of 1 to 20,000. A quantitative volumetric determination of manganese with ferrocyanide has also been developed. No prior separations are necessary.

POTASSIUM ferrocyanide is used extensively for titration of zinc. Because more than two dozen metals form precipitates or colored complexes with ferrocyanide, its use as an analytical reagent is rather limited. It was found that ferrocyanide was a very selective precipitant for manganese and zinc if (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid, EDTA) and other complexing agents were used. In the presence of (ethylenedinitrilo)tetraacetic acid, ferrocyanide formed a specific deep blue color with ferric iron and a specific bluish turbid solution with ferrous iron. In the presence of (ethylenedinitrilo)tetraacetic acid, copper(II) was reduced by ferrocyanide, which formed a brownish red soluble complex with copper(I).

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REAGENTS AND INSTRUMENTS

Potassium ferrocyanide solution, 0.05*M*, stored in a brown bottle.

(Ethylenedinitrilo)tetraacetic acid solution, 5%. Five grams of the disodium salt of (ethylenedinitrilo)tetraacetic acid were dissolved in 100 ml. of water.

Nitrilotriacetic acid, 5%. Five grams of nitrilotriacetic acid were dissolved in 100 ml. of water.

Thiosulfate solution, 5%. Five grams of sodium thiosulfate pentahydrate were dissolved in 100 ml. of water.

Fluoride solution, 5%. Five grams of potassium fluoride were dissolved in 100 ml. of water. This solution should be stored in a waxed bottle.

Potassium ferricyanide solution, 1%, freshly prepared.

Indicator solution, 1%. One gram of diphenylamine was dis-

solved in 100 ml. of glacial acetic acid. This solution should be freshly prepared.

Manganese chloride solution, 0.1*M*. The solution was standardized by the complexometric method (*2*).

Zinc chloride solution, 0.1*M*. The solution was prepared by dissolving pure zinc in 1 to 1 hydrochloric acid and diluted with an appropriate amount of water.

Other reagents used were reagent grade.

Coleman spectrophotometer and Beckman pH meter.

QUALITATIVE REACTION

One drop of the solution containing 1000 p.p.m. of metal was treated with 1 drop of 5% (ethylenedinitrilo)tetraacetic acid solution, or 1 drop of another complexing agent solution, 1 drop of glacial acetic acid, and 1 drop of 1% potassium ferrocyanide solution. A positive test was claimed if a precipitate or different coloration was formed upon addition of ferrocyanide. The results in Table I show that only silver(I), iron(II), manganese(II), zinc(II), and zirconium(IV) were precipitated with ferrocyanide in the presence of (ethylenedinitrilo)tetraacetic acid and other complexing agents. Nitrilotriacetic acid (NTA) showed a masking effect on the interfering ions similar to that of (ethylenedinitrilo)tetraacetic acid, except that mercury(II) was precipitated with ferrocyanide in the presence of nitrilotriacetic acid.

EFFECT OF pH ON PRECIPITATION OF MANGANESE AND ZINC

Most bi- and trivalent metals are precipitated with ferrocyanide in acid medium. In order to consider the complexing ability of (ethylenedinitrilo)tetraacetic acid as a masking agent, the highest pH value permissible for the precipitation of manganese and zinc with ferrocyanide in the presence of (ethylenedinitrilo)tetraacetic acid was determined.

To 10 ml. of 0.1*M* manganese or zinc solution, 70 ml. of water and 20 ml. of 5% (ethylenedinitrilo)tetraacetic acid solution were added. The pH of the mixture (originally approximately 3.9) was adjusted to about 9.5 with 10% sodium hydroxide. No precipitate was formed when 1 ml. of potassium ferrocyanide was added. The absorbance (turbidity) of the solution was measured at 600 mμ using the Coleman spectrophotometer after adjusting the pH of the solution with dilute hydrochloric acid. Both manganese and zinc were precipitated with ferrocyanide at a pH from 1 to 3.

Table I. Effect of Complexing Agents on Reactions of Metals with Ferrocyanide

Metal	Complexing Agent Added				
	None	EDTA	Thiosulfate	Fluoride	NTA
Antimony(III)	+	−			−
Bismuth(III)	+	−			−
Cadmium(II)	+	−			−
Cerium(III)	+	−			−
Cobalt(II)	+	−			−
Copper(II)	+	R(S)	−		R(S)
Gadolinium(III)	+	−			−
Gallium(III)	+	−			
Germanium	+	−			−
Indium(III)	+	−			−
Iridium(III)	−	−			−
Iron(II)	+	+		+	+
Iron(III)	B(S)	B(S)		−	B(S)
Lanthanum(III)	+	−			−
Lead(II)	+	−			−
Manganese(II)	+	+	+	+	+
Mercury(II)	+	−			+
Molybdate	R(S)	−			
Neodymium(III)	+	−			−
Nickel(II)	+	−			−
Palladium(II)	Y(S)	Y(S)			Y(S)
Ruthenium(III)	G(S)	Y(S)			
Samarium(III)	+	−			
Scandium(III)	+	−			−
Silver(I)	+	+	−	+	+
Tellurium(IV)	B(S)	−			
Thorium(IV)	+	−			
Titanium(IV)	+	−			−
Tungstate	Y(S)	−			−
Uranium(VI)	R(S)	−			−
Vanadate	Y(S)	−			−
Yttrium(III)	+	−			
Zinc(II)	+	+	+	+	+
Zirconium(IV)	+	+	+	−	+

+ precipitate; − no precipitate; (S) solution; R red; B blue; Y yellow; G green.

Table II. Titration of Manganese with Ferrocyanide in Presence of Other Metals

Metals Added, Mg.	Manganese, Millimoles		
	Taken	Found	Error, %
Al(III), 10	0.500	0.500	0.0
		0.487	−2.6
		0.493	−1.4
Fe(III), 5	0.500	0.504	+0.8
	1.000	1.014	+1.4
		0.998	−0.2
Ca(II), 10	1.000	0.998	−0.2
		1.000	0.0
		1.000	0.0
Mg(II), 10	1.000	0.994	−0.6
		1.007	+0.7
		0.982	−1.8
Ba(II), 10	1.000	1.000	0.0
		0.998	−0.2
		0.994	−0.6
Sr(II), 10	1.000	0.998	−0.2
		1.014	+1.4
Ni(II), 10		1.007	+0.7
	1.000	0.982	−1.8
WO_4^{--}, 10	0.500	0.480	−4.0
		0.490	−2.0
Pb(II), 10	0.500	0.500	0.0
		0.504	+0.8

TITRATION OF MANGANESE WITH FERROCYANIDE

According to the reactions previously described, it seems that manganese may be titrated with ferrocyanide in the presence of other metals which can be masked by the complexing agents. Glacial acetic acid was used for adjusting the pH of solution because it gave a pH of about 2.3 to 2.5.

To an aliquot of manganese solution containing from 0.5 to 2 millimoles of manganese(II), an excess amount (20 ml.) of 5% (ethylenedinitrilo)tetraacetic acid solution, 70 ml. of water, 20 ml. of glacial acetic acid, 4 drops of 1% potassium ferrocyanide solution, and 4 drops of diphenylamine were added. The mixture was titrated with 0.05*M* potassium ferrocyanide solution. The end point was from purple to colorless or light yellow. Alternatively, the end point was from colorless or light yellow to purple if the solution was back-titrated with a standard manganese solution after the addition of an excess amount of ferrocyanide.

The results shown in Table II were obtained using the back titration method, which gave a better end point.

DISCUSSION

Although potassium ferrocyanide is widely used for the titration of zinc, lack of specificity made it unsatisfactory for detecting zinc in the presence of other interfering metals. It was felt that greater specificity would be obtained if the interfering metals could be masked by the addition of complexing agents. If (ethylenedinitrilo)tetraacetic acid or nitrilotriacetic acid, thiosulfate, and fluoride are used as the complexing agents, only manganese and zinc are precipitated with ferrocyanide under the conditions described. The use of citric acid, tartaric acid, and thiourea for masking the interfering metals was unsatisfactory. Zinc could not be titrated with ferrocyanide at pH 2.5 in the presence of (ethylenedinitrilo)tetraacetic acid using ferricyanide and diphenylamine as the indicator. However, a satisfactory end point was obtained when the (ethylenedinitrilo)tetraacetic acid was omitted. This is possibly explained by the fact that the presence of small amounts of zinc or manganese ions is necessary for the oxidation of the diphenylamine by ferricyanide. At pH 2.5, zinc is so strongly complexed by (ethylenedinitrilo)-tetraacetic acid that the concentration of free zinc ion is too small for the ferricyanide to oxidize the indicator. On the other hand, (ethylenedinitrilo)tetraacetic acid does not complex manganese strongly at pH 2.5 and there is a sufficient amount of

manganese ion present for the oxidation of the indicator by ferricyanide, thereby making the quantitative titration possible.

Other methods of detecting the end point of the zinc determination were attempted in place of the diphenylamine and ferricyanide. Variamine blue (*1, 3*) was tried, but proved unsatisfactory because it took 10 to 15 seconds after the addition of each drop of the ferrocyanide solution for the color to become stable. An attempt was made to measure the turbidity of the solution containing zinc ferrocyanide precipitate and (ethylenedinitrilo)-tetraacetic acid. For the complete precipitation of small amounts of zinc in the presence of much excess of (ethylenedinitrilo)tetraacetic acid, a longer time of standing or a large amount of ferrocyanide was required. Manganese, if present, formed a precipitate almost immediately with the ferrocyanide in the presence of (ethylenedinitrilo)tetraacetic acid.

Other interferences were encountered in the end point determination for the manganese titration: Though cobalt(II) is not precipitated by ferrocyanide at pH 2.5, the red colored cobalt(III)–(ethylenedinitrilo)tetraacetate complex is formed in the acetic acid medium upon the addition of ferricyanide (*5*). Therefore manganese cannot be titrated with ferrocyanide using diphenylamine and ferricyanide as the indicator when cobalt is present. Interfering coloration caused difficulty when manganese was titrated in the presence of molybdate or uranyl ion.

The manganese and zinc can be titrated with ferrocyanide in the presence of (ethylenedinitrilo)tetraacetic acid at pH 2.5 by a high frequency method (*4*). The high frequency method gives two breaks in the titration of a mixture of manganese and zinc. The ferrocyanide precipitates the zinc first and the manganese next. Further investigation of a better means of detecting the end point for both zinc and manganese determinations is needed.

ACKNOWLEDGMENT

The author is indebted to H. Flaschka for providing variamine blue.

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RECEIVED for review April 8, 1955. Accepted July 18, 1955.

Determination of Titanium and Mixtures of Iron and Titanium with Electrolytically Generated Ceric Ion

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Titanium sulfate solutions were reduced in a Jones reductor, caught in saturated cerous sulfate solutions, and titrated coulometrically with electrolytically generated ceric ion under an atmosphere of nitrogen. Using the sensitive amperometric end-point procedure it was possible to determine amounts of this ion ranging from 50 γ to 5 mg. with an accuracy of within ±0.6%. Larger samples than this cannot be determined readily, because of the insolubility of titanium sulfate in the generating medium. Mixtures of iron and titanium containing amounts of titanium from 0.013 to 0.16 meq. and amounts of iron from 0.06 to 0.12 meq. can be determined with an accuracy of within ±0.66% or better. The titanium can be determined in this mixture with an accuracy of within ±0.6% or better, and the iron within ±0.80% or better. The mixture was passed through a Jones reductor, caught in a solution containing at least 90% of the amount of ceric ion calculated to be required for the titration of titanium in the sample, and then titrated in an atmosphere of nitrogen. This procedure necessitates a prior rough knowledge of the amount of titanium in the mixture, but if the ceric sulfate is not present initially, the results are in error.

IN VOLUMETRIC analysis, trivalent titanium is titrated with standard solutions of ferric iron, methylene blue, potassium permanganate, or ceric sulfate. The most widely recommended procedure is the one given by Scott (*5*) which involves titration with standard permanganate. From a consideration of the standard potentials of the titanous-titanic couple and of the cerous-ceric pair, it would appear that the titration of titanium with ceric ion should be excellent. The reaction would be expected to be rapid, quantitative, and the detection of the equivalence point accurate. The only information, however, concerning this titration that has been published is by Takeno (*9*), who reduced quadrivalent titanium with a zinc amalgam (Jones reductor) and then, in an inert atmosphere, titrated the trivalent titanium solution with standard ceric sulfate. The reduction of the titanium was performed with near boiling solutions, although there appears to be no adequate reason for this, because the reduction is just as complete when carried out in the cold.

The determination of titanium has recently become more important, and since ceric ion can be generated coulometrically very readily at a platinum anode (*6*) it was decided to study the coulometric determination of this metal.

The application of coulometric titrations has been limited almost exclusively to the determination of one species of ion in solutions of a single substance. Because it was found possible to determine titanium coulometrically with ceric ion and it was known that iron could be easily determined by the same method (*6*), the coulometric determination of both of these metals in the presence of one another was studied since they are found together frequently.

The standard potentials of the titanous-titanic and ferrous-ferric couples are sufficiently separated (about 1 volt difference) to give two breaks in a potentiometric titration curve with a standard oxidant. Shippy (*7*) titrated mixtures of these two substances with standard permanganate and obtained good results. Under his experimental conditions the formal potentials of the two systems are about 650 mv. apart, which is more than adequate for complete and accurate determination of both metals. Shippy passed a hot solution of the sample through a Jones reductor, catching it in 1 to 1 sulfuric acid. The warm, reduced solution was titrated with permanganate until the titanium end point was reached using methylene blue as an indicator. The solution was then cooled to room temperature and the iron

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Table I. Coulometric Titrations of Titanium with Electrolytically Generated Ceric Ion

Titanium Added, Mg.	Current, Ma.	Time, Min.	Titanium Found, Mg.	Error	
				Mg.	%
5.085	13.34	12.788	5.081	−0.004	−0.08
5.052	13.34	12.747	5.064	+0.012	+0.24
5.027	13.82	12.290	5.058	+0.031	+0.61
5.012	16.65	10.144	5.030	+0.018	+0.35
4.989	13.84	12.136	5.002	+0.013	+0.26
1.011	3.737	9.111	1.014	+0.003	+0.30
1.002	3.729	9.003	0.9999	−0.002	−0.20
1.008	3.620	9.324	1.005	−0.003	−0.30
1.000	3.774	8.901	1.000	+0.000	+0.00
0.9990	3.779	8.883	0.9998	+0.0008	+0.08
0.5593	1.490	12.618	0.5599	+0.0006	+0.11
0.5035	1.506	11.268	0.5054	+0.0019	+0.38
0.5075	1.495	11.429	0.5089	+0.0014	+0.27
0.5040	1.459	11.662	0.5067	+0.0027	+0.54
0.5114	1.445	11.787	0.5073	−0.0041	−0.80
γ	μa.		γ	γ	
49.86	202.9	8.219	49.67	−0.19	−0.38
49.76	202.8	8.265	49.92	+0.16	+0.32
50.14	202.2	8.336	50.20	+0.06	+0.11
49.79	208.9	7.961	49.53	−0.26	−0.52

titrated with 1,10-phenanthroline ferrous sulfate as indicator. As his recommended procedure is perfectly straightforward and the potential breaks in the actual titration were excellent, this procedure was adapted to the coulometric determination of these two metals with ceric ion, using the sensitive end-point procedure for the detection of the two equivalence points.

APPARATUS

The apparatus for the coulometric titrations was that described in a previous publication (*4*).

The titration cells were weighing bottles of 30- or 70-ml. capacities, depending upon the size of the sample taken. Each cell was covered with a rubber stopper provided with openings for the four electrodes, a gas inlet, and the Jones reductor.

Jones reductors of three sizes were employed for the reduction of the titanium and iron samples. The one selected for a given determination depended upon the size of the sample. These reductor columns were made of soft glass tubing, flared at one end, and fitted with a standard taper stopcock at the other; the tip of the stopcock outlet was drawn out to a fine orifice. The dimensions of these reductors were: large reductor, 30 cm. in length with an internal diameter of 0.7 cm.; medium reductor, 33 cm. long with a 0.5-cm. internal diameter; small reductor, 21 cm. long with a 0.4-cm. internal diameter.

A No. 7664 Leeds and Northrup pH meter was used to detect the end points with the potentiometric titrations and to preset the potentials of the indicator circuit when using the sensitive end-point procedure.

All potentials in this work were measured against the lead amalgam–lead sulfate reference electrode, as recommended by Cooke, Reilley, and Furman (*3*).

SOLUTIONS

The cerous sulfate solution was prepared by saturating 1.0*N* sulfuric acid with reagent grade cerous sulfate trihydrate (G. Frederick Smith Chemical Co.).

The standard titanium sulfate solution was prepared as follows. Titanium dioxide of reagent grade from the Amend Drug and Chemical Co. was placed in a porcelain crucible and ignited for half an hour at red heat with a Meker burner. Weighed amounts of the oxide, with 8 grams of Baker and Adamson ammonium sulfate of reagent grade were dissolved in 20 ml. of concentrated sulfuric acid. It was necessary to heat this mixture to boiling in order to effect dissolution. The resulting solution was a pale yellow. This solution was evaporated to about 10 ml. to reduce the concentration of sulfuric acid in the final solution. The concentrated solution was cooled and then made up to 250 ml. in a volumetric flask, using distilled water only.

In one instance a titanium solution was standardized in order to determine whether the ignited titanium dioxide could be employed directly as a standard. This standardization was carried out by reducing the titanium sulfate solution in a Jones reductor, catching the reduced species in a 5% solution of ferric sulfate in 3.0*N* sulfuric acid, and then titrating the ferrous ions produced with standard ceric sulfate, which was standardized against arsenious oxide. *o*-Phenanthroline ferrous sulfate was used as an indicator. The concentration of titanium as calculated from the weight of titanium dioxide taken was 4.008 mg. per ml., whereas the value obtained from the standardization procedure was 4.005 mg. per ml. Other solutions were prepared and their concentrations were calculated from the weight of the pure titanium dioxide.

A standard ferric sulfate solution was prepared by dissolving a weighed amount of Baker and Adamson, reagent grade ferric sulfate nonahydrate in 1*N* sulfuric acid and diluting it up to volume. This solution was standardized by passing it through a Jones reductor and then titrating it, under an atmosphere of nitrogen, with standard ceric sulfate, using *o*-phenanthroline ferrous sulfate as an indicator. This solution was found to be 0.06027*N* with respect to iron.

DETERMINATION OF TITANIUM

Procedure. The size of the actual reductor column that was used depended upon the amount of titanium being determined. It was washed first with boiling 5% sulfuric acid and then with water until it no longer decolorized dilute permanganate. Next a stream of pure nitrogen was forced through it for 5 to 10 minutes in order to remove all traces of oxygen. While the reductor was being deaerated the potential of a previously deaerated solution of cerous sulfate was adjusted to 1.250 volts *vs.* the lead amalgam–lead sulfate reference electrode, by generating ceric ions until the galvanometer in the indicator circuit showed that no current was flowing in the indicator system—i.e., the impressed and the solution potentials were identical.

One milliliter of deaerated 1.0*N* sulfuric acid was then added to the reductor column, followed by the sample of titanium. The titanium was kept in the reductor column for 10 minutes by closing the stopcock at the bottom of the reductor column. At the end of this period the sample was drained into the titration cell and the column was washed with 3.0 ml. of 1.0*N* sulfuric acid and 3.0 ml. of water. Generation of ceric ion was begun and continued until the indicator galvanometer again showed that the two potentials were equal. In the immediate vicinity of the end point it was necessary to add the current in small increments and to allow a short period of time for equilibrium to become established; usually 2 or 3 minutes were adequate.

For samples that contained 50 γ of titanium or less, the working indicator electrode was a 1-cm. (*2*) platinum-iridium foil instead of the platinum wire that had been used for the larger samples.

The results of a series of titrations on various amounts of titanium performed according to this procedure are presented in Table I.

Discussion. The data in Table I show that samples of titanium from 50 γ to 5.0 mg., or from about 1.0 microequivalent to 0.1 meq. can be titrated successfully with coulometrically generated ceric ion with an accuracy of within ±0.60%.

The potential that was impressed across the indicator electrodes for the sensitive end-point procedure was determined by means of a potentiometric titration. At first it was found that two breaks appeared in the potential curve for this titration and that the solution was pale yellow, after the reduced titanium solution had been added to the titration cell. It was believed that this was caused by traces of titanium peroxide that might have been formed in the reductor column. The subsequent deaeration of all solutions and of the reductor column itself eliminated the second break in the curve and the coloration of the solution.

Initial determinations of titanium using cold 1*N* sulfuric acid as the wash solution gave results that were in error by ±3 to 10%. Neither more concentrated acid as a wash solution nor the use of a boiling wash solution improved the results. It was decided finally that this error was caused by incomplete reeduction of the titanium. Allowing the titanium solution to remain in contact with the zinc amalgam for 5 minutes gave improved results, which were only from 0.2 to 1.4% too low. This time of contact was increased to 10 minutes, and accurate results were obtained. Shippy (*7*) in his work with titanium, maintains that it is necessary to permit a near-boiling solution of titanium to remain in the Jones reductor for 15 minutes in order to achieve

Table II. Successive Coulometric Titration of Titanous and Ferrous Ions with Ceric Ions

Titanium			Iron			Total Iron and Titanium		
Added, meq.	Found, meq.	Error, %	Added, meq.	Found, meq.	Error, %	Added, meq.	Found, meq.	Error, %
0.01369	0.01330	−2.85	0.1211	0.1213	+0.17	0.1348	0.1346	−0.15
0.01432	0.01423	−0.63	0.1211	0.1213	+0.17	0.1354	0.1353	−0.08
0.02629	0.02641	+0.45	0.07913	0.07955	+0.52	0.1054	0.1060	+0.57
0.02785	0.02791	+0.22	0.07962	0.08025	+0.80	0.1075	0.1082	+0.64
0.05206	0.05209	+0.06	0.05243	0.05228	−0.29	0.1045	0.1044	−0.11
0.05258	0.05254	−0.08	0.05243	0.05248	+0.09	0.1050	0.1050	+0.00
0.1058	0.1052	−0.56	0.02043	0.02049	+0.29	0.1262	0.1257	−0.40
0.1088	0.1087	−0.09	0.01205	0.01198	−0.58	0.1208	0.1207	−0.08
0.1048	0.1056	+0.76	0.06087	0.06122	+0.57	0.1657	0.1668	+0.66

complete reduction; however, these extreme conditions were found to be unnecessary in this investigation.

A few attempts were also made to determine samples of titanium that were smaller than 50 γ. Using the smallest of the reductor columns, two determinations on 25-γ samples gave results that were 3.6 and 7.9% too high. Samples of this size normally require more than ordinary precautions in order to obtain accurate results, but since the purpose of this study was merely to show that the coulometric determination of titanium was feasible over a wide range of concentrations, rather than to develop a microprocedure, no further work was done on samples smaller than 50 γ. However, there appears to be no adequate reason why they could not be analyzed.

Experiments were made with titanium samples of 10 mg. or larger. In these cases the results were always too high by about 10%. It is possible that this error or apparent decrease in the current efficiency, might have been introduced through the dilution of the generation solution by the addition of large volumes of the samples and wash solutions. In order to minimize these effects it becomes necessary to work with reasonably concentrated titanium solutions and as small amounts of washing as possible.

DETERMINATION OF MIXTURES OF IRON AND TITANIUM

Procedure. Twenty-five milliliters of the cerous sulfate solution were placed in the 70-ml. titration cell and deaerated with nitrogen for 20 minutes. During this time the Jones reductor of the medium size was washed with hot, 1.0*N* sulfuric acid and water until it no longer decolorized very dilute permanganate. The reductor was then placed in the opening provided for it in the stopper of the titration cell, with the tip extending just below the level of the liquid in the cell. Next the reductor column was deaerated for 5 minutes by forcing a stream of tank nitrogen through it. During this time the potential of the generating solution was adjusted to 1.300 volts, *vs.* the lead amalgam half-cell, by generating ceric ion. One milliliter of deaerated 1*N* sulfuric acid was added to the reductor followed by the titanium-iron sample, which was allowed to remain in contact with the zinc amalgam for 5 to 10 minutes. During the period of contact, ceric sulfate was generated in the titration vessel. The amount of ceric ion generated was approximately 90% of the calculated amount for the titration of the sample of titanium. The stopcock on the reductor was opened, and the iron-titanium sample permitted to flow into the titration vessel. This was washed through by approximately 5.0 ml. of 1.0*N* sulfuric acid. The impressed potential was lowered to 0.725 volts for the titanium end point, and generation of ceric ion continued until the galvanometer indicated that no current was flowing. Near the end point itself, the current must be added, in small increments and a few minutes allowed after each addition for completion of the reaction, as indicated by a cessation of downward drift in the galvanometer readings. The impressed indicator potential was increased then to the equivalence potential selected for the ferrous-ceric titration, 1.300 volts, and generation continued until once more no current flowed in the indicator system, again adding increments of current and waiting in the vicinity of the end point. The amounts of titanium and iron present, respectively, were calculated from the times required to reach the first end point and from the first end point to the second one, and the value of the current.

The results of a series of determinations of varying ratios of these two elements are presented in Table II.

DISCUSSION

Initial titration without prior generation of ceric sulfate gave results that were correct within 1% for the iron, but were from 15 to 23% too low for the titanium. In these titrations 0.638 volt was selected as the end-point potential to be applied for the titanium titration and 1.232 volts as that for the end point of the iron titration. These values had been selected from a potentiometric titration of a mixture of the two ions. No explanation could be found for this error since the conditions employed were identical with those that Shippy (*7*) had successfully used for the mixture, and also with those that were successful for the determination of titanium alone.

In the successive pairs of experiments, the pregeneration times for forming the ceric solution to receive the reduced solution were 1.50, 3.00, 6.00 minutes, and in the last three experiments 13.00 minutes. In all cases the pregeneration current was between 11.64 and 11.81 ma.

The length of time that the mixture remained in the reductor column was increased to 10 minutes and decreased to 0 minutes, the amount of wash solution varied, and the end-point potential for the titration of titanium altered. None of these changes in the operating conditions gave accurate results. Although some of them improved the accuracy of the titanium determination, they increased the error in the determination of iron to 5% or greater.

A second potentiometric determination was carried out, allowing several minutes to elapse after each generation period before taking the potential reading. The resulting potential-time curve showed three breaks, with the new break between the two previously observed. The mid-point potential of this new break was about 0.800 volt. A study was then undertaken to ascertain which solution contained this unknown third substance that was being titrated. It was found, by varying the amount of titanium solution taken, that the unknown substance was in this solution, and that the amount of it was very roughly proportional to the amount of titanium that was taken. It could be possible that this is again a titanium peroxide; from a consideration of the standard potential of the peroxide system in acid medium it is positioned correctly between the ferrous-ferric and the titanous-titanic potential. Where this peroxide might have originated could not be established (if it was titanium peroxide at all), as it was thought that all of the solutions and the reductor had been thoroughly deaerated. Also to ensure complete reduction of the sample, the titanium had been permitted to remain in contact with the zinc amalgam for 5 minutes or more.

Bricker and Sweetser (*1*) and Sill and Peterson (*8*), when titrating mixtures of uranium and iron with ceric sulfate, observed that the results of the uranium determination were consistently low. The explanation given by both sets of authors for this phenomena is that the presence of iron, or any other multivalent ion, causes an "induced oxidation" of the uranium. It is possible that an analogous induced oxidation could be occurring in this work with titanium and iron.

Bricker and Sweetser (*1*) overcame this error in their determination of uranium by initially adding sufficient ceric sulfate so that their uranium and iron mixtures were always caught in this and not in pure sulfuric acid. They found that if about 90 to 97% of the amount of ceric sulfate required for the titration of uranium was added to their titration vessel before the mixture being determined was introduced, excellent results were obtained for both substances. Therefore, this practice was adopted in the

work with titanium and iron. Before the samples were drained into the electrolysis cell from the Jones reductor, 90% of the theoretical quantity of ceric ion required for the titration of titanous ion was generated coulometrically, so that the reduced solution was caught in this and not in the pure cerous sulfate.

This procedure necessitates knowing, within a reasonable range, the concentration of titanium in the sample. This means that either a preliminary titration must be performed, without the initial generation of ceric ion, to obtain a very rough idea as to the amount of substance present, or the information must be obtained by some other means.

The data in Table II show that in mixtures of iron and titanium, amounts of titanium ranging from 0.013 to 0.16 meq. can be determined with an accuracy of within ±0.6% or better. In these solutions 0.06 to 0.12 meq. of iron can be determined with an accuracy of within ±0.80% or better. The total amount of the sample present can be established within a range of 0.66% or better.

Traces of dissolved oxygen may account for the limits of precision and accuracy that are observed. Sulfuric acid is suspected of possible contamination by traces of redox systems. Redistillation of the acid does not eliminate fluctuations in the reagent blanks that are observed in various procedures.

CONCLUSIONS

Amounts of titanium alone can be determined coulometrically with ceric sulfate over fairly wide ranges of concentration with an accuracy of within ±0.6%. There appears to be no reason why samples smaller than one microequivalent of titanium could not be determined, provided that the precautions necessary for microcoulometric titrations are undertaken. Since the limiting factor with amounts of titanium larger than 0.1 meq. appears to be the solubility of titanium sulfate, there is no reason why samples of this magnitude should have to be encountered. Through appropriate dilution of the sample solution, the amount of titanium to be determined can be brought within the range of concentrations found to be satisfactory in this work.

Mixtures of titanium and iron can be determined coulometrically with electrolytically generated ceric ion with reasonably good accuracy and precision. However, unless there is knowledge of the approximate amount of titanium in the sample, it is necessary to run a preliminary coulometric titration, and in a subsequent titration to generate about 90% of the required ceric ion before addition of the sample to be analyzed.

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RECEIVED for review March 24, 1955. Accepted July 20, 1955. Based upon a dissertation submitted by Robert V. Dilts in partial fulfillment of requirements for degree of doctor of philosophy at Princeton University, 1954.

Direct Determination of Acetic Acid in Acetic Anhydride

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This investigation was undertaken to develop a rapid and accurate method for the determination of acetic acid in acetic anhydride. It has been extended to other acids in their anhydrides and other acids in acetic anhydride. Two methods are described which depend on the reaction of a tertiary amine (triethylamine) with the free acid in the anhydride. The first method depends on the color change of methyl red at the end point, while the second measures the temperature rise when the reagent is added to the sample. A precision of ±0.07% absolute over the range 0.5 to 3.5% acid was obtained for the visual method and ±0.09% absolute for the thermometric method over the range 0.8 to 5.5% acid. Potentiometric titration of acetic acid in anhydride using combinations of glass, calomel, gold, tungsten, silver, silver–silver chloride, platinum, and antimony electrodes was unsuccessful.

THE literature lists only a few methods which can be used for the determination of acids in anhydrides. Nicolas and Burel (*2*) used aniline to produce the anilide from the anhydride, thus providing a method for calculating the acid and anhydride by titrating the sample before and after anilide formation. Smith and Bryant (*4*) also performed two titrations, one with methanolic sodium methylate and one with aqueous caustic. From these data, the acid and anhydride can be calculated. As both of these methods are indirect, they are not very satisfactory for determining small amounts of acid in anhydride. Siggia and Floramo (*3*) used potentiometric titration of the acid with a tertiary amine in a solvent such as acetone. Their method is limited to acids with a pK_a 3 or greater. Thus, acetic acid cannot be determined by their procedure. The visual titrimetric and thermometric methods presented here permit the determination of acids with dissociation constants at least as small as that of benzoic acid (6×10^{-6}); they also reduce the analytical time and tend to eliminate the lack of precision arising from the difference in large numbers in the Nicolas-Burel or Smith-Bryant methods.

In the visual method triethylamine in benzene is used as titrating agent. Acetic anhydride, as the solvent, appears to enhance the acidity of the acetic acid to the point where it will yield a color change with methyl red. The molarity of the triethylamine-benzene reagent is determined by titration against standard hydrochloric acid, and consequently, the reagent must be free of primary and secondary amines. The purification step is relatively simple and involves treating the triethylamine with excess acetic anhydride to remove primary and secondary amines, neutralizing the excess anhydride with sodium hydroxide, and extracting the amine into benzene. The benzene-amine mixture is dried by distillation, which removes the benzene-water azeotrope. The amine-benzene azeotrope is then collected, diluted with dry benzene, and standardized. The titration of a sample is made to an end point which matches the color of methyl red in benzene.

The thermometric method is entirely empirical and involves

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Table I. Precision of Visual Method for Determination of Acetic Acid in Acetic Anhydride

Sample No.	% AcOH Found	Av.	σ
1	0.633		
2	0.581	0.606	0.044
3	0.558		
4	0.652		
5	1.435		
6	1.476	1.484	0.071
7	1.581		
8	1.446		
9	2.323		
10	2.120	2.284	0.102
11	2.346		
12	2.346		
13	3.277		
14	3.419	3.364	0.075
15	3.325		
16	3.434		

Standard deviation for all samples 0.070% absolute.

Table II. Recoveries of Added Acetic Acid by Visual Method

AcOH Added, Grams	AcOH Found, Grams[a]	Δ
1.65	1.55	+0.10
2.36	2.38	−0.02
3.42	3.51	−0.09

[a] Average of four results.

the measurement of the change in temperature when reagent grade triethylamine is added to acetic anhydride in a Dewar flask. Since the method is empirical, any water and/or primary and secondary amines in the reagent will have the same effect on the standards as on the samples and consequently no purification of the reagent is needed.

A potentiometric study of the reaction was attempted using a Beckman Model H-2 pH meter and all possible combinations of glass, calomel, gold, tungsten, silver, silver–silver chloride, platinum, and antimony electrodes. The only combination which was found to show any promise was glass-silver. However, the breaks obtained were not reproducible. No potentiometric study of any system except acetic acid–anhydride was made.

VISUAL METHOD

Reagents. Triethylamine in benzene, 2*M*. Dry the contents of a 500-gram bottle of C.P. triethylamine with potassium hydroxide pellets overnight. Decant into a 1-liter flask and cautiously add 25 ml. of acetic anhydride. (This will generally provide a sufficient excess of anhydride.) Reflux the mixture for 30 minutes. Allow to cool. Add 125 ml. of 10*M* sodium hydroxide with constant stirring. (The small temperature rise which accompanies this addition is an indication that an excess of acetic anhydride was used.) Allow to stir for 10 minutes. Pour the mixture into a separatory funnel and add 500 ml. of benzene. Shake thoroughly, and draw off and discard the water layer. Wash the benzene layer with another 125 ml. of sodium hydroxide. Transfer the triethylamine in benzene solution to a distilling flask fitted with a simple condenser set for distillation. Distill at least 200 ml. and discard (temperature >81° C.). Replace the receiver and condenser with dry apparatus and distill until 100 ml. remain in the pot. Protect the receiver with an Ascarite-Drierite tube at all times. Dilute the distillate with an equal volume of dry (<0.05% water) benzene. Standardize the triethylamine solution by titrating potentiometrically with 0.5*N* standard aqueous hydrochloric acid to a pH of 4 to 6. Store the reagent in a dry glass-stoppered bottle or in an automatic buret protected with an Ascarite-Drierite tube.

Methyl red indicator. Dry acetonitrile over anhydrous magnesium sulfate for an hour and saturated the dry solvent with methyl red indicator. Filter the solution and store in a closed bottle to prevent water contamination.

Recommended Procedure for Visual Method. Prepare a solution for color comparison by adding 5 drops of the indicator solution to 100 ml. of benzene in a 250-ml. Erlenmeyer flask. Stopper the flask. Weigh a sample of anhydride containing 5 to 60 meq. of acid into another 250-ml. Erlenmeyer flask. Add 5 drops of methyl red indicator solution per 100 ml. of sample and titrate immediately with triethylamine-benzene reagent until the solution color matches that of the indicator in benzene. The color is stable in benzene, but it will change rapidly in acetic anhydride after the end point has been reached.

Discussion of Visual Method. Initial studies concerned the stiochiometry of the reaction. By titrating known amounts of acetic acid in anhydride it was determined that 1 ml. of base was equivalent to 0.4184 gram of acetic acid. Standardization of the base against aqueous hydrochloric acid showed it to be 2.2294*M*. From these data it can be shown that the ratio of acetic acid to triethylamine is 3.13 to 1. Thus, within experimental error, the equation for the reaction appears to be

$$3AcOH + N(C_2H_5)_3 \rightarrow N(C_2H_5)_3.3AcOH$$

No attempt was made to characterize the product further. Kaufman and Singleterry (*1*) gave an excellent bibliography on this type of problem and indicated a precedent for this stoichiometry.

The indicator, methyl red, was chosen after consideration indicated that the indicator must contain no groups which are readily acetylated such as hydroxyl or primary and secondary amino groups. Other azo indicators and the indicator quinoline blue (1,1′-diisoamyl-4,4′-quinocyanine iodide) were also tried as was a mixed indicator methyl red–quinoline blue; none was suitable. With the exception of methyl red all the indicators tried were already in the basic form when added to the anhydride mixture or would give no color change in the medium.

Table I shows the precision of the visual method over the range 0.6 to 3.4% acetic acid. The standard deviation for the sixteen results is 0.07% absolute.

Table II indicates the accuracy of the method in terms of acid recovered from known samples.

An extension of the method was made to the determination of acetic acid in acetic anhydride–hydrocarbon samples. In this

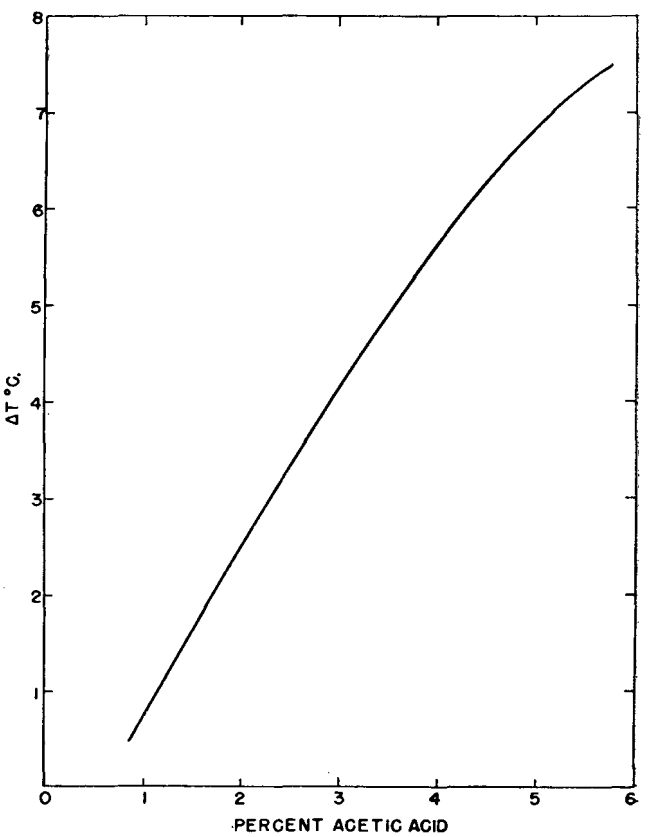

Figure 1. Standard curve for thermometric determination of acetic acid in acetic anhydride

case acetic anhydride containing a previously determined amount of acetic acid was added to the hydrocarbon, and the anhydride layer was titrated with triethylamine-benzene reagent without separation from the hydrocarbon. The results obtained on some standard samples are given in Table III. In this case or in others where a solvent might be used to dilute the anhydride sample, the solvent must be dry (<0.1% water).

The method appears suitable for the determination of other acids in acetic anhydride. Studies were made in which benzoic or propionic acid was added to acetic anhydride of known acid content. Table IV shows the recoveries. The results obtained in each case are slightly high but are within the precision of the method.

THERMOMETRIC METHOD

Reagents. Triethylamine, reagent grade.

Recommended Procedure for Thermometric Method. Prepare a standard curve of ΔT *vs.* per cent of acid in the following manner. Pipet 20 ml. of anhydride containing a known amount of acetic acid into a small Dewar flask. (A nonstandard Dewar 1 inch in internal diameter and 4 inches long was used in this work, but a standard 200-ml. Dewar flask will work equally well if double the amounts of sample and amine specified are used.) A thermometer with a range of 20° to 50° C., graduated in 0.1° C., is used and the temperature is estimated to hundredths of a degree. Using rubber bands, attach a glass stirring rod flattened at one end to the thermometer. Insert the thermometer and stirrer in the Dewar flask and read the temperature. The actual immersion requirement of the thermometer is unimportant, as the sample will always be at the same level. Adjust the temperature of the reagent grade triethylamine to that of the anhydride ±1° C. Deliver 1.2 ± 0.1 ml. of the reagent rapidly into the Dewar flask by means of a small marked pipet with a pressure bulb. Move the thermometer and stirring rod up and down a few times to mix the reactants. Read the highest temperature attained. Plot the value of ΔT against the known per cent of acid for the various acid concentrations. A typical curve is shown in Figure 1. To analyze a sample, pipet 20 ml. into the same Dewar flask used for setting up the standard curve, read the temperature, add the triethylamine, and note the temperature rise. Refer the temperature rise to the graph to obtain the per cent of acid in the anhydride.

Discussion of Thermometric Method. The thermometric method is based on the same reaction with triethylamine as the visual method and has been applied over the range 0.5 to 6% acetic acid in acetic anhydride. It can probably be extended to somewhat higher values by using more triethylamine. This, of course, will change the curve. The method is empirical, and as such, conditions used for establishing the working curve must be adhered to exactly in sample analysis. Solution of triethylamine in acetic anhydride appears to have a negative temperature coefficient. This effect may limit the lower value of acetic acid determinable. In the authors' equipment the negative value of the temperature change begins at 0.5% of acetic acid. They did not obtain any anhydride with less than this amount of acid and consequently cannot give any indication of the applicability of the procedure below this value. The optimum range of the procedure as given is from 0.8 to 5.5%. Table V indicates a precision of ±0.09% absolute for the method. This is about the same as the precision of the visual method which was used to determine the composition of the standard samples. Recoveries were excellent as is also shown in Table V. Variation in the initial temperature, provided sample and reagent are within ±1° C., between 24° and 32° does not affect the results.

Although it is not felt necessary to purify the triethylamine for the thermometric method, and experience has shown no difference between bottles from different suppliers, it is advisable to check a few points on the curve whenever a new supply of triethylamine is used. The temperature rise is dependent on the heat capacity of the system, and consequently, it is important to calibrate each Dewar flask and thermometer-stirrer system used.

The thermometric method was also applied to determinations of benzoic or propionic acid in acetic anhydride. The results are in excellent agreement with those obtained by the visual method as shown in Table VI.

The thermometric method provides an advantage over the visual method on samples that are dark in color.

Preliminary experiments using other liquid acid-anhydride systems (propionic and butyric) indicate that both the visual and thermometric methods will work. The precision of the visual method is reduced due to trouble in detecting the color change at the end point. This is more serious for the butyric system than for the propionic system. The precision of the thermometric method should be about the same in these systems as in the acetic acid–anhydride system, although the determination of the amount of acid present for the original calibration may present some difficulties.

Neither of the methods is applicable for determining acids in anhydrides if amines are present in the anhydride.

Table III. Recovery of Acid from Anhydride-Hydrocarbon Mixtures

Vol. Hydrocarbon, Ml.	Vol. Ac_2O, Ml.	AcOH Added, Grams	AcOH Found, Grams	Δ
100	100	1.26	0.90	+0.36
100	100	0.92	0.97	−0.05
100	100	1.09	1.06	−0.03

Table IV. Determination of Benzoic or Propionic Acid in Acetic Anhydride by Visual Method

Acid Added, Meq./Gram Anhydride	Acid Found, Meq./Gram Anhydride	Calcd. Acid, Meq./Gram Anhydride[a]
0	0.137	...
0.062 Benzoic	0.217	0.199
0.157 Benzoic	0.312	0.294
0.168 Propionic	0.330	0.305

[a] Acid in Ac_2O + acid added.

Table V. Data on the Thermometric Method for Determination of Acetic Acid in Acetic Anhydride

Known AcOH, %	% AcOH Read from Graph: Range of 5 determinations	% AcOH Read from Graph: Av. of 5 determinations
0.86	0.845–0.895	0.863
1.11	1.08–1.12	1.095
1.54	1.41–1.51	1.468
2.11	2.08–2.17	2.134
2.33	2.30–2.33	2.310
3.92	3.78–3.89	3.832
4.60	4.60–4.84	4.688
5.80	5.49–6.10	5.79

Standard deviation of all results ±0.088% absolute.

Table VI. Data on Determination of Propionic or Benzoic Acid in Acetic Anhydride

Calcd. Acid Present, Meq./Gram Anhydride[a]	Acid Found Visual Method, Meq./Gram Anhydride	Acid Found Thermometric Method, Meq./Gram Anhydride
0.199	0.217	0.213
0.294	0.312	0.308
0.305	0.330	0.280

[a] Acid in Ac_2O + acid added.

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RECEIVED for review April 22, 1955. Accepted May 26, 1955. Presented before the Division of Analytical Chemistry at the 128th Meeting of the AMERICAN CHEMICAL SOCIETY, Minneapolis, Minn.

Analysis of Residues Obtained on Treatment of Ancient Base Silver Alloys with Nitric Acid

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The usual treatment of samples of ancient base silver alloys with nitric acid often leaves residues composed of gold and impure metastannic acid. Silver chloride may also be present in the residues if corroded alloys are being analyzed. Since procedures for the analysis of such complex residues were not included in the usual schemes for the complete analysis of base silver alloys, two such procedures were devised. These were tested on an alloy of known composition and on synthetic mixtures. The test analyses showed that the individual components of the complex residues could be determined with an absolute error not exceeding 0.2 mg. The addition of these procedures to the usual schemes for analyzing base silver alloys now makes possible the complete accurate analysis of ancient base silver alloys.

AN ANCIENT base silver alloy usually contains a small proportion of gold and tin in addition to the silver, copper, and other metals soluble in nitric acid. Consequently the residue obtained on treatment of a sample with nitric acid contains gold as well as impure metastannic acid. If the alloy is corroded, silver chloride is usually present, and this also remains in the insoluble residue. The following procedures give accurate results for the analysis of these complex residues. Procedure I is for a residue from an uncorroded alloy, and Procedure II for one from a corroded alloy that is known or suspected to contain silver chloride. These procedures presuppose that a sample of about 1 gram has already been treated with nitric acid in the usual way, and that the insoluble residue has been collected on paper and washed repeatedly with hot dilute nitric acid.

PROCEDURE I

Method. Place the filter paper and residue in a weighed porcelain crucible and dry for 1 hour at 110° C. Burn off the paper at the lowest possible temperature, and then ignite over a Meker burner or in an electric muffle at 900° C. to constant weight.

Thoroughly mix the residue in the crucible with about 15 times its weight of powdered ammonium iodide and place the crucible with its contents in an electric muffle previously adjusted to 475° C. After 15 minutes remove and cool. Moisten the residue that now remains with 2 drops of concentrated nitric acid, and evaporate to dryness. Ignite over a Meker burner or in an electric muffle at 900° C. to constant weight. From the loss in weight due to the volatilization of the stannic oxide, calculate the percentage of tin.

Treat the residue that remains in the crucible with 5 ml. of concentrated hydrochloric acid and warm over a water bath until all action ceases. Dilute the solution with an equal volume of water, allow the residue to settle, and decant off the clear solution, preferably with the aid of a filter stick. Wash the residue by decantation with at least four 5-ml. portions of warm water. Add the decantate and washings to the original filtrate containing the metals soluble in nitric acid. Evaporate the wash water remaining in the crucible and ignite to constant weight. The weight of this final residue should be that of the gold in the sample.

To confirm the result for gold, which may be slightly high because of the presence of other insoluble and nonvolatile matter, such as silica, first treat the final residue with 10 ml. of cold 10% aqua regia, filter the solution through paper, and wash with warm water. Add 2 ml. of concentrated sulfuric acid to the filtrate and evaporate until fumes of sulfur trioxide are abundantly evolved. Dilute to 50 ml., add 25 ml. of saturated oxalic acid solution, and warm and stir until the gold is coagulated. Filter through close-grained paper and wash with warm water. Ignite in a porcelain crucible to obtain the weight of the gold.

Discussion. The use of sublimed ammonium iodide is highly recommended because this avoids the necessity of correcting for the nonvolatile matter ordinarily present in the reagent grade salt in variable proportions. Furthermore, the sublimed salt is directly obtained in a desirable finely divided state.

A suitable apparatus for the sublimation of ammonium iodide consists of a horizontal 100-cm. length of 35-mm. borosilicate glass tubing fitted at one end with a stopper containing an inlet tube for the introduction of tank nitrogen. In use a convenient quantity of reagent grade salt is placed in the tube near the stoppered end, the air is swept out of the tube by a stream of nitrogen, and the tube is heated with a gas burner placed under the salt until it has sublimed to the cool part of the tube. The stream of nitrogen should be so adjusted that no salt sublimes between the inlet tube and the heated salt and no salt is swept out the open end. For greater convenience in removing the sublimate, the tube may be provided with a few joints, though the use of a long glass scraper is almost equally convenient. Tests on samples of the salt so prepared showed that it does not contain weighable amounts of nonvolatile matter. When the sublimed salt is used, the additional step in the procedure that provides for the confirmation of the weight of the gold may usually be omitted.

Experiments showed that gold, even when finely divided, is not attacked when heated with ammonium iodide at 475° C. In these experiments, 30- to 40-mg. quantities of gold were dissolved in aqua regia in weighed crucibles, the solutions were evaporated to dryness, and the residues were ignited at 900° C. to produce the finely divided gold. After weighing, about 1 gram of ammonium iodide was mixed with the gold in each of the crucibles and volatilized at 475° C. The crucibles and their contents were again ignited at 900° C. and weighed. No changes in weights of gold beyond the normal weighing errors could be detected.

The leaching with hydrochloric acid serves to dissolve the cupric oxide and the ferric oxide usually present as impurities in the stannic oxide. The use of cold dilute aqua regia instead of the usual hot concentrated mixture is more convenient from the standpoint of manipulation and shortens the time of evaporation. It is prepared by diluting freshly made aqua regia with 9 times its volume of water. Experiments showed that the cold dilute solvent rapidly and completely dissolves metallic gold when it is in a finely divided state, as it is in these residues. The recommended method of reducing the gold to the metallic state usually produces a precipitate that may be filtered without the aid of macerated paper, though this may be used as an additional precaution.

When less accurate results are sufficient, the whole procedure may be abbreviated and made much more rapid by omitting the treatment with ammonium iodide, leaching the original weighed residue with cold dilute aqua regia, and determining the weight of the residue remaining after this treatment. For convenience the original residue is treated in the crucible with 10 ml. of cold 10% aqua regia and washed by decantation using a filter stick. The weight of the residue that remains is then determined by evaporating the residual wash water and heating to constant weight. The weight of the tin is calculated from the weight of

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this second residue, which should consist of impure stannic oxide, and the weight of the gold is the difference between the weights of the two residues. Experiments showed that cold aqua regia of this concentration does not dissolve stannic oxide. When the tin content of the sample is very small—i.e., not more than about 2 mg.—this shortened procedure yields accurate results.

PROCEDURE II

Method. Place the filter paper and residue in a weighed porcelain crucible and dry for 1 hour at 110° C. Burn off the paper at the lowest possible temperature and complete the removal of the carbon by placing the crucible in an electric muffle adjusted to 500° C.

After the crucible has cooled, add 2 drops of concentrated nitric acid and evaporate to dryness. Add 10 ml. of a nearly saturated solution of ammonium iodide to the crucible and allow it to remain in contact with the residue for about 15 minutes with occasional agitation. Filter the solution through paper, transferring it with small portions of the concentrated ammonium iodide solution, preferably added from a dropper or small pipet, and catching the filtrate in a 250-ml. beaker. Wash the residue on the paper with at least six additional small portions of the concentrated ammonium iodide solution, and finally with sufficient water to remove all the ammonium iodide from the crucible and paper. Treat the paper and residue by Procedure I, using the original crucible for the drying and ignition.

Add 10 ml. of concentrated sulfuric acid to the above filtrate and evaporate on a hot plate until fumes of sulfur trioxide have been evolved for an hour. Cool the beaker and contents, add 50 ml. of water, warm the solution, filter through paper, and wash thoroughly with warm water. Add dilute hydrochloric acid to the filtrate until precipitation is complete. Warm and stir the solution until the precipitate has coagulated. Collect the silver chloride in a weighed filter crucible, and wash, dry, and weigh in the usual way. Add a solubility correction of 0.5 mg. to the weight of the dried silver chloride to give the weight of silver chloride originally present in the alloy.

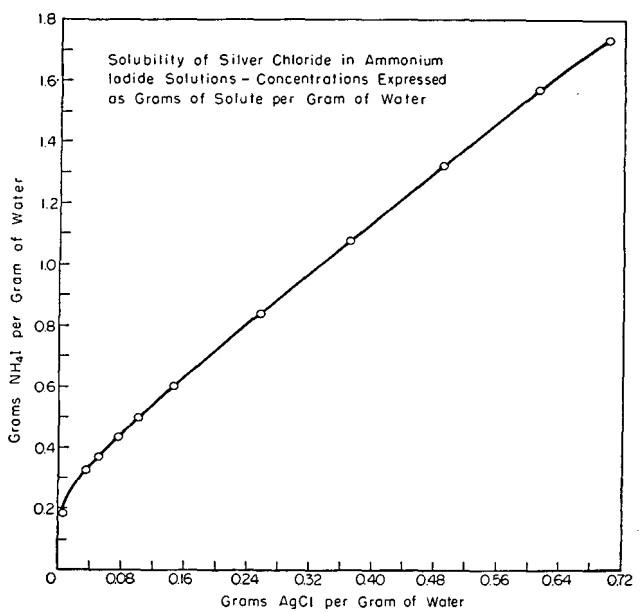

Figure 1

Discussion. Ignition before treatment with the ammonium iodide solution is necessary to dehydrate the metastannic acid to a considerable degree, so that it is not peptized by the salt solution during filtration. The temperature of ignition is restricted to 500° C., about the lowest at which the carbon of the filter paper may be burned off, because the silver chloride is not only readily reduced to silver by contact with carbon at higher temperatures but reacts with and fuses into the glaze of the crucible so that it cannot conveniently be recovered. The ignition at 500° C. must be done with free access to air, and even under these conditions some silver may be formed. The purpose of adding nitric acid after ignition is to convert any such silver to silver nitrate so that it can react with the ammonium iodide solution added in the next step. It is not practicable to collect the residue in a filter crucible and thus avoid the possibility of reduction by carbon, since the finely divided metastannic acid clogs the pores of the filter.

That silver chloride is readily dissolved by ammonium iodide solutions of sufficiently high concentration is indicated by Figure 1, which summarizes the results of a series of solubility determinations. For these determinations solutions were made up from accurately weighed quantities of ammonium iodide, silver chloride, and water, brought to 25° C. in a constant temperature bath, and slowly titrated with water to the first appearance of an opalescence as indicated by the Tyndall effect from a transverse light beam. The total weight of water for a saturated solution containing any of the various combinations of weights of ammonium iodide and silver chloride was the sum of the weight of water originally taken plus that added by titration. Closely reproducible results were obtained.

An ammonium iodide solution nearly saturated at room temperatures contains 1.7 grams of salt for each 1.0 ml. of water. Cold concentrated ammonium iodide solutions do not react with stannic oxide or partly dehydrated metastannic acid, at least during the short time of contact recommended in this procedure.

It is important not to wash with water until all the silver solution is in the filtrate, for dilution of this solution may cause precipitation of silver iodide on or in the filter. Although the silver may be quantitatively precipitated by dilution of the filtrate with a very large volume of water, it is not feasible to end the determination by this means because the large volume and the very finely divided precipitate of silver iodide thus obtained make filtration very difficult. Furthermore, this precipitate will also contain a small proportion of silver chloride.

The treatment with sulfuric acid oxidizes most of the iodide to free iodine, and the subsequent evaporation removes this iodine and any unconverted hydriodic acid. Filtration after the treatment with sulfuric acid is necessary to remove the sulfur formed from the reduction of the sulfate. As many as 3 hours of warming and occasional stirring may be needed to coagulate properly the silver chloride, which usually precipitates in a finely divided form.

The reason for the solubility correction is that some silver chloride is dissolved by the nitric acid solution and washings that

Table I. Analysis of Alloy

Component	Calcd. Composition, %	Composition by Analysis, % Detn. I	Detn. II
Au	0.50	0.53	0.51
Sn	4.78	4.79	4.74
Ag	46.91	46.70	46.75
Cu	47.81	47.85	47.93
All	100.00	99.87	99.93

Table II. Analysis of Synthetic Mixtures

Component	Mixture No.	Taken, Mg.	Found, Mg.	Error, Mg.
AgCl	I	9.6	9.8	+0.2
	II	10.7	10.8	+0.1
Au	I	21.9	22.0	+0.1
	II	15.9	16.1	+0.2
Sn	I	40.4	40.3	−0.1
	II	49.2	49.4	+0.2
Cu	I	71.3	71.2	−0.1
	II	97.9	97.7	−0.2
Fe	I	2.7	2.9	+0.2
	II	3.1	3.3	+0.2
All	I	145.9	146.2	+0.3
	II	176.8	177.3	+0.5

must be used when a sample of an alloy containing silver chloride is first dissolved for analysis. In experiments in which pure silver chloride and synthetic mixtures containing silver chloride were treated with nitric acid under conditions simulating those that would be used for dissolving such an alloy, the loss of silver chloride was found to range from a minimum of 0.3 mg. to a maximum of 0.8 mg., with an average of 0.5 mg., the recommended correction.

TEST ANALYSES

In order to test Procedure I on an alloy of known composition, one was prepared from 24-karat gold, fine silver of 99.99% purity, electrolytic copper, and reagent grade tin. These ingredients were weighed out on an analytical balance and fused in a graphite crucible under conditions that caused no loss of metal. Metallic lithium was used to deoxidize the melt which was thoroughly stirred before being poured into a mold. After removal of the rough surface metal, the ingot was reduced to chips in a milling machine, and these were thoroughly mixed to obtain the analytical samples. The calculated composition of the alloy is shown in Table I along with the results of duplicate analysis in which the gold and tin were separated and determined by Procedure I and the other components by conventional procedures. It will be seen that the results for gold and tin are very satisfactory and those for the other two metals acceptable.

Procedure II was tested on intimate mixtures containing silver chloride that were prepared from accurately weighed quantities of the several components. It will be seen from Table II that satisfactory results were obtained from the silver chloride as well as for the other components.

RECEIVED for review January 19, 1955. Accepted June 10, 1955. Abstracted from a dissertation submitted by Charles D. Oviatt to The Graduate School of The Ohio State University in partial fulfillment of the requirements for the Ph.D. degree, 1954.

Determination of Mixed Phthalic Acid Isomers in Alkyd Resins

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The meta and para isomers of phthalic acid have recently attained commercial significance in alkyd resin manufacture. An analytical method for measuring each of the three phthalic acid isomers in mixture consists of a special saponification technique to recover the acids from resin solution, followed by hydrolysis in methanol solution and measurement of the absorptivity at three ultraviolet wave lengths. Analytical control can be exercised on the new compositions.

FOR a number of years *o*-phthalic anhydride has been used in the manufacture of alkyd resins and these resins in paint vehicles have been measured by determination of phthalic anhydride content. Recently, the meta and para isomers, also known as isophthalic and terephthalic acid, have attained commercial significance and may be utilized in alkyd resin manufacture. Investigations were undertaken to determine the effect of these two isomers on existing methods of analysis for *o*-phthalic acid and to devise methods of analysis for each isomer.

Two quantitative methods for determining *o*-phthalic acid specifically in the presence of other dicarboxylic acids are found in the literature. In these methods, the phthalic acid is measured by its absorption in the ultraviolet region at 276 mμ (*2*) or by the weight of the lead salt formed in glacial acetic acid (*3*). These two methods with slight modification also appear as ASTM methods (*1*). The latter is not affected by the presence of iso- and terephthalic acids and can be used to measure *o*-phthalic acid or anhydride in alkyd resins without interference. All phthalic acid isomers absorb strongly in the ultraviolet region at 276 mμ, so that the spectrophotometric method in its present form is unsuitable. However, it was found that each isomer shows a distinctive absorbance curve throughout the ultraviolet range; the iso- and terephthalic acids show strong absorption at the shorter wave lengths and secondary absorption at the longer wave lengths. Figure 1 shows the absorptivities of 1 to 1 methanol-water solutions, made 0.1*N* in hydrochloric acid, of the three phthalic acids plotted against the wave length in millimicrons. Since the isomers show different points of maximum absorption at the longer wave lengths, it is possible to treat mixtures of the acids as three-component systems. The wave lengths chosen for the analysis of the isomeric phthalic acids, 275, 281, and 287 mμ, are points at which the spread between the curves is large but not so large as to give absorbance readings which are either very high or low. The use of long wave lengths is considered advisable in order to minimize the interference that would be caused by the presence of any water-soluble organic contaminants that might be present in the saponification product of alkyd resins.

PROCEDURES

Calibration. The absorbances of the three phthalic acids must be determined at 275, 281, and 287 mμ. The isophthalic and terephthalic acids used were obtained from Eastman Kodak Co., catalog numbers 3233 and 640, respectively. The Beckman spectrophotometer, Model DU, was used with 1-cm. cells. Because of the low solubility in water of isophthalic and terephthalic acids, a 1 to 1 methanol-water mixture was used as the solvent throughout. To calibrate, 25 mg. of each acid are dissolved in 250 ml. of absolute methanol by refluxing. To the methanol solution are added 5 ml. of concentrated hydrochloric acid, and the solution is diluted to 500 ml. with distilled water, thus giving a final concentration of 50 mg. of acid per liter of solution. The absorbance of each solution is determined at the three wave lengths using a slit width of 0.6 mm. and a 1 to 1 methanol-water mixture, made 0.1*N* in hydrochloric acid, as a blank, following the method of reversing the cells as proposed by Shreve and Heether (*2*) in the original spectrophotometric method for determining *o*-phthalic acid. The absorptivity of each acid at each wave length is calculated, using the equation

$$a = \frac{A}{bc}$$

where a is the absorptivity at the particular wave length measured; A is the average absorbance of the acid solution being measured at the same wave length; b is the cell length in centimeters; and c is the concentration expressed in grams of acid per liter.

Analysis of Acid Mixtures. The analytical data in Table I were obtained by applying the following procedure to known mixtures of the isomeric phthalic acids.

Mixtures totaling a maximum of 50 mg. of acid are refluxed with 50 ml. of absolute ethyl alcohol. When dissolved, 10 ml. of dry benzene are added, followed by 3 ml. of 2*N* alcoholic potassium hydroxide. After refluxing for 1 hour, 150 ml. of dry benzene are added, the flask is stoppered, and the contents are cooled with water and then filtered through a Gooch crucible, benzene being used for transferring and washing the precipitate. The residue is given a final ether wash and dried at 105° C.

The dried salts are dissolved in water and diluted to 100 ml. A 10-ml. aliquot of this aqueous solution is then diluted to 100 ml. with 1 to 1 methanol-water, made 0.1*N* in hydrochloric acid, and the absorbance of this solution is determined at 275, 281, and 287 mμ as in the calibration procedure.

For this three-component system, the following equations apply:

$$A_{275} = b(a_{275,i}c_i + a_{275,o}c_o + a_{275,t}c_t)$$

$$A_{281} = b(a_{281,i}c_i + a_{281,o}c_o + a_{281,t}c_t)$$

$$A_{287} = b(a_{287,i}c_i + a_{287,o}c_o + a_{287,t}c_t)$$

where A, a, b, and c have the significance as in the calibration procedure and the subscripts i, o, and t stand for isophthalic, *o*-phthalic, and terephalic acid, respectively.

Table I. Comparison of Theoretical and Experimental Values of c_i, c_o, and c_t

Mixture No.	Isophthalic Acid, Mg.		*o*-Phthalic Acid, Mg.		Terephthalic Acid, Mg.	
	Taken	Found	Taken	Found	Taken	Found
1	15.8	15.4	19.5	20.2	15.4	16.5
2	25.8	26.6	20.1	19.7	4.9	4.8
3	20.0	19.5	20.0	19.8	10.6	10.5
4	4.7	5.1	39.9	39.9	4.9	5.6
5	10.0	9.7	30.9	30.4	10.3	10.7
6	20.1	19.2	10.0	9.9	21.0	20.7
7	40.7	39.8	10.0	10.3	0.0	−0.7
8	9.8	10.7	39.7	39.2	0.0	+0.2
9	0.0	0.3	39.0	38.9	10.8	11.4
10	0.0	−1.0	30.9	31.2	20.0	20.6
11	50.2	50.3	0.0	+1.4	0.0	−0.5
12	0.0	1.8	0.0	−0.8	18.9	18.4
13	40.3	39.2	4.5	4.9	5.4	5.4
14	40.1	40.3	0.0	0.2	10.4	9.6
15	0.0	1.2	49.6	48.7	0.0	−0.2

The absorptivities experimentally determined in this case are shown in Table II. When they are inserted into the above three equations and the equations solved simultaneously, the following solutions for c_i, c_o, and c_t are obtained, using cells of 1-cm. length:

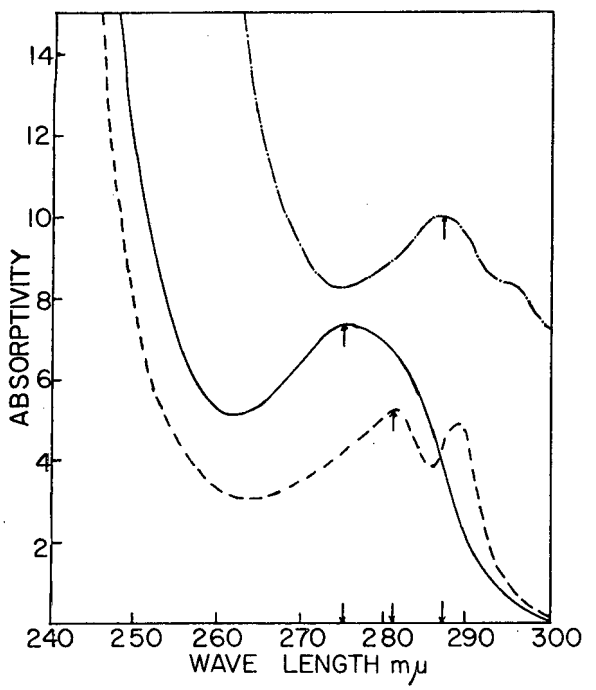

Figure 1. Ultraviolet spectra

——— *o*-Phthalic acid
- - - - - *m*-Phthalic acid
–·–·–· *p*-Phthalic acid
In 1:1 methanol: water made 0.1*N* in hydrochloric acid

Table II. Absorptivities of Isophthalic, *o*-Phthalic, and Terephthalic Acids as Determined at 275, 281, and 287 Mμ and Slit Width of 0.6 Mm.

Wave Length, Mμ	a_i	a_o	a_t
275	4.26	7.31	8.30
281	5.26	6.71	9.02
287	4.47	3.99	9.98

$$c_i = -0.975A_{275} + 1.254A_{281} - 0.3223A_{287}$$

$$c_o = 0.383A_{275} - 0.170A_{281} - 0.165A_{287}$$

$$c_t = 0.283A_{275} - 0.493A_{281} + 0.310A_{287}$$

Some analytical results are given in Table I where known and experimental values of c_i, c_o, and c_t are compared.

Analysis of Alkyd Resins. In analyzing alkyd resins, a sample estimated to contain 0.100 to 0.500 gram of total phthalic acid is weighed into a 500-ml. Erlenmeyer flask and dissolved in 10 ml. of dry benzene. When dissolved, 100 ml. of 0.5*N* alcoholic potassium hydroxide (made with absolute ethyl alcohol) are added, and the mixture is agitated occasionally at room temperature until precipitation begins. It is then warmed at 45° C. in a bath or oven, for at least 4 hours. An air condenser is then attached and the sample is refluxed for 1 hour; 150 ml. of dry benzene are added, and the flask is stoppered and cooled, and allowed to stand for 30 minutes or longer. It is then filtered through a Gooch crucible, benzene being used for transferring and washing the precipitate. The residue is given a final ether wash and dried at 105° C. The dried salts are dissolved in water and diluted to 250 ml. An aliquot which contains 5 mg. total phthalic acids is withdrawn, transferred to a 100-ml. volumetric flask, and diluted with 1 to 1 methanol-water, made 0.1*N* in hydrochloric acid, and the absorbance of this solution is determined at 275, 281, and 287 mμ as in the calibration procedure. The concentration of each acid can be calculated as above. The percentage of each acid present in the alkyd resin can be calculated from the equation

$$\% \text{ acid} = \frac{c \times 2500}{\text{sample weight in grams} \times \text{fraction solids} \times \text{aliquot size in milliliters}}$$

The "fraction solids" refers to the original resin solution.

DISCUSSION

The weight of the potassium salts of the acids obtained by saponification in ethyl alcohol cannot be used to estimate the weight of total acid because isophthalic acid always precipitates with entrained alkali, so that the weight yields are higher than theoretical. In the usual saponification techniques, this entrainment will include some of the oils or fatty acids and, if sufficiently high, may cause some error in the determinations. The entrainment of the latter is minimized by the modified technique as described, by allowing the precipitated salts to form slowly. These properties were studied by the addition of alcohol solutions of iso- and terephthalic acids to alkyd resins of known *o*-phthalic content, prior to saponification.

It is estimated that in alkyd resin analysis, the accuracy for the determination of the ortho and para isomers will be ±1%; for isophthalic this will be nearer ±1.5%.

For the analysis of materials known to contain one specific acid or where the identity of the acids present is known, the use of wave lengths other than the three chosen for this work might be advantageous. For example, the shoulder at 295 mμ on the terephthalic acid curve is subject to little interference from the ortho- and iso-acids. However, the intended purpose of these investigations is for the analysis of materials of unknown composition in which the three acids may appear singly or in a variety

of combinations. The three wave lengths were chosen in the interest of permitting routine analysis of such materials with a minimum of trial-and-error dilutions to obtain suitable absorbance readings, and with adequate although not necessarily optimum accuracy.

Small samples of pure acids were used in these investigations because of the low solubility of terephthalic acid in ethyl alcohol.

The usual qualitative test used to detect alkyd resins by condensing the dried film with phenol to form phenolphthalein does not give positive results with resins made only with isophthalic or terephthalic acids.

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RECEIVED for review March 24, 1955. Accepted May 19, 1955.

Determination of Sulfur in Rubber Vulcanizates

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A combustion method for rubber which determines the sulfur evolved at 480° to 500° C. is compared with the fusion and the zinc-nitric acid methods. In the combustion method an interaction occurs between the sulfur and the fillers present in the rubber. The effect of particular fillers with respect to this interaction is discussed. A value representative of the organically bound sulfur in an extracted specimen can be obtained by the combustion method when no reaction takes place between the sulfur and the fillers during combustion. The fusion method yields results in good agreement with the total sulfur added in compounding the rubber samples. Except in the presence of barium compounds, the zinc-nitric acid method likewise determines total sulfur. When barium compounds are present, barium sulfate is formed during the oxidation, and low values are obtained for the total sulfur.

THE determination of organically bound sulfur in rubber compounds presents a problem to the analytical chemist. A rubber vulcanizate may contain sulfur that is present in the free state, sulfur that is combined with the rubber or in other organic compounds, and sulfur in inorganic compounds.

The free sulfur can be determined after its removal by extraction. The sulfur that is combined with the rubber and other organic material and the sulfur contained in the inorganic fillers are usually determined together. A separate determination of the sulfur present in the inorganic fillers is then made in order to calculate the amount of sulfur that is organically combined.

Combustion methods are often used to determine sulfur, and the rapidity and accuracy of such methods make them suitable for many types of analyses. At temperatures of 1350° C. and higher, the total sulfur in rubber vulcanizates can be determined (*2*). The rubber technologist, however, is usually more interested in organic sulfur than in total sulfur. Accordingly, an investigation of the combustion method at lower temperatures was undertaken. The compounds used in this study were also analyzed by the fusion and by the zinc-nitric acid methods for comparative purposes.

EXPERIMENTAL

A combustion method for the determination of sulfur in rubber may be divided into three phases: decomposing the specimen, collecting the evolved gases, and determining the amount of sulfur evolved.

Decomposing the Specimen. A rubber hydrocarbon can be distilled from a rubber compound below red heat. Wall (*7*) in work on the thermal decomposition of rubbers for mass spectrometric investigations used a temperature of 400° C. in a high vacuum, because lower temperatures did not completely decompose the polymers. In analysis of GR-S carbon black masterbatches (*5*, *6*), 550° C. is used to remove the polymer in determining the ash and the carbon black. At 550° C. any lithopone used in rubber compounds decomposes slightly with the evolution of hydrogen sulfide, which leads to high results for sulfur; at temperatures of 400° C. pyrolysis of the rubber always may not be completed during the time allowed. Therefore, a temperature of 480° to 500° C. appears to be optimum for the purpose of this investigation. The combustion of the rubber sample was accomplished by using the two unit electric organic combustion furnace shown in Figure 1. The 8-inch unit was placed at the exit end of the combustion tube and was maintained at a temperature of 850° to 900° C. The 12-inch unit which was placed next to the smaller unit was maintained at a temperature of 480° to 500° C. The upper part of each unit was hinged to facilitate installing the combustion tube and the thermocouples. An unglazed porcelain McDanel combustion tube, 30 inches long, $1^1/_4$-inch outside diameter, 1-inch inside diameter, with a standard tapered tip and equipped with a sample inserter was found to be very satisfactory.

To obtain a clean decomposition, it was necessary to adjust the specimen size to a minimum compatible with the precision desired, to control the rate of decomposition of the specimen so as to avoid minor explosions, and to use sufficient oxygen to burn completely the volatilized organic material in order to avoid having soot enter the absorber. The specimens contained approximately 0.15 gram of hydrocarbon; and since the compounds contained about 65% hydrocarbon by weight, 0.25-gram specimens were used throughout this work.

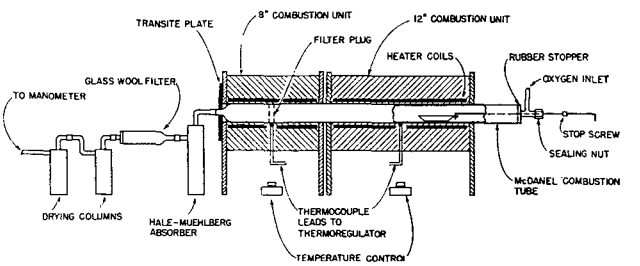

Figure 1. Assembly of apparatus for combustion method

A specimen of ground rubber vulcanizate was accurately weighed, transferred to a 10 × 75 mm. test tube, and tapped into the bottom with a glass rod. The test tube was laid in a porcelain combustion boat, which was placed in the cool portion of the combustion tube with the open end of the test tube facing the outlet end of the combustion tube. The sample injector was inserted into the large end of the combustion tube, the oxygen flow was adjusted to 1100 cc. per minute, and the combustion boat was pushed into the center of the 12-inch unit, which was maintained at about 500° C. The dense vapors that accompanied the initial decomposition of the specimen displaced the

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oxygen around the specimen and burned at the mouth of the test tube. Thus, the rubber was permitted to decompose in an oxygen-poor atmosphere without a localized temperature rise.

Collecting the Evolved Gases. As the distillation of the rubber hydrocarbon occurred at a relatively low temperature, the gases were passed through a high temperature zone to ensure complete combustion. This was accomplished with the 8-inch furnace unit maintained at about 900° C. A close-fitting porous ceramic plug may be used in the high temperature zone to filter the gas or to present more surface to facilitate oxidation, but is not essential.

Since sulfur trioxide was present in the evolved gases, the exit end of the combustion tube was maintained at a temperature of at least 200° C. as suggested by Hale and Muehlberg (*4*). This was accomplished by placing the tube so that the outlet tip was about 2 inches from the edge of the hot zone. A Transite baffle with a hole just large enough to admit the thick portion of the tip was mounted on the end of the furnace to reduce the heat loss in the furnace and protect the operator from the heat while manipulating the absorber.

A Hale-Muehlberg absorber, lubricated with heavy silicone grease, was connected to the McDanel combustion tube by means of a standard taper joint. The absorber was charged with a solution prepared just prior to use by mixing 4 ml. of 5% hydrogen peroxide solution, 18 ml. of 10% sodium chloride solution (to prevent foaming during combustion), and 18 ml. of distilled water. The 5% hydrogen peroxide solution was prepared by diluting 30% ACS grade hydrogen peroxide that did not contain a stabilizer. This type of absorber, or even a battery of them, did not collect completely the sulfur trioxide, which was seen passing through in the form of a fog. This loss amounted to about 4% of the evolved sulfur. However, when a glass tube containing a filter of dry glass wool was attached to the outlet end of the tower, this vapor was quantitatively collected. Approximately 10 grams of ordinary glass wool, previously leached in a warm 0.5% hydrochloric acid solution and washed with distilled water until the washings were neutral to methyl red, was used to pack the filter tube. The exit end of the filter tube was fitted with a rubber stopper containing a short length of glass tubing to provide a connection to the drying columns and flow meter.

Determining the Amount of Sulfur Evolved. After 10 minutes the oxygen flow was shut off, the boat was removed from the combustion tube, and the two absorbers were disconnected. The solution used to absorb the gases was quantitatively removed from the tower and glass tubing, and particular attention was paid to thorough rinsing of the fritted disk and the inside surface of the connecting tubes. The glass wool filter was rinsed by mounting the glass tube on a small suction flask and washing it with 125 ml. of distilled water while using gentle suction. This was combined with the solution already collected in a 400 ml. beaker, four drops of methyl red indicator were added, and the solution was titrated with standardized 0.02*N* sodium hydroxide. The filter tube was prepared for the next run by rinsing with acetone and drying with compressed air.

RUBBER COMPOUNDS

Four rubber batches were carefully prepared on a parts per weight basis to represent the various types of mixes and concentrations of sulfur indicated in Table I. Each of these batches was divided into four groups, and to each group was added one of the four selected fillers. The fillers included carbon black (Thermax), calcium carbonate (Whiting), barium carbonate, and lithopone.

Table I. Composition of Compounds

Batch designation	1	2	3	4
Original mixes, parts by weight				
Pale crepe	100	100	100	100
Sulfur	0.625	4.13	7.17	10.5
Tetramethylthiuramdisulfide [bis(dimethylthiocarbamoyl)]	0.3	...	...	...
Phenyl-2-naphthylamine	1	1	1	1
Diphenylguanidine	...	...	1	...
Lead oxide	...	10	...	...
Zinc oxide	5	..	...	...
Fillers added[a]	50	50	50	50
Total, parts by weight	156.925	165.13	159.17	161.5
Free sulfur added, %	0.50[b]	2.50	4.50	6.50
Cure: Time, min.	30	30	30	60
Temp., ° C.	140	150	150	160

[a] Filler Identity

	Compound Designation			
Carbon black	1*A*	2*A*	3*A*	4*A*
Calcium carbonate	1*B*	2*B*	3*B*	4*B*
Barium carbonate	1*C*	2*C*	3*C*	4*C*
Lithopone	1*D*	2*D*	3*D*	4*D*

[b] Includes 0.16 gram of sulfur from organic accelerator.

The 16 rubber compounds, whose designations are listed in Table I, were analyzed for sulfur by the fusion method and the zinc-nitric acid method described in ASTM Standards on Rubber Products (*1*) and the Federal Test Method Standard (*3*). They were also analyzed by the combustion method described herein. The residues remaining after the combustion of the compounds in groups *A*, *B*, and *D* were analyzed for sulfur by oxidation with a bromine-nitric acid solution and precipitation as barium sulfate. Those of group *C* were analyzed by the fusion method since the wet oxidation procedure is inoperative in the presence of barium carbonate.

Table II. Sulfur Determined by Combustion Method

Compound	No. of Detn.	% Sulfur (Av.)	Std. Dev.
1*A*	4	0.39	0.033
2*A*	4	1.67	0.037
3*A*	5	4.49	0.078
4*A*	3	6.35	0.036
1*B*	4	0.30	0.031
2*B*	4	1.52	0.029
3*B*	5	4.32	0.062
4*B*	4	6.19	0.146
1*C*	5	0.27	0.038
2*C*	7	1.45	0.052
3*C*	7	4.13	0.047
4*C*	2	5.77	0.021
1*D*	11	0.56	0.125
2*D*	22	1.99	0.089
3*D*	8	4.54	0.085
4*D*	6	6.39	0.139

RESULTS BY COMBUSTION METHOD

Table II gives the average values for the sulfur evolved during combustion from each of the 16 compounds and also the standard deviations for the individual determinations. An analysis of these standard deviations indicates the existence of two levels of precision. Determinations made on samples of groups *A*, *B*, and *C*, which when compounded contained no inorganic sulfur, gave a standard deviation of the order of 0.04% sulfur. Determinations made on samples of group *D*, which contained lithopone, gave a standard deviation of approximately 0.11% sulfur. Thus, the presence of lithopone appears to cause somewhat poorer reproducibility of results by this method of analysis.

A study of the amounts of sulfur found by combustion, as listed in Table II, reveals systematic differences in evolved sulfur in the presence of different types of inorganic fillers. The lowest values of evolved sulfur are obtained in samples containing barium carbonate, group *C*. The values for the samples containing calcium carbonate (*B*), carbon black (*A*), and lithopone (*D*), exceeded those found for the samples containing barium carbonate, on the average, by 0.18, 0.32, and 0.47%, respectively. These differences in evolved sulfur are not appreciably influenced by the amount of added free sulfur.

Except for two of the compounds containing lithopone, the evolved sulfur is less than the added free sulfur. This fact, together with the observed differences in evolved sulfur for different fillers, can be interpreted in terms of varying degrees of combination of the added sulfur, exclusive of added inorganic sulfur, with the filler. Further evidence regarding this point is obtained from the study of the interaction between sulfur and fillers during combustion.

INTERACTION BETWEEN SULFUR AND FILLERS

Specimens from each of the rubber compounds were dissolved in hot *o*-dichlorobenzene (*3*), and the remaining inorganic residues

were analyzed for total sulfur by the fusion method. Thus, the amount of inorganic sulfur formed during vulcanization was determined. Also the residues from the combustion of each vulcanizate were analyzed for sulfur by a wet oxidation procedure, except in group *C*, where the fusion method was used. By comparing these two sets of values one can evaluate the degree of interaction between sulfur and fillers during combustion. These data are listed in Table III.

Examination of the values in column I leads to the conclusion that except in compounds containing lead oxide, very little sulfur combines during vulcanization as inorganic sulfur. For compounds in batch 1, which were compounded with only 0.5% of free sulfur, the formation of zinc sulfide during vulcanization did not occur to any appreciable extent. On the other hand, a comparison of the values in columns I and II indicates that sulfur combines to an appreciable extent during combustion with zinc, lead, calcium, and barium compounds present in the vulcanizate. The largest amount of combination with the filler takes place in those samples containing lead oxide. When barium carbonate is present a moderate amount of combination takes place. The least combination occurs in samples which contain only carbon black, compounds 3*A* and 4*A*.

For compounds 1*D*, 3*D*, and 4*D*, which contain sulfide from the added lithopone, less sulfide is recovered after combustion than was present initially. The results for sulfur determined by the combustion method and listed in Table II show that these three samples evolved more sulfur than the corresponding samples of groups *A*, *B*, and *C*. Compounds 1*D* and 3*D* were the only two that evolved more sulfur than was originally added in the form of free and organic sulfur. These results indicate that inorganic sulfides liberate some sulfur during the combustion.

Compound 2*D* differs from the other members of group *D* in that more sulfide sulfur is found in the residue from combustion than was added in preparing the sample. Apparently the sulfur that combines with the lead more than compensates for that evolved from the lithopone.

Table III. Interaction between Sulfur and Fillers during Combustion

Compound	Inorganic Sulfur Added in Compounding Samples, %			Sulfur Found in Residue, %	
	Sulfide	Sulfate	Total	I[a]	II[b]
1*A*	0	0	0	0.00	0.16
2*A*	0	0	0	0.69	0.60
3*A*	0	0	0	0.01	0.02
4*A*	0	0	0	0.03	0.07
1*B*	0	0	0	0.02	0.30
2*B*	0	0	0	0.03	1.02
3*B*	0	0	0	0.01	0.12
4*B*	0	0	0	0.02	0.14
1*C*	0	0	0	0.01	0.29
2*C*	0	0	0	0.62	1.12
3*C*	0	0	0	0.01	0.27
4*C*	0	0	0	0.04	0.73
1*D*	3.10	3.05	6.15	6.15	2.92
2*D*	2.94	2.90	5.84	6.50	3.18
3*D*	3.05	3.01	6.06	6.13	2.84
4*D*	3.01	2.96	5.97	5.96	2.72

[a] Total sulfur determined in residue remaining after dissolving vulcanized rubber in *o*-dichlorobenzene.
[b] Sulfide sulfur in nonvolatile residue remaining from the combustion analysis. For group *C*, inorganic sulfur was determined by the fusion method.

Table IV. Comparison of Values for Sulfur Found by Different Analytical Procedures

Compound	Sulfur Added, %				Sulfur Found, %			
	Free	Sulfide	Sulfate	Total	Fusion method	Zn + HNO_3 method	Combustion method + residue	Combustion method
1*A*	0.50	0	0	0.50	0.44	0.47	0.55	0.39
2*A*	2.50	0	0	2.50	2.53	2.36	2.27	1.67
3*A*	4.50	0	0	4.50	4.49	4.45	4.51	4.49
4*A*	6.50	0	0	6.50	6.41	6.39	6.42	6.35
1*B*	0.50	0	0	0.50	0.46	0.48	0.60	0.30
2*B*	2.50	0	0	2.50	2.43	2.26	2.54	1.52
3*B*	4.50	0	0	4.50	4.55	4.44	4.44	4.32
4*B*	6.50	0	0	6.50	6.50	6.36	6.33	6.19
1*C*	0.50	0	0	0.50	0.50	0.01	0.56	0.27
2*C*	2.50	0	0	2.50	2.55	0.01	2.57	1.45
3*C*	4.50	0	0	4.50	4.38	0.11	4.50	4.13
4*C*	6.50	0	0	6.50	6.59	1.40	6.50	5.77
1*D*	0.50	3.10	3.05	6.65	6.66	3.62	3.48	0.56
2*D*	2.50	2.94	2.90	8.34	8.33	5.27	5.17	1.99
3*D*	4.50	3.05	3.01	10.56	10.58	7.58	7.38	4.54
4*D*	6.50	3.01	2.96	12.47	12.14	9.39	9.11	6.39

COMPARISON OF METHODS

Table IV lists the sulfur added in compounding each sample and the amount of sulfur found by the three methods included in this study.

Specimens from each rubber compound were analyzed by the fusion method to determine the total sulfur present; by the zinc-nitric acid method to obtain a combined value for the free, organically bound, and sulfide sulfur; and by the combustion method to determine the sulfur evolved during combustion. In the column headed Combustion method and residue, the results in Table II and those in column II of Table III have been combined.

In interpreting this table the following points should be borne in mind. For the compounds of groups *A* and *B*, which contained no barium, the values obtained by the fusion method, zinc-nitric acid method, and combustion plus residue procedure can be compared with the total amount of sulfur added. In the zinc-nitric acid method, the presence of barium carbonate leads to low and erroneous results as the barium sulfate formed during the oxidation phase of the analysis is not determined. The results of group *C* can be explained on this basis. For the compounds of this group, a comparison of the combustion plus residue values and those obtained by the fusion method can be made with the total amount of sulfur added since no sulfate was added in preparing these samples. For the compounds of group *D*, both the zinc-nitric acid method and the combustion plus residue procedure determine all but the sulfate sulfur.

An analysis of the data shows that the fusion method is free of systematic errors and that its reproducibility is reflected by a standard deviation of 0.1% sulfur. The zinc-nitric acid method, where applicable, tends to give results that are low, on the average, by 0.09% sulfur. Its reproducibility is characterized by a standard deviation of 0.1% sulfur, which is comparable to that of the fusion method. The values obtained by the combustion plus residue procedure appear to be free of systematic errors for the compounds of groups *A*, *B*, and *C*. For compounds of group *D* this procedure yields values which are consistently low (on the average, by 0.23% sulfur), when compared to the sulfur added, exclusive of sulfate sulfur.

The values listed in the last column represent the sulfur evolved during combustion. Column II in Table III lists the inorganic sulfur formed during the course of the combustion. For the compounds of groups *B*, *C*, and *D* where reaction takes place with the filler during combustion, the combustion method is only semiquantitative. Among the compounds of group *A*, samples 3*A* and 4*A* contained only carbon black as a filler and satisfactory results were obtained by the combustion method for these samples. For samples 1*A*, and 2*A* which contained zinc oxide and lead oxide, respectively, the combustion method yielded low results.

In conclusion, a value representative of the organically bound sulfur can be obtained by the combustion method for extracted samples that contain only carbon black as a filler and when zinc oxide and lead oxide are both absent. For the other samples a combination of extraction,

a total sulfur method and the *o*-dichlorobenzene solution procedure (*3*), appears to be required for determining organic sulfur reliably.

ACKNOWLEDGMENT

The authors wish to thank John Mandel of the Testing and Specifications Section for his statistical analysis of the data.

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RECEIVED for review April 5, 1955. Accepted May 26, 1955.

Densities and Refractive Indexes for Ethylene Glycol—Water Solutions

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The lack of agreement among previously published data indicated the need for precise analytical data for mixtures of ethylene glycol (1,2-ethanediol) and water. Investigation of glycol purification by vacuum distillation defined the necessity for extreme care in preventing contamination with trace amounts of oxidation products. Densities at 25° C. and refractive indexes at 20° C. were determined for mixtures of highly purified ethylene glycol and water. The density data provide a basis for analysis to within ±0.11% by weight, whereas refractive index determinations yield analyses accurate to within ±0.03 weight %. Refractive index is not a linear function of weight per cent composition, as is commonly assumed for this system.

IN THE course of a recent distillation study involving the ethylene glycol–water system a precise analytical method was of critical importance. Variations in published values for the density and refractive index of purified ethylene glycol suggested the presence of trace impurities, since the range of values was considerably in excess of that which would be expected from a series of independent determinations on identical materials. Therefore, purification by vacuum distillation was studied from the standpoint of identification and subsequent elimination of trace contaminants. Once a satisfactory distillation procedure was found, densities and refractive indexes were determined for the aqueous glycol system over the entire composition range.

PURIFICATION OF MATERIALS

Technical grade, 99.5%, ethylene glycol was fractionated at 7 to 10 mm. of mercury absolute pressure and with a reflux ratio of 10 to 1 in a nitrogen-blanketed, adiabatically operated, packed column (1 inch in diameter and packed to a depth of 36 inches with glass helices $^3/_{16}$ inch in diameter). Only the middle third of the constant boiling point distillate was collected. Reproducibility of the product was adequate as evidenced by the constancy of the physical properties. Of eleven lots thus purified, the densities at 25° C. varied from 1.1098 to 1.1099 grams per ml. with the average being 1.10982 grams per ml., as compared to previously published values which range from 1.1097 (*6*) to 1.1110 (*4*) with the most probable value, according to Curme and Johnston (*3*), being 1.10986 grams per ml. Despite all precautions taken to prevent water contamination, the purified glycol analyzed (by Karl Fischer reagent) 0.07 ± 0.01% (weight) water. Correcting for the presence of water by extrapolation resulted in an average density of 1.10988 grams per ml. for the purified ethylene glycol. Similarly, the refractive index, n_D^{20}, varied from 1.43179 to 1.43182 with the higher value being the most common. Extrapolating to zero water content led to a value of 1.43188 for purified glycol as compared to previously published values ranging from 1.4304 (*1*) to 1.43192 (*10*).

In general, there are two primary sources of glycol contamination, both of which tend to lower density and refractive index values, and which could well account for the wide range of reported values for density and refractive index. The most obvious is the almost unavoidable presence of small quantities of water; in much of the early literature no water determinations were reported and probably this accounts for many of the discrepancies. The second class of contaminants consists of oxidation products: aldehydes and acids. It was shown during the first attempts at purification that the oxidation reaction was platinum catalyzed; however, trace amounts of aldehydes were detected (Schiff base) even when all-glass equipment was used. Repeated purging of the distillation equipment with oxygen-free nitrogen prior to start-up eliminated the formation of analyzable quantities of aldehydes. Contamination by this mechanism would be a distinct possibility in much of the previously published work and very probable in those cases where an air bleed was introduced into the still pot to promote smooth boiling.

Water used in the preparation of the glycol solutions used in this work was freshly distilled and had a specific conductivity of the order of 10^{-5} ohm^{-1} cm.$^{-1}$ As an added precaution, the water was boiled to remove dissolved gases and then cooled without agitation immediately prior to use.

PREPARATION OF SOLUTIONS

Solutions of known composition were prepared by injecting approximate amounts of purified glycol (0.07% water) and water into dried, stoppered 60-ml. vaccine bottles. The exact compositions were determined by weighing to 0.1 mg. Hypodermic syringes used to transfer the glycol were dried at 110° C. and then allowed to cool in a desiccator until used.

Since the smallest amount of either component was 2.7 grams, the compositions were known to at least 1 part in 27,000 or 0.004 weight %.

DENSITY AND REFRACTIVE INDEX DETERMINATIONS

Density measurements were made in 10-ml. Weld-type, capped, specific gravity bottles which had been calibrated with boiled, distilled water. Temperature control was obtained by partially submerging the bottles in a 5-gallon constant temperature bath controlled at 25.00° ± 0.03° C. The maximum error resulting from uncertainties in the volume calibration of the specific gravity bottles would be ±0.00009 gram per ml. However, strict adherence to a standardized procedure resulted in duplicate measurements having a maximum deviation of .000011 gram per ml.

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All weighings were reduced to values in vacuo and the relative densities at 25° C. were calculated in grams per milliliter. Expressed in these units, the density is numerically equal to the specific gravity at 25° C. relative to water at 3.98° C.

Refractive index measurements were made with a Bausch and Lomb Precision oil refractometer, with water controlled at 20.00° ± 0.02° C. circulating through the prism blocks. The temperature coefficient for the refractive index of water is 0.0001 unit per degree Centigrade; thus, in order to obtain readings accurate to the limit of the instrument (0.00003 unit), temperature fluctuation could be as much as 0.3° C. Similarly for glycol, the temperature coefficient is 0.00026 unit per degree Centigrade (*3*), necessitating temperature control to only 0.1° C. Duplicate analyses never differed by more than 0.00003 unit, provided the sample was injected between the closed prisms by means of the hypodermic syringe. Earlier work on pure glycol showed that the refractive index decreased as rapidly as 0.00012 unit per minute if exposed to room air at approximately 35% relative humidity.

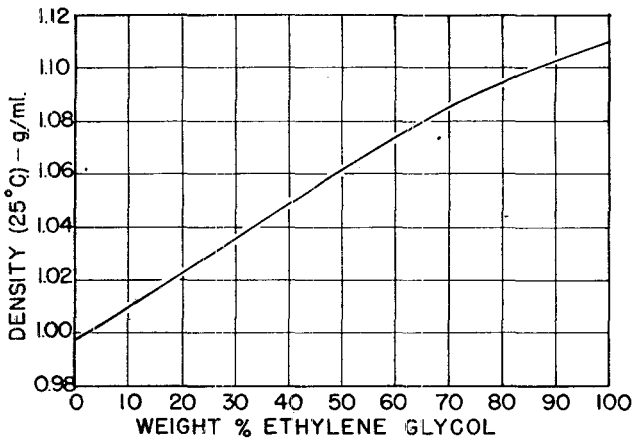

Figure 1. Density-composition data

Ethylene glycol–water solutions

Experimental data are listed in Table I. Large scale plots were drawn for both density and refractive index (Figures 1 and 2), from which the smoothed values at even composition increments given in Table II were obtained.

LIMITATIONS OF DATA

Analyses based on density determinations accurate to within ±0.0001 gram per ml. will be good only to ±0.1 weight %. Further, the comparatively involved manipulations required increase the chance of experimental error considerably over that likely with refractive index determinations which are inherently more accurate (±0.03%). Then, too, refractive index determinations have the obvious advantages of requiring smaller samples and much less time per sample. The primary requisite for precise analysis by refractive index is that of preventing sample contact with moist air; the use of a hypodermic syringe is strongly recommended.

The experimental density data agree closely with those of Spangler and Davies (*11*) except over the composition range from 50 to 70 weight % of glycol. A large scale plot of their data indicates slight breaks at 54 and 69% glycol in an otherwise smooth curve.

DISCUSSION

The density-composition curve determined in this work is very slightly concave upward over the range 0 to 20 weight % of glycol, and concave downward over the remainder of the composition range. This double curvature appears to be characteristic of aqueous glycol systems in that it is indicated in the work of Spangler and Davies (*11*) on the ethylene glycol–water system at 25° C., Cragoe (*2*) on ethylene glycol and water at 20° and 15.6° C., MacBeth and Thompson (*7*) on aqueous propylene glycol at 25° C., and the same authors (*8*) on aqueous diethylene glycol at 25° C. That a volume change occurs upon mixing ethylene glycol and water is indicated by the lack of a linear relation between density and volume per cent composition.

The refractive index–composition curve is also S-shaped, being concave upward from 0 to 40 weight % of glycol. This is contrary to the relatively common assumption (*3*) that refractive index varies linearly with weight per cent composition for this particular system. Analyses based on a straight line between the refractive indexes of water and purified glycol would result in a maximum error of 1.6 weight % at about 75% of glycol.

As with the density data, the characteristic shape of the refractive index curve is indicated for the ethylene glycol–water system in the data of Spangler and Davies (*11*) and Romstatt (*9*) and for aqueous propylene and diethylene glycols by the work of MacBeth and Thompson (*7*, *8*).

Table I. Experimental Data

Composition, Weight % Ethylene Glycol	Refractive Index, n_D^{20}	Absolute Density, Grams/Ml. at 25° C.
0	1.33300	...
5.44	1.33810	1.0038
15.14	1.34750	1.0163
24.85	1.35724	1.0294
36.58	1.36925	1.0449
48.05	1.38107	1.0597
56.65	1.38990	1.0701
66.53	1.39990	1.0813
76.13	1.40939	1.0910
80.97	1.41407	1.0954
85.50	1.41835	1.0993
89.23	1.42184	1.1023
94.45	1.42668	1.1062
99.93	1.43182	1.1098

Table II. Smoothed Data

Composition, Weight % Ethylene Glycol	Refractive Index, n_D^{20}	Absolute Density, Grams/Ml. at 25° C.
0	1.33300	0.99707 (*5*)
10	1.34242	1.0096
20	1.35238	1.0229
30	1.36253	1.0364
40	1.37275	1.0495
50	1.38313	1.0620
60	1.39336	1.0740
70	1.40340	1.0850
80	1.41315	1.0945
90	1.42262	1.1029
100	1.43188	1.1099

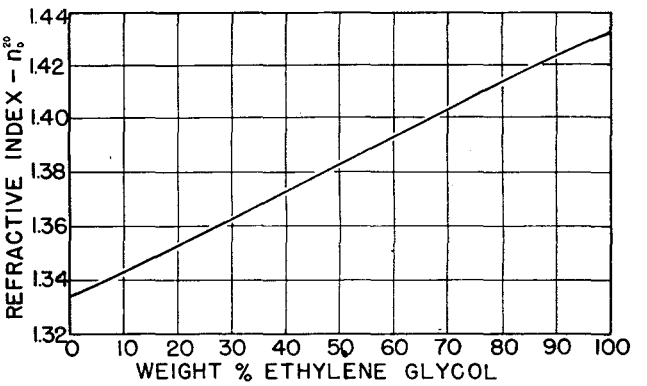

Figure 2. Refractive index–composition data

Ethylene glycol–water solution

ACKNOWLEDGMENT

This work, representing a portion of E. T. Fogg's doctoral research, was supported by a grant awarded to A. R. Thompson

by the Committee on the Advancement of Research of the University of Pennsylvania.

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RECEIVED for review February 8, 1955. Accepted June 3, 1955.

Determination of Halogen in Organic Compounds

Potentiometric Microtitration with Silver–Amalgamated Silver Electrode System

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The potentiometric titration of halide with silver nitrate, employing the silver–amalgamated silver electrode system of Clark, has been adapted to the microdetermination of chlorine, bromine, and iodine in organic compounds. This electrode system lends itself with advantage to micro work, because it is easy to construct, can be made very small in size, and gives a sensitive indication of the end point even in titrations with very dilute solutions. Procedures are given which provide a convenient and rapid analysis of organic compounds on a micro scale, where 1 to 4 mg. of sample are available. If the substance contains ionizable halogen, it may be titrated directly in alcoholic solution; compounds with nonionic halogen are decomposed most conveniently prior to the titration by the catalytic dry combustion method. The procedures described have been applied to the determination of halogen in standard organic substances with a precision of 3 parts per thousand.

THE direct determination of halogen in organic compounds invariably involves the measurement of halide ion, usually after preliminary decomposition of the organic substance. The determination of halide on a micro scale by gravimetric means is a tedious and lengthy procedure, and hence titrimetric methods are preferred for routine analysis. Of the volumetric methods for the determination of halide, those employing visual indicators suffer from a lack of end point sharpness, because of the high dilution of the sample and titrant which must necessarily be used. The potentiometric titration of chloride, bromide, and iodide with silver nitrate is, however, applicable to very dilute solutions and lends itself well to the determinations required in microanalytical work.

The potentiometric titration of halide with silver nitrate is commonly carried out with a metallic silver indicator electrode and a reference electrode of the conventional type, such as silver–silver chloride or calomel, which is connected to the titration cell by a halogen-free salt bridge. For the titration of halide on a microanalytical scale, where approximately 0.5 mg. of the ion is to be measured, the volume of the solution must be kept small in order to minimize end-point errors due to solubility of the precipitated silver halide. Consequently, the electrodes used must also be kept small in size. Moreover, the insertion of a salt bridge into the titration vessel is often inconvenient and introduces a potential source of error in contaminating the solution with traces of halide or loss of the ion by diffusion into it.

In 1926 Clark (*1*) suggested a bimetallic type of electrode system for the titration of chloride with silver nitrate, which has none of the disadvantages which make electrode pairs of the conventional kind awkward for micro work. This electrode pair consists of a plain silver electrode and one of amalgamated silver. This system was later adapted by Cunningham, Kirk, and Brooks (*2*, *3*) to the ultramicrotitration of chloride in biological fluids. The latter authors found that in drop-scale potentiometric titrations, using a pair of tiny silver and amalgamated silver electrodes, microgram quantities of chloride could be determined with an accuracy of about 0.5%.

This silver and amalgamated silver electrode system deserves a wider application in the determination of small quantities of halide than it appears to have enjoyed. It is extremely simple to construct and can be made to fit any requirements of size. It gives a sensitive indication of the end point in the titration of bromide and iodide, as well as chloride. The authors have adapted a modification of the procedure of Kirk and his coworkers to the routine elementary analysis of organic compounds, where quantities of 1 to 4 mg. of sample are available, and have found that it offers a rapid and convenient method which is capable of a high degree of precision.

APPARATUS

Electrodes. Prepare two silver electrodes by heating one end of two 5-cm. lengths of 18-gage silver wire in a flame for a few seconds to fuse a ball of metal about 2 mm. in diameter onto it. Clean one of the electrodes by rubbing it with emery cloth and immersing it briefly in dilute nitric acid (1 to 1) containing a little sodium nitrite until a rapid evolution of gas occurs. Amalgamate the other electrode by dipping it into a pool of mercury covered with dilute nitric acid. Polish the amalgamated end by rubbing it with tissue until it is coated with a uniform shiny layer of mercury. The amalgamated electrode should be allowed to stand for about an hour before use, but thereafter is serviceable for a day or two, after which it should be cleaned with emery cloth and nitric acid and reamalgamated. Between titrations the electrodes are wiped clean with tissue or filter paper and rinsed with distilled water.

Voltmeter. A sensitive vacuum-tube potentiometer such as a Beckman Model G pH meter or Fisher Titrimeter is required.

Buret. A 5-ml. microburet calibrated in hundredths of milliliters is used. It is mounted so that the buret tip is immersed beneath the surface of the liquid in the titration cell.

Titration Cell and Stirrer. Small beakers of 10- or 15-ml. capacity are suitable titration vessels. Brown glass titration

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vessels, cut from brown vials of appropriate size, protect the precipitated silver halide from light and are to be preferred if the titration is carried out in a brightly lighted room. Stirring is accomplished by a magnetic stirrer with a small stirring bar of approximately 1.5-cm. length. Stirring bars of the proper size are easily constructed by sealing a small wire nail into 3-mm. outside diameter glass tubing. An asbestos pad beneath the titration vessel is desirable in order to prevent temperature changes in the sample solution during the titration, because the magentic stirrer may become warm in operation.

Table I. Analyses of Standard Substances

Compound	Sample Weight, Mg.	% Halogen Theory	Found	Diff.
Aniline hydrobromide	2.167	45.92	45.78	−0.14
	1.917		46.26	+0.32
	2.235		45.67	−0.25
Dibenzylethanolamine hydrochloride	3.112	12.77	12.72	−0.05
	3.257		12.74	−0.03
	2.711		12.73	−0.04
	2.930		12.79	+0.02
Benzylamine hydrobromide	2.113	42.49	42.30	−0.19
	2.021		42.50	+0.01
2-Chlorobenzoic acids[a] (Natl. Bur. Stds. No. 144)	1.735	22.64	22.68	+0.04
	1.228		22.60	−0.04
p-Chlorobenzoic acid[a]	1.642	22.64	22.32	−0.32
	1.599		22.58	−0.06
p-Chloroacetanilide[a]	1.544	20.90	20.93	+0.03
	1.582		20.71	−0.19
p-Bromoacetanilide[a]	1.892	37.30	36.91	−0.39
	2.176		37.21	−0.09
	1.942		37.27	−0.03
2,7-Dichloroquinoline[a]	0.937	35.78	35.72	−0.06
	0.907		35.77	−0.01
2-Iodobenzoic acid[a] (Natl. Bur. Stds. No. 145)	2.131	51.15	50.90	−0.25
	2.283		51.10	−0.05
	2.141		50.96	−0.19
	0.802		51.00	−0.15

[a] Titration preceded by combustion in oxygen.

Microcombustion Apparatus. For the determination of halogen in compounds requiring preliminary combustion, the compound is burned in oxygen in a conventional Pregl-type halogen and sulfur combustion tube with glass spiral (*4*, *5*). (The tube employed in this work was 60 cm. in over-all length and contained a spiral 16 cm. long.) Two platinum "star contacts" serve as catalyst; these are maintained at approximately 750° C. by a microcombustion furnace of the usual kind.

REAGENTS

Silver Nitrate, 0.00282*N*. Dissolve 0.4891 gram of primary standard silver nitrate, dried at 140° C., in water and dilute to exactly 1 liter. Each milliliter of the solution is equivalent to 0.100 mg. of chloride, 0.225 mg. of bromide, or 0.358 mg. of iodide. If desired the silver nitrate may be standardized against a standard sodium chloride solution as follows: Take a 2-ml. aliquot of standard sodium chloride solution, containing approximately 0.4 mg. of chloride, and add 5 ml. of 95% ethyl alcohol and 0.5 ml. of dilute sulfuric acid (1 to 10). Titrate as described in the procedure for compounds with ionizable halogen.

Sodium Carbonate Solution. A 5% aqueous solution.

Hydrazine Sulfate Solution. A saturated aqueous solution.

DETERMINATION OF IONIZABLE HALOGEN

If the organic compound being analyzed is an acid halide, amine hydrohalide, or the like, in which the halogen is readily ionizable, it may be titrated directly in an acidified alcoholic solution of the substance. The functioning of the electrodes is not impaired by the presence of the organic radical or amine cation, nor by high concentrations of alcohol. The latter is an advantage, as many amine hydrohalides of high molecular weight have only a low solubility in water. The presence of alcohol increases the sensitivity of the voltage change at the equivalence point, hence solution of the sample in alcohol is to be preferred even though the compound may be readily soluble in water alone.

Procedure. Weigh a sample of the organic substance of such size to contain 0.2 to 0.45 mg. of chloride (or an equivalent amount of bromide or iodide). Transfer it to the titration vessel, dissolve it in 5 ml. of 95% ethyl alcohol, and add 0.5 ml. of dilute (1 to 10) sulfuric acid. Insert the electrodes and stirrer, and the buret so that the tip of the latter dips below the surface of the solution. Carry out the potentiometric titration with 0.00282*N* silver nitrate in the usual way, taking voltage readings after the addition of each increment of the standard solution. In the vicinity of the equivalence point, take readings after each 0.01 ml. of silver nitrate, allowing 30 seconds for equilibrium to be attained before reading the voltmeter. Continue the titration until an abrupt increase in voltage has been passed and the voltage has become constant or begun to decrease.

DETERMINATION OF NONIONIC HALOGEN

If the organic substance contains nonionizable halogen, it must be decomposed and the halogen converted to inorganic halide before determination. This can be done either by fusion with sodium peroxide in the micro Parr bomb, or by catalytic dry combustion in oxygen. Although the titration is applicable after either method of decomposition, the authors have found the dry combustion procedure to be more convenient and rapid, because the manipulation involved is easier. In this procedure, the compound is burned in oxygen in the conventional Pregl combustion tube with glass spiral and platinum star contacts (*4*). The resulting halogen is absorbed on the spiral, which is wet with an alkaline solution containing a reducing agent to convert to halide any free elementary halogen that may be formed. Hydrazine sulfate (*5*) has been found to be a reducing agent particularly suited to the proposed method, because it requires no decomposition before the ensuing potentiometric titration.

Procedure. Prepare an alkaline absorption solution by mixing 6 ml. of 5% sodium carbonate solution with 6 drops of a saturated solution of hydrazine sulfate. Support the combustion tube in a vertical position, immerse its tip in the absorption

Table II. Analyses of Research Samples

Compound	% C Theory	Found	Diff.	% H Theory	Found	Diff.	Sample Weight, Mg.	% Halogen Theory	Found	Diff.
$C_{16}H_{11}ClO_2$[a]	71.06	70.99	−0.07	4.10	4.10	0.00	2.324	13.10	12.84	−0.26
$C_{25}H_{28}O_5N.HCl$	65.85	65.79	−0.06	5.75	5.82	+0.07	5.048	7.78	7.71	−0.07
$C_{22}H_{24}ON_2.2HCl$	71.62	71.34	−0.28	6.83	6.78	−0.05	4.178	9.62	9.46	−0.16
$C_{24}H_{23}O_4N.HCl$	67.52	67.42	−0.10	5.67	5.64	−0.03	3.427	8.31	8.26	−0.05
$C_{16}H_{16}OClN.HCl$	61.94	61.76	−0.18	5.48	5.74	+0.26	3.289	11.42[b]	11.44	+0.02
							1.728	22.84[c]	22.71	−0.13
$C_{13}H_{21}O_2N.HCl$	60.10	60.21	+0.11	8.54	8.70	+0.26	2.641	13.65	13.66	+0.01
$C_{20}H_{21}O_3N.HCl$	66.75	66.62	−0.13	6.16	6.31	+0.15	3.406	9.86	9.79	−0.07
$C_{20}H_{25}O_4N.HCl$	63.23	63.21	−0.02	6.90	7.16	+0.26	3.070	9.33	9.22	−0.11
$C_{17}H_{21}ON.HCl$	69.71	70.00	+0.29	7.60	7.20	−0.40	3.024	12.15	12.08	−0.07
$C_{27}H_{43}Cl_2N_2O_3P$	59.44	59.10	−0.34	7.95	7.77	−0.18	2.373	13.00	12.74	−0.26
$C_{15}H_{11}Cl_4OFe$[a]	44.49	44.42	−0.07	2.91	2.74	+0.17	0.920	35.03	35.27	+0.24

[a] Titration preceded by combustion in oxygen.
[b] Only one of the two chlorines ionizable. Direct titration.
[c] Both chlorines determined after combustion of the compound.

solution, and carefully suck it up until the liquid covers the entire spiral and extends about 5 mm. above it. Allow the liquid to drain from the tube, leaving the spiral wet. Mount the tube in the combustion train [see (*4* or *5*) for details of arrangement of the train], insert the platinum contacts, and introduce a platinum boat carrying the sample. (The sample should be of the size indicated in the procedure for analysis of compounds containing ionizable halogen.) Clamp a titration vessel of about 15-ml. capacity over the exit end of the combustion tube. Carry out the combustion of the compound in the usual way (*4*, *5*) in an oxygen stream of approximately 5 ml. per minute. Conduct the combustion slowly, allowing 20 or 25 minutes for this operation. If the sample is an iodine derivative, free elementary iodine may condense in the portion of the tube just beyond the exit end of the furnace and must be driven down into the alkaline solution on the spiral by careful heating with a small flame. At the end of the combustion, remove the tube from the furnace and allow it to cool while oxygen is swept through. Then dismount the tube, remove the boat and platinum contacts, and wash the absorption solution into the 15-ml. titration vessel with 48% ethyl alcohol by first sucking up 5 ml. of the wash liquid over the spiral, and then rinsing down the tube from the top with two 2.5-ml. portions. Add 0.5 ml. of 1 to 10 sulfuric acid to the contents of the beaker and titrate as described in the preceding procedure.

ANALYTICAL RESULTS

In the titration of aliquots of alkali halide standard solutions, the precision of the potentiometric titration, expressed as relative standard deviation, was found to be 0.2%, or 2 parts per thousand. There is no observable difference in the precision of the titration for the three different halides, although the voltage change at the equivalence point is greater and somewhat more abrupt for bromide and iodide than it is for chloride.

In Table I are shown the results of the application of the procedures described to the analysis of pure organic compounds, which are employed in this laboratory as microanalytical standards. For these data, the relative standard deviation from the theoretical values is 0.3%, or a precision of 3 parts per thousand. Table II presents data obtained in the analysis of some research compounds which were submitted to this microanalytical laboratory for routine elementary analysis. These and similar data obtained in the routine analysis of many other research samples have shown the procedures described to be applicable without modification or difficulty to the determination of halogen in organic substances of a variety of structural types.

DISCUSSION

Figure 1 shows typical titration curves obtained with chloride, bromide, and iodide. Shortly after the beginning of a titration, the voltage becomes essentially constant and remains so until approximately 5% before the equivalence point. A change in voltage then begins to occur, followed by an abrupt rise as the end point is reached and exceeded. The initial change in voltage, generally a decrease, serves as a warning of the approach of the end point. This is almost always true in the case of bromide or iodide, where the drop is quite marked, but in the titration of chloride the preliminary decrease in voltage may be slight or replaced by a gradual rise. As the titration is continued beyond the end point, the voltage soon begins to decrease again, after which it eventually becomes nearly constant.

The drop in voltage readings, which is often noted to precede and follow the abrupt rise that marks the equivalence point, is due to the fact that in this system the potentials of both electrodes respond to changes in silver (and hence chloride) ion concentration, but do so at different rates. The measured voltage of the cell is thus the potential difference between two electrodes of variable potential. This difference becomes greatest as the equivalence point is reached and exceeded slightly, but may reach minimum values shortly before and after it. The shape of the titration curve depends on the ion which is being titrated and the physical condition of the electrodes.

The amalgam electrode is positive with respect to the plain silver one. The potentials of a given pair of electrodes are not reproducible from titration to titration, but this is of no importance to the potentiometric determination of halide. The voltage rise near the equivalence point is of the order of 30 to 60 mv., being smallest in the titration of chloride and greatest with iodide.

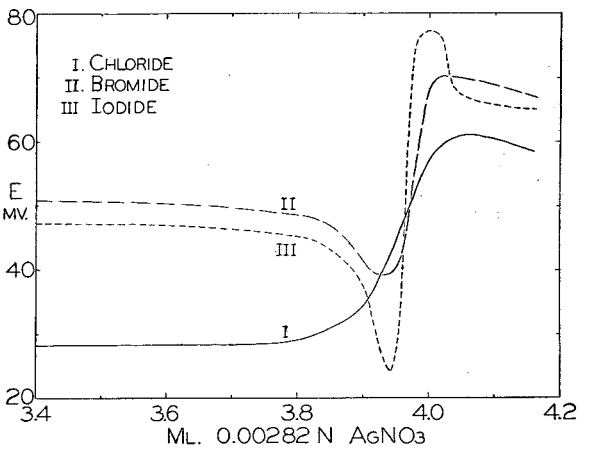

Figure 1. Typical titration curves

The end-point volume of titrant is most conveniently located as the point of inflection of the titration curve—that is, the mid-point of the approximately straight portion. It can also be located as the maximum on the $\Delta E/\Delta$ml. *vs.* volume plot, as is customary in potentiometric titrations. With iodide and bromide, a sharp maximum at the equivalence point is obtained on such a plot, but with chloride these curves often show several maxima near the end-point volume. However, the location of the end point as the point of inflection on the titration curve is easily accomplished with accuracy. The entire abrupt rise in voltage occurs over a range of 0.1 ml. (much less with bromide and iodide), and hence it is not difficult to estimate the mid-point on the rising portion of the curve to within a few thousandths of a milliliter.

ACKNOWLEDGMENT

The authors are indebted to Patricia L. Paynter of the Microanalytical Laboratory, University of Virginia Department of Chemistry, who performed many of the analyses in the course of this study. J. Jack Kirkland was holder of a fellowship in the John Lee Pratt Trace Analysis Laboratory, University of Virginia. This support is gratefully acknowledged.

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RECEIVED for review April 25, 1955. Accepted June 29, 1955.

Rapid Determination of Traces of Iron and Copper in Acrylonitrile

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Methods have been developed for rapid colorimetric determination of mutually occurring copper and iron in commercial acrylonitrile in concentrations as low as 0.05 p.p.m. The color reactions and absorbance measurements are carried out in the acrylonitrile phase, and thus prior treatment of the sample, such as decomposition, is not required. This approach precludes the introduction of contaminating metals, which frequently results in high blank corrections in the wet-decomposition methods generally applied to monomers. The specificity of the reagents (2,2′-biquinoline for copper and *o*-phenanthroline for iron) and the procedures used make it possible to determine either metal in the presence of a preponderance of the second, and there is no appreciable interference from a several hundredfold excess of the cyanide ion, present either as free hydrocyanic acid or as cyanhydrin—e.g., lactonitrile.

THE influence of trace amounts of metals upon the polymerization rate of acrylonitrile is well known (*7*). A few parts per million of metallic impurities, specifically cupric and ferric ion, cause a severalfold increase in the polymerization rate of acrylonitrile, when the persulfate-thiosulfate redox system is used (*1*). As the variability in the level of these trace metallic impurities affects the polymerization rate, a relatively high concentration of cupric ion is usually added to the polymerization system to swamp the natural variation of trace metal impurities. In fact, it has been reported that a few parts per million of copper make it necessary to polymerize acrylonitrile at a low temperature in order to maintain uniform molecular weight (*8*). Thus, it became of interest to develop rapid methods for the determination of traces of copper and iron impurities in acrylonitrile.

The customary dry-ashing and wet-decomposition methods were briefly investigated. Both methods are slow, lack the desired precision and accuracy, and unless especially purified reagents are used, iron contamination is hard to avoid. The wet-decomposition method for copper gave accurate, reproducible values with known amounts of copper; however, the time requirement was still a major disadvantage. Therefore, other methods for determining iron and copper were sought.

APPARATUS AND MATERIALS

Beckman Model H2 pH meter. Beckman Model B spectrophotometer with 50-mm. cells.

Hydroxylamine hydrochloride (Matheson), 10 grams dissolved in copper- and iron-free water and diluted to 250 ml.

o-Phenanthroline (G. F. Smith Chemical Co.), 0.40 gram dissolved in 100 ml. of C.P. methanol.

Glacial acetic acid.

Methanol, C.P.

Ammonium hydroxide, C.P.

2,2′-Biquinoline (G. F. Smith Chemical Co.), 0.10 gram dissolved in 500 ml. of C.P. methanol.

Standard copper solution, 0.1965 gram of cupric sulfate pentahydrate dissolved in water and diluted to 500 ml. This standard, containing 100 p.p.m. of cupric ion, was further diluted with water to give various copper concentrations. Aliquots of the various copper solutions were added to acrylonitrile to prepare the calibration curve.

Standard iron solution, 0.1000 gram of analytical grade iron wire (99.85%) dissolved in dilute nitric acid. After boiling off the nitrogen dioxide fumes, it was diluted to 1 liter. This standard, containing 100 p.p.m. of ferric ion, was further diluted with water to give various concentrations of iron. Aliquots of the various standard iron solutions were added to acrylonitrile to prepare the calibration curve.

The water used in making dilutions was passed through a mixed-bed ion exchange resin to ensure iron- and copper-free blanks. The acrylonitrile used was made metal-free by distilling from an acidic 8-quinolinol solution after a reflux period of 30 minutes. Only the heart cut was used.

DETERMINATION OF IRON

o-Phenanthroline is one of the most sensitive colorimetric reagents for iron (*4*, *17*). Its use in trace iron determinations is well described by Fortune and Mellon (*4*) and others. This reagent was used for all iron investigations.

Because many of the metal-organic complexes—for example, dithizones and 8-quinolates—are soluble in organic solvents, it was assumed that the ferrous-phenanthroline complex could be developed and its absorbance measured directly in acrylonitrile. Subsequent investigations revealed that the determination of iron in acrylonitrile medium gave more rapid, reproducible, and accurate values than did the conventional decomposition methods. The variables affecting the determination were also studied.

The color development in acrylonitrile was found to be essentially complete in 25 minutes, after pH adjustment. Increasing the time for color development did not increase the accuracy.

The pH range of 2 to 9 has been recommended for iron determination using *o*-phenanthroline reagent (*4*). This work indicated that an apparent pH of 4.5 to 6.5 gave a stable color and permitted maximum color development.

As the wave length of some colored complexes shifts in organic media, the maximum absorbance was investigated and found to be the same as in water—i.e., 508 mμ.

The literature (*4*) states that a fivefold excess of copper does not interfere in iron determination if the pH is adjusted to between 2.5 and 4.0. Copper is reported to interfere somewhat at a pH of 5.86; laboratory studies confirmed this. Copper interferes, owing to a yellow color at the higher pH. However, acrylonitrile containing a tenfold excess of copper did not give an interference beyond the precision of the method, as is shown in Table I.

As all commercial acrylonitrile is manufactured with hydrogen cyanide, its effect on iron determination was investigated.

Table I. Effect of Copper and pH upon Iron Determination

Fe Known, P.P.M.	Cu Added, P.P.M.	pH	Fe Found, P.P.M.
0.20	2.00	3.3	0.24
		5.5	0.27
		2.5	0.21
	0.20	4.5	0.21
		5.5	0.21

Table II. Effect of Cyanide upon Iron Determination

Fe Known, P.P.M.	HCN Added[a], P.P.M.	Fe Found, P.P.M.
0.10	200	0.02
	300	0.02
	275[b]	0.10
0.20	5	0.22
	10	0.22
	25	0.20
	50	0.21
0.30	275[b]	0.30
0.40	500	0.07
0.50	500	0.08
0.60	275[b]	0.60
	500	0.20

[a] Cyanide added as NaCN.
[b] HCN added as lactonitrile.

Mellon and Fortune (*4*) had noted that cyanides interfered with the colored iron-phenanthroline complex—for example, with 2 p.p.m. of iron, 10 p.p.m. of cyanide was reported to give a 2% interference.

Known amounts of cyanide (from sodium cyanide and from lactonitrile) were added to acrylonitrile containing a known iron content. As seen in Table II, a several hundred-fold excess of free cyanide ion over iron did not result in any appreciable decrease in the iron found. Lactonitrile did not interfere even with a 2000-fold excess over iron, evidently because of the failure of lactonitrile to dissociate appreciably at the pH used (*14*). The cyanide was added in all cases before the phenanthroline reagent.

Procedure. To a clean 250-ml. iodine flask, add 125 ml. of acrylonitrile (free from suspended matter). Add 20 ml. of C.P. methanol, 10 ml. of 4% hydroxylamine hydrochloride solution, and 5 ml. of 0.4% *o*-phenanthroline solution, and shake vigorously. Use iron-free water (125 ml.) as a blank and treat it the same as the acrylonitrile sample. Adjust the blank and samples to an apparent pH of 5.0 to 6.0 with dropwise addition of dilute ammonium hydroxide and glacial acetic acid, if needed. After 30 minutes read the absorbance of the solution at 508 mμ, using 50-mm. cells and the blank as a reference. Convert the absorbance to iron concentration by means of a predetermined calibration curve. The calibration curve found for iron in acrylonitrile follows Beer's law in the range 0 to 2 p.p.m.

DETERMINATION OF COPPER

The literature contains many references to reagents pertaining to trace copper analysis. Sandell (*15*) lists the common reagents and their limitations. A brief investigation of the sodium diethyl dithiocarbamate method (*16*) for the desired range of copper gave poor results. As many improved colorimetric methods for copper have been recently developed, other recent literature methods were examined—for example, copper in dyes and organic chemicals can be accurately determined with zinc dibenzyldithiocarbamate after wet decomposition (*13*). The same reagent in carbon tetrachloride can be used to extract and determine traces of copper in acidified beer and cider (*19, 20*).

The reagent 2,2′-biquinoline is reported to detect 0.01 p.p.m. of copper and the cuprous-biquinoline complex is stable and unaffected by light, heat, or air (*2*). In addition, the complex is soluble in acetic acid, benzyl and amyl alcohols, chloroform, and ethyl acetate, and the reagent is specific for cuprous ion. The reagent is reported 10 times more sensitive than sodium diethyl dithiocarbamate and is more stable and has less interference than dithizone (*9*). The complex is unstable in a highly acid medium (pH 3), and can be easily extracted from water with *n*-amyl alcohol.

Cheng and Bray (*3*) determined traces of copper in soils by use of biquinoline and found that it gave slightly more accurate and precise values on known standards than their improved sodium diethyl dithiocarbamate method.

Gillis, Hoste, and others (*5, 6, 11, 12*) studied the cuprous-biquinoline complex and confirmed that biquinoline was specific for copper. They also found that the color is stable between pH 2 and 9 for a least 72 hours and that Beer's law holds up to 40 p.p.m. of copper. Biquinoline has been used to determine as little as 0.02 p.p.m. of copper in plants, animal tissues, blood, water, lampblack, steel, and ores.

Because of the success of the determination of iron directly in acrylonitrile, a similar method using biquinoline was applied for the determination of copper and certain variables were investigated.

The color of the solution develops immediately after pH adjustment; however, in the case of acrylonitrile containing more than 5 p.p.m. of cyanide, the color develops more slowly. A period of 30 minutes will usually suffice for full color development. An apparent pH higher than 5.5 or less than 3.0 causes fading of the color (not shown in the table). A pH over 6.5 causes disappearance of the color. The most desirable pH found was 3.5 to 5.0 (Table III) and the apparent pH was more stable if a slight excess of ammonium hydroxide was added and the pH brought into the proper range with acetic acid.

Table III. Effect of pH upon Copper Determination

Cu Known, P.P.M.	pH	Cu Found, P.P.M
2.0	3.4	2.00
	4.6	2.00
	5.0	2.00
	5.5	1.55
	6.5	0.00
0.20	4.9	0.22

Table IV. Effect of Hydrocyanic Acid upon Copper Determination

Cu Known, P.P.M.	HCN Added, P.P.M.	Cu Found, P.P.M. Immediately	Cu Found, P.P.M. After standing 30 min.
0.40	5[a]	0.39	0.41
	25[a]	0.35	0.41
	50[a]	0.28	0.40
0.20	11[b]	0.21	...
0.30	11[b]	0.29	...
0.40	11[b]	0.40	...

[a] Cyanide added as NaCN.
[b] Cyanide added as lactonitrile.

The maximum absorbance for the complex in acrylonitrile-methanol medium was found to be 540 mμ.

The literature (*10*) states that iron does not interfere in copper determination with biquinoline. Laboratory data (not shown) confirmed that a tenfold excess of iron over copper causes no interference.

Cyanide was found by Cheng and Bray (*3*) and Gillis (*5*) to interfere in the biquinoline method for copper; however, the interference level was not given. Investigation of cyanide interference proved that a hundred-fold excess of free cyanide did not cause interference, provided at least 30 minutes were allowed for color development. A 50-fold excess of cyanide as lactonitrile did not affect the color development. Table IV gives the results found. The absorbance of copper-biquinoline complex in acrylonitrile did not change upon standing overnight, even if 50 p.p.m. of cyanide were present.

Procedure. Place 125 ml. of acrylonitrile (free of suspended solids) in a clean 250-ml. iodine flask. Pipet 10 ml. of 4% hydroxylamine hydrochloride and 25 ml. of 0.02% biquinoline into the flask, and shake vigorously. Use 125 ml. of C.P. methanol as a blank and treat it the same as the acrylonitrile sample. Adjust the apparent pH to 3.5 to 5.0 with dropwise addition of ammonium hydroxide and acetic acid. Read the absorbance at 540 mμ using 50-mm. cells and the blank as a reference. Convert the absorbance to copper concentration by use of a predetermined calibration chart. The calibration curve for copper in acrylonitrile follows Beer's law from 0 to 2 p.p.m.

Work by Smith and Wilkins (*18*) indicates that 2,9-dimethyl-4,7-diphenyl-1,10-phenanothroline is superior to biquinoline as a specific copper reagent. However, the reagent was unavailable commercially at the time of this work and was not investigated.

SUMMARY

Traces of iron and copper in acrylonitrile can be determined directly, and without prior treatment. Application of these procedures to routine samples indicates that the reproducibility of both methods is within ±0.03 p.p.m. These methods have been found applicable to the determination of these metals in other organic liquids, and with minor modifications should be readily adaptable for many other monomers and solvents.

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RECEIVED for review April 23, 1955. Accepted July 13, 1955. Division of Analytical Chemistry, 127th Meeting ACS, Cincinnati, Ohio, 1955.

Colorimetric Submicromethod for Determination of Ammonia

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The blue color, formed when sodium phenate is added to a solution of ammonia that has been treated with hypochlorous acid, forms the basis of a method for the determination of submicro amounts of ammonia. The method has been used for the determination of the molecular weight of compounds containing nitrogen which is easily transformed into ammonia. Coupled with the cyanohydrin reaction it can be utilized for the determination of the average molecular weight of aldehydes, such as sugars and certain polysaccharides possessing a free reducing group. The procedure might lead to the simplification of protein determination by the Kjeldahl method.

IN THESE investigations into polysaccharides the authors sought to devise a method for determining the reducing group and, hence, the average molecular weight of these substances by a chemical method.

The reaction between an aldehyde and the elements of hydrocyanic acid, known to proceed to completion under certain conditions according to the law of mass action with simple aldoses, has formed the basis of these studies (*4, 5*). In the formation of polysaccharide cyanohydrins of high molecular weight the amount of combined cyanide is relatively small, and its determination requires an extremely sensitive method unless unlimited amounts of polysaccharide are available. The difficulty has been overcome by the use of cyanide labeled with radioactive carbon-14 (*5*).

The blue color produced by the action of phenol and hypochlorous acid upon ammonia (*2, 6–9*) can be used to determine fairly accurately extremely small quantities of ammonia.

The density of color developed by dilute solutions of ammonium chloride (measured by an Evelyn colorimeter and filter 620) was found to be a linear function of the concentration of ammonia from 0 to 8×10^{-8} mole of ammonia per ml. The slope of this linear function, moles of ammonia per milliliter per absorbance, however, was best determined by saponifying an aliquot of a standard solution of purified acetamide containing about 0.0002 gram of acetamide with ammonia-free base. The solution was steam distilled with steam generated from a dilute solution of sulfuric acid and the ammonia-containing distillate collected in about 15 ml. of ammonia-free distilled water. About 75 to 100 ml. of distillate was collected and the exact volume determined by weighing. This standard ammonia solution was then used to determine the slope of the linear relationship between concentration of ammonia and color intensity. Acetamide as a primary standard was determined by saponification, steam distillation into standard acid, and back-titration with standard base using methyl red indicator.

The slope has been found to be independent of the concentration of either the phenol or the chlorine water reagent. The slope obtained, however, depends upon the success with which ammonia has been removed from the saponifying base. The problem of its removal has not yet been solved and consequently the slope relationship must be redetermined every time fresh base is prepared.

In contrast to the phenol-hypochlorite reaction (*2, 6, 9*) no heating is necessary, as the reaction takes place quickly at room temperature. In addition, more color is produced with the phenol-chlorine water than with the phenol-hypochlorite reagent.

The color disappears only very slowly, 1% approximately in 24 hours. It is possible to detect 5×10^{-10} mole of ammonia per ml. of solution. By comparison, Nessler's reagent has only one tenth of this sensitivity and, moreover, the color must be read shortly after its development. The values of slope obtained by four acetamide determinations differed from their average value by about 2%. The accuracy of this method is therefore within ±2%.

PREPARATION OF REAGENTS

Ammonia-Free Distilled Water. This reagent is prepared by distilling distilled water from a dilute solution of sulfuric acid in an all glass apparatus.

Hypochlorous Acid Reagent. Chlorine is bubbled into ice-cold distilled water until solid chlorine hydrate forms. The approximate chlorine content determined by the iodide-thiosulfate method should thereby exceed the required minimum value, about 0.08*M* chlorine. The molarity should, however, be determined approximately before use by adding 10 ml. of 5% potassium iodide solution and titrating the liberated iodine with 0.2*M* sodium thiosulfate.

The required minimum chlorine content is determined by plotting the maximum color development obtained for a given ammonia solution against the strength of the hypochlorous acid reagent (Figure 1).

A suitable ammonia solution which gives a maximum color development of about 50% transmittance is prepared by adding one drop of 0.4*M* ammonium chloride solution to 500 ml. of ammonia-free distilled water.

Potassium iodide, 5% aqueous solution.

Sodium thiosulfate solution, 0.2*M*, 5 grams of sodium thiosulfate (hypo) per 100 ml. of solution.

Sodium Phenate Reagent. A cool solution of sodium hydroxide, 7.2 grams (0.18 mole), in 300 ml. of ammonia-free water is added to commercial phenol, 16.7 grams (0.0178 mole), and shaken until the latter is dissolved.

Manganous chloride solution, 0.003*M*.

Ammonia-Free Sodium Hydroxide Solution. Sodium hydroxide pellets are dissolved in ammonia-free water to give a 20% solution. The amount of ammonia in commercial sodium hydroxide varies from sample to sample. This ammonia cannot be removed effectively by dissolving the hydroxide in distilled water and passing ammonia-free steam through the solution. Several experiments with different samples of sodium hydroxide revealed that a steady diminishing rate of ammonia removal is obtained (Figure 2).

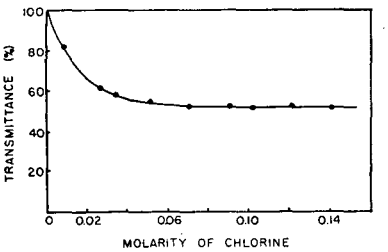

Figure 1. Color development of standard ammonia solution *vs.* strength of hypochlorous acid reagent

Reaction time 5 minutes

However, if sodium sulfide is added to the base, all the ammonia is removed with the first 50 ml. of distillate. This suggests that the above retention of ammonia in commercial sodium hydroxide is due to the presence of heavy metal ammonia complexes. Unfortunately a small amount of hydrogen sulfide is obtained in the distillate which can react with the hypochlorous acid reagent and interfere with the color development.

Heavy metals may also be removed by sodium silicate. Since sodium silicate forms slowly by the etching of the glass, this accounts for the observed gradual removal of the ammonia on steam distillation of hydroxide solutions.

If a simple method can be discovered for the preparation of ammonia-free base, an absolute method of determination of submicro quantities of ammonia may become available. The slope relationship moles of ammonia per milliliter per absorbance, will be independent of the preparation of the reagents.

Color Development. The solutions are added to the colorimetric tubes in the following order: 10 ml. of the unknown ammonia solution are mixed with 1 ml. of hypochlorous acid reagent. The initial reaction between the chlorine and ammonia is complete in 1 minute or less with 0.10*M* chlorine. At lower concentrations more time is required. After 5 minutes, 1 ml. of phenate reagent and one drop of manganous chloride reagent are added. After shaking the tubes, the color develops rapidly and reaches a maximum intensity in about 3 minutes. The intensity of color was measured in an Evelyn colorimeter using a No. 620 filter.

APPLICATION OF THE METHOD

Molecular Weight of Gluconamide. This experiment is representative of many carried out with pure D-gluconamide (melting point 144°) $[\alpha]_D^{23}$ +31° in water (*c*, 2.0), after recrystallization from ethyl alcohol. D-Gluconamide, 0.01 gram, was dissolved in 49.75 grams of ammonia-free water and 9.92 grams of solution withdrawn for saponification with ammonia-free sodium hydroxide (10 ml.). The weight of distillate collected was 110.30 grams. Since an absorbance of 0.491 was developed from 10 ml. of distillate, the molecular weight was calculated from the relationship

$$\text{Mol. wt. gluconamide} = \frac{\frac{0.01}{49.75} \times 9.92}{1.95 \times 10^{-7} \times 0.491 \times 110.30} = 188.8$$

(where 1.95×10^{-7} is the slope)

Calcd. for $C_6H_{13}O_6N$: mol. wt. = 195

Molecular Weight of Glucose. The following is representative of many experiments carried out with D-glucose. Pure anhydrous α-D-glucose (melting point 146°) $[\alpha]_D^{23}$ +52.5° equilibrium value in water (*c*, 2.0), 0.000386 gram in 1 ml. of solution (obtained by diluting a weighed sample of glucose by weight not volume for greater accuracy), potassium cyanide, 0.1 gram in 1 ml. of solution, and 2 ml. of 0.4*N* acetic acid (ammonia-free reagents) were heated in a water bath at 40° C. for 3 hours (*4*). The solution was acidified with 3 ml. of 0.4*N* acetic acid, and the hydrogen cyanide was removed by blowing air through the solution for 1 hour. (The air used for this and other experiments was blown through phosphoric acid to remove possible ammonia contamination.) The cyanohydrin was saponified with ammonia free sodium hydroxide (10 ml., 20%) and the distillate (114.57 grams) investigated for its ammonia content; the absorbance was 0.367.

Because the cyanide decomposes slowly to give ammonia, a reaction blank was run for these determinations.

$$\text{Mol. wt. glucose} = \frac{0.000386}{1.60 \times 10^{-7}(0.367 \times 114.57 - 29.0)} = 185$$

(where 29.0 is average blank of 6 determinations)

Calcd. for $C_6H_{12}O_6$: mol. wt. = 180

Apparent Average Molecular Weight of Laminarin. Duplicate runs were made as follows: A mixture of laminarin, 0.00673 gram in 1 ml. of solution, potassium cyanide, 0.1 gram, in 1 ml. of solution, and 2 ml. of 0.4*N* acetic acid was heated in a water bath at 40° C. for 20 hours. The solution was acidified, freed from hydrogen cyanide, saponified, and steam distilled as before.

The reaction is incomplete after 3 hours. The result after 42 hours is approximately the same as that obtained after 20 hours. The cyanide blank, however, becomes so great after 42 hours that the results are no more than an indication of the chain length.

As the absorbances obtained from the distillates in all cases were too great to be read with accuracy, aliquots were taken and diluted. Calculations show:

	Weight			
	Distillate	Aliquot	Diluent	Absorbance
Laminarin				
i.	104.24	20.33	70.02	0.297
ii.	108.83	19.85	68.87	0.276
Blank				
i.	114.10	19.33	68.35	0.240
ii.	115.50	19.90	62.53	0.258

Laminarin

i. $\Delta = 0.297 \times 70.02 \times 104.24/20.33 = 106.5$

ii. $\Delta = 0.276 \times 68.87 \times 108.83/19.85 = 104.2$

Av. = 105.3

Blank

i. $= 0.240 \times 68.35 \times 114.10/19.33 = 96.8$

ii. $= 0.258 \times 62.53 \times 115.50/19.90 = 93.5$

Av. = 95.1

$$\text{Mol. wt. laminarin} = \frac{0.00673}{1.60 \times 10^{-7}(105.3 - 95.1)} = 4130$$

$$\text{Chain length (av.)} = \frac{4130}{162} = 25 \text{ anhydroglucose units (approx.).}$$

This result is in reasonably good agreement with a repeating unit of 20 as found by methylation studies (*1*, *3*).

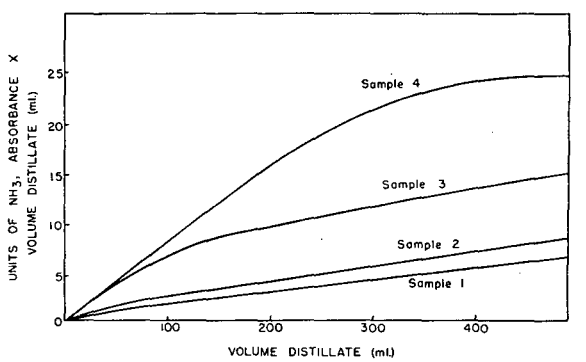

Figure 2. Evolution of ammonia during steam distillation of different samples of hydroxide solutions

Samples 1 to 4

Apparent Average Molecular Weight of Corn Amylose. Corn amylose cyanohydrin prepared as described for laminarin (reaction time 70 hours) was isolated by acidification with acetic acid followed by precipitation with ethyl alcohol (3 volumes). The amylose cyanohydrin was dialyzed for 7 days against distilled water, precipitated with ethyl alcohol, and dried, and the nitrogen

content determined as above. The apparent average molecular weight of the corn amylose in duplicate experiments was found to be 32,000 and 39,000.

SUMMARY AND CONCLUSION

Although the use of the hypochlorite–sodium phenate reaction is difficult to control for the quantitative determination of ammonia (*6*), a method using hypochlorous acid gives reliable results for the determination of minute amounts of ammonia.

The procedure gives good results with simple nitrogen-containing compounds such as acetamide and D-gluconamide, which liberate ammonia directly upon treatment with alkalies. The method could be employed in conjunction with micro or submicro Kjeldahl determinations.

In conjunction with the Kiliani cyanohydrin reaction the method has been used for the determination of the apparent average molecular weight of the polyglucosan and laminarin, which was found to correspond to about 25 anhydroglucose residues.

As the Kiliani condensation requires considerable time to reach completion, hydrolysis of the cyanide ion must be taken into account. The effect is too large to be corrected for accurately by a blank determination; and it seems necessary to remove ammonium ions produced by hydrolysis of cyanide ions by some chemical method, or by a physical method such as dialysis. However, the polysaccharide cyanohydrin can be purified by precipitation with alcohol as in the case of amylose cyanohydrin. Water-insoluble polysaccharides such as cellulose cyanohydrin can be filtered off and washed to remove contaminating ammonium ions.

ACKNOWLEDGMENT

The authors wish to thank the E. I. du Pont de Nemours & Co. for a grant, which defrayed the expenses of this work and provided a fellowship for P. G. Scheurer.

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RECEIVED for review January 28, 1955. Accepted June 10, 1955. Paper No. 3310 Scientific Journal Series, Minnesota Agricultural Experiment Station.

Absorptiometric Microdetermination of Total Sulfur in Rubber Products

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The 1.0 to 2.5% of sulfur normally present in rubber products is oxidized with concentrated nitric acid–bromine reagent, followed by perchloric acid in the presence of excess lead nitrate. Sulfur as lead sulfate is precipitated and washed with acetone. The lead sulfate is dissolved in 50% hydrochloric acid and absorbance of the lead chloride complex is recorded at 270 mμ. Sulfur is calculated on the basis of the measured lead content of the precipitate. The high sensitivity puts the method in the micro range. An experienced analyst can analyze 40 to 50 samples a day. Precision and accuracy are comparable to those of the conventional barium sulfate gravimetric method at the low sulfur concentrations normally found in rubber products.

THE literature contains numerous references to determination of total sulfur in rubber products as barium sulfate by gravimetric, volumetric, turbidimetric, or nephelometric methods. The latter methods were developed in the course of a search for a more rapid procedure than that afforded by standard gravimetric methods.

Recently an amperometric titration has been applied for the indirect semimicrodetermination of sulfur in organic compounds after precipitation as lead sulfate (*8*). However, digestion of the sample in a sealed Carius tube has serious disadvantages for routine control of sulfur in rubber products, and the range of sulfur concentration (about 1 to 6 mg. of sulfur) recommended requires rubber samples of above 50 mg.

A new combustion apparatus for automatic determination of sulfur in steel has been applied successfully to rubber (*2*), but the cost of this single-purpose instrument precludes its use in most rubber laboratories.

Perchloric acid has been used to speed up oxidation of rubber products during determination of sulfur (*9*). Published methods have all been for work on a macro scale, where the possible explosion hazard has retarded general acceptance of this strong oxidant in routine analysis of rubber. Even with the more rapid oxidation provided by perchloric acid, the conventional gravimetric barium sulfate procedure is used to precipitate the sulfur. This limits the speed of analysis.

A very sensitive absorptiometric method for determining lead (*4*) depends on the strong selective ultraviolet light absorbance near 270 mμ of the lead chloride complex formed in strong hydrochloric acid solutions.

A rapid oxidation with nitric and perchloric acids on a safe micro scale has been developed and is reported here. Sulfur is insolubilized and precipitated as lead sulfate with acetone. Absorptiometric measurement of lead as the lead chloride complex in 50% hydrochloric acid provides data for calculating sulfur concentration.

EQUIPMENT, REAGENTS, AND PROCEDURE

Equipment. Sample test tubes, such as 15 × 100 mm. lipless culture tubes, of 12- to 15-ml. volume.

Capillary-tipped pipet connected to a water pump vacuum source. It may be made by drawing out the lower end of a standard pipet over a hot flame until it is of capillary size, with thin walls and an internal bore of 1 to 2 mm.

Standard high speed semimicrocentrifuge capable of holding sample tubes.

Finger stall of pure gum rubber, or a finger from a rubber glove, extracted with a solution of 5% hydrochloric acid in acetone.

Beckman DU quartz ultraviolet spectrophotometer with ultraviolet accessories, or a similar instrument.

Roller-Smith microbalance, torsion type, of 25-mg. capacity with V-shaped pan, or similar balance weighing to 0.01 mg.

Reagents. CONCENTRATED NITRIC ACID–BROMINE. Add reagent grade bromine to concentrated reagent grade nitric acid, in

an all-glass dropper bottle with clean rubber or Tygon bulb. Have a layer of excess bromine on the bottom at all times.

PERCHLORIC ACID, 70 to 72% reagent grade used as received. It is conveniently stored in an all-glass dropper bottle with either rubber or Tygon bulb. Store away from heat and handle only a small volume at a time, keeping the main part of the reagent in the original bottle in the storeroom. A 1-pound bottle is sufficient for over 2000 analyses.

ACETONE, reagent grade. This must be redistilled if a brown residue is present after evaporation of the acetone following the precipitation and washing step.

HYDROCHLORIC ACID, 50% by volume. A convenient method of preparation is to mix equal volumes of concentrated hydrochloric acid (35 to 38% reagent, specific gravity 1.1778 to 1.1923 range) and distilled water, using the same volumetric flask to measure the acid and water.

LEAD NITRATE, 10%. Dissolve 10.0 grams of lead nitrate in water and dilute to 100 ml.

Calibration. The average specific absorbance or K value (also termed absorptivity) of lead at 270 mμ is 54.0. The exact figure should be determined for each instrument for greatest accuracy.

Prepare a stock solution containing 1000 p.p.m. of lead by dissolving 0.160 gram of reagent grade lead nitrate in distilled water in a 100-ml. volumetric flask and diluting to volume. Pipet 10.0 ml. into a second 100-ml. volumetric flask and dilute to volume for a 100 p.p.m. lead standard. Use a 50-ml. buret to deliver different volumes between 0.5 and 8.0 ml. of the 100 p.p.m. standard into a clean glass Erlenmeyer flask. With a second 50-ml. buret, add enough distilled water to make exactly 10.0-ml. total volume of lead solution. Pipet 10.0 ml. of concentrated hydrochloric acid reagent into the flask. Shake well and measure absorbance of the 50% hydrochloric acid solution at 270 mμ in 1.00-cm. quartz sample cells. Calculate K_{Pb} as follows:

$$K_{Pb} = \frac{A_{270 m\mu}}{b\,c}$$

where A is instrument absorbance at 270 mμ

b is internal cell thickness and is neglected as long as it is 1.00 ± 0.005 cm.

c is concentration of lead in grams per liter at the dilution for which absorbance is measured—e.g., 100 p.p.m. $\times$ 4 ml./20 ml. = 20 p.p.m. or 0.020 gram per liter

The K value to be used is the average of the data over the range of linear instrument absorbance (usually considered 0.1 to 1.8 A).

Procedure. Weigh 1 to 3 mg. of well milled representative sample. Sheet as thin as possible and weigh to 0.01 mg. on a suitable microbalance. Check the zero point of the balance carefully before and after any series of weighings. Brush the sample directly into the labeled sample tube, add 0.25 ml. of 10% lead nitrate solution from a 10-ml. buret, then add about 1 ml. of prepared concentrated nitric acid–bromine reagent and 3 drops (1 ml.) of 70% perchloric acid reagent. Do not add bumping stones of any kind.

Place a 3-inch square of sheet asbestos, about 1/16 inch thick, on a hot plate at 300° to 320° C. under a well ventilated hood. Immediately place a 150-ml. beaker containing as many as six sample tubes on the asbestos, and heat until completely oxidized (about 30 to 60 minutes). When the perchloric acid solution is essentially colorless and no particles of unoxidized sample are present on the walls of the tube (a trace of carbon black may be ignored), remove the beaker from the asbestos and place it directly on a bare hot plate at 300° to 320° C. for about 10 minutes. The tube should fume copiously and the liquid condensation level should rise at least to within 0.5 inch of the top of the tube to be sure all lead nitrate is converted to lead perchlorate. Do not allow to dry; if this occurs, the sample may be recovered by fuming again with 2 drops of perchloric acid. As long as the residue is wet with liquid acid on cooling, no heat decomposition of lead perchlorate present should result. Lead perchlorate will decompose to form some acetone-insoluble lead chloride, if heated excessively in the absence of free perchloric acid.

Remove the beaker and tubes from the hot plate and place on a cool surface. If desired, accelerate cooling with a blast of compressed air. When tubes are at room temperature and cold to the touch (acetone poured into hot perchloric acid may result in a dangerous explosion), rapidly pour about 10 ml. of acetone reagent into all tubes in the beaker. Cover the index finger with a finger stall and "stopper" each tube. Shake just enough to mix acetone and acid residue. Lead sulfate will be present as a turbid suspension, but if only a trace of sulfur is present—e.g., 0.1%—this turbidity may not always be apparent. Always wipe the finger stall on edge of sample tube to minimize possible loss of a trace of sulfur.

Fill a 150-ml. beaker about two thirds full with hot water at 50° to 60° C., but not be so hot that the acetone boils. Immediately immerse as many as six sample tubes in this water bath and allow the beaker to stand at room temperature for 10 to 15 minutes. Remove tubes from bath, and centrifuge in a semimicrocentrifuge for 1 to 2 minutes. Remove from centrifuge. With a capillary-tipped pipet connected by rubber tubing to a water pump vacuum drawing down a sink, withdraw all but about 0.3 to 0.5 ml. of acetone from each tube. Always hold the tube in a vertical position, and lower the pipet tip in the center of the tube. Be careful not to jar the tube when removing it from the centrifuge. Wash the precipitated lead sulfate twice more with about 10 ml. of acetone, centrifuging 1 to 2 minutes each time. Three of these small tubes may conveniently be handled at the same time during the washing operation when acetone is drawn off and added.

After removal of the third addition of acetone add 2 drops of water to each tube and place the beaker with tubes on the 300° C. hot plate to evaporate to dryness. Reduce the temperature if bumping occurs, but complete drying at high temperature for 5 to 10 minutes. Acetone absorbs in the ultraviolet and must be removed completely. When dry, remove from the hot plate, and cool in an air blast. The residue should be white at this point. If any trace of brown coloration is apparent, heat the tubes with the beaker at 550° C. for 15 minutes until the residue is white. Then proceed as usual.

When tubes are cool, carefully pipet 10.0 ml. of exactly 50% hydrochloric acid reagent into the sample tubes. With a finger stall over the finger to act as a stopper, shake the tubes a few seconds until all the lead sulfate is in solution. Inspect the bottom of the tube for undissolved material. If there is difficulty in dissolving the precipitate (or if white side-wall stock with acid-insoluble titanium dioxide is being analyzed), add a few small silicon carbide bumping stones and shake vigorously. In the case of white side-wall stock, or other material where acid-insoluble matter is present, centrifuge the sample to throw down the acid-insoluble material.

Sometime during this solution operation, place the Beckman DU spectrophotometer in operation with the hydrogen tube. An instrument slit width of about 1.1 mm. with sensitivity set near the middle of its range has proved satisfactory. Rinse a 1.00 ± 0.005-cm. quartz sample cell twice with about 2 ml., and finally pour in 3 to 4 ml. of the sample solution. Record absorbance at 270.0 mμ against a 50% hydrochloric acid blank. If absorbance is above 1.80, pour the contents of the cell into a 10-ml. glass-stoppered graduated cylinder and dilute to twice its volume accurately with 50% acid (a 1 to 1 dilution).

Calculate the per cent sulfur in the sample as follows, with a spectrophotometer cell thickness of 1.00 cm. A reagent blank normally amounts to about 0.010 absorbance or less, which is about 0.01 to 0.02% sulfur. Therefore, the experienced analyst may neglect the reagent blank, except for the most exacting work.

$$\%\ \text{sulfur} = \left(\frac{A \times \frac{S(32.07)}{Pb(207.2)} \times 100}{K_{Pb} \times c\ (\text{mg./ml.})} \right) = \frac{A_{270 m\mu} \times 0.155 \times 100}{54.0 \times (\text{wt. mg./10 ml. acid})}$$

For 10.0 ml. of acid this reduces to

$$\%\ \text{sulfur} = \frac{(2.87)\,A_{270\,m\mu}}{\text{mg. of sample}}$$

If 1 to 1 volume dilution is necessary (20-ml. volume), the factor is doubled and is 5.74.

EXPERIMENTAL WORK

Evaluation of the procedure for sample preparation used for semimicro amperometric titration (*1*) proved that the amount of residual nitric acid was a critical point in working on a micro scale with low-sulfur compounds. A significant amount of lead sulfate is dissolved by the residual nitric acid.

Use of potassium chlorate on a micro scale, following the conventional ASTM macromethod (*5*), oxidized the sample com-

pletely in 5 to 10 minutes. However, it proved too unreliable for routine analysis, as excess lead nitrate, excess acid, degree of dryness of residue, and washing technique were critical.

Use of perchloric acid was investigated as a final resort. Even this gave erratic results until the precipitation technique with an organic solvent was developed. This proved the answer to the recovery problem.

High and erratic results may be obtained, if fuming sulfuric acid is near when the sulfur is being determined.

Oxidation. The oxidation is carried out in the presence of excess lead nitrate. Normally about 10 mg. is sufficient for up to 5 mg. of rubber stocks, but about 25 mg. is added to ensure complete precipitation of sulfur as lead sulfate for diverse materials. It is convenient to add the lead nitrate from solution; the consistent amount added in this manner tends to result in more accurate data and to give a more reproducible blank.

Oxidation of natural rubber with fuming nitric acid is almost instantaneous and violent, and may lead to loss of sulfur. Consequently, the more slowly acting concentrated acid is used for all elastomers and other materials. Though concentrated nitric acid will not decompose butyl stocks, this elastomer and similar hard-to-oxidize materials are oxidized completely when the high boiling (200° C.) perchloric acid content increases as the nitric acid evaporates. As long as analyses are carried out on a micro scale, this is a safe practice, but larger samples of butyl polymer—e.g., 100 mg.—may oxidize with explosive violence, once perchloric acid becomes concentrated by evaporation, unless fuming nitric acid is first used to decompose the polymer. Perchloric acid should never be used indiscriminately for oxidation purposes.

The heavy bromine gas retains and oxidizes volatile sulfur compounds formed in the initial stages of oxidation. Though it has been said that iodic acid is superior to bromine for this purpose (*9*), the amount of sulfur lost when bromine is used in the presence of excess lead nitrate has proved negligible for routine analysis of rubber products and compounding materials.

Unless perchloric acid volume is above 2 drops, incomplete oxidation of sulfur may result in some cases. A range of 3 to 5 drops of added perchloric acid gave essentially the same sulfur recovery with natural rubber stocks but in some cases use of 5 drops gave somewhat lower recoveries—e.g., 95 to 98% of 3-drop recovery. Safety precautions, as well as lead sulfate solubility, require use of the minimum volume that will produce complete oxidation, which is about 3 drops (0.1 ml.).

The only really critical point about oxidation with the specified amount of perchloric acid on a micro scale is possible evaporation to dryness. In such a case the heat-sensitive lead perchlorate present decomposes to form lead chloride, that is insoluble in acetone and will lead to false high figures for sulfur recovery. The sample may be recovered by reheating to fumes with 2 drops of perchloric acid.

Precipitation. Perchlorates of most cations are soluble in alcohol and acetone. Potassium perchlorate is the only salt essentially insoluble in these solvents (*4*). The precipitation of lead sulfate through its insolubility in organic solvents offered a novel means of separation from interfering perchlorate salts. Several other solvents were investigated, including water-miscible alcohols (ethanol and methanol), higher ketones (methyl ethyl ketone and methyl isobutyl ketone) and ether (dioxane) alone, as well as water-immiscible hydrocarbons (*n*-hexane) mixed with acetone.

When 3 drops of perchloric acid were used, the alcohols all gave low recoveries, on the order of 60 to 90% of that of reagent grade acetone. The acid in dioxane dissolved practically all the lead sulfate. Methy ethyl ketone gave recoveries only a little lower than acetone, but methyl isobutyl ketone precipitated lead salts other than lead sulfate in the second wash, once acid concentration was reduced by the first wash. Mixtures of hydrocarbons and acetone offered no advantage over acetone alone.

Early work with acetone was carried out with specially prepared essentially anhydrous acetone. However, it was later proved that, though the water content of the acetone must be controlled, reagent grade acetone can be used. In fact, 1 to 2% of water added to reagent grade acetone may at times be beneficial. The presence of a trace of water inhibits the oxidation of anhydrous and reagent grade acetone, which normally results in development of a yellow coloration as the acetone stands in the presence of perchloric acid. However, this solvent oxidation does not normally appear to affect the quantitative recovery.

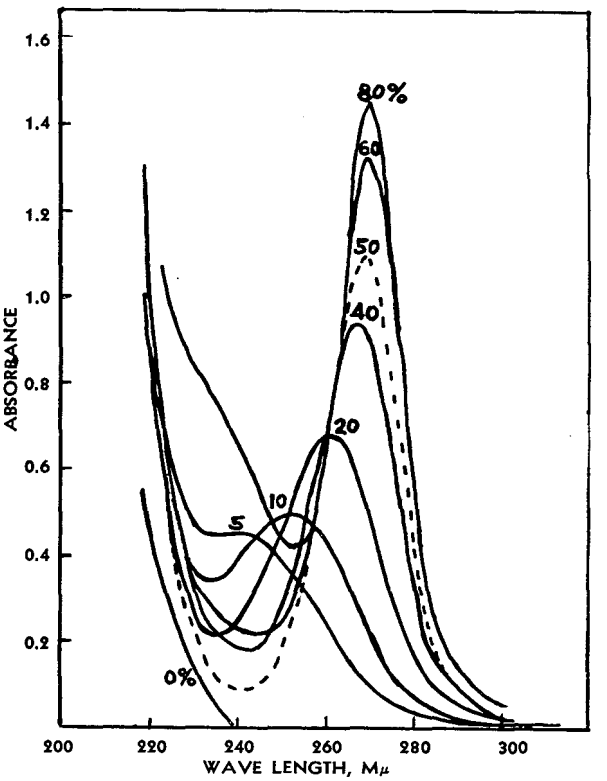

Figure 1. Effect of acid concentration on absorbance of lead

20 p.p.m. lead
Range 0 to 80% HCl
- - - 50% HCl

When acetone is first poured into the residual cold perchloric acid after sample digestion, an exothermic reaction results due to heat of solution. This has not proved hazardous even with such a large excess as 20 drops of perchloric acid, which would mean a final concentration of about 10% perchloric acid in acetone. On the micro scale involved, only 2 drops (0.07 ml.) of excess perchloric acid are diluted to about 8 ml., which is a concentration of less than 1% perchloric in acetone. Consequently it is felt that no hazard is involved in adding acetone to cold perchloric acid, as done here on a micro scale.

Paradoxically, heating the acetone in hot water at below the boiling point tends to force complete precipitation more efficiently than cooling in an ice bath. With high-sulfur materials, the additional recovery due to heating the acetone may not always be apparent, and immediate centrifuging may be justified in many cases. However, a definite increase in sulfur recovery was noted on several occasions following a period of heating the acetone, particularly with butyl stocks, so this step is incorporated in the procedure. Heating a reagent blank in the same manner for extended periods did not alter the blank. Therefore, such heating does not decompose the acetone-soluble perchlorate salts.

Interferences. Lead and iron salts and even barium perchlorate are very soluble in acetone and are probably all dissolved

when the first acetone is added to precipitate the insoluble lead sulfate (the sulfur could be precipitated as barium sulfate in the same manner with excess barium chloride). However, a residual volume of about 0.5 ml. remains in the tube each time after the clear acetone is drawn off following centrifuging. This makes necessary two more dilutions of the residue to reduce the blank to negligible proportions. A correction for background absorbance was necessary when potassium chlorate or nitric acid alone was used as the oxidant, but the acetone solubility of interfering perchlorates eliminates all possible interference. Even lead present in a stock as the hard-to-dissolve oxide, and acid-insoluble barium sulfate will be converted to perchlorates and be removed by the acetone with quantitative recovery of sulfur as lead sulfate.

This procedure offers a sensitive and rapid method for determining lead in rubber products and compounding materials. The sample is wet or dry ashed, then taken up in 50% hydrochloric acid. The absorbance at 270 mμ is measured and per cent lead is calculated using a specific absorbance, K, of 54.0. A simple mathematical correction may be made for any background interference due to iron.

Acetone absorbs strongly in the ultraviolet spectral region of interest and must be removed completely by evaporation before the 50% acid is added. Some lots of reagent grade acetone had to be redistilled, as they left a brown residue when the acetone was evaporated to dryness after the final wash. This residue absorbs and gives high results for sulfur. The brown material was apparently a high boiling oxidized impurity formed by oxidation with perchloric acid. It could be removed by heating the sample tubes at 550° C. for 15 minutes. However, it is best eliminated by redistilling the reagent grade acetone to ensure purity. Addition of 2 drops of water to each sample before evaporation to dryness will reduce such interference.

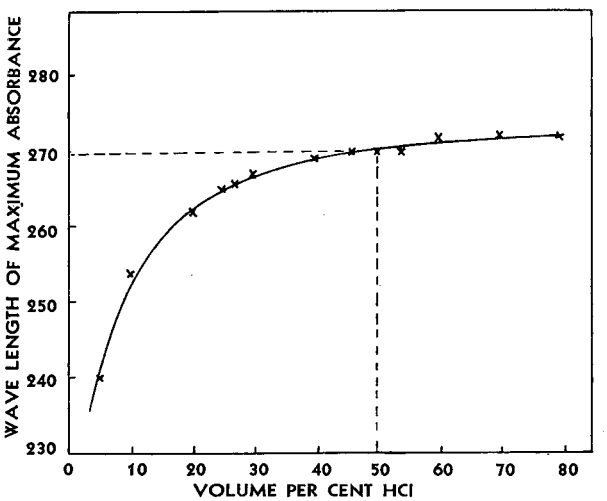

Figure 2. Shift of absorbance maximum with acid concentration

Standard micro chemical practice would call for use of a filter stick or some method of filtration for each sample, but the vacuum pipet is much faster and easier to handle. Recoveries are some times a little low—e.g., 98% of theory—for inorganic salts where no sulfur is likely to be lost by oxidation. This may be attributed to the slight solubility of lead sulfate in the acidic acetone.

Solution. It has been shown (*7*) that the intensity and selectivity of ultraviolet absorbance of lead in aqueous hydrochloric acid change drastically with change in acid concentration. This was investigated and the data are illustrated in Figure 1. The maximum is sharp and symmetrical at an acid concentration above about 40%.

The shift of wave length of maximum absorbance toward longer wave lengths is observed in Figure 2 to be an asymptotic function of the acid concentration. Above about 40% acid the shift in wave length is slow, and at 50% acid concentration the wave length of the maximum (270 mμ) will be essentially constant. Its location will not be affected by small changes in acid concentration, which might occur, owing to normal variation in normality of the concentrated reagent grade hydrochloric acid used.

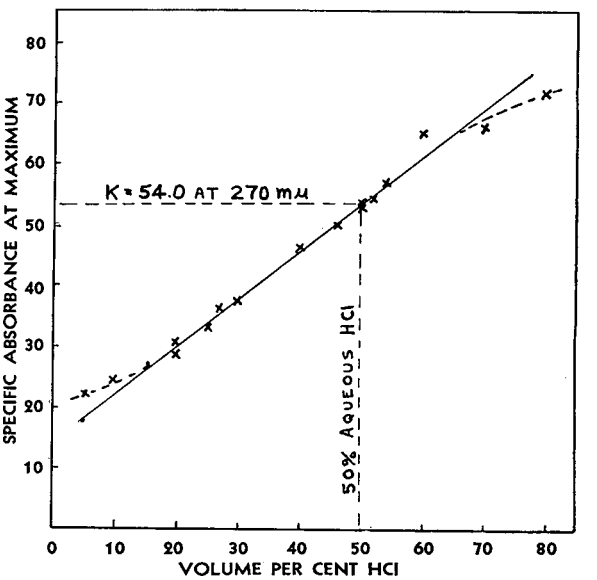

Figure 3. Effect of acid concentration on specific absorbance of lead

20 p.p.m. lead

Figure 3 shows that the intensity of absorbance, or specific absorbance, K at the maximum is a linear function of acid concentration in the range of about 20 to 60 volume % hydrochloric acid. The K value of 50% acid is 54.0 at 270 mμ, but in 25% acid it drops to 34.0 at 264 mμ. The high (50%) acid concentration is preferred because the greater sensitivity makes possible a smaller sample, which is rapidly oxidized with a minimum amount of perchloric acid, and any interfering absorbance is reduced in relative intensity to negligible proportions by the strong lead absorbance. Use of a weaker acid—e.g., 25%—may prove advantageous for purified materials of high sulfur content. However, dilution to larger volumes of 50% acid than the 10 ml. used here is preferred because of the greater accuracy possible at high sensitivity.

The concentrated ($12N$) hydrochloric acid (considered 37% hydrochloric acid with a range of 35 to 38%) as used to prepare a 50% acid mixture by volume would result in a range of 17.5 to 19% hydrochloric acid content in a 1 to 1 mixture. During development of the procedure, no abnormal results were obtained which could be attributed to variations in hydrochloric acid concentration. For determination of sulfur in rubber products, no correction was made for variation in hydrochloric acid content of the concentrated acid. For the most accurate work, the hydrochloric acid content should be checked carefully and kept constant.

Any chemically inert material can be used as a finger stall for stoppering the sample tubes containing acid. Rubber provides a better seal for acetone washing, but polyethylene film is satisfactory for 50% aqueous acid. Pure gum rubber is satisfactory for both acetone and acid, if extracted with both solvents before

Table I. Determination of Organic and Inorganic Sulfur in Rubber Compounding Materials

Compounding Material	Sulfur, % Theoretical	Sulfur, % By analysis	Sulfur, % Av.	Mean Deviation, %	Recovery, % of Theory
Inorganic Sulfur Compounds					
Sodium sulfate	22.6	21.8, 22.0	21.9	±0.1	97
Zinc oxide		0.30, 0.34	0.32	±0.02	
Barium sulfate	13.7	13.4, 13.1	13.2	±0.2	96.5
Organic Sulfur Compounds					
Sulfur (commercial rubber grade)	99.5	96.2, 99.4, 98, 100	98.4	±1.3	99 (97–101)
Accelerators					
MBTS (purified)[a]	38.6	36.2, 36.4, 37.9, 38.5	37.3	±0.9	96.7 (94–100)
TMTD (purified)[b]	53.4[c]	54.0, 52.6, 54.8, 53.4 52.0	53.4	±0.8	100 (97–103)
Softeners					
Petroleum oils (rubber grade)					
1.39% by O_2 bomb		1.40, 1.28	1.34	±0.06	
0.20% by O_2 bomb		0.11, 0.13, 0.13	0.13	±0.01	
Coal tar origin (Bardol)		0.57, 0.64	0.60	±0.04	
Asphalt origin (Paraflux)		0.86, 0.80	0.83	±0.03	
Carbon Blacks					
SRF (furnace)		0.044, 0.043, 0.064	0.050	±0.009	
HMF (furnace)		0.12, 0.14	0.13	±0.01	
FEF (furnace)		0.67, 0.64	0.66	±0.02	
HAF (furnace)		0.29, 0.35	0.32	±0.03	
SAF (furnace)		0.47, 0.45	0.46	±0.01	
EPC (channel)		0.15, 0.17	0.16	±0.01	

[a] Benzothiazyl disulfide.
[b] Tetramethyl thiuram disulfide.
[c] Chemical analysis by ASTM procedure gave 51.8, 51.5, and 52.9% sulfur in TMTD, average recovery of 97.7%.

Table II. Total Sulfur in Compounded Rubber Stocks by Absorptiometric Method

Stock Type	Polymer Type	Gravimetric Sulfur % Range	Gravimetric Sulfur % Av.	Absorptiometric Sulfur, % Range	Absorptiometric Sulfur, % Av.	Mean Deviation, %	Recovery[a], %
Standard	GR-S	1.28 to 1.34	1.31	1.28 to 1.38	1.32	±0.04	101
Standard	Natural	2.05 to 2.12	2.08	2.12 to 2.19	2.16	±0.04	104
Tire tread	Natural		1.72	1.85 to 1.93	1.89	±0.03	110
Foam rubber	Natural		2.62	2.52 to 2.61	2.56	±0.03	98
White side wall	Natural		2.02	1.91 to 1.98	1.95	±0.02	97
Tire tread	Mixed GR-S and natural		2.18	2.14 to 2.15	2.15	±0.01	99
Tire tread	GR-S (LTP)		1.53	1.50 to 1.57	1.54	±0.04	100
Hose (10% $CaCO_3$)	Neoprene		0.60	0.59 to 0.68	0.62	±0.04	103
Cement	Chlorinated rubber		0.66	0.72 to 0.80	0.76	±0.03	115

[a] % recovery by absorptiometric method relative to recovery by ASTM gravimetric method, % absorptiometrically × 100/% gravimetrically.

use. However, any possibility that acid will extract iron from compounded rubber must be avoided, as ferric chloride absorbs in the ultraviolet. The uncovered finger should never be used to stopper the sample tubes whether acetone or acid is the solvent. Safety preparations for handling lead recommended that the analyst wash his hands thoroughly after determining total sulfur by this method.

APPLICATIONS

The results obtained by the perchloric acid method represent true total sulfur and are comparable to those by the sodium carbonate fusion method (*1*). Only in critical work, or in application to materials other than rubber products, need possible loss of volatile sulfur be taken into consideration.

The results are not strictly comparable to those of the standard ASTM gravimetric method, as perchloric acid will recover sulfur from barium sulfate, which is filtered off as acid-insoluble in the regular method. Both methods determine sulfur in acid-soluble inorganic sulfates, such as sodium and calcium sulfates. The combustion method does not determine inorganic sulfate, as does this wet oxidation procedure.

If there is need to distinguish between inorganic and organic sulfur, the material can be ashed prior to determination of sulfur by wet oxidation on the ash. However, for the normal tire and tube stock, results are also in close agreement with the standard gravimetric barium sulfate procedure following nitric acid oxidation.

Analyses in Rubber Chemistry. The data of Table I illustrate the wide application of the perchloric acid method to both inorganic and organic rubber compounding materials. The following modifications of the regular perchloric acid procedure for analysis of these compounding materials are recommended

CARBON BLACK. Weigh 5 to 10 mg. of pelletized black and digest with a minimum amount of perchloric acid (3 to 5 drops). Add 3 drops, and if oxidation is incomplete, add 1 or 2 more as required for a colorless wet residue.

INORGANIC SALTS. For high-sulfur salts, such as barium sulfate, weigh 1 to 2 mg. and digest with 3 drops of perchloric acid. Dilute to 25 to 100 ml. with 50% hydrochloric acid as needed. For low-sulfur inorganic salts, such as zinc oxide, make the final 50% acid volume up to 10 ml. as usual.

PETROLEUM OILS (rubber grade, essentially nonvolatile at 100° C.). Weigh 5 to 10 mg. (1 small drop) and digest with a minimum amount of perchloric acid (3 to 5 drops). Dilute to 10 ml. with 50% acid, with further dilution as needed for high-sulfur oils. Weighing of softener oils on a microbalance is facilitated by taking up the sample on a weighed cotton cord, cutting off the part of the cord with the sample, and dropping the cord and all into a sample tube. The cord must be essentially sulfur-free.

ORGANIC COMPOUNDS. For sulfur below 3%, proceed as for rubber analysis. For high sulfur, such as tetramethyl thiuram disulfide accelerator, dissolve 1 to 5 mg. in 100 ml. of chloroform or other suitable solvent. Pipet an aliquot of 1 to 5 ml. into a sample tube and evaporate just to dryness at low temperature (70° C. oven or water bath). Proceed as for rubber analysis, and include the dilutions of the chloroform solution in the final calculations. Alternatively, the final acid volume can be made up to 100 ml. instead of 10 ml.

The data of Table II illustrate the application of the method to a wide variety of complex compounded rubber products. No difficulty was experienced in analysis of natural, GR-S, butyl, neoprene, or chlorinated rubber-base polymer stocks. Insolubility of titanium dioxide in white side-wall tire stock requires an extra centrifugation, to remove turbidity, but no other modification was necessary for any stock analyzed.

Other Methods of Measurement. The amount of lead sulfate precipitate can be measured in different ways—polarographic, volumetric, gravimetric, turbidimetric, or nephelometric techniques. The volumetric technique might involve the well known, but difficult, visual end point titration with tetrahydroquinone indicator, or amperometric titration (*8*). However, the ultraviolet absorptiometric procedure is considered equal or superior in accuracy and reliability to any of these methods. In addition, its high sensitivity makes possible analyses on a micro scale.

It was desired to demonstrate that the procedure for sample preparation with perchloric acid and acetone could be used to advantage with other methods of measurement. Results of a

gravimetric semimicroprocedure are given in Table III. Some modifications were necessary for the gravimetric method. Sample size ranged from 5 to 10 mg. for high-sulfur compounds and from about 75 to 100 mg. for low-sulfur compounded rubber products. Sulfur content was kept below 5 mg., which means a weighable lead sulfate recovery below 50 mg. Amount of reagent per sample was increased to 100 mg. of lead nitrate and 1 to 2 ml. of concentrated nitric acid, followed by 3 or 4 ml. of fuming nitric acid added in two or three treatments. Perchloric acid was added up to 10 drops for organic compounds containing no carbon black, and up to 20 drops for compounded rubber stocks. Volume of acetone wash was maintained at about 10 ml. per wash, but four washes were used.

Close attention to complete disintegration of rubber products with fuming nitric acid prior to addition of perchloric acid is essential. No trouble was encountered with perchloric acid oxidation of natural and GR-S rubber stocks. However, the relatively large (100 mg.) sample of hard-to-oxidize butyl rubber may oxidize with explosive violence unless completely disintegrated before the nitric acid has evaporated. A preliminary swelling in chloroform and evaporation of the chloroform prior to acid treatment were used to speed oxidation of butyl rubber in nitric acid.

If a significant amount of acid-insoluble material from the sample is present, it is necessary to dissolve the lead sulfate in 50% hydrochloric acid, centrifuge, and weigh the sample tube again to correct for the acid-insoluble. No correction was made for any trace of acid-insoluble material in the data of Table III. Absence of any significant turbidity after dissolving the lead sulfate will show where such a correction is unnecessary.

Petroleum Chemistry. The general application of this method to fields other than rubber chemistry is suggested by successful analysis of petroleum oil softeners. The somewhat lower results obtained may indicate the presence of volatile sulfur compounds, but agreement of the perchloric acid and oxygen bomb methods is close enough to make the wet oxidation satisfactory for routine analyses. Regular analysis by oxygen bomb methods could be speeded by using lead nitrate and measuring the lead sulfate formed by the absorptiometric method outlined here. For critical analyses where volatile materials are concerned, the sample may be oxidized by the Carius sealed tube method in the presence of lead nitrate. Perchloric acid is then added when the tube is opened and the regular procedure outlined here is followed thereafter.

Absolute Structural Analyses. The method appears to be of value for absolute determination of sulfur content in securing data for chemical formula reconstruction. Analysis of purified tetramethyl thiuram disulfide and high purity sulfur shows an average of 99 to 100% recovery. The perchloric acid procedure outlined here has been simplified for rapid routine determination of sulfur and some precision and accuracy have been sacrificed to speed up the analysis. Modifications, such as a somewhat larger sample with 100 ml. or more of exactly 50% acid, should make the accuracy comparable to that of other methods now in use. A preliminary slow oxidation with nitric acid and excess bromine at room temperature prior to heating, or use of conventional Carius or bomb techniques, should eliminate loss of trace amounts of volatile sulfur compounds.

Accuracy. The accuracy is difficult to prove in analysis of rubber products because sulfur is present in several compounding materials (see Table I). To prepare a standard stock of known true total sulfur content would require a prohibitive amount of work, involving determination of sulfur in every material added to the stock. However, a practical alternative is to consider only added sulfur as such and that present in accelerators added, in calculating theoretical per cent sulfur. Analysis of data of Table IV indicated the absorptiometric method recovers 97 to 108% of theoretical. This is good, considering the many factors affecting the theoretical per cent sulfur.

Table III. Gravimetric Sulfur as Lead Sulfate by Perchloric Acid Oxidation Semimicromethod

Stock	Sulfur Theoretical, %	S by $BaSO_4$ (ASTM), %	Gravimetric Lead Sulfate: Sample, mg.	$PbSO_4$, mg.	Sulfur, %	Recovery, % of Theoretical
Compounded Elastomer Stocks						
Natural tire tread	2.14	2.08	75.0	15.3	2.16	97.2
		(2.05 to 2.12)	100.8	19.1	2.01	
					Av. 2.08	
GR-S tire tread	1.26	1.31	104.7	12.6	1.27	103
		(1.28 to 1.34)	92.6	11.6	1.33	
					Av. 1.30	
Butyl tube	1.38	1.35	112.4	13.4	1.27[a]	89
			92.8	10.9	1.24[a]	
			102.0	11.2	1.17	
			97.2	11.5	1.25	
					Av. 1.23	
Elastomer Compounding Materials						
MBTS (purified)	38.6		2.82	10.1	37.9	95
			5.24	17.9	36.2	
			10.19	34.6	36.0	
					Av. 36.7	
TMTD (purified)	53.4	51.5	2.42	12.1	53.0	98.6
		52.9	5.03	24.8	52.2	
		51.8				
		Av. 52.1			Av. 52.6	
Sulfur (commercial of high purity)	100	...	2.68	26.1	103	101
			4.56	43.1	100	
			4.95	47.4	101	
			2.29	22.8	105	
			2.17	20.8	101	
			3.22	29.6	98	
					Av. 101	

[a] Analysis was completed even though these two of four samples showed explosive oxidation near end of oxidation. Explosive oxidation occurred because of failure to swell samples in chloroform as final procedure for butyl requires:

Precision. The data of Table IV illustrate the precision of the absorptiometric micromethod for analysis of three complex compounded cured rubber stocks. Work of seven different analysts, most of them unfamiliar with the new technique, shows a mean deviation from average of ±0.06% for the natural and butyl stock and only ±0.02% for the GR-S stock. Familiarity with the procedure should give duplicate determinations that check well within ±0.1% deviation for analysis of rubber products.

Absolute accuracy is here best measured by determination of sulfur on purified organic and inorganic materials. Recoveries of 97% or higher of available sulfur in pure compounds are observed in Table I. Similar or higher recoveries are obtained for organic compared to inorganic compounds, which indicates there is probably no loss of sulfur through volatilization of the organic material. The slightly low recoveries for these pure materials may be attributable to a very slight solubility of lead sulfate in perchloric acid in the presence of acetone. The absorptiometric recovery is a little higher than the gravimetric recovery for both rubber stocks and purified tetramethyl thiuram disulfide accelerator.

A more practical basis for establishing accuracy is to determine relative accuracy by comparing recoveries for the same sample by the conventional gravimetric and absorptiometric methods. This was done for the GR-S and natural stocks of Table IV. Average recoveries by the gravimetric method are about 97% of those by the absorptiometric method. However, agreement of the results is so close that the two methods may be considered of comparable accuracy for all practical purposes.

Table IV. Precision and Relative Accuracy of Absorptiometric Micromethod

		Sulfur Found, %		
Analyst		Natural	GR-S	Butyl
1		2.10	1.40	1.30
		2.06		1.36
	Av.	2.08		1.33
2		2.10	1.29	1.38
		2.06	1.33	1.43
		2.09	1.29	1.35
	Av.	2.08	1.30	1.39
3		2.19	1.27	1.46
		2.25	1.39	1.43
			1.39	
	Av.	2.22	1.35	1.44
4		2.10	1.46	...
		2.15	1.26	
	Av.	2.12	1.36	
5		2.12	1.39	1.34
		2.12	1.35	1.37
	Av.	2.12	1.37	1.36
6		2.01	...	1.20
		2.04		1.16
	Av.	2.03		1.18
7		1.88	1.39	1.38
		1.94	1.38	1.35
	Av.	1.91	1.38	1.36
Summary of Data				
Av. for sample, %		2.09	1.36	1.34
Max. deviation		−0.18	−0.06	−0.16
Mean deviation		±0.06	±0.02	±0.06
Comparison of ASTM Gravimetric and Absorptiometric Methods				
Av. absorptiometric S, %		2.09	1.36	1.34
Theoretical S as compounded, %		2.14	1.26	1.38
Av. gravimetric S, %		2.04	1.31	...
Ratio $\frac{\text{absorptiometric S, \%}}{\text{theoretical S, \%}}$		0.98	1.08	0.97
Ratio $\frac{\text{absorptiometric S, \%}}{\text{gravimetric S, \%}}$		1.02	1.04	...

DISCUSSION

The only special equipment required for this analysis is an ultraviolet spectrophotometer capable of making absorbance readings at 270 mμ. The Beckman DU spectrophotometer used here is by no means a single-purpose instrument in the rubber laboratory. Its application to identification of accelerators (*6*) and antioxidants (*3*) and for quantitative determination of accelerators (*5*) has been described. All other equipment and reagents are standard equipment in the average rubber control laboratory.

The method has all the advantages of a micromethod but eliminates most of the disadvantages involving tedious manipulation. Work on a micro scale makes possible a significant saving in time, cost of glassware, and amount of reagents required. Volume of oxidizing reagents is here measured in drops, rather than milliliters as in the macromethod. This micromethod is sensitive to contaminants, and glassware should be kept scrupulously clean and stored protected from laboratory dust.

The sample size is so small that the rubber chemist may often question the homogeneity of the sample. However, rubber is one of the best dispersing mediums known, and under normal conditions after cured stocks have been milled the homogeneity has never proved a problem. On the other hand, the sample can be so minute that the method may be used to detect incomplete disperson of sulfur of sulfur-bearing materials in rubber products, when it is not homogenized on a mill.

The method is considerably more rapid than any conventional procedure used for determination of sulfur in rubber products. An experienced analyst can complete an analysis in duplicate in about 2 hours, with less than 1-hour working time. Several samples may be conveniently run simultaneously with only a nominal increase in total time for analysis. In the rubber industry there is normally no need for a total sulfur procedure that will yield results in less than 3 to 4 hours. Therefore, this method is a practical solution to the long-standing problem of a more convenient and rapid determination of total sulfur in rubber products.

ACKNOWLEDGMENT

The author acknowledges the help of Jack Z. Falcon with development work. His interest in perchloric acid as an oxidant aided materially in developing the final procedure.

Many analysts of the Firestone analytical laboratories helped to determine the precision of the method and obtained data on its application to diverse rubber compounding materials. The greater part of the gravimetric barium sulfate data in tables was secured by R. O. Lankenau.

The permission of the Firestone Tire and Rubber Co. to publish this paper is gratefully acknowledged.

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RECEIVED for review April 20, 1955. Accepted July 14, 1955.

Redox Determination of Tervalent and Total Cobalt in Presence of Excess Tungstate

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Total cobalt can be determined readily in the presence of excess tungstate, molybdate, and iron by a volumetric redox method. The end point (dichromate *vs.* ferrous) can be observed potentiometrically in the presence of precipitated tungstic acid by employing platinum electrodes and a polarizing voltage. The method can be adapted to determination of tervalent cobalt in the presence of bivalent cobalt. The latter may be found from the difference between the total and tervalent cobalt determinations.

THIS paper describes how total cobalt or, alternatively, tervalent cobalt can be determined easily in the presence of excess tungstate. Bivalent cobalt may be found from the difference between the two determinations. Molybdate and iron do not interfere. Ammonia or amines may cause the results to be slightly low for tervalent cobalt when bivalent cobalt is also present.

The procedures are modifications of Sarver's quick volumetric method (*9*), which utilized a redox indicator. It is well known that diphenylamine and diphenylbenzidine indicators are precipitated by traces of tungstate, and that diphenylaminesulfonic acid may be employed when moderate amounts of tungstate are present (*8, 10, 13, 14*). In the present study satisfactory results were obtained with Sarver's original method (*9*), using a diphenylamine sulfonate and solutions of the concentrations cited below, when the gram-atom ratio of tungsten to cobalt was less than 4 to 1 (weight ratio less than 12.5 to 1). When the proportion of uncomplexed tungstate was higher, the indicator was unsatisfactory. It appears that in such circumstances the indicator is rapidly adsorbed on the colloidal tungstic acids formed in acidic solutions. (If several drops of indicator were added just before the final end point, a very vague color change could sometimes be observed; but the considerable indicator blank was then very variable.) Under these conditions a sharp end point can be observed potentiometrically by using platinum electrodes and a polarizing voltage. The end point, which can be conveniently determined with a Serfass electron ray titrimeter, must be approached by addition of reducing agent, rather than from the other direction. This technique, involving no determination of an indicator blank (*14*), gives an excellent end point even when the final solution contains large amounts of precipitated tungstic acid.

In certain procedures, such as the estimation of chromium and vanadium (*12, 14, 15*), diphenylamine sulfonate indicator can be used in the presence of excess tungstate, provided the latter is held in solution as a complex phosphate or fluoride. It was evident in the authors' experiments that phosphate was unable, under the conditions of the reaction, to hold the tungsten in solution (*14*). The methods which have been recommended for the formation of the tungstate-fluoride complex are not appropriate in the cobalt determinations. It has been shown (*12*) that the tungsten-fluoride complex contains some tungsten in an oxidation state lower than +6 when the complex is formed by oxidation of solutions containing tungsten in lower states of oxidation. It was evident that such lower states exist in the cobalt determination. It was evident that such lower states exist in the cobalt determination because intense tungsten-blue colors formed in the basic solution upon addition of the standard ferrous solution. Treatments, as with persulfate (*12, 14*), which are necessary to complete the oxidation of tungstate-fluoride complex, cannot be used once the standard ferrous solution has been added. That all of the uncomplexed tungstate was reoxidized in the procedure described below, was evident from the bleaching of the color upon acidification and from the fact that the results were not high.

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EXPERIMENTAL

Chloropentamminecobalt(III)dichloride, $[CoCl(NH_3)_5]Cl_2$ (theoretical cobalt = 23.53%), was chosen as a source of cobalt (*5*) because it is a good primary standard for that element (*4, 5*). Cobalt was determined in three samples, each weighing approximately 0.6 gram, by evaporating to dryness with a little sulfuric acid, igniting, and weighing the cobalt sulfate ($CoSO_4$) formed (*4, 11*). Found, per cent cobalt: 23.50, 23.52, 23.54; average, 23.52.

Three samples, each containing enough cobalt to oxidize 15 to 20 ml. of 0.1*N* standard ferrous solution in the later operation, were accurately weighed into 500-ml. Erlenmeyer flasks which had standard-taper ground-glass necks. To each flask was added over six times as many moles of sodium orthotungstate dihydrate, $Na_2WO_4.2H_2O$, as the number of moles of complex salt already therein.

Total cobalt was determined in each mixture according to the following modification of Sarver's method: Twenty milliliters of water were added to dissolve the compounds. Five milliliters of 6*N* sulfuric acid were added, followed by 4 grams of solid sodium perborate, and then 20 ml. of 6*N* sodium hydroxide. The flask was immediately fitted with a standard-taper ground-glass stopper holding a 40-ml. dropping funnel. [The lower tip of the funnel was sealed to the bottom of the stopper in such a way that no pocket of gas was above the funnel's tip. The ground-glass parts were lubricated with a high vacuum silicone, because greases and waxes (*9*) melt and leak during the later operations.] The mixture in the flask was boiled for 5 minutes with the funnel stopcock open. After the mixture was cooled, a small additional lump of sodium perborate was added, to reoxidize any cobalt which had been reduced by ammonia before all of that gas was boiled away (*7*). The mixture was then boiled for another 10 minutes. A little boiled distilled water was added to the dropping funnel to prevent air from being sucked back into the flask when the heating was discontinued. These operations precipitated the cobalt as cobaltic hydroxide, decomposed the excess perborate, and expelled all free oxygen from the flask. It was removed from the flame and shaken to allow the superheated water to form steam, and the stopcock was closed just when the flask began to suck the water from the funnel. Excess standard 0.1*N* Mohr's salt solution was at once added to the dropping funnel from a buret, and this solution was admitted cautiously to the flask in such a way that no air entered. The content of the flask was swirled, and 35 ml. of 6*N* sulfuric acid were immediately admitted through the dropping funnel. The content was swirled again and 10 ml. of 25% phosphoric acid were added. The cobaltic hydroxide dissolved immediately and completely upon acidification. Air was then admitted and the funnel was rinsed into the flask. The solution had the typical pink color of cobaltous ion. The solution was transferred to an 800-ml. beaker to which excess standard 0.1*N* potassium dichromate was added. The mixture was back-titrated with more 0.1*N* Mohr's salt to the potentiometric end point. Found, per cent cobalt: 23.52, 23.25, 23.45; average, 23.41.

Two more samples of the same size were weighed out and mixed with sodium orthotungstate dihydrate as before. To one of these about 0.2 gram of cobaltous chloride hexahydrate was added. Both were analyzed for tervalent cobalt by using exactly the same procedure, except that no additions of perborate were made. In both cases 23.00% tervalent cobalt was found. That these results are a little low is probably attributable to the slight reduction of cobaltic hydroxide by ammonia which is known to occur in basic solution (*7*). No such effect was noted when analyzing compounds which contained no amine or ammonia.

A series of heteropoly tungstocobaltates (*3*), containing both bivalent and tervalent cobalt, was analyzed by these methods. In some cases the proportion of tungstate was several times that cited above, but the end point was always sharp. The substances are not good primary standards, but the results showed excellent consistency. Examples: percentages calculated for $Cs_6H[Co^{+2}Co^{+3}W_{12}O_{42}].13H_2O$: cesium, 19.8; tungsten, 54.8; water, 6.04; cobalt (tervalent), 1.47. Found: cesium, 19.9; tungsten, 54.9; water, 6.00; cobalt (tervalent), 1.47. (Total cobalt is always a little high in compounds of this anion because the method of preparation leaves them impure in that respect.) Percentages calculated for $K_4H_5[Co^{+3}W_{12}O_{42}].18H_2O$: potassium, 4.57; tungsten, 64.5; water, 10.8; cobalt (tervalent), 1.72. Found: potassium, 4.60; tungsten, 64.5; water, 10.5; cobalt (tervalent), 1.79. The method is applicable in the presence of molybdates also. Percentages calculated for $(NH_4)_3H_5[Co(OH)(MoO_4)_5].3H_2O$ (2,6): cobalt, 5.97; nitrogen, 4.25; molybdenum, 48.6. Found: cobalt, 5.96; nitrogen, 4.42; molybdenum, 48.3. Percentages calculated for $(CH_3NH_3)_6[(CoO_6Mo_6O_{15})_2].18H_2O$ (1,2, 15): cobalt, 4.81; nitrogen, 3.42; molybdenum, 47.0. Found: cobalt, 4.80; nitrogen, 3.53; molybdenum, 46.7. A sample of the ammonium salt of the last-mentioned anion (1,2,6,15) gave 4.74% cobalt by the 1-nitroso-2-naphthol method and 4.74% cobalt by the method here described.

ACKNOWLEDGMENT

Helpful advice was received from Donald Cooke, Arno Heyn, and Wallace McNabb.

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RECEIVED for review January 28, 1955. Accepted May 3, 1955.

Spectrophotometric Determination of Phosphorus as Molybdovanadophosphoric Acid

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The molybdovanadophosphoric acid method for the spectrophotometric determination of phosphorus has been extensively reviewed. The optimum concentrations of acid, vanadium(V), and molybdenum(VI) were determined by factorial experiment. The optimum color development occurs in solutions which are 0.4*M* in acid, 0.02 to 0.06*M* molybdenum(VI), and 1.0 to 4.0m*M* vanadium(V). The optimum range is 3 to 20 p.p.m. of phosphorus pentoxide for 1-cm. cells. Dichromate is the only serious interference, but can be eliminated by volatilization of the chromium as chromyl chloride.

AN EFFORT is currently being made to review critically the analytical methods employed in this laboratory, with the intention of replacing any which may have become outdated, by more rapid or accurate procedures. The two requirements for routine control analyses are speed and a reasonable degree of accuracy. With these needs in mind, the literature was reviewed for methods of analysis for phosphorus. Various spectrophotometric procedures have been proposed and employed for the determination of phosphorus. Because spectrophotometric procedures offer the speed and accuracy mentioned as requisites for a routine analysis, these procedures were examined in greater detail.

In common with arsenic, germanium, and silicon, phosphorus forms a yellow heteropoly acid with excess molybdate. These heteropoly acids may be utilized directly in spectrophotometric procedures or they may be reduced to molybdenum blue with an increase in sensitivity. While either of these methods can be used for phosphorus determination, they are subject to serious interference if arsenic, silicon, or germanium is also present. As the samples treated in this laboratory are ores and ore products, all of these interferences may be encountered. Therefore, the heteropoly methods, as such, were not considered further. Various workers have attempted to make the molybdophosphoric acid method more selective by extracting the complex into an organic solvent. The literature on this subject has been recently reviewed by Wadelin and Mellon (*17*). However, the reagents which are most selective are difficult to separate from the aqueous phase, and the other proposed reagents are not selective enough to justify the extra time involved in performing the extraction.

Another popular spectrophotometric method for phosphorus is based on the formation of the yellow molybdovanadophosphoric acid as originally proposed by Misson (*13*). This method seems to be the most specific spectrophotometric method proposed for phosphorus, and has been used for the determination of phosphorus in steels and iron ores (*3, 5, 7–10, 13, 14, 16, 18*); in uranium metal, uranium oxides, and uranium phosphate (*1, 12*); and in phosphate rock (*2, 6*), limestone (*4, 15*), and biological materials (*11*). It was decided to investigate the possible application of this method to the routine determination of phosphorus in uraniferous ores and ore products. A review of the literature reveals a surprising lack of conformity in the procedures for developing the color of molybdovanadophosphoric acid. Therefore, it was necessary to make a critical study of the optimum conditions for color development before the method could be employed. The results of this study and a modified procedure are reported in this paper.

Table I. Absorbance of Blanks

V, m*M*	Mo, *M*	0.2*M* $HClO_4$ Duplicates	0.2*M* $HClO_4$ Av.	0.4*M* $HClO_4$ Duplicates	0.4*M* $HClO_4$ Av.	0.6*M* $HClO_4$ Duplicates	0.6*M* $HClO_4$ Av.	0.8*M* $HClO_4$ Duplicates	0.8*M* $HClO_4$ Av.
0.4	0.01	0.065, 0.062	0.0635	0.007, 0.018	0.0125	0.020, 0.012	0.0160	0.014, 0.011	0.0125
0.4	0.02	0.075, 0.075	0.0750	0.031, 0.027	0.0290	0.014, 0.020	0.0170	0.012, 0.020	0.0160
0.4	0.04	0.087, 0.081	0.0840	0.081, 0.074	0.0775	0.038, 0.052	0.0450	0.029, 0.034	0.0315
0.4	0.06	0.095, 0.095	0.0950	0.084, 0.082	0.0830	0.062, 0.074	0.0680	0.046, 0.040	0.0430
1.0	0.01	0.084, 0.087	0.0855	0.017, 0.021	0.0190	0.027, 0.035	0.0310	0.027, 0.030	0.0285
1.0	0.02	0.104, 0.100	0.1020	0.042, 0.042	0.0420	0.026, 0.037	0.0315	0.031, 0.026	0.0285
1.0	0.04	0.117, 0.117	0.1170	0.103, 0.100	0.1015	0.054, 0.053	0.0535	0.059, 0.047	0.0530
1.0	0.06	0.130, 0.131	0.1305	0.111, 0.108	0.1095	0.075, 0.078	0.0765	0.068, 0.067	0.0675
2.0	0.01	0.093, 0.087	0.0900	0.054, 0.054	0.0540	0.039, 0.028	0.0335	0.027, 0.027	0.0270
2.0	0.02	0.146, 0.141	0.1435	0.074, 0.075	0.0745	0.069, 0.062	0.0655	0.043, 0.063	0.0530
2.0	0.04	0.170, 0.165	0.1675	0.148, 0.133	0.1405	0.118, 0.098	0.1080	0.087, 0.087	0.0870
2.0	0.06	0.190, 0.181	0.1855	0.155, 0.145	0.1500	0.128, 0.118	0.1230	0.072, 0.101	0.0865

Table II. Absorbance of Samples

V, m*M*	Mo, *M*	0.2*M* $HClO_4$ Duplicates	0.2*M* $HClO_4$ Av.	0.4*M* $HClO_4$ Duplicates	0.4*M* $HClO_4$ Av.	0.6*M* $HClO_4$ Duplicates	0.6*M* $HClO_4$ Av.	0.8*M* $HClO_4$ Duplicates	0.8*M* $HClO_4$ Av.
0.4	0.01	0.775, 0.798	0.7865	0.744, 0.761	0.7525	0.602, 0.656	0.6290	0.373, 0.422	0.3975
0.4	0.02	0.828, 0.842	0.8350	0.788, 0.788	0.7880	0.751, 0.770	0.7605	0.724, 0.741	0.7325
0.4	0.04	0.835, 0.842	0.8385	0.830, 0.830	0.8300	0.760, 0.807	0.7835	0.770, 0.786	0.7780
0.4	0.06	0.845, 0.845	0.8450	0.845, 0.843	0.8440	0.794, 0.821	0.8075	0.784, 0.800	0.7920
1.0	0.01	0.795, 0.810	0.8025	0.775, 0.812	0.7935	0.713, 0.755	0.7340	0.524, 0.575	0.5495
1.0	0.02	0.856, 0.877	0.8665	0.790, 0.812	0.8010	0.773, 0.788	0.7805	0.788, 0.790	0.7890
1.0	0.04	0.867, 0.876	0.8715	0.860, 0.857	0.8585	0.784, 0.818	0.8010	0.823, 0.797	0.8100
1.0	0.06	0.868, 0.866	0.8670	0.868, 0.880	0.8740	0.820, 0.847	0.8335	0.830, 0.812	0.8210
2.0	0.01	0.810, 0.841	0.8255	0.794, 0.834	0.8140	0.773, 0.785	0.7790	0.607, 0.606	0.6065
2 0	0.02	0.910, 0.926	0.9180	0.825, 0.875	0.8500	0.840, 0.835	0.8375	0.782, 0.798	0.7900
2.0	0.04	0.930, 0.920	0.9250	0.910, 0.896	0.9030	0.875, 0.873	0.8740	0.830, 0.875	0.8525
2.0	0.06	0.945, 0.955	0.9500	0.927, 0.923	0.9250	0.870, 0.891	0.8805	0.814, 0.874	0.8440

Table III. Absorbance of Samples − Blanks

V, m*M*	Mo, *M*	0.2*M* $HClO_4$ Duplicates	0.2*M* $HClO_4$ Av.	0.4*M* $HClO_4$ Duplicates	0.4*M* $HClO_4$ Av.	0.6*M* $HClO_4$ Duplicates	0.6*M* $HClO_4$ Av.	0.8*M* $HClO_4$ Duplicates	0.8*M* $HClO_4$ Av.
0.4	0.01	0.710, 0.736	0.7230	0.737, 0.743	0.7400	0.582, 0.574	0.5780	0.359, 0.421	0.3900
0.4	0.02	0.753, 0.766	0.7595	0.757, 0.760	0.7585	0.737, 0.746	0.7415	0.712, 0.719	0.7155
0.4	0.04	0.748, 0.761	0.7545	0.749, 0.756	0.7525	0.722, 0.755	0.7385	0.741, 0.752	0.7465
0.4	0.06	0.750, 0.756	0.7530	0.761, 0.767	0.7640	0.734, 0.747	0.7455	0.738, 0.739	0.7385
1.0	0.01	0.711, 0.723	0.7170	0.758, 0.791	0.7745	0.686, 0.720	0.7030	0.496, 0.545	0.5205
1.0	0.02	0.752, 0.777	0.7645	0.748, 0.770	0.7590	0.747, 0.751	0.7490	0.757, 0.764	0.7605
1.0	0.04	0.750, 0.759	0.7545	0.757, 0.757	0.7570	0.730, 0.765	0.7475	0.764, 0.750	0.7570
1.0	0.06	0.738, 0.735	0.7365	0.757, 0.772	0.7645	0.745, 0.769	0.7570	0.762, 0.745,	0.7535
2.0	0.01	0.717, 0.744	0.7305	0.740, 0.780	0.7600	0.724, 0.757	0.7405	0.550, 0.579	0.5635
2.0	0.02	0.764, 0.785	0.7745	0.751, 0.800	0.7755	0.771, 0.773	0.7720	0.739, 0.722	0.7305
2.0	0.04	0.760, 0.755	0.7575	0.762, 0.763	0.7625	0.757, 0.775	0.7660	0.743, 0.788	0.7655
2.0	0.06	0.755, 0.774	0.7645	0.772, 0.778	0.7750	0.742; 0.773	0.7575	0.742, 0.773	0.7575

APPARATUS AND REAGENTS

Absorbance measurements were made with a Beckman Model DU quartz spectrophotometer using matched 1.00-cm. Corex cells.

All chemicals were of analytical reagent purity.

A standard solution of phosphate was prepared by dissolving 1.917 grams of dried potassium dihydrogen phosphate (KH_2PO_4) in deionized water and diluting to 1 liter. This solution was standardized gravimetrically, in triplicate, and was found to contain 1.01 mg. of phosphorus pentoxide per ml. as soluble phosphate.

A stock solution of 0.20*M* molybdenum(VI) was prepared by dissolving 35.3 grams of ammonium molybdate tetrahydrate in deionized water and diluting to 1 liter.

A stock solution of 0.020*M* vanadium(V) in 0.4*M* perchloric acid was prepared by dissolving 1.17 grams of ammonium metavanadate in 400 ml. of deionized water, acidifying with 25 ml. of 8*M* perchloric acid, and diluting to 500 ml.

A stock solution of 8*M* perchloric acid was prepared by diluting 345 ml. of 70 to 72% perchloric acid to 500 ml.

RECOMMENDED PROCEDURE

Solid Samples. Weigh out a sample which contains 0.3 to 2.0 mg. of phosphorus pentoxide into a platinum dish. Cover the sample with hydrofluoric acid, and add 5 or 10 ml. more. Add 5 ml. 70% perchloric acid, and evaporate to dense fumes of perchloric acid. Dilute, and transfer the solution to a 100-ml. volumetric flask, filtering off any residue.

Liquid Samples. Pipet an aliquot which contains 0.3 to 2.0 mg. of phosphorus pentoxide into a 250-ml. beaker. Neutralize any excess acid or base to the phenolphthalein end point with concentrated sodium hydroxide or perchloric acid, respectively. Add 5 ml. of 70% perchloric acid, and evaporate to dense fumes of perchloric acid. Dilute, and transfer the sample to a 100-ml. volumetric flask.

Samples Suspected to Contain Chromium. To the fuming perchloric acid solution of the sample add about 1 gram of sodium chloride. If red fumes of chromyl chloride are observed, rinse down the sides of the beaker or platinum dish, bring to fumes again, and add another gram of sodium chloride. Repeat this procedure until fumes of chromyl chloride are no longer observed. Dilute, and transfer the sample to a 100-ml. volumetric flask.

To the sample in the 100-ml. volumetric flask add exactly 10 ml. of the vanadium stock solution, mix, and add 20 ml. of the molybdenum stock solution. Dilute to the mark and mix well. After 15 minutes, measure the absorbance at 400 mμ (0.055-mm. slit) against a reagent blank.

EXPERIMENTAL

Color-Developing Agents. Until 1948, the vanadate and molybdate were added as separate reagents (*10*), but then Barton (*2*) proposed the use of a mixed reagent containing both the color developing agents. Since then most other workers have recommended the mixed reagent (*1*, *6*, *12*). Gee and Dietz (*6*) found the reagent stable for only a week in warm weather, but Anderson and Wright (*1*) claim that the reagent is stable for a "long period." During development work on the method separate reagents were employed. Once a final procedure was developed, the possibility of using a mixed reagent was examined. However, the mixture of vanadate and molybdate is soluble only at high acid concentrations. Since excess acid was found to result in poor color development, it was considered preferable to continue to use the separate reagents.

Concentration of Acid, Vanadate, and Molybdate. The various final molar concentrations of acid which have been recommended are 0.20 (*6*), 0.53 (*10*), 0.64 (*1*), and 0.80 (*2*, *12*). If the acid concentration is too low, a yellow vanadium molybdenum complex (*6*, *10*) or a precipitate (*14*, *18*) forms. If the acid concentration is too high, the color development is slow or incomplete (*1*, *10*, *18*). Nitric acid has been most widely used, but Kitson and Mellon (*10*) claim that sulfuric, hydrochloric, or perchloric acid is also satisfactory. In the present work perchloric acid was chosen, as nitric acid may contain dissolved colored oxides of nitrogen and sulfuric or hydrochloric acid may give rise to colored complexes with cations. The optimum final concentration of vanadate has generally (*2*, *10*, *12*, *15*) been specified as 2.0 to 2.2m*M*, although 1.7m*M* (*1*) and 0.4 to 1.2m*M* (*6*) have been employed. While some excess of vanadate is required for full color development, too large an excess simply adds to the absorbance of the blank. There is no unanimity as to the optimum molybdate concentration. The following final molar concentrations have been recommended: 0.006 to 0.018 (*6*), 0.028 (*10*), 0.038 (*15*), 0.045 (*1*), and 0.057 (*2*, *12*). Gee and Dietz (*6*) attempted to evaluate the nature of the complex acid, and concluded that the ratio of vanadium to phosphorus is 1 to 1 and that the ratio of molybdenum to phosphorus is at least 14 to 1. The complex was observed to be relatively strong, so that no great excess of reagent should be required to develop full color.

Kitson and Mellon (*10*) commented that the reagents used by previous workers were usually too concentrated, and merely served to contribute to background absorbance.

Some means was needed to determine the optimum concentration or range of concentrations out of the various recommendations which have been mentioned in the literature. A factorial experiment was designed to study the effect of the following variables on the color development: acid concentration at levels of 0.2, 0.4, 0.6, and 0.8*M*; vanadium concentration at levels of 0.4, 1.0, and 2.0m*M*; and molybdenum concentration at levels of 0.01, 0.02, 0.04, and 0.06*M*.

Table IV. Two-Way Tables for Absorbance of Blanks

		Acid Molarity			
	Mo, *M*	0.2	0.4	0.6	0.8
Av. of 6 analyses at 3 vandium levels	0.01	0.0797	0.0285	0.0268	0.0227
	0.02	0.1068	0.0485	0.0380	0.0325
	0.04	0.1228	0.1065	0.0688	0.0572
	0.06	0.1370	0.1142	0.0892	0.0657
	Av.	0.1116	0.0744	0.0557	0.0445

		Vanadium Concn., m*M*		
	$HClO_4$, *M*	0.4	1.0	2.0
Av. of 8 analyses at 4 molybdenum levels	0.2	0.0794	0.1088	0.1466
	0.4	0.0505	0.0680	0.1048
	0.6	0.0365	0.0481	0.0825
	0.8	0.0258	0.0444	0.0634
	Av.	0.0481	0.0673	0.0993

		Molybdenum Concn., *M*			
	V, *mM*	0.01	0.02	0.04	0.06
Av. of 8 analyses at 4 acid levels	0.4	0.0261	0.0342	0.0595	0.0723
	1.0	0.0410	0.0510	0.0813	0.0960
	2.0	0.0511	0.0841	0.1258	0.1363
	Av.	0.0394	0.0564	0.0889	0.1015

To run a determination of each combination of these variables required 48 samples and 48 blanks (4 levels of acid × 3 levels of vanadium × 4 levels of molybdenum). Duplicates were run at each condition thus raising the number of solutions required to 192. The samples all contained 2 mg. of phosphorus pentoxide per 100 ml. of final volume. The absorbance of both the samples and the blanks was measured at 400 mμ (0.055-mm. slit) using deionized water as the reference standard so that the effect of change in concentration of reagents could be observed on both samples and blanks. The results of the experimental work are presented in Tables I, II, and III. The variables are summed over one at a time—i.e., the results for all conditions of one variable are summed at each combination of the remaining two—and the data are grouped in the form of two-way tables in Tables IV, V, and VI.

Without any further statistical analysis, the following conclusions can be reached from the results:

Absorbance of Blank. Increasing acidity causes the absorbance of the blank to diminish, while increasing molybdenum and vanadium concentration cause the absorbance to increase.

Absorbance of Sample. Again, increasing acid concentration causes a decrease in absorbance, while increasing vanadium and molybdenum concentration causes an increase in absorbance.

Absorbance of Sample Minus Blank. When the absorbance is measured versus a reagent blank, maximum absorbance is obtained in 0.4*M* acid, and increasing the vanadium concentration at least up to 2.0m*M* causes an increase in absorbance. However, over the range of 0.02 to 0.06*M* molybdenum there is no difference in the average absorbance of the samples even though the acid and vanadium concentrations were varied over the entire range chosen for the factorial experiment. Obviously the optimum molybdenum concentration lies anywhere in the range of 0.02 to 0.06*M*. The optimum acid concentration is 0.4*M*, but the optimum vanadium concentration was not explicitly revealed in the experiment. In order to determine the optimum concentration of vanadium a series of solutions containing 20 p.p.m. of of phosphorus pentoxide, a final concentration of 0.4*M* acid, 0.04*M* molybdenum, and 0.4 to 10m*M* vanadium was prepared. The absorbance of these solutions was determined against an appropriate blank at 400 mμ (0.055-mm. slit). A region of constant absorbance was observed over the range of 1.0 to 4.0m*M* vanadium. As a result of these studies it was decided to use final concentrations of 0.4*M* perchloric acid, 0.04*M* molybdenum, and 2.0m*M* vanadium in the recommended procedure.

Order of Addition of Reagents. There seems to be no doubt that if reagents are added separately, the order of addition should be acid, vanadate, and molybdate as recommended by Kitson and Mellon (*10*). Any variation in this order will cause the formation of other complexes or precipitates which are only slowly converted to the molybdovanadophosphoric acid.

Color Stability. Using the recommended procedure, the maximum color intensity was developed at once, decreased about 1.3% in 15 minutes, and then remained constant for at least 2 hours.

Table V. Two-Way Tables for Absorbance of Samples

		Acid Molarity			
	Mo, *M*	0.2	0.4	0.6	0.8
Av. of 6 analyses at 3 vanadium levels	0.01	0.8048	0.7867	0.7140	0.5178
	0.02	0.8732	0.8130	0.7928	0.7705
	0.04	0.8783	0.8638	0.8195	0.8135
	0.06	0.8873	0.8810	0.8405	0.8190
	Av.	0.8609	0.8361	0.7917	0.7302

		Vanadium Concn., m*M*		
	$HClO_4$, *M*	0.4	1.0	2.0
Av. of 8 analyses at 4 molybdenum levels	0.2	0.8263	0.8518	0.9046
	0.4	0.8036	0.8317	0.8730
	0.6	0.7451	0.7872	0.8427
	0.8	0.6750	0.7423	0.7732
	Av.	0.7625	0.8032	0.8483

		Molybdenum Concn., *M*			
	V, m*M*	0.01	0.02	0.04	0.06
Av. of 8 analyses at 4 acid levels	0.4	0.6413	0.7790	0.8075	0.8221
	1.0	0.7198	0.8093	0.8353	0.8480
	2.0	0.7563	0.8488	0.8886	0.8998
	Av.	0.7058	0.8124	0.8438	0.8566

Table VI. Two-Way Tables for Absorbance of Samples Minus Blanks

		Acid Molarity			
	Mo, *M*	0.2	0.4	0.6	0.8
Av. of 6 analyses at 3 vanadium levels	0.01	0.7235	0.7581	0.6738	0.4916
	0.02	0.7662	0.7643	0.7541	0.7355
	0.04	0.7555	0.7573	0.7506	0.7563
	0.06	0.7513	0.7678	0.7516	0.7498
	Av.	0.7491	0.7619	0.7325	0.6833

		Vanadium Concn., *mM*		
	$HClO_4$, *M*	0.4	1.0	2.0
Av. of 8 analyses at 4 molybdenum levels	0.2	0.7475	0.7431	0.7567
	0.4	0.7537	0.7637	0.7682
	0.6	0.6996	0.7391	0.7590
	0.8	0.6476	0.6978	0.7045
	Av.	0.7121	0.7359	0.7471

		Molybdenum Concn., *M*			
	V, m*M*	0.01	0.02	0.04	0.06
Av. of 8 analyses at 4 acid levels	0.4	0.6078	0.7437	0.7480	0.7490
	1.0	0.6787	0.7582	0.7540	0.7528
	2.0	0.6988	0.7631	0.7628	0.7636
	Av.	0.6618	0.7550	0.7549	0.7551

Temperature Dependence of Color. Several investigators who applied the molybdovanadophosphoric acid method to steel analysis (*5*, *8*) found the color intensity to be temperature dependent. However, Hill (*9*) demonstrated that the change in color with change in temperature is actually due to the background absorbance of iron in solution, and the complex itself is temperature stable at least at normal room temperature.

Optimum Wave Length. According to Barton (*2*) the spectra of the complex acid and reagent blank both show increasing absorbance as the wave length is decreased toward the ultraviolet. The largest spread between the two spectra is at 400 mμ. However, in steel analysis a longer wave length was usually

chosen to diminish interference from iron. The authors' observations on the spectra confirmed those of Barton, so that all absorbance measurements were made at 400 mμ in this study.

Calibration Data. Using the recommended procedure, duplicate solutions of known concentration in the range 2.5 to 50 p.p.m. of phosphorus pentoxide were prepared. Beer's law was obeyed over the entire range. The average absorbance index (per parts per million) was 0.0372 and the coefficient of variation was ±0.9%. The range of most accurate spectrophotometric measurements was 3 to 20 p.p.m. of phosphorus pentoxide.

Study of Interferences. The possible interference of 60 different ions was evaluated by Kitson and Mellon (*10*), and several other authors have examined the interferences. In general, it may be said that reducing agents should be avoided, because they may reduce the complex to molybdenum blue. Ions which complex molybdenum, such as oxalate, tartrate, and citrate, tend to bleach the color. These interferences are all removed in the recommended sample preparation. Kitson and Mellon (*10*) claimed that chloride and fluoride also bleach the color, but Gee and Dietz (*6*) and McCutchen, Robinson, and House (*12*) reported that chloride and fluoride do not interfere. Tests in this laboratory indicate that chloride does not interfere up to concentrations of 500 p.p.m. but that fluoride does interfere, causing erratic readings at concentrations of 10 p.p.m. or more. Therefore, any fluoride used in the decomposition of the sample should be removed by evaporation and fuming with perchloric acid.

Table VII. Removal of Dichromate Interference

Taken, Mg.		P_2O_5 Found, Mg.	Treatments Necessary
P_2O_5	$Cr_2O_7^{--}$		
1.00	1.0	1.00	1
1.00	5.0	1.00	1
1.00	10	1.01	2
1.00	25	1.03	3

The interference of dichromate is well known (*10*) and results from the fact that the color of this ion resembles very closely that of the molybdovanadophosphoric acid. This interference was found to be directly proportional to dichromate concentration with each part per million of dichromate equivalent to 0.14 p.p.m. of phosphorus pentoxide. Since the interference of dichromate is so serious, a study was made to devise a means of separating dichromate and phosphate. The simplest procedure seemed to be a volatilization of the undesired chromium as chromyl chloride from hot perchloric acid. In order to determine whether this proposed method is applicable, mixtures of 1.00 mg. of phosphorus pentoxide and various amounts of dichromate were prepared and treated according to the recommended procedure. However, when the samples came to dense perchloric acid fumes, about 1 gram of sodium chloride crystals was added to the fuming acid. Red fumes of chromyl chloride were observed. The sides of the beakers containing the mixtures were rinsed down, the solutions were evaporated to fumes of perchloric acid again, and the treatment with sodium chloride was repeated. This procedure was continued until no dichromate color was visible in the solutions and no chromyl chloride color was visible in the fumes. Then the color-developing agents were added according to the recommended procedure, and the solutions were spectrophotometrically analyzed for phosphorus pentoxide. The results of this experiment, shown in Table VII, indicate satisfactory removal of dichromate.

Accuracy and Precision. Some indication of the precision of the method was given in the low coefficient of variation for the average absorbance index obtained in the calibration data. In order to test the accuracy of any method it is usually necessary to analyze samples of known composition. No standard samples approximating the materials analyzed in this laboratory, were available. Therefore, the method was tested by two other approved analytical techniques. The first technique was to analyze leach liquors from typical ores treated in this laboratory, and then add known concentrations of phosphorus pentoxide to the leach liquors to determine whether an accurate analysis of the amount added could be obtained. This procedure afforded a test of precision and accuracy, as without reproducibility the amount of phosphorus pentoxide added could not be determined accurately. The results of these analyses are summarized in Table VIII. The added amount of phosphorus pentoxide was determined with an accuracy within 1 to 2%, which is acceptable for a spectrophotometric procedure. The second test of the method was a comparison of the proposed spectrophotometric method with the "alkalimetric volumetric" method for phosphorus. Although a comparison with a gravimetric procedure might have been more significant, the volumetric method was being used in the laboratory at the time. A series of leach liquors was analyzed by both procedures and the results are compared in Table IX. The average deviation between the two methods was 2.8%.

Table VIII. Test of Precision and Accuracy

Sample	P_2O_5 Concn., Mg. per Ml. Duplicates		Av.	P_2O_5 Added, Mg.	P_2O_5 Recovered, Mg. Duplicates	Av.	% Error
1	0.187,	0.192	0.190	1.00	1.01, 1.01	1.01	+1.0
2	14.5,	14.5	14.5	1.00	1.02, 1.00	1.01	+1.0
3	15.1,	15.4	15.2	1.00	1.01, 1.01	1.01	+1.0
4	25.8,	25.6	25.7	1.00	1.04, 0.99	1.02	+2.0

Table IX. Comparison of Volumetric and Spectrophotometric Methods

Sample	Grams P_2O_5 per Liter Volumetric	Spectrophotometric	% Deviation[a]
SER 106	2.07	2.00	−3.4
SER 103	1.78	1.71	−3.9
JBB 651	0.602	0.593	−1.4
JBB 615	0.479	0.458	−4.4
JBL 801	0.425	0.416	−2.1
JBL 807	0.806	0.799	−0.9
JBB 704	10.9	10.5	−3.6
JBB 636	0.722	0.751	+4.0
NBS 120[b]	35.3% P_2O_5[c]	34.7% P_2O_5	−1.7

[a] Volumetric analysis taken as standard.
[b] National Bureau of Standards phosphate rock.
[c] Certified value.

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RECEIVED for review March 11, 1955. Accepted June 8, 1955. The Raw Materials Development Laboratory is operated by the National Lead Co., Inc., for the Atomic Energy Commission. Work carried out under Contract No. AT(49-6)-924.

Rapid Colorimetric Determination of Benzidine

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A rapid colorimetric procedure for the determination of benzidine by oxidation with permanganate in the presence of nitric acid is presented. The reproducibility of results is unaffected by small amounts of hydrochloric acid; however, the quantity of permanganate solution required is critical. The average deviation between this method and a gravimetric procedure is ±0.19 gram per liter for solutions containing 19 to 25 grams per liter of benzidine dihydrochloride. Results may be duplicated on the same sample with a precision average of ±0.13 gram per liter.

A RAPID procedure for the quantitative determination of benzidine in the presence of excess hydrochloric acid was required for process control in the course of the development of a new electrochemical recorder paper (*9*). Gravimetric (*5, 6, 16*), volumetric (*13, 14, 17*), colorimetric (*4, 8*), and electrometric (*1, 11*) methods were too lengthy for this process or required the absence of excess halogen (*7*).

The oxidation of benzidine in acid medium produces a greenish yellow color (*2, 12*), the intensity of which conforms with Beer's law on dilution. The new method is a modification of the photometric procedure for the determination of permanganate using benzidine (*15*), and is based upon the light absorption characteristics of an aliquot sample of the benzidine solution which is oxidized with potassium permanganate in the presence of nitric acid.

APPARATUS

Photelometer, Cenco-Sheard-Sanford, equipped with a 410- to 490-mμ filter and two 1-cm. absorption cells.

Volumetric flask, 1 liter.

Two pipets, 1.0 ml., Mohr type, graduated in 0.1-ml. units.

Calibrated medicine dropper.

REAGENTS

Benzidine dihydrochloride, C.P., obtained from Matheson, Coleman and Bell, Inc.

Nitric acid, specific gravity 1.42.

Hydrochloric acid, specific gravity 1.18.

Potassium permanganate solution, 0.400%, prepared according to Fales and Kenny (*3*), except that a sintered-glass filter crucible was used in place of a Gooch asbestos mat.

PROCEDURE

A sample of benzidine solution containing about 4.6 mg. of benzidine dihydrochloride (0.20 ml. for a 2.3% solution), and containing 3 ml. of hydrochloric acid per liter of solution, is pipetted into the 1-liter volumetric flask which contains 900 to 950 ml. of distilled water. Four drops (0.11 to 0.16 ml.) of nitric acid are added from a calibrated medicine dropper and the sides of the flask are washed down with distilled water. The flask is then swirled to mix the acid and the benzidine solution.

By means of the second pipet, 1.00 ml. of 0.400% potassium permanganate solution is added to the flask, the sides are again washed down, and the flask is swirled. The flask is then filled to the mark with distilled water and the contents are mixed well. A 1-cm. absorption cell is filled with the greenish yellow solution and placed in the photelometer. Exactly 8 minutes after the addition of the permanganate solution the per cent light transmittance of the colored solution is read against the light transmittance of distilled water. The concentration of benzidine dihydrochloride in grams per liter is read directly from a calibration curve (Figure 1) prepared from a series of solutions of benzidine dihydrochloride of known concentration. The time required for a single analysis is about 10 minutes.

The absorption cell containing the benzidine solution must be cleaned frequently with cleaning solution. Otherwise a film, which is not removed by rinsing with water, eventually forms on the glass and significantly changes the light transmittance.

PREPARATION OF CALIBRATION CURVE

Solutions were made up containing 19, 21, 23, and 25 grams of benzidine dihydrochloride, and 3 ml. of concentrated hydrochloric acid in 1 liter of water. These four solutions were analyzed gravimetrically in triplicate by modification of the method of Marsden and Pollard (*10*) as follows:

A 20-ml. aliquot of the benzidine solution was pipetted into a 50-ml. beaker containing 5 ml. of sodium sulfate solution (6.4 grams per 100 ml. of distilled water). The mixture was stirred, allowed to stand 15 to 30 minutes in an ice-water bath, then filtered through a fine porosity sintered-glass filter crucible which had been previously brought to constant weight by washing with cold 80% ethyl alcohol and drying at 75° C. The white precipitate of benzidine sulfate was washed free of salts with cold 80% ethyl alcohol, and the crucible was dried for 1 hour at 75° C., cooled for 0.5 hour in a desiccator, and weighed. The washing, drying, and cooling were repeated until the crucible reached constant weight. From the weight of benzidine sulfate found in the aliquot, the concentration of benzidine dihydrochloride in the original solution was calculated.

The four standardized solutions were then analyzed for benzidine by the colorimetric procedure given above. The per cent light transmittance was plotted on semilogarithm graph paper against the grams per liter of benzidine dihydrochloride in the solution from which the 0.20-ml. aliquot was taken (Figure 1).

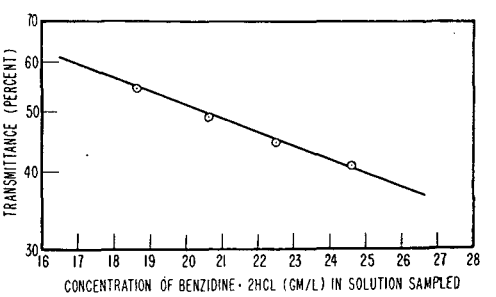

Figure 1. Calibration curve

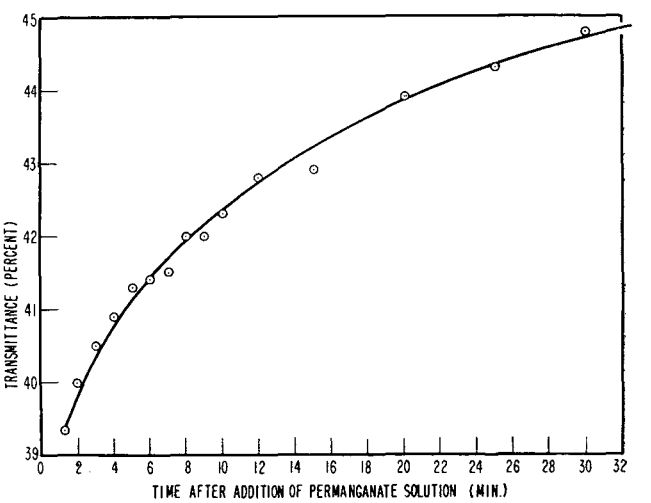

Figure 2. Stability of colored solution

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This serves as the working curve. The authors did not find it necessary to construct a new calibration curve each time a new permanganate solution was prepared.

DISCUSSION

The effects of several variables were investigated for their influence upon the color formation and quantitative application of the procedure. These factors included the stability of the colored solution, concentration of nitric and hydrochloric acids, and the concentration of the permanganate solution.

Stability of Colored Solution. The per cent transmittance of the greenish yellow solution varies with time, as shown in Figure 2. For this reason it is essential that all photelometer readings be made at exactly the same time after addition of the permanganate solution.

Consideration of the curve indicates that the time of photelometer reading may be arbitraily chosen. The authors considered 8 minutes as the minimum period of time required for the performance of the necessary manipulations.

Table I. Effect of Nitric Acid

Expt. No.	HNO_3 Added, Ml.	Transmittance, %
1	0.03	43.6
2	0.05	43.9
3	0.08	44.8
4	0.11	45.7
5	0.13	45.7
6	0.16	45.7
7	0.18	46.3
8	0.21	46.8
9	0.26	46.8
10	0.40	47.0

Acid Concentration. Hydrochloric acid, in concentrations of 0.1 to 0.6%, has no appreciable effect upon the per cent transmittance of the colored solution. The optimum concentration of added nitric acid is 0.11 to 0.16 ml. per liter; the per cent transmittance is constant over this range as shown in Table I.

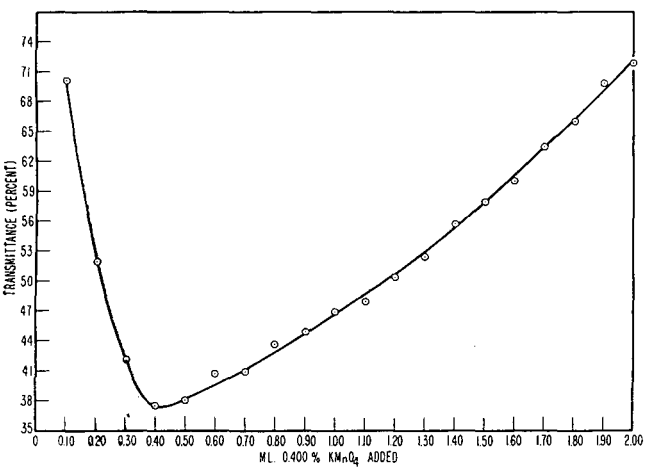

Figure 3. Effect of concentration of permanganate

Concentration of Permanganate. The variation of per cent transmittance of a solution containing 22.8 grams per liter of benzidine dihydrochloride with increasing amounts of added 0.400% permanganate solution is shown in Figure 3. It can be seen that the validity of the method is dependent upon the accurate addition of a critical amount of permanganate solution.

Comparison of Results. Comparative data obtained from the colorimetric method and the modified procedure of Marsden and Pollard (*10*) are presented in Table II. The greatest difference between procedures was 0.5 gram per liter of benzidine dihydrochloride, and the average difference was ±0.19 gram per liter. These analyses were performed at intervals over an extended period of time, during which the temperature varied between 20° and 35° C., indicating that normal variations in room temperature have no apparent effect upon the reproducibility of results.

The precision of the method is shown in Table III. The average deviation from the mean values ranged from ±0.07 to ±0.23 gram per liter. Results may be duplicated on the same sample with a precision average of ±0.13 gram per liter.

Table II. Comparison of Colorimetric and Gravimetric Methods

	Benzidine·2HCl, G./L.		
Expt. No.	Grav.	Color.	Difference, G./L.
1	22.4	22.9	+0.5
2	13.1	13.1	0.0
3	20.3	20.5	+0.2
4	22.4	22.0	−0.4
5	21.5	21.7	+0.2
6	21.9	22.0	+0.1
7	22.0	22.2	+0.2
8	22.3	22.2	−0.1
9	21.9	21.9	0.0
10	20.9	21.1	+0.2
11	19.9	19.8	−0.1
12	22.9	22.9	0.0
13	22.1	22.5	+0.4
14	22.8	22.5	−0.3
Av. difference			±0.19

Table III. Precision of Method

Sample No.	No. of Detns.	Av. Benzidine Dihydrochloride Found, G./L.	Range, G./L. Min.	Range, G./L. Max.	Av. Deviation from Mean, G./L.
1	5	22.4	22.3	22.6	±0.12
2	3	19.5	19.1	19.7	±0.23
3	3	19.7	19.6	19.9	±0.10
4	3	20.9	20.6	21.2	±0.23
5	3	21.8	21.6	21.9	±0.10
6	3	23.2	22.9	23.5	±0.23
7	4	24.0	24.0	24.0	0.00
8	3	24.9	24.8	25.0	±0.07
9	3	25.5	25.4	25.7	±0.10
Total av. deviation, g./l.					±0.13

ACKNOWLEDGMENT

The authors are indebted to Curtis R. Singleterry for many helpful comments and suggestions.

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RECEIVED for review October 5, 1954. Accepted May 5, 1955.

Vacuum Fusion Analysis of Titanium, Zirconium, and Molybdenum

Determination of Oxygen by the Iron Bath Technique

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In the vacuum fusion method, the oxygen in a metal sample is converted to carbon monoxide and measured. In one of the two main modifications of the method, a molten iron bath in a graphite crucible is the reaction medium. Satisfactory results are obtained only when the sample dissolves in a fluid iron bath. A major source of error in vacuum fusion analysis is solidification of the iron bath due to excessive carbon content. At 1800° to 1900° C., the iron bath may solidify in an hour or two. Solidification can be circumvented by adding fresh iron to the bath at the same time samples are added, in effect supplying each sample with its own bath. Results are corrected for the known oxygen content of the iron added to the bath. Up to four samples are analyzed per crucible.

VACUUM fusion is an analytical method for the total oxygen content of metals. The oxygen in a metal sample is made to react with carbon to form carbon monoxide, which is collected and measured. Two main modifications of the method are the "iron bath" technique (*1, 9, 11*) and the "Walter" or "dry" technique (*13*).

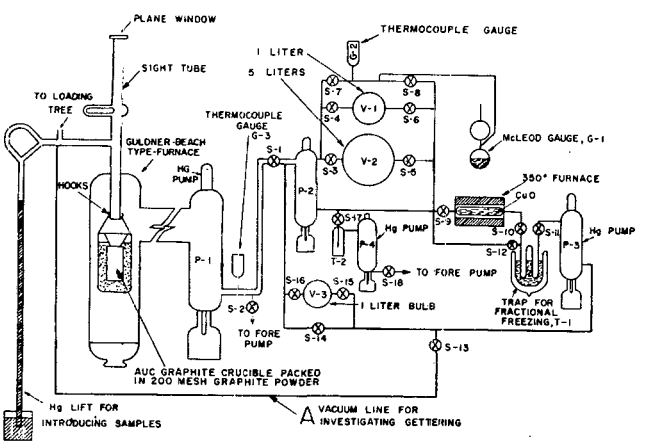

Figure 1. Schematic diagram of vacuum fusion apparatus

In the iron bath technique, a molten iron bath in a graphite crucible is the reaction medium. The bath dissolves even the most refractory metals and promotes contact between carbon and those metals which do not dissolve carbon readily. While this appears to be a general method for all types of metals, the dry method is ordinarily preferred for the analysis of titanium and zirconium. Poor recovery of oxygen by the iron bath technique led Walter to invent the dry technique (*13*). On the other hand, Derge (*1*), Sloman (*9*), and Stanley (*11*) have reported satisfactory results for titanium and zirconium by the iron bath technique.

The need of this laboratory has been a method for all types of metals. While the iron bath technique appears to meet this requirement, there is the question of why this method has given incomplete recovery of oxygen in zirconium and titanium for some workers. Preliminary experiments on the determination of oxygen in zirconium by the iron bath method gave results a factor of 10 lower than the known oxygen content of prepared samples. Therefore, a study has been made of the factors responsible for low recovery in the iron bath method when applied to zirconium and titanium.

Solidification of the iron bath due to high carbon content has been shown to be a major factor preventing quantitative recovery of oxygen by this method. Solidification is aggravated by the high operating temperature required for refractory metals. The oxides present in a sample cannot be reduced unless they come in contact with the bath. Thus, when a sample does not dissolve in the bath, a low value for its oxygen content is obtained. The more thoroughly the bath is outgassed, the more trouble is likely to be experienced with solidification.

A modification of the iron bath technique which ensures that the sample dissolves in the bath has been developed. The method has proved satisfactory for the determination of oxygen in titanium, zirconium, and molybdenum.

APPARATUS

A schematic diagram of the apparatus is shown in Figure 1. The general features of vacuum fusion apparatus are discussed in a review article by Yeaton (*14*).

Furnace. The air-cooled, quartz furnace is similar to that described by Guldner and Beach (*2*).

Special precautions to avoid packing are required in the introduction of the 200-mesh graphite insulating powder around the crucible (*7*). No color due to thermal radiation from properly introduced powder is detectable at a crucible temperature of 2400° C.

The crucible is heated by a 10-kw., 500-kc. electronic induction heater. Approximately half the rated power is required to reach 2400° C.

Pumping System. The furnace gases are removed through a pumping line, 2 inches in diameter and 2 feet long, by a single-stage mercury pump. The net pumping speed of pump and vacuum line was determined by letting gas into the furnace at a known rate through line *A* (Figure 1) and measuring the equilibrium pressure. The measured rate is 40 liters per second at a pressure of 4 microns.

A trap between the furnace and *P*-1 (not shown) can be cooled with liquid nitrogen to reduce the mercury pressure in the furnace. Use of this trap is avoided except when necessary to quench a mercury discharge because the mercury vapor normally present in the furnace retards clouding over of the viewing window. [At room temperature, the mean free path of iron atoms in saturated mercury vapor is less than the dimensions of the furnace. Iron vapor from the crucible must diffuse through several mean free paths before reaching the window. Consequently, most of the iron vapor which enters the sight tube is diverted to the walls before reaching the window. When the trap is cooled, the mean free path of iron atoms is greater than the distance from the crucible to the window, and most of the iron atoms which leave the crucible within the angle subtended by the window condense on its inner surface. The transmission of the window has been observed to decrease appreciably in a matter of minutes under these conditions.]

Analytical System. The gas from *P*-1 is collected in the 1- or 5-liter bulbs, *V*-1 and *V*-2, by a mercury diffusion pump similar to that described by Naughton and Uhlig (*6*). Small gas samples are collected in the volume (650 ml.) of the system exclusive of bulbs.

The geometry of the analytical system is such as to maximize the pumping speed for evacuation of *V*-1 and *V*-2 and for circulation of gas through the copper oxide tube. The stopcock, *S*-3, which controls the evacuation of *V*-2, the largest volume in the analytical system, forms the high vacuum inlet to pump, *P*-2.

Eck and Krebs, Types 5044 and 5050, precision-ground, hollow

plug stopcocks are used throughout. All stopcocks have a bore of 6 mm. except *S*-1, which has a bore of 10 mm. The design of these stopcocks is such that the plugs are seated by atmospheric pressure. The constricted portion is short (ca. 2 mm.) so that the stopcocks offer very little resistance to the flow of gas. They are greased with Apiezon grease N, by a method which ensures that no air is trapped by the grease.

The gas is analyzed by oxidizing the carbon monoxide to carbon dioxide in a copper oxide filled trap at 300° C. The condensable gas is collected in liquid nitrogen trap, *T*-1. After oxidation is complete, noncondensable gas is discarded and the liquid nitrogen is replaced by methanol slush (*3*). The gas volatile at the melting point of methanol is considered to be carbon dioxide.

The copper oxide is prepared by oxidizing 0.005-inch copper wire, in place, by heating to 400° C. in a stream of oxygen. Copper oxide prepared in this way offers very little resistance to the flow of gas (*4*). Approximately 95% of the gas is oxidized on the first pass. The residual gas is continuously recycled until the recorder attached to *G*-2 indicates that the pressure has leveled off. Complete oxidation of the gas sample takes no more than 5 minutes, approximately twice the time required to pump the gas through the copper oxide.

The McLeod gage, *G*-1, is used for quantitative pressure measurements. The thermocouple gages, *G*-2 and *G*-3, are used to indicate trends in the pressure.

PROCEDURE

Sample Preparation. Titanium and zirconium samples are cut to weigh approximately 0.25 gram on a water-cooled abrasive wheel. These samples are cleaned of surface oxide by washing in dilute hydrofluoric acid or by abrading with a file. They are then rinsed with ethyl alcohol and dried in a filter flask under nitrogen.

Molybdenum samples are cut to weigh approximately 1 gram on a water-cooled abrasive wheel. These samples are washed in warm C.P. acetone to remove grease, abraded with a file to remove surface oxide, rewashed in warm C.P. acetone, and dried under nitrogen, as specified by the ONR Subcommittee on Analysis of Molybdenum for Small Traces of Gas.

Iron standards are prepared by swaging the 1-inch diameter rods obtained from the National Bureau of Standards (*12*) to $^{6}/_{16}$ inch in diameter, followed by centerless grinding to $^{1}/_{4}$ inch in diameter, according to the method of McGeary, Stanley, and Yensen (*4*). Pieces weighing 1 to 2 grams are cut from these rods on a water-cooled abrasive wheel. Surface oxide is removed immediately before use by washing in an ethyl alcohol–hydrochloric acid solution, followed by rinsing in ethyl alcohol and drying under nitrogen.

Pieces of tin weighing 1 to 2 grams, for addition to the bath to reduce gettering, are cut from C.P. stick tin. Surface oxide is removed by trimming off all of the original surface with wire cutters. The samples are then washed in ethyl alcohol and dried under nitrogen.

Outgassing of Crucible and Preparation of Iron Bath. After the furnace has been pumped to below 1 mm. of pressure, the temperature is slowly raised to 2300° C. and held for about 5 hours, or until the blank at 1800° C. is less than 5 micron-liters per hour. The temperature is lowered to about 1400° C. and 10 grams of iron are added to form a bath. The iron is outgassed at 1800° C. until the blank rate is 20 to 40 micron-liters per hour. Every effort is made to minimize the outgassing time to avoid saturating the bath with carbon. Outgassing of the bath must be completed in less than 1 hour if solidification is to be avoided. Outgassing time for the bath is held to a minimum by spending extra time on the initial outgassing of the crucible and by using a pure, vacuum-melted iron containing less than 0.005% of oxygen and 0.001% of carbon for the bath. A minimum of oxygen, therefore, must be removed to obtain a good blank, and all of the carbon in the final bath must come from the crucible: As the heating time with iron in the crucible is held to a minimum, the iron does not excessively attack the crucibles.

When the iron bath has been outgassed sufficiently, the temperature is lowered to 1200° C. and 1 to 2 grams of tin are added. The temperature is then raised to 1800° C. The system is ready for the analysis of samples within a few minutes.

Blank. The blank is checked before each sample is run. In order to reduce the time at 1800° C., this is done by observing the slope of the record of the reading of thermocouple gage, *G*-2, while stopcock *S*-17 is closed. The slope can be measured with sufficient accuracy about 2 minutes after closing *S*-17, thus making it feasible to check the blank before and after each sample.

The composition of the blank is determined by analyzing the gas collected in 10 minutes. Approximately 90% of the blank is carbon monoxide.

Analysis of Samples. The temperature is lowered to approximately 1600° C. and the sample, together with 1 to 3 grams of NBS No. 4 steel containing 0.002% of oxygen, is added. After the initial burst of gas, the temperature is raised to 1800° C. Lowering of the temperature is essential for titanium to avoid loss of sample by spattering; zirconium and molybdenum can be added at higher temperature without loss, but, for uniformity, the temperature is lowered for all samples.

The NBS No. 4 steel is added to make sure the sample fluxes with iron. Gas is analyzed after collecting for 30 minutes. Then, a sample of NBS No. 4 steel is analyzed to check the recovery of gas. Recovery of less than the nominal amount of gas from the NBS steel indicates the loss of gas by gettering. Recovering of more than the nominal amount indicates incomplete recovery of the gas from the original sample. Accordingly, the excess gas is added to that for the sample, and additional NBS No. 4 standards are analyzed until the excess oxygen is negligible.

Additional tin and iron are added to the crucible between samples in the same way as before the first sample. Up to four samples are analyzed in a single crucible.

DISCUSSION

The quantitative removal of oxygen from a sample consists of the following three steps: intimate mixing of sample with carbon at high temperature, reduction of oxides present in the sample by carbon, and removal of gas evolved by sample from furnace. These three steps are so intimately connected that a completely independent study of each does not appear to be practicable.

Intimate Mixing of Sample with Carbon at High Temperature. In the iron bath technique, the solid sample is dissolved in a molten bath of iron in a graphite crucible. The iron contains carbon from the crucible in solution. Oxygen present in titanium, zirconium, and molybdenum can be determined at lower temperatures when the sample is dissolved in an iron bath than when the sample is in direct contact with carbon (*9*). In order for the sample to dissolve in the bath, it must come into contact with fluid iron when it is dropped into the crucible.

It is not particularly easy to tell whether the bath is fluid before the sample is introduced. The inside of the crucible approximates a black body, making it difficult to distinguish detail at the surface of the melt. However, for an instant after the sample is dropped, some detail can be observed because the sample is at a lower temperature than the contents of the crucible. If the sample is watched closely as it falls, a splash can be seen if the bath is fluid. Boiling of the bath can be observed as gas is evolved. Also, for some time after the addition of tin, boiling can be observed due presumably to the evaporation of tin. If the bath is solid, the outline of the sample can be observed for some time until it reaches bath temperature and blends into the background.

Early results on a sample of zirconium containing 1.0% of oxygen were low by a factor of 10. Examination of the ingots taken from the apparatus showed that the samples had not mixed with the iron bath. In one case, it was possible to pick the intact sample from a hollow it had made in the surface. As the sample had made an impression in the surface, it appeared that the bath could not have been completely rigid. It seemed that the melt was relatively viscous or that a scum had formed on the surface preventing the sample from coming in contact with the liquid iron. The appearance of the ingot suggested the latter.

Since electromagnetic stirring disturbs the surface of the melt, it was thought that such stirring might be effective if the trouble were due to a surface film. Stirring would promote intimate contact of the sample with carbon and, also, the elimination of gas from the melt. Comparison of apparatus and techniques of laboratories which have reported success in the analysis of titanium and zirconium by the iron bath method revealed the use of induction heaters operating at approximately 30 kc. (*1, 9, 11*), almost a factor of 20 lower than that used in this laboratory. Calculation indicated that the current density at the inner surface of a graphite crucible, 5 mm. thick, is 11% of the value at the outer surface at 500 kc., and 58% at 30 kc. It seemed possible

that the field can stir the contents of the crucible at the lower frequency but not at the higher frequency. The calculations were checked by melting tin in a graphite crucible at each frequency. At 30 kc., the field stirred the tin, but at 500 kc., it did not. (No stirring of an iron bath by the field at the operating temperature at either frequency has been observed in this work.)

In an experiment performed to test the effectiveness of the field in stirring the bath, a sample of zirconium which contained 0.036% oxygen was dropped into the iron bath which was being heated at the time by the 500 kc. oscillator. Approximately 0.008% of oxygen was found, the remaining 0.028% oxygen apparently did not react with carbon. The 500 kc. oscillator was then replaced by a portable 30 kc. oscillator in the expectation that the bath would be stirred by the lower frequency current. It was reasoned that if stirring were important, the change, from a power supply which did not stir the bath to one which did, would cause a strong evolution of gas. However, no such strong evolution of gas was observed. It was concluded that the bath had solidified and thus resisted stirring.

This experiment revealed that the bath was in such a state that reduction of the oxides present in the sample could not take place.

In another experiment, 0.04% of oxygen was found in a sample of zirconium known to contain approximately 1.0% of oxygen. At the end of this determination, the apparatus was left overnight with all pumps running so that no gas would come in contact with the crucible or its contents. On the following day, the crucible was reheated to 1800° C. until a good blank was obtained, and a sample of NBS No. 4 steel was added to check the efficiency of recovery. The amount of gas recovered was far in excess of that contained by the steel. It was approximately equal to the total to be expected from the zirconium and the steel.

This result has been interpreted as follows: The zirconium sample dissolved only partly, if at all, in a bath which was already solid or covered with a thin solid layer. Oxide which came in contact with carbon on the surface of the bath reacted to form carbon monoxide equivalent to the original value of 0.04% oxygen. The zirconium sample must have been largely intact when the crucible was outgassed on the second day. The steel standard melted immediately when added, forming a molten pool on top of the original bath, dissolving the zirconium and some of the original bath. This allowed oxide to come in contact with dissolved carbon. The resulting carbon monoxide came from both the NBS No. 4 sample and the zirconium. Further experiments have substantiated this explanation.

Previous workers (*1, 9, 11*) have reported that recovery tends to decrease as the concentration of refractory metal in the bath increases. Since the experiment described above was performed with a fresh bath, high concentration of refractory metal could not have caused the low recovery.

Analysis of ingots has revealed that they contain as much as 10 to 12% of carbon. With the bath in contact with the graphite crucible, the concentration of carbon apparently increases until the melting point of the bath reaches the operating temperature. If the bath is held at a high temperature too long, it solidifies. Iron must be added in order to keep it fluid. Work which indicates that solidification starts at the surface of the bath is described by Smith (*10*).

It is pertinent to ask why some analysts have obtained satisfactory results without making special allowances for solidification. Derge (*1*) and Sloman (*9*) used larger crucibles with correspondingly more iron in the bath. The larger ratio of volume to surface tends to prolong the period during which the bath is fluid. Derge (*1*) recommends the addition of sizable amounts of iron and tin between samples. This procedure is common in the analysis of other nonferrous metals, but usually for the stated purpose of keeping the concentration of the metal being analyzed below a certain critical value (*8*). This remaking of the bath between samples may reduce difficulties due to solidification if the newly added iron is allowed only a short period to outgas. However, if the operator is meticulous about obtaining a good blank after such addition, solidification may occur.

Stanley, von Hoene, and Wiener (*11*) recommend wrapping the sample with iron foil for the purpose of preventing the sample from wetting the crucible wall if the two come into contact as the sample falls into the bath. It is suggested that the use of iron foil is successful partly because of the stated purpose and partly because it assists the sample to dissolve in the bath.

The large amount of carbon found in the iron bath suggests an explanation for the increase in blank when iron is added to an empty crucible. The extra gas probably is liberated from the graphite as it dissolves in the iron. Even after outgassing at 2400° C., appreciable amounts of gaseous elements remain in the graphite. Assuming that all of the oxygen is recovered from the 5 to 10% graphite which dissolves in a 10-gram iron bath in 1 hour, the 20 to 40 micron-liter per hour blank rate at 1800° C. corresponds to 0.001 to 0.004% of oxygen in the graphite.

A crucible which would not dissolve in the bath would be ideal. Known amounts of carbon could be added and the process could be controlled precisely. In the absence of such a crucible, the problem of maintaining a fluid bath has been attacked by minimizing the outgassing time and temperature. Thorough outgassing of the empty crucible and high purity iron for the bath

Table I. Vacuum Fusion Results on Zirconium with 1% Nominal Oxygen

	% Oxygen Found	
Before solidification discovered	0.04 0.09	
Technique modified to circumvent solidification, but final technique not worked out	0.80 0.82 0.7	
Using final procedure	0.95 0.90 0.91	Crucible 1
	0.89	Crucible 2
	0.90 0.94	Crucible 3
	0.87 0.86	Crucible 4
Av. (last 8 results) *s* (standard deviation)	0.90 0.03	

Table II. Results of Vacuum Fusion Analyses of Titanium Cooperative Samples Analyzed for ONR Metallurgical Advisory Committee on Titanium

	% Oxygen				
Sample Designation	WA2	WA9	WA10	WA12	WA12 (2nd sample)
Temperature not lowered before adding samples to bath. Low results due to loss of sample by spattering	0.170(2) 0.299(8)	0.125(4) 0.080(5)[a]	0.134(3) 0.149(7)	0.318(1)[a] 0.147(6)	...
Samples analyzed by final procedure. Only these results included in averages.	0.256(9)[a] 0.256(10) 0.274(12)	0.127(11) 0.118(15) 0.125(19)	0.115(13) 0.123(14)[a] 0.117(18)	0.296(16) 0.320(17) ...	0.312(20)[a] 0.310(21) 0.314(22) 0.313(23)[a] 0.314(24)
Av. 0.262 Calcd. *s*[b]		0.123	0.118	0.308	0.313 0.002

[a] New crucibles used for samples 1, 5, 9, 14, 19, 20, 23. (Numbers in parenthesis after each result indicate order in which samples were analyzed.)
[b] Standard deviations are calculated for no less than five replicates.

aid greatly in minimizing both time and temperature for outgassing. When it is necessary to outgas the bath at 1800° C., the outgassing time is held below 1 hour. It is impracticable to use an iron bath in a graphite crucible at temperatures above 1800° C. because solidification takes place so rapidly. Determinations run at temperatures above 1800° C. are probably, in reality, examples of the dry or Walter technique rather than the iron bath technique.

Reduction of Oxides Present in Sample by Dissolved Carbon. For each metal oxide, there is a minimum temperature below which reduction by carbon is slow, or does not occur at all. For molybdenum, zirconium, and titanium, Sloman has reported these temperatures to be 1500°, 1640°, and 1770° C., respectively (*9*). While it is desirable to operate at the lowest practical temperature for any given sample, all samples containing these metals have, for the sake of uniformity, been run at 1800° C. Pure molybdenum can be analyzed at a much lower temperature than this, but Sloman's figures for the minimum temperature for reduction indicate that the presence of alloying elements, particularly titanium, may cause large errors in determinations run at the minimum temperature for pure molybdenum.

At a given temperature, the rate of reduction of oxide in a sample depends on the availability of carbon to react—i.e., on the thoroughness of mixing of sample with iron—and on the rate at which carbon monoxide escapes from the bath once it is formed. The gas liberated at the surface can be expected to be pumped away rapidly; however, gas liberated below the surface is under the hydrostatic head of the iron above it. This head can be of the order of several millimeters of mercury, depending on how well the sample is mixed with the bath. When the bath is viscous, the effective head may be considerably increased. This is another reason why fluidity of the bath is essential.

Removal of Gas Evolved by Sample from Furnace. An additional factor which can cause low results for titanium and zirconium is a tendency of condensed films of these metals to serve as getters. Previous workers have indicated that the addition of tin to the bath improves recovery of gas. It has been suggested that the tin evaporates from the bath and covers over active films of other metals, and that it aids the evolution of gases from the bath.

In an effort to evaluate the magnitude of the gettering problem, the recovery of carbon monoxide was investigated by letting precisely known amounts into the furnace through line *A* to simulate gas evolved from the crucible. The carbon monoxide was introduced at approximately the same rate as gas is evolved from the crucible during a determination. The carbon monoxide was collected and analyzed as if it had come from a metal sample. This technique allows the recovery to be checked far more accurately than the recovery of gas from a standard can be checked, and allows gettering losses to be separated from losses which occur in the bath.

This experiment has been performed several times after obtaining satisfactory oxygen values for NBS steel samples. On these occasions, the loss of gas in passing through the furnace was less than 1%.

After a zirconium determination which gave a low result, a sample of NBS steel was analyzed and the amount of oxygen found was less than the usual value for this material. At this point, 100 micron-liters of carbon monoxide were introduced into the analytical system and measured. The carbon monoxide was transferred to the bulb, *V*-3. Then, while collecting gas in the same way as if a sample were being analyzed, and with the furnace at operating temperature, this sample of carbon monoxide was allowed to flow from *V*-3 into the furnace through line *A*. Only 85 micron-liters of carbon monoxide were recovered. The remainder must have been absorbed by active surfaces within the furnace. About half a gram of tin was added to the crucible, and again 100 micron-liters of carbon monoxide were passed into the furnace. This time, 100 micron-liters of carbon monoxide were collected. These results support the theory that the tin improves recovery by covering over active surfaces which would otherwise absorb the evolved gases. The possibility that the tin also makes the bath more fluid or that it acts as a carrier for the gases from the sample as it boils out of the bath cannot be excluded. However, the authors have found that it is very difficult to maintain even a moderate concentration of tin in the bath at the operating temperature.

Table III. Results of Vacuum Fusion Analysis of Molybdenum Cooperative Samples Analyzed for ONR Advisory Committee on Molybdenum

Molybdenum Sample	% Oxygen Found
4C	0.0060
	0.0060
	0.0055
12D	0.0064
	0.0061
	0.0067
Av.[a]	0.0061
s[a]	0.0004

[a] On basis of these results, 4C and 12D are considered to be identical.

RESULTS

Typical results obtained by the modified iron bath technique are shown in Tables I, II, and III.

Table I lists results obtained on a sample of zirconium prepared to contain 1.0 ± 0.1% oxygen by arc melting a mixture of zirconium oxide and zirconium metal. The first two results are typical of those obtained before the method was modified to ensure that the bath was fluid. The next three results were obtained after it had been shown that solidification was causing low results. The technique was modified to the extent that additional iron was added to the bath before the sample, in order to fluidize the bath (which was observed to have solidified before the addition of the new iron).

The last eight results were obtained by the fully modified procedure as described earlier. The marked change in the efficiency of recovery of oxygen between the first and last groups of samples is attributed to the improvement in the fluidity of the bath.

Table II lists the vacuum fusion results obtained for a group of titanium samples analyzed as a part of the cooperative program of ONR Metallurgical Advisory Committee on Titanium. NBS cooperative steel samples (*12*) were analyzed after each sample. The correct value was obtained in each case. Loss of sample due to spattering was discovered while the first eight samples were being run. After the eighth sample, the procedure was modified to include the step of lowering the temperature to 1600° C. before adding the sample. All of the results have been included in the table; however, the first eight have been rejected in making up the averages because spattering could have occurred for any of these samples.

Only in the case of WA-12 were enough samples analyzed to allow calculation of a meaningful standard deviation. The results for this sample happen to be more consistent than for the other samples; thus, the calculated standard deviation for this sample indicates as much as five times better reproducibility than usual.

Table III lists the results obtained for a group of molybdenum samples analyzed as part of the cooperative program on the ONR Committee on Molybdenum.

During the course of work on these and other samples, it was found that once the problem of bath solidification had been recognized, and steps had been taken to minimize the outgassing time at 1800° C., recovery of gas improved greatly. After the gas from the sample has been collected, the excess gas which is collected when the first sample of NBS No. 4 steel is analyzed has been negligible in most cases. This suggests that this step could

be omitted. However, the practice of analyzing a sample of NBS No. 4 steel after each sample has been continued because it gives a good indication of the reliability of the results.

ACKNOWLEDGMENT

The assistance given by many members of the Metallurgy Research Department, General Electric Co., is acknowledged. In particular, thanks are due to R. L. Hadley for preparing the "1% oxygen" zirconium and A. U. Seybolt for metallographic examination of the "1% oxygen" zirconium. L. B. Bronk of the Analytical Chemistry Unit performed the carbon analyses.

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RECEIVED for review December 10, 1954. Accepted May 24, 1955. Presented before the Division of Analytical Chemistry at the 126th Meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y.

Vacuum Fusion Analysis by the Iron Bath Technique

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An investigation of the reason for the increased viscosity of the iron bath used in vacuum fusion analysis reveals that the cause is precipitation of graphite as a solid phase. The iron bath becomes semisolid within 2 hours at 1800° C. A mechanism for the precipitation of graphite is proposed.

McDONALD, Fagel, and Balis (*1*) have shown that a major source of error in vacuum fusion analysis by the iron bath technique is the tendency of the bath to become very viscous, or solidify. In an attempt to determine the cause for this behavior the investigation outlined below was undertaken.

To a graphite crucible previously outgassed at 2300° C. were added 20 grams of low-carbon, low-oxygen iron. The temperature was raised to 1800° C. and held for 2 hours, then cooled to room temperature. A second experiment was performed in the same manner, except that a temperature of 2000° C. was used. Both experiments were carried out in vacuum. It required about 7 minutes for the melt to cool to a temperature of 1135° C., where solidification should be complete. Macrographs and micrographs of sections through each ingot are shown in Figures 1 to 5.

Examination of these photographs reveals a high concentration of large graphite flakes near the top of both ingots with a much higher concentration occurring in the 2000° C. melt. The finer graphite flakes are believed to have been formed during cooling of the melt. There is no evidence of any iron carbide in any of the micrographs of either heat. The very heavy concentration of graphite flakes near the top of the melt would cause this region to become very viscous or pasty. Such a condition at the top of the bath would not allow dropped samples to penetrate into the bath. The presence of the entrapped gas bubble in the 2000° C. melt is further evidence of the high viscosity near the top of the bath.

It is possible to account for this particular distribution of graphite as follows: When the iron is heated in contact with graphite, the bath becomes saturated with carbon. Because of thermal gradients in the bath, loss of iron by evaporation, and radiation losses, some free graphite is either precipitated or carried to the top of the bath. Once formed, these particles can continue to grow, as thermal gradients will supply more carbon to the growing graphite flakes.

The importance of not holding the iron bath at high temperatures for long periods of time can thus be understood. As it has been shown by Sloman, Harvey, and Kubuschevski (*3*) and Mallett and Griffith (*2*) that many of the most stable oxides can be reduced at temperatures of 1800° C. and less, there is actually no need to exceed this temperature. Sloman (*3*) has shown by thermodynamic calculations that, with the possible exception of thorium, all vacuum fusion work could be done below 1800° C.

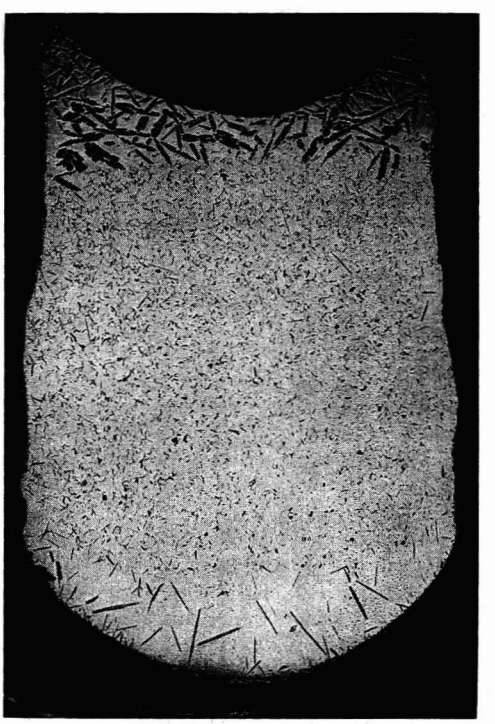

Figure 1. Macrograph of section through iron ingot melted in graphite crucible

Held 2 hours at 1800° C., ×4

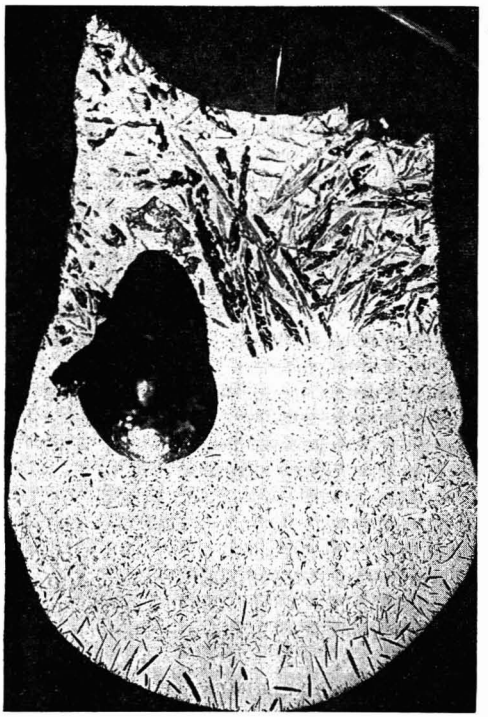

Figure 2. Macrograph of section through iron ingot melted in graphite crucible

Held 2 hours at 2000° C., ×4

Figure 3. Micrograph of top section of iron ingot held in a graphite crucible

Two hours at 2000° C. showing large graphite flakes, ×25

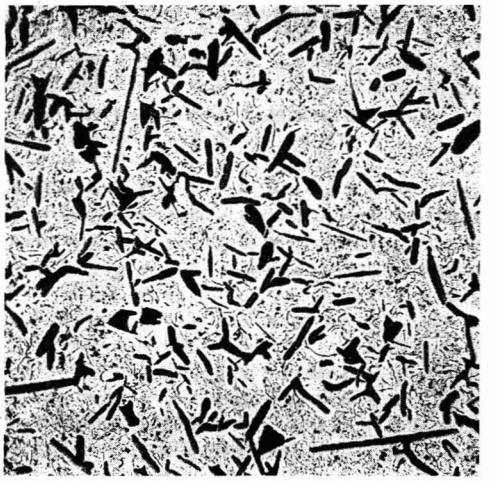

Figure 4. Micrograph of center section of iron ingot held in a graphite crucible

Two hours at 2000° C. showing fine graphite, ×25

Figure 5. Micrograph of bottom section of iron ingot held in graphite crucible

Two hours at 2000° C., ×30

Outgassing of the initial iron bath can easily be done in a short time at 1800° C., especially if iron of low oxygen content is used as a charge.

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RECEIVED for review December 10, 1954. Accepted May 24, 1955.

Potentiometric Method for Karl Fischer Titrations

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Two platinum electrodes, one bright and one platinized, permit potentiometric titrations using the Karl Fischer-Johansson procedure with an accuracy equal to the dead-stop technique.

TO MINIMIZE the effects of the hygroscopicity of the Karl Fischer reagent (*3*), Johansson (*6*) suggested the use of two solutions essentially forming the reagent *in situ* (*8*). The sulfur dioxide–pyridine-methanol component serves as sample solvent and a relatively stable, nonhygroscopic iodine solution as titrant.

The methods that have been used for end-point detection in this titration have been described by Mitchell and Smith (*7*). For colored solutions visual indication is usually impractical and electrical methods are usually employed. The most common of these include the dead-stop technique with polarized platinum electrodes as described by Faulk and Bawden (*2*) or utilizing self-polarizing electrode systems such as that of platinum-tungsten as discussed by Almy, Griffin, and Wilcox (*1*) and Willard and Fenwick (*9*). Heinemans (*5*) has used a platinum-glass electrode system for both direct and indirect titrations.

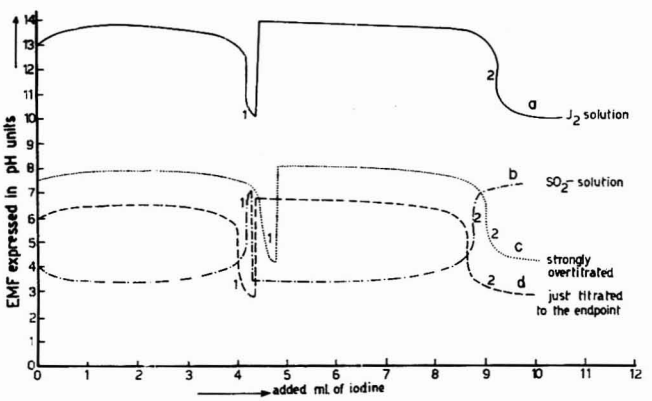

Figure 1. E.m.f. change during titration using different solutions in reference electrode

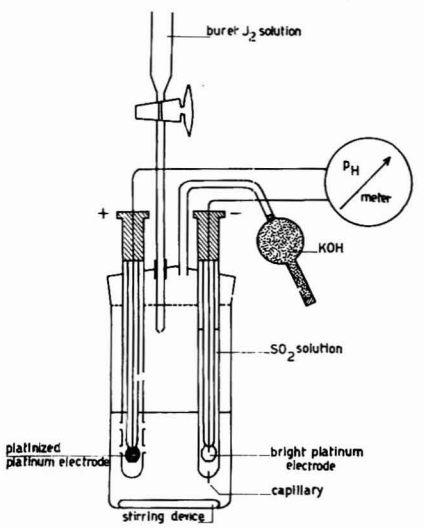

Figure 2. Design of titration cell and electrodes used

In the authors' laboratory it has been found convenient to use two platinum electrodes, with a laboratory pH meter, for titrations involving the Johansson technique. The bright platinum electrode to serve as reference is placed in a tube containing the sulfur dioxide solution. Electrical contact with the solution to be titrated is made through a thin asbestos fiber sealed through the tube wall. The platinized platinum indicator electrode is placed in a second glass tube, perforated at the bottom, to minimize stirring effects. At the equivalent point an e.m.f. change of about 150 mv. occurs. This is comparable to the e.m.f. change occurring in titrating sodium bisulfite with 0.1*N* iodine in water.

Other reference electrode solutions may be used. Figure 1 illustrates titrations with (*a*) iodine-methanol; (*b*) sulfur dioxide–pyridine-methanol; (*c*) solution (*b*) just titrated to an end point with iodine; and (*d*) solution (*b*) with a large excess of iodine. Solution (*b*) was found most convenient to use, as it is one of the components of the Johansson technique. Initial voltages with this reference solution are relatively constant.

EXPERIMENTAL

Final design of the titration cell and electrodes used is shown in Figure 2 and the completely assembled apparatus is pictured in Figure 3. The direct titration technique was used (no back titration). The following reagents were used. Solution A: dissolve 100 grams of dry sulfur dioxide in a mixture of 500 ml.

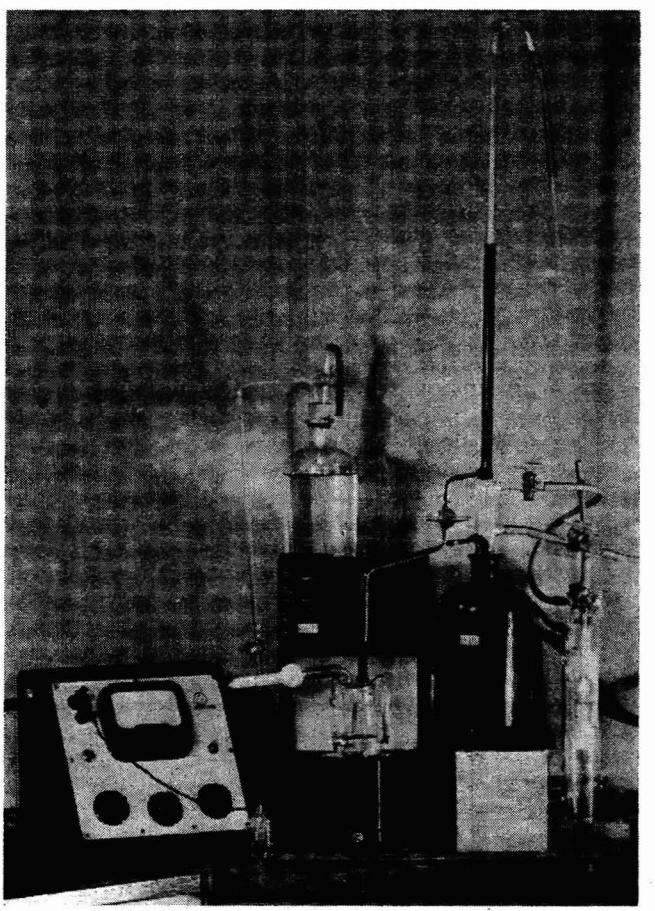

Figure 3. Completely assembled apparatus

Table I. Titration of Water

H_2O in Sample Taken, Mg.	Water Found, Mg.	Difference	
		Mg.	%
29.8	29.7	−0.1	−0.34
32.2	32.4	+0.2	+0.62
33.1	32.9	−0.2	−0.60
38.6	38.5	−0.1	−0.26
39.7	39.8	+0.1	+0.25
40.1	40.0	−0.1	−0.25
41.0	41.2	+0.2	+0.49
42.6	42.5	−0.1	−0.24
43.5	43.4	−0.1	−0.23
46.4	46.2	−0.2	−0.43
50.2	50.2	0.0	0.00
51.5	51.9	+0.4	+0.78
98.6	98.1	−0.5	−0.51

of anhydrous methanol and 500 ml. of anhydrous pyridine. Solution B: dissolve 50 grams of resublimed iodine in 1 liter of anhydrous methanol.

Procedure. Place 30 to 50 ml. of Solution A in the titration vessel, start the stirrer, and rapidly titrate with Solution B until a sharp inflection is noted on the vacuum tube voltmeter. Continue the titration dropwise until the increased voltage remains constant for at least 1 minute. Add the sample to be tested and repeat the titration with Solution B, recording the volume used. This is equivalent to the moisture in the sample taken. Solution B is best standardized by titrating a known weight of a stable hydrate (*4*) and should be checked daily.

RESULTS

The precision attainable is evident from Table I.

ACKNOWLEDGMENT

The authors wish to thank J. R. H. van Nouhuys, director of the Fibre Research Institute, T.N.O., for permission to publish the results of this investigation, and are indebted to Nicolaas de Hart of this institute for his assistance with the analytical work.

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RECEIVED for review March 15, 1954. Accepted May 23, 1955.

Turbidimetric Method for the Determination of Yeast Mannan and Glycogen

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The interaction of concanavalin-A, a globulin extracted from jack bean meal, with glycogen and with yeast mannan has been utilized for the determination of these two polysaccharides. It is suggested that the reaction between concanavalin-A and glycogen could be used for the determination of alpha-amylase activity.

DURING a study of the purification of yeast invertase it was desirable to have a method which would permit the estimation of small concentrations of yeast mannan (yeast gum). Although yeast mannan was generally detected by means of its precipitation with alkaline Fehling solution (*7*), this method lacked the sensitivity necessary for the determination of small amounts. Sumner and O'Kane (*9*) had reported that a jack bean globulin, concanavalin-A, was effective in precipitating not only invertase but also yeast mannan. This observation was utilized to develop a simple method for the determination of yeast gum. The method was also found to be suitable for the determination of glycogen.

METHODS

Preparation of Concanavalin-A Solution. A partially purified concanavalin-A solution suitable for determining yeast mannan and glycogen was prepared as follows (*6*, *9*). Twenty grams of jack bean meal (Arlington Chemical Co., Yonkers, N. Y.) was stirred with dilute sodium chloride (2%, 200 ml.) for about 10 minutes and then centrifuged for 10 minutes at 2000 r.p.m. The turbid supernatant solution was treated with 2 ml. of 2*M* acetate, pH 4.2, and after standing for an hour was centrifuged. The solution was warmed to 50° to 55° C. with continual stirring, and after standing at room temperature for 0.5 hour was centrifuged. Generally, the solution was clear at this point, but occasionally it was slightly turbid, in which case 2 or 3 ml. of 0.1% glycogen in water was added, and after standing in the refrigerator overnight the solution was filtered or centrifuged.

The clear solution was treated with 15 ml. of a 7% aqueous solution (15 ml.) of poly(vinyl alcohol) (Elvanol, Grade 71-24, Du Pont Co.) and the reagent kept in the refrigerator until ready for use. The solution (pH 6.0) is stable for several months when refrigerated.

It is possible to re-use the concanavalin-A reagent several times; it should be clarified before being re-used. With some solutions little change in absorbance was noted when they were treated with a standard glycogen solution even after the reagent had been used several times. Generally the reagent develops a slight sediment on standing longer than several days, but this is much less in solutions prepared by acetate and heat treatment than in those prepared under neutral conditions. Lyophilization of the acetate- and heat-treated preparations gives products which are stable at room temperature and which dissolve completely in 1% saline to yield solutions which are stable when refrigerated.

Preparation of Yeast Mannan and Standard Liver Glycogens. The yeast polysaccharide was obtained from baker's yeast autolyzate (*4*) by precipitation with alkaline Benedict solution and subsequent purification according to the method described by Haworth, Hirst, and Isherwood (*5*). The product was dried in vacuo at 56° C. and was slightly green. The color, presumably due to bound copper, could not be removed by dialysis against distilled water or against 0.01*N* acetic acid, but was removed by passage over a cation exchange resin (Amberlite IR-120, Rohm & Haas Co.). The addition of concanavalin-A reagent to a standard weight of the yeast mannan before and after passage through Amberlite IR-120 produced the same absorbance. Hence traces of copper do not influence the analytical results. The specific rotation of the yeast mannan was $[\alpha]_D^{25}$ +88° in water (*c*, 1.0), in agreement with the value reported previously (*5*).

The sample of human liver glycogen used as a standard in this study was purified according to the procedure previously described (*2*), and the rabbit liver glycogen was fractionated with alcohol (*3*), after a preliminary purification, as for the human liver glycogen. Both samples had a specific rotation of $[\alpha]_D^{23}$ +195° in water (*c*, 0.5).

Turbidimetric Procedure. One milliliter of an aqueous solution of yeast mannan containing 10 to 100 γ or of glycogen containing 100 to 1000 γ is mixed at once with 9 ml. of concanavalin-A solution at room temperature. After standing for 10 minutes, the absorbance of the solution is determined in an Evelyn colorimeter using a No. 420 filter. A blank is prepared by using water in place of the polysaccharide solution.

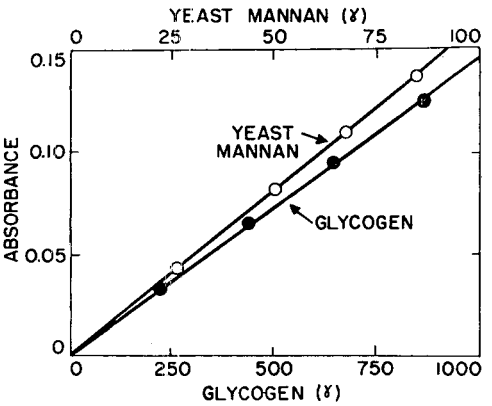

Figure 1. Interaction of concanavalin-A with glycogen and with yeast mannan

A standard curve for comparing the polysaccharide concentration of the unknowns is prepared with standard yeast mannan or with standard normal human or rabbit liver glycogen, using the concentration ranges noted above (see Figure 1).

RESULTS AND DISCUSSION

The preparation of crystalline concanavalin-A was first described by Sumner (*8*). More recently a simplified method has been reported (*6*). While the precipitation of both glycogen and yeast gum was effected (*9*), presumably by the use of crystalline concanavalin-A, in this study it was found that a relatively crude solution of concanavalin-A was suitable for the determination of these polysaccharides. The procedure as finally developed makes use of both heat and acetate for the removal of inert protein.

Heating the crude jack bean meal extract to 70° to 72° C. left much of the concanavalin-A in an "active" form, though the decrease in activity of the concanavalin solution was related to the temperature to which it was heated. The use of acetate buffer of pH 4.1 to 4.5 (the value is not critical) in conjunction with heating produced a solution with greater precipitating power than the use of either acetate or heat alone (see Table I). The use of both heat and acetate apparently removes more inert protein than the use of either condition alone. Thus, protein precipitation occurred when the acetate-treated extracts were heated to 71° C., and also when acetate was added to the heat-treated extracts.

To obtain reaction mixtures which give consistent readings turbidimetrically, it is necessary to operate under conditions which do not cause rapid precipitation. The most convenient range for glycogen was found to be between 0.1 and 1.0 mg. and for yeast mannan, 10 to 100 γ per ml. (The sensitivity of the concanavalin-A reagent toward glycogen and yeast mannan may be increased by the use of a more concentrated concanavalin-A reagent.) Even at these concentrations it is advisable to add a stabilizer such as poly(vinyl alcohol) to prevent precipitation during the test. Concentrations of polysaccharides higher than those indicated should not be used; otherwise precipitation will take place even in the presence of the stabilizer. An excess of polysaccharide acts as a protective colloid (see Figure 2), and at a yeast mannan concentration of 20 mg. per ml., the reaction mixture with concanavalin-A remains clear for approximately 30 minutes. Thereafter, the mixture shows an opalescence which increases slowly, but even after standing overnight the mixture remains only moderately opalescent and shows no suspension or precipitate.

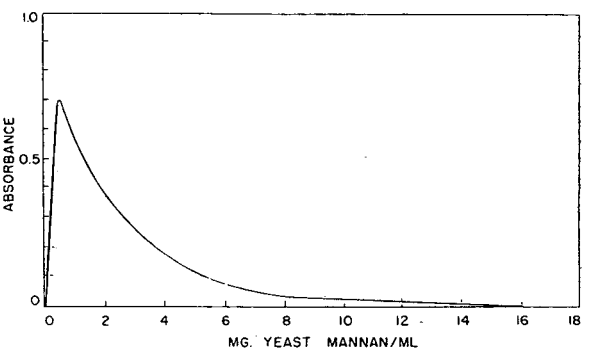

Figure 2. Interaction of yeast mannan with concanavalin-A

Absorbance measured after 10 minutes

Glycogen behaves somewhat differently, for at a concentration of 60 mg. per ml. it gives a precipitate after shaking for a short while with concanavalin-A. On the other hand, the addition of glycogen to 2% yeast mannan does not result in the formation of a precipitate with concanavalin-A. Moreover, dilution of a clear mixture of 2% yeast mannan–concanavalin-A with saline does not promote precipitation whereas the addition of one volume of concanavalin-A solution results in the formation of a turbidity within a minute or two and precipitation within several minutes.

The control of pH is important in the reaction between the two polysaccharides and concanavalin-A. A pH of 5 to 6 is optimal, whereas pH values either lower or higher result in decreasing absorbances of the glycogen–concanavalin-A reaction mixtures. Under alkaline conditions the concanavalin-A reagent produces little or no turbidity with glycogen. Similarly, no turbidity occurs at pH values of 2 to 3 when a concanavalin-A reagent prepared by acetate and heat treatment is used, but when the reagent is prepared under neutral conditions and at room temperature, bringing the pH to 4 to 5 results in the precipitation of much inert protein. Thus, it is best to prepare the reagent by the use of acetate to eliminate the possibility of erroneous results from this source of inert protein precipitation.

It appears that proteins do not influence the concanavalin-polysaccharide reaction. The addition of an equal part or more of blood-serum protein to a solution of glycogen does not interfere with the reaction of the latter with the concanavalin-A reagent.

Table I. Effect of Acetate and Heating on Preparation of Concanavalin-A Extract[a]

Vol. 2*M* Acetate (pH 4.1) Added, Ml.	Temp., ° C.	Soln. after Centrif.[b]	Absorbance after Adding 1 Ml. of 1% Glycogen Soln.
1.0	67	Clear	0.24
1.5	25	Slightly turbid	0.24
1.0	45	Slightly turbid	0.48
0.0	71	Clear	0.17
1.5	45	Opalescent	0.45
1.0	71	Clear	0.19

[a] Volume of extract in each case was 50 ml. and contained 2 ml. of 5% poly(vinyl alcohol). Extracts were prepared by treating Jack Bean meal with 10 parts by weight of 2% saline solution and after mixing for 10 minutes, centrifuging and then treating the centrifugate with 5% poly(vinyl alcohol) solution as indicated above.
[b] 10 minutes at 2500 r.p.m.

Furthermore, it was possible to determine the amount of yeast mannan in an invertase preparation which contained 85% protein with an accuracy equivalent to that involving acid hydrolysis of the mannan followed by determination of the reducing sugar liberated. It is thus possible to determine the concentrations of glycogen or yeast mannan in the presence of protein and consequently the determinations are greatly simplified, since isolation of the polysaccharides is unnecessary.

Determinations of yeast mannan in the presence of glycogen may be accomplished simply by adding diluted saliva to the mixture of polysaccharides. (Such a mixture is obtained when yeast is extracted by dilute alkali.) When the glycogen has been destroyed, the addition of concanavalin-A reagent then measures the yeast mannan which is not affected by the salivary α-amylase. Pretreatment of the glycogen-yeast mannan mixture with salivary α-amylase before addition of the concanavalin-A reagent is not necessary, as α-amylase is active even in the presence of the reagent. The precipitating ability of glycogen is generally destroyed within a few minutes, and only the turbidity caused by the interaction of yeast mannan with concanavalin-A remains.

By use of the above method, it is possible also to determine the amount of glycogen in admixture with yeast mannan. The turbidity produced by the mixture with the concanavalin-A reagent is determined first, and then that produced by the α-amylase treated reaction mixture. The difference between the two results gives the glycogen concentration.

It will be apparent that the concanavalin-A reagent could be utilized for the detection and determination of α-amylase activity.

The reaction of concanavalin-A with glycogen or yeast mannan requires an intact molecule, since when either polysaccharide is oxidized with periodate, the products no longer react with the concanavalin-A reagent (*3*). Reduction of these periodate-treated polysaccharides produces polyalcohols (*1*) which likewise show no reaction with concanavalin-A.

All glycogen specimens tested thus far including those from plant, vertebrate, and invertebrate sources give precipitates while the various starches and mannans, other than yeast mannan, which were examined, failed to give precipitates with the concanavalin-A reagent (*3*).

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RECEIVED for review January 17, 1955. Accepted April 19, 1955. This paper (No. 3296, Scientific Journal Series, Minnesota Agricultural Experiment Station) forms part of a thesis presented by J. A. Cifonelli to the graduate faculty of the University of Minnesota in partial fulfillment for the Ph.D. degree, 1952.

Determination of Iron Pentacarbonyl in Commercial Carbon Monoxide

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In the course of experimental work in which carbon monoxide from steel containers was used, the presence in the gas of a substance which appeared on qualitative test to be iron pentacarbonyl [$Fe(CO)_5$] was noted. Further investigation led to the development of a specific, rapid, and accurate method for its determination, with particular application to its presence as an impurity in commercial carbon monoxide. The gas sample to be analyzed, in volume up to 10 liters, is passed through traps in which the pentacarbonyl is condensed or absorbed, then measured spectrophotometrically at 235 mμ in methanol solution. An average accuracy within 1.3% is obtained in analyses of samples holding a minimum of 0.04 mg. of iron pentacarbonyl. The concentrations present in commercial carbon monoxide in steel cylinders have been found to range from 0.16 to 18 mg. per liter.

THE formation of carbonyl in carbon monoxide and other technical gases held under pressure in metal cylinders, has been investigated by others (*1, 9, 11, 14*). In extensive studies carried out by Pohland and Harlos (*14*), the commercial gases examined were air, carbon dioxide, nitrogen, oxygen, hydrogen, and carbon monoxide. Of these, only cylinders of the latter two were found to contain iron pentacarbonyl (in about 10 out of 50 cylinders). The conditions under which carbon monoxide reacts with iron to form the carbonyl, and the physical and chemical properties of the latter, were apparently first studied by Roscoe and Scudder (*15*) and Mond and Quincke (*13*), and subsequently by many others (*2, 4, 6, 7, 18, 19*). An extensive bibliography comprising over 500 titles and abstracts on the metal carbonyls has been compiled by Croxton (*3*). However, very little of that literature is applicable to the treatment of the present paper based on more recent developments in instrumentation.

Of the relatively few procedures described for the estimation of iron carbonyl compounds (*1, 3, 8–12, 14*), most have been based on the analysis of the iron component. For the determination of the carbonyl in concentrations likely to be found in gas cylinders, a rapid, precise, and specific method of analysis based on the ultraviolet absorption characteristics of iron pentacarbonyl was developed.

METHOD

Apparatus. For determination of iron pentacarbonyl in pure carbon monoxide or in any gas mixture, the apparatus shown in Figure 1 is used. The gas to be analyzed is contained in a sample tube (tonometer), *S*, of known volume (1 or 2 liters). Water or saturated sodium chloride solution is used as displacement fluid. Tube *S* is attached to the 3-way T-bore stopcock, *T*, which provides connection with *N*, a cylinder of water-pumped nitrogen (free of organic matter), and with a trap.

When the sample contains less than 2 mg. of iron pentacarbonyl per liter it is preferable to use a condensation cold trap consisting of the tube 1 immersed in a solid carbon dioxide–Cellosolve mixture, *C*, held in a Dewar cylinder. The tube is a standard taper midget impinger vessel (*5*) of 30-ml. capacity (obtainable from H. S. Martin and Co., Evanston, Ill., M-14050). A narrow band of lubricant (high vacuum grease, Dow Corning) is applied to the upper portion of the ground glass joint. When larger volumes (>2 liters) of sample are taken, to obtain carbonyl in amount sufficient for accurate analysis, the gas sample is passed from its cylinder directly through a glass flow meter (Emil Greiner, Type G914C) connected to *T*. All connections (such as *R*) are made with minimal exposure of the rubber tubing (pure gum, amber) to the gas.

When the sample contains more than 2 mg. of iron pentacarbonyl per liter, the all-glass trap unit of the three collection

tubes 2, 3, and 4 is used (at connection *R*). Each of these tubes, which are like tube 1 except that they are equipped with fritted-glass bubblers, contains approximately 20 ml. of absolute methanol.

Procedure. With the condensation (or less frequently, the methanol) trap in place, the system is purged of air by the passage through it of 200 to 500 cc. of nitrogen from *N*. Stopcock *T* is then turned to admit the flow of sample gas through the trap tube (or tubes), at a rate of flow of 40 cc. per minute. Samples holding less than 2 mg. of iron pentacarbonyl per liter may be passed through the cold (or methanol) trap at a correspondingly faster rate, up to 250 cc. per minute. Finally, 200 cc. of nitrogen are passed to purge the system. Throughout the analysis, sample tube and all other parts of the system are shielded from light. This may be done simply by covering the apparatus with an opaque cloth, which permits observation when necessary.

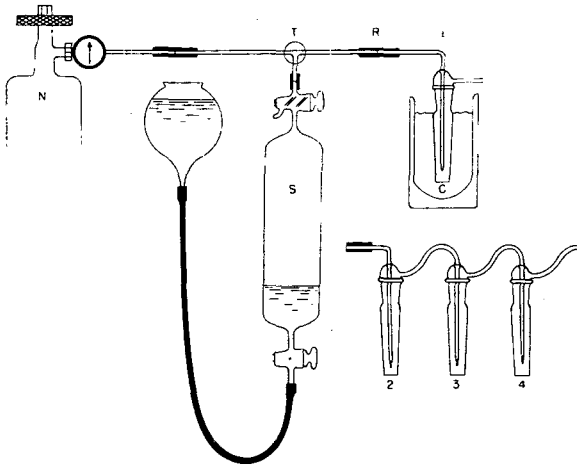

Figure 1. Train for analysis of iron pentacarbonyl by cold trap condensation or methanol absorption

The condensation trap, 1, is removed from the system, together with the connecting rubber tubing, *R*, which is closed by the application of a spring clamp. The tube is warmed almost to room temperature by being held in flowing tap water for half a minute. Tubing *R* is removed, the parts of tube 1 are separated at the ground joint, and all surfaces exposed to contact with the gas sample are washed with absolute methanol. Washings and rinsings are transferred to a 20- or 25-ml. volumetric flask and diluted to volume with the solvent.

When the methanol absorption unit (Figure 1, tubes 2, 3, and 4) is used, the contents of each trap, together with the methanol washings of the tube and the lower extremities of the bubblers, are transferred to a 25-ml. flask. Alternatively, the pooled contents of the three tubes may be transferred to a 100-ml. flask. Further dilution of any aliquot volume (from cold or methanol trap) may be made to obtain carbonyl concentrations within the desirable range for measurement—i.e., 0.2 to 5.0 mg. of iron pentacarbonyl per 100 ml. of solution. When very small amounts of carbonyl are trapped, in order to obtain a concentration of at least 0.1 mg. per 100 ml., dilution may be made to volume directly in the graduated trap tube.

Several precautions must be taken. All solutions are handled with minimal exposure to light. Volumetric flasks or trap tubes, the contents of which are not immediately analyzed, are covered with metal foil. All glassware, trap tubes, volumetric flasks, and especially spectrophotometer cuvettes must be kept scrupulously clean. Evaporation of the solvent may leave iron residues of carbonyl not removable by detergents, but requiring the use of acid—e.g., 10% hydrochloric acid—for their dissolution. As an index of cleanliness, blank analyses performed once daily with nitrogen gas in place of the gas sample, should show no absorbance whatever for the trap methanol compared with the pure solvent.

Spectrophotometric Determination of Iron Pentacarbonyl. Measurements of the absorbance of the trap solutions are made, with the Beckman DU spectrophotometer with hydrogen source, at 235 mμ, with a 1.8-mm. slit width in 10-mm. path covered (or stoppered) cells. The iron pentacarbonyl concentrations are read from the standard curve (Figure 2) obtained as described below.

The calculations are as follows:

Mg. of iron pentacarbonyl in gas sample = $0.01 \times R \times V$ where *R* is the concentration reading in *terms of milligrams* per 100 ml. of solution, and *V* corresponds to the total (diluted) final volume (in milliliters) of the trap contents.

Mg. of iron pentacarbonyl per liter sample = $0.01\ RV/S$ where *S* = volume (in liters) of gas sample analyzed. Example. A gas sample of 0.991 liter (0° C., 760 mm. of mercury) was passed through the cold trap. Analysis of the 25 ml. of trap washing solution, diluted to twice the volume, showed a concentration of 3.17 mg. of iron pentacarbonyl per 100 ml. of solution (absorbance, 1.619). Therefore

Mg. of iron pentacarbonyl per liter of sample =

$$\frac{0.01 \times 3.17 \times 50}{0.991} = 1.60$$

Water vapor in the gas sample will also be trapped in the impinger tubes. Under extreme conditions, this may affect the results. Thus, the moisture from a 10-liter sample of gas saturated with water may be sufficient to dilute the methanol by 1% leading to a reduction in the absorbance by 0.005 unit. Although an effect of this magnitude has not been observed in the present work, its occurrence in the form of a reading for the blank should be taken into account as a correction to the analytical values when these are low (absorbance <0.2).

EXPERIMENTAL

Ultraviolet Spectral Absorption Curves. These were found for the absorbance of iron pentacarbonyl in methanol at three concentrations (1.11, 1.79, and 2.78 mg. per 100 ml.) at 25° C. (Figure 3). They indicate a maximum absorbance at 230 to 240 mμ, the same as that of Sheline's (*17*) curve for a solution of 0.37 mg. per 100 ml. in iso-octane (2,2,4-trimethylpentane).

Standard Concentration Curve. This was obtained from solutions prepared by the delivery of redistilled iron pentacarbonyl into absolute methanol contained in a tared volumetric flask; the latter was weighed after the addition of the carbonyl, then filled to volume. The reverse procedure of delivering the sample into the dry container and weighing, prior to dilution with methanol did not give sufficiently reproducible results in this laboratory. The relationship (milligrams of iron pentacarbonyl

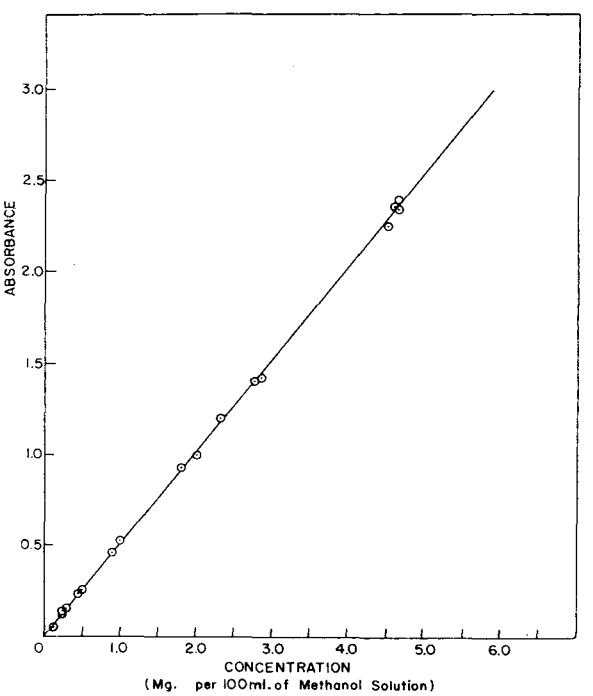

Figure 2. Ultraviolet absorption spectrum of iron pentacarbonyl at 25° C.

***A*, *B*, and *C* represent concentrations of 1.11, 1.79, and 2.78 mg. per 100 ml. in methanol, respectively**

per 100 ml. of solution = absorbance × 1.957) established at 235 mμ and indicated in Figure 2 has been repeatedly confirmed and follows Beer's law within the range of 0.2 to 5.0 mg. per 100 ml. of solution.

In the foregoing experimental work with standard solutions, the results obtained were the same for both untreated and redistilled iron pentacarbonyl. Only slight changes in absorbance were found when solutions of the pentacarbonyl in methanol were exposed to light of normal intensity at room temperature for approximately 24 to 48 hours. Stability of such duration, following which appreciable and irregular changes in absorbance were noted, was in marked contrast to the rapidly deleterious effect of light and air on the undiluted iron pentacarbonyl liquid.

Tests of Accuracy and Precision. In establishing the validity of the method, the use of accurate mixtures of iron pentacarbonyl in carbon monoxide or other gases would have been most desirable. However, difficulty was encountered in preparing such gas samples, presumably owing to the reactivity of the carbonyl with the oxygen of the air and its gradual decomposition in the presence of light. Recourse was had, therefore, to a simpler procedure for obtaining validating data.

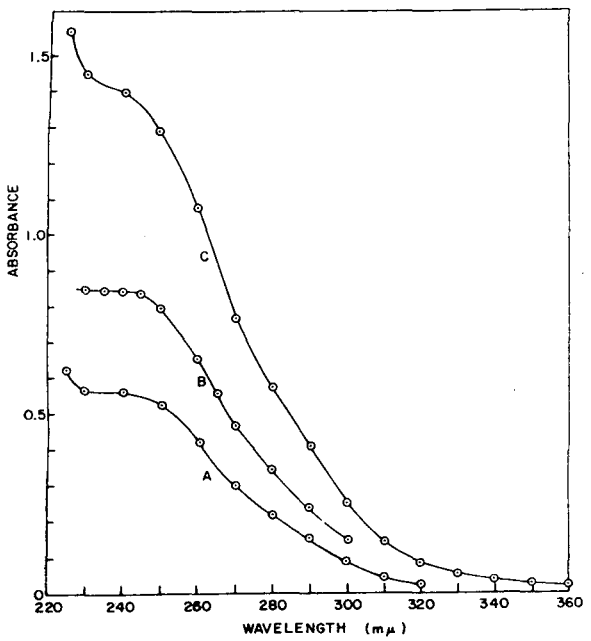

Figure 3. Concentration-absorbance curve for iron pentacarbonyl in methanol at 25° C. measured at 235 mμ

Another impinger unit (tube *X*), exactly like tube 1, was inserted between stopcock *T* and a cold or methanol trap (Figure 1). Nitrogen was then passed through the train as usual. Meanwhile, a small amount of liquid carbonyl (11 to 117 mg.) was introduced into a previously tared, nitrogen-filled, 5-ml. specimen tube (with cover), which was then promptly reweighed. The nitrogen flow through the train was stopped, and the uncovered specimen tube was quickly inserted into the impinger tube, *X*, which was promptly reattached to the train with its bubbler tip in the carbonyl-containing tube. The nitrogen gas flow was resumed, carrying with it the carbonyl as vapor from the vaporization tube to the trap.

Another group of experiments was designed to test the method under conditions of analysis involving smaller quantities (<2 mg.) of iron pentacarbonyl in gas mixture. Cognizance was taken of the relative inaccuracy of weighing smaller samples as contrasted with the accuracy with which such amounts could be handled in the form of progressively diluted methanol solutions, and of the effect of air and/or light on the liquid pentacarbonyl as compared with the relative stability of the compound in methanol solutions.

A carbonyl solution of 100 mg. per 20 ml. in methanol was prepared, from which further dilutions were made. A desired volume, 1 ml. or less, was delivered directly into tube *X*, and a suitable volume of nitrogen was passed over it, somewhat more than sufficient to evaporate the liquid completely for transfer to the trap (cold or methanol) next in line of flow.

In this way, an attempt was made to simulate the operating conditions in the analysis of a gas sample, to the extent that the carbonyl was vaporized, liberated, and absorbed or condensed at a uniform rate over the entire period of passage of gas through the apparatus.

RESULTS

Recovery Experiments. Table I gives data on the recovery of vaporized iron pentacarbonyl from a stream of nitrogen passed over known amounts of the compound in concentrated liquid form or in methanol solution, as described above.

Samples 1 to 10 represent 10 consecutive experimental recoveries by methanol trap absorption of weighed quantities (117 to 11 mg.) of pure iron pentacarbonyl. The average recovery of content was 99.4 ± 0.6%. The accuracy and precision obtained under these conditions were good, inasmuch as varying low values might be expected as a result of decomposition and loss during the weighing process and the subsequent manipulations. Recoveries by cold trap condensation of approximately 10-mg. quantities, under these conditions, whereby 1 to 2 liters of nitrogen were passed through the apparatus in 25 minutes, were somewhat less satisfactory (94%). The cold trap is apparently relatively inadequate

Table I. Recovery of Known Amounts of Iron Pentacarbonyl Vaporized in Nitrogen

Sample No.	N_2 Gas Passage			$Fe(CO)_5$				
	Time, min.	Rate, cc./min.	Volume, cc.	Taken, mg.	Vol. of methanol soln., ml.	Transfer rate, mg./mln.	Found, mg.	Recovery, %
				By Methanol Trap Absorption				
1	25	35	875	117.0	Undil.	4.68	118.0	100.9
2	25	35	875	58.5	Undil.	2.34	57.4	98.1
3	25	35	875	49.1	Undil.	1.96	48.9	99.6
4	25	35	875	43.4	Undil.	1.74	43.3	99.8
5	25	35	875	43.4	Undil.	1.74	43.2	99.5
6	25	35	875	41.4	Undil.	1.66	41.0	99.0
7	25	35	875	34.9	Undil.	1.40	34.7	99.4
8	25	35	875	28.1	Undil.	1.12	28.0	99.6
9	25	35	875	19.2	Undil.	0.77	18.9	98.4
10	25	35	875	11.4	Undil.	0.46	11.4	100.0
Average								99.4
				By Cold Trap Condensation				
11	25	35	875	1.68	0.2	0.067	1.58	94.1
12	25	70	1750	1.68	0.2	0.067	1.67	99.6
13	25	70	1750	1.68	0.2	0.067	1.62	96.4
14	10	215	2150	1.128	0.2	0.113	1.121	99.4
15	10	225	2250	1.128	0.2	0.113	1.122	99.5
16	40	225	9000	1.128	0.2	0.028	1.120	99.4
17	12.5	72	900	1.013	0.2	0.08	1.013	100.0
18	12.5	72	900	1.013	0.2	0.08	1.009	99.6
19	40	225	9000	1.013	1.0	0.025	1.006	99.3
20	25	35	875	0.971	0.2	0.04	0.969	99.8
21	40	225	9000	0.282	0.8	0.007	0.275	97.6
22	40	225	9000	0.0376	1.0	0.001	0.0376	100.0
Average								98.7

Table II. Iron Pentacarbonyl Content of Commercial Gases from Steel Cylinders

Cylinder No.	Gas Compn., % CO	Analysis No.	Vol., Cc. (Uncorrected) Cylinder gas taken	Vol., Cc. (Uncorrected) Diluted with N_2 to	Method of Analysis Flow time, min.	Method of Analysis Flow rate, cc./min.	Method of Analysis Trap	$Fe(CO)_5$ Found Sample Analyzed Vol., %	$Fe(CO)_5$ Found Sample Analyzed Total mg.	Cylinder gas, mg./liter[a]
1	98.5	1	1115		25	45	Cold	1.84×10^{-2}	1.60	1.61
		2	1115		25	45	Cold	1.83×10^{-2}	1.58	1.60
		3	1115		25	45	Cold	1.82×10^{-2}	1.59	1.59
		4	1115		28	40	Methanol	1.80×10^{-2}	1.59	1.57
		5	1027		1 hr. tonometer. 113 ml. methanol			1.81×10^{-2}	1.50	1.58
		6	113	1120	1 hr. tonometer. 20 ml. methanol			1.90×10^{-3}	0.168	1.65
		7	100	1000	25	40	Methanol	1.91×10^{-3}	0.155	1.67
		8	100	8200	205	40	Methanol	2.26×10^{-4}	0.150	1.62
		Average								1.61
2	98.1	9	250		7	35	Methanol	1.33×10^{-1}	2.73	11.6
3	98	10	1000		4	250	Methanol	2.10×10^{-1}	17.3	18.4
4	98	11	4000		16	250	Methanol	1.83×10^{-3}	0.60	0.16
5	0.085 in air	12	10000		40	250	Methanol	0.00	0.00	0.00
		13	9400		40	235	Cold	3.5×10^{-6}	0.0027	0.0003
		14	9400		40	235	Cold	3.5×10^{-6}	0.0027	0.0003
6	0.05 in air	15	4000		16	250	Methanol	$<6.9 \times 10^{-5}$	<0.023	<0.006
7	0.01 in air	16	10000		40	250	Methanol	0.00	0.00	0.00

[a] At 0° C., 760 mm. of mercury.

for the quantitative isolation of carbonyl transferred at a rate of passage higher than the maximum indicated in Table I, column 7, samples 11 and 12.

Samples 11 to 22 represent experimental recoveries by cold trap absorption of smaller quantities (1.68 to 0.038 mg.) of iron carbonyl in methanol solution. The average recovery of content was 98.7 ± 1.4%. On the other hand, recoveries by methanol trap absorption of similar amounts, under these conditions, whereby 5 to 9 liters of nitrogen were passed through the apparatus in 40 to 250 minutes, were not so satisfactory (96%). Apparently, the passage through the methanol trap tubes, of a volume of nitrogen far in excess of that required to evaporate the carbonyl solution, results in some loss of carbonyl by aeration.

The experimental conditions under which the results of Table I were obtained are only an approximation to the analysis of gas containing iron pentacarbonyl vapor. Nevertheless, although the results involve errors arising from the experimental difficulties mentioned in addition to analytical errors, they serve to indicate the adaptability of the method over a wide range of concentration. The accuracy maintained at extremely low concentrations is shown by the cold trap recoveries for samples 21 and 22, corresponding to 3.8×10^{-4} and 5.1×10^{-5}% by volume of iron pentacarbonyl as gas, respectively. The latter is in confirmation of a limiting value for accurate results by the present method, calculated from the requirement of minimum spectrophotometric absorbance of 0.1 (80% transmittance). This would be given by a minimum of 0.04 mg. of iron carbonyl in 20 ml. of methanol. This amount obtained from a maximum gas sample volume of 10 liters, would represent a concentration of 4.6×10^{-5}% by volume (0.46 p.p.m.).

Analysis of Cylinder Gases. Table II shows the results, obtained by the method described, for analyses of commercial gases drawn from steel cylinders. Of especial interest are the eight analyses of gas from cylinder 1. In this series of determinations, both cold and methanol traps were used, and some of the samples were diluted with nitrogen gas. As an independent check, two analyses were also done by a less convenient method: A tonometer (sample tube *S*, Figure 1) containing known volumes of gas sample and methanol was agitated in a shaking machine for a time long enough for the liquid to absorb all of the carbonyl in the gas; the solvent was then analyzed spectrophotometrically. The common basis of comparison, the value for the concentration of carbonyl in the undiluted cylinder gas (Table II, last column), indicates good agreement among the results for this varied group of analyses (cylinder 1). The mean deviation of ±1.6% from the average value (1.61 mg. per liter) is nearly the same obtained for the recovery results with comparable samples (lower half of Table I). Furthermore, the result for sample 8, cylinder 1, indicates the applicability of the methanol trap absorption to the accurate analysis of gas of low carbonyl content (2.26×10^{-4}% by volume). Finally, this series of direct analyses of gas from cylinder 1 serves to bear out the suggestion made above in connection with the results of recovery experiments (at least for the lower range < 2 mg. of iron pentacarbonyl per liter)—namely, that discrepancies in methanol and cold trap results arise mainly from experimental, rather than analytical, errors.

DISCUSSION

The range of concentrations of iron pentacarbonyl in commercial carbon monoxide (98%) extends to values (Table II) which exceed the highest found in the literature [4 mg. per liter for 1% carbon monoxide in hydrogen (*11*)]. On the other hand, the fact that none of the gases containing carbon monoxide in low concentration (cylinders 5 to 7) has shown more than a trace of carbonyl under these conditions of analysis calls for further examination. Obviously, dilution of "pure" carbon monoxide gas would result in a proportionate decrease of any iron carbonyl present. Thus, 0.1 to 0.01% carbon monoxide mixtures made from the pure gas taken from cylinders such as are typified in Table II would contain from 0.018 to 0.001 mg. of iron carbonyl per liter. Furthermore, for dilutions made with air, the presence of oxygen would result in a reduction of these iron carbonyl concentrations to trace levels, such as have been found (Table II, cylinders 5 to 7).

On the other hand, if carbon monoxide which contains iron carbonyl be diluted with certain unreactive gases, to the exclusion of air or oxygen, the carbonyl concentration, if not increased by in situ formation in a steel cylinder, will, in any container, be maintained at the dilution level. The presence of the iron carbonyl may be of quantitative significance in the analyses of such gas mixtures for carbon monoxide by the iodine pentoxide method or in any other procedure in which the gas sample is bubbled through concentrated sulfuric acid in its passage through an absorption or reaction train. The result will be a quantitative decomposition by the acid, of carbonyl to carbon monoxide (*4, 9, 14*), with an increase in the carbon monoxide of the gas mixture by a volume five times that of the carbonyl in gaseous form. The possibility of a discrepancy on this account, between the carbon monoxide concentration of the gas mixture and the value found by analysis, may be avoided by successive passage of the gas through concentrated sulfuric acid and water prior to use.

Obviously, when experimental carbon monoxide-containing gas mixtures contaminated with iron carbonyl are used for breathing purposes, these considerations may be of importance not only from analytical but also from experimental and toxicological aspects. Such mixtures, at the carbon monoxide levels of less than 0.1% used in physiological and blood gas equilibrium studies (*16*), will usually be air dilutions, the carbonyl contents of which

have been found to be vanishingly low, for reasons discussed above. On the other hand, in gases of higher carbon monoxide content and in freshly prepared air mixtures, when there has not been sufficient time for appreciable decomposition of carbonyl by oxygen, the concentration of the former may be significant. In any case, as no information is available concerning allowable toxic limits of iron pentacarbonyl when gases containing this contaminant are used for breathing, they should be passed successively through concentrated sulfuric acid and water as suggested above.

ACKNOWLEDGMENT

The authors are indebted to the late Martin Shepherd and to Shuford Shuhmann, Gas Section, National Bureau of Standards, for generous portions of their analyzed stock supply of commercial (Du Pont) carbon monoxide.

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RECEIVED for review August 17, 1954. Accepted June 4, 1955.

Simple Photoelectric Polarimeter

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An attachment which can convert a photoelectric colorimeter to a polarimeter employs two polaroid plates and eliminates the need for an angular scale. From successive readings of transmittance values, the angle of rotation is calculated. Its performance has been evaluated with solutions of known rotation, and by comparison with a visual saccharimeter. The reproducibility of rotation measurements is about 0.03°. Multiplication of the result by a conversion factor is necessary.

PREVIOUSLY reported photoelectric polarimeters have been adaptations from traditional visual models. In these instruments an end point setting was required, followed by the reading of an angular scale. A precise angular scale is expensive. In general, the photoelectric cell (or cells) simply replaced the eye as a recorder of a null point or a minimum transmittance (*2–5, 7–9, 12, 14–17*).

In 1937 Landt and Hirschmueller (*10*) reported a radically different and ingenious design for a photoelectric polarimeter which eliminates the need for an angular scale and which employes inexpensive Herotar polarizing filters. Their design was based on optical principles first employed in 1849 for studies of magnetic rotation of infrared radiation by de la Provostaye and Desains (*13*), who used two calcite rhombs set 45° apart with a thermopile to receive the ordinary ray. The angle of rotation could be calculated from readings of the galvanometer first without and then with the magnetic field applied across the transmittance medium which was flint glass.

Landt and Hirschmueller gave no reports on the sensitivity and reproducibility of their instrumental design in either their paper (*10*) or their patent claim (*11*). The authors of this paper decided, therefore, to construct an instrument of similar design and test its usefulness and precision.

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DESIGN OF A ONE-PHOTOCELL POLARIMETER

The design schematically represented in Figure 1 is essentially the same as that described by Landt and Hirschmueller (*10, 11*). The alternating current from a Raytheon constant voltage regulator after being stepped down by a variable voltage transformer supplies energy for a tungsten lamp (50-watt, 6- to 8-volt Mazda). The light from the lamp's filament passes through an infrared filter (Corning, light-shade Aklo), is then collimated by a lens, and finally filtered through a color filter which transmits a band of wave lengths between 575 to 605 mμ with maximum transmittance at 590 mμ. This corresponds closely with the D line of sodium.

The resulting beam which is almost parallel and nearly monochromatic, then passes through two polaroid plates (factory-matched pair of high-transmittance polaroid plates) and the solution cell, and strikes the face of a Weston Photronic photocell.

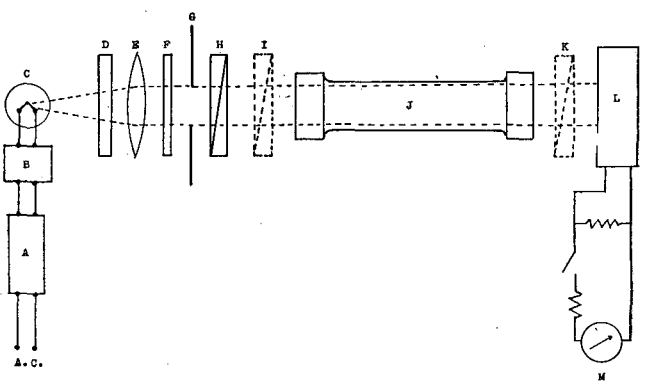

Figure 1. Schematic diagram of single-cell polarimeter

A. Voltage regulator
B. Transformer
C. Lamp, 6- to 8-volt Mazda
D. Heat-absorbing filter
E. Lens
F. Color filter
G. Aperture
H. Polarizing polaroid plate
I. Analyzing polaroid plate (adjacent position)
J. Polarimeter tube
K. Alternate position for analyzer polaroid
L. Photovoltaic cell
M. Galvanometer with variable sensitivity shunt

The current from the photocell is measured on a galvanometer with variable sensitivity.

The first polaroid plate functions as polarizer and remains in fixed position. The second polaroid as analyzer is set at 45° to the polarizer and can occupy the alternate positions before and after the solution cell in the light path. The analyzer is also mounted to permit its rotation through 90° to give an angle of 135° to the polarizer.

THEORY OF THE DESIGN

If the polaroids were adjacent and their axes parallel, the diminution in intensity would be due entirely to light absorption by the media in the optical path.

According to the Bouguer-Lambert-Beer law:

$$I = K_1 I_0 10^{-\Sigma\epsilon} \quad (1)$$

where K_1 is the reflection loss factor and $\Sigma\epsilon$ gives the sum of all sources of extinction. From Malus' law:

$$I = K_2 I_0 \cos^2\theta \quad (2)$$

where θ is the angle between the axes of crossed polaroid plates. If then the axes of the polarizer and analyzer differ by angle θ but they remain in adjacent positions ahead of the solution cell, it follows that:

$$I = K_3 I_0 10^{-\Sigma\epsilon} \cos^2\Theta \quad (3)$$

If now the analyzer be moved to its alternate position so the solution cell is between it and the polarizer, the effective angle becomes the resultant of θ and of α, the angle of rotation of the solution in the cell, hence:

$$I_\alpha = K_3 I_0 10^{-\Sigma\epsilon} \cos^2(\Theta \pm \alpha) \quad (4)$$

Dividing Equation 4 by 3 and assuming proportionality of current reading R to light intensity:

$$\frac{R_\alpha}{R} = \frac{\cos^2(\Theta \pm \alpha)}{\cos^2\Theta} \quad (5)$$

From the law of Malus it can be shown by the standard methods of differential calculus that $d\Theta/dI$ is minimum when Θ is 45°. Or, $dI/d\Theta$ is maximum when Θ is 45°. Therefore:

$$\frac{R_\alpha}{R} = \frac{\cos^2(45 \pm \alpha)}{\cos^2 45} = \frac{\cos^2(45 \pm \alpha)}{0.500} \quad (6)$$

Equation 6 is equally valid when θ is 135° or 225° or 315°. Solving for α, the equation may be restated:

$$\alpha = {}^1/_2 \cos^{-1}[(R_\alpha/R) - 1] - 45 \quad (7)$$

Although R_α/R may be less than unity, and result in a negative value for the bracketed factor, such negative values are perfectly admissible, provided α is not greater than 45° because then $\cos^{-1}[(R_\alpha/R) - 1]$ will always have values between 90° and 180°. A table of values of α corresponding to various R_α/R ratios covering the rotation range of 0° to 10° has been found very useful.

POLARIZED LIGHT EMISSION BY THE LAMP

The initial model had the analyzer in fixed position with the polarizer movable. It proved impossible to obtain reproducible results from settings of the polarizer at 45° and 135° to the analyzer. It was found that with a single polaroid plate in the beam of light there were two maximum and two minimum photocell readings evenly distributed over the 360° arc of rotation of the polaroid plate. Identical results were obtained with a Nicol prism. Investigation of various lamps revealed as much as 15% difference between maximum and minimum settings. No lamp was found which did not show this effect. The 6- to 8-volt Mazda automobile lamp showed the least polarization. Willey (*17*) reported the observation of two maxima and two minima and attributed this effect to the light which was not polarized by the polaroid plates. He estimated the unpolarized light to amount to only 2% however. From this hypothesis, all lamps should give identical effects, and the maxima and minima should not be observed when a Nicol prism is substituted.

It was found that by setting a fixed polarizer to the angle of minimum photocell response and then setting a movable analyzer at 45° to the polarizer, reproducible results could then be obtained for 45° and 135° settings of the analyzer. It would appear therefore that the only feasible arrangement is fixed polarizer and movable analyzer.

OPERATION

Following the setting of the polarizer as described above, the analyzer is inserted and rotated to the position giving a maximum photocell response. This corresponds to approximately zero angle between the axes of the two polaroids. From Equation 2 it can be seen that when the analyzer is rotated 45° the photocell response must drop to one half of the value for 0°, because cos $45 = ({}^1/_2)^{1/2}$. Following this preliminary adjustment, fine adjustment can be continued until identical readings are obtained on movement of the analyzer through 90°. During this adjustment, the solution cell contains only water.

The solution cell is a 1-dm. Bausch & Lomb spectrophotometer absorption cell with removable end plates. The cell aperture is 26 mm.

Six optically active solutions ranging from 8 to 75% sucrose were prepared from roughly weighed, commercial sucrose to permit comparison of this instrument with a Bausch & Lomb quartz-wedge saccharimeter.

Each solution was observed first in the photoelectric polarimeter, where four readings were taken: two at 45° and two at 135°. The same solution was then placed in the 2-dm. saccharimeter tube and six settings were obtained, using unfiltered tungsten lamp illumination, and averaged together. A plot of values of α from the photoelectric polarimeter against ${}^1/_2 V°$—i.e., one half of the saccharimeter reading expressed in degrees Ventzke to con-

Table I. Data Obtained with Single-Cell Polarimeter Showing Reproducibility and Comparison with Saccharimeter

Soln. No.	Photoelectric Polarimeter (Circular Degrees), 1-Dm. Tube: 45°-setting	135°-setting	Av.	Saccharimeter[a] (Ventzke Degrees), 2-Dm. Tube	½ V° × 0.370	±Δ
1	1.55	1.55	1.53	7.8	1.45	0.08
	1.49	1.52				
2	2.81	2.67	2.79	14.8	2.74	0.04
	2.81	2.67				
3	5.83	5.86	5.83	31.7	5.86	0.03
	5.83	5.80				
4	8.01	7.95	8.00	43.3	8.01	0.01
	7.98	8.04				
5	9.54	9.39	9.50	51.2	9.47	0.03
	9.48	9.57				
	13.66	13.73	13.66	74.4	13.76	0.10
	13.56	13.79				
	Average deviation					0.05

[a] Each result is the average of six independent readings.

Table II. Showing Reproducibility of Two-Cell Polarimeter

Run	Slide-Wire Readings R	Slide-Wire Readings R_{45}	Slide-Wire Readings R_{135}	$R\alpha/R$ 45°	$R\alpha/R$ 135°	Angular Rotation, Degrees α	Average, Degrees
A	50	60.9		1.218		−6.30	−6.15
	50		39.6		0.792	−6.00	
B	50	60.9		1.218		−6.30	−6.15
	50		39.6		0.792	−6.00	
C	50	61.0		1.220		−6.36	−6.18
	50		39.6		0.792	−6.00	
D	50	59.5		1.190		−5.48	−6.18
	50		38.1		0.762	−6.88	

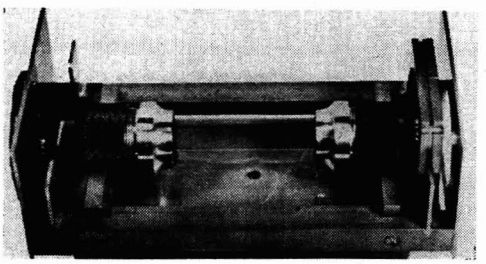

Figure 2. Adapter assembled

vert to 1-dm. basis—was linear and the value of the slope was 0.370. Browne (*1*) gives 0.398 as the conversion factor from degrees Ventzke, using unfiltered Wellsbach mantle light, to circular degrees measured with the D line of sodium. Table I shows the reproducibility of photoelectric polarimeter settings and also compares the observed average values of α with corresponding calculated values from saccharimeter settings.

Linearity over a rotation range of 14° with an average deviation of about 0.05° describes the performance of this one-photocell instrument. It became very clear that much of the uncertainty in readings was due to line voltage fluctuations which affected the readings in spite of the presence of the voltage regulator.

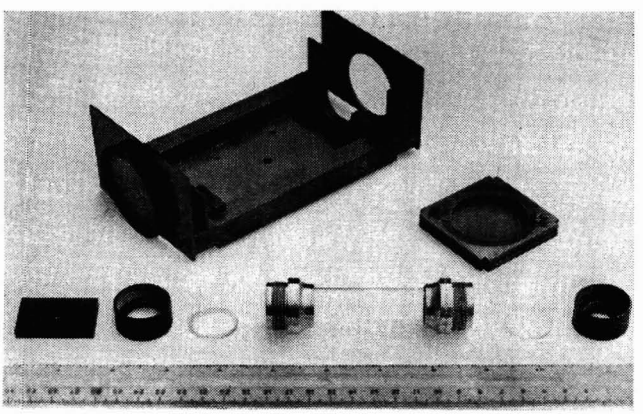

Figure 3. Adapter disassembled

A power supply consisting of two batteries in parallel was substituted for the alternating current supply and although a slight improvement in stability of light intensity was noted, the discharge rate to produce adequate light intensities was so high that the over-all precision was not noticeably improved.

DESIGN OF A TWO-PHOTOCELL POLARIMETER

A two-cell, balanced circuit instrument seemed to be the answer for line voltage fluctuations. It was clear that the instrument described above became a primitive photoelectric colorimeter upon removal of the polaroid plates. It seemed reasonable, therefore that a stable photoelectric colorimeter might be converted to a polarimeter. A Lumetron, Model 402-E, photoelectric colorimeter was available and it proved easy to construct a simple adapter for insertion into the cell compartment of this instrument.

The ultimate design of the adapter is shown by Figure 2, a photograph of the assembled adapter. Figure 3 shows the adapter disassembled, and Figure 4 shows the adapter in position in the cell compartment of the Lumetron photocolorimeter.

The polarizer polaroid is fastened to the left end plate of the adapter. The aperture in this plate is 6 mm. The analyzer polaroid is mounted in a square brass plate with a 4-cm. aperture, slotted on the four edges. This plate fits snugly into the vertical positioning braces at the two ends of the adapter and at either end, the analyzer may be held at the four positions, 90° apart.

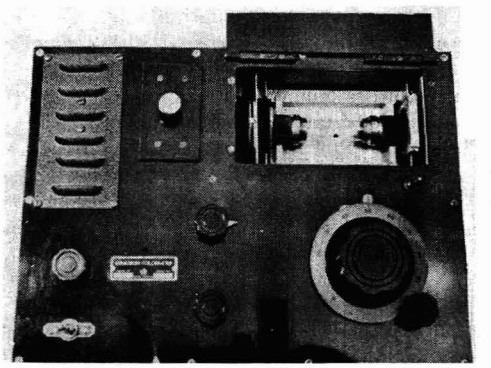

Figure 4. Adapter in well of Lumetron colorimeter

The rectangular Bakelite plate seen at the extreme left in Figure 3 has an aperture of 9 mm. This plate was inserted in the slot ahead of the reference photocell of the Lumetron to decrease the light striking the photocell and hence simplify the balancing at a setting of 50 on the slide wire. It was necessary to use an initial setting less than full scale because half of the readings, R_α, were greater than the initial reading. The slide-wire readings on the Lumetron are read as per cent transmittance. The ratio of two transmittance values will equal the ratio of transmitted light intensities with identical incident intensity:

$$\frac{R_\alpha}{R} = \frac{T_\alpha}{T} = \frac{I_\alpha/I_0 \times 100}{I/I_0 \times 100} = \frac{I_\alpha}{I} \qquad (8)$$

It therefore follows that the theoretical principle of this two-cell design is basically the same as that summarized earlier in Equation 7.

The light filter used was a Farrand interference filter which has maximum transmittance at 597 mμ with a half-band width of 15 mμ. This gave nearly monochromatic light of wave length near that of the sodium D line.

RESULTS

Table II shows the results of four independent observations on the same solution of levulose. In run D, the analyzer was deliberately rotated several degrees from its initial setting at approximately 45° to the polarizer. This instance of obtaining the same average as with the earlier setting agrees with the general conclusion finally adopted that this 45° setting is not critical in the present design.

Table II also illustrates the rule for the sign of the rotation. Here the ratio at 45° (clockwise on the optical axis from lamp to photocell) is greater than unity and the ratio at 135°—i.e., 90° clockwise movement—is less than unity which corresponds to levorotation. Dextrorotation gives the reverse order of ratio magnitudes.

Table III. Measurement of Rotation by Substances of Known Optical Activity

Sample	α Obsd.	α Calcd.	Calcd./Obsd.
d-Tartaric acid	1.35	1.39	1.030
Sucrose (B.S.)	1.69	1.735	1.026
Dextrose (B.S.)	2.11	2.165	1.026
Dextrose (B.S.)	2.84	2.93	1.030
Maltose	3.56	3.65	1.026
Lactose	6.78	6.98	1.026
Resolving agent[a]	8.10	8.31	1.026
Resolving agent[a]	8.46	8.64	1.022
Maltose	14.30	14.76	1.026
Average			1.026

[a] Resolving agent is *d*-α-bromodicamphor sulfonate.

To test the linearity of this polarimeter design, rotation observations (the average of two determinations, one at 45° and one at 135°) were made on a series of 26 optically active solutions with arbitrary concentrations, and compared with average readings obtained on the saccharimeter previously described using a 1-dm. tube in both instruments. This series consisted of three aqueous solutions of *d*-tartaric acid, six aqueous solutions of *d*-α-bromodicamphor sulfonate, two aqueous solutions of maltose four aqueous solutions of levulose, two aqueous solutions of lactose, six aqueous solutions of dextrose, one aqueous solution of sucrose, and two ethyl alcohol solutions of turpentine. The quartz-wedge saccharimeter was illuminated by a sodium vapor lamp. Ten readings were taken on each solution and averaged together.

Figure 5 is a plot of the saccharimeter readings against the photoelectric polarimeter readings. A clearly linear relation holds up to 15°. The points marked by arrows are levorotations, all the remainder are dextro. The slope of the straight line as determined by the method of least squares, is 3.05 ± 0.08. The reciprocal, which gives the conversion factor, is 0.328. This is to be compared with the factor 0.346 cited by Gibb (*6*) for sodium light.

To test for accuracy, photoelectric polarimeter readings were taken on a series of precisely prepared solutions of purified, optically active compounds. Two were Bureau of Standards samples. The rotations of these solutions were calculated from the known specific rotation values. Table III gives the results along with a column of ratios of calculated to observed values. The average value for the correction factor is 1.026 which in the light of present knowledge must be considered as an empirical correction.

DISCUSSION

There are several obvious sources of error: The illumination is neither monochromatic nor the same wave length as the D line of sodium, the polaroid plates do not completely polarize the light, the beam of light is not parallel, fatigue effects in the two photocells might not cancel since the reading photocell is illuminated with polarized light and the reference photocell receives unpolarized light, and temperature was not controlled any closer than 25° ± 3°.

Further investigation is planned along these lines: observations with controlled temperatures, attempts to improve the precision by means of Nicol prisms and other types of photocells, and attempt to construct a similar adapter which could fit into the optical path of a photoelectric spectrophotometer to produce a device for rotatory dispersion observations.

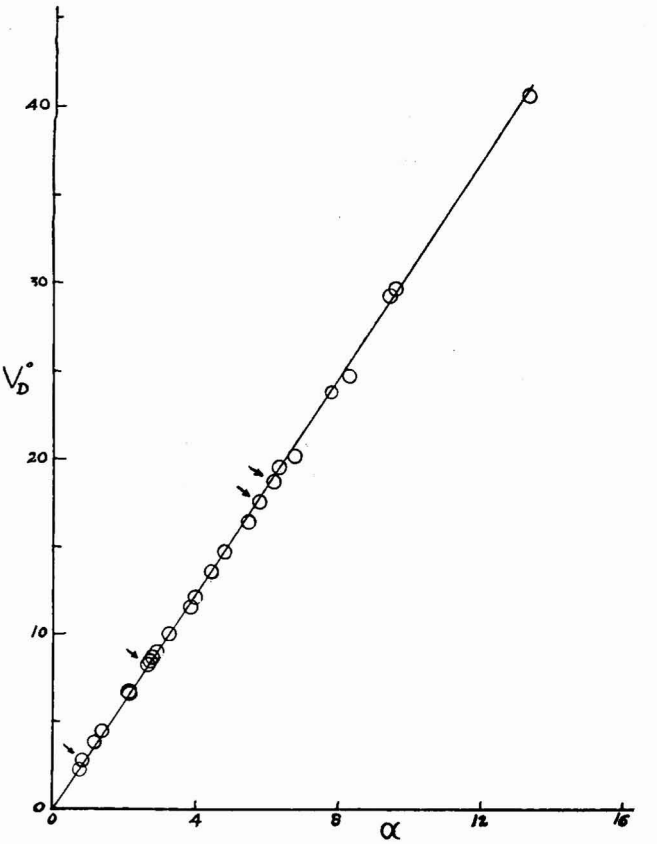

Figure 5. Plot of saccharimeter readings *vs.* photoelectric polarimeter readings for 26 optically active solutions

ACKNOWLEDGMENT

The authors wish to express appreciation for the valuable assistance of David Blaikley, the Tulane University mechanician.

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RECEIVED for review May 8, 1955. Accepted June 11, 1955. Presented in part before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1951. Based on portions of the M.S. theses of W. H. Dyre and A. Spell.

Analysis of Iron Pickle Liquor by Means of Ion Exchange

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The method of determining total cation concentration by the conversion of the cations present in solution to hydrogen ion, using a sulfonic acid type of cation exchange resin, is applicable to solutions of high initial acidity. This technique, combined with a standard volumetric iron determination, has been successfully used in the rapid, routine analysis of iron pickle liquor solutions for both their iron and their acid content.

IN A recent study (*1*), the necessity of following both the acid and the iron concentration of an iron pickle liquor arose. Such solutions are commonly 1 to 2*M* in sulfuric acid and may contain as much as 30 grams of iron(II) per liter. Their analysis is complicated by the interference of the high concentrations of iron with the detection of the end point in the determination of the acidity of the solution. To obviate this, the possibility of determining the iron content of one portion of the solution to be analyzed by the usual volumetric methods and, in a second portion of the same solution, of converting the iron(II) to hydrogen ion by means of the hydrogen form of a sulfonic acid cation exchange resin followed by titration to determine the total cation content of the solution suggested itself. This latter method has been commonly used in inorganic analysis for the determination of total cations (*2*). Little information is available, however, concerning the feasibility of such a cation determination in solution of as high an acidity as those found in pickle liquor problems. As acids in the concentration range of 1 to 2*M* are frequently used to remove other cations from ion exchange resins in regeneration procedures, a study of the ability of the resin to retain iron under these conditions was required before the method could be applied to the present analytical problem. The results of this study are reported herein.

PROCEDURE

From the sample to be analyzed a series of aliquots, of size estimated to give convenient titrations, is taken. One set of aliquots is used for the determination of iron (ferrous and total, if required) by any of the standard methods. In the second set of aliquots a total cation determination is made by the following procedure.

Convert a portion of a sulfonated, cross-linked polystyrene resin (20- to 50-mesh) such as Amberlite IR-120 to the hydrogen form using 10 volumes of 10% sulfuric acid per volume of resin. Rinse the converted resin free of excess acid with deionized or distilled water until the effluent is neutral to methyl red indicator.

Prepare 25-ml. beds of this hydrogen-form resin in glass-stopcock burets using a small plug of glass wool and a few milliliters of acid-washed sea sand to support the resin bed. Backwash the resin column to remove air and drain off the excess water to the top of the resin bed. Pass an aliquot of the solution to be analyzed through the resin at a rate slower than 5 ml. per minute, maintaining the resin bed full of liquid at all times. Rinse the bed with 50 ml. of deionized or distilled water, combining the rinse with the sample effluent. Titrate the entire effluent or a known aliquot thereof for acid content, using phenolphthalein as the indicator. The initial acidity of the sample is calculated by subtracting the milliequivalents of iron, calculated as hydrogen ion, from the total acidity as determined after ion exchange treatment.

EXPERIMENTAL RESULTS

Before a routine method for the determination of the total cation concentration of a pickle liquor type of solution utilizing ion exchange could be established, the ability of the resin to remove iron from such solutions had to be investigated. The limitations imposed by the interaction of bed dimensions, flow rate, and influent concentration were determined in a set of runs using a synthetic pickle liquor as the sample. This consisted of a standardized ferrous sulfate solution containing 30 grams of iron per liter, acidified with a known amount of sulfuric acid, and mixed with an equal quantity of a standardized 10% sulfuric acid solution. This mixture represented a 1 to 1 dilution of an average pickle liquor. The iron leakage, when 20-ml. portions of this solution were passed through various amounts of resin at two flow rates, was determined colorimetrically. Results are shown in Table I. From these it was apparent that with a 25-ml. resin bed and an iron influent of 15 grams per liter, satisfactory reduction of the iron leakage to 2 parts per 1000 parts of influent could be obtained at flow rates of 5 ml. per minute or less. This combination of flow rate, bed size, and maximum iron concentration was adopted for all subsequent work.

Having established the conditions for the removal of iron from the acid solution, the determination of the conditions for the recovery of the acid from the resin bed remained. For this, 50-ml. samples representing two different dilutions of synthetic pickle liquor were used. The sample effluent and the washings were collected in 25-ml. aliquots and each aliquot was titrated for acid content. Results are given in Table II. The major portion of the acid is removed from the column by the first 50 ml. of wash water, but 75 ml., or three-bed volumes, are required for maximum recovery.

To test the method evolved from the above experiments a series of solutions of varying, known ratios of iron to acid was analyzed by the outlined procedure. Results are summarized in Table III. As these analyses were intended to simulate routine analysis, only 50 ml. of water were used as the rinse solution. Therefore, as a general trend, the total milliequivalents of acid in the effluent is slightly less than that of the influent. Leakage of iron, which would not be determined in routine analysis, was checked by colorimetric analysis. It was found to be a negli-

Table I. Iron Leakage as a Function of Flow Rate and Bed Depth

Influent Concn., Grams Fe/Liter	Influent Acidity, *N*	Sample Volume, Ml.	Bed Size, Ml.	Flow Rate, Ml./Min.	Leakage Fe, Grams/Liter
15.0	1.424	20.0	10	5	2.08
15.0	1.424	20.0	25	5	0.026
15.0	1.424	20.0	50	5	0.017
15.0	1.424	20.0	10	10	3.87
15.0	1.424	20.0	25	10	0.077
15.0	1.424	20.0	50	10	0.017

Table II. Elution of Acid from Resin Bed

Sample	I	II
Influent concn.		
Fe, meq.	10.80	5.40
Acid, meq.	29.04	14.52
Total, meq.	39.84	19.92
Sample effluent concn., meq.		
1st 25 ml.	9.64	4.64
2nd 25 ml.	19.88	9.68
Wash effluent concn., meq.		
1st 25 ml.	10.11	5.24
2nd 25 ml.	0.28	0.15
3rd 25 ml.	0.10	0.04
Total meq. recovered	40.01	19.75

Table III. Typical Analyses

Influent	Concn.	Sample Taken				
Fe, N^a	Acid, N	Fe, meq.[a]	Acid, meq.	Total meq.[a]	Recovered Total, meq.[a]	Leakage, Fe, N^a
0.2148	0.578[4]	10.74	28.92	39.66	40.0	0.000216
0.1074	0.289[2]	10.74	28.92	39.66	39.6	0.000036
0.2148	0.306[4]	10.74	15.32	26.06	25.8	0.000036
0.2148	0.0922	10.74	4.61	15.35	15.2	<0.000007
0.2148	0.0344	10.74	1.72	12.46	12.5	<0.000007
0.1074	0.561[6]	5.37	28.08	33.44	33.2	0.000144
0.0215	0.547[4]	1.074	27.37	28.44	28.4	<0.000007
0	0.544[0]	0	27.20	27.20	27.2	0

[a] All normalities calculated as acid.

gible fraction of the influent iron in all cases. As would be expected, the magnitude of the leakage was directly proportional to the acid content of the influent.

In routine analysis this method has consistently given results of greater than 1 part per 1000 accuracy, as shown in Table III. Greater accuracy may be obtained at the expense of speed by decreasing the rate of flow, using resin of smaller particle size, and/or increasing the volume of the water wash. With this increased care accuracy of the order of 3 parts per 1000 is possible. In addition to the present case where only iron(II) and acid were present in the solution, the method has been successfully applied to the determination of copper(II) and iron(II) mixtures of high acid content. Presumably it could be adapted to other similar situations where the analysis of high concentration of both metal cations and the acid is required.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Diana Kenny for her assistance in the experimental phases of this work.

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RECEIVED for review May 14, 1954. Accepted June 30, 1955. Presented before the Analytical and Microchemical Group of the Philadelphia Section AMERICAN CHEMICAL SOCIETY, April 15, 1955.

Determination of Nicotine, Nornicotine, and Total Alkaloids in Tobacco

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This study was undertaken to provide the industry with a more rapid procedure for the analysis of total alkaloids, nicotine, and nornicotine-type alkaloids in tobacco. According to the proposed method an alkaline tobacco mixture is extracted with benzene-chloroform solution and aliquots of the extract are titrated with standard perchloric acid. The fact that secondary base-type alkaloids present in tobacco may be quantitatively acetylated provided a means of determining nornicotine-type alkaloids in the presence of nicotine. Ammonia may be eliminated as an interferant in the proposed scheme of analysis.

NICOTINE in tobacco products may be determined gravimetrically as the silicotungstate (*1–3, 6, 8*); volumetrically, after liberation and extraction of the free base (*7, 11, 16, 19*); spectrophotometrically, after isolation of the alkaloid by steam distillation (*21*); and colorimetrically (*13, 22*). None of these procedures will distinguish nornicotine from nicotine. Since the literature on the determination of nicotine and related alkaloids is voluminous, the references listed are representative rather than inclusive.

Bowen and Barthel (*5*) and Markwood (*14*) described methods for separating nicotine and nornicotine by reaction of nornicotine with nitrous acid. Larson and Haag (*12*) proposed a colorimetric procedure for the simultaneous determination of these two alkaloids, and among others Houston (*9*) has presented a chromatographic technique for separation and determination of nicotine and nornicotine.

The silicotungstate methods are time-consuming and tend to give erroneous results when appreciable ammonia or ammonium salts are present (*15*). Jeffrey (*10*) compared several alkaloid methods and found that there was satisfactory correlation of results between the conventional procedures investigated when nicotine was the predominant alkaloid, but noted considerable discrepancy of results when mixed alkaloid types of tobacco were analyzed. The Houston chromatographic technique (*9*) gives precise results and provides a means of determining both nicotine and nornicotine. However, this procedure is not readily adaptable for the routine analysis of a large number of samples.

As it was believed that an extraction and titration procedure would provide the most satisfactory approach to a simplified and rapid analysis, the Garner titration method (*7*) appeared to be a suitable starting point for the investigation. In the Garner method an alkaline tobacco paste is allowed to stand overnight in contact with petroleum ether or a similar type of solvent, an aliquot of the hydrocarbon phase is extracted with standard acid, and the nicotine is determined by titration with standard alkali. Although this method is simple, it, too, is time-consuming and involves an indirect titration.

By the proposed procedure it was found possible to remove the alkaloids from an alkaline tobacco mixture with a benzene-chloroform solution after only a 15-minute extraction period, and to titrate the alkaloids in an aliquot of the hydrocarbon extract directly with standard perchloric acid in acetic acid. It was found also that nornicotine could be quantitatively acetylated with acetic anhydride, and after acetylation, only one half of the nornicotine equivalent was titrated in the nonaqueous system. This, therefore, provided a means of determining nicotine and nornicotine in the same solution.

A number of alkaloids and alkaloid derivatives have been identified in tobacco. Although the principal alkaloids in commercial tobacco are nicotine and nornicotine, related species and hybrid tobaccos may have correspondingly larger amounts of other alkaloids, especially anabasine. Since alkaloids other than nicotine or nornicotine are present in many tobaccos, it was thought preferable to designate the tertiary amine alkaloids, nicotine-type alkaloids, and the primary and secondary amine alkaloids, including anabasine, nornicotine-type alkaloids. Hence in this work unless definitely specified, the connotation "nicotine" includes the tertiary amine alkaloids, and "nornicotine" includes the primary and secondary amine alkaloids.

Table I. Recovery of Nicotine and Nornicotine from Aqueous Solutions

Alkaloid Added, Mg.		Alkaloid Recovered			
		Nicotine		Nornicotine	
Nicotine	Nornicotine	Mg.	%	Mg.	%
50.8	34.6	50.9	100.20	33.5	96.82
50.8	34.6	50.6	99.61	34.8	100.58
50.8	17.3	50.4	99.21	16.2	93.64
101.7	34.6	100.6	98.92	33.9	97.98
		Mean	99.48		97.26

Table II. Recovery of Nicotine and Nornicotine When Added to Tobacco

Alkaloid Added, Mg.		Alkaloid Recovered			
		Nicotine		Nornicotine	
Nicotine	Nornicotine	Mg.	%	Mg.	%
50.8	15.1	50.4	99.21	14.9	98.68
28.8	16.7	28.6	99.30	16.4	98.20
28.8	16.7	29.4	102.08	16.4	98.20
28.8	16.7	28.7	99.65	16.5	98.80
		Mean	100.06		98.47

REAGENTS, SOLUTIONS, AND APPARATUS

Acetic acid, A.C.S. grade.

Acetic anhydride, A.C.S. grade.

Barium hydroxide, octahydrate, A.C.S. grade.

Celite analytical filter aid, Johns-Manville Corp.

Perchloric acid, 70 to 72% A.C.S. grade.

Potassium acid phthalate, primary standard grade.

Benzene-chloroform solution. Mix 900 ml. of benzene with 100 ml. of chloroform.

Barium hydroxide solution. A saturated aqueous solution.

Crystal violet indicator. Dissolve 1 gram of crystal violet in 100 ml. of glacial acetic acid.

Perchloric acid solution, 0.025*N*. Dilute 2.1 ml. of 72% perchloric acid to 1 liter with glacial acetic acid. Standardize the perchloric acid solution against potassium acid phthalate according to the procedure of Seaman and Allen (*17*).

Precision-Shell titrator with modified calomel and glass electrodes was used for the potentiometric titrations.

Modified calomel electrode. Replace the saturated aqueous potassium chloride in a fiber-type calomel electrode with a methanol solution saturated with potassium chloride.

Wrist-action shaker, Model BB, Burrell Corp., Pittsburgh, Pa.

PROCEDURE

Accurately weigh 2.5 to 3.5 grams of finely ground tobacco into a 250-ml. glass-stoppered Erlenmeyer flask. Add approximately 1 gram of granular barium hydroxide and 15 ml. of barium hydroxide solution. Swirl the flask until the tobacco is thoroughly wetted, adding more barium hydroxide solution if necessary. Pipet 100 ml. of benzene-chloroform solution into the flask, stopper tightly, and agitate vigorously for 15 minutes using a shaking machine, or for 20 minutes if shaken by hand. Add approximately 2 grams of Celite, swirl flask until the filter aid is well dispersed, allow the two liquid phases to separate, and filter the majority of the hydrocarbon layer through Whatman No. 2 filter paper into a second flask. Pipet 25-ml. aliquots of the filtrate into each of two 125-ml. Erlenmeyer flasks. Pass a stream of air over the surface of the solution in the first flask for 5 minutes to remove any free ammonia that might be present in the extract. Add 0.5 ml. of acetic anhydride to the second flask. To each flask, add 1 drop of crystal violet indicator and titrate to a green end point with the 0.025*N* perchloric acid. If the nornicotine content is found to be as high as 25% of the nicotine content, acetylate another 25-ml. portion of the filtrate, add 25 ml. of acetic acid, and titrate potentiometrically to obtain the equivalence point.

Calculations.

$$\% \text{ total alkaloids, as nicotine} = \frac{V_1 \times N \times 32.45}{\text{wt. of sample (moisture-free basis)}}$$

$$\% \text{ nicotine} = \frac{(2V_2 - V_1) \times N \times 32.45}{\text{wt. of sample (moisture-free basis)}}$$

$$\% \text{ nornicotine} = \frac{2(V_1 - V_2) \times N \times 29.64}{\text{wt. of sample (moisture-free basis)}}$$

where

V_1 = milliliters of perchloric acid required to neutralize non-acetylated aliquot

V_2 = milliliters of perchloric acid required to neutralize acetylated aliquot

N = normality of perchloric acid solution

EXPERIMENTAL

Solvents and Extraction. Several solvents were investigated as alkaloid extractants. These included petroleum ether, benzene, iso-octane, and chloroform. Benzene proved to be more satisfactory as an extracting solvent than the other hydrocarbons investigated. The indicator end point was sharper in benzene solution, and of the solvents tested only chloroform was more efficient as an alkaloid extractant. Chloroform alone was not investigated further as an extracting solvent because of its high specific gravity. A solvent with specific gravity less than 1 would be much easier to separate from a mixture. A solution of 9 parts of benzene and 1 part of chloroform was found to have the advantages of both of these solvents.

To test the efficiency of this benzene-chloroform solution as an alkaloid extractant, known mixtures of nicotine and nornicotine were made alkaline and extracted with benzene-chloroform solution, and the alkaloids were determined as outlined in the preceding method. Table I indicates the recovery of these alkaloids from aqueous solutions.

Equally good recovery was obtained when known mixtures of these alkaloids were added to tobacco and analyzed by the described method. This is demonstrated by the data in Table II. Tobacco samples without added alkaloid were analyzed simultaneously to serve as blanks for these determinations.

Acetylation. Treatment of primary and secondary amines with acetic anhydride to yield neutral actylation products in nonaqueous systems has been reported (*18*, *20*). Since nornicotine contains both a secondary and tertiary amine group, it was believed that the secondary amine group would acetylate in benzene-chloroform solution.

When acetic anhydride was added to a benzene-chloroform solution of nornicotine, allowed to react for several minutes, and titrated with perchloric acid, exactly one half of the equivalent was titrated, whereas the total equivalent was titrated in a non-acetylated solution. This acetylation of nornicotine takes place rapidly at room temperature. Nicotine, being a tertiary base, is unaffected by the acetic anhydride treatment. The amide formed on acetylation of nornicotine caused a false visual end point in the subsequent titration, so that it was necessary to ascertain the equivalence point potentiometrically. This amide formation also affected the visual end point in tobacco samples where the nornicotine content was as high as 25% of the total alkaloid. Hence all tobacco samples in which the nornicotine content is 25% or more of the total alkaloid content should be titrated potentiometrically after the acetylation. Samples in which the nornicotine content is less than 25% of the total alkaloid may be titrated satisfactorily by means of the indicator end point procedure.

Potentiometric Titrations. Although suitable titration curves were obtained with the regular glass-calomel electrode system in benzene-chloroform solutions, much more satisfactory curves were obtained if 25 ml. of glacial acetic acid were added to the benzene-chloroform solutions prior to titration, and if the calomel electrode was modified by replacing the aqueous potassium chloride with methanolic potassium chloride.

Table III. Reproducibility of Extraction Procedure as Determined by Analysis of Flue-Cured and Burley Tobacco Samples

Sample	% Total Alkaloids as Nicotine	Sample	% Total Alkaloids as Nicotine
Flue cured No. 588	2.44	Burley No. 589	4.09
	2.43		4.10
	2.43		4.10
	2.44		4.11
	2.43		4.09
	2.40		4.10
	2.42		4.12
	2.43		4.11
	2.44		4.10
	2.46		4.13
Mean	2.43		4.11
Standard deviation	0.016		0.013

Table IV. Reproducibility of Extraction Procedure for Alkaloids in Tobacco

Total Alkaloid as Nicotine, %	Nicotine, %	Nornicotine, %	Total Alkaloid as Nicotine, %	Nicotine, %	Nornicotine, %
2.24	2.08	0.15	4.68	4.41	0.25
2.26	2.11	0.14	4.69	4.42	0.25
2.56	2.34	0.20	4.52	4.18	0.31
2.56	2.34	0.20	4.51	4.17	0.31
1.65	1.50	0.15	3.80	3.62	0.16
1.66	1.50	0.14	3.85	3.67	0.16
1.16	1.04	0.11	4.15	3.88	0.24
1.15	1.01	0.13	4.21	3.94	0.25
3.05	2.83	0.21	2.96	2.73	0.21
3.05	2.87	0.16	2.98	2.76	0.20
5.38	5.09	0.27	0.60	0.54	0.05
5.38	5.09	0.26	0.60	0.55	0.05

Standard deviation for total alkaloids = 0.017
Standard deviation for nicotine = 0.021
Standard deviation for nornicotine = 0.012

The potentiometric end point of the acetylated benzene-chloroform–acetic acid solutions was found to be −535 to −540 mv. with the methanol modified calomel electrode. This equivalence point is sufficiently reproducible to warrant the use of an automatic titrator, or of titrating to a definite millivoltage with standard titrators.

Effect of Ammonia. The presence of ammonia in the hydrocarbon extract would lead to erroneous results in the nicotine-nornicotine determination. It was believed that any ammonia absorbed during the extraction could be removed by aspirating the hydrocarbon solution for a short time prior to titration. Such aspiration would probably not remove any of the alkaloids. To test this and to ascertain the magnitude of the error due to the presence of ammonia, the following steps were taken. Known solutions of ammonium sulfate were made alkaline then extracted with benzene-chloroform solution. One aliquot of the extract was titrated directly; the second aliquot was transferred to a 125-ml. Erlenmeyer flask and a gentle stream of air was passed over the surface of the solution for 5 minutes, then titrated. In addition, known amounts of nicotine and nornicotine were treated in the same manner to ascertain whether aspiration would cause volatilization of either alkaloid. Approximately 1 to 2% of the added ammonia was extracted by the benzene-chloroform solution and this was effectively removed by the specified aspiration technique. None of the alkaloids was volatilized in a 5-minute aspiration period.

Ammonia will react quantitatively with acetic anhydride to yield acetamide, a nonbasic compound in the nonaqueous media, but since acetic anhydride will also react with nornicotine, acetylation would not be an effective means of eliminating ammonia as an interferant in this particular analysis.

Because such a small quantity of ammonia is absorbed by the extracting solution, total alkaloids as nicotine can be determined with a fair degree of accuracy by direct titration of an aliquot of the hydrocarbon extract without removing the ammonia.

Precision of Method. A detailed study of the precision and accuracy of the method was not made because only a limited amount of nornicotine was available for this investigation. Some measure of the precision was obtained by analyzing ten samples from each of two known aqueous nicotine solutions.

One solution was prepared so that each aliquot contained 28.40 mg., the approximate amount of nicotine which would be found in the analysis of a low alkaloid tobacco. The average recovery for the ten analyses was 28.39 mg., with a standard deviation of 0.36. The second solution was prepared so that each aliquot contained 199.6 mg., the approximate amount of nicotine which would be found in the analysis of a high alkaloid tobacco. The average recovery for these ten analyses was 199.30 mg. with a standard deviation of 0.50.

In addition, a flue-cured and burley tobacco sample were each analyzed ten times for total alkaloid content as nicotine by the described method. Results of these determinations are given in Table III.

Table IV indicates the precision obtained on twelve duplicate analyses of burley tobacco samples for total alkaloid, nicotine, and nornicotine content.

Comparison with Other Alkaloid Procedures. Table V compares the results for total alkaloids with those obtained by the AOAC silicotungstate method (*1*). The Bowen-Barthel steam distillation apparatus (*4*) was used for alkaloid isolation in the AOAC method.

These data indicate that both methods are equally reliable.

A comparison of results obtained by the Houston chromatographic procedure (*9*) and the extraction procedure on a series of tobacco samples is shown in Table VI.

Table V. Total Alkaloids in Tobacco as Determined by Extraction and AOAC Silicotungstate Methods

Per Cent Total Alkaloids as Nicotine	
Extraction method	AOAC method
6.03	5.99
4.71	4.74
3.12	3.15
3.59	3.60
0.60	0.67
1.16	1.22
3.53	3.52
2.97	2.95

Table VI. Comparison of Extraction Procedure with the Houston Chromatographic Procedure

Extraction Procedure, %			Houston Procedure, %		
Nicotine	Nornicotine	Total	Nicotine	Nornicotine	Total
1.68	0.16	1.84	1.61	0.27	1.88
2.85	0.25	3.10	2.78	0.38	3.16
4.30	0.37	4.67	4.16	0.46	4.62
5.54	0.45	5.99	5.65	0.39	6.04
2.96	0.17	3.13	2.75	0.36	3.11
2.85	0.21	3.06	2.71	0.39	3.10
0.43	1.34	1.77	0.38	1.25	1.63
0.39	1.44	1.83	0.35	1.38	1.73

DISCUSSION

The method has been used successfully in this laboratory for more than 2 years and has been found to offer advantages of simplicity and specificity over a number of other alkaloid procedures in use at present.

Some difficulty was experienced in obtaining reproducible results if the barium hydroxide was too finely ground. The reason for this is not readily apparent; therefore, it is recommended that the barium hydroxide be used as obtained from suppliers.

Nicotine and nornicotine were found to be dibasic in all of the nonaqueous systems investigated. A perchlorate precipitate separated in each of the hydrocarbon solutions when titrated with

perchloric acid, but did not interfere with the discernment of the equivalence point. The addition of acetonitrile to the titration solutions prevented precipitate formation; however, as commercial grade acetonitrile is slightly basic, it was necessary to apply a blank correction when it was used. In analyzing tobacco samples the indicator end point was found to be sufficiently sharp not to warrant the continued use of acetonitrile. However, acetonitrile addition to the titration solutions would be advantageous in the assay of commercial nicotine solutions.

It is believed that this procedure offers several advantages as a routine procedure for alkaloid analyses. All of the alkaloids are removed from the tobacco in a 15-minute extraction period, the hydrocarbon extract may be titrated directly, thus eliminating the acid extraction and indirect titration steps necessary in other titration procedures, no distillation is required as in the gravimetric and spectrophotometric procedures, ammonia may be eliminated as an interfering material in the analysis, and a means is provided for determining tertiary and secondary alkaloids in the same solution.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Abner Eisner, Eastern Regional Research Laboratories, for a supply of nornicotine picrate; to Forrest Houston, University of Kentucky, for furnishing some of the samples and analyses used in this study; to Frank B. Wilkinson, Tobacco Branch of the Agricultural Marketing Service, U. S. Department of Agriculture, for permission to use data obtained on samples submitted by his committee; to Joel P. Clingman and Herman H. Musselwhite for the silicotungstate analyses; and to Jacquelyn B. Conrad for her assistance in the precision studies.

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RECEIVED for review January 3, 1955. Accepted May 21, 1955.

Volumetric Determination of Zirconium

An Ethylenediamine Tetraacetate Method Involving Back-Titration with Bismuth

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Zirconium can be determined in acid solution by addition of excess ethylenediamine tetraacetate, followed by back titration with bismuth nitrate. Thiourea is used as the indicator. Very few metals form complexes which are strong enough to interfere with the method. Most complexing anions, including sulfate, phosphate, oxalate, thiocyanate, and tartrate, do not interfere. Fluoride (except in low concentrations) interferes but can be removed easily by fuming with perchloric or sulfuric acid. Slight modification of the procedure permits determination of zirconium in the presence of moderate amounts of thorium, titanium, niobium, or tantalum without separation.

FOR many years macro quantities of zirconium have been determined by tedious, time-consuming gravimetric methods. Recently, however a number of titrimetric procedures have been proposed. Kolthoff and Johnson (*4*) reported an amperometric titration of zirconium, thorium, tin, and uranium with *m*-nitrophenylarsonic acid. White (*8*) developed an alkalimetric method for zirconium following the quantitative isolation of zirconium mandelate. Dhar and Das Gupta (*1*) determined zirconium by either colorimetric or volumetric means following precipitation with oxalohydroxamic acid. Fritz and Fulda (*3*) developed a selective method in which zirconium is titrated with disodium ethylenediamine tetraacetate [disodium (ethylenedinitrilo) tetraacetate EDTA] using Eriochromecyanine indicator. Milner and Phennah (*5*) isolated the zirconium in zirconium-uranium alloys by means of mandelic acid, then added excess EDTA and back-titrated the excess with ferric chloride using salicylic acid indicator. Very recently Olson and Elving (*6*) published a procedure for the amperometric titration of zirconium with cupferron.

In the new method, excess EDTA is added to the sample, forming a stable complex with zirconium. The excess is then titrated with bismuth nitrate using thiourea indicator. The pH, indicator concentration, etc., are the same as described recently for the titration of bismuth (*2*). Few metal cations interfere because zirconium and bismuth form stronger complexes with EDTA than most metals, and in the back-titration bismuth does not react with the thiourea indicator until the metal-EDTA complexes which are much weaker than bismuth have been broken. Another important advantage is that zirconium can be determined in the presence of such anions as phosphate, sulfate, thiocyanate, tartrate, and small amounts of fluoride.

EFFECT OF ANIONS

Zirconium can be titrated in the presence of chloride, nitrate, perchlorate, tartrate, and thiocyanate without any difficulty.

Table I. Titration of Zirconium in Presence of Various Anions

Added	Ratio—$\frac{\text{Anion}}{\text{Zr}}$	Zr Taken, Mg.	Zr Found, Mg.	Difference, Mg.
CNS^-	10:1	43.2	43.2	±0.0
F^-[a]	20:1	43.2	43.0	−0.2
	2:5	23.1	23.1	±0.0
	4:5	23.1	23.1	±0.0
	2:1	9.0	9.0	±0.0
PO_4^{---}	1:1	23.1	23.0	−0.1
	2:1	23.1	23.1	±0.0
SiO_3^{--}	1:1	43.2	43.1	−0.1
	2:1	23.1	23.1	±0.0
SO_4^{--}	4:1	23.1	23.2	+0.1
	4:1	23.1	23.1	±0.0

[a] In each case fluoride concentration was 4 mg. per 100 ml. or less. Concentration of thiourea used was double that recommended in general procedure.

If sulfate is present, a precipitate is formed when excess EDTA is added and the pH is adjusted to 2.0 in preparation for the back-titration with bismuth. Extensive boiling will not completely dissolve this precipitate and the results for zirconium are low. Formation of a precipitate can be avoided by adding tartrate before the EDTA. Results for zirconium are still low unless the solution is heated to boiling after the EDTA has been added and the pH adjusted to approximately 2.0. Apparently the sulfate or mixed sulfate-tartrate complex with zirconium is only slowly converted to the zirconium-EDTA complex at room temperature. If excess EDTA is added to a solution of zirconium chloride at room temperature, a large amount of sulfate may then be added without any interference.

Another way to titrate zirconium successfully in samples containing sulfate is to add a measured excess of EDTA, adjust the pH to about 6, boil to convert quantitatively to the zirconium-EDTA complex, cool, adjust the pH to 2.0, and titrate. This scheme may also be employed for titration of zirconium in the presence of phosphate or oxalate.

Fluoride, in concentrations up to 4 mg. per 100 ml., does not interfere with the titrimetric determination of zirconium. Higher concentrations of fluoride cause indistinct end points and low results for zirconium. Apparently the total fluoride concentration, rather than the ratio of fluoride to zirconium, is the critical consideration with regard to interference.

In the method of Fritz and Fulda (*3*) substances such as colloidal silica absorb the zirconium-indicator complex and make the end point difficult or impossible to detect. The presence of moderate amounts of colloidal silica does not interfere in any way with the present method.

Data for individual titrations of zirconium in the presence of various anions are presented in Table I.

EFFECT OF METAL CATIONS

Although several of the divalent metals form extremely stable complexes with EDTA, none of these was found to interfere appreciably. The slight error observed when cobalt is present may be caused by partial oxidation to the very stable cobalt(III)-EDTA complex. Titration of zirconium in the presence of aluminum is very tedious because the aluminum-EDTA complex is broken only very slowly during the back-titration with bismuth. To avoid this enough cupric ion is added before the EDTA so that the cupric-EDTA complex will be formed instead of the slow-reacting aluminum complex.

When thorium or titanium is present, a very gradual end point is obtained and the determination is not a success. However, a considerable amount of the thorium-EDTA complex is replaced by the bismuth during the back titration. This indicates that both zirconium and bismuth form more stable complexes than does thorium [log K for thorium is 23.2 (*7*)]. It is possible to determine zirconium in the presence of limited amounts of thorium or titanium by adding a large amount of ammonium sulfate as a masking agent.

Niobium and tantalum cause difficulty in almost all analytical methods for zirconium. Niobium and tantalum cannot be kept in a true solution unless a complexing agent is present; precipitation of the acid earths brings down appreciable quantities of zirconium. A completely clear solution can be obtained by dissolving a zirconium sample containing niobium or tantalum in hydrofluoric acid plus nitric acid. Sulfuric acid is then added and the solution is evaporated to white fumes. The sulfuric acid solution is cooled, a strong tartrate solution is carefully added, and the analysis is completed by the method used for titration of zirconium in the presence of sulfate.

Small amounts of iron(III) and tin(IV) are titrated quantitatively along with zirconium. If the concentration of iron is too high, the yellow color of the ferric-EDTA complex interferes with detection of the end point. When more than about 20 mg. of tin are present, precipitation occurs and incorrect results are obtained.

Data for individual titrations of zirconium in the presence of various metal ions are given in Tables II and III. The interfering metal ions are also noted in Table II and the formation constants for the metal-EDTA complexes are given where such information is available. In Table IV results are given for the analysis of some zirconium alloys.

REAGENTS AND SOLUTIONS

Ammonium Tartrate. Prepare a 10% aqueous solution of diammonium tartrate.

Bismuth Nitrate, 0.05M. Dissolve 10 grams of bismuth metal in nitric acid. Carefully dilute with distilled water until a permanent turbidity appears. Immediately add nitric acid in small portions until the solution clears. Repeat this alternate addition of water and nitric acid until the solution is diluted to 1 liter. Standardize by titration against EDTA solution using thiourea indicator.

EDTA, 0.05M. Dissolve 17 grams of reagent grade disodium ethylenediamine tetraacetate [disodium(ethylenedinitrilo)tetraacetate] in 1 liter of water. Standardize against pure zinc metal using Eriochromeblack T indicator and pH 10 buffer (ammonia–ammonium chloride).

Zirconium Chloride, 0.05M. Dissolve 16 grams of zirconium chloride octahydrate (which contain less than 100 p.p.m. of hafnium) in 1 liter of 5% hydrochloric acid. Standardize gravimetrically by ignition to the oxide.

Thiourea, practical grade.

All other chemicals used were of reagent grade.

APPARATUS

All titrations were performed using a 10-ml. buret which could be read easily to within 0.01 ml. Magnetic stirring was employed. A pH meter equipped with standard glass and calomel electrodes was used for all pH measurements.

PROCEDURES

General Procedure. Take a sample or an aliquot containing 13 to 36 mg. of zirconium, and adjust the volume to about 100 ml. Add 10.00 ml. of 0.05M EDTA and adjust the pH to 2.0 with dilute ammonia or dilute perchloric acid. Add 1.3 grams of thiourea, and titrate with 0.05M bismuth nitrate to the first yellow color due to the bismuth-thiourea complex. If the bismuth nitrate solution contains much excess acid it may be necessary to readjust the pH to 2.0 when most of the required bismuth has been added.

Sulfate or Phosphate Present. Add 10.00 ml. of 0.05M EDTA, and 10 ml. of 10% ammonium tartrate, and adjust the pH to about 2.0. Boil for about 5 minutes, then cool and dilute to 100 ml. Adjust the pH to 2.0, add 1.3 grams of thiourea, and titrate with 0.05M bismuth nitrate.

Fluoride Present. Add 5 ml. of 72% perchloric acid, evaporate to fumes, and fume for 5 minutes. Dilute to 100 ml., and

titrate according to the general procedure using 2.5 grams of thiourea. Alternatively evaporate to fumes of sulfuric acid, then follow the procedure given above for sulfate.

Niobium or Tantalum Present. Adjust the sample size so that less than 20 mg. of niobium and tantalum are present. Dissolve the sample in hydrofluoric acid or in hydrofluoric-nitric acid, then add 5 ml. of sulfuric acid, and evaporate to fumes. Thoroughly cool, then slowly add 10 ml. of 10% ammonium tartrate. Complete the determination as outlined in the procedure given above for sulfate.

Thorium or Titanium Present. Follow the general procedure, but adjust the sample size so that less than 25 mg. of thorium or 5 mg. of titanium are present. After adding the EDTA, add 3 grams of solid ammonium sulfate, then adjust the pH to 2.0, add 1.3 grams of thiourea, and titrate with 0.05*M* bismuth nitrate.

Tin Present. Take a sample or aliquot which contains 13 to 23 mg. of zirconium and not more than 20 mg. of tin. If the solution is strongly acidic, adjust the pH to about 0.5 with ammonia. Add exactly 10.00 ml. of 0.05*M* EDTA and follow the general procedure. Calculate the results as millimoles of zirconium plus tin.

Table II. Titration of Zirconium in Presence of Various Metal Ions

Added	Ratio Metal:Zr	pK of Metal-EDTA Complex (7)	Zr Taken, Mg.	Zr Found, Mg.	Difference, Mg.
Ag^{+}	1:1	...	43.2	43.0	−0.2
	2:1		43.2	43.1	−0.1
Al^{+++}	1:1	16.13	43.2	43.3	+0.1
As(III)	1:1		43.2	Interferes	
Ba^{++}	1:1	7.76	43.2	42.8	−0.4
	2:1		43.2	42.8	−0.4
Be^{++}	1:1	...	43.2	43.4	+0.2
	2:1		43.2	43.2	+0.0
Ca^{++}	1:1	10.96	43.2	43.1	−0.1
	2:1		43.2	43.1	−0.1
Cd^{++}	1:1	16.46	43.2	43.0	−0.2
	2:1		43.2	43.0	−0.2
Ce^{+++}	1:1	15.98	43.2	43.6	+0.4
	2:1		21.6	21.8	+0.2
Co^{++}	1:1	16.31	43.2	43.5	+0.3
	2:1		43.2	43.6	+0.4
Cu^{++}	1:1	18.80	43.2	43.0	−0.2
Fe^{+++}	1:1	25.1	43.2	Interferes	
Hg^{+}	1:1	...	43.2	Interferes	
Hg^{++}	1:1	21.8	43.2	43.0	−0.2
	2:1		43.2	43.1	−0.1
K^{+}	1:1	...	43.2	43.0	−0.2
	2:1		43.2	43.1	−0.1
La^{+++}	1:1	15.50	23.1	22.8	−0.3
Li^{+}	1:1	2.79	43.2	43.0	−0.2
	2:1		43.2	43.3	+0.1
Mg^{++}	1:1	8.69	43.2	43.2	±0.0
	2:1		43.2	43.0	−0.2
Mn^{++}	1:1	14.04	43.2	43.0	−0.2
	2:1		43.2	43.1	−0.1
Mo(VI)	1:1		43.2	Interferes	
Na^{+}	1:1	1.66	43.2	43.1	−0.1
	2:1		43.2	43.1	−0.1
Nb(V)	2:5	...	23.1	23.1	±0.0
	4:5		23.1	23.0	−0.1
Ni^{++}	1:1	18.62	43.2	Interferes	
Pb^{++}	1:1	18.04	43.2	43.7	+0.5
	2:1		43.2	43.4	+0.2
Sb(III)	1:1	...	21.6	20.5	−1.1
Sr^{++}	1:1	8.63	43.2	43.0	−0.2
	2:1		43.2	43.3	+0.1
Sn(II)	1:1	...		Interferes	
Ta(V)	1:1	...	21.6	21.9	+0.3
	1:1		21.6	21.9	+0.3
Th^{++++}	2:5	23.2	23.1	23.0	−0.1
	1:1		23.1	23.6	+0.5
Ti^{+++++}	1:1	...	9.3	9.6	+0.3
	2:3		14.6	14.5	−0.1
	1:2		18.8	18.7	−0.1
	2:5		23.1	23.2	+0.1
UO_2^{++}	1:1	...	43.2	43.2	±0.0
	2:1		43.2	43.0	−0.2
Zn^{++}	1:1	16.50	43.2	43.4	+0.2
	2:1		43.2	43.2	±0.0

Table III. Titration of Zirconium-Tin Mixtures

Zr, Mg.	Sn, Mg.	Zr + Sn, Millimole Taken	Zr + Sn, Millimole Found	Difference, Millimole
23.1	12.0	0.355	0.353	−0.002
23.1	12.0	0.355	0.352	−0.003
23.1	21.3	0.434	0.425	−0.009
23.1	22.0	0.440	0.438	−0.002
23.1	22.5	0.444	0.437	−0.007

Table IV. Determination of Zirconium in Alloys

Alloy	Stated Composition, % Zr	Results, % Zr: Standard lab. methods	Results, % Zr: EDTA method
Zr-Zn	14–18	...	16.4
Zr-Zn	15	13.4[a]	13.2
Zr-Th	65	...	63.8
Zr-Zn-Mg	60–80	76.1[b]	78.8
Zr-Mg	...	87.5[b]	87.1
Zr-S	88	...	85.9
Zr-Zn	...	9.07[a]	10.3
ZrF_4	54.9	54.4[c]	55.0
$Zr(SO_4)_2 \cdot 4H_2O$	25.9	26.0[c]	25.7
Zr-Nb	91	...	89.0
Zr-Nb	71	...	69.0

[a] Mandelic acid method.
[b] Remelting and weighing.
[c] Pyrohydrolysis.

DISCUSSION

Hafnium will be titrated along with zirconium. The results obtained will be millimoles of zirconium plus hafnium. When millimoles are converted to weight per cent, the amount of hafnium present must be taken into account. If the zirconium-hafnium ratio is not known, it is usually nearly correct to assume that 2% of the combined metals titrated is hafnium. The "average" atomic weight is then (0.98 × 91.2) + (0.02 × 178.6) or 93.0.

The new method compares favorably with other titrimetric procedures which have been proposed for zirconium. It is more convenient and rapid than titrimetric methods which require preliminary isolation of a precipitate. It compares favorably with the method of Fritz and Fulda (*3*) in accuracy and selectivity and has the additional advantage that sulfate, phosphate, thiocyanate, and certain colloids such as hydrated silica do not interfere. An advantage of the Fritz and Fulda method (*3*) is that zirconium can be titrated in the presence of large amounts of iron. Iron interferes in the new method, but the interference is quantitative if the amount of iron present is not too great.

The selectivity and accuracy of the new method is approximately comparable to the amperometric titrimetric procedure of Olson and Elving (*6*). Their method does not require removal of fluoride but has the disadvantage that the titrant employed (cupferron) is unstable in solution.

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RECEIVED for review February 17, 1955. Accepted July 5, 1955. Presented before the Division of Analytical Chemistry at the 127th Meeting of the AMERICAN CHEMICAL SOCIETY, Cincinnati, Ohio. Contribution No. 395. Work performed in the Ames Laboratory of the Atomic Energy Commission.

Flame Photometric Determination of Chloride in Sea Water

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This investigation involves expansion of the flame photometric technique to the quantitative determination of chloride in sea water. By modifying the "Beilstein chloride test" a determination of chlorides in the range of 0.02 to 0.50*M* has been accomplished. The source of copper is copper nitrate which is introduced directly into the chloride solution; when aspirated into the hydrogen flame the CuCl band systems appear. By measurement of the CuCl band peak of the *D* system, which occurs at 435.4 mμ, the intensity of the emission is found to be proportional to the concentration of the chloride present. Although the sensitivity is not very good, the method provides a useful extension to existing methods for the determination of chlorinity in more concentrated solutions.

FLAME photometric determinations have been quantitatively applied in the analysis of many cations in various substances during the past 10 years. Other aspects of these determinations have included viscosity effects and particle size affecting the determinations of sodium, potassium and calcium as studied by Caton and Bremner (*4*). However, very little work has been done on the quantitative estimation of anions, except for studies in which the anionic interferences in relation to metallic cation emissions were investigated (*1–3*, *5*, *11*, *14*). Recently Dippel, Bricker, and Furman (*6*) applied the flame photometric technique in the quantitative determination of phosphates by measuring the depression of the calcium emission. Band spectra have been used to a certain extent in flame photometry such as, for example, in the determination of calcium by using the calcium oxide band head at 554 mμ. The CuCl band system which occurs in the blue region has been used extensively in the qualitative identification of chlorides in the form of the "Beilstein chloride test." This flame test is dependent on the appearance of the CuCl bands of which the 435.4-mμ band of the *D* system is the most prominent (*8*, *15*). The possibility of a quantitative application of this test was first noted in a publication by Honma and Smith (*10*), who used the spectrographic technique and photographed the CuCl spectra. By modifying the Beilstein test, a flame photometric method for the determination of chlorine in sea water has been developed.

APPARATUS AND REAGENTS

Photometer. The photometer used was the Beckman DU spectrophotometer equipped with a Model 9290 oxyhydrogen burner attachment.

Hood. The flame photometer was placed in an efficient hood and, as a further precaution, a filter system was devised to collect the oxidation products of the flame, particularly noxious copper combustion products. A 2-foot stack equipped with copper heat radiators and a glass wool filter paper collector at the top was placed about 4 inches over the burner. A positive displacement air pump operating at 14 cubic feet per minute sucked the combustion gases and oxidation products through this filter system.

Manometer. A water manometer was placed between the fuel pressure gage and the burner to get more accurate readings of the hydrogen pressure, since the investigation was made at very low hydrogen pressures.

Reagents. All reagents used were C.P. grade chemicals.

Synthetic Sea Water. The standard solutions of chlorides were prepared from a stock solution of synthetic sea water prepared by referring to the table of ionic concentrations of natural sea water given by Sverdrup (*16*). Modification involved the replacement of all chloride values with equivalent amounts of nitrates. The synthetic sea water stock solution consisted of:

Compound	Grams per Liter
Na_2SO_4	3.917
$NaHCO_3$	0.192
NaBr	0.0823
$Mg(NO_3)_2.6H_2O$	13.411
$Ca(NO_3)_2.4H_2O$	2.360
KNO_3	0.981
$Sr(NO_3)_2$	0.0317
$NaNO_3$	34.0635

Copper Solution. The 1.26*M* copper solution was prepared by dissolving 304.45 grams of cupric nitrate trihydrate in distilled water and making up to 1 liter in a volumetric flask. This was then filtered through a fine sintered-glass funnel to remove the insoluble materials.

PROCEDURE

Preparation of Standard Chloride Solutions. Chloride solutions ranging from 0.025 to 0.50*M* were prepared by adding to each 10-ml. volumetric flask 5 ml. of 1.26*M* copper nitrate solution, 2.5 ml. of synthetic sea water stock, and 2.5 ml. of the appropriate standard sodium chloride solution. Each standard solution prepared corresponded to a 1 to 4 dilution of the natural sea water except for the chloride value. A blank solution in which distilled water was used instead of the chloride solution was also prepared at the same time. These solutions constituted standards for the determination of chlorinity in sea water. About 0.5 hour after preparation in some of the higher chloride samples, a faint cloudy precipitation started which did not settle appreciably even after 24 hours. When shaken thoroughly, this cloudiness did not impair the flame photometric readings. The precipitate was later identified as amorphous basic cupric chloride.

Preparation of Sea Water. The sea water unknown was prepared in a manner similar to that used for the standard. Five milliliters of 1.26*M* copper nitrate were pipetted into a 10-ml. volumetric flask, 2.5 ml. of sea water were added, and the solution was made up to volume with distilled water. Both standards and unknown solutions were run off immediately on the flame photometer.

Flame Photometric Technique. Data for the calibration curve were obtained by running the standard chloride solutions prepared with the synthetic sea water matrix and copper nitrate solution. All measurements were made at the wave length 437 mμ. The oxygen pressure was maintained at 13 pounds per square inch and hydrogen pressure at 1 inch of water as indicated by the manometer. Because of the low sensitivity a slit width of 0.34 mm. was selected. At this low hydrogen pressure there was considerable gas pressure fluctuation which was barely observable on the manometer and not detectable with the Beckman gage. However, these small changes in the hydrogen pressure were evident on the flame photometric readings. Even a change of $^1/_{16}$ inch in the manometer reading caused considerable error. First, the approximate concentration of the unknown chloride was obtained and the standards necessary for bracketing it were run off. In the final determination the unknown solution was run off between two standard solutions at most 0.04*M* apart. The solutions were run off consecutively as fast as an accurate reading could be obtained. Usually three repetitions were sufficient. The procedure was repeated for the next sample.

DISCUSSION

Results. The reproducibility of the method was checked by using the burning procedure adopted for the determination of chlorinity in synthetic sea water. Table I shows the reproducibility data obtained.

Accuracy of the determinations was obtained by comparing the flame photometric results with the Mohr titration of chlorides (*12*). The results of a determination of chlorinity in sea water are given in Table II.

Table I. Reproducibility of Method

M Found	
0.220*M* NaCl	0.140*M* NaCl
0.222	0.142
0.220	0.142
0.218	0.140
0.224	0.139
0.220	0.141
0.218	0.140
0.222	0.138
0.221	0.142

Table II. Determination of Chloride in Sea Water[a]

Sample	Mohr's Method, *M* Cl^-	Flame Method, *M* Cl^-
Sea water No. 2	0.1368	0.137
	0.1375	0.134
	0.1378	0.136
	0.1379	0.133
		0.135
Av.	0.1375	0.138

[a] Chloride value of sea water is for 1 to 4 dilution and includes bromide and iodide.
Standard deviation is 0.0018 or 1.3% for flame method.

Production of CuCl Spectra. Preliminary investigations on the satisfactory production of CuCl spectra were first attempted with the original Beilstein's method using a copper wire ring with a copper screen. All results were unsatisfactory. It was necessary to find another means of introducing the copper ions into the flame and this was successfully accomplished by dissolving copper nitrate in the chloride solution. Figure 1 shows the CuCl spectra obtained at the most intense portion of the blue region after a scan of that region. The flame spectra of copper nitrate and copper sulfate are also shown. The peak emission occurs at 437 mμ shifted to the right from the literature value of 435.4 mμ (*15*). To produce the desirable spectra of optimum signal to noise ratio, considerable adjusting of the hydrogen was necessary, since the energy of the flame and the quantity of hydrogen introduced presented difficulties. The competing reactions of interest in this flame are:

$$Cu + O_2 = CuO^* \text{ emits in the same region as CuCl}$$
$$Cu + H_2 = CuH^* \text{ emits in the same region as CuCl}$$
$$Cu + Cl = CuCl^* \text{ desirable at maximum intensity}$$

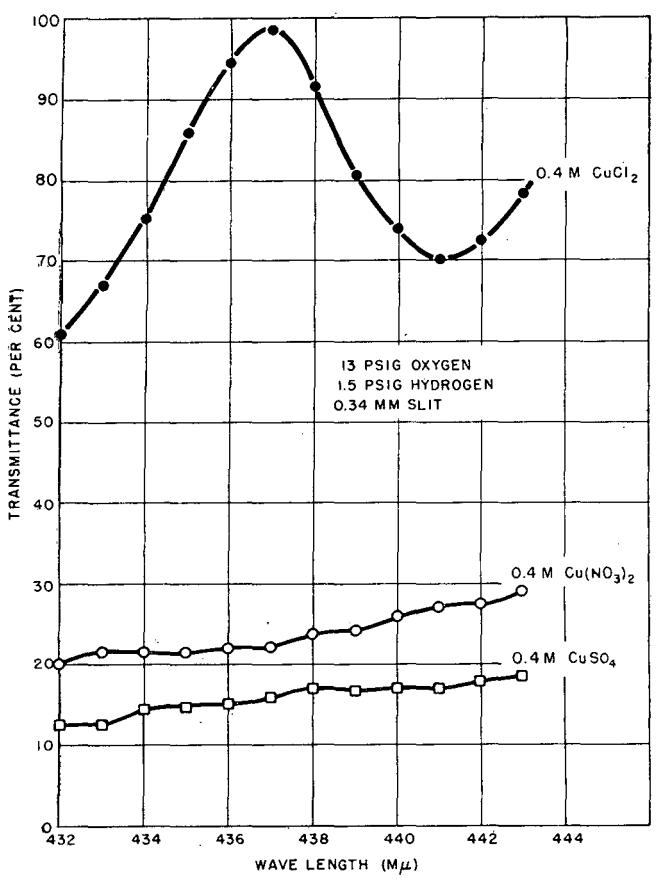

Figure 1. Spectrum of 0.4*M* copper chloride

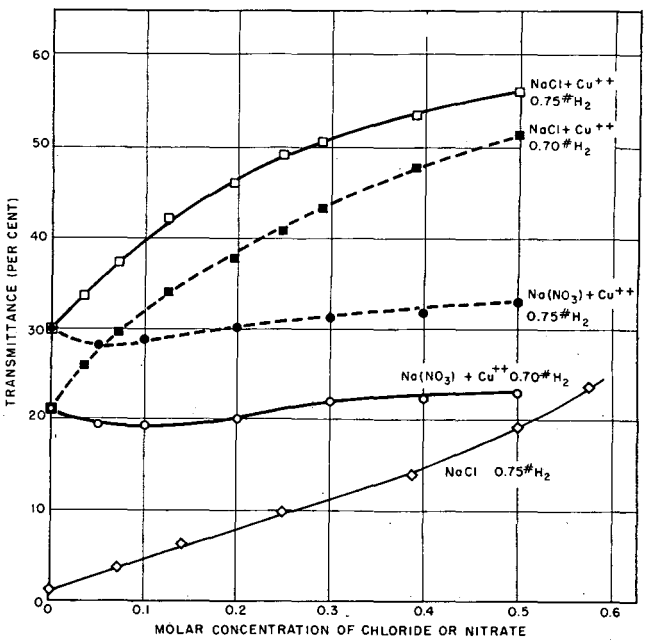

Figure 2. Calibration curve for sodium chloride at 437 mμ

Of these reactions the first two constitute the background. The dissociation energies listed are CuO, 4.5 e.v.; CuH, 2.89 e.v.; and CuCl, 3.0 e.v. (*9*). From the energy consideration a relatively cool flame would reduce the background of the CuO system (*7*, *13*). However, the oxygen pressure cannot be reduced since the atomization of the sample as well as the copper ion concentration in the flame are dependent on this pressure. But by lowering the hydrogen pressure, the flame energy is reduced and the formation of the CuH background is also minimized at the same time, since the concentration of hydrogen is decreased in the flame. Therefore, by maintaining a normal oxygen pressure and a very low hydrogen pressure just sufficient for a flame 0.5 inch high, the flame energy was enough to excite the desired spectra, although there still was considerable background emission.

Effect of Some Variables. Of all the variables involved, the hydrogen pressure was the most critical. A minute change in the pressure gave considerable differences in the CuCl emission. Figure 2 shows the calibration curve for the chloride in the form of sodium chloride. A slight difference in hydrogen pressure resulted in the two curves, one run at 0.7 and the other at 0.75 pound per square inch gage of hydrogen.

To find the effect of copper concentration on the CuCl spectra, a series of experiments was performed in which increments of copper nitrate were added to known concentrations of chloride. The chloride concentration was the same for each solution in the series. A parallel family of curves including the curve for the background emission of copper nitrate was formed in this study and when the background was subtracted from the appropriate points in each curve a horizontal family of curves past the 1 to 1 ratio of copper to chlorine was obtained. As seen in Figure 3,

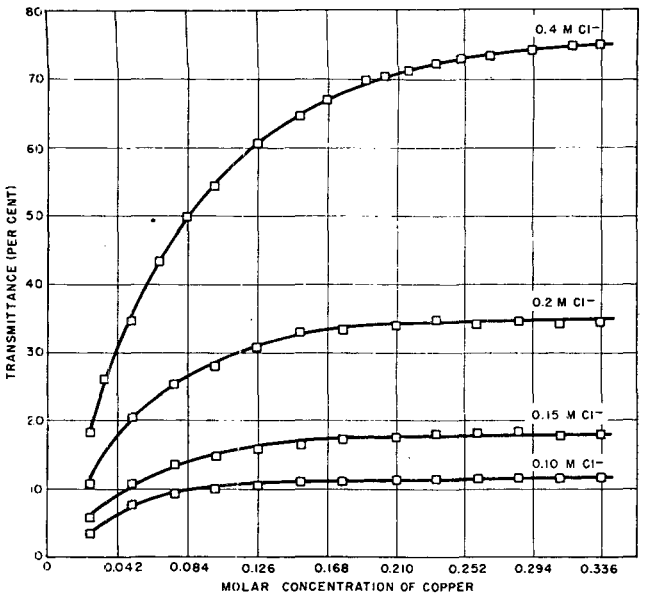

Figure 3. Effect of copper nitrate on copper chloride emission at 437 mμ (corrected for background)

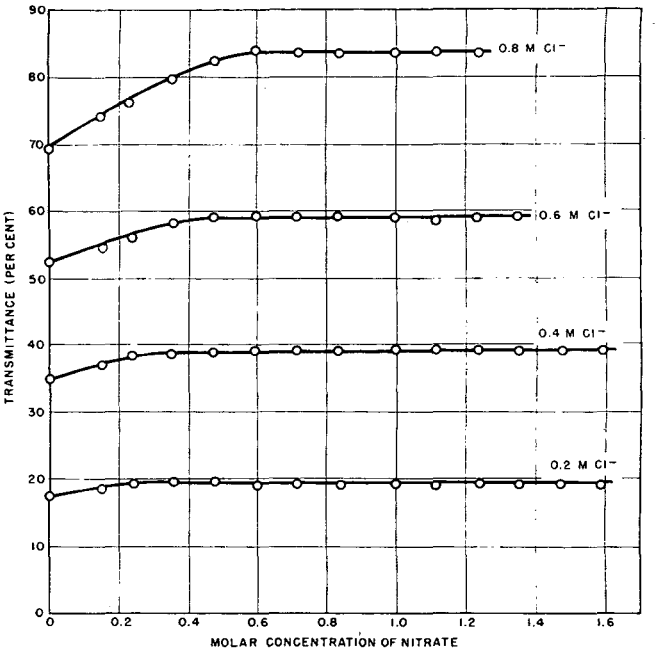

Figure 4. Effect of nitric acid on copper chloride emission

the corrected curves were parallel to the abscissa indicating that excess copper beyond the 1 to 1 ratio had no effect on the CuCl emission over the range investigated.

Since copper was added in the form of the nitrate to develop the CuCl flame bands, the nitrate concentration became important in its effect on these spectra. A study was conducted by adding increments of nitric acid to the cupric chloride solution. The cupric chloride concentration was kept the same for each solution in the series and only the nitrate concentrations were changed. The changes in pH were neglected. The maximum emissions occurred when the nitrate to chloride ratio reached 1.0, from which point the emissions remained constant as seen in Figure 4. By referring to the first point on each curve (shown at the ordinate) which represents the cupric chloride solution without the nitrate, an evaluation of the nitrate enhancement may be obtained. In the 0.2*M* chloride solution there was an 8.6% increase, for the 0.4*M* chloride a 10.6% increase, for the 0.6*M* chloride a 11.4% increase, and for the 0.8*M* chloride a 20% increase in the CuCl emission over that for the standard cupric chloride solutions. The addition of cupric nitrate then served two purposes: It added the required copper ions to the solution and it enhanced the CuCl emission, which was desirable since the sensitivity was low.

Some Interferences on CuCl Spectral Emission. Carbon compounds interfered in the radiative range of the CuCl. Thus, gases such as acetylene could not be used as the fuel because of the strong emissions of the CH and C_2 band systems at 438 mμ. Because of the presence of small amounts of carbonaceous materials in the natural sea water, bicarbonate was added to the synthetic sea water which was used as the matrix for the standards. The presence of copper in the solution had an inhibitive effect on the background emissions of the other metallic cations in the synthetic sea water. Particularly in the case of sodium there was considerable reduction in its background interference. The sulfate had an increasing inhibitive effect on the CuCl emission, with increasing concentrations of sulfate. A 0.4*M* chloride with 0.2*M* sulfuric acid reduced the emission 17.9% and for a 0.4*M* sulfuric acid, the repression was 44.2%.

Calibration Curves of Other Chlorides. Hydrochloric acid which does not have any metallic cation presented the idealized case for the production of CuCl spectra. Neglecting the pH effect, a flame of much higher potential (4 pounds per square inch gage of hydrogen) was used in this determination without undue interferences. Aluminum chloride behaved very similarly. Satisfactory calibration curves were also obtained for magnesium and calcium chlorides but at 0.75 pound per square inch gage of hydrogen. Only very low hydrogen pressures gave satisfactory curves for potassium and ferric chlorides similar to that used for sodium chloride. Generally, with the exception of iron, the excitation potential of the cation gave an excellent indication as to the hydrogen pressures necessary for the determination of the chloride (the higher the excitation potential the more hydrogen could be used). Figure 5 shows the ferric chloride and the hydrochloric acid calibration curves.

Limit of Determination. The limit of determination with any accuracy for the chloride in its sodium salt is 0.02*M* chloride and,

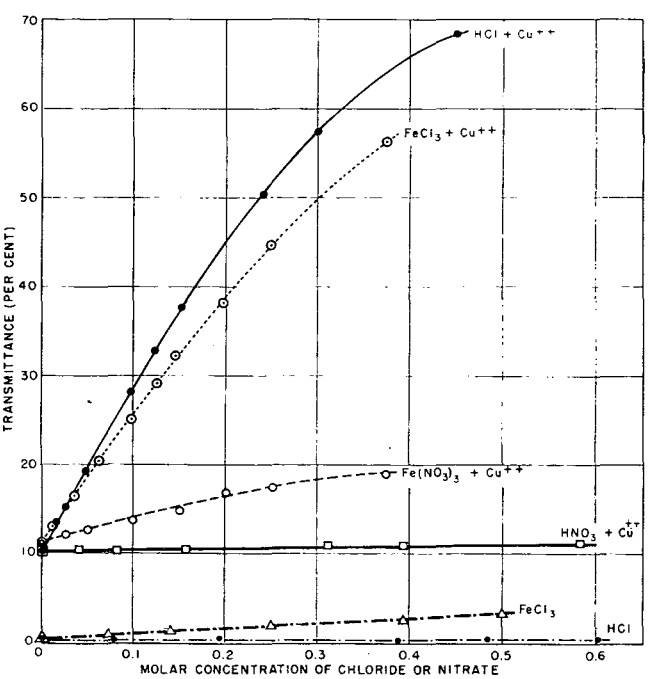

Figure 5. Calibration curves for ferric chloride and hydrochloric acid at 437 mμ

in the idealized cases for such substances as hydrochloric acid or aluminum chloride, the limit is 0.01*M* chloride. In the case of sea water the chlorinity is high and by dilution to 1 to 4 the optimum working range of 0.05 to 0.4*M* chloride was used for this determination. The reading required for a chloride determination by the burning procedure can be obtained in about 1 minute if all solutions are previously prepared.

The method fails to distinguish between the chloride, bromide, or the iodide. The *B* and *C* band systems of CuBr and the *D* and *E* band systems of CuI emit in the same region as the chloride. However, the concentrations of the bromide and iodide in sea water are below the detection limit of the method and so for practical purposes only the chloride value is obtained.

The sensitivity leaves much to be desired and may be improved by using a more sensitive detection system, but even with this improvement it is insensitive compared with the determination of metallic elements. However, for the more concentrated chloride solutions this method should provide a useful extension to the existing methods of chloride analysis.

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RECEIVED for review November 12, 1954. Accepted June 22, 1955.

Spectrophotometric Determination of Pyruvic Acid by the Salicylaldehyde Method

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To determine pyruvic acid in effluents from basic ion exchangers, the salicylaldehyde method for the determination of pyruvic acid was modified and the color reaction studied in detail.

IN AN investigation of the sorption and elution of pyruvic acid using basic ion exchangers, it became desirable to determine the acid according to a spectrophotometric method. Straub (*4*) proposed the salicylaldehyde method for determining pyruvic acid. Sodium hydroxide and a solution of salicylaldehyde in ethyl alcohol were added to the pyruvic acid. The method was revised by Delrue, Devis, and Villano and Rota (*2, 3, 5*), but the results showed poor agreement with Beer's law and the color was not stable.

The present paper deals with a modification of Straub's method. The results are in agreement with Beer's law and the color intensity after the initial 2 hours remains constant for at least another hour.

Braunstein (*1*) reported that all compounds containing a CH_3CO- group linked directly to a hydrogen or carbon atom give a positive salicylaldehyde reaction. Among substances interfering with the determination of pyruvic acid by the salicylaldehyde method are thus acetaldehyde, acetone, diacetyl, levulinic acid, and acetoacetic acid, while formaldehyde, oxalacetic acid, ketobuturic acid, and α-ketoglutaric acid do not interfere. According to experiments by the present author, bisulfite ions and formaldehyde in large amounts prevented to some extent the development of the color.

APPARATUS, REAGENTS, AND SOLUTIONS

Beckman quartz spectrophotometer, DU.
Pyruvic acid, Merck, redistilled twice.
Salicylaldehyde, Merck, for the determination of ketones.
Sodium hydroxide solution, 250 grams per liter.

RECOMMENDED PROCEDURE

In a 50-ml. volumetric flask, 5 ml. of sodium hydroxide solution are added to the sample that must not contain more than 0.01 meq. of pyruvic acid. The solution is diluted with water to about 35 ml. and 0.5 ml. of salicylaldehyde is added. The flask is shaken for a few minutes, 10 ml. of sodium hydroxide solution are added, and the volume is adjusted to 50 ml. with water. About 2 hours after the addition of the color reagent the extinction is read against a reagent blank at 456 mμ. The amount of pyruvic acid in the sample can be evaluated from a calibration curve.

Table I. Effect of Time

Amount of Pyruvic Acid, Meq.	Extinction After, Hours					
	0.5	1	2	3	5	24
0.002	0.194	0.201	0.206	0.204	0.200	0.185
0.003	0.296	0.302	0.310	0.307	0.300	0.281
0.004	0.391	0.407	0.410	0.410	0.395	0.372

EXPERIMENTAL

Some preliminary experiments with the color reagent dissolved in ethyl alcohol were performed. Beer's law was found to be obeyed if the color intensity was read after 30 minutes. The results also showed that the presence of ethyl alcohol affected the consistency of the readings.

Pure salicylaldehyde was used in the following experiments, in which the conditions affecting the development of the color were investigated systematically. The experiments were performed according to the description given above unless otherwise stated.

In order to determine the time for developing the color, three samples were allowed to stand at room temperature and the extinction was read at various intervals (Table I). The color

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intensity, after about 2 hours, was constant for at least another hour.

The influence of the temperature was determined by treating 0.008 meq. of pyruvic acid with color reagent at various temperatures (Table II). The experiments showed that the best color development was obtained at room temperature.

Table II. Effect of Temperature

Temp., ° C.	Extinction
20	0.810
50	0.728
75	0.570
100	0.565

Table III. Effect of Sodium Hydroxide Concentration

Sodium Hydroxide Added, Ml.	Extinction
5	0.065
10	0.178
15	0.202
20	0.199
30	0.204

The amount of sodium hydroxide was varied by adding different volumes of sodium hydroxide solution to a sample containing 0.002 meq. of pyruvic acid (Table III). The results showed that at least 15 ml. of the sodium hydroxide solution must be added for complete color development.

To determine the optimum concentration of color reagent, 0.002 meq. of pyruvic acid was treated with varying amounts of salicylaldehyde (Table IV). At least 0.5 ml. of salicylaldehyde was needed for maximum extinction as can be seen from the figures.

The extinction curves versus wave length at four different concentrations (0.008 to 0.001 meq.) of pyruvic acid were determined. The curves had a maximum at 456 mμ, independent of the pyruvic acid concentrations. Because experiments showed that the reagent blank had a considerable light absorption at 456 mμ, the readings were made against a reagent blank.

Table IV. Effect of Salicylaldehyde Concentration

Salicylaldehyde Added, Ml.	Extinction
0.1	0.131
0.3	0.184
0.5	0.203
0.7	0.202
0.9	0.181

Experiments with 0.0005 to 0.02 meq. of pyruvic acid were carried out to get a calibration curve. Beer's law was found to be valid up to an extinction of 1.0, allowing a maximum amount of 0.01 meq. of pyruvic acid to be determined according to the calibration curve. The relative error in the range of 0.0005 to 0.01 meq. was less than 2 %.

ACKNOWLEDGMENT

The author is greatly indebted to Olof Samuelson for valuable suggestions during the work and to Nils Karlson for his assistance.

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RECEIVED for review November 23, 1954. Accepted July 5, 1955.

Determination of Oxygen in Sodium and in Sodium-Potassium Alloy by the Butyl Bromide Method

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Modifications are suggested for the butyl bromide method for determining oxygen in sodium. Tools for breaking the glass capsule and for cutting the sodium are described. Chromatographic purification of the organic reagents by use of silica gel was found to be effective and far more rapid than earlier purification methods. Data are included to show the effectiveness of this type of purification, along with the appropriate drying agents. Liquid sodium-potassium alloy can be handled by initially freezing the alloy and then controlling the speed of reaction by the temperature. After the potassium has reacted, the sodium reaction is hurried by increasing the content of the butyl bromide.

IMPETUS has been given to the study of impurities in sodium, because the liquid metal is to be used as a heat transfer agent in a new reactor designed by North American Aviation's Nuclear Engineering and Manufacturing Department. Koenig and Vandenberg (*3*) presented liquid sodium as a noncorrosive coolant, and the corrosive properties are attributed to impurities, chiefly oxygen, potassium, calcium, and magnesium (*9*). Further information was contained in a report in *Chemical and Engineering News* (*1*).

Two general methods for the determination of oxygen in sodium have been described. In the first, the original apparatus of Pepkowitz and Judd (*5*) (mercury method) was modified by Williams and Miller (*11*) and improved results were reported by the originators (*5*) when purified helium gas was used to purge the apparatus. From laboratory tests, Silverman and Bradshaw (*8*) found that the sodium-potassium bubbler purification method (*6*) reduced the oxygen content of the helium gas from 5 to 0.1 p.p.m. The Williams and Miller (*11*) modification has been used successfully at this laboratory by C. T. Young (*7*), and the difficulties encountered have to do only with sample preparation. The sodium sample must be obtained in a special thin glass container that can be broken in the Pepkowitz setup.

The second method for oxygen determination is that of White, Ross, and Rowan (*10*) (butyl bromide method). This procedure has practical advantages in that test samples of sodium metal may be obtained in glass capsules or in metal capsules which are glass-enclosed and samples of bulk (as in 1-pound cans) sodium may be obtained. This report suggests practical methods for breaking the glass capsules, a minor change in the analytical procedure, and desirable improvements in the preparation of

Table I. Water Content of Dried Reagents, P.P.M.

Method of purification Distillation Drying agent	None	H_2SO_4 Dist. Na	H_2SO_4 Dist. $CaSO_4$	H_2SO_4 Dist. $CaCl_2$	Gel Dist. Na	Gel Dist. $CaCl_2$	Gel No P_2O_5	Gel No Gel	Gel Dist. Gel	Gel No Al_2O_3
Butyl bromide	175 182		30 30	6		50		74	70	0, 0, 0 0, 0, 0
Decalin	10	2 4					0, 0 0, 0 0, 0			
Hexane	15	3					0, 0 0, 0 0, 0			

the reagents. Precautions and modified directions for the determination of oxygen in sodium-potassium alloy are also included.

BREAKING THE GLASS CAPSULES

White, Ross, and Rowan (*10*) place the sodium-in-glass sample in a tube (with reinforced bottom) containing a sufficient amount of butyl bromide and hexane to cover the sample adequately. The glass sample is then crushed by a heavy glass rod.

The glass capsule usually has a drawn-out end with a melted knob, formed when the capsule is sealed. A modified channel lock pliers, with jaws bent at right angles to the handle and with handle extensions, is recommended for breaking this end of the glass capsule (under hexane). In the process of breaking the capsule, the handle of the pliers is parallel to the vertical axis of the containing beaker or test tube, and the glass capsule is at a 45° angle with the same axis.

The container is filled with dry hexane, to a height of about 1 inch above the glass capsule. A dry, inert gas blanket must be used to prevent pickup of moisture by the reagents. The drawn-out end of the glass capsule is pointed upward, and is easily snapped off with the channel lock pliers without much effort. Depending on the size of capsule and its sodium content, other breaks may be made in the capsule at convenient times. The opening of the capsule by cracking, instead of by pounding, saves occasional breakage of the container and loss of sample.

After the sample is opened, the initially large volume of hexane used to cover the unbroken capsule is no longer necessary, and excess liquid is decanted off until the height of hexane is about 1 inch above the broken glass. The remaining volume of hexane is estimated, and 40% of this volume of dry butyl bromide is added. This is the minimum per cent volume of butyl bromide in hexane which will react with the metallic sodium, at a reasonable speed. From here, the procedure is as described (*10*).

CUTTING AND SAMPLING SODIUM METAL UNDER DECALIN OR HEXANE

The sodium metal, obtained after breaking a capsule, may be in pieces of such volume that reaction with bromide would be slow. It is possible to press out the lumps with the flattened end of a stirring rod, but a more convenient way is to use "cutting" pliers. The pliers are of the long-nose type with sharpened edges, and have extended handles. The sodium metal is easily cut into small pieces, under hexane.

This type of pliers is very useful when sampling a large mass of sodium metal, such as a 1-pound sample, under Decalin or hexane. The exposed portion of the sodium mass may be removed, and the smaller chunks may then be cut with the special pliers.

PURIFICATION OF REAGENTS

White, Ross, and Rowan (*10*) suggest preliminary washing of the reagents with sulfuric acid, which is a time-consuming affair. It is here recommended that a chromatographic purification of the reagents, and the storing of reagent over selected drying agents are much to be preferred. No blanket of gas was maintained over the reagents. Karl Fischer determinations showed no moisture pickup. Available tanks of argon and helium gas contained 5 p.p.m. of oxygen, and hydrogen contained about 2 p.p.m.

In this manner, distillation, sulfuric acid washing, and subsequent neutralization are not required.

PREPARATION OF CHROMATOGRAPHIC COLUMN

Mix silica gel (28 to 200 mesh) with Celite in the volume ratio of 5 to 1. Plug a glass chromatographic column about 4.5 cm. in diameter and 40 cm. in length (500-ml. dispensing buret) at the lower end with glass wool, wash the silica gel–Celite mix with anhydrous ethyl ether, then slurry the mix into the column with the anhydrous ether. Cover the drained mix with glass wool. Cap the column to keep out dust and moisture.

Hexane. Run hexane through the column (discarding the first 50 ml.) at the rate of about 2 liters per hour. Store over anhydrous phosphorus pentoxide. Check the moisture content by the Karl Fischer method (*2, 4*) after 24 hours of contact (Table I). The hexane should not contain more than 0.5 p.p.m. of water. Make a sulfuric acid wash test for unsaturates after every fourth liter. Contamination of the column is usually noted visually. Discard the column when the contamination covers one half of the column.

When a good grade of hexane is available, the column may be used to clean 15 to 20 times its volume of hexane.

The presence of heptane in the hexane is not detrimental.

Decalin. Treat exactly the same as hexane (Table I).

Butyl Bromide. A double column is required. Pass the butyl bromide through the column at the rate of 2 liters per hour. Pass the eluant through a second column of similar size. Store over freshly activated alumina. Check the moisture content by the Karl Fischer method after 24 hours of contact (Table I). The butyl bromide should not contain more than 0.5 p.p.m. of water. Make a sulfuric acid test after every fourth liter. Contamination of the column will be noted visually. Discard the column when the contamination covers one half of the column.

This procedure for purifying the butyl bromide is obviously far superior to the sulfuric acid method.

DETERMINATION OF OXYGEN IN SODIUM-POTASSIUM SAMPLES BY BUTYL BROMIDE METHOD

Attach rubber or Tygon tubing, oven-dried glass tubing, and a long Drierite tube to a helium tank and allow the gas to flow for about 15 minutes. Make a preliminary check for moisture pickup in dry hexane, using the Karl Fischer method.

Place the very clean glass-enclosed sample of sodium-potassium alloy in a reaction vessel (*10*) (or a 500-ml. tall-form beaker which has been sealed to a borosilicate glass tube of the same diameter) and add sufficient dry hexane to reach a height of about 1 inch above the glass capsule. Cover the mouth of the reaction vessel with dried aluminum foil. Pierce the foil with the lead-in tube from the helium tank.

Allow the gas to flow for several minutes before placing the reaction vessel assembly in liquid nitrogen or in an acetone–dry ice bath. Cool the hexane to about −10° C., and allow the vessel to remain in the bath for several minutes more until the sodium-potassium is solid. Temporarily remove the foil cover and use the tongs to clip the end from the glass capsule. Continue the gas flow throughout the entire operation. Lower the level of hexane in the vessel by pipetting off the excess hexane. Estimate the volume of the remaining hexane and add butyl bromide, equivalent to 10% of the volume. Remove the reaction flask from the cooling bath and allow it to warm in air.

At about 0° C. the metal surface of the sodium-potassium alloy turns blue and the eutectic sodium-potassium alloy (melting point, −10° C.) is liquid. Gradually expose more metal surface by agitating or cracking the capsule. Reaction bubbles will be noted at about +10° C. As the reaction becomes somewhat rapid at 40° C., the temperature is maintained at 30° to 35° C. by infrequent cooling of the mixture in the dry ice bath. The entire sample will soon turn blue, and white particles of insoluble potassium bromide will separate. The action will slow, even at 40° C., when only sodium metal is left. At this time add

another portion of butyl bromide equal to three times the volume of the previous addition, and with the aid of heat and agitation, run the reaction to completion as for sodium metal (*10*). Keep the vessel covered whenever possible and continue the flow of helium until the reaction has been completed.

DISCUSSION OF RESULTS

Eutectic sodium-potassium alloy is Na:2K in mole ratio or 22 and 78% by weight, respectively. The alloy (*1*) is liquid at room temperature. While sodium metal requires a 40% butyl bromide mixture, eutectic sodium-potassium alloy reacts rapidly at lower temperatures, with 10 to 25% butyl bromide mixture. When the reaction slows at 35° C. with only a 10% butyl bromide mix, it is to be assumed that the remaining unreacted metal is sodium and that the potassium has been consumed. Aside from the necessary cooling precautions, eutectic sodium-potassium alloy is dissolved more smoothly than sodium alone.

The composition of the sodium-potassium alloy must be known before the oxygen content is calculated. If a weighed sample is obtainable, the percentage composition may be calculated from the total bromide content and the weight of sample, by titration with acid, by conversion to salts, or by similar methods.

ACKNOWLEDGMENT

The two sets of pliers were partly designed and constructed by William Taylor.

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RECEIVED for review November 1, 1954. Accepted July 5, 1955.

Spectrophotometric Determination of Methoxyl

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Methoxyl is hydrolytically cleaved to methanol, the latter oxidized to formaldehyde, and the formaldehyde determined colorimetrically after condensation with chromotropic acid. This method is specific for the methoxy group in comparison with the Zeisel reaction. Applications to several types of compounds are tabulated and comparisons with theoretical values indicate the method is accurate. A modification is shown for application on a micro scale.

THIS investigation was undertaken to develop a methoxyl determination applicable to research problems concerned with the study of some alkaloids and compounds isolated from alcoholic beverages.

In general, the determination of methoxyl utilizes the Zeisel reaction in which the methoxyl is converted to methyl iodide. The methyl iodide is quantitatively absorbed and converted to silver iodide, ionized iodide, or iodate. Then, the iodide or iodate is determined by a standard method. The use of sulfuric acid for hydrolytically cleaving the methoxy group, as employed in this investigation is not new; alkalies and mineral acids have been used in hydrolysis and fusion processes to convert methoxyl to methanol in many natural products such as wood (*8*), pectin (*11*), and lignin (*9*). The methanol produced under these conditions has been determined by specific gravity, colorimetry, and several other means. Pavolini and Malatesta (*12*) employed satisfactorily to nine alkaloids and six phenols this same type of hydrolysis in their determination of methylenedioxyl and methoxyl, utilizing warm 80% phosphoric acid to convert methylenedioxyl to formaldehyde and warm concentrated sulfuric acid for the hydrolysis of methoxy groups to methanol. The methanol was oxidized to formaldehyde with potassium dichromate, and the formaldehyde produced from both reactions was determined with Nessler's or Tollen's reagent.

The value of chromotropic acid as a reagent for the detection of formaldehyde was demonstrated by Eegriwe (*7*). Bricker and Johnson (*4*) developed the test into a quantitative procedure and discussed a number of the factors affecting color development. In addition Bricker and Vail (*6*) extended the use of chromotropic acid to the microdetermination of formaldehyde, in the presence of large concentrations of various organic compounds. Further, Bricker and Roberts (*5*) described the determination of end unsaturation in organic compounds by converting the end carbons to formaldehyde and using the chromotropic acid condensation product for a quantitative measure of the doubly bonded carbon. Boos (*3*) utilized permanganate solution to oxidize methanol to formaldehyde and determined the latter colorimetrically after condensation with chromotropic acid. Beyer (*2*) adapted the procedures of Bricker and Johnson (*4*) and Boos (*3*) to the determination of methanol in distilled spirits. Based on a review of methods for the colorimetric determination of methanol via formaldehyde, Mathers (*10*) specified the conditions necessary, in the various stages of the procedure, to determine methanol with reproducibility from the formaldehyde–chromotropic acid condensation product.

EXPERIMENTAL

Apparatus. Volumetric flasks and pipets.

Simple distillation apparatus.

Beckman Model DU spectrophotometer with cuvettes, 1-cm. square.

For microdeterminations. Total condensation, variable take-off type distillation column with a packed section 1 × 20 cm. lagged with a silvered vacuum jacket. The packing material consists of single turn glass helices.

Reagents. Standard methanol solution (conforming to A.C.S. specifications), 20 mg. per 100 ml. in 5.5% v./v. ethyl alcohol.

Ethyl alcohol (U.S.P.), 5.5 to 6% v./v.

Chromotropic acid (Eastman Kodak Co. No. P. 230) solution (4,5-dihydroxy-2,7-naphthalenedisulfonic acid), 1 gram per 25 ml. of water. Prepare fresh daily.

Potassium permanganate (A.C.S.) solution, 3 grams of potassium permanganate plus 15 ml. of 85% phosphoric acid (A.C.S.) diluted to 100 ml. with distilled water.

Sodium bisulfite, C.P.

Sulfuric acid (A.C.S.) concentrated.

Procedure. Introduce a weighed sample (approximately 0.1 gram) into a 250-ml. flask and attach to an efficient reflux condenser. Through the condenser add about 10 ml. of concentrated sulfuric acid (double the quantity of sulfuric acid for larger samples) and heat to fumes of sulfur trioxide for 5 minutes. Cool the reaction mixture and dilute with 75 ml. of water, added through the condenser. Again cool the solution, remove the condenser, attach a simple distilling head, and distill *about* 45 ml. of the liquid into a 50-ml. volumetric flask containing 3 ml. of 95% ethyl alcohol and make to the mark with water. Pipet 1 ml. of this solution into a 50-ml. volumetric flask, set in an ice bath, and add 2 ml. of chilled permanganate solution. Allow oxidation to take place for 30 minutes at ice bath temperature, then destroy the excess oxidant with approximately 0.2 to 0.3 gram of sodium bisulfite. To the clear solution add 1 ml. of chromotropic acid solution followed by the slow addition of 15 ml. of concentrated sulfuric acid with swirling. Set the open flask in a 55° to 65° C. water bath for 30 minutes. Dilute the solution with water, cool to room temperature, and dilute to volume with water. Prepare a reference blank and a standard methanol color by treating, respectively, 1 ml. of 5.5 to 6% ethyl alcohol and 1 ml. of the standard methanol solution in the above manner beginning with the oxidation step. Read the absorbance of the sample and standard methanol at 570 mμ versus the reference ethyl alcohol blank.

The absorbance is directly proportional to the quantity of formaldehyde, which in turn is proportional to the methoxyl and methanol, respectively. However, the color intensity is also a function of temperature, and thus, it is necessary to read the absorbances of both sample and standard methanol solution at nearly identical temperatures.

Calculations. The quantity of methoxyl is calculated by the following formula:

$$\frac{A_s}{A_m} \times F \times M \times R = \text{wt. \% of methoxyl}$$

A_s is absorbance of sample.
A_m is absorbance of methanol standard.
F is dilution factor of sample.
M is per cent by weight of methanol in standard.
R is molecular weight ratio of methoxyl to methanol.

MICRODETERMINATION

Introduce a weighed sample of approximately 1 mg. of sample into a 100-ml. round-bottomed flask, attached to a reflux condenser. Add 10 ml. of concentrated sulfuric acid and heat to fumes of sulfuric acid for 5 minutes. Then cool and add about 40 ml. of water through the reflux condenser. Remove the condenser, attach the flask to the distilling column, and place the solution under total reflux for 20 minutes. Maintaining a reflux ratio of 30 to 1, collect 2 ml. of distillate. Pipet 1 ml. of distillate into a 10-ml. volumetric flask and add 1 drop of 95% ethyl alcohol. Cool the flask in an ice bath, add 2 ml. of chilled permanganate solution, and allow oxidation to proceed for 30 minutes. Destroy the excess permanganate with sodium bisulfite, add 1 ml. of chromotropic acid solution, and 6 ml. of concentrated sulfuric acid, and heat the mixture for 30 minutes in 55° to 65° C. water. Then cool the flask, fill to the mark with sulfuric acid, and read the absorbances at 570 mμ versus a reference blank prepared with 1 drop of ethyl alcohol. Treat 0.2 ml. of standard methanol solution in the same manner as the sample. Calculate the methoxyl content of the sample according to the preceding equation.

DISCUSSION

The oxidation of methanol by acid permanganate solution is an equilibrium type reaction in which less than a 50% yield of formaldehyde is present under equilibrium conditions. For this reason it is one of the most critical steps in the procedure (*10*). Methanol and ethyl alcohol are oxidized to the respective aldehydes and acids with the possibility of the former giving some carbon dioxide. The acetaldehyde produced from the ethyl alcohol interferes with the determination of formaldehyde to some extent by giving a yellow colored solution, which has some absorption at 570 mμ. Interference due to acetaldehyde is minimized by the use of a reference blank in which an identical quantity of ethyl alcohol is treated in the same manner as the sample.

The oxidation of the methanol can be carried out in the absence of ethyl alcohol, but especial care must be taken to have both sample and permanganate at approximately ice bath temperature. This tends to prevent the oxidation of methanol to formic acid or carbon dioxide even though a large excess of oxidant is present. In Table I are shown results of the determination of methoxyl, both with and without the addition of ethyl alcohol to some of the samples. More accurate values were obtained by oxidations in the presence of ethyl alcohol; however, in work not reported there was some indication that the absence of ethyl alcohol might be preferable when the content of methanol in the distillate was high, about 4 to 5%, and again in samples where only a few micrograms of methanol were present.

Table I. Results of Methoxyl Determinations

Compound	Methoxyl, Weight %: Calculated	Found
Anisaldehyde, $C_8H_8O_2$ (*p*-methoxybenzaldehyde)	22.8	22.8
α-Chloroisobutyraldehyde dimethyl acetal, $C_6H_{13}O_2Cl$	40.6	40.2
α-Methoxyisobutyraldehyde 2,4-dinitrophenylhydrazone, $C_{11}H_{14}O_5N_4$	11.0	10.8
Methyl Cellosolve, $C_3H_8O_2$ (2-Methoxyethanol)	40.7	40.4 40.6
Cocaine hydrochloride, $C_{17}H_{22}O_4NCl$ (benzoyl methyl ecgonine)	9.1	8.9 8.8
Codeine sulfate hydrated, $C_{36}H_{54}O_{15}N_2S$ (methyl morphine)	7.9	7.5[a] 7.8
Codeine sulfate hydrated, $C_{36}H_{54}O_{15}N_2S$ (microdetermination)	7.9	7.5[a] 8.0 7.8
Methyl α-chloroisobutyrate, $C_5H_9O_2Cl$	22.7	22.9
Methylcellulose, $C_7H_{12}O_5$ (monomethyl ether)	17.6	17.5
Methyl methacrylate, $C_5H_9O_2Cl$	31.0	30.6
Pectin	..	3.5[a] 3.5
Vanillin, $C_8H_8O_3$ (4-hydroxy-3-methoxybenzaldehyde)	20.4	20.2 20.2
Glycine	0	0
Glycerol	0	0
Ethylene glycol	0	0
p-Dimethylaminobenzaldehyde	0	0
Lactic acid	0	0
Tartaric acid	0	0
Methyl iodide	21.8	7.6
Glycolic acid (hydroxyacetic acid)	40.8	24.7

[a] Oxidized in absence of ethyl alcohol.

Although no attempt was made to apply the microdetermination to samples other than codeine sulfate, it is believed the method is applicable to any compound containing methoxyl.

The esters and acetal were readily hydrolyzed by distillation from 10% sulfuric acid solution, other compounds were hydrolytically cleaved by heating with concentrated sulfuric acid on a steam bath for 1 to 2 hours, while a few compounds required heating to fumes of sulfur trioxide to ensure complete hydrolysis. Therefore, the latter step was required to make the procedure general.

Beroza (*1*) has demonstrated that methylenedioxyl or other labile methylene groups can be converted to formaldehyde by hydrolytic cleavage of the molecule; therefore, compounds containing this group interfere with methoxyl determinations. In this work two compounds shown to interfere with the test were glycolic acid and methyl iodide. This was not unexpected, because any compound which can be decarboxylated or hydrolyzed to methanol will yield positive tests by this reaction. From the data presented in Table I it is apparent that the methoxy group in a rather wide variety of compounds can be determined accurately.

Methoxyl was determined on several different types of materials and some of these substances were specifically selected, because of their difficult solubility in the solvents ordinarily used in the Zeisel reaction. The results show that hydrolytic cleavage was accomplished in concentrated sulfuric acid. The simplicity of the apparatus and the speed with which the determination can be made are added advantages of this method. No interference is offered by other alkoxy or alkimide groups in the determination of

the methoxy group by this method, whereas the Zeisel reaction is not specific for the methoxy group.

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RECEIVED for review February 18, 1955. Accepted July 26, 1955.

Spectrophotometric Method for Determining Hydroxylamine Reductase Activity in Higher Plants

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A rapid, simple color test for hydroxylamine has been adapted for the quantitative determination of micromolar amounts of hydroxylamine in biological materials. The hydroxylamine reacts quantitatively with an excess of 8-quinolinol to form the stable 5,8-quinolinequinone-5-(8-hydroxy-5-quinolylimide). When measured spectrophotometrically at its absorption peak, 705 mμ, this compound obeys Beer's law over the range of 0 to 5 × 10^{-2} millimole of hydroxylamine per ml. of solution. The procedure has been applied successfully for the determination of hydroxylamine reductase activity in soybean leaves.

PREVIOUS procedures for the determination of hydroxylamine have been reported by Blom (*2*), Endres and Kaufmann (*5*), and Csaky (*4*). These procedures involve the oxidation of hydroxylamine to nitrous acid, which is then determined colorimetrically by the Rider and Mellon (*7*) or Shinn (*8*) procedures. Although these methods are sensitive for the resulting nitrous acid formed, the oxidation of the hydroxylamine is neither specific nor simple. Nason and others (*6*) have demonstrated the presence and the requirements of hydroxylamine reductase in soybean leaves using Csaky's (*4*) method for the determination of hydroxylamine.

Colter and Quastel (*3*) have recently reported a manometric procedure for the determination of hydroxylamine, which depends upon the oxidation of hydroxylamine by manganese dioxide to produce nitrous oxide. This method, however, is not very sensitive and is also rather involved.

Berg and Becker (*1*) have reported a very sensitive and specific qualitative color test for hydroxylamine. This test has now been applied quantitatively to biological materials for the determination of micromolar quantities of hydroxylamine. The hydroxylamine reacts quantitatively with an excess of 8-quinolinol in the presence of ethyl alcohol and sodium carbonate to form the stable 5,8-quinolinequinone-5-(8-hydroxy-5-quinolylimide) designated as Indooxine. This compound exhibits a very prominent adsorption peak at 705 mμ (Figure 1).

REAGENTS

8-Quinolinol Solution. Dissolve 1.0 gram of 8-quinolinol (Eastman Kodak Co.) in 100 ml. of absolute ethyl alcohol. Keep tightly stoppered.

Sodium Carbonate Solution. Sodium carbonate, C.P., 1.0*M* solution.

Trichloroacetic Acid Solution. Water solution, 12% by weight.

Manganese Chloride Solution. Manganese chloride, C.P. 0.001*M* solution.

Reduced Diphosphopyridine Nucleotide Solution. Reduced diphosphopyridine nucleotide (Sigma Chemical Co.), 3 × 10^{-4}*M* solution. Keep refrigerated.

Phosphate Buffer Solution, pH 6.8. Adjust the pH of a 0.05*M* monobasic sodium phosphate solution to pH 6.8 by the addition of 0.05*M* dibasic sodium phosphate solution. Keep refrigerated.

Hydroxylamine Standard Solution. Dissolve 0.0695 gram of dry, recrystallized hydroxylamine hydrochloride, C.P., in water and dilute to 1 liter. Take a 250-ml. aliquot of this solution, adjust to pH 3.0 with 0.01*N* hydrochloric acid, and dilute to 1 liter. This solution contains 0.25 micromole of hydroxylamine per milliliter, and is stable for several days.

PROCEDURE

Hydroxylamine Standard Curve. In a 15 × 125 mm. test tube place up to 1.0 ml. of the hydroxylamine standard solution (0.00 to 0.25 micromole of hydroxylamine), 1.0 ml. of the 0.05*M* phosphate buffer, pH 6.8, and water to bring the volume to 2.8 ml. Add 0.2 ml. of the trichloroacetic acid solution. Follow with 1.0 ml. of the 8-quinolinol solution and swirl gently. Next, add 1.0 ml. of the 1.0*M* sodium carbonate solution, shake vigorously, and stopper before placing in a boiling water bath for 1 minute to develop the green color. On removal from the water bath, cool for 15 minutes, and then read in the Beckman DU spectrophotometer at 705 mμ using matched 1-cm. Corex cuvettes. Carry out simultaneously a blank determination which contains everything but hydroxylamine and set at 100% *T*. Within this concentration range (0.00 to 0.25 micromole of hydroxylamine), the Beer-Lambert law is obeyed, giving a

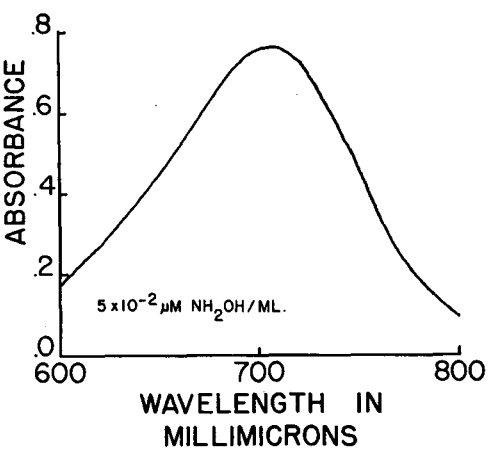

Figure 1. Absorption spectrum of Indooxine

Table I. Effect of Hydroxylamine Concentration on Absorbance

Hydroxylamine Concentration $\times 10^{-2}$ μM per Ml.	Absorbance at 705 mμ				
	I	II	III	IV	Av.
1	0.146	0.154	0.151	0.153	0.151
2	0.305	0.304	0.315	0.314	0.310
3	0.442	0.451	0.449	0.457	0.450
4	0.599	0.599	0.592	0.594	0.596
5	0.720	0.762	0.742	0.749	0.743

Table II. Soybean Hydroxylamine Reductase Activity

Time, Minutes	Plant Extract Absorbance at 705 mμ	Boiled Plant Extract Absorbance at 705 mμ	Hydroxylamine Loss Corrected, 10^{-2} μM/Ml.
0	0.621	0.747	0.85
5	0.429	0.683	1.70
10	0.328	0.622	1.95
15	0.270	0.599	2.20

straight line when the absorbances are plotted against the hydroxylamine concentrations (Table I). The color, under these conditions, develops completely in 15 minutes and is stable for at least another 30 minutes.

Plant Tissue Extract. Grind 1 gram of leaves from young soybean seedlings in a cold mortar with 1.0 gram of washed sand and 10.0 ml. of cold 0.05M phosphate buffer pH 6.8. Strain the resulting brei through cheesecloth, centrifuge in the cold at 20,000 times gravity for 10 minutes, decant, and use the supernatant solution directly for hydroxylamine reductase assay.

Hydroxylamine Reductase Assay. In a 15 × 125 mm. test tube, place 1.0 ml. of the hydroxylamine standard solution (0.25 micromole of hydroxylamine), 1.0 ml. of the 0.05M phosphate buffer, pH 6.8, 0.1 ml. of the 3 × $10^{-4}M$ reduced diphosphopyridine nucleotide solution, 0.1 ml. of the 0.001M manganese chloride solution, 0.3 ml. of the plant tissue extract, and water to make a final volume of 2.8 ml. Incubate in a 30° C. water bath. Stop the enzyme action by the addition of 0.2 ml. of the trichloroacetic acid solution. Follow with the addition of 1.0 ml. of the 8-quinolinol solution, and swirl gently. Add 1.0 ml. of the 1.0M sodium carbonate solution, shake vigorously, stopper, and place in a boiling water bath for 1 minute. Centrifuge for 5 minutes at 3000 times gravity, cool for 10 minutes, decant into matched 1-cm. Corex cuvettes, and read in the Beckman DU spectrophotometer at 705 mμ. Carry out a blank determination which contains the enzyme system minus the hydroxylamine, and set at 100% T (Table II). To correct for any nonenzymatic hydroxylamine decomposition at pH 6.8 in the presence of the plant tissue extract, concurrently carry out a determination containing boiled plant tissue extract.

DISCUSSION

To ensure reproducible results, accurate volumetric measurements of all additions, especially the hydroxylamine, along with identical experimental procedure and experimental conditions are absolutely essential. The last step of the reaction of hydroxylamine with 8-quinolinol to form Indooxine is an oxidative step; consequently, the test tubes must be shaken vigorously after the addition of the 1.0M sodium carbonate solution to ensure complete atmospheric oxidation.

Above a pH of 6.8, hydroxylamine decomposes rapidly. Even at pH 6.8, under the enzyme conditions specified here, there is a significant hydroxylamine decomposition in 10 minutes (Table II). Rather large amounts of nitrous acid, glutathione, and ascorbic acid have been found to enhance the decomposition of hydroxylamine at pH 6.8, but these concentrations are considerably in excess of any concentrations normally found in plant tissues. In the crude plant tissue extracts used in these experiments there is also a slight loss of the hydroxlyamine bound to the large amount of precipitated protein.

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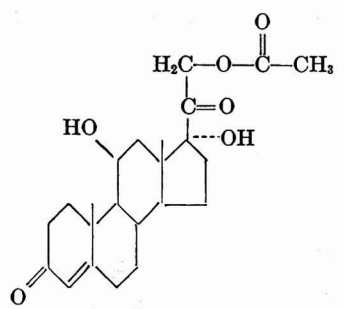
Received for review May 6, 1955. Accepted July 18, 1955.

CRYSTALLOGRAPHIC DATA

99. Hydrocortisone Acetate

Contributed by JOHN W. SHELL, The Upjohn Co., Kalamazoo, Mich.



Structural Formula for Hydrocortisone Acetate

Crystals of hydrocortisone acetate suitable for microscopic and x-ray study are readily obtained from an acetone-water solution. This compound is only slightly soluble in most organic solvents, except dimethylformamide, in which it is exceedingly soluble. There is no evidence of polymorphism, although an unstable solvate does form from dimethylformamide solutions.

Crystal Morphology
Crystal System and Class. Monoclinic, sphenoidal.
Form and Habit. Tabular, showing forms {100}, {001}, {110}, {011}, and {031}.
Axial Ratio. $a:b:c$ = 0.649:1:0.648.
Beta Angle. 102.3°.

X-Ray Diffraction Data
Cell Dimensions. a = 8.85 A.; b = 13.64 A.; c = 8.84 A.
Formula Weights per Cell. 2.
Formula Weight. 404.49; 404.57 (x-ray).
Density. 1.289 (flotation); 1.288 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
8.82	4	3.15	8
7.33	9	2.82	4
6.71	5	2.67	4
6.11	5	2.59	3
5.63	8	2.45	4
5.26	10	2.27	1
4.79	5	2.18	1
4.39	4	2.09	1
4.07	7	2.04	1
3.48	5		

OPTICAL PROPERTIES

Refractive Indices. $\alpha = 1.543 \pm 0.002$, $\beta = 1.589 \pm 0.002$, $= 1.627 \pm 0.002$.
Optic Axial Angle. $2V = 83^1/_2°$ (calcd.).
Dispersion. $r > \nu$, strong.
Optic Sign. Negative.
Optic Orientation. $Y = b, Z \wedge a = 16°$, in obtuse beta.
Molecular Refraction. $\sqrt[3]{\alpha\beta\gamma} = 1.586$; $R = 103.9$ (calcd.), 105.3 (obsd.).

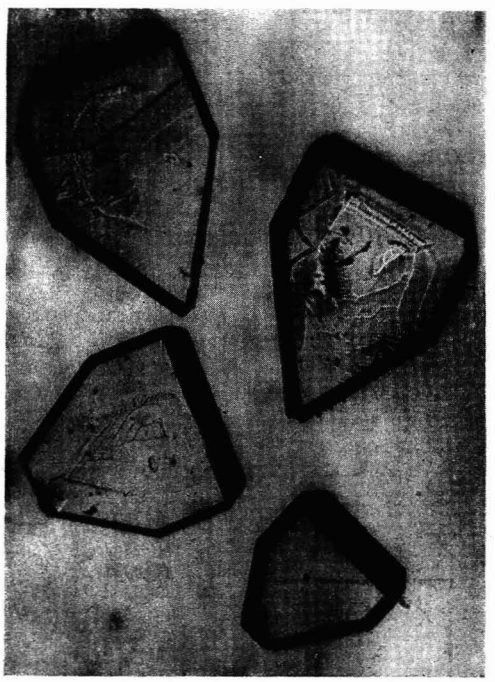


Figure 1. Hydrocortisone acetate

From acetone-water

The specific rotation, $[\alpha]_D$, of the hydrocortisone acetate used for these determinations is +164° (dioxane). Typical crystals from acetone-water are shown in Figure 1, 75×. Figure 2 shows the crystals from fusion after seeding at the edge of the cover glass (dark area), 150×. The melting point is 224° C.; the melt does not recrystallize unless seeded. Orthographic projections are shown in Figure 3.

The unstable solvate, obtained when hydrocortisone acetate is crystallized from dimethylformamide, is partially characterized by the following data. Because of this compound's instability, further characterization has not been made.

Crystal System and Class. Orthorhombic, rhombic dipyramidal.
Forms Present. Unit prism, {110}; brachy pinacoid, {010}; macro dome, {101}.
Axial Ratio. $a:b:c = 0.614:1:0.312$.
Cell Dimensions. $a = 15.2$ A.; $b = 24.74$ A.; $c = 7.71$ A.

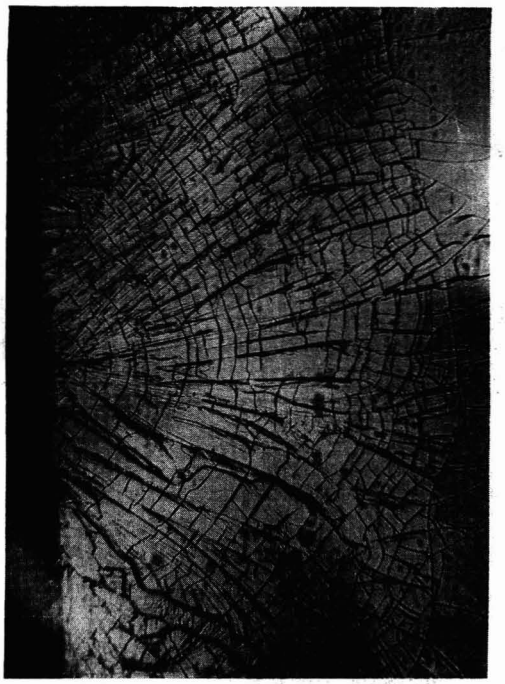


Figure 2. Hydrocortisone acetate growing from melt

Seeded at edge (dark area) with crystals from acetone-water

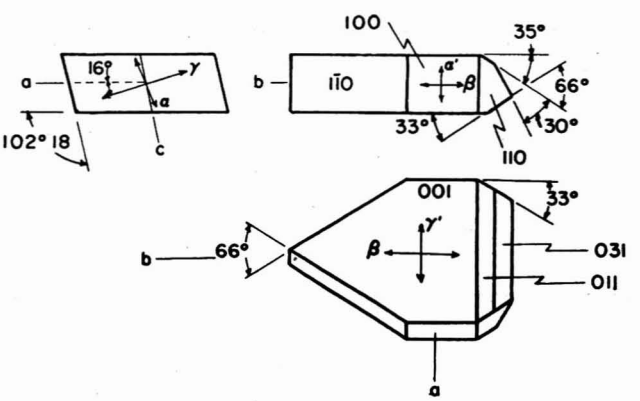


Figure 3. Orthographic projection of hydrocortisone acetate

Refractive Index (5893 A., 25° C.). $\alpha = 1.558$.
Optic Sign. Positive.
Dispersion. $\nu > r$, very strong.
Optic Axial Angle. Very small.
Optic Orientation. $X = c$, $Y = a$.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Research, Armour Research Foundation, Illinois Institute of Technology, Chicago 16, Ill.

Society for Analytical Chemistry

ABOUT 130 analytical chemists were present at I.C.I. Nobel Division Works, Stevenson, Scotland, May 20, for the Symposium on Gas Chromatography, arranged jointly by the Physical Methods and Microchemistry Groups with the Scottish Section of the Society for Analytical Chemistry. Two sessions were held, at which the following papers were presented.

Gas-Liquid Chromatography. A. J. P. MARTIN, National Institute for Medical Research, Mill Hill, London.

The use of a gas as the mobile phase of a chromatogram has two main advantages over the corresponding use of a liquid. The mobility being about 100 times greater permits the use with a practicable pressure head of very long and thin columns at a high rate of flow. Secondly, the coefficient of diffusion being about 10,000 times greater, similar efficiency per unit length of column is attainable in spite of this high rate of flow.

A further advantage of the use of a gas is that detection of a vapor in a gas is in general much easier than detection of a solute in a solvent. Though a number of methods of detecting vapors in gases have been developed over many years, much development remains for the particular purposes of the gas chromatogram. A description was given of the author's gas density meter.

Gas chromatography is a convenient method not only for analysis but also for the preparation of small amounts of pure substances.

The chromatographic behavior provides a ready means of estimating free energies and heats of solution. Since the state of the dilute vapor is practically ideal, interpretation of the chromatographic behavior in terms of the structure of the molecule and the interaction with the solvent is much simpler than in the case of the ordinary partition chromatogram, and as data accumulate a real contribution to the knowledge of the relatively weak forces involved in solution should be possible.

Vapor-Phase Chromatographic Analysis of Hydrocarbon Mixtures. D. E. CHALKLEY, Physical Chemistry Group, Billingham Division, Billingham.

Vapor-phase chromatography is a method well suited to the analysis of complex hydrocarbon mixtures, either on its own or in conjunction with other physical methods such as infrared and mass spectrometry. Techniques have been developed for the analysis of hydrocarbons in the range methane (boiling point −161.5° C.) to triisopropylbenzene (boiling point 240° C.). Examples were given of their application both in the laboratory and for process monitoring of plant streams.

The C_1 to C_4 hydrocarbons have been the subject of a special investigation of the gas-liquid partition technique. Their relative retention volumes over a large number of stationary liquid phases were recorded to illustrate the separations possible when different types of solubility forces are brought into play.

Finally, various aspects of the accurate quantitative analysis of hydrocarbon mixtures have been investigated using a thermal conductivity cell as a detector and hydrogen as a carrier gas. Results were compared with those of the mass spectrometer and some comparisons made of the accuracy and sensitivity of the two methods.

Techniques Used in a Study of the Boron and Silicon Hydrides. A. B. LITTLEWOOD, Merton College, Department of Inorganic Chemistry, Oxford University.

The paper outlined the more important general properties of the boron and silicon hydrides, and the methods used for their preparation and manipulation. The apparatus which is being used for their separation and analysis by gas chromatography was discussed with examples. An account was given of the methods used for the identification of fractions from the chromatographic columns, in particular by effusiometry and pyrolysis.

Adsorption and Partition Methods. C. S. G. PHILLIPS, Merton College, Oxford University.

Work in the author's laboratory was outlined, using both partition and adsorption methods. Some details of apparatus were given, and the use of glass thermal-conductivity cells and of the surface potential detector was discussed. Results obtained with partition chromatography were used to illustrate the value of fully corrected retention volumes both as a basis for an absolute scheme of analysis and for thermodynamic calculations. The paper concluded with a general review of published work on adsorption gas chromatography (including both elution and displacement techniques) and a comparison of the displacement and elution methods.

Rapid Chromatographic Method for Determination of Bromine-Inert Impurities in Ethylene. N. H. RAY, Research Department, Alkali Division, Winnington.

A simplified chromatographic method for the rapid determination of small amounts of nonolefinic impurities (other than carbon dioxide) in ethylene has been developed. The principle is as follows: A measured sample of the gas (25 or 50 cc.) is carried in a current of pure carbon dioxide through a column which is in two parts. The first is packed with a solid absorbent consisting of charcoal impregnated with bromine, which absorbs the ethylene and also retains the ethylene dibromide formed. The unabsorbed impurities pass into the second section of the column packed with activated charcoal, where they are separated. The issuing gases are collected in a micronitrometer over caustic potash solution and appear in two or more discrete fractions, with a sufficient interval between them to allow of separate measurement. The first fraction contains any hydrogen, oxygen, nitrogen, carbon monoxide, and methane present in the sample; the second consists of ethane only, and any higher paraffins appear in subsequent fractions. The complete determination occupies only 5 to 20 minutes, and the smallest detectable concentration of any one impurity is 0.01% (on a 50-cc. sample). Sufficient bromine-impregnated charcoal can be accommodated in the column to last for about 30 analyses, and this reagent can be safely handled without any special precautions.

The summer meeting of the Western Section of the society was held in Gloucester on May 20 and 21. Keith Morgan, Brasenose College, Oxford, addressed the meeting, speaking on "The Role of Iodine in Analytical Chemistry."

The physical and chemical properties of iodine are far from ideal for an analytical oxidizing agent. The importance of iodine and of iodine-containing reagents in analytical chemistry depends largely on a convenient estimation procedure. The reliability, yet complexity, of those reactions commonly used for this purpose present problems of considerable interest. The reactions between iodine and thiosulfate, iodine and arsenite, iodine and alkali, and iodide and iodate were discussed.

Second Ottawa Symposium on Applied Spectroscopy

THE Second Ottawa Symposium on Applied Spectroscopy was held at the Physical Metallurgy Research Laboratories, Department of Mines and Technical Surveys, Ottawa, Canada, September 14 to 16. Abstracts of the papers presented are given here.

Performance Report on the Spectromet. J. H. JURMAIN, Baird Associates, Inc., Cambridge, Mass.

The Spectromet, a new direct-reading spectrometer designed to be independent of atmospheric environment, was introduced early in 1955. Performance characteristics of the instrument and its analytical capabilities on problems thus far treated were presented. It was demonstrated that automatic monitoring, even in unstable environments, provides greater optical stability than earlier monitoring methods.

Performance of Direct-Reading Attachment to the Hilger Medium Quartz Spectrograph. Hilger & Watts Ltd., London, England. Read by J. E. BURGENER, Technical Service Laboratories, Toronto, Ontario.

Recent data obtained with the direct-reading attachment were presented and its performance was assessed.

Use of the Jarrell Ash Direct Reader. F. A. MCNALLY, Jarrell Ash Co., Newtonville 60, Mass.

The JACO Atomcounter is a compact self-contained direct-reading spectrometer. The optical arrangement allows wide versatility in line selection and provides the user with additional photomultiplier space for future expansion. Working curves can be extended to higher concentrations by a 10-position switch which changes the termination pin of the counting tubes. This increases the working curve slope while maintaining extended range of concentration.

Latest data show that the direct reader can fulfill a number of needs. Data on precision and accuracy were given.

Analytical Applications of the Ebert Spectrograph with Order Sorter. RICHARD K. BREHM, Jarrell Ash Co., Newtonville 60, Mass.

The use of the Ebert grating mounting with the order sorter at intermediate and high dispersion settings for difficult analyses was discussed. The effect of dispersion on sensitivity in typical cases was considered and the term "optimum dispersion" introduced. Conditions for the determination of thorium in uranium and carbon in steel were given.

What Are We Worth? DAVID L. FRY, Research Laboratories Division, General Motors Corp., Detroit, Mich.

This paper reports the factors which have to be considered in determining the cost of operating a spectrographic laboratory, the ways in which a spectrographic laboratory can save a company money, and some examples of the monetary value of a spectrographic laboratory.

Production Control Using Direct-Reading Spectrographic Equipment. H. M. PARKHURST, Aluminum Co. of Canada, Ltd., Arvida, Que.

Extensive changes in laboratory operations at the Aluminum Co. of Canada's Arvida Works were brought about by the conversion from photographic to quantometric analysis. The paper describes the changes and their effects on production quality control and on certain plant operations.

Simple Concentric Jet Apparatus for Controlling the Direct Current Arc. W. H. CHAMP, Geological Survey, Ottawa, Ont.

Construction of a simple jet apparatus modified after Stallwood [Stallwood, B. J., *J. Opt. Soc., Amer.*, **44**, 171(1954)] was described. Reasons for design features were given. Principles of operation were discussed, with accounts of experimental work from which they were derived. Preliminary recommendations were made regarding optimum operating conditions for use of the jet-controlled direct current arc in a number of analytical applications.

Quantitative Analysis of Steel Using Powdered Samples with the Air-Jet Source. R. F. STURROCK, Mines Branch, Ottawa, Ont.

The air-jet source developed by Stallwood has been applied to the analysis of steel samples. Conventional internal standard techniques were used to prepare working curves for a number of elements. These were presented, with data on the accuracy and reproducibility obtained with the method.

Fundamental Techniques of Spectrochemistry. B. J. STALLWOOD, Clarkson College of Technology, Potsdam, N. Y.

A brief historical sketch was given of developments leading up to the widespread use of modern photoelectric omission spectrometers in the metal industry. Successful applications of the same basic procedures in the analysis of complex alloys and nonmetallics were recalled to illustrate the importance of the internal standard principle. While discussing the limitations of existing methods and the possibilities of extending their scope, an attempt was made to distinguish between principles of fundamental interest and those of more limited application.

Quantitative Spectrochemical Determination of Lithium in Ores. J. F. GURNEY AND N. RUDNIK, Technical Service Laboratories, Toronto, Ont.

A description was given of a procedure developed to determine lithium. Potassium chloride is used as a buffer and enhancer and cupric oxide as an internal standard.

Spectrographic Determination of Palladium in Doré Metal. N. TEMINGAS AND W. CHARLES COOPER, Canadian Copper Refiners, Ltd., Montreal East, Que.

A convenient spectrochemical procedure for the determination of small amounts of palladium in Doré metal containing varying amounts of copper and gold has been devised. Copper in excess of 4 parts per thousand was found to produce a marked enhancement of the palladium and silver line intensities, thereby prohibiting the use of a single working curve for the estimation of palladium. This enhancement increased with increasing copper concentration and was more pronounced on the silver 3469.206 line than on the palladium 3421.24 line. A number of working curves were prepared, each corresponding to a definite copper concentration. In the analysis of a Doré metal sample the palladium-silver and copper 2961.165–silver 2929.351 line intensity ratios were determined, the copper-silver ratio serving to indicate the position of the working curve. The influence of gold on the palladium and silver line intensities was studied and was found to be significant only when the gold exceeded 100 parts per thousand. Amounts of palladium determined by this procedure ranged from 25 to 200 p.p.m.

Transient Intensities. J. K. HURWITZ, Mines Branch, Ottawa, Ont.

Experiments were described in which transient intensities (sparking-off effects) were observed in the spectra of zinc-base and nickel-base alloys. These intensities were explained in terms of the physical properties of the alloys and of the discharge.

Flame Photometry. W. R. INMAN, Mines Branch, Ottawa, Ont.

Accurate flame photometry demands rigidly controlled flame temperature and atomization and elimination of or compensation for interferences. Interferences are due to foreign ions in the solution, nature of the solvent, and various other factors. A brief description of some flame photometers was given. Methods of compensating for interferences were discussed. Data were presented to show that remarkably accurate determinations of alkali and alkaline earth elements are possible.

X-Ray Spectrography. R. J. TRAILL, Geological Survey, Ottawa, Ont.

A brief account was given of the general principles, instrumentation, and applications of x-ray spectrography. This method has been used for several years by the Geological Survey of Canada for the partial analysis of minerals and ores. Some of the problems and techniques of x-ray spectrography were discussed and examples of analyses cited.

Polarography. C. H. MCMASTER, Mines Branch, Ottawa, Ont.

A brief outline of the polarographic method of analysis was given, and the limitations of the method were discussed. At the Mines Branch Analytical Laboratories the polarograph is used mainly for the determination of trace quantities of metals in alloys and ores. Some examples of these determinations were presented. Although much information is available regarding the half-wave potentials of inorganic ions in various media, it is still essential in most cases for the chemist to develop a method applicable to each particular problem.

Radiochemical Analysis. G. H. FAYE, Mines Branch, Ottawa, Ont.

Two analytical methods involving the use and measurement of artificial radioactivity were discussed: neutron activation analysis and isotope dilution analysis. Neutron activation analysis is primarily a method for detecting and quantitatively determining small quantities of impurities by reason of the characteristic radioactivity which is induced in the impurity, when the sample is irradiated by a beam of thermal neutrons. Isotope dilution analysis is an example of the use of radioactive isotopes as tracers. This technique is used when a complex system is to be analyzed for one of its constituents and especially when the chemical behaviors of the constituents of the mixture are very similar.

Mass Spectrometry. R. K. WANLESS, Geological Survey, Ottawa, Ont.

The general design and construction of the mass spectrometer were outlined briefly. As a result of recent refinements to the ion source and collector assemblies, the scope and usefulness of the instrument have been greatly increased. A list of elements that may be analyzed and the methods employed to produce the required positive ions were presented. The application of the mass spectrometer to the problem of geologic age determination was discussed.

Fluorescence and Absorption Spectroscopy Applied in Studies of Laboratory Products, Isolated and Purified by Chromatography and Electrophoresis. M. O'L. CROWE AND A. WALKER, State of New York Department of Health, Albany, N. Y.

Laboratory products that have been studied include normal and poliomyolitis human immune blood serum and serum proteins, beef-heart antigens, cephalin and lethicin from beef brain, carbohydrates of the pneumococcus, diphtheria toxin and toxoid, cardiolipin, viruses, antibiotics, fluorescent stains, and pigments synthesized by microorganisms. Several pigments not previously reported to be synthesized by the diphtheria bacillus and the tubercle bacillus have been identified by spectroscopic data combined with other physical characteristics.

Spectrographic Microvolume Analysis. J. K. HURWITZ, Mines Branch, Ottawa, Ont.

Examples were given of the application of the microvolume technique to segregates in metals and alloys.

Automatic Apparatus for Fluoride Distillation

Wesley B. Estill[1] and Loren C. Mosier, Ozark-Mahoning Co., Tulsa, Okla.

SINCE the advent of the Willard and Winter distillation procedure (*6*) for separating fluoride from numerous interfering substances prior to analysis, considerable effort has been put forth to decrease the time necessary for distillation (*2, 4*) and/or make the distillation operation more automatic (*1, 3, 5*). Both improvements would in the end save man-hours, and give more reproducible results.

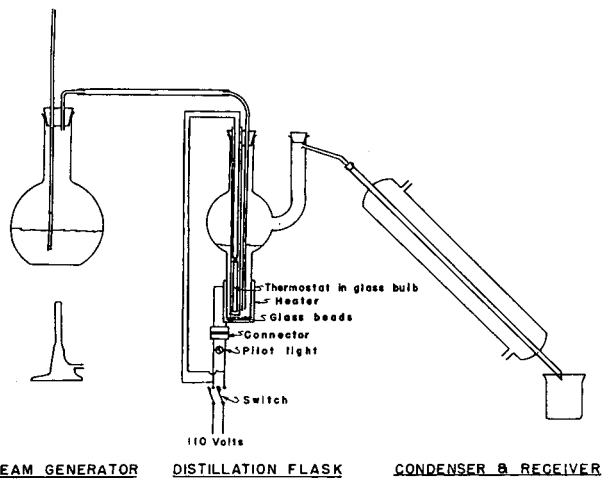


Figure 1

Probably the greatest obstacles to general use of automatic control for fluoride distillation have been the high cost of fabricating a satisfactory unit and the addition to the basic apparatus of a large amount of extra equipment which makes the operation of cleaning equipment and changing acid somewhat laborious.

The automatic unit designed by the authors is shown in Figure 1.

The glass-enclosed thermostat (Fenwal No. 17000 is recommended), which can be adjusted to a predetermined value, is connected in series with the heating coil. At 135° C. this thermostat is capable of controlling the temperature within ±1° C. As soon as the desired distillation temperature is reached, the steam valve is opened to allow the steam to enter the perchloric acid. Aside from the fact that it is necessary to open the steam valve manually the distillation is completely automatic.

The distillation flask is custom-made for the unit. The heating coil was fabricated by the authors to draw about 100 watts. The pilot light is installed in parallel with the heating coil to facilitate initial temperature adjustment.

Although there may be some bumping in the liquid at the outset, this ceases when steam is introduced and no perchloric acid has been carried over to interfere in the subsequent determination of fluoride, as occasionally occurred before adoption of this unit, when distillation temperatures were allowed to go too high. The small side tube through which the vapors pass to the condenser is made to protrude about 2 mm. through the wall of the larger side arm. This feature of construction, the fact that the liquid level is kept low in the distilling flask, and the close temperature control effected by the thermostat have appeared to eliminate distillation difficulties completely (Table I). The effects of the presence of other compounds were assumed to be the same as in other types of fluoride distillation equipment.

The rate of steam generation is not critical, if the distillation flask is not cooled below its operating temperature.

[1] Present address, Oak Ridge, Tenn.

Table I. Titration of Fluoride

Direct Titration without Distillation				Titration after Distillation in Proposed Still			
Titration	F, mg., std.	Factor, mg. F^-/ml. $Th(NO_3)_4$	Deviation from mean	Titration	F, mg., std.	Factor, mg. F^-/ml. $Th(NO_3)_4$	Deviation from mean
2.55	5	1.962	−0.032	2.50	5	2.000	+0.010
2.52	5	1.987	−0.007	2.53	5	1.978	−0.012
2.50	5	2.000	+0.006	2.54	5	1.970	−0.020
2.50	5	2.000	+0.006	2.52	5	1.985	−0.005
2.53	5	1.978	−0.016	2.49	5	2.008	+0.018
2.53	5	1.978	−0.016	2.53	5	1.978	−0.012
3.03	6	1.982	−0.012	3.00	6	2.000	+0.010
3.00	6	2.000	+0.006	2.99	6	2.008	+0.018
2.96	6	2.028	+0.034	3.02	6	1.987	−0.003
3.00	6	2.000	+0.006				
2.99	6	2.015	+0.021		Mean	1.990	±0.0120
3.01	6	1.995	+0.001				
	Mean	1.994	±0.0136				

LITERATURE CITED

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(5) Willard, H. H., and Winter, O. B., IND. ENG. CHEM., ANAL. ED., **5**, 7 (1933).
(6) Willard, H. H., Toribara, T. Y., and Holland, L. N., ANAL. CHEM., **19**, 343 (1947).

PRESENTED before the Tri-Sectional Meeting, AMERICAN CHEMICAL SOCIETY, Bartlesville, Okla., October 17, 1953.

Digester and Filter for Preparing Extract Solutions from Solids

George R. Van Atta and Jack Guggolz, Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, Calif.

THE accompanying figure depicts a type of apparatus found useful for digesting or extracting solids such as plant materials which require repeated lixiviations with fresh portions of solvent or reagent solution. Its use avoids the bothersome transfer of the entire digestion mixture to a separate filter for withdrawal of each successive extract solution and subsequent return of the drained solids to a digestion vessel for the next extraction. As either lixiviation or digestion is accomplished in a single vessel, hot extract solutions can be drained from residual solids with but little cooling. Liquid losses by vaporization during digestion or extraction can be prevented by use of a reflux condenser.

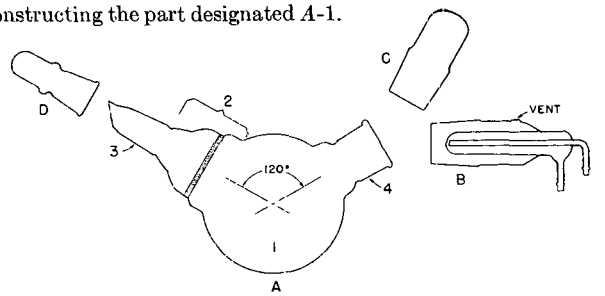
This apparatus is similar in principle to the commonly used microbeaker filter described by von Bergkampf [Bergkampf, E. S. von, *Z. anal. Chem.*, **69**, 321–41 (1926)]. The present design, however, was developed for work primarily with macro samples. To this end a spherical flask is employed and contributes the ruggedness needed in a larger unit. Furthermore, absence of square corners where solids could tend to lodge facilitates admixture of the charge and recovery of undissolved residual solids. In the authors' experience use of standard-taper glass joints and the accessories shown are also desirable features of the present design.

The sample and a portion of the extractant are placed in flask *A*-1 and mixed by swirling. For operations that involve long periods of soaking at room temperature the apparatus is stoppered with caps *C* and *D*. Cold-finger condenser *B* is used for hot digestion or extractions. The apparatus is heated in either a steam bath or heating mantle.

To filter and withdraw extract solution the ground drip-tip joint, *A*-3, is connected in an upright position to a three-necked flask or other suitable receiver, which in turn is connected to a source of suction.

After the residual solids are drained, they are dislodged from the surface of the fritted-glass filter disk by swirling with a fresh portion of extractant. To facilitate this operation the wall of the Büchner-type glass funnel, *A*-2, should be as short as is consistent with convenience in sealing the funnel to the flask, *A*-1.

The apparatus can be built in any size desired. Flasks having capacities of 300 to 3000 ml. have been used in this laboratory for constructing the part designated *A*-1.



The principal parts of the unit from which the accompanying figure was drawn to scale were made from glassware items of the following stock sizes: capacity of flask *A*-1, 2000 ml.; diameter of fritted disk in funnel *A*-2, 80 mm.; standard-taper through joint *A*-3, 34/45; outside standard-taper joint *A*-4, 50/50.

These sizes are mentioned here only for the sake of illustration. The relative proportions of the elements of the apparatus can be varied to suit individual requirements. Ruggedness and speed of filtration are favored, however, if the size of *A*-2 is large rather than small in relation to *A*-1. Büchner-type glass funnels fitted with "coarse" fritted disks have been found suitable in constructing this type of apparatus for work with ground plant materials. Removal of spent solids after extraction is facilitated if an oversized joint is used to replace the original flask neck at *A*-4.

Typical Experiment. A 20-gram sample of commercially dehydrated alfalfa meal was defatted by extraction under reflux in a 2000-ml. device of this type. Five 30-minute digestions with separate 450-ml. portions of a mixture of benzene and absolute ethyl alcohol (9 to 1) were made on a steam bath. After each digestion the solvent was removed by suction and the meal was rinsed with 50 ml. of the hot solvent mixture. After the fifth extraction and rinse, air was pulled through the meal until all of the solvent was evaporated. A total of 7.9% of the weight of the meal was removed by this solvent.

The dry, bleached, defatted meal was then extracted twice with 80% ethyl alcohol in the same manner as with the benzene–ethyl alcohol. With the two extractions, 18.6% of the weight of the original meal was removed.

Heated Sample Inlet System for Mass Spectrometry

V. J. Caldecourt, The Dow Chemical Co., Midland, Mich.

HEATED sample inlet system has been developed to permit the loading of weighed amounts of liquids or solids. The solids may be materials volatile at the temperature of the reservoir, or nonvolatile materials that contain volatile components which are to be measured. The system is constructed of glass, Teflon, and stainless steel, and therefore can be used up to about 250° C.

Figure 1 shows the reservoir, loading port, and pump-out valve. The loading port is sealed by a Teflon slug 0.188 inch in diameter and 0.25 inch long. The port was drilled and reamed with a standard-taper pin reamer, so that the diameter at the top of the port is 0.188 inch. The reservoir is a 5-liter sphere fitted with a 1-inch glass pipe joint.

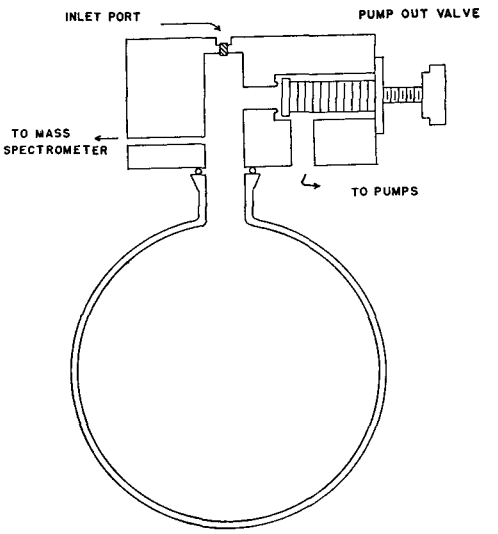


Figure 1. Sample inlet system

The sample-loading device is shown in Figure 2. To load a sample, the two Teflon parts and the split sleeve are weighed. Then the cup is filled with sample. A cup made by drilling a No. 50 hole $^1/_8$ inch deep will hold 5 to 7 mg. of liquid. After the cup has been filled, it is pressed into the split sleeve, and the blank slug is pressed down on top of it. The assembly is then weighed and the sample weight found by difference. Next, the split sleeve is placed in the recess of the entry port and the pusher shown in Figure 2 is used to force the sealing plug and sample cup into the reservoir and force the blank slug into the port to serve as a new vacuum seal. After a number of samples have been loaded, the reservoir is removed and a clean sphere is substituted.

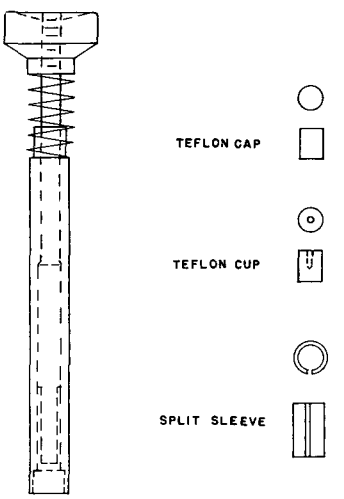


Figure 2. Sample loader

This method of sample loading has proved convenient and useful. It has a limitation, however, in that a small amount of air is introduced with the slugs. About one half of this seems to be trapped and carried in with the slugs. The other half appears to have been dissolved or adsorbed by the Teflon, and is released in a few seconds after the slugs are introduced. In a typical sample, loading 7 mg. of toluene would give a reservoir pressure of about 500 microns in a 5-liter volume at 200° C. In the loading process, about 0.003 mg. of air would be introduced. So far, very few samples encountered have reacted with air at this temperature.