ANALYTICAL CHEMISTRY

AUGUST 1956

More Comprehensive Analytical Books Needed

REFERENCE works now appearing in the field of analytical chemistry are very likely to be composed of sections written by experts who are more or less guided by an editor in chief, whose main function is to round up the authors for the publisher and to see that they get their contributions in on time.

The early classics in analytical chemistry, of course, dealt mainly with inorganic systems and the methods described were gravimetric and volumetric. They were, however, masterpieces of scientific writing and philosophy and were an important part of the scientific literature of chemistry of the time. With the growth and resulting complexity of science over the intervening years, definitive books have tended to deal with more and more specialized topics, simply because the scientist is no longer a master of the total subject. We wonder if such a happy state will ever again be possible.

It was not too many years ago that the physicist had his field well cataloged and, in fact, was feeling so complacent that some of the younger men were saying: There is no exciting future, so why go into physics? Chemists and physicists alike were teaching the relative indivisibility of the atom, and the chemist was having all the fun and playing with the reactions possible in the outermost orbits.

Well, we all know, and have lived through, the cataclysmic years of atom smashing, which has had a very significant effect on analytical chemistry and the techniques which it now uses. For one thing, it has resulted in the development of a group of specialists in analytical chemistry, because no one can hope at the present pace to master the whole subject of analysis. This is the reason why books are generally written around a special technique such as infrared or polarography. In fact, it may be so specialized and academic that the author does not attempt to show how the technique can be applied to analysis.

It would seem, then, that the phase through which we are now passing—the editor-specialist combination—may be a happy one: At least it brings the techniques together and shows how they apply to the solution of analytical problems. We are still in the phase where the specialist thinks in terms of solving all problems with his special technique, and such an approach, if not corrected, can be costly and sometimes sterile. However, as long as these approaches are new and have not established themselves in final form as to instrumentation, they must remain in the hands of the specialist.

In the years to come, when the various techniques are established, we should then approach the subject of an analysis as the concertmaster approaches the instruments at his command in achieving a final result. Then, too, we hope that at least an author or two will appear capable of dealing with the subject of analysis as an integrated and mature part of the scientific method.

Unfortunately, we do not find the climate in our universities conducive to such growth. In fact, specialization among our young professors is very much the style. Where there is youth there is hope, and we do know a few in the field who appreciate the larger problem about which we are concerned. Over the years let us not underestimate the importance of this more mature approach to analysis.

Turning to industry and the applied side of analysis, we find that in many industrial laboratories the various specialized approaches to analysis are not even coordinated. This, in some measure, is due to the competitive spirit of individual specialties. We suspect, however, that there is no one strong enough and with a sufficient knowledge of the application of these specialties to analysis to guide and lead a more coordinated group. Lest we be set down as dreamers, we are glad to record that there are others here and abroad who are giving some thought to the larger scope which analysis must play in the future. Lectures, scientific papers, and books all can contribute to the fulfillment of these aims.

We trust that some editor in chief, as the result of his present experience, may in his more mature years have gained sufficient wisdom, knowledge, and inspiration to write a definitive work on how the various specialized techniques should be integrated in solution of analytical problems so that the most adequate answer is obtained quickly. This is a challenging task, but we believe it is not impossible if the writer thinks in terms of analytical chemistry and not as a specialist.

Catalog of Infrared Spectra for Qualitative Analysis of Gases

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A catalog of infrared spectra of 66 gases and vapors is presented, which should increase the usefulness of the infrared technique for qualitative analysis of unknown gas mixtures. A chart is also given as an aid to rapid identification of the constituents of an unknown sample. Threshold values revealed by the chart show the sensitivity of the infrared method under specific conditions for the various gases and vapors reported. The methodologies illustrated should be applicable to more extensive cataloging of gases and vapors.

THE usefulness of infrared spectrophotometry as a tool for analysis of gases is well known. That there is a need for specially suitable catalogs of infrared spectra of gases for use in qualitative analysis is also generally recognized. If an analyst (or spectroscopist) has to deal with only a few gases, the matter of reference spectra is not complex or difficult, but if he is to analyze unknown samples with a wide variety of molecules, an extensive, reliable, and convenient catalog of gas spectra is necessary.

Spectra now available in the literature fail to meet the analyst's requirements in a variety of ways: The wave length ranges may be too narrow or entirely outside the one of interest; temperatures and pressures for the reported spectra may be radically different from those needed; resolution or other instrumental conditions may be greatly different from those the analyst must use. Many of the available spectra are obsolete because of improvements in instrumentation which have taken place since the spectra were recorded. In many cases the purity of the samples on which the spectra in this paper were determined was superior to that of materials used for earlier work.

The need for assembling an up-to-date and reliable catalog of infrared spectra of gases first became apparent in the authors' laboratory when it was desired to apply infrared techniques to the analysis of gases from several kinds of combustion processes. There have been indications that the spectra presented will be of considerable interest to many analysts in the same or related fields. Some groups or "families" of compounds were found to be exceptions to the more general case, in that their infrared spectra have been recently recorded with modern equipment and reported for conditions generally suited to analytical purposes. Halogenated hydrocarbons comprise such a family (*3, 8, 14, 15, 17, 18*), and, consequently, only a few of these materials have been included in this paper.

For quantitative determinations the analyst, for many reasons, will need to construct his own working curves (*3, 6*). This paper deals chiefly with qualitative aspects and presents a catalog of 66 gases and vapors which can serve as a good basis on which to build a more extensive collection of reference data. All the spectra were acquired recently using modern equipment of a type now in very wide use. A table and a chart are provided which demonstrate techniques for systematizing the reference spectra for work on unknown gas samples.

Because of the extent of the field of investigation, and consequently the time and economic limitations involved, this catalog must be far from complete. The original intention was to assemble only a nucleus of about 20 spectra of some of the more common gases, but as the work progressed, various extensions of the project were found desirable. As presented, the catalog is largely oriented toward materials which might be present as products of combustion from fuel used in rockets or as the residual gases from such fuels. A need was also encountered for a few spectra of the vapors from liquids which have vapor pressures much lower than 1 atm. at room temperature. Some of these spectra are included in the catalog.

Very little information is available in the literature which is of value to the analyst in estimating the amount of a specific gas which he can expect to detect by infrared examination under specified conditions. This paper presents a guide for such estimates based on extensive determinations of threshold values for the gases of the catalog. These thresholds are sorted into a convenient number of intensity classifications and presented in chart form. It is hoped that many will find this chart useful and will be able to extend its value in their own field by adding to it data of their own on gases not already recorded thereon.

EXPERIMENTAL

Origin and Purification of Samples. It was desirable that all the samples used for the catalog be of such a high degree of purity that no peaks due to impurities would be detectable. This purity goal was achieved for most of the samples, but was in a few cases economically unfeasible. A number of the gases were generated and purified in the authors' laboratory by well-known procedures or by methods indicated in the references cited. Some samples could be obtained commercially in a satisfactory pure grade. Some could be sufficiently improved by simple fractionation procedures. Still others required extensive purification. Assurance regarding purity was in some instances provided by mass spectrographic analysis. Many spectra were judged to be completely valid by comparisons made against unpublished spectra of materials of known purity which were graciously made accessible to the authors from the following: Phillips Petroleum Co., Bartlesville, Okla.; Bureau of Mines, Bartlesville, Okla.; Union Oil Research Center, Brea, Calif.; Richfield Oil Corp., Wilmington, Calif.; Shell Development Co., Emeryville, Calif.; Standard Oil Co., Richmond, Calif.; Wyandotte Chemicals Corp., Wyandotte, Mich.; and Commercial Solvents Corp., Terre Haute, Ind.

Because of their importance in combustion studies, spectra of nitrosyl chloride and propyne were included in the catalog, although the materials available were not as pure as desired. A fractionation procedure improved the purity of the nitrosyl chloride as originally received, but the contaminant (nitrogen dioxide) could not be completely eliminated in this manner. The propyne was the best commercially available; no attempt was made to remove the acetylene which it contained. For qualitative analysis these known impurities do not invalidate the spectra.

Peaks known or suspected to be due to impurity are marked by an asterisk (*) on the spectra presented. There may be minor peaks due to impurities which were unsuspected and consequently not marked. In qualitative work the absence of such markings will be of no moment because only selected peaks which are of high or medium intensity are utilized, and all minor peaks which might be due to impurities are disregarded. Carbon dioxide was frequently encountered as a troublesome impurity.

In many cases the samples were dried on introduction to the infrared cell by passage through a suitable drying agent. In general, the analyst must take care in the selection of a drying agent so as to avoid possibility of reaction with some gas present in a mixture being analyzed. For cataloging work where one is deal-

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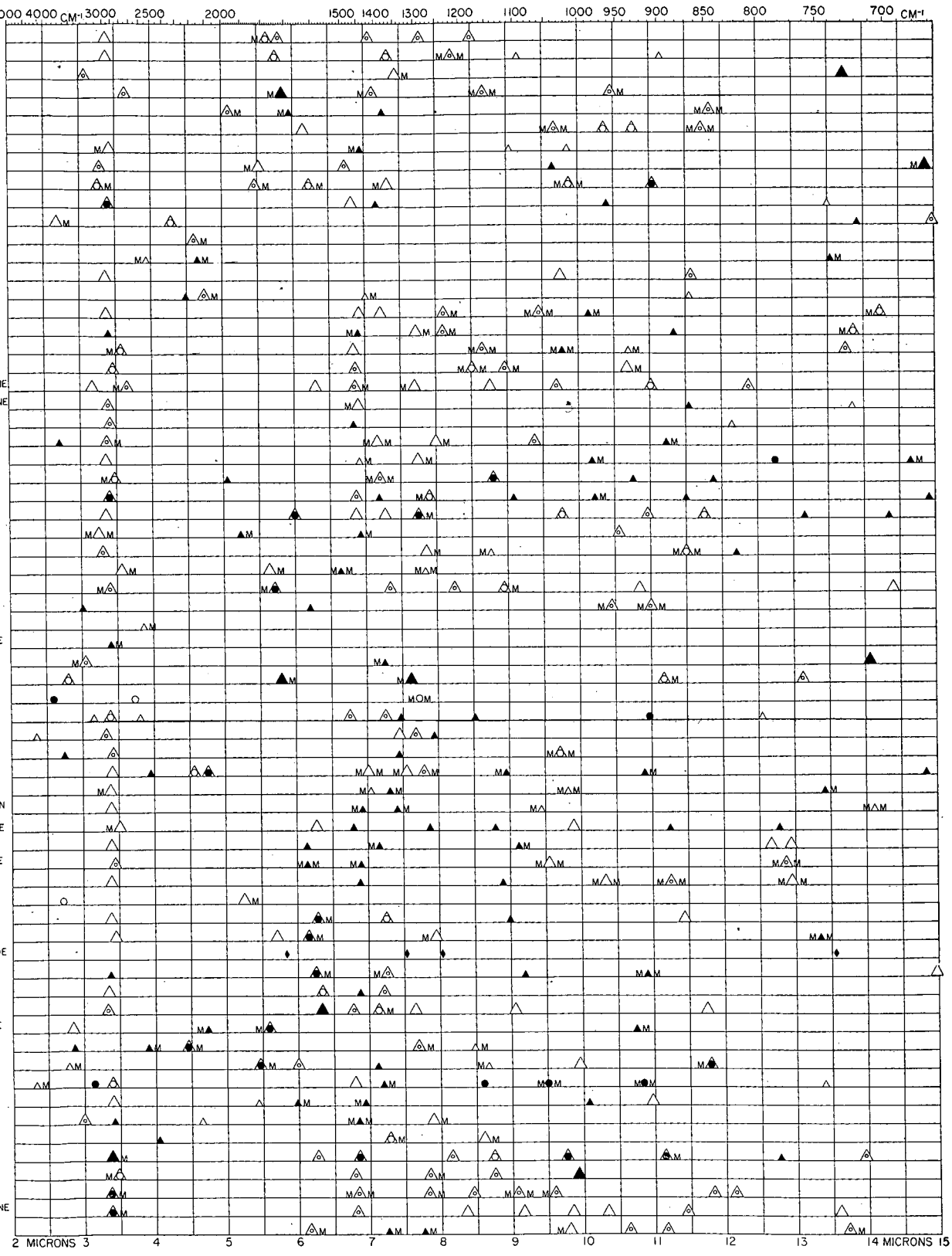


Figure 1. Chart of classified threshold values

Percentage intervals shown for each symbol represent approximately equal logarithmic divisions except for first and last categories

Table I. Source, Purification, and Vapor Pressures of Gases Used

Spectrum No.	Compound	Source and Grade	Purification[a]	Drying Agent	V.P. (Satn.) Mm. Hg at 27° C.	*P*, Mm. Hg, Highest Used
1.	Acetic acid	Baker & Adamson; reagent	Redist.	$CaCl_2$	17	12
2.	Acetone	Eastman Kodak; spectro	None	$Ba(OH)_2$, $CaSO_4$	235	235
3.	Acetylene	Authors' lab.; $CaC_2 + H_2O$	.....	NaOH, $CaSO_4$	710+	704
4.	Acrolein	Matheson; tech. (heated)	None	$CaSO_4$	300	228
5.	Allene	Air Reduction Co.; res. labs.	None	Ascarite	710+	701
6.	Ammonia	Matheson; anhydrous, 99.9%	Redist., Na	.....	710+	700
7.	Azomethane	Authors' lab.[b]	.....	$CaCl_2$, BaO	710+	130[cc]
8.	Benzene	Merck; thiophene-free	None	None	105	100
9.	1,3-Butadiene	Phillips; res.[c]	None	$CaCl_2$	710+	708
10.	*n*-Butane	Phillips; res.	None	NaOH, $CaCl_2$	710+	705
11.	Carbon dioxide	Authors' lab., comm. cylinder	None	$CaCl_2$	710+	702
12.	Carbon monoxide	Matheson; 95%+	None	NaOH, $CaCl_2$	710+	714
13.	Cyanogen	Authors' lab.; $HgCl_2 + Hg(CN)_2$	By cold trap	None	710+	350
14.	Cyclopropane	Matheson; 99.5%+	None	NaOH, $CaCl_2$	710+	709
15.	Diazomethane	Authors' lab.[d]	.....	.....	710+	130[dd]
16.	1,1-Dichloroethane	Matheson, Coleman & Bell; tech.	Redist.	$CaSO_4$	245	245
17.	1,2-Dichloroethane	Eastman Kodak; White label	Redist., CaO	.....	88	82
18.	Dimethylamine	Authors' lab.[e]	.....	.....	710+	704
19.	Dimethyl ether	Matheson; 99.9%+	None	Ascarite, P_2O_5	710+	704
20.	1,1-Dimethylhydrazine	Authors' lab.[f]	.....	.....	[aa]	147
21.	1,2-Dimethylhydrazine	Authors' lab.[g]	.....	.....	77	48[cc]
22.	Ethane	Matheson; 95%+	None	Ascarite, $CaSO_4$	710+	705
23.	Ethyl alcohol	Shell; absolute	None	$Ba(OH)_2$, $CaSO_4$	65	65
24.	Ethyl chloride	Matheson; U.S.P., 99.99%	None	Ascarite, P_2O_5	710+	706
25.	Ethyl ether	Mallinckrodt; anhydrous	None	$Ba(OH)_2$, $CaSO_4$	550	550
26.	Ethyl mercaptan	Eastman Kodak; White label	None	$CaCl_2$	555	550
27.	Ethyl nitrate	Eastman Kodak; White label	None	$CaCl_2$	[aa]	66
28.	Ethylene	Matheson; C.P., 99.9%+	None	NaOH, P_2O_5	710+	705
29.	Ethylene oxide	Eastman Kodak; White label	None	None	710+	701
30.	Formaldehyde	Authors' lab.; by heating paraformaldehyde	Cold trap	None	710+	100
31.	Formic acid	Baker & Adamson; C.P.	Redist.	None	42	38
32.	Hydrazine	Fairmont; comm. 94.7%	Redist.[h]	.....	ca. 15	15[d]
33.	Hydrogen bromide	Matheson; anhydrous, 99.1%+	None	None	710+	710
34.	Hydrogen chloride	Authors' lab.; NaCl, HCl, H_2SO_4	.....	H_2SO_4, P_2O_5	710+	705
35.	Hydrogen cyanide	Authors' lab.; KCN + dil. H_2SO_4	.....	H_2SO_4, P_2O_5	710+	707
36.	Hydrogen nitrate	Authors' lab.; $KNO_3 + H_2SO_4$	Redist.	.....	[aa]	65
37.	Hydrogen sulfide	Authors' lab.; Al_2S_3 + water	.....	$CaCl_2$, P_2O_5	710+	702
38.	Isobutane	Matheson; C.P., 99.0%+	None	NaOH, $CaSO_4$	710+	708
39.	Methane	Matheson; C.P., 99.0%+	None	NaOH, $CaCl_2$	710+	702
40.	Methyl alcohol	Mallinckrodt; absolute	None	$CaSO_4$	135	135
41.	Methyl azide	Authors' lab.; $NaN_3 + (CH_3)_2SO_4$[i]	.....	.....	710+	698
42.	Methyl chloride	Matheson; 99.5%+	None	Ascarite, P_2O_5	710+	704
43.	Methyl mercaptan	Matheson; 99.0%+	None	$CaCl_2$	710+	710
44.	Methylene dimethylhydrazine	Authors' lab.[j]	.....	.....	[bb]	50[cc]
45.	Monoethylamine	Matheson; anhydrous, 96%+	None	Ascarite, $CaSO_4$	710+	704
46.	Monomethylamine	Matheson; anhydrous, 96.5%+	None	Ascarite, $CaSO_4$	710+	704
47.	Monomethylhydrazine	Authors' lab.[k]	.....	.....	55	42[cc]
48.	Nitric oxide	Matheson; 98%	Redist.	None	710+	706
49.	Nitroethane	Eastman Kodak; White label	Redist.	None	22	21
50.	Nitrogen dioxide	Matheson; 98%	Redist.	None	710+	702
51.	Nitrogen pentoxide	[l]	.....	.....	[bb]	19
52.	Nitromethane	Eastman Kodak; pract.	Redist.	None	37	35
53.	1-Nitropropane	Matheson, Coleman & Bell; pract.	Redist.	None	11	8.5[cc]
54.	2-Nitropropane	Matheson; pract.	Redist.	None	19	19
55.	Nitrosyl chloride	Matheson; 93%+	Redist.	None	710+	710
56.	Nitrous oxide	Matheson; 98%+	Redist.	P_2O_5	710+	701
57.	Phosgene	Matheson; 99.5%+	Redist.	None	710+	710
58.	Propane	Phillips; Res.	None	NaOH, $CaCl_2$	710+	709
59.	Propylene	Matheson; C.P., 99.0%+	None	$CaCl_2$, $CaSO_4$	710+	710
60.	Propyne	Farchan Research Lab.; res.	None	Ascarite	710+	704
61.	Sulfur dioxide	Matheson; anhydrous, 99.7%+	None	$CaCl_2$	710+	704
62.	Tetramethylhydrazine	Authors' lab.[m]	.....	.....	[aa]	90[cc]
63.	Tetramethyltetrazene	Authors' lab.[n]	.....	.....	[aa]	6.5
64.	Trimethylamine	Authors' lab.[o]	.....	.....	710+	700
65.	Trimethylhydrazine	Authors' lab.[p]	.....	.....	[aa]	106[cc]
66.	Vinyl chloride	Matheson; 99.8%+	None	$CaCl_2$	710+	700
67.	Illustrative sample	Authors' lab.	None	None	710+	705[dd]

[a] Other than treatments for removal of CO_2 and moisture.
[b] (*19*).
[c] According to label, contained 0.02 % by weight of tertiary butyl catechol.
[d] Made from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide; (*2*).
[e] Made from Eastman Kodak Co., White label dimethylamine hydrochloride, treated with NaOH, and redistilled from BaO.
[f] Made from Westvaco 1,1-dimethylhydrazine, converted to sulfate, recrystallized from absolute ethyl alcohol, treated with NaOH, and redistilled using high degree of fractionation.
[g] (*1*).
[h] (*7*).
[i] (*9*).
[j] (*13*).
[k] Made from Eastman Kodak Co., White label methylhydrazine sulfate, recrystallized from 80% ethyl alcohol, treated with saturated solution of NaOH, and redistilled from NaOH pellets.
[l] Spectrum supplied by Naval Ordnance Laboratory, University of Minnesota; (*16*).
[m] (*5*).
[n] (*20*).
[o] Made from Eastman Kodak Co., White label trimethylamine, converted to hydrochloride, recrystallized from absolute ethyl alcohol, treated with NaOH, and redistilled from NaOH pellets.
[p] (*4*).

[aa] Not found in literature; believed to be substantially the same as pressure used in preparing spectrum.
[bb] Not found in literature; believed to be considerably higher than pressure used in preparing spectrum.
[cc] Plus nitrogen to give about 710 mm. total pressure.
[dd] Plus helium to give about 710 mm. total pressure.

ing with a single, relatively pure gas, this selection will not be a severe problem in most cases. However, there are gases for which no suitable drying agent was found. On unknown samples the drying problem becomes much more critical, and in so far as possible the use of chemical agents is to be avoided. Cold traps may be adequate for drying in some instances. If a drying agent must be used, the analyst may find it advantageous to try several different types in alternative determinations as a check on the possibility of interfering reactions.

Sources of samples, methods of preparation or purification, vapor pressures and initial pressure are given in Table I. By "initial" pressure is meant the highest pressure used for each gas, and for those samples on which more than one pressure was used, the highest was lettered *A* on the spectrum. Except as noted in Table I and in the captions accompanying each spectrum, no inert pressurizing gas was added to the cell when the catalog spectra were produced. Most of the values for vapor pressure were read from charts (*12*). The drying agents listed in Table I are those which were found to be satisfactory; the list does not imply that other reagents would be incompatible.

Spectroscopic Procedures. All the catalog spectra were prepared by means of a Perkin-Elmer Model 21 infrared spectrophotometer, using the following standard conditions unless otherwise indicated in the catalog: sodium chloride optics, 10-cm. cell with sodium chloride windows, and a wave length range of 2 to 15 microns. In most cases the spectrum was first recorded at a pressure of about 705 ± 5 mm. of mercury for gases (the equivalent of 1 atm. at the location of the instrument) and near saturation pressure for vapors. In case satisfactory resolution was obtained at this initial pressure, the curve was not lettered; if unresolved areas appeared, these were resolved by scans at suitably reduced pressure and the curves were lettered for identification. Certain exceptions regarding initial pressures and other departures from the standard conditions are indicated in the catalog of spectra. For some of the vapor samples (especially those of low vapor pressure, such as acetic acid, acrolein, 1-nitropropane) it was found desirable to use an initial pressure somewhat below saturation pressure in order to avoid recording of peaks of the liquid spectrum produced by condensation on the cell windows. Initial pressures for azomethane and cyanogen were considerably below saturation, simply because difficulties were encountered in preparing larger amounts of purified samples. Mass spectrographic analysis showed the cyanogen sample from which the spectrum was derived to be of high purity (except for a trace of hydrogen cyanide), whereas larger samples turned out to be much less pure.

The catalog is largely self-explanatory. Resolution settings of the instrument were selected so that adequate resolution was obtained at all areas run at reduced pressures; the slit widths were reduced until no further improvement in resolution was obtained. Values shown for resolution are those of the slit scheduling scale (arbitrary) of the Perkin-Elmer Model 21 instrument and are useful only to those who have the same type of instrument. It was not an objective of this paper to determine any of the peak positions with great precision, but it is believed that the calibration and other instrumental control was such that all the sharp peaks are located with an accuracy of ±0.03 micron.

Threshold Values. In addition to a catalog which shows the peak locations, the analyst has real need for information regarding the intensities of the various peaks. He should have knowledge not only about the relative intensities but also the absolute intensities; that is, for every gas of interest he must know the detectability limit for each of the useful peaks.

Threshold values were therefore determined for all the gases of the catalog. These were assembled into a table (not reported), classified into a number of suitable categories, and then reported in chart form (Figure 1). For the determination of the amount detectable, a conservative criterion was used. In order to be detectable, a peak was required to be several times as intense as the noise irregularities and in general of the order of 2% transmittance for the sharpest peaks and, on a sliding scale based on "spread," up to about 6% for the broadest areas of absorption. These detectable limits were verified by plotting the log I_0/I *vs.* pressure. All the threshold data for gases and values for some of the vapors were determined on a pressure-broadened basis—that is, an inert gas (nitrogen, helium, or air) was added to the cell to bring the pressure up to about 700 mm. for all infrared scans. A technique of progressive dilutions was advantageous in that relatively simple pressure measuring devices (ordinary manometer or Dubrovin gage) were adequate.

Because of sorption effects, threshold values for a number of the vapors could not be reliably determined by the progressive dilution method; therefore, a second method was used. In such cases the values were obtained by starting with a clean evacuated cell, adding a small amount of the vapor (by measurement of pressure), and making a scan. The cell was then recleaned (including renewal of stopcock grease and testing of the cell alone as a blank) and a sample at a different pressure was taken for the next scan. Plots of several such runs at moderately low concentrations (0.5 to 8.0 mm.) were extrapolated linearly to find the detectable limits. For this technique no inert gas was added—that is, pressure-broadening effects were neglected. It is believed that for these vapors the pressure-broadening aspects may be safely disregarded (*6, 11*).

Figure 2 shows the results for the 5.71-micron peak of formic acid obtained by each of the above described procedures. The symbol P_1 refers to the first procedure, and P_2 to the second For this vapor the P_1 results would be highly misleading.

In addition to being a succinct means of presentation, the chart of Figure 1 provides a convenient first approach to examination of the spectrum of an unknown gas. Comparisons between peaks of the unknown with those of the catalog gases can be rapidly made. Also, areas in which overlapping can occur are easily discernible, and due allowance can be made in interpreting peaks appearing in such areas.

The symbols are positioned to indicate the wave length at which the intensity measurements were made. The "% by volume" ranges for amounts detectable were based on local atmospheric pressure (705 ± 5 mm.); for locations having an appreciably different barometric pressure, the values would be subject to some adjustment. For single sharp peaks the vertexes of the triangular symbols will be found to indicate very closely the wave lengths of the catalog peaks. For double peaks, for areas having numerous fine structure peaks—e.g., ammonia—or for broad bands, the wave length had to be selected on a somewhat arbitrary basis. Sometimes, in the case of double peaks that were closely spaced, the approximate center of the doublet area was selected. At other times, when two peaks were about equal in persistence at pressures approaching the threshold level, one or the other was chosen at random. When one of two peaks was more persistent than the other, the more sensitive one was favored. For broad bands the center was used, but this could only be an approximate location. For fine structure areas, the selection of one or two of the strongest peaks was most arbitrary. Because of this necessary variation in the site of threshold measurement, the threshold wave lengths should not be expected always to correspond precisely with the appearance of the peaks as presented in the spectra.

Some care was taken in selecting appropriate intensity symbols. The number of classifications is thought to be about optimum and the symbols portray intensity by size, configuration, and degree of blackness. In order to avoid cluttering of the chart by entering too many peaks of about equal intensity the symbol *M* was utilized to indicate areas having "multiple" peaks.

Special Procedures for Certain Gases. Spectrum No. 15, diazomethane. Because of the explosive hazard in handling diazomethane, its spectrum was produced on a mixture of about 1 part of diazomethane and 4 parts of helium.

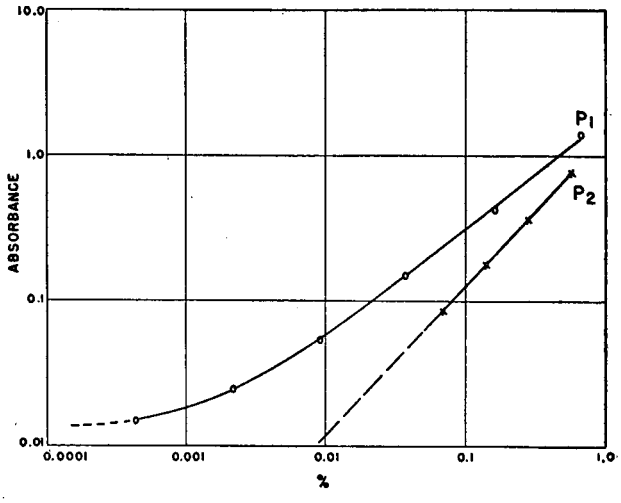
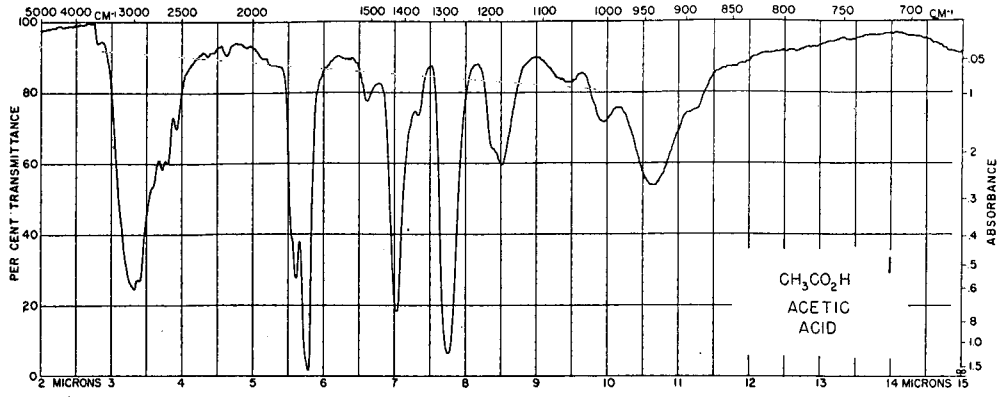
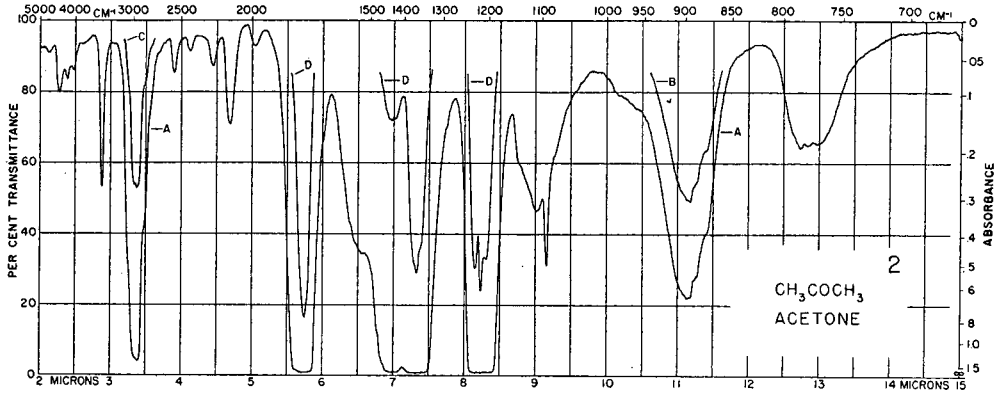
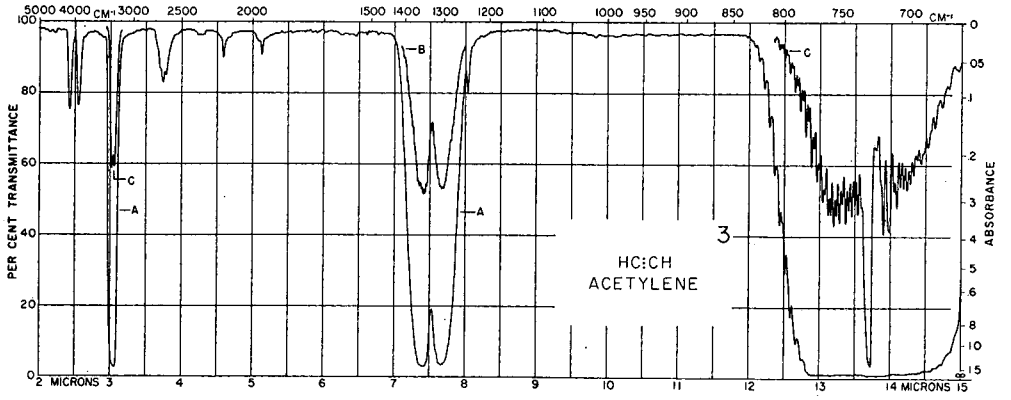
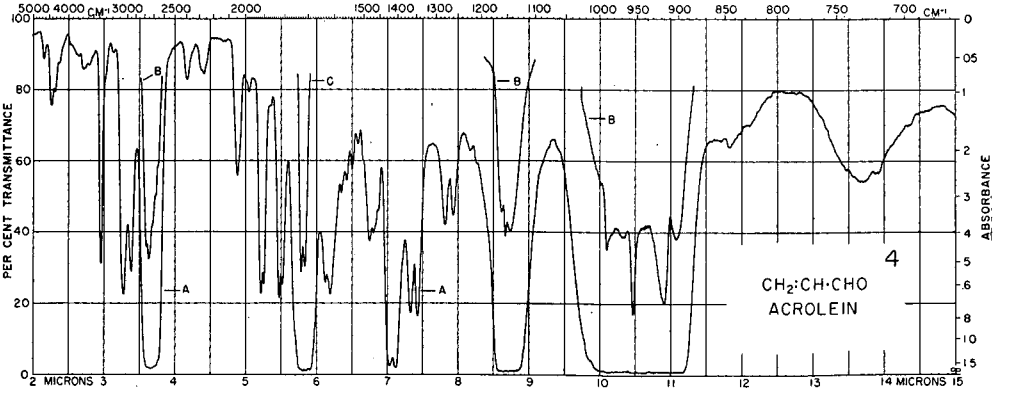


Figure 2. Comparison of P_1 and P_2 procedures with formic acid at 5.71 microns

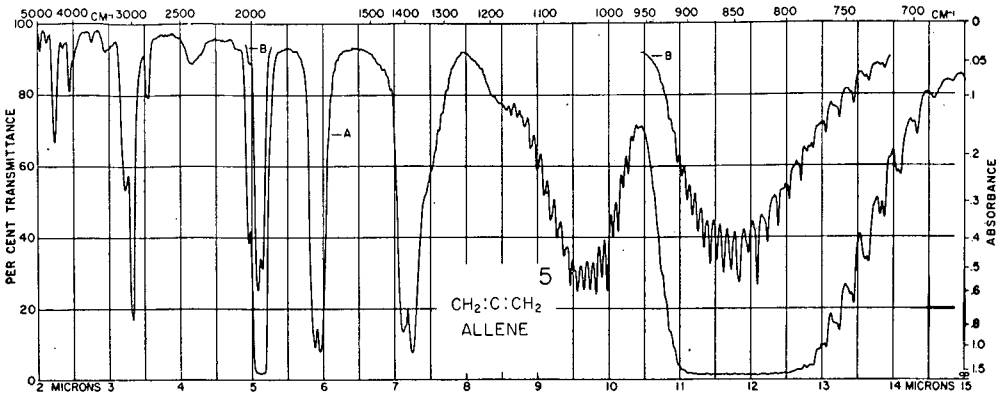


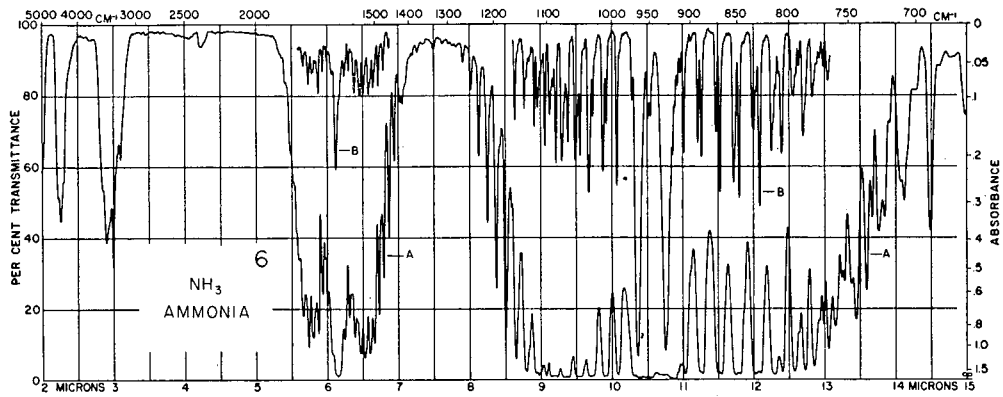


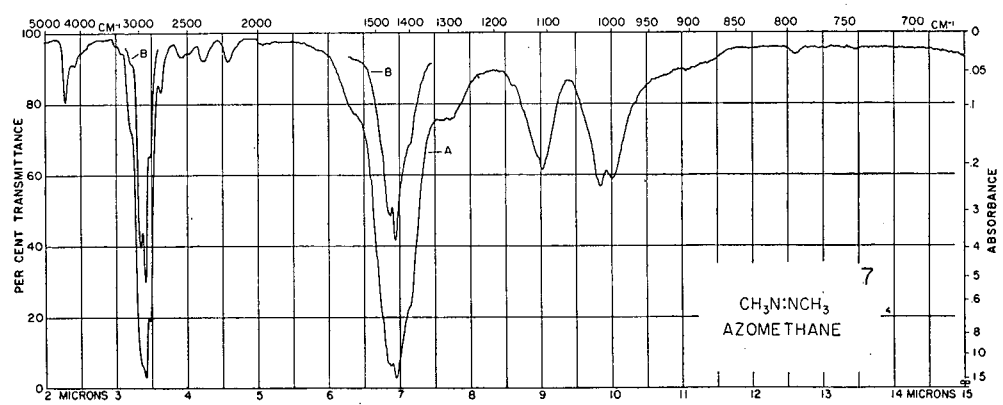


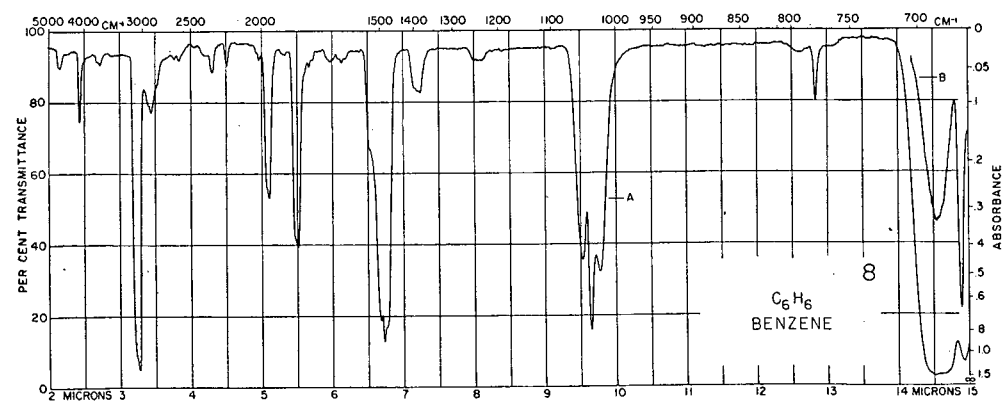


Curve letter															
Pressure, mm.	No. 1	12	No. 2	*A*	*B*	*C*	*D*	No. 3	*A*	*B*	*C*	No. 4	*A*	*B*	*C*
Resolution		927		235	110	45	13		704	148	59		228	26	6
				927	900	900	900		927	900	900		927	927	927



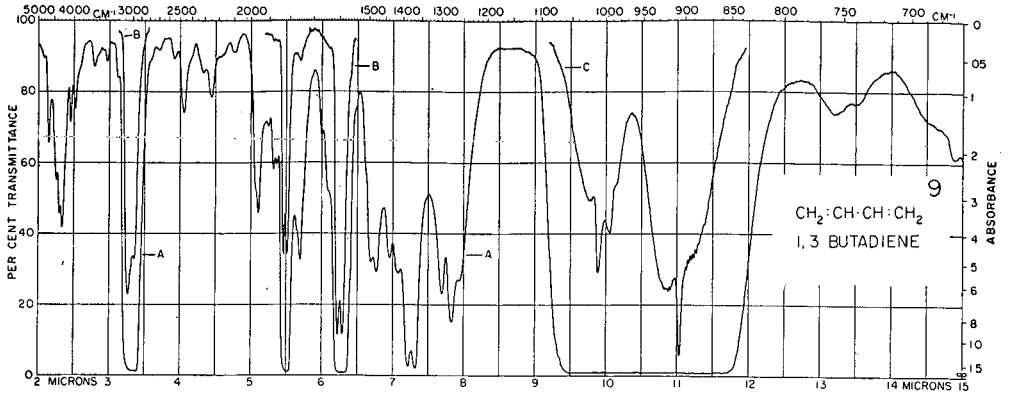


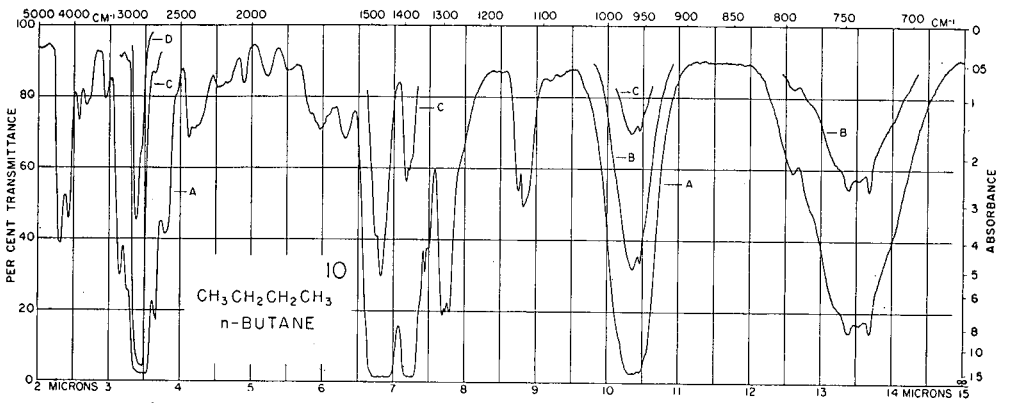


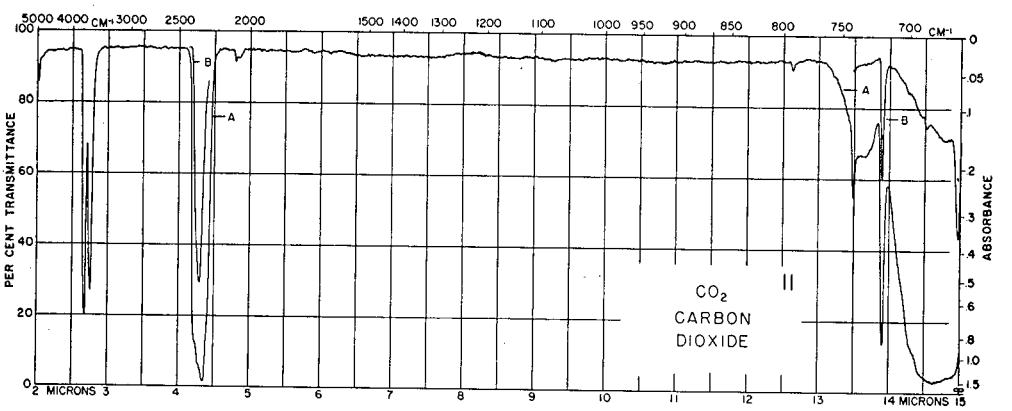


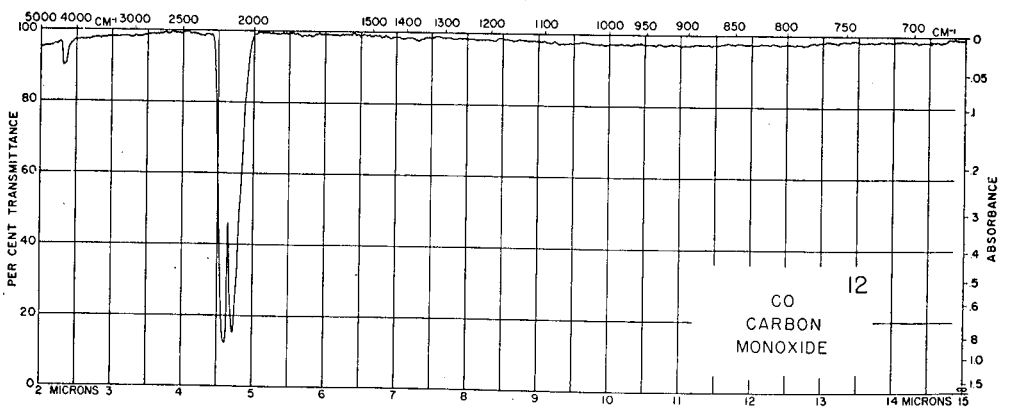
Curve letter		*A*	*B*		*A*	*B*		*A*	*B*		*A*	*B*
Pressure, mm.	No. 5	701	38	No. 6	700	45	No. 7	130[cc]	30[cc]	No. 8	100	5
Resolution		927	927		927	900		927	927		927	900

[cc] See Table I, [cc]

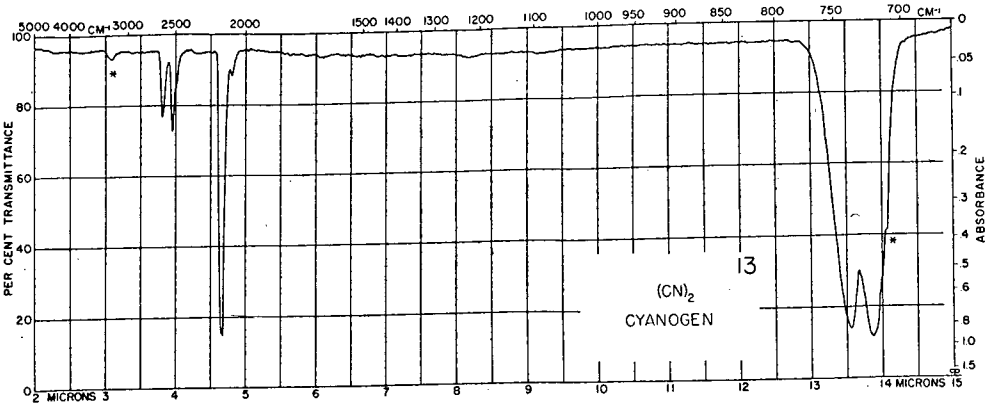


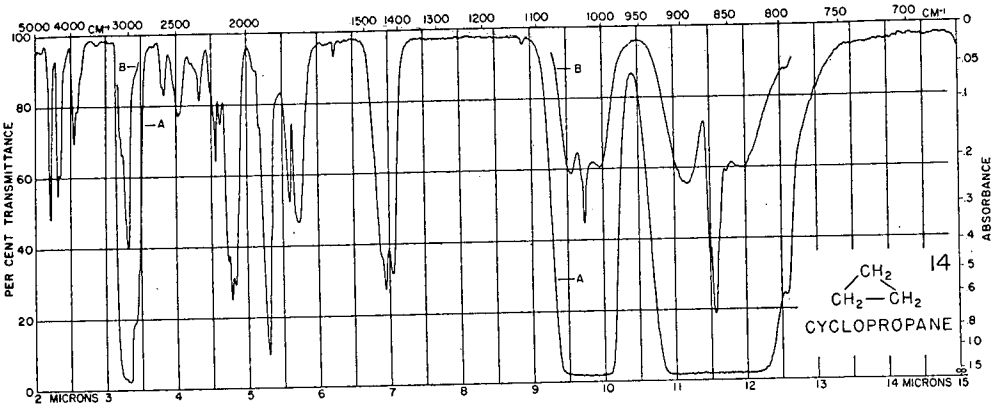


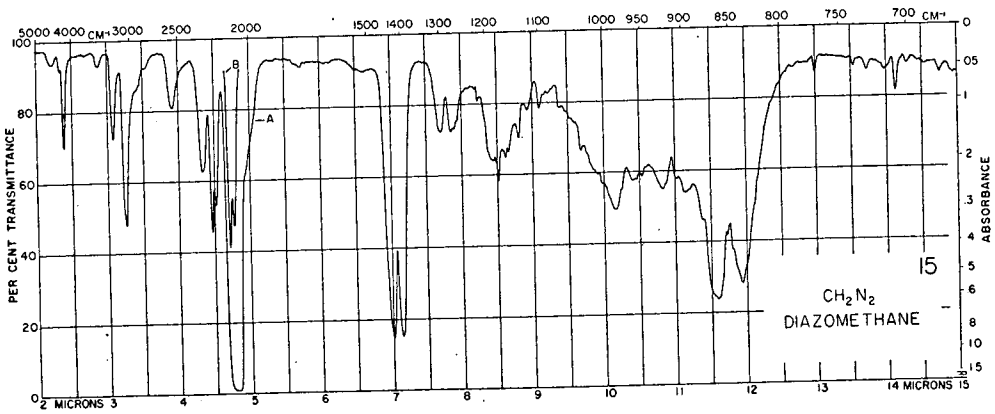


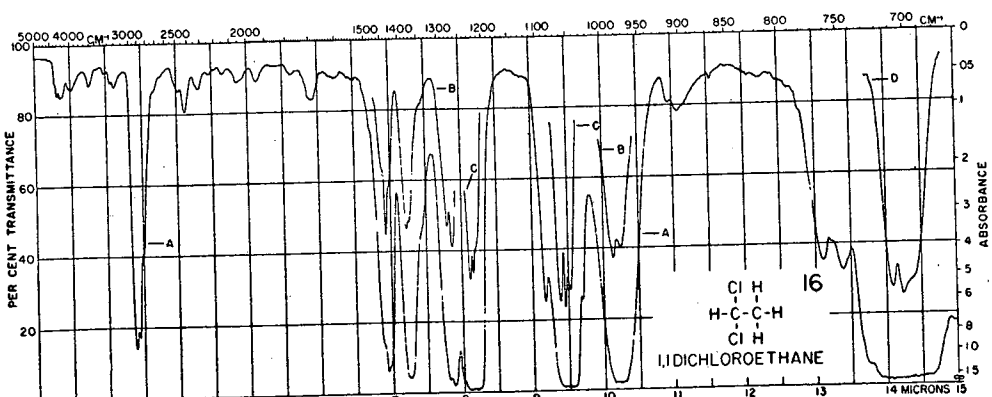


Curve letter		A	B	C	D
Pressure, mm.	No. 9	708	71	29	
Resolution		927	900	900	
Pressure, mm.	No. 10	705	209	58	6
Resolution		927	900	900	900
Pressure, mm.	No. 11	702	70		
Resolution		927	900		
Pressure, mm.	No. 12	714			
Resolution		927			





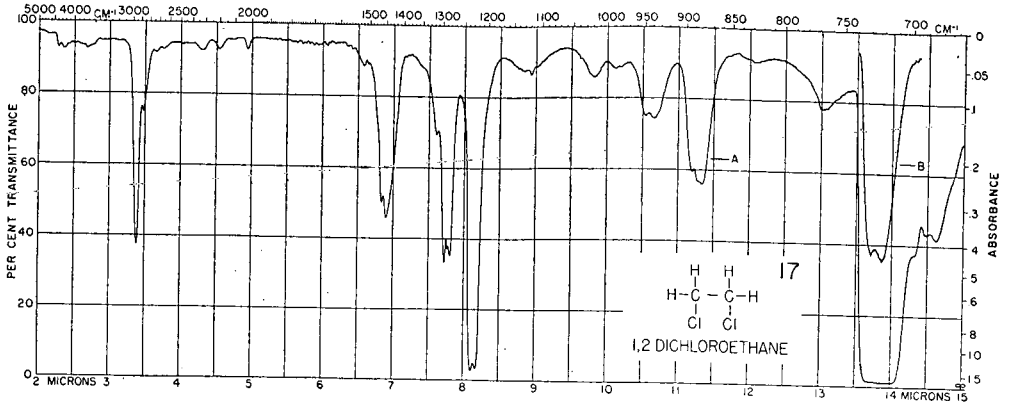


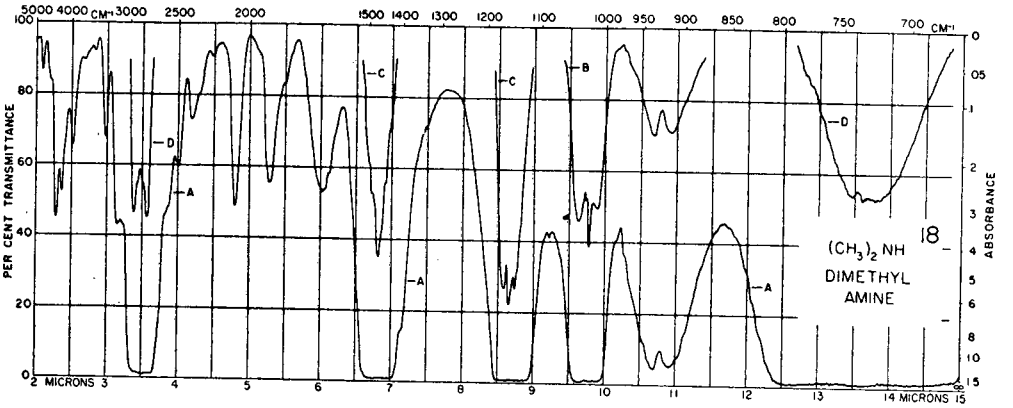


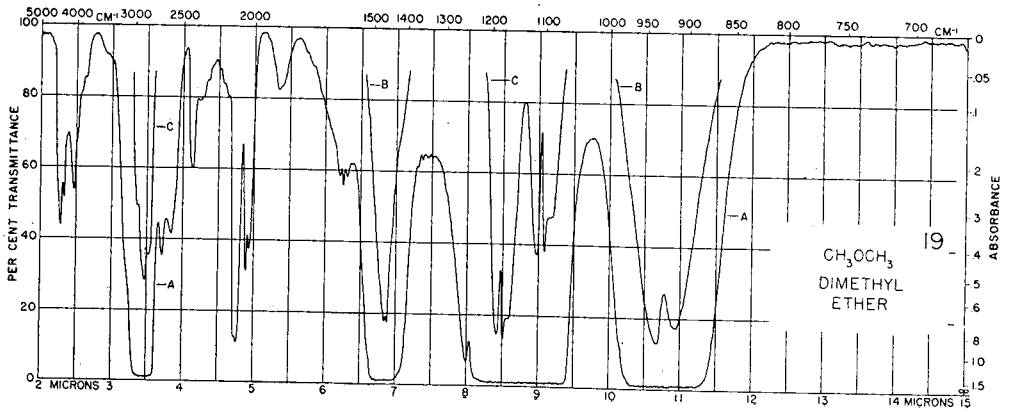
Curve letter															
Pressure, mm.	No. 13	350	No. 14	*A* 709	*B* 42	No. 15	*A* 130*dd*	*B* 13*dd*	No. 16	*A* 245	*B* 44	*C* 19	*D* 8
Resolution		927		927	900		927	927		927	927	927	927

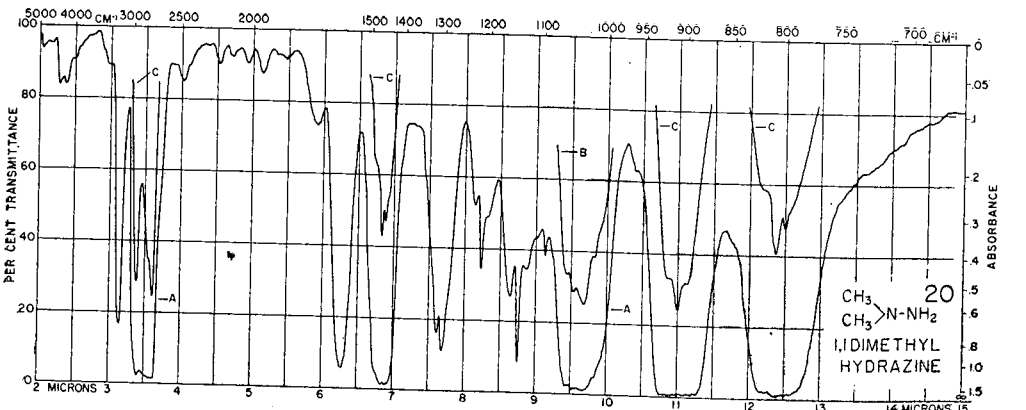
* Absorbance at about 3.05 and 14.05 μ probably due to trace of HCN.

dd See Table I, *dd*

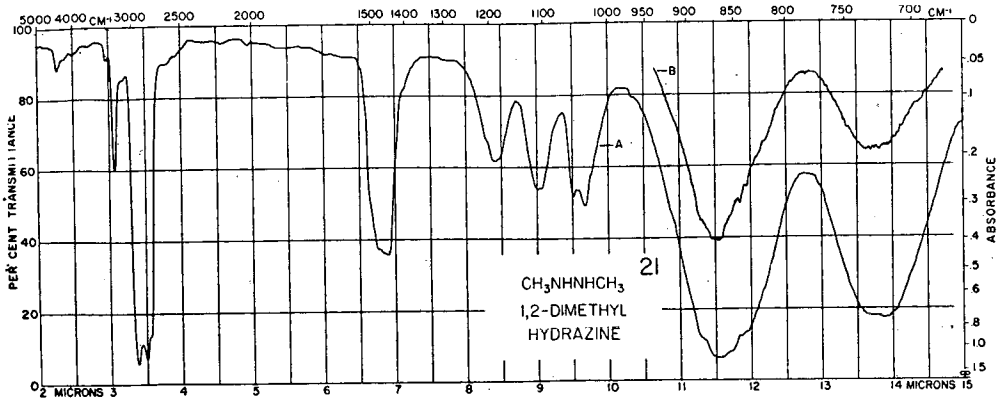


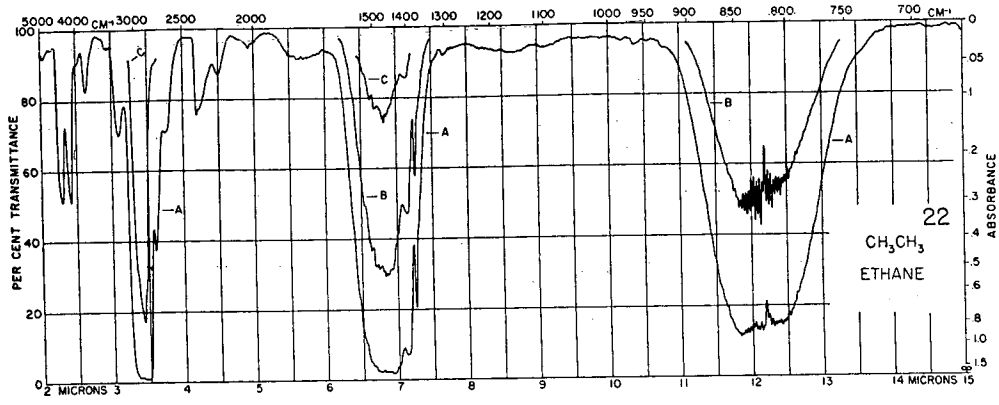


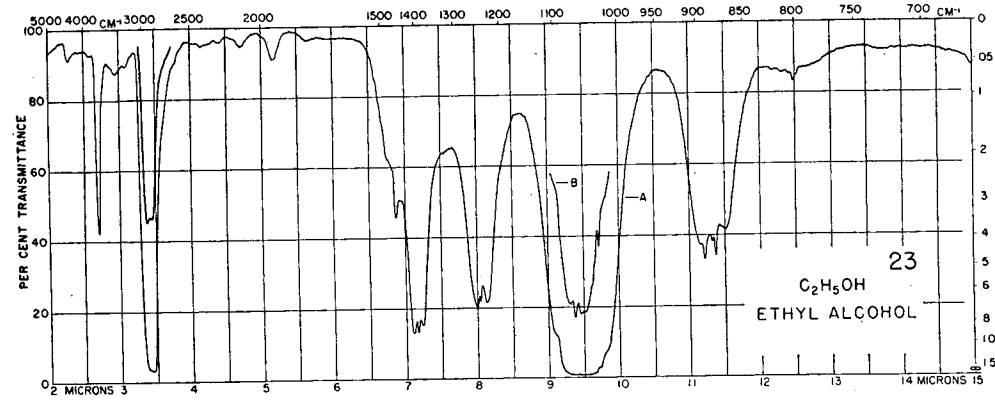


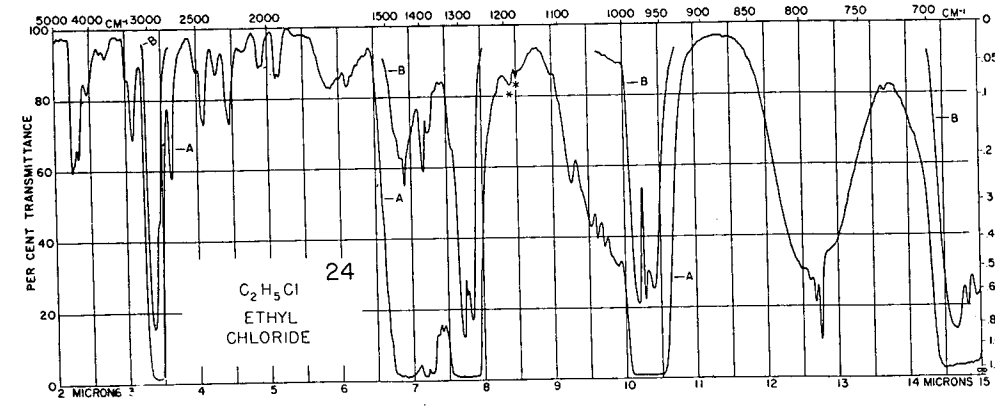


Curve letter		A	B	C	D
Pressure, mm.	No. 17	82	5		
Resolution		927	900		
	No. 18	704	96	43	12
		927	900	900	880
	No. 19	704	80	18	
		927	900	900	
	No. 20	147	30	10	
		927	927	927	





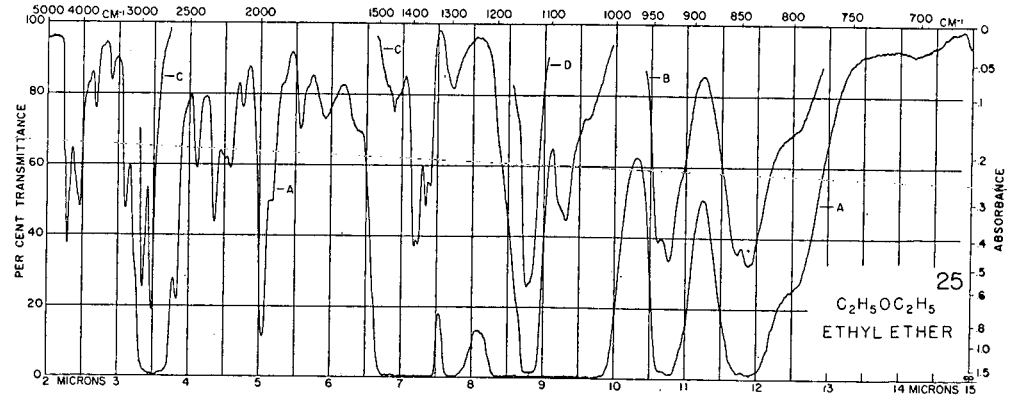


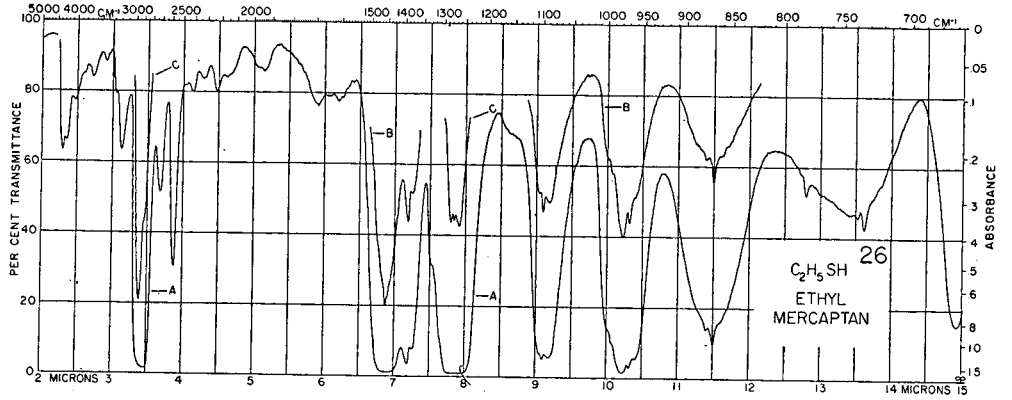


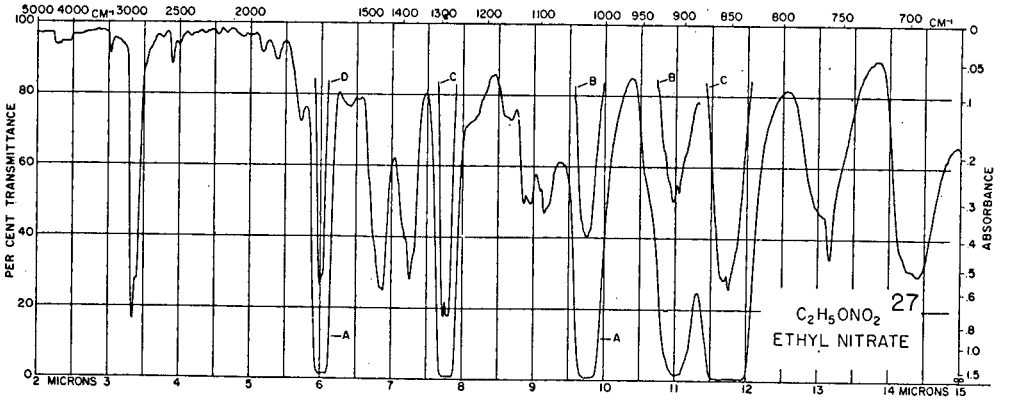
Curve letter																			
Pressure, mm.	No. 21	*A*	*B*	No. 22	*A*	*B*	*C*	No. 23	*A*	*B*	No. 24	*A*	*B*
Resolution		48[cc]	14[cc]		705	226	50		65	20		706	59
		927	900		927	900	900		927	927		927	900

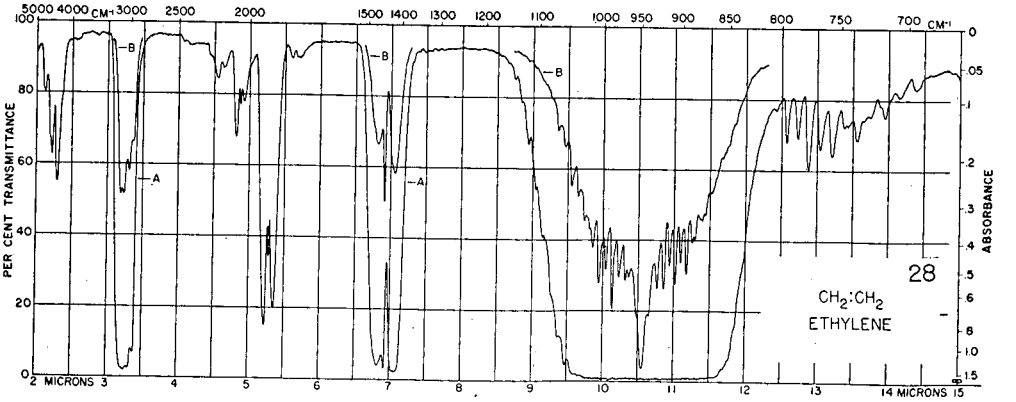
[cc] See Table I, [cc]

* Absorbance at 8.4 and 8.5 μ probably due to trace of impurity.

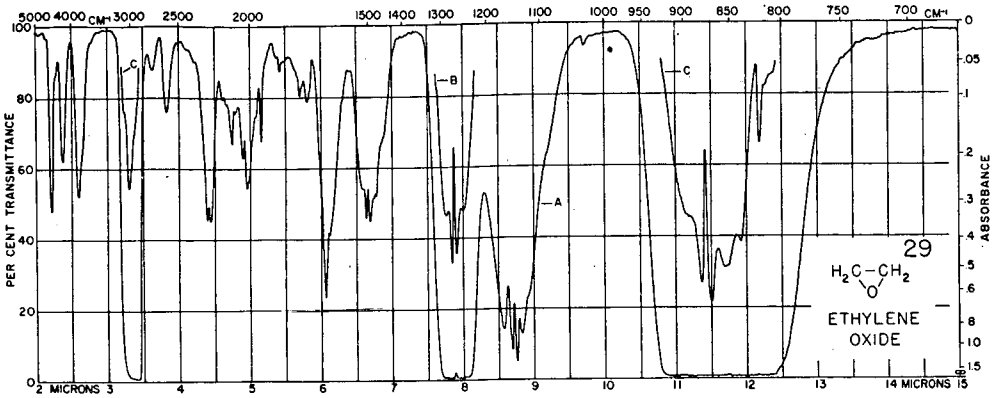


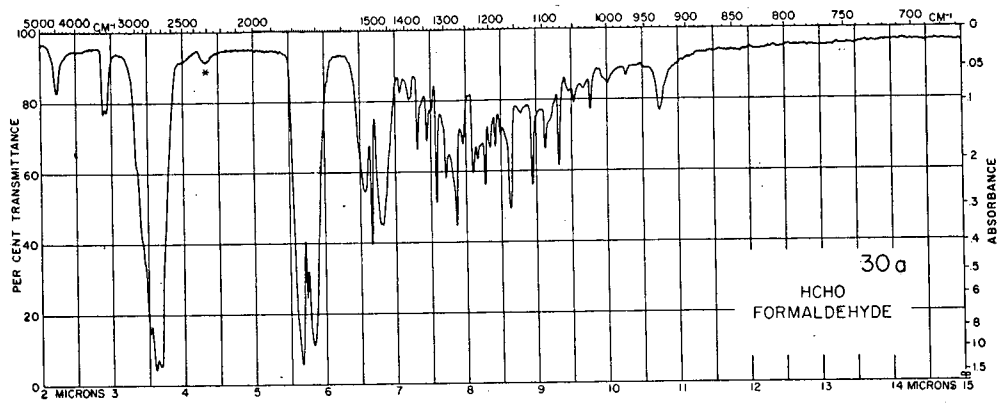


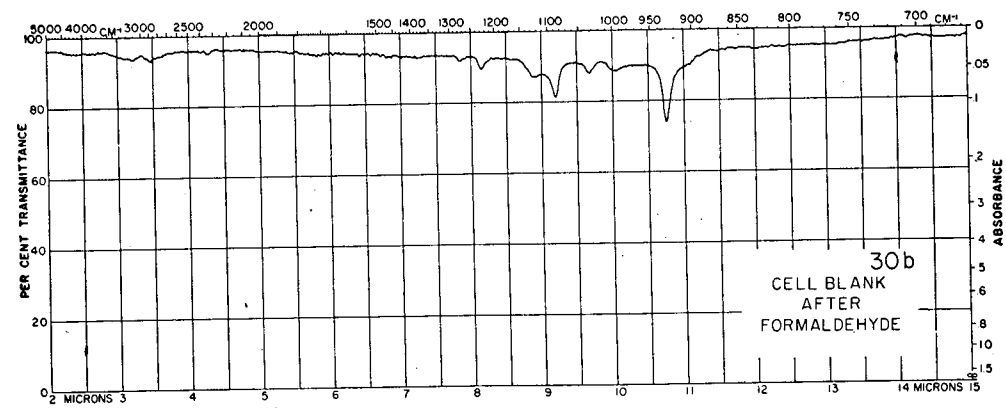


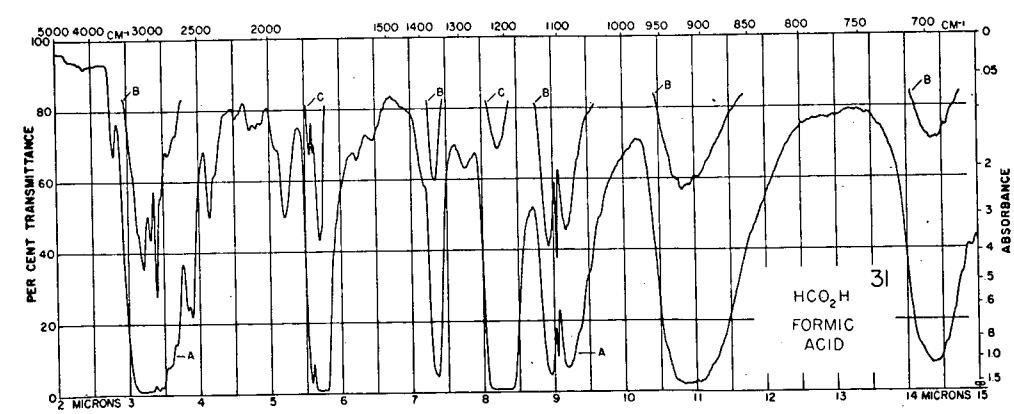


Curve letter		*A*	*B*	*C*	*D*		*A*	*B*	*C*		*A*	*B*	*C*	*D*		*A*	*B*
Pressure, mm.	No. 25	550	160	14	5	No. 26	550	123	29	No. 27	66	6	3	1	No. 28	705	87
Resolution		927	927	927	927		927	900	900		927	900	900	900		927	927







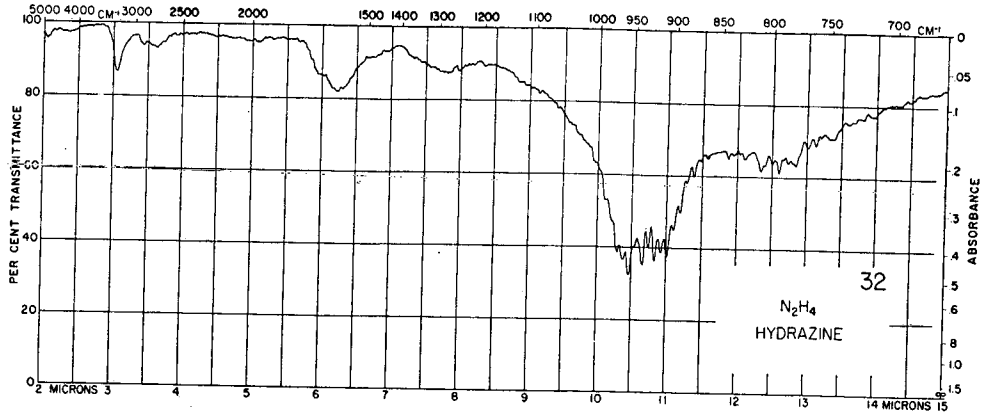


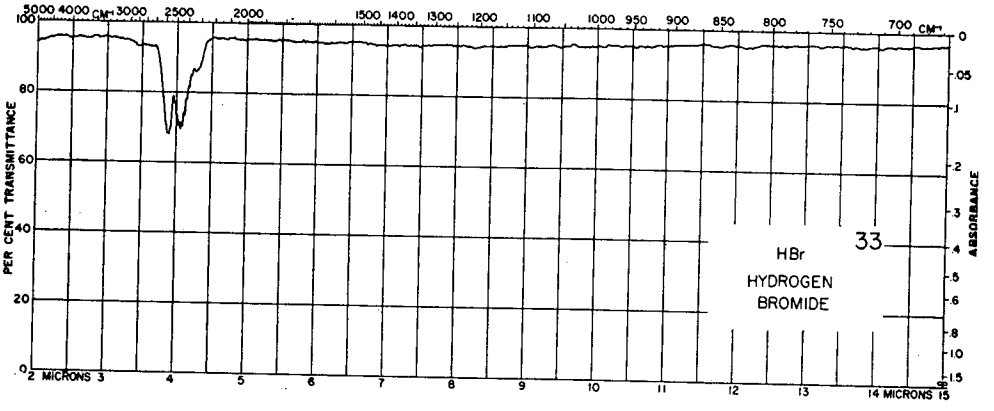
Curve letter		A	B	C
Pressure, mm.	No. 29	701	82	23
Resolution		927	900	900

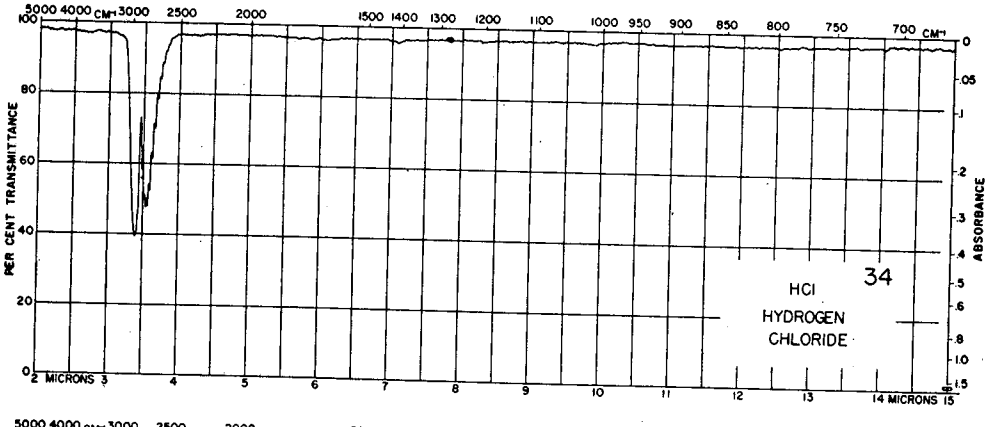
		A	B
	No. 30, a	100	
		927	

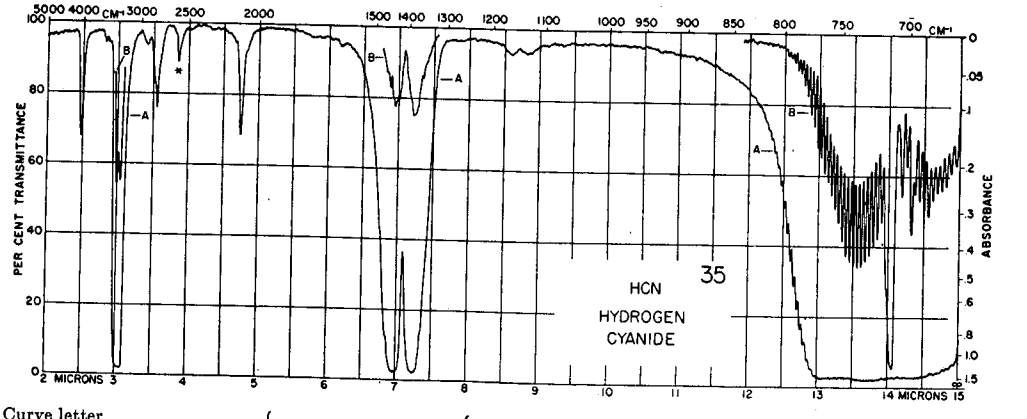
		A	B	
	No. 31	38	8	2
		927	927	927

* Absorbance at about 4.3 μ due to impurity; may be CO_2.









Curve letter
Pressure, mm.
Resolution

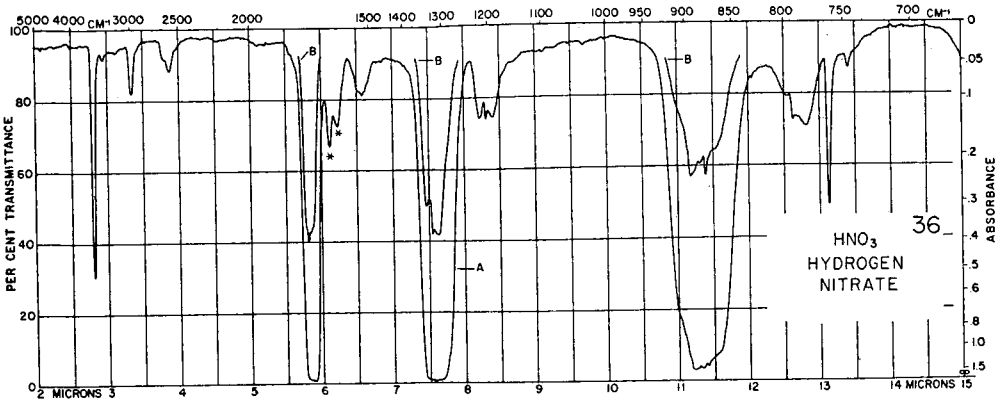
No. 32 { 15*dd* / 927
dd See Table I, *dd*

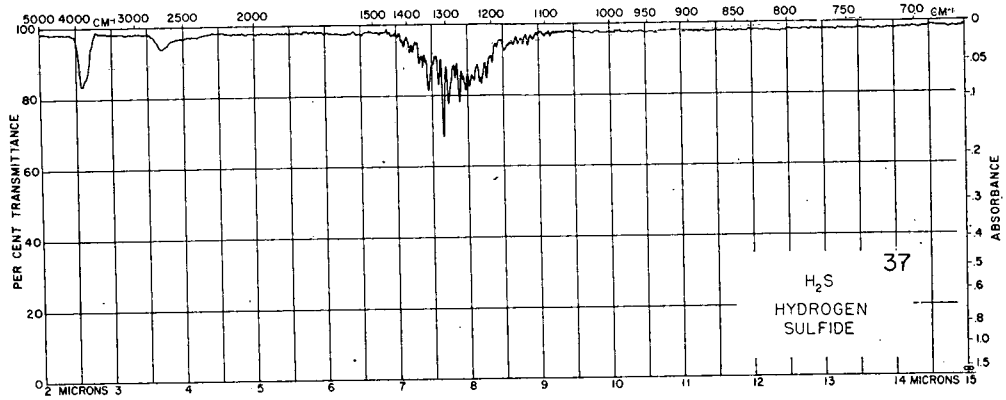
No. 33 { 710 / 900

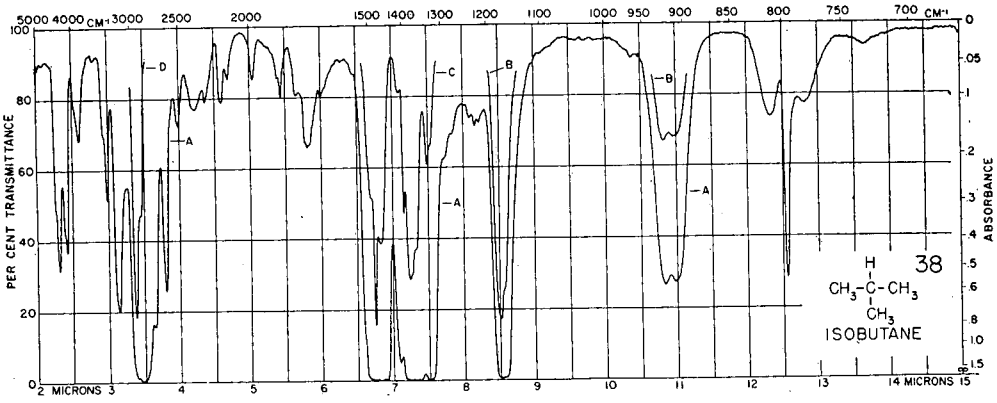
No. 34 { 705 / 927

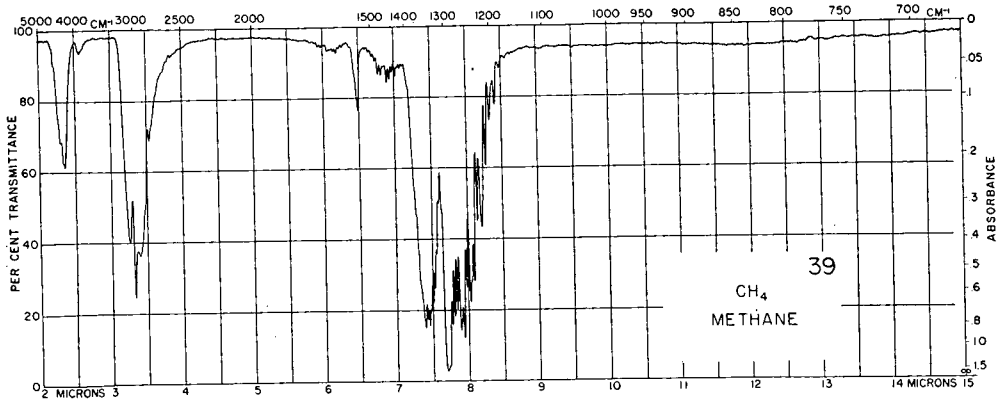
No. 35 { *A*: 707 / 927; *B*: 56 / 900

*Absorbance at about 3.8 μ probably due to impurity.



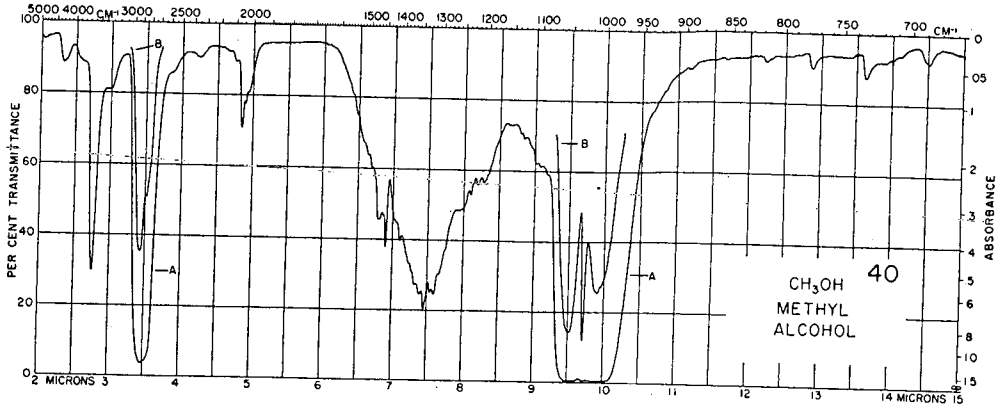


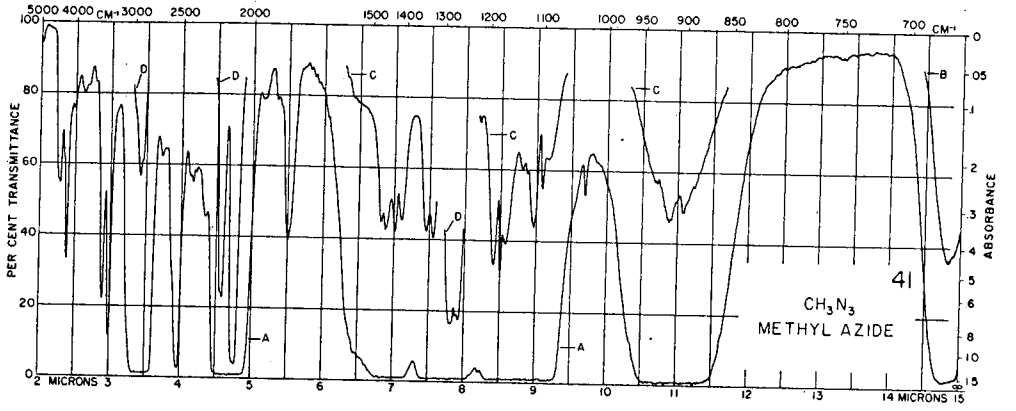


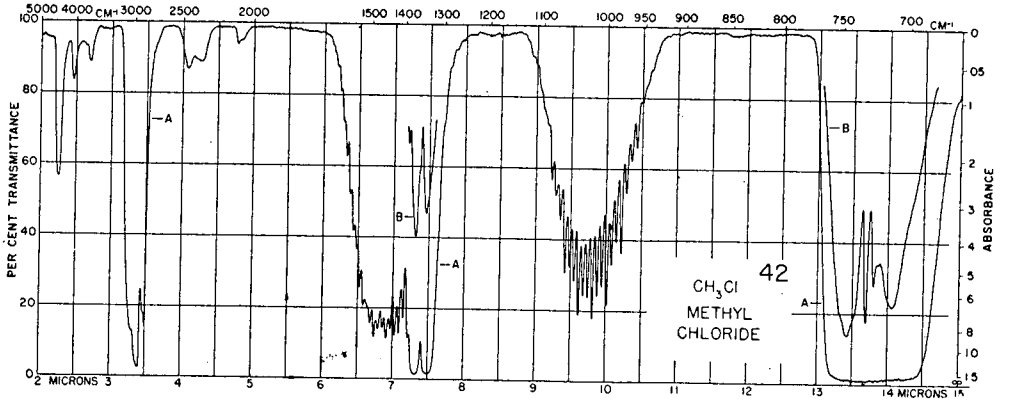


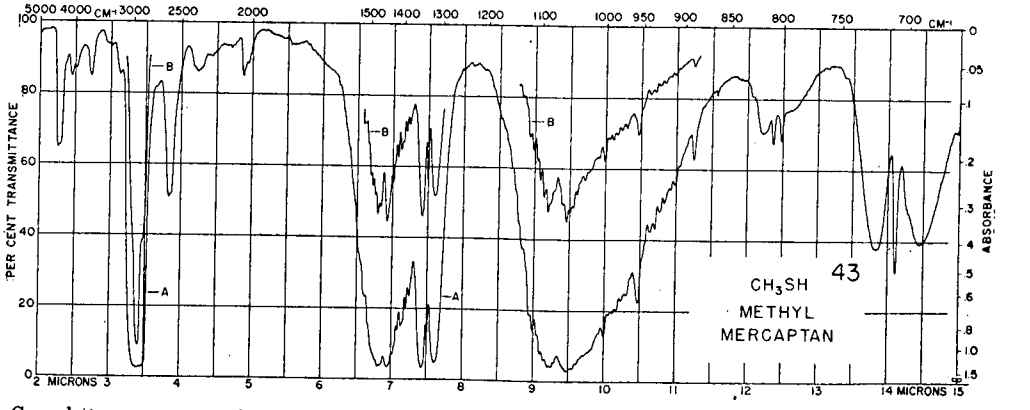
Curve letter		A	B			A	B	C	D	
Pressure, mm.	No. 36	65	3	No. 37	702	708	196	51	12	No. 39 702
Resolution		927	927		927	927	927	900	900	927

* Absorbance at about 6.1 and 6.25 μ probably due to NO_2.

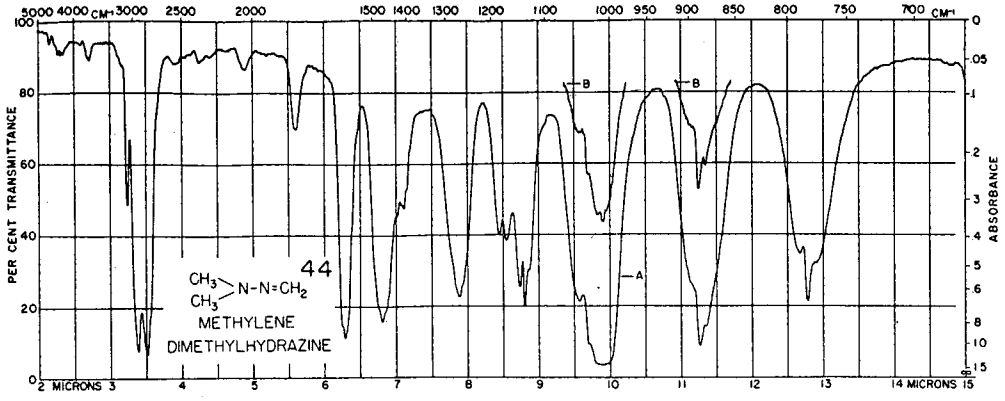


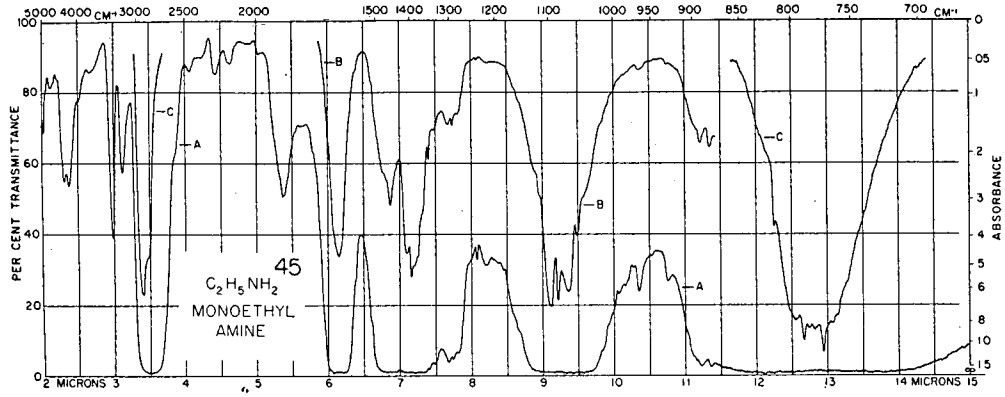


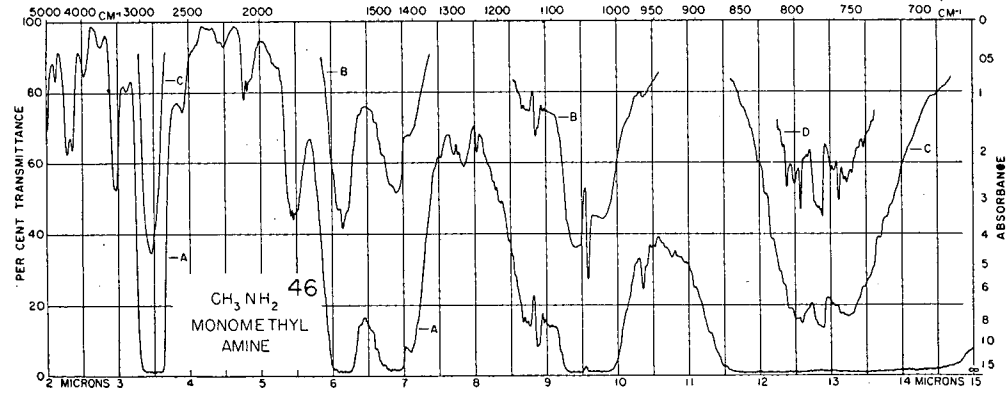


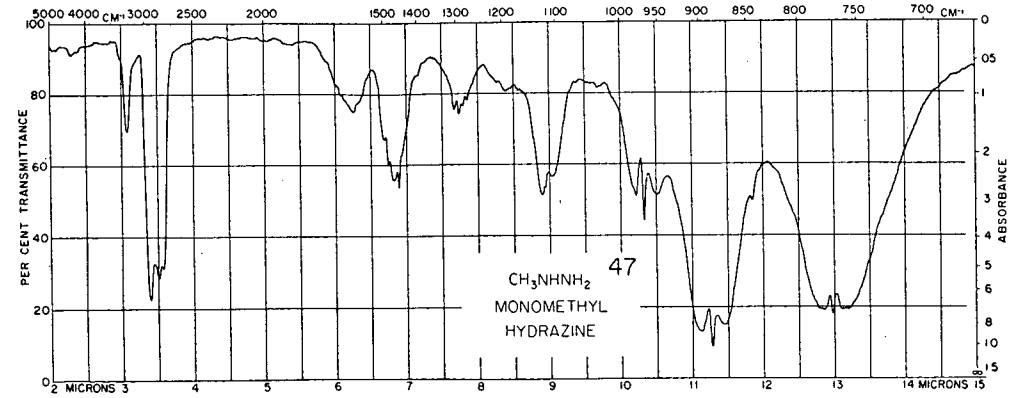


Curve letter															
Pressure, mm.	No. 40	*A*	*B*	No. 41	*A*	*B*	*C*	*D*	No. 42	*A*	*B*	No. 43	*A*	*B*
Resolution		135	24		698	100	51	15		704	89		710	140
		927	927		927	927	927	927		927	927		927	927



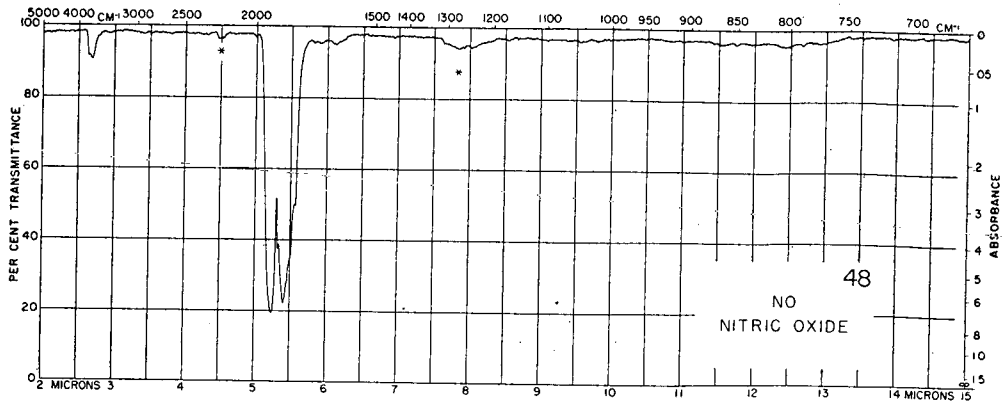


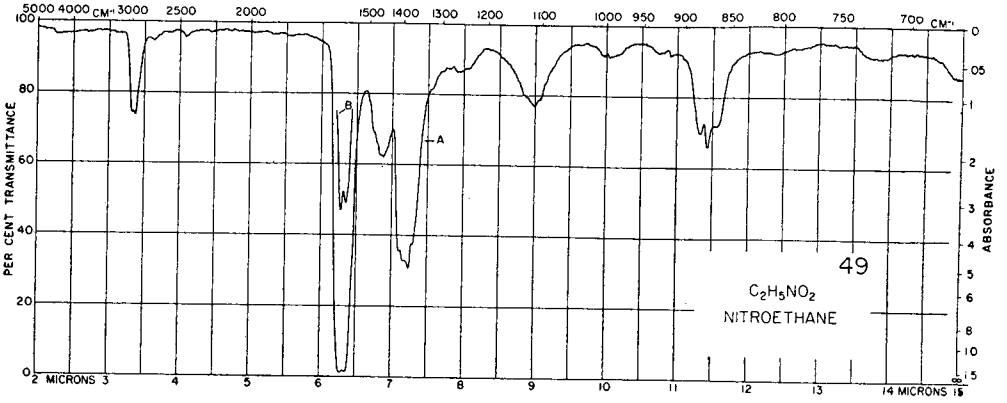


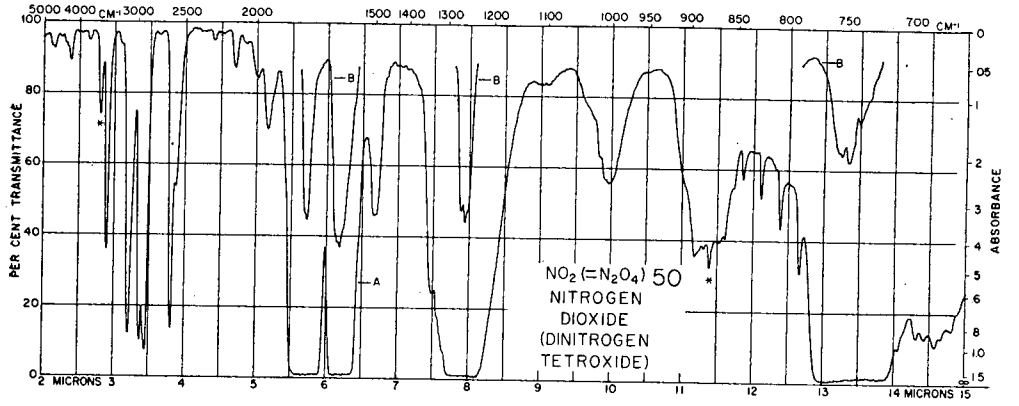


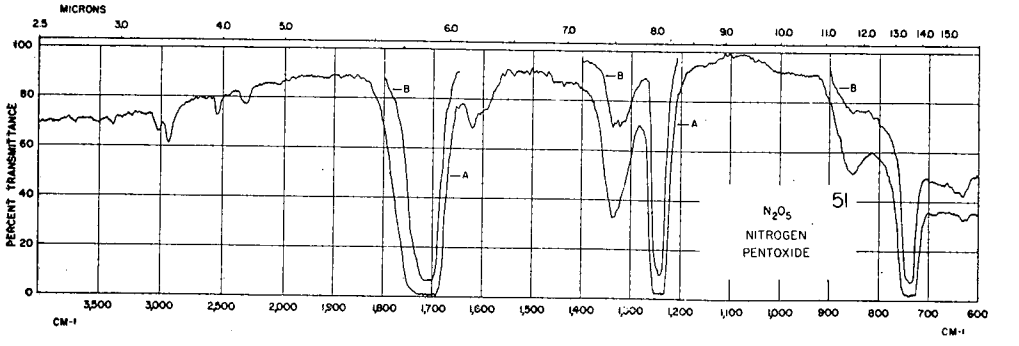
Curve letter		A	B	C	D
Pressure, mm.	No. 44	50[cc]	9[cc]		
Resolution		927	900		
	No. 45	704	70	22	
		927	900	880	
	No. 46	704	119	35	11
		927	900	900	880
	No. 47	42[cc]			
		927			

[cc] See Table I, [cc]







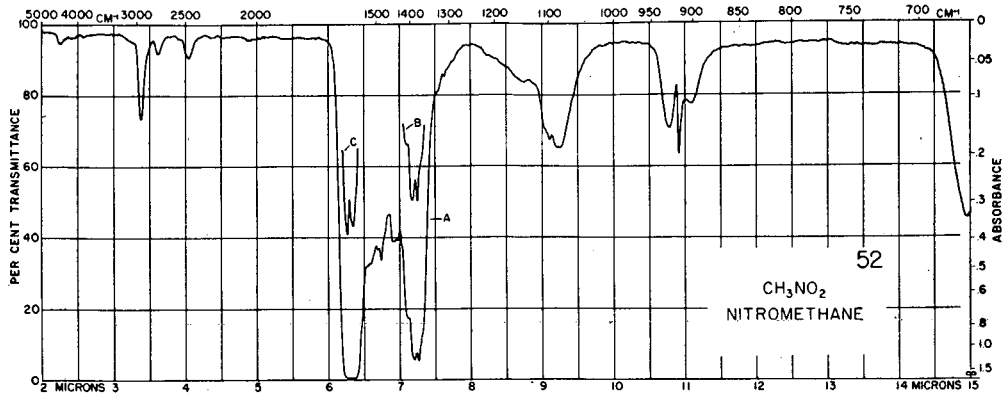


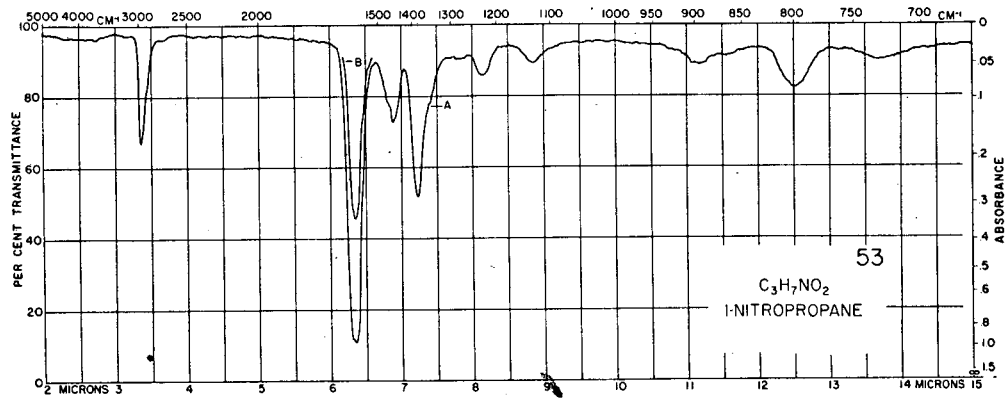
Curve letter		No. 48	No. 49		No. 50		No. 51	
Pressure, mm.		706	A 21	B 1.4[cc]	A 702	B 16	A 19	B 4
Resolution		927	927	927	927	927	927	927

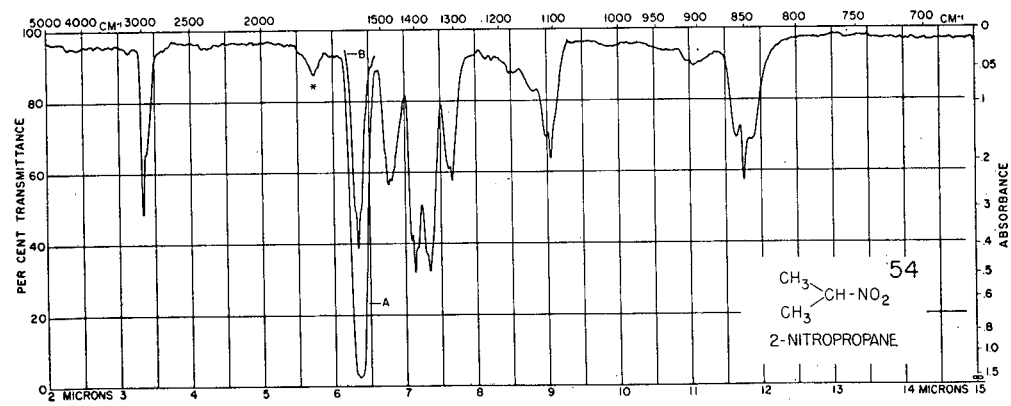
* Absorbance at about 4.5 and 7.8 μ probably due to N_2O.

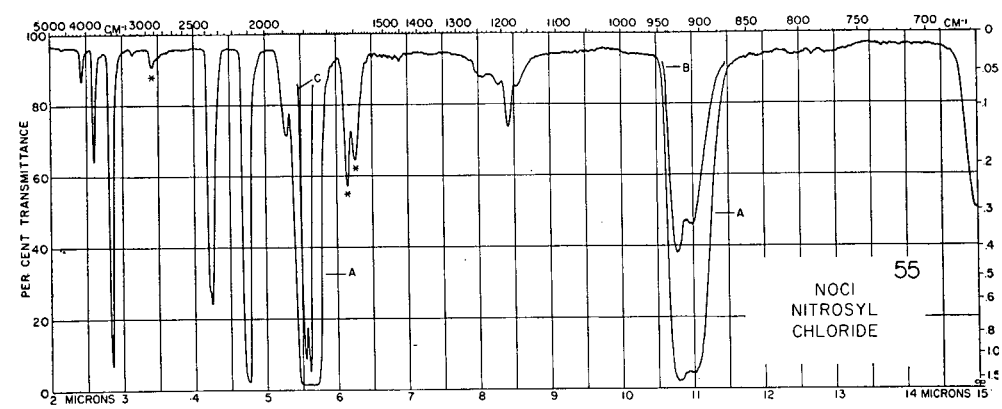
[cc] See Table I, [cc]

* Absorbance at about 2.8 and 11.35 μ probably due to HNO_3.









		A	B	C
Curve letter				
Pressure, mm.	No. 52	35	4[cc]	1.4[cc]
Resolution		927	927	927

[cc] See Table I,[cc]

		A	B
Curve letter			
Pressure, mm.	No. 53	8.5[cc]	2.3[cc]
Resolution		927	927

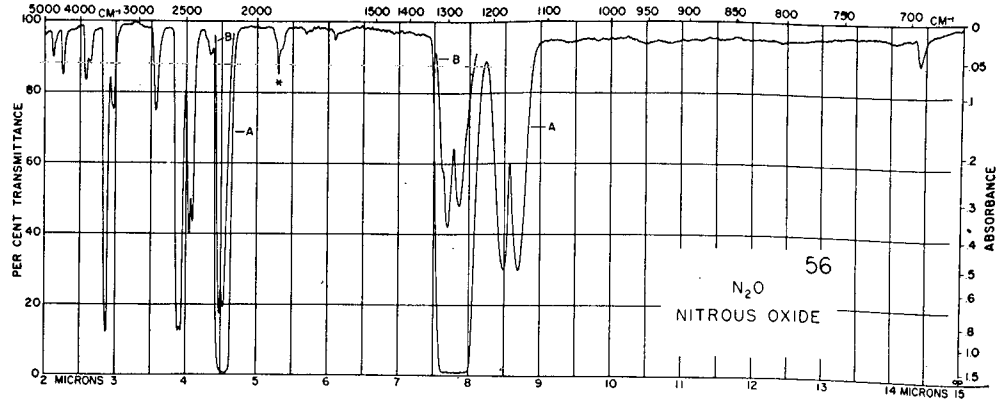
[cc] See Table I,[cc]

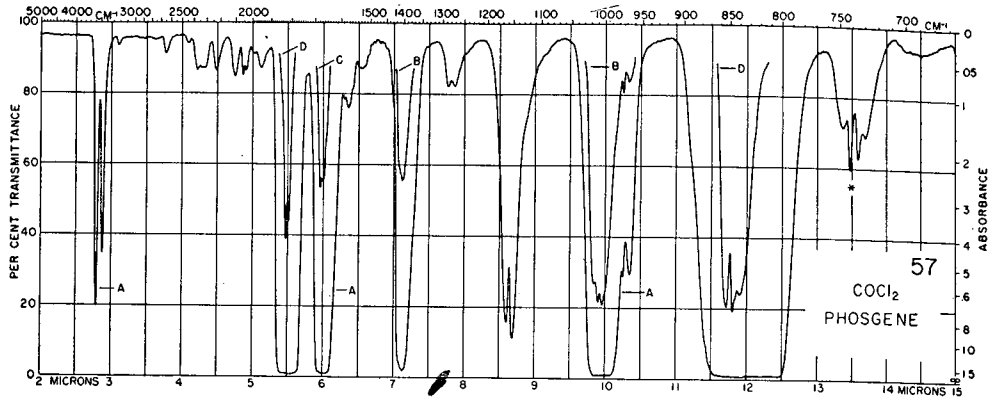
		A	B
Curve letter			
Pressure, mm.	No. 54	19	2
Resolution		927	927

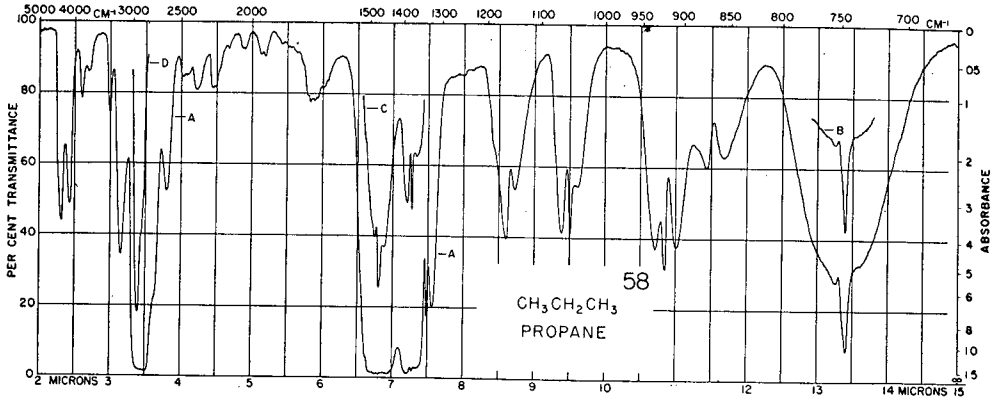
* Absorbance at about 5.73 μ may be due to impurity.

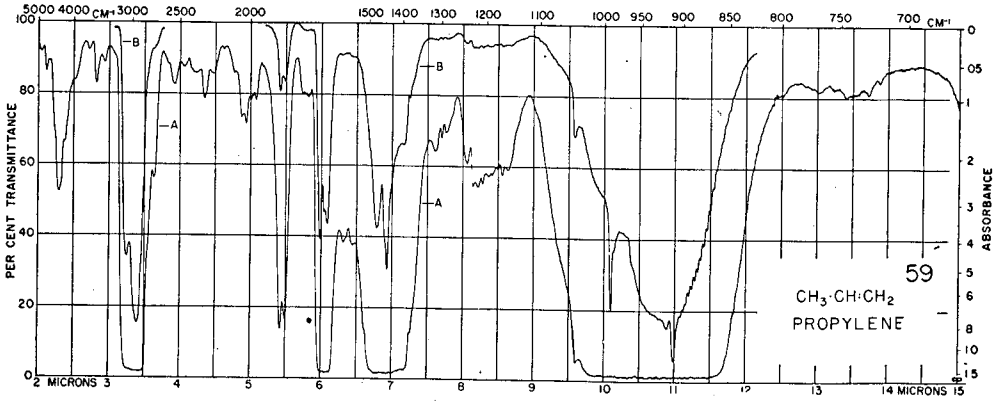
		A	B	C
Curve letter				
Pressure, mm.	No. 55	710	158	2
Resolution		927	927	927

* Absorbance at about 3.4, 6.1, and 6.25 μ probably due to NO_2.





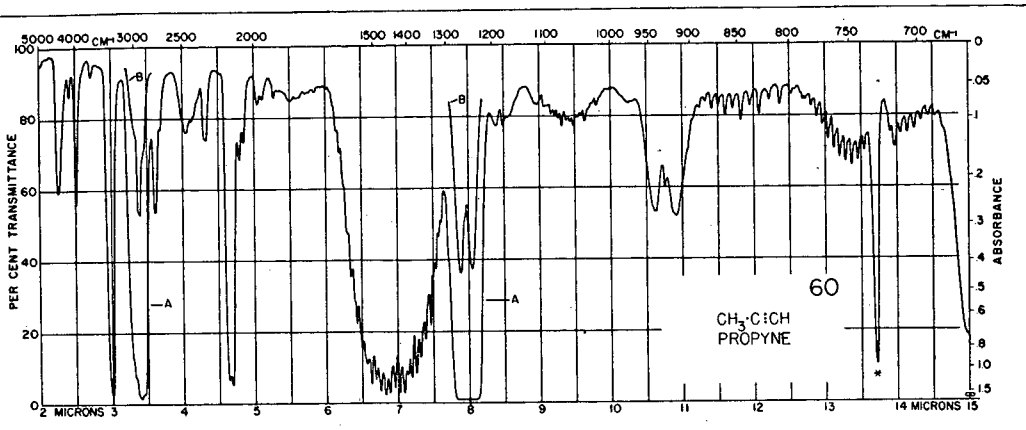


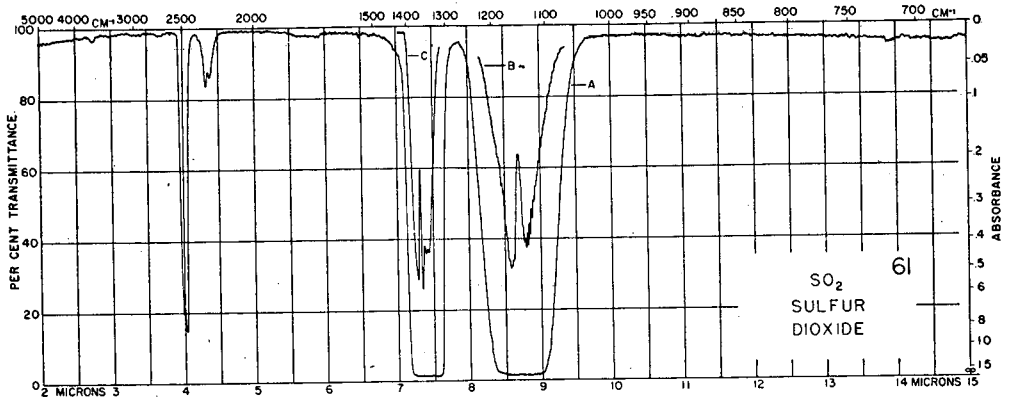


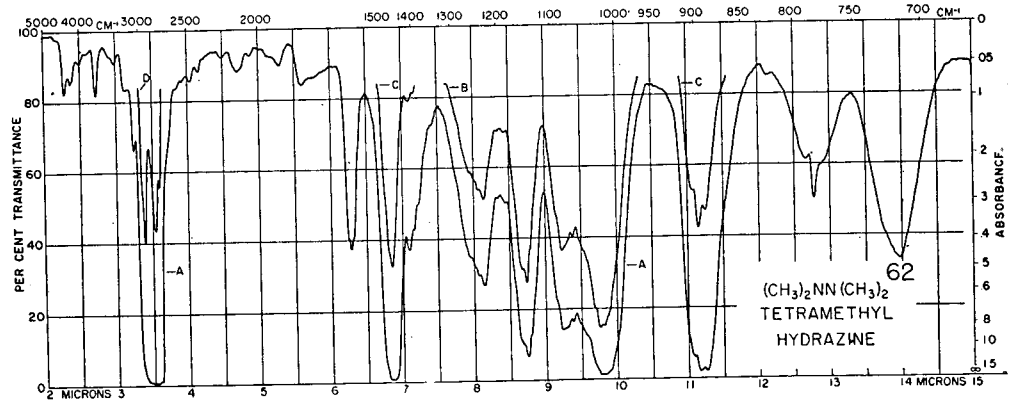
Curve letter		A	B	C	D
Pressure, mm.	No. 56	701	69		
Resolution		927	900		
Pressure, mm.	No. 57	710	80	17	1.5
Resolution		927	927	927	927
Pressure, mm.	No. 58	709	211	94	15
Resolution		927	900	900	900
Pressure, mm.	No. 59	710	80		
Resolution		927	900		

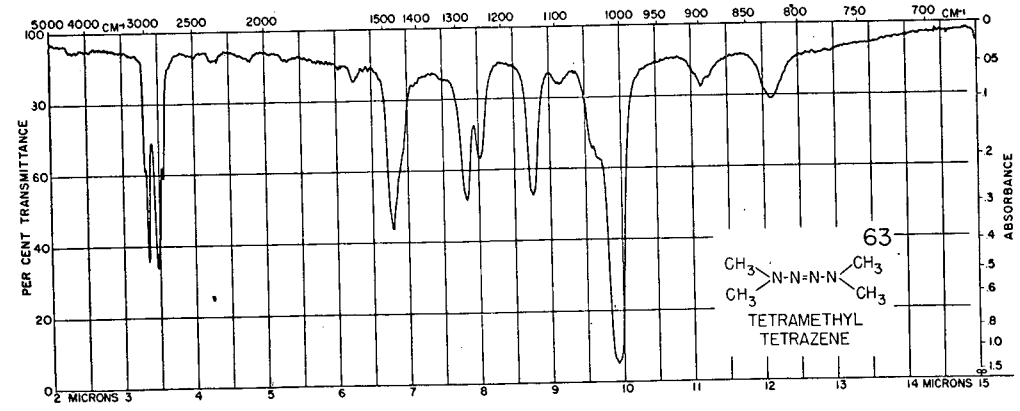
* Absorbance at about 5.3 μ probably due to NO.

* Absorbance at about 13.5 μ may be due to impurity.





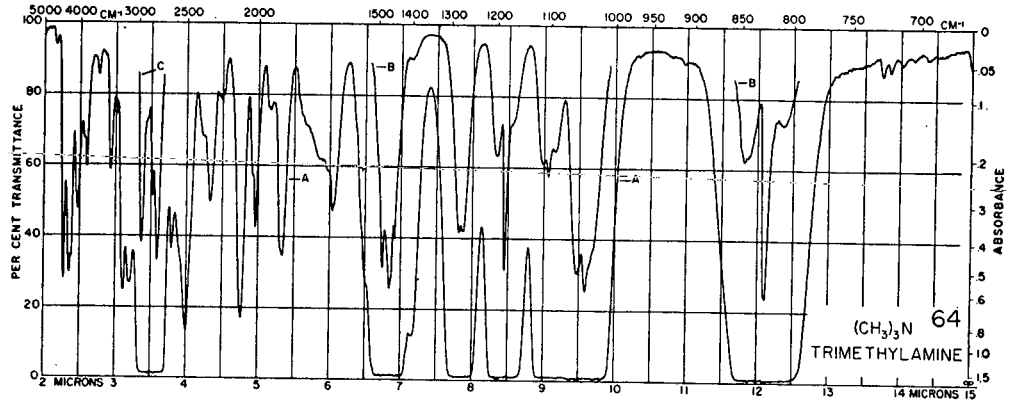


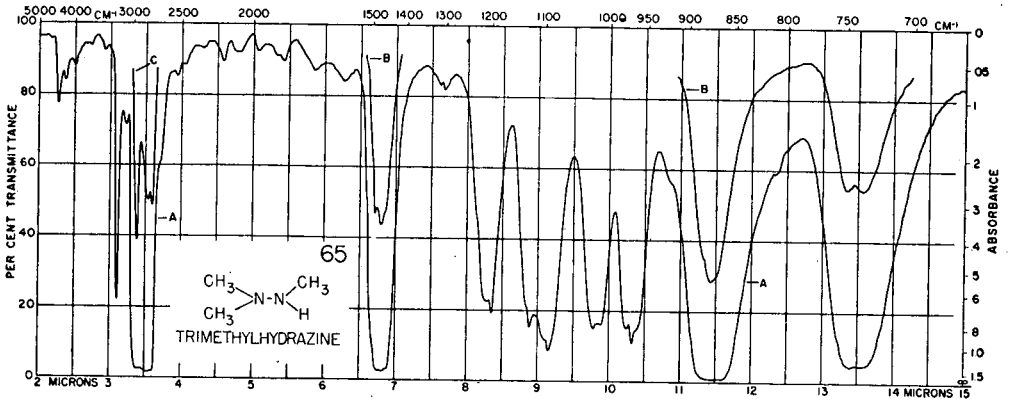


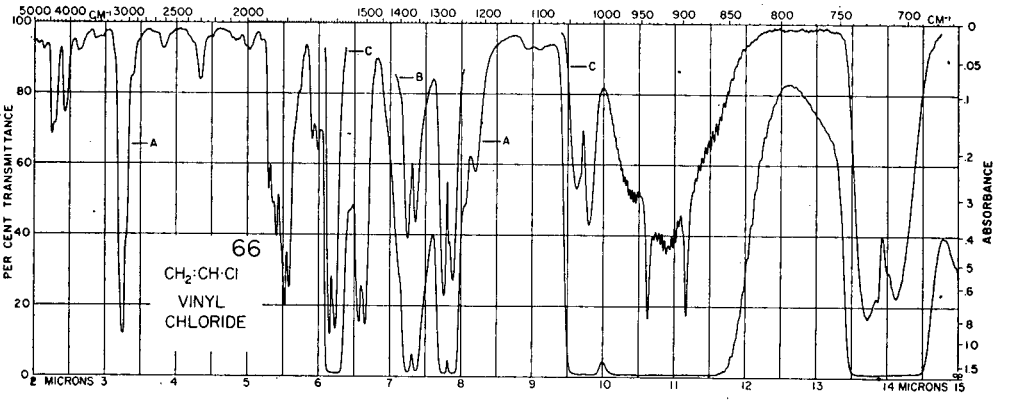
Curve letter																			
Pressure, mm.	No. 60	*A*	*B*	No. 61	*A*	*B*	*C*	No. 62	*A*	*B*	*C*	*D*	No. 63	6.5					
Resolution		704	51		704	68	20		90[cc]	27[cc]	7[cc]	2.5[cc]							
		927	927		927	900	900		927	927	927	927		927					

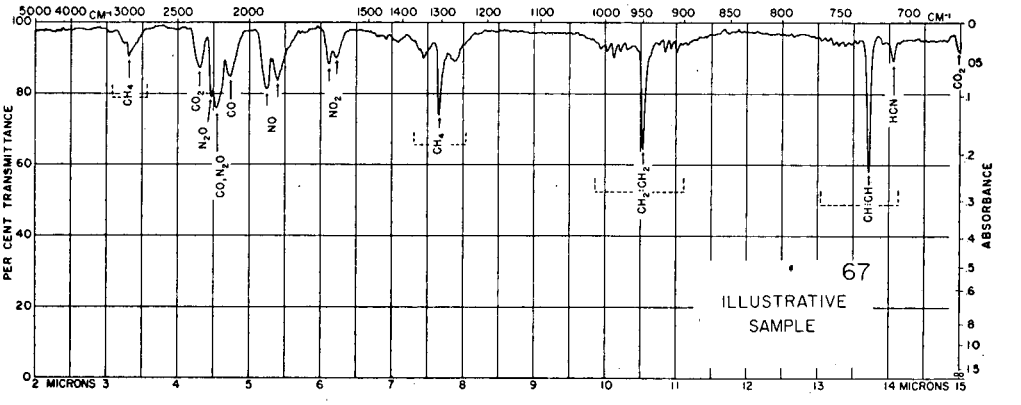
* Absorbance at about 13.7 μ due to acetylene, present in considerable amount.

[cc] See Table I,[cc]









Curve letter		A	B	C		A	B	C		A	B	C		
Pressure, mm.	No. 64	700	25	8	No. 65	106[cc]	15[cc]	6.5[cc]	No. 66	700	117	35	No. 67	705[dd]
Resolution		927	927	927		927	927	927		927	900	900		927

[cc] See Table I, [cc]

[dd] See Table I, [dd]

Spectra: No. 21, 1,2-dimethylhydrazine; No. 44, methylene dimethylhydrazine; No. 47, monomethylhydrazine; No. 62, tetramethylhydrazine; No. 65, trimethylhydrazine. In order to minimize decomposition effects, spectra of the subject materials were determined on initial samples of less than the atmospheric or saturation pressure.

Spectra No. 30, *a* and *b*, formaldehyde. In high concentrations formaldehyde polymerizes rapidly at room temperature. The polymer is a white solid which deposits on cell windows to give undesirable infrared absorption peaks (sometimes referred to as artifacts). Even at the 100-mm. pressure used for the catalog of this paper, and with the time for obtaining the spectrum kept at a minimum, some artifacts developed. No. 30, *b*, is reported to show the magnitude of the false absorbance and thus to provide corrections for No. 30, *a*. A lower concentration which would eliminate the need for such a correction was not feasible because the absorbance would be too drastically reduced.

Spectrum No. 32, hydrazine. The spectrum for hydrazine vapor was obtained at a temperature of about 45° C. using a cell with sodium chloride windows. At a room temperature of 27° C. windows of this material are attacked by hydrazine to give undesirable absorption effects. That the increased temperature did not produce appreciable changes in the hydrazine spectrum as compared to one at 27° C. was evidenced by several scans made at room temperature with a cell equipped with very thin polyethylene windows. The spectrum at the elevated temperature was reported in preference to others because the blank cell scan made with all polyethylene films available showed a number of unwanted absorptions.

Spectra: No. 36, hydrogen nitrate; No. 50, nitrogen dioxide. For the spectra of hydrogen nitrate and nitrogen dioxide, special conditions were necessary. These gases, even when dry, attack rubber tubing, stopcock grease, mercury, Glyptal (window cement), and sodium chloride windows. Hence, the cells used for these samples were equipped with silver chloride windows cemented to the cell body with a minimum amount of Fluorolube grade HG (Hooker Electrochemical Co.). An all-glass apparatus was used so that the gas introduced into the cell was never in contact with grease, flexible tubing of any kind, or mercury. Because the proportions of nitrogen dioxide and dinitrogen tetroxide in a mixture are dependent upon temperature, the relative heights of the infrared peaks vary with the temperature at which the spectrum is obtained (*10*, *16*, *21*, *22*).

Spectrum No. 51, nitrogen pentoxide. It will be noted that the scales and proportions of the spectrum of nitrogen pentoxide differ slightly from those of the other spectra. This spectrum was transcribed and then photoreduced from one supplied by the Naval Ordnance Laboratory of the University of Minnesota and is published here with the permission of its authors. Although the recording has a somewhat different appearance from the others in the catalog, it was produced on the same model instrument and under about the same conditions as those used by the authors of this paper for hydrogen nitrate and nitrogen dioxide. The cell had silver chloride windows and was 7.8 cm. in length. The temperature during the scan was 23° C. Because nitrogen pentoxide is subject to rapid decomposition (particularly when in high concentration), the spectra were produced at the decreased concentrations shown; the scans were made at a moderately fast rate and a separate filling was used for each concentration (*16*).

DISCUSSION

Perhaps the utility of the basic data provided in this paper may be best visualized by examination of an illustrative example such as that shown as spectrum No. 67. Nine gases are readily identifiable in the spectrum of this synthetic unknown. The amounts of these gases varied from 0.03% (hydrogen cyanide and acetylene) to 3.0% (carbon monoxide). The remainder of the sample was helium. By comparison of spectrum No. 67 with the peaks symbolized in Figure 1, one is able to select very quickly the gases which could be responsible for the peaks of the spectrum. Confirmation is then made by checking the unknown against the spectra of the likely individual gases. The example shown is a favorable one regarding overlapping difficulties. In applying infrared to general unknowns the need for some kind of separation prior to scanning will become evident in many laboratories. Fractional distillation will often prove helpful. Considerable progress has recently been made in combining the techniques of infrared spectroscopy and gas chromatography, and the discrepancies between the two procedures are being resolved.

Although the illustrative sample contained nitrogen dioxide, it was held in a cell equipped with sodium chloride windows. No damage occurred to the windows in this case. In another sample containing about four times as much nitrogen dioxide the windows were attacked slightly. The illustrative sample contained about 0.05% nitrogen dioxide; the sample which attacked the windows contained about 0.2%. These data give some indication of the amount of nitrogen dioxide which may be tolerated without window damage. It should be remembered that, although nitric oxide itself will not affect salt windows, it will be converted to damaging nitrogen dioxide if any oxygen is present. The usual supply of nitrogen contains sufficient oxygen to cause formation of the dioxide, whereas helium usually does not. If helium is not available, nitrogen can be used as a diluting gas in samples containing nitric oxide, provided the nitrogen is first treated for removal of the trace of oxygen.

The threshold data provided in this paper show that infrared spectroscopy is a relatively sensitive tool for many gases, but that it is not suitable for others. No useful infrared peaks are observable in the 2- to 15-micron range for elements such as hydrogen, nitrogen, oxygen, fluorine, chlorine, and helium (*11*). The spectrum for hydrogen sulfide is a notably weak one and hydrogen chloride and hydrogen bromide yield only one moderately strong band in the sodium chloride range. Pressures above 1 atm. or cells of greater length than the one used for this work (10 cm.) may be useful in some cases, but these procedures may increase the difficulty of overlapping and have the disadvantage of requiring a much larger amount of sample.

ACKNOWLEDGMENT

Many coworkers in the Chemistry Division of the Research Department at the Naval Ordnance Test Station assisted in accumulating the data of this paper. The authors are especially grateful to two members of this group, H. W. Kruse and Walter F. Edgell (who was on leave from Purdue University). Much inspiration and encouragement in carrying out the project came from Wallace C. Brode and E. C. Creitz of the National Bureau of Standards; R. M. Badger of the California Institute of Technology; and I. C. Hisatsuni and Bryce Crawford, Jr., of the University of Minnesota. Permission to publish the paper was granted by W. B. McLean, Technical Director of the U. S. Naval Ordnance Test Station, China Lake, Calif.

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RECEIVED for review March 21, 1956. Accepted May 2, 1956. Presented in part, Division of Analytical Chemistry, 129th meeting, ACS Dallas, Tex., April 1956.

Effect of Time on Fluorescing Power of Estrogenic Steroids

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The fluorescing power of four estrogenic steroids has been found to fall off with time, if certain concentrations of sulfuric acid are used for sensitizing and dilution. Because under these conditions the background fluorescence of the urinary extract is constant, correction can be made for impurities.

IN STUDYING the ability to fluoresce, induced by sulfuric acid treatment of various steroids, it was noted that under the conditions used in this laboratory the fluorescing power of estrone, 17 β-estradiol, estriol, and 17 α-ethinylestradiol decreased with the progress of time after the sensitizing reaction. Diethylstilbestrol (α,α'-diethyl-4,4'-stilbenediol) did not exhibit this phenomenon. Other investigators (*1, 10*) report constancy of fluorescence with time, but they differ in their procedure, in that they dilute less after the sensitizing reaction and use stronger acid. It is believed that the decay observed may be due to the lower viscosity of the diluted solutions. Diczfalusy (*4*) observed such a decrease and therefore chose other conditions for his assay. However, it was noted that purified urinary extracts have a background fluorescence due to residual impurities, which under the conditions of analysis remain essentially constant with time. Hence by the measurement of the fluorescence of the solutions at different times the apparent level of estrogen content can be corrected for impurities. Should this "background" fluorescence turn out to be indicative of the presence of other compounds related to estrogen metabolism, new methods based on further refinement in fractionation, together with a combination of fluorometry and colorimetry, would need to be evolved.

Table I. Comparison of Sensitizing Conditions

Goldzieher (Bates-Cohen)	Strickler and Coworkers (Engel)
0.2 ml. 1 to 20 alcohol-toluene	0.1 ml. 1 to 19 alcohol-toluene
1 ml. "90%" sulfuric acid (90 ml. c.p., 10 ml. water)	0.5 ml. (1 ml.) 90 wt. % sulfuric acid
80° C., 10 minutes	10 min., water bath, 90°, 100° (ovens 100–103° 10–12 min.)
Water bath	
Dilute with 5 ml. "65%" acid (65 ml. c.p., 35 ml. water) (Bates-Cohen use 6 ml.)	Dilute with 3.5 ml. (7 ml.) 65 wt. % acid
Fluorescence stable $\geq$ 24 hr.	Fluorescence readings fall off with time
Final mixture ~76 wt. % sulfuric acid	Final mixture ~68 wt. % sulfuric acid
Viscosity, $\eta \simeq 15$	Viscosity, $\eta \simeq 9$

Sulfuric acid, c.p., taken as 96 wt. %, sp. gr. 1.836

Table II. Example of Time Study Calculations

	Estrone			17 β-Estradiol			Estriol		
	0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.	0 hr.	24 hr.	48 hr.
G_a	152.8[a]	73.0	52.0	141.3	111.5	98.5	93.3	48.3	31.0
G	56.3	42.3	39.0	50.3	46.5	44.5	33.3	21.3	17.5
γ		0.21	0.21		0.15	0.16		0.36	0.34
In aliquot C (calcd.)		36.0			36.5			12.2	

Specimen E-55-138 purified by ccd.

[a] Galvanometer readings are referred to a reading of 35 for the standard. Where solutions were too concentrated, the sensitivity controls were adjusted to some smaller value and the observed readings were calculated to the bases of 35.

Studies by the authors of Goldzieher's (*10*) conditions of assay indicate, in accordance with his report, that the fluorescing power of pure estrogen is indeed constant with time. There is, however, no opportunity by his method to correct for the presence of impurities. Cognizance of the falling off of the fluorescence power with time lends additional precision to the determination of these substances.

EXPERIMENTAL

A direct-reading Farrand Fluorometer (Farrand Optical Co., New York, N. Y.) with quartz optics was used. Improved stability was attained by the use of an electronic voltage regulator, Stabiline Model IE5 1005 (Superior Electric Co.). The smallest apertures (No. 5 or 6) were chosen in order to limit the action of the ultraviolet light on the solutions. Uranyl acetate, 0.1% in 5% acetic acid, was used as the reference standard for readings with the 490-mμ secondary filter, and uranyl acetate, 0.04% in water, was used with the 524-mμ secondary filter. Solutions of sodium fluorescein, 0.0005% in 0.0001*N* sodium hydroxide, and quinine sulfate, 0.01% in 0.1*N* sulfuric acid, were usually read before each assay in order to check for any deterioration of the ultraviolet lamp or phototube. The primary (source) filters that were used were 435-mμ interference type (Farrand) and 365-mμ glass (Corning No. 5860). The secondary filters were 490-mμ interference (Baird) backed with glass (Corning No. 4303), and 524-mμ interference type (Farrand). These choices of filters were based on recommendations of the manufacturers of the instrument and of Engel and his associates (*6, 11*).

The countercurrent distribution machine used was the glass type, 10-ml. lower phase, two banks of 50 tubes each (H. O. Post Co.). The contents of the countercurrent tubes after partition were evaporated at 0.4 to 0.8 mm. of mercury with controlled cooling. This procedure appears to be desirable because of removal of the easily volatile phenols.

METHODS

The initial preparation of urinary extracts was essentially that of Engel (*7*). However, hydrochloric acid was used for hydrolysis, and, to minimize losses of estriol, the final extraction of estrogens with ether was made from the solution saturated with sodium bicarbonate rather than with the carbonate-bicarbonate buffer. At this point, countercurrent distribution for exploration of a sample was sometimes carried out with 65 transfers and 50 tubes, using the system 70% methanol–30% water/40% chloroform–60% carbon tetrachloride (*8*). Just before countercurrent distribution the sample was usually partitioned between 50 ml. of benzene and 50 ml. of 0.3*M* sodium carbonate (single-stage Mather). The estrone and estradiol remain primarily in the benzene. The carbonate phase, after acidification and saturation with sodium bicarbonate, was re-extracted with ether. The countercurrent distribution then proceeded in one bank, but in two parts.

Various procedures were tried, which will be discussed elsewhere (*19*). In recent work done at this laboratory, the estradiol and estrone were separated by a simple countercurrent distribution procedure using only five tubes and the same system of solvents. The estradiol is removed to the extent of 81% (at $K_2 = 1$) in eight withdrawals, two more withdrawals are discarded, and the estrone is then present in tubes 1, 2, 3, and 4 to the extent of 85% ($K_1 = 0.2$). (K is the distribution coefficient.) Tube 0, which contains much pigment, is discarded. The estriol was likewise removed in the same type of distribution, but the solvents used were those employed by Bauld (*2*) in his Celite system (70% methanol–30% water/ethylene chloride) and Z =

0.5—i.e., only 5 ml. of upper phase are used in each transfer. Samples may be subjected to purification with a Bauld column, but the authors have found that recovery is lower.

Further purification of the urinary extracts is desirable. Estrone and estradiol were separately purified on Celite–sodium hydroxide columns, essentially according to Bitman and Sykes (*3*). Columns in this laboratory were 10 mm. in inside diameter. The first 5 ml. of eluate benzene (total 80 ml.) were discarded in the case of estrone. For estradiol the first 55 ml. of eluate were discarded (total 170 ml.). At assay, a single distribution of the estriol fraction between 40% ethyl acetate–60% cyclohexane/31.6% ethanol–68.4% water (mixture TG) removed sufficient impurity into the lower layer so that aliquots of the upper phase could be used. K for estriol was found to be 0.83.

Such separations are good starting points for bioassay as well as for fluorometry, since the impurity level is considerably reduced. In bioassay, the separation of the three estrogens is desirable. This is of importance because, as shown in this and other laboratories, varying proportions of the three biologically active estrogens will exhibit potentiation or depression, depending upon the mixtures of the estrogens employed (*5*, *12*).

PREPARATION FOR FLUOROMETRY

The estrogens were sensitized by heating the residues which were obtained on evaporation with 0.1 ml. of toluene–absolute alcohol (19 to 1) and 0.5 ml. of 90 weight % sulfuric acid for 10 minutes in a boiling water bath (*7*). Other temperatures and concentrations of acid have been tried. On cooling, the solutions were diluted with 3.5 ml. of 65 weight % acid. In the case of urinary extracts, chilling, followed by filtration through sintered glass and/or centrifugation, seemed desirable in order to avoid any traces of turbidity. The clear solutions were then transferred to cuvettes and heated at 50° for 45 minutes prior to being read immediately after their removal from the oven. The fluorometric readings were completed in a few seconds and the tubes were then capped and stored in a cupboard until the next day.

The amounts of acid employed are compared in Table I to those of two other investigators (*1*, *10*), who indicated that a constancy of fluorescence for over 24 hours is obtained. Evidently the difference lies in the fact that the present authors used dilution to a greater extent and with weaker acid. The conditions followed were those published by Engels' group (*7*). The falling off of the fluorescence with time is considered to be dependent on the concentration of the acid solutions. The method of making up the solutions is therefore detailed here.

Du Pont C.P. concentrated sulfuric acid, specific gravity 1.841–1.844, is used. The 90 weight % acid is made up with 409 ml. of the concentrated acid and 46 ml. of water. The diluent acid (65 weight %) is made up from 231 ml. of concentrated acid and 200 ml. of water. These are cooled in an ice bath when being prepared.

The differences in viscosity (*13*) listed in Table I may afford an explanation of the observed differences. In the less viscous solutions there is a fall-off of sensitized molecules, which is perhaps due to an increased frequency of collisions. There is, consequently, an opportunity to distinguish between the stable background (impurity) fluorescence and that due to the estrogen under the conditions of the investigation. This is not true when the higher acid concentrations are used.

TIME STUDY OF FLUORESCENT (SULFURIC ACID-SENSITIZED) ESTROGENS

The procedure followed in this laboratory involves measuring the time decrease of the fluorescence in material obtained from a given sample, and noting the degree of fluorescence daily for 3 or 4 days (Table II). The samples were purposely exposed to the ultraviolet excitation for only the brief period necessary to obtain a galvanometer deflection each day. The decrease of fluorescence observed was therefore not due to the lability of the sensitized steroids to the exciting radiation. Further heat treatment (50°) was applied in order to clarify the solutions. This did not affect the previously observed decrease in fluorescence. It was not due to oxygen diffusion into the samples. The fluorescence decrease indicated instead that the sensitized estrogens shifted structure in some manner, so that the population of sensitized molecules fell off in a manner analogous to decay of radioactive substances. Hence, the differential equation $dG/dt = -A'G$ was applied to the data. Here G is the galvanometer reading corrected for blank and/or impurity, t is the time, and A' is a constant. Integration of this equation yields

$$\ln G_t = \ln G_0 - A't: \log_{10} G_t = \log_{10} G_0 - At \qquad (1)$$

Consequently, the data should lie on straight lines on a semilog plot (cf. Figure 1). Numerous other experiments, including those in which every precaution was taken to avoid traces of turbidity (filtration, heating at 50°) indicate that this equation is rigidly applicable only with some sets of data. The decay in many cases follows a more complex law.

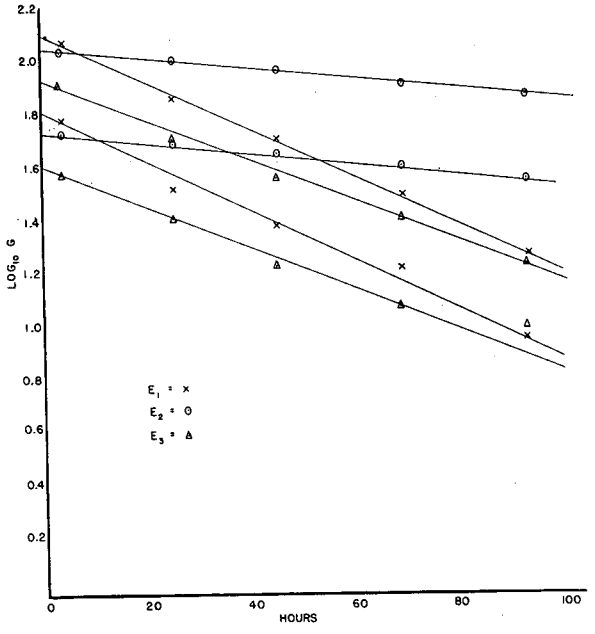

Figure 1. Semilog plot of galvanometer readings *vs.* time for pure estrogens

Upper line of each pair, 1.0 γ; lower line, 0.5 γ. Samples heated at 50° just before reading. Filters P435/S490. E_1 = estrone; E_2 = 17β estradiol; E_3 = estriol

The filter system used here was P435/S490. The relation is stoichiometric, for 0.5-γ lines parallel 1.0-γ lines. It was observed in this laboratory that 17 α-ethinylestradiol also gives fluorescence which falls off with time. Slaunwhite, Engel, and coworkers (*16*) observed that 17 α-ethinylestradiol did not give a linear response with concentration and the authors have confirmed this. The compound gives a pink color with an absorption curve peaking at 530 mμ (*18*). Appreciable self-absorption would therefore be expected at either 490 or 524 mμ.

On examining some samples with the filter system P435/S524 it was noted that the fluorescence readings also fell off with time, as did the values obtained on correcting for fluorescence (at equal blank readings) at P365/S524 according to Finkelstein (*9*).

It was found, for estrogenic urinary extracts prepared essentially according to Engel (*7*), that the impurity contribution to the

Table III. Approximate Constancy of Background Fluorescence Contrasted with Fall Off of That of Internal Standard

Specimen and Fraction (Aliquots)	Corrected Galvanometer Reading					
	Reading	Time, hr.	Reading	Time, hr.	Reading	Time, hr.
E-55-427; estradiol	23.5	2.5	23.3	20.1	22.3	44.2
+ 1 γ 17β-estradiol	153.7	2.5	115.3	20.1	105.0	44.2
E-55-408; estriol[a]	32.2	2.5	33.0	20.1	32.0	44.2
+ 1 γ estriol	87.7	2.5	47.2	20.1	39.5	44.2
Pooled male urine; estrone	19.5	2.0	17.0	22.0	15.3	70.0
+ 1 γ estrone	184.5	2.0	93.5	22.0	34.8	70.0
Gonadectomized true hermaphrodite						
E-55-376[b]; estrone	5.8	2.3	5.2	20.0	5.5	44.0
+ 1 γ estrone	121.5	2.3	39.2	20.0	18.7	44.0
E-55-327[b]; estriol	17.3	2.3	18.7	20.0	21.2	44.0
+ 1 γ estriol	55.3	2.3	37.8	20.0	30.7	44.0

[a] Finally cleaned up on Celite–sodium bicarbonate–benzene column.
[b] See Table IV.

fluorescence may be considered to be a constant, C, invariable with time (cf. Table III). This is deduced from the readings listed and many other observations on extracts of different purity, and representing different physiological states. As purification proceeds, the value of C decreases, so that the values of γ from time studies and from the initial readings ("apparent" estrogen) converge. Thus, some of the values in Table IV are nearly the same. E-55-376 gave a large apparent peak of estrone and estriol on exploratory countercurrent distribution but none by time study (*19*). This was before intensive purification. The equation based on this assumption is:

$$G = \frac{\gamma E}{Q} + C \qquad (2)$$

where G is the galvanometer reading, γ the number of micrograms of a particular estrogen in the aliquot, and E the reading of 1 γ of pure estrogen. Q is the quench coefficient as defined by $Q = \frac{fE}{G_a - G}$, where f is the number of micrograms of pure estrogen added to an equal aliquot, and G_a is the resulting galvanometer reading (*19*). All galvanometer readings are corrected for reagent blank. If Equation 2 is written at two different times—e.g., 0 and t—then on solving for γ, C drops out and

$$\gamma = \frac{f(G_0 - G_t)}{[(G_a - G)_0 - (G_a - G)_t]} \qquad (3)$$

and

$$(1 + f\gamma)\frac{\delta G}{\delta t} = f\gamma \frac{\delta G_a}{\delta t}$$

Here Q has been replaced by its definition at each time. Data illustrating the application of this principle are shown in Tables II and III.

DISCUSSION

The use of conventional acid hydrolysis throughout this work is justified on the basis that only by restricting the type of processing could one become at all familiar with the types of impurities likely to be encountered. The countercurrent distribution procedures were designed to deal with observed interference. Despite these and supplementary procedures, it would appear that the use of time studies yields the lowest and most convincing values. Thus, in the case of male urine, the Engel group (*17*) has never demonstrated any estrogen by redistribution in different solvents. The time study values from the authors' preliminary experiments indicate the presence of either none or low levels of estrogen in male urine. The materials used by Engel may have been subject to deterioration, because he employed repeated redistribution and quench corrections may not have been applied.

Some correlation with structure is evident from time study measurement. Dehydration at positions 16 and 17 in the steroid ring would convert estriol to estrone. In the sensitization with sulfuric acid, dehydration probably occurs at these positions. Consequently, the fluorescent complex obtained from estriol should be similar to that obtained from estrone. Indeed, the time decay slopes of the two are similar. 17 β-Estradiol and 17 α-ethinylestradiol, which differ from the other two at the five-membered ring, have different slopes. 17 β-Estradiol shows less fluorescent decay than the other three estrogens. Diethylstilbestrol, another synthetic estrogen but not of the steroid family, gives very mild fluorescence and does not decrease with time. It may be concluded that the presence of the phenolic structure alone is insufficient to give strong fluorescence under these conditions (cf. *16*). Recently it has been found in this laboratory that the methyl ethers of estrone, 17 β-estradiol, and estriol show fluorescence and time decay comparable to the free phenols.

So far, no decrease of fluorescing power with time comparable to those of the estrogens has been observed with other steroids. Dehydroisoandrosterone, 3 α-, 20 α-preganediol, 17-hydroxypregnanolone, desoxycorticosterone, and compound F do not show the decrease in fluorescence with the same technique. The conditions of Linford (*14*) were also used with slight modification (0.1 ml. of alcohol with concentrated sulfuric acid at 60° for 1 hour). No marked fall in fluorescence was noted using P435/S524 filters.

Countercurrent distribution, Celite chromatography, and fluorometry were used to assay a number of urines. In Table IV apparent estrogen is referred to as that estrogen computed from the ratio of the galvanometer reading of the unknown and the standard, computed without time studies—i.e., at or near zero hours. These values are similar in magnitude to those of Migeon (*15*), using different countercurrent systems and fluorometry.

Table IV. Comparison of Apparent Estrogen Values with Those from Time Studies

Specimen	Estrone		Estradiol		Estriol	
	Apparent	Time study	Apparent	Time study	Apparent	Time study
E-55-346	6.7	6.0	1.0	≤0.09	20.4	1.3
E-55-553	0.3	0.1	0.4	≤0.2	8.4	≤0.5
E-55-327	0.3	≤0.09	0.6	≤0.2	6.1	≤0.7
E-55-376	0.4	≤0.09	0.4	≤0.2	3.5	≤0.6
E-55-319	0.2	≤0.1	0.8	≤0.2	10.0	≤0.7
E-55-291	0.9	0.6	..	0.0	3.8	≤0.7
E-55-285	1.3	1.0	0.7	≤0.1	9.6	≤1.0
E-55-243	2.2	1.4	2.5	≤0.1	18.5	6.5
E-55-218	1.4	1.3	0.5	≤0.2	14.4	≤0.5
E-55-233	3.1	2.7	1.3	≤0.3	13.5	≤0.4
E-55-367	2.0	1.6	0.8	≤0.1	14.6	5.5
E-55-644	9.4	9.6	2.3	≤0.3	37.2	12.8

However, these values appear too high. Time studies give lower values, as indicated in the table. The use of additional steps, such as chromatography and redistribution of the samples in an additional system of solvents, increases the sensitivity of the assays. It also reduces the high values of "apparent" estrogens, and consequently the contrast between the apparent estrogen and the result by the time study method is reduced.

SUMMARY AND CONCLUSIONS

The fluorescence of estrone, 17 β-estradiol, and estriol decreases with time under the experimental conditions. This is achieved by diluting with weaker acid than is employed by those investigators who report constancy of fluorescence. Decrease of fluorescence with time is observed not only at 490 mμ (secondary) but at 524 mμ and after the use of the "correction" formula of Finkelstein. However, fluorescence of associated impurities in the urinary extract remains constant. A procedure for the assay of estrogens

in such extracts based on these observations is described. The levels of estrogen thus obtained are lower and appear to be more reasonable than those calculated from the initial readings (apparent values). Some of the specimens show simple logarithmic decay—e.g., like Figure 1—others indicate a more complex behavior.

A system, based on known methods of countercurrent distribution, chromatography, and redistribution, is given for the necessary preliminary purifications. These steps have been tested only on extracts prepared by conventional (hydrochloric acid) hydrolysis.

The fluorescence of estrone and estriol falls off at similar rates, as might be expected from their chemical structures, while 17 β-estradiol and 17 α-ethinylestradiol do not show the same rates of fluorescent decay. The methyl ethers of estrone, 17 β-estradiol, and estriol behave like the free phenols.

Diethylstilbestrol, dehydroisoandrosterone, 3 α-, 20 α-pregnanediol, 17-hydroxypregnanolone, desoxycorticosterone, and compound F do not show decrease of fluorescence with time under identical conditions nor with a somewhat different technique of excitation.

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RECEIVED for review February 1, 1956. Accepted April 28, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 1955. Assisted in part by a grant-in-aid from the Council on Pharmacy and Chemistry of the American Medical Association and from the Pennsylvania Division of the American Cancer Society to Robert C. Grauer, M. D.

Analysis of the Aromatic Fraction of Virgin Gas Oils by Mass Spectrometer

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A short-cut method for the semiquantitative determination of the major compound types in the aromatic fraction of virgin gas oils has been developed using the high molecular weight mass spectrometer. A prior separation of the sample into saturate and aromatic fractions by percolation over silica gel is required. The discussion gives details of the techniques and assumptions involved in deriving a calibration matrix. This analysis includes the major aromatic and sulfur types—benzenes, naphthenebenzenes, dinaphthenebenzenes, naphthalenes, acenaphthenes, acenaphthylenes, phenanthrenes, pyrenes, chrysenes, benzothiophenes, dibenzothiophenes, and naphthobenzothiophenes—in a 600° to 1000° F. virgin gas oil. These are assumed to be the major hydrocarbon and sulfur compound types in other virgin gas oils. The accuracy of the method is discussed in terms of properties measurable by other techniques, such as per cent of carbon present in aromatic rings and sulfur content.

THE story of the application of the mass spectrometer to the analysis of hydrocarbon fractions during the past decade is one of a steady increase in scope in terms of boiling range. The earliest applications, in 1945, were to the analysis of gaseous mixtures (*17*). In the late forties the mass spectrometer was utilized for the component analysis of the lower boiling fraction of gasolines (*4, 6, 13*). In the early fifties, schemes for the compound-type analysis by mass spectrometry of the heavier boiling fractions of gasolines and even of kerosine and light heating oil fractions were described (*3, 12*). About this same time O'Neal (*15*) and Brown (*2*) independently described improved sample introduction systems for vaporizing compounds in the heavy gas oil boiling range which, coupled with increased resolution of the instrument, permitted them to make semiquantitative analyses in this range, with emphasis on the analyses of waxes. No mass spectrometric method has yet appeared in the literature, however, for the determination of the aromatic compound types in the gas oil boiling range.

In 1954 Lumpkin and Johnson (*11*) described the identification of some of the major hydrocarbon and sulfur compound types occurring in a heavy petroleum gas oil (aromatic fraction). They emphasized the significance of sulfur compounds in the composition of certain gas oils. Although previous workers in the field of gas oil analysis, notably those employing physical property methods for structural group analysis (*5, 9, 10, 14*), recognized that these compounds were present, very meager data were available as to the types of sulfur compounds present in the gas oil boiling range and even fewer data were available with regard to their physical properties. This lack of knowledge resulted in a neglect of the effect of sulfur compounds on most structural group analyses. The identification of the major hydrocarbon and sulfur compound types in a high-sulfur gas oil described in the previous work demonstrated that the number of these types was not prohibitively large for the development of a compound type analysis. The present paper presents the results of a natural extension of that work and describes the development of a method for the compound-type analysis of the aromatic fractions of virgin gas oils.

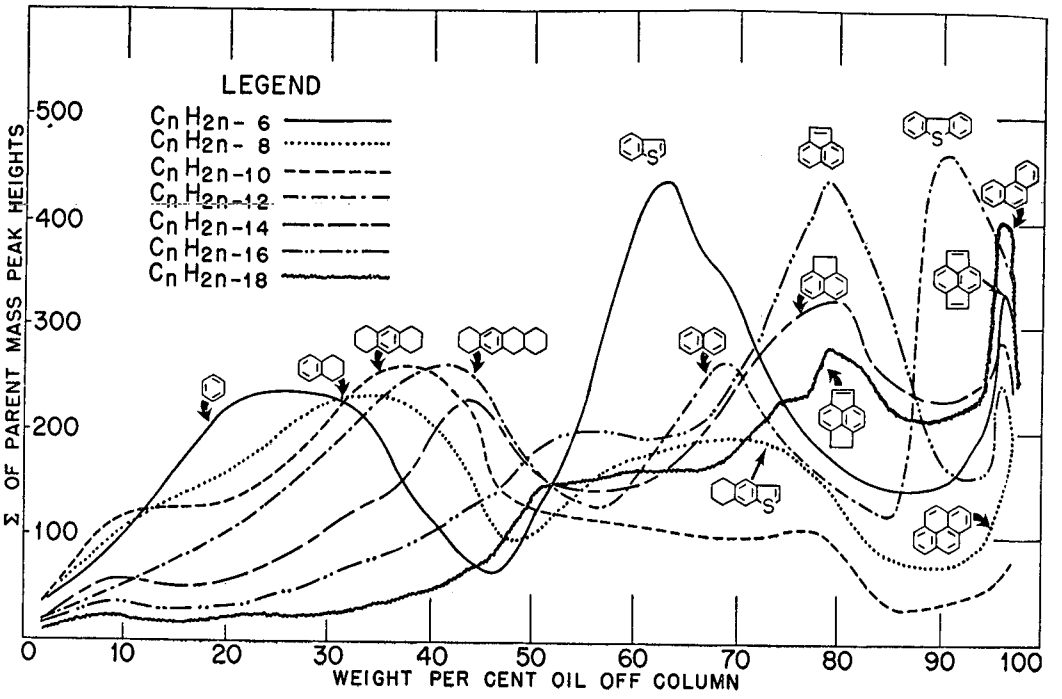

Figure 1. Identification of compound types in aromatic fraction A of heavy gas oil

DISCUSSION

The equipment employed for obtaining the mass spectral data has been described (*11*).

A sample of a heavy gas oil (boiling range about 600° to 1000° F.) was separated chromatographically in the preparation of samples for instrument calibration. Although this separation was discussed in detail previously, it is repeated here, because it is necessary for a full understanding of the method of development of the present analysis.

In the first separation silica gel was used as an absorbent. A vertical glass column consisting of an upper section (5 feet by 2 inches) and a lower section (1 foot by 1/4 inch) was packed with Davison 28-200-mesh silica gel. Fifty grams of the heavy gas oil was diluted with 10 ml. of benzene to reduce the viscosity of the sample and added to the top of the packed column. A large volume of iso-octane was passed through the column to elute a saturate portion which amounted to 52.4 weight % of the original sample. Most of the saturates were removed by the iso-octane before any of the aromatics began to come off the column. A 10% benzene–90% iso-octane solution was then used as an eluent. The portion of the sample that could be removed with this eluent, 30.6 weight %, was collected as aromatic fraction A. All the oil that could be eluted with 100% benzene, 14.3 weight %, was collected as aromatic fraction B. The remainder of the sample, 2.7 weight %, was displaced from the column with ethyl alcohol. Fractions A and B were then charged separately to columns packed with Alcoa F-20 alumina gel. A series of graded solvents was used to elute the samples from the columns, beginning with iso-octane and following with mixtures of benzene and iso-octane. In each case a particular solvent was used until the rate of the sample removal became very slow. Approximately 2% cuts, based on the charge, were taken. Ultraviolet and mass spectra and total sulfur contents were obtained on selected fractions.

Mass spectra of the fractions from the alumina gel percolation were obtained using the constant-volume pipet method of sample introduction (*1*). All the peak heights were read and plotted in various combinations to facilitate the compound type identification. One of the most illuminating methods of presenting the mass spectral data is illustrated by Figure 1. Here the sum of the parent masses in the parent mass region for the hydrocarbon series C_nH_{2n-6}, -8, -10, etc., to $_{n-18}$ for each fraction was plotted as a function of the position of the fraction in the alumina gel percolation. The first 50% of the percolation of fraction A was shown by ultraviolet to consist predominantly of single ring aromatics—i.e., benzenes, indanes, Tetralins, and dinaphthenebenzenes. This simplifies the interpretation of the various peaks occurring in the 0 to 50% portion of Figure 1. Thus, the first peak in the solid line occurs because of the presence of benzene hydrocarbons. The first peak in the dotted line (the C_nH_{2n-8} series) shows the presence of molecules containing one benzene and one naphthene ring. These may be indanes, Tetralins, or benzenes with a naphthene ring not condensed with the aromatic nucleus. However, the solid curve (C_nH_{2n-6}) shows a second peak at about the 65% region on the abscissa. A study of the fragment masses occurring in this region and of the sulfur distribution curve showed that this peak is due to benzothiophenes. Similarly, the first peak in the C_nH_{2n-12} curve (at 40%) is due to molecules containing one benzene and three naphthene rings, the second peak (at 70%) is due to naphthalenes, and the third peak (at 90%) is due to dibenzo- or naphthothiophenes. The peak at 44% in the C_nH_{2n-14} curve could be attributed to tetranaphthenebenzenes and/or thiophenes. The fragment masses and sulfur contents of cuts in this region indicate that this material is predominantly tetranaphthenebenzenes. There is no evidence of significant concentrations of thiophenes, although the condensed thiophenes are found in abundance. Details on the identification of these and the other hydrocarbons and sulfur compound types shown in Figure 1 are given in the previous paper (*11*).

The compound types shown in Figure 2 (fraction B) were identified in a similar manner. Two rather unusual compound types are shown. The first occurs in the C_nH_{2n-6} series of parent masses. The indicated nuclear molecular weight is 176, since the first predominant fragment is 189. The evidence is strong for a compound type of general formula C_nH_{2n-20} having the structure shown in Figure 2. Similarly, acepyrenes are indicated to be present (see the C_nH_{2n-8} curve). The first 15% off the column in the case of fraction B was found to be sulfides, by using the iodine-complex method developed in these laboratories (*7*, *8*).

After the major compound types in the aromatic portion of one gas oil had been identified, and assuming that these are also the major compound types present in other virgin gas oils of about the same boiling range, it was desired to see whether any char-

acteristic fragment masses or series of masses could be found for each type. Generally this was possible, as the same characteristic fragment masses which were used to identify the various compound types were sufficiently unique to serve also as a means of measuring each of these compound types in a complex mixture of all of them. One of the most serious deficiencies of the method is the fact that the trinaphthenebenzenes contribute generally to the same fragment series as the naphthalenes. Similarly, tetranaphthenebenzenes contribute to the same series as the acenaphthenes. No simple method of overcoming these deficiencies has as yet been devised.

Twelve compound types were selected as being most important in this gas oil and the characteristic masses chosen for each of these types are given in Table I. Because of the difficulties mentioned in the previous paragraph, several of these mass groupings undoubtedly measure more than one compound type. In the subsequent discussion and tables the first type shown represents the whole group, because the data in Figures 1 and 2 indicate them to be the predominant types. The masses found to be most characteristic of the aromatic types were those beginning with the nucleus plus one CH_2 group and extending to the nucleus plus three or four CH_2 groups. Because of the differences in the nuclear molecular weights, additional compound types can be included in the method—with parent masses alone only seven series are available. The peak heights of the characteristic masses shown are summed and treated as single coefficients. As in most multicomponent analyses, to determine the 12 compound types shown it is necessary to have coefficients for each of the 12 compound types for each of the 12 characteristic series, or a total of 144 key and interference coefficients. Attempts to evaluate these coefficients from the mass spectra of pure compounds either run in these laboratories or published by API Research Project 44 were hampered not only by a lack of sufficient samples of some compound types but more seriously by the complete absence of any representatives of certain important compound types, notably the sulfur compounds. It was necessary, therefore, to evaluate most of the coefficients from the percolation cuts themselves.

In order to obtain the key coefficients it was assumed that in any percolation fraction in which a component was known to predominate, the ratios of the sums of the parent masses for each of the seven possible series to the total of the sums are a direct measure of the concentrations of the compound types. Available data on pure compounds indicate that this is a reasonable assumption when one is dealing with narrow percolation fractions as in the present case. Making the further assumption that none of the other compound types present in a given fraction interferes with the fragment mass sum of the predominant compound type, one can then calculate the key coefficient for the major compound type in that fraction. This is actually done for several fractions containing high concentrations of a given compound type in order to obtain average coefficients. It is necessary to obtain such an average, as it has been observed that percolation over alumina gel tends to separate each compound type according to the degree of substitution on the nucleus and the molecular weight.

By plotting the characteristic fragment mass sums against weight per cent percolated it is possible to make reasonable estimates of most of the interference coefficients. For example, because benzenes are almost completely separated from naphthalenes and the other condensed ring types, the major portion of the sums for the characteristic series of these types in the benzenes concentrates can be attributed with reasonable certainty to benzene interference. The most uncertain interferences are

Table I. Characteristic Mass Sums for Major Compound Types in Aromatic Fraction of Virgin Gas Oils

Compound Type	Mass Series
Benzenes	Σ 91 = 91 + 105 + 119 + 133
Indanes and/or Tetralins	Σ 117 = 117 + 131 + 145 + 159
Dinaphthenebenzenes	Σ 129 = 129 + 143 + 157
Naphthalenes and trinaphthenebenzenes	Σ 141 = 141 + 155 + 169
Acenaphthenes and other naphthenenaphthalenes	Σ 167 = 167 + 181 + 195 + 209
Acenaphthylenes and dinaphthenenaphthalenes	Σ 165 = 165 + 179 + 193 + 207
Phenanthrenes, anthracenes, and trinaphthenenaphthalenes	Σ 191 = 191 + 205 + 219
Pyrenes	Σ 215 = 215 + 229 + 243
Chrysenes	Σ 241 = 241 + 255 + 269
Benzothiophenes	Σ 147 = 147 + 161 + 175
Dibenzothiophenes and/or naphthothiophenes	Σ 197 = 197 + 211½ + 225
Naphthobenzothiophenes	Σ 247 = 247 + 261½ + 275

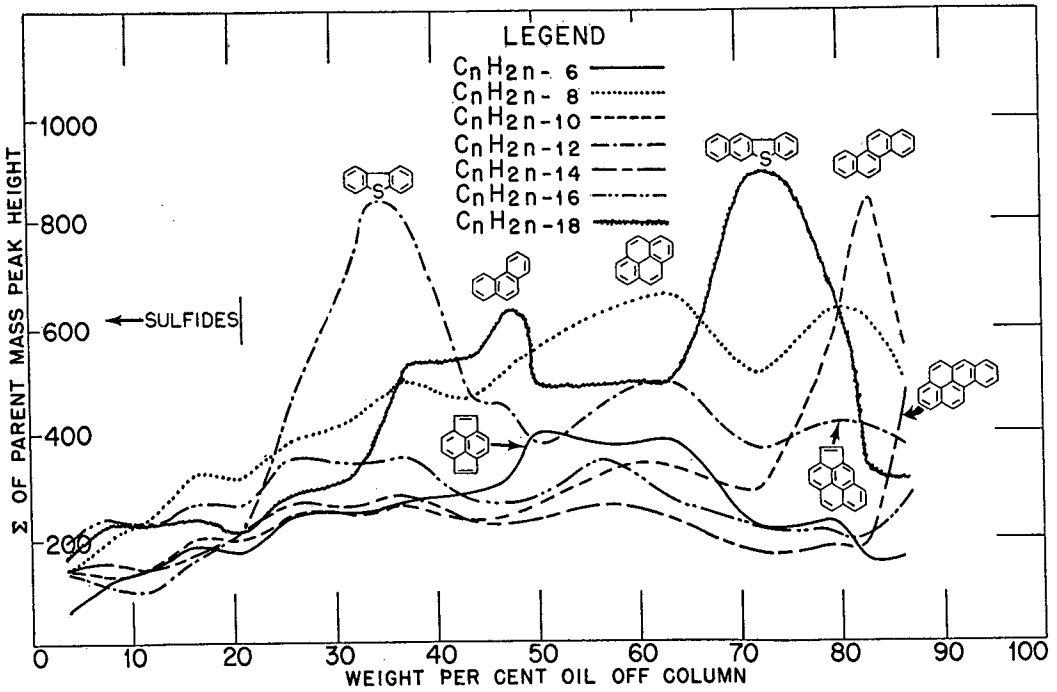

Figure 2. Identification of compound types in aromatic fraction B of heavy gas oil

Table II. Calibration Data for Analysis of Aromatic Fraction of Virgin Gas Oils by Mass Spectrometer

Mass Series[a]	Benzenes	Indanes	Dinaphthenebenzenes	Naphthalenes	Acenaphthenes	Acenaphthylenes	Phenanthrenes	Pyrenes	Chrysenes	Benzothiophenes	Dibenzothiophenes	Naphthobenzothiophenes	Diagonal
Σ 91	1.0000	0.0500	0.0500	0.0500	0.0140	0.0140	0.0140	0.0200	0.0200	0.0540	0.0110	0.0230	3500
Σ 117	0.0500	1.0000	0.0500	0.0320	0.0100	0.0100	0.0100	0.0200	0.0200	0.0300	0.0080	0.0230	3100
Σ 129	0.0500	0.0500	1.0000	0.0670	0.0170	0.0170	0.0110	0.0330	0.0330	0.0610	0.0110	0.0330	1800
Σ 141	0.0420	0.0420	0.0420	1.0000	0.0230	0.0230	0.0050	0.0120	0.0120	0.0230	0.0050	0.0120	4300
Σ 167	0.0610	0.0610	0.0610	0.0610	1.0000	0.0610	0.0180	0.0180	0.0120	0.0610	0.0180	0.0120	1650
Σ 165	0.0880	0.0880	0.0880	0.0880	0.0880	1.0000	0.0880	0.0260	0.0260	0.0880	0.0880	0.0260	1130
Σ 191	0.0670	0.1330	0.1780	0.2220	0.3780	0.3560	1.0000	0.0440	0.0440	0.2670	0.2220	0.0890	450
Σ 215	0.1860	0.1860	0.1860	0.2330	0.2330	0.2330	0.4650	1.0000	0.4650	0.2330	0.2790	0.2330	430
Σ 241	0.1540	0.1150	0.1150	0.0770	0.0770	0.0770	0.1350	0.0960	1.0000	0.0770	0.1150	0.1920	520
Σ 147	0.1600	0.1440	0.1440	0.0800	0.0400	0.0400	0.0160	0.0240	0.0240	1.0000	0.0080	0.0240	1250
Σ 197	0.1040	0.1040	0.1040	0.1300	0.1560	0.1560	0.1300	0.0390	0.0390	0.1430	1.0000	0.0390	770
Σ 247	0.0360	0.0360	0.0360	0.1460	0.1950	0.1950	0.2440	0.1220	0.1220	0.1460	0.1950	1.0000	410

[a] Refer to Table I for definition of mass series.

those for compound types which are only partially separated. In these cases it is necessary to refer to pure compound data to estimate interference coefficients.

In this manner, the 144 calibration coefficients were obtained from the percolation fractions from the heavy gas oil. These are shown in Table II. Application of these data to other gas oils is based on the assumptions (1) that these compound types are also the major compound types present in the aromatic fraction of other virgin gas oils of about the same boiling range, and (2) that the distribution of individual compounds making up each type is sufficiently similar in various gas oils so that the same coefficients will apply to these oils. The validity of these assumptions cannot be proved, but experience in the analysis of the gasoline boiling range stocks leads to the belief that they are reasonable.

APPLICATIONS

Generalized Method. In the application of the present analysis it is necessary to obtain a prior separation of the gas oil under study into saturate and aromatic fractions. This is accomplished by means of a silica gel procedure reported by Watson (*18*). The mass spectrum of the aromatic fraction is obtained and its composition is calculated employing the matrix given in Table II. As it has been shown (*8*) that sulfides are present in many gas oils, it is necessary to know the sulfide content. This is determined by the iodine-complex method as mentioned above. The weight per cent of sulfide sulfur thus determined is converted to weight per cent of sulfides from the average molecular weight of the sample (assuming one sulfide atom per molecule). The sulfides appear in the aromatic fraction from a silica gel percolation but do not contribute significantly to the characteristic masses chosen for the procedure. The analysis obtained by the mass spectral procedure is normalized to the weight per cent of aromatics found by silica gel minus the determined weight per cent of sulfides.

Analysis of Hydrodesulfurization Feeds and Products. Research into the hydrodesulfurization of high-sulfur gas oils has been hampered to some extent by a lack of knowledge of the sulfur compound types present in these stocks. The method described in this paper was applied to the feed to and products from the desulfurization of a high-sulfur stock at two severity levels to determine the sulfur compound types present in the original gas oil and the changes in these types effected by hydrodesulfurization. The results are shown in Table III. It is of significance that the sulfides are readily converted (none appearing in either of the two products), whereas the benzothiophenes appear to be the most difficult to convert under the conditions of these runs. The sulfur content of each of the stocks as determined by the Dietert method is compared at the bottom of the table with the sulfur content calculated from the mass spectral analysis. Excellent agreement is obtained; thus considerable confidence can be placed in the accuracy of the method as applied to these samples. However, the feed stock employed in this desulfurization operation is very similar to the one from which the calibration coefficients were derived.

Table III. Mass Spectral Analyses of a Heavy Gas Oil before and after Desulfurization

Stock, Compound Type, %	Feed, as Is	Gas Oil Product from Run, Feed Basis	
		A	B
Saturates[a]	52.8	46.2	47.8
Benzenes	6.4	7.8	7.2
Indanes	3.2	3.7	3.4
Dinaphthenebenzenes	3.8	3.0	2.6
Naphthalenes	0.9	0.8	0.6
Acenaphthenes	3.7	3.6	3.2
Acenaphthylenes	5.5	5.3	4.7
Phenanthrenes	3.2	3.0	3.0
Pyrenes	0.8	1.5	1.3
Chrysenes	2.0	0.9	0.6
Benzothiophenes	3.7	2.1	1.3
Dibenzothiophenes	6.1	2.4	0.8
Naphthobenzothiophenes	1.4	0.3	0.1
Sulfides[b]	6.5	0.0	0.0
% S (actual)	1.68	0.46	0.20
% S (calculated from analysis)	1.64	0.44	0.21

[a] Determined by silica gel.
[b] Determined by iodine complex.

Analysis of Wasson Crude Oil Concentrates. The finding that sulfides and sulfur compounds formed by condensation of the thiophene nucleus with various aromatic nuclei are the major sulfur compound types in one virgin gas oil represents the first time that any concrete information, with regard to the predominant sulfur compound types in the gas oil boiling range, has been available. This information was made available to API Research Project 48, which requested that an attempt be made to determine the sulfur compound types present in the higher boiling ranges of Wasson crude oil, one of the crudes under exhaustive study by the project. To facilitate obtaining this information, workers at Project 48 topped a sample of the total crude to 150° C. under isothermal conditions (100° C. maximum, a few seconds' contact time) and then deasphalted the topped crude oil. The deasphalted, topped crude was then separated over alumina gel into nine fractions. Eight of these fractions were analyzed by the method described above, with the results shown in Table IV. The ninth fraction was too heavy for satisfactory analysis and also contained considerable amounts of oxygen and nitrogen compounds. The fair agreement between the observed and calculated sulfur contents indicates that sulfides and thiophene types comprise the major portion of the sulfur compounds. Because of the wide boiling range of the stock, the best possible results cannot be expected. Nevertheless, this information should aid Project 48 in planning its sample procurement program. The results of this particular study have already been reported by Project 48 (*16*). The data contained herein have been revised somewhat.

Analysis of Other Virgin Gas Oils. The mass spectrometer analyses of a number of virgin gas oil aromatic fractions from a wide variety of crude sources, both domestic and foreign, are shown in Table V. One check on the accuracy of the data may be obtained from a comparison of the actual sulfur content as determined by combustion methods and that calculated from the

analysis itself, using the measured average molecular weight of the sample. In most cases good agreement between the two sulfur values is obtained; however, several high-sulfur (and generally high molecular weight) stocks show fairly serious disagreement. These high-sulfur stocks presumably contain appreciable concentrations of some sulfur compound types which are not determined by the method. This of course points out an area for improvement in the mass spectrometer method and warns that results in these cases should be used with caution.

Another means of checking on the accuracy of the mass spectrometer analyses is to compare the values for C_A (percentage of carbon occurring in aromatic rings) as calculated from the mass spectrometer analyses with C_A values obtained by other more conventional methods such as the *n-d-M* method of van Nes and van Westen (*14*) These data are also shown in Table V. The agreement between the *n-d-M* method and the mass spectrometer leaves much to be desired in most of these samples. Nuclear magnetic resonance has also been applied in these laboratories to the determination of C_A content of aromatic fractions of virgin gas oils (a method to be published in the near future), and values obtained by the nuclear magnetic resonance technique are also shown in Table V. In this case good agreement is obtained between the mass spectrometer (MS) and the nuclear magnetic resonance (NMR) methods. Because these are completely independent methods and show such good agreement on this wide variety of samples, it is believed that they are accurately measuring the C_A content of these samples. The *n-d-M* method may be in error because of the difficulty in correlating refractive index, density, and molecular weight on such complex mixtures as are represented by these samples. It is planned to make a comparison of the mass spectrometer, nuclear magnetic resonance, and *n-d-M* methods for measuring C_A the subject of a future communication from these laboratories.

ACKNOWLEDGMENT

The authors acknowledge with gratitude the contributions of C. S. Langford, G. R. Taylor, C. R. Middleton, Joe Dzilsky, R. R. Burch, and H. W. Kinsey in performing the greater portion of the experimental work in these investigations. They also gratefully acknowledge helpful discussions with R. B. Williams, who developed the nuclear magnetic resonance method.

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RECEIVED for review December 12, 1955. Accepted May 14, 1956. American Society for Testing Materials, Committee E-14, San Francisco, Calif., May 1955.

Table IV. Results of Mass Spectral Study of Percolate Fractions of Topped Wasson Crude Oil

	Percolation Fractions from API 48 Alumina Gel Run 124-S[a]							
	1	2	3	4	5	6	7	8
Charge (output basis), wt. %	13.1	13.4	12.6	5.6	1.7	11.3	13.9	11.2
Compound type, wt. %								
Saturates	100.0	100.0	100.0	100.0	75.7	0.0	0.0	0.0
Aromatics								
1 ring	0.0	0.0	0.0	0.0	22.0[b]	94.4	26.4	0.0
2 rings	0.0	0.0	0.0	0.0	0.0	0.0	23.2	9.8
3 rings	0.0	0.0	0.0	0.0	0.0	0.0	11.2	23.4
4+ rings	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5
Thiophenes								
1 ring	0.0	0.0	0.0	0.0	2.3[b]	3.2	0.0	0.0
2 rings[c]	0.0	0.0	0.0	0.0	0.0	0.0	22.3	4.7
3 rings[d]	0.0	0.0	0.0	0.0	0.0	0.0	2.5	29.0
4+ rings	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sulfides[e]	0.0	0.0	0.0	0.0	0.0	2.4	14.4	9.6
Other S compounds[f]								
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Wt. % S calcd.[g]	0.000	0.000	0.000	0.000	0.29	0.72	5.01	5.55
Wt. % S obsd.	0.013	0.006	Nil	0.025	0.229	0.858	4.73	6.11

[a] See (*16*).
[b] Estimated from ultraviolet.
[c] Benzothiophenes.
[d] Dibenzothiophenes or naphthothiophenes.
[e] Free S, mercaptans, disulfides, and polysulfides below limits of detectability of polarograph.
[f] Iodine complex method.
[g] Average molecular weight estimated to be about 250 from mass spectra.

Table V. Analyses of Gas Oil Aromatic Fractions from Various Crude Sources

Compound Type, Wt. %	Crude Source						
	Louisiana	Iraq	Canada	South America	East Texas	West Texas	California
Benzenes	11.8	18.0	15.4	12.2	10.6	11.1	8.7
Indanes, etc.	8.9	8.3	8.5	7.3	7.4	6.0	8.4
Dinaphthenebenzenes	12.9	8.3	9.4	7.9	11.9	7.7	7.7
Naphthalene	4.6	2.7	2.0	1.7	5.2	1.8	3.1
Acenaphthenes	8.2	5.1	9.1	8.3	12.4	6.1	10.8
Acenaphthylenes	17.6	10.4	19.0	15.8	24.9	11.6	10.4
Phenanthrenes	15.0	2.8	13.8	9.7	14.6	7.1	7.4
Pyrenes	11.0	0.8	6.1	3.4	5.4	3.8	4.2
Chrysenes	3.1	0.7	3.2	1.8	3.0	3.4	5.3
Benzothiophenes	0.4	14.4	3.3	10.2	0.0	9.9	5.9
Naphthothiophenes	1.2	19.6	4.1	14.2	2.3	14.4	5.7
Benzonaphthothiophenes	4.1	3.9	2.9	0.0	0.0	5.0	0.6
Sulfides	1.2	5.0	3.2	7.5	2.3	12.1	21.8
S, wt. %							
Measured	0.55	3.98	1.39	3.42	0.49	4.63	2.86
Calculated	0.60	3.94	1.17	3.06	0.40	3.68	3.15
C_A							
MS	42.0	32.2	38.0	38.7	38.6	35.8	32.5
NMR	41.3	36.4	39.0	40.0	39.5	36.0	32.7
n-d-M	50.6	37.7	46.6	48.4	49.0	46.5	33.9

Determination of Unsaturated Hydrocarbons by Low Voltage Mass Spectrometry

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A method of determining unsaturates in petroleum naphthas gives quantitative information concerning the compound types present and the compounds belonging to these types. Interference from paraffins and naphthenes is negligible. The analysis is performed with a mass spectrometer operated with the ionizing voltage adjusted to form only the molecule ions of the unsaturates in the sample analyzed. The method is applicable to the analysis of catalytic cracked naphthas, virgin naphthas and hydroformates, and propylene polymers.

THE mass spectrum of a polyatomic compound obtained in the conventional way—that is, with ionizing voltage in the range 50 to 70 volts—is complex, consisting of ions of many masses (m/e values). In general, the mass spectrum may be thought of as consisting of the molecule ion (parent ion) and fragment ions. If, however, the ionizing voltage is adjusted so as to be larger than the ionization potential of the molecule but smaller than the lowest appearance potential, the mass spectrum will contain only one peak, that of the molecule ion. The existence of small peaks due to the carbon-13 content of the molecule is ignored here as trivial.

This simplification of the mass spectrum has, if it could be properly exploited, certain obvious advantages for both qualitative and quantitative analysis. For example, in a mixture of compounds with roughly the same ionization potentials and lowest appearance potentials, each peak in the mass spectrum of the mixture obtained under the conditions outlined above would represent the presence in the mixture of isomers of a given compound type and molecular weight. The compound types and molecular weights could then be qualitatively identified by the most cursory inspection of the mass spectrum, and, if appropriate calibration data were available, quantitative calculations of the concentrations of the mixture components could be made quickly with no more equipment than a slide rule. In particular, the complicated matrix calculations necessitated by fragmentation interference effects would be eliminated.

These considerations have long been recognized but not much used. In 1942, Taylor (*5*) applied the method to the determination of simple inorganic gases—e.g., nitrogen, oxygen, carbon monoxide, and carbon dioxide—and Stevenson and Wagner (*4*) used it to analyze mixtures of isotopically enriched paraffins. Similarly, it has been fairly general practice to measure free radicals formed by pyrolyzing the gas entering the ionization chamber of a mass spectrometer by operating at ionizing voltages above the ionization potential of the radical but below the ionization potential of the gas from which the radical is produced. However, in the analysis of complex mixtures of compounds such as those found in petroleum products problems are encountered which are not present in the case of the more simple mixtures referred to above. This paper describes a method for the determination of olefins and aromatics, which overcomes these problems.

From an inspection of a table of hydrocarbon ionization potentials, it may be seen that the ionization potentials of olefins and aromatics are generally at least a volt lower than the ionization potentials of paraffins, if the comparison is made between olefins and paraffins with equal numbers of carbon atoms. For example, Honig (*3*) finds that the ionization potentials of the normal paraffins range from 13.04 e.v. for methane to 10.19 e.v. for n-$C_{10}H_{22}$, while the values for the straight-chain 1-olefins range from 10.62 e.v. for ethylene to 9.51 e.v. for $C_{10}H_{20}$. Furthermore, the variation in ionization potentials of molecules containing five or more carbon atoms (the start of the naphtha range) is relative small. Thus, in going from n-C_5H_{12} to n-$C_{10}H_{22}$, the ionization potential drops from 10.55 to 10.19 e.v., and in going from 1-C_5H_{10} to 1-$C_{10}H_{20}$, it drops from 9.66 to 9.51 e.v. The more extensively the olefin $\rangle C{=}C\langle$ group is substituted, the lower the ionization potential. The ionization potential data on branched paraffins are somewhat sketchy, and the data on naphthenes are for practical purposes nonexistent, but theoretical calculations made in this laboratory by Franklin (*2*) indicate that the ionization potentials of paraffins are not dependent upon the structure of the molecule and that naphthene ionization potentials are of about the same magnitude as those of paraffins. The ionization potentials of aromatics are in general even lower than those of olefins, starting at 9.4 to 9.5 e.v. for benzene and dropping as the ring hydrogens are replaced by alkyl groups (*1*). The ionization potentials of polynuclear aromatics are lower still. Under these circumstances, one might reasonably expect to find an ionizing voltage which will ionize only olefins and aromatics, and this expectation is realized in actual practice.

The range of ionizing voltages wherein only molecule ions are formed—that is, the voltage difference between the ionization

Table I. Molecule-Ion Intensities of Various Hydrocarbons at an Ionizing Voltage of 6.90 Volts

Compound	Observed Intensity[a]	Compound	Observed Intensity[a]
Aromatics		Cyclic Olefins	
Benzene	1050	3- or 4-Methyl-1-cyclopentene	396
Toluene	1620	Cyclohexene	868
o-Xylene	1890	2,4-Dimethyl-1-cyclopentene	1330
1,2,3-Trimethylbenzene	2130	1-Methyl-1-cyclohexene	1380
1,3-Diethylbenzene	1250	4-Methyl-1-cyclohexene	728
Average	1588		940
Olefins		Diolefins	
2-Methyl-2-pentene	1000	1,2-Pentadiene	387
1-Hexene	220	*trans*-1,3-Pentadiene	2360
2-Hexene	335	1,4-Pentadiene	165
2,3-Dimethyl-2-butene	1830	2-Methyl-1,3-butadiene	1740
1-Heptene	157	1,5-Hexadiene	0
2-Heptene	96	2,3-Dimethyl-1,3-butadiene	1880
2-Methyl-2-hexene	800	Average	1089
1-Octene	95		
2,3-Dimethyl-2-hexene	1480		
2,4,4-Trimethyl-2-pentene	488		
1-Nonene	95		
1-Decene	79		
Average	556		

[a] Expressed as chart divisions (1 chart division = 1×10^{-15} ampere ion current).

potential and the lowest appearance potential—also exhibits a dependence upon compound type. The data in the literature upon which generalizations must be based are relatively scanty and refer only to compounds of relatively low molecular weight, but to the extent that they are representative, they indicate that the range for aromatics is greatest, on the order of 4 e.v., followed by olefins with values from 1 to 3 e.v., which in turn are followed by paraffins with values from 0 to 1 e.v. These facts, taken in conjunction with the above-mentioned relationships between ionization potential and compound type, indicate that it should be possible to set the ionizing voltage so as to ionize, but not fragment, the unsaturates in a mixture of saturates and unsaturates.

EXPERIMENTAL

All the work described was done with a Westinghouse Type LV mass spectrometer modified by replacing the original mass spectrometer tube with one manufactured by J. A. Hipple. This tube, except for engineering details, is similar to the Westinghouse tube. The ion currents were measured with a Brown recording electrometer, which has a sensitivity of 1×10^{-15} ampere per chart division. Magnetic scanning was used.

Except for some preliminary work, the ionizing electron current, the ionizing voltage, and the ion drawout potential (pusher potential) were maintained at 9.5 μa., 6.90 volts, and 1.9 volts, respectively (hereafter referred to as standard low voltage conditions). The electron current was set at 9.5 μa. in order to achieve the highest possible ionization sensitivity compatible with the operating characteristics of the instrument, and the ion drawout potential was set at 1.9 volts as a continuation of a long-standing practice which is known from experience in measuring ionization and appearance potentials to give satisfactory results. In choosing a value for the ionizing voltage, it was necessary to strike a compromise between too high a value, which would give a high ionization sensitivity but undesirable amounts of fragmentation and naphthene and paraffin ionization, and too low a value, which would decrease the ionization sensitivity unduly. The value of 6.90 volts was chosen on the basis of trial and error experience and seems to work well. This is the voltage applied between the filament and the ionization chamber of the mass spectrometer, but it is not the actual voltage of the ionizing electrons, as they receive additional energy contributions from the ion drawout electrode, the thermal energy of the filament, etc. No attempt was made to control the ion source temperature, and, as a consequence, it varied between about 175° and 200° C., depending upon the state of the filament. However, as the only ions formed are molecule ions, such control is not so important as in high voltage mass spectrometry, in which ion fragmentation plays a major role; furthermore, sensitivity variations due to temperature fluctuations were accounted for by a sensitivity calibration procedure described later.

Samples were charged to the instrument by means of the conventional constant-volume pipet technique.

Because molecular ionization cross sections decrease sharply with decreasing ionizing voltage below about 50 volts, perhaps the first problem to be considered in developing a low voltage analytical method is whether ion currents of sufficient intensities for the purposes of practical analysis can be achieved. Experience shows that this is possible and for illustration the molecule-ion intensities of various compounds belonging to several compound types are listed in Table I. These compounds were chosen to include both favorable and unfavorable examples, and thus the table defines rather well the range of intensities likely to be found with unsaturated hydrocarbons. The intensities of the aromatics are uniformly high, running between about 1000 and 2000 divisions. As two divisions are clearly distinguishable above instrument noise, aromatic concentrations on the order of 0.1% are detectable. The olefin intensities exhibit a wide variation, the smallest intensities, on the order of 100 divisions, occurring for the 1-olefins, and the largest, about 1500 divisions, for compounds containing the RR′C=CR″R‴ structure. However, even for the less sensitive compounds, concentrations of about 1 to 2% should be detectable. The intensities of cyclic olefins and diolefins are intermediate between those of aromatics and acyclic olefins. Compounds that produce a vanishingly small amount of parent ions even at high voltages—1,5-hexadiene, for example—are not detectable, but, fortunately, the number of these is small.

Table II. Temporal Variations in Intensity of 2,4,4-Tri-methyl-1-pentene Ions

Date	Intensity (Chart Divisions)
8/27/54	487
8/30/54	367
9/2/54	370
9/2/54	378
9/2/54	408
2/3/54	422
9/7/54	456
9/8/54	449
9/10/54	431
9/13/54	Filament replaced
9/13/54	630
9/14/54	615
9/15/54	615
9/16/54	586

The volume of sample used in obtaining these data was constant, although this and the corresponding pressure in the mass spectrometer reservoir were never actually measured. The volume used—i.e., the volume of the constant-volume pipet—was as large as possible compatible with the restriction that linearity must exist between ion intensity and sample volume. This volume was determined empirically. The position of the auxiliary magnet (electron beam collimating magnet) is of critical importance. Small changes in the position of the auxiliary magnet can effect a two- or threefold change in the magnitude of the ion currents without bringing about any perceptible change in the ratio of collected electron current to emitted electron current. The authors' practice is to adjust the position of the auxiliary magnet so as to give the largest possible ion currents compatible with the maintenance of a satisfactory ratio of collected to emitted electron current.

The second problem to be considered in the development of low voltage mass spectrometry is that of the long-term stability and reproducibility of the apparatus and method. At the standard low voltage operating conditions, the ionization efficiency curves—that is, the variation in the ion currents with the electron voltage—are very steep, and as a consequence, small changes in the ion source conditions, and particularly voltages, result in relatively large changes in the ion currents. For example, changing the ionizing voltage from 6.90 to 6.80 volts reduced the intensity of 2,4,4-trimethyl-1-pentene ions from 607 to 515 chart divisions—i.e., about a 20% change. By contrast, at the usual high voltage conditions (50 to 70 volts), the ionization efficiency curves exhibit a broad maximum, and the ion currents are relatively insensitive to voltage variations.

In the course of the work, it soon became apparent that the instrument sensitivity depends upon many factors, some of which are not controllable, and that rather marked variations in the sensitivity with time can be expected. The sensitivity is greatly affected by the electron voltage, and this includes not only the voltage applied between the filament and the ionization chamber, but also the ion drawout voltage, as this contributes to the energy of the electrons in the ionization chamber. These voltages must be controlled as closely as possible, but unavoidable errors in making the chosen settings will give rise to small variations in the sensitivity. Among the uncontrollable factors may be listed ion source deposits, variations in the work function of the filament, and, most important, the position of the auxiliary magnet.

To illustrate the day-to-day variations in sensitivity, there are given in Table II representative intensities of 2,4,4-trimethyl-1-pentene measured over about a 3-week period. The variations were large, particularly when the new filament was installed in the instrument, and it is clear that some means of compensating for the changes must be applied if quantitative work is to be

possible. Consequently, the 2,4,4-trimethyl-1-pentene ion intensities have been used as a measure of the instrument sensitivity at any given time, and it is assumed that the intensities of ions from other compounds will vary in proportion to the variations in the 2,4,4-trimethyl-1-pentene intensity. If this assumption be valid, multiplication of the observed intensity of an ion of interest by the ratio of the corresponding 2,4,4-trimethyl-1-pentene intensity to some arbitrarily chosen constant 2,4,4-trimethyl-1-pentene intensity should give an intensity value for the ion of interest which is independent of the actual instrument sensitivity at the time the measurement was made. The choice of 2,4,4-trimethyl-1-pentene as a sensitivity-calibrating substance was based on the fact that its ionization intensity at the standard low voltage conditions stands roughly midway between the highest and lowest intensities found with olefins. The intensities of ions of interest are corrected to a 2,4,4-trimethyl-1-pentene intensity of 400 chart divisions, which was chosen arbitrarily for convenience. Intensities thus corrected are referred to as being corrected to standard sensitivity.

To determine whether such a one-term correction factor adequately compensates for sensitivity changes, a test mixture consisting of equal volumes of five aromatics (benzene, toluene, *o*-xylene, 1,2,3-trimethylbenzene, and 1,3-diethylbenzene) and four olefins (2,3-dimethyl-2-butene, 2-methyl-2-hexene, 2,4,4-trimethyl-1-pentene, and 3-ethyl-4-octene) was prepared, and its low voltage mass spectrum measured daily. The average results obtained over a typical 1-month period are listed in Table III, from which it can be seen that the ion intensities corrected to standard sensitivity are satisfactorily constant, with an average deviation from average of about 3%. During this period the filament was replaced without materially affecting the ion intensities. Similar results have been obtained in subsequent operations.

Table III. Temporal Variations in Test Mixture

Compound	Av. Intensity (Chart Divisions)	Av. Deviation (Chart Divisions)	Deviation, %
2,4,4-Trimethyl-1-pentene	370	23	6.2
C_6 olefins	109	4.8	4.4
Benzene	107	6.4	6.0
C_7 olefins	59	1.6	2.7
Toluene	170	5.1	3.0
C_8 olefins	44	1.5	3.3
C_8 benzenes	205	5.3	2.6
C_9 benzenes	251	6.2	2.5
C_{10} olefins	33	1.1	3.4
C_{10} benzenes	143	4.3	3.0
Total olefins	243	9.6	3.8
Total aromatics	869	19.6	2.3

A third problem to be considered is that, while theoretical considerations indicated that unsaturates could be ionized selectively and without significant amounts of fragmentation, the point should be established experimentally. The amounts of fragmentation to be expected at a given low voltage can best be determined by measuring the mass spectra of a large number of unsaturated compounds. In the initial work, the authors deemed it excessively time-consuming to make an extensive survey of the low voltage mass spectra of compounds and proceeded on the basis of satisfactory results of the observations of the behavior of mixtures and a relatively small number of pure compounds. As the work proceeded, it became clear that satisfactory low voltage quantitative analyses could be made, with the result that the extensive pure compound survey became unnecessary for practical purposes. All observations lead to the conclusion that the amount of fragmentation of unsaturates occurring under standard low voltage conditions is in general not significant. Fragmentation producing radical ions—i.e., those of odd mass—is trivial, for such ions can immediately be distinguished from the molecule ions of interest. A certain amount of such fragmentation has been observed, particularly with compounds containing a group such as *tert*-butyl, which can form an ion of relatively low energy. Of greater importance is fragmentation producing molecule ions of lower molecular weight, both in the parent mass region and at lower masses, for in an analysis of a mixture such fragment ions could not be distinguished from the parent molecule ions of other compounds. In the course of the work, low voltage scans of the parent mass regions have been made for what is probably a representative number of unsaturated compounds, and no evidence of fragmentation in the parent mass region has been observed. More worrisome is the possibility of fragmentation involving the decomposition of an olefin molecule ion to form the molecule ion of an olefin smaller by two or three carbon numbers. The energies required for such reactions are not very large, and the reactions conceivably might occur to some extent. However, experience indicates that the extent must in actuality be small and unimportant for practical purposes.

Table IV. Molecule-Ion Intensities of Naphthene Hydrocarbons at Ionizing Voltage of 6.90 Volts

Intensities expressed as chart divisions (1 chart division = 1×10^{-15} ampere ion current)

Compound	Observed Intensity
C_5	
Cyclopentane	2
C_6	
Cyclohexane	24
Methylcyclopentane	5
C_7	
Methylcyclohexane	31
1,1-Dimethylcyclopentane	5
trans-1,2-Dimethylcyclopentane	12
C_8	
trans-1,2-Dimethylcyclohexane	43
1,1-Dimethylcyclohexane	29
1,1,2-Trimethylcyclopentane	16
cis, cis, cis-1,2,3-Trimethylcyclopentane	24
C_9	
1,1,3-Trimethylcyclohexane	20
n-Butylcyclopentane	23
C_{10}	
tert-Butylcyclohexane	15

To determine the amount of naphthene ionization to be expected under the standard low voltage operating conditions, the molecule-ion intensities of a number of C_5 to C_{10} naphthenes were determined (Table IV). A certain amount of ionization of the naphthenes occurs, the intensities ranging from 2 to 43 chart divisions. If the data for naphthenes and olefins of the same carbon number are compared (see Tables I and IV), the naphthene intensities are as much as 20 to 30% of the olefin intensities for certain C_7, C_9, and C_{10} compounds. However, perhaps the fairest comparison can be obtained from the average naphthene and olefin intensity. The average intensity of the naphthenes listed in Table IV is 19 divisions, while that of the olefins listed in Table I is 556 divisions—i.e., the naphthene ionization is about 3% that of the olefins. To determine whether naphthene and paraffin ionization at standard low voltage conditions would constitute a problem in a practical analysis, determinations were made of the C_5 to C_{10} mass spectra of three cuts in the paraffin-naphthene fraction of a silica gel percolation of catalytic naphtha. The sum of the intensities of all the ions formed (paraffin, naphthene, and some fragmentation) was in each case 32 chart divisions, but the sum of the olefin intensities in a cut taken from the middle of the olefin fraction of the percolation was 694 divisions. Thus, the paraffin-naphthene ionization was about 5% that of the olefins and in a sample undergoing analysis one might expect the experimentally observed olefin

concentrations to be erroneously high by about 5% of the paraffin-naphthene concentration. As the olefin analysis will be applied mainly to samples with relatively high olefin contents, errors from this source will usually be negligible.

QUANTITATIVE ANALYSES

Analysis of Synthetic Mixtures. To gain insight into the accuracy possible with the low voltage method, a number of simple synthetic mixtures were analyzed, and, for illustrative purposes, results of two such analyses made under standard low voltage conditions are given in Tables V and VI. The samples were prepared by mixing appropriate volumes of the different compounds by means of a 1.0-ml. graduated pipet. It is suspected that some of the errors shown in Table VI for 2,3-dimethyl-2-butene should be attributed to an error in volume measurement, for the experimentally determined concentration of this compound is low in all three olefin blends and by approximately the same relative amount. The results of the toluene determination are very good, and those for the olefin determinations are considered to be satisfactory, particularly as the experimental total olefin concentrations are correct to within 2% or less. The satisfactory results for the olefins tend to support the contention that the olefin-olefin and olefin-naphthene interferences are, at least in the mixtures investigated, negligible. These analyses are relatively uncomplicated, but even so, the results were considered encouraging.

Table V. Quantitative Analyses of Toluene-*n*-Heptane Synthetic Binaries

Toluene in Sample, %	Peak Height (Chart Divisions)	Toluene (MS), %	Deviation, %
100	2450		
80	1940	79.2	−1.0
60	1490	60.8	+1.3
40	960	39.2	−2.0
20	491	20.0	0
			Av. 1.1

Table VI. Quantitative Analyses of Olefin Synthetic Blends

Compound	% in Sample	% (MS)	% Deviation
Blend 1			
2,3-Me_2C_4-2	33.3	30.4	−8.7
2-MeC_6-2	33.3	34.6	+3.9
2,3-Me_2C_6-2	33.3	34.4	+3.3
		Sum 99.4	Av. 5.3
Blend 2			
2,3-Me_2C_4-2	20.0	18.7	−6.5
2-MeC_6-2	20.0	20.4	+2.0
2,3-Me_2C_6-2	60.0	59.8	−3.3
		Sum 98.9	Av. 3.9
Blend			
2,3-Me_2C_4-2	16.7	15.3	−8.4
2-MeC_6-2	16.7	19.3	+9.6
2,3-Me_2C_6-2	16.7	16.8	+0.6
Iso-octane	25.0	...	
Methylcyclohexane	25.0	...	
		Sum 51.4	Av. 6.2

Analysis of Catalytic Cracked Naphthas. A promising application of low voltage mass spectrometric (LVMS) analysis, which was selected for initial study, is to cracked naphtha fractions for the determination of the olefin and aromatic hydrocarbon types. It was visualized that such an analysis would yield a quantitative molecular weight breakdown of the following compound types: olefins, cyclic olefins plus diolefins, cyclic diolefins, benzenes, indanes, indenes, and naphthalenes. Each of these compound types falls in a separate mass series—i.e., C_nH_{2n} (masses 70, 84, 98, ...) for olefins; C_nH_{2n-2} (masses 68, 82, 96, ...) for cyclic olefins plus diolefins, etc. Other compound types belonging to the several mass series exist—for example, the acetylenes belong to the C_nH_{2n-2} series—but it was thought that the probability of finding them in the cracked naphtha would be negligible.

One of the more difficult and time-consuming problems in developing the analysis was the preparation of calibration standards, particularly for the olefin types. Calibration data are readily obtained for compound types that have only one isomer in a given molecular weight range—for example, cyclopentene, benzene, toluene, and naphthalene. In addition, sufficient information is available concerning the isomer distribution of certain compound types at certain molecular weights to permit the preparation of synthetic blends for calibration—e.g., the distribution of the C_5 acyclic olefins in catalytically cracked materials is well known. The composition of the C_6 fraction is also fairly well known, and data are available on the composition of C_8 and C_9 benzene fractions.

However, to obtain calibration data for the olefins above the C_5 range, it was decided to prepare olefin fractions (preferably of one carbon number) from a representative catalytic naphtha. The naphtha employed was obtained from the plant catalytic cracking unit when an average gas oil feed mixture was cracked under normal operating conditions. This naphtha was percolated over Davison Code 950 silica gel to prepare an olefin concentrate for subsequent distillation. The olefin concentrate was distilled in a 40-plate distillation column at 30 to 1 reflux ratio and 2% cuts were obtained. Low voltage mass spectra were obtained on each of these cuts in order to determine the molecular weight distribution. On the basis of this information the cuts were recombined to produce the narrowest possible carbon-number spread, taking into account the following reservation.

It has been shown that the several isomers of a given compound type differ appreciably in their sensitivities, and therefore it is desirable that the isomer distribution in any calibration blend correspond as closely as possible to that which will be encountered in an actual sample. In the catalytic naphtha there conceivably will be several different types of olefins, and at the higher carbon numbers there will be a number of isomers of the different types. The boiling points of these numerous compounds will in general be different, with the result that the boiling range of the olefins of a given carbon number will be broad, and indeed oftentimes overlap the range of the compounds with one more and one less carbon atom.

In order to minimize discrimination against possibly important isomers in the upper or lower portion of the boiling range, the number of distillation cuts recombined was in some cases large enough to produce a blend containing small amounts of material with one carbon number above and below the carbon number group desired for calibration. These blends were then divided into two portions. One portion was retained for calibration of the low voltage mass spectrometer; the second portion was hydrogenated and the product analyzed by high voltage mass spectrometry to determine the relative amounts of paraffins and naphthenes. Ultraviolet spectroscopy showed that in none of the blends was there a significant concentration of conjugated diolefins, and it was assumed that this indicated also a low concentration of nonconjugated diolefins. Therefore, the saturates found in the hydrogenated blends were considered to come almost exclusively from acyclic mono-olefins. Similarly it could be inferred from the low voltage mass spectra of the distillation cuts that the concentrations of cyclic diolefins plus dicyclic olefins in the blend were small, and thus it was assumed that the naphthenes found in the hydrogenated blends came from cyclic olefins.

This procedure yields satisfactory calibration standards for acyclic and cyclic olefins when the blends can be made up to contain only one carbon number. However, as the high-voltage analysis gives a measure of only the total concentrations of acyclic

Table VII. Calibration Data for Analysis of Olefin and Aromatic Types by Mass Spectrometer at Low Voltage

Mass No.	C No.	Compound Type: Acyclic olefins	Cyclic olefins	Benzenes	Indanes	Naphthalenes
		Intensity, Chart Divisions				
68	5		460			
70		795				
78				1010		
82	6		830			
84		945				
92				1560		
96	7		1010			
98		675				
106				1860		
110	8		980			
112		525				
118					1575	
120	9			2030		
124			870			
126		465				
128						1875
132					1580	
134	10			2000		
138			720			
140		425				
142						1875[a]
146					1580[a]	
148	11			1300[a]		
152			550			
154		390				
156						1875[a]
160					1580[a]	
162	12			1000[a]		
166			400[a]			
168		370[a]				

[a] Extrapolated.

and cyclic olefins, for blends containing more than a single carbon number, a correction has to be applied to obtain the concentrations of these compound types as a function of carbon number. To do this, any available low voltage calibation data were utilized further to analyze the blend. For example, blend 1 contained nothing but C_5 acyclic and cyclic olefins, and these could be determined accurately by the method described above and the low voltage calibration coefficients obtained. However, blend 2 was predominantly C_6 acyclic and cyclic olefins but contained a small amount of C_5 acyclic and cyclic olefins. The low voltage mass spectrum of this blend was obtained, and the concentrations of C_5 cyclic and acyclic olefins were calculated using the sensitivity coefficients obtained from blend 1. The concentration of these C_5 olefins was subtracted from the olefin concentrations obtained by the high voltage analysis on the hydrogenated blend, and the difference was taken to be the concentration of the C_6 cyclic and acyclic olefins in the blend.

For blends containing components at three different carbon numbers, an additional step was required. It was found that the variation in the sensitivity coefficient for a given compound type as a function of molecular weight is regular, and consequently, the technique described above for blends containing components with two carbon numbers was extended to the blends containing components of three carbon numbers, taking as approximate low voltage sensitivity coefficients for the components of the highest carbon number values extrapolated from the known values at lower carbon numbers. In this manner it was possible to obtain calibration data for acyclic and cyclic olefins through the C_{12} range. The calibration coefficients thus determined are shown in Table VII, along with olefin calibration data obtained from pure compounds and synthetic blends. Also included in this table are data for the various aromatic series of interest, obtained for the most part from pure compounds and synthetic blends.

In application, the method is straightforward. Under standard operating conditions, a known amount of the standard olefin, 2,4,4-trimethyl-1-pentene is charged to the instrument and its ion intensity is determined. This is then pumped out of the instrument, and a charge of the sample to be investigated is introduced and scanned over the entire spectral region where peaks are expected to be found. The standard compound is then run again. The intensities of the standard compound before and after the scan are read and averaged. The intensities of the ions in the sample spectrum are measured and adjusted to standard sensitivity. By using the calibration data shown in Table VII and the intensities corrected to standard sensitivity, one can calculate the composition of the unknown mixture simply by forming ratios.

Table VIII. Analyses of Catalytic Naphthas

	Liquid Volume %: Plant Naphthas: C. Cracker 1 Light	C. Cracker 1 Heavy	C. Cracker 2 Light	C. Cracker 2 Heavy	Pilot Unit Naphthas: Paraffinic[a]	Desulfurized high sulfur[a]	High sulfur[a]	Av. sweet[a]
Acyclic olefins								
C_5	14.3		10.0		32.1	10.6	8.8	15.6
C_6	10.3		12.3	0.1	19.6	8.3	7.6	10.1
C_7	7.5		8.4	1.1	8.5	6.5	6.8	5.6
C_8	5.3	0.7	4.9	3.0	4.9	5.2	6.1	3.4
C_9	3.1	3.0	2.3	3.3	2.7	3.4	4.6	2.0
C_{10}	1.2	4.7	1.0	3.1	1.7	2.7	3.5	0.9
C_{11}		5.0		3.0	1 1	2 1	2.5	0.9
C_{12}		3.6		2.2		1.4	1.6	0.3
Cyclic olefins								
C_5	1.3		0.9		1.0	1.4	1.4	1.2
C_6	3.6		3.6		1.6	3.6	3.2	3.2
C_7	5.0		4.8	0.9	1.9	4.5	4.8	4.3
C_8	3.7	0.5	3.1	2.0	1.9	4.0	4.3	3.6
C_9	1.9	2.1	1.5	2.1	1.1	2.9	2.9	2.1
C_{10}	0.6	2.5	0.4	1.5	0.4	1.7	1.6	0.9
C_{11}		2.4	0.1	1.5	0.4	1.5	1.4	0.6
C_{12}		1.8		1.1		1.0	0.7	0.3
Benzenes								
C_6	0.5		0.5		0.5	1.1	0.7	0.6
C_7	2.6		2.6	0.8	0.5	2.3	1.6	1.4
C_8	4.7	3.1	4.3	5.6	1.4	3.7	3.8	3.7
C_9	2.5	11.6	3.1	11.0	1.8	4.0	4.9	4.8
C_{10}	0.7	10.4	1.0	9.6	1.1	2.6	3.7	3.0
C_{11}		7.0	0.2	7.4	0.7	1.9	2.4	2.0
C_{12}		1.8		2.0		0.6	0.7	0.5
Indanes								
C_9	0.1	1.0	0.2	0.8	0.1	0.4	0.4	0.3
C_{10}	0.2	4.7	0.3	3.8	0.2	1.5	1.5	1.3
C_{11}		4.4	0.1	3.9	0.2	1.3	1.5	1.4
C_{12}		1.0		0.9		0.2	0.3	0.2
Naphthalenes								
C_{10}		1.5		1.3	...	0.5	0.4	0.4
C_{11}		0.6		0.4				
C_{12}								
Totals								
Acy. olefins	41.7	17.0	38.9	15.8	70.6	40.2	41.5	38.8
Cyc. olefins	16.1	9.3	14.4	9.1	8.3	20.6	20.3	16.2
Total olefins	57.8	26.3	53.3	24.9	78.9	60.8	61.8	55.0
FIA (olefins)	59.5	29.5	55.9	26.0	77.5	62.0	61.3	56.5
Totals								
Benzenes	11.0	33.9	11.7	36.4	6.0	16.2	17.8	16.0
Indanes	0.3	11.1	0.6	9.4	0.5	3.4	3.7	3.2
Naph.	0.0	2.1	0.0	1.7	0.0	0.5	0.4	0.4
Total aromatics	11.3	47.1	12.3	47.5	6.5	20.1	21.9	19.6
FIA (aromatics)	11.6	47.0	12.4	47.0	6.3	21.6	24.2	21.4

[a] Gas oil feed stock.

The method has been applied to light and heavy catalytic naphthas produced in the plant and to catalytic naphthas produced in pilot unit operations. The averages of triplicate runs on a number of naphthas are summarized in Table VIII. Fluorescent indicator adsorption (FIA) analyses have also been performed on each of the naphthas in duplicate in order to check the degree of accuracy being obtained by the mass spectrometer analysis. These results are also shown in Table VIII. Excellent agreement between the mass spectrometer and fluorescent indicator adsorption analyses is obtained in all cases.

Table IX. Precision of Mass Spectrometer and Fluorescent Indicator Adsorption Methods for Total Olefins and Total Aromatics

	Liquid Volume %									
	Total Olefins					Total Aromatics				
	FIA		MS			FIA		MS		
Run	1	2	1	2	3	1	2	1	2	3
Sample										
Light naphtha—cat cracker 1	60.0	59.0	59.7	56.8	57.2	11.0	12.2	11.4	11.9	11.4
Heavy naphtha—cat cracker 1	30.1	28.8	27.6	26.9	24.7	46.5	47.4	45.8	49.4	46.2
Light naphtha—cat cracker 2	55.8	56.0	54.0	51.8	54.3	12.2	12.6	12.3	11.3	13.1
Heavy naphtha—cat cracker 2	26.3	25.7	25.6	25.6	23.7	47.0	47.0	47.0	47.6	47.4
Naphtha from pilot unit										
Paraffinic feed	78.2	76.9	78.6	80.6	76.9	6.4	6.1	6.7	6.9	6.5
Desul. high sulfur	61.3	62.7	62.6	61.9	57.5	23.0	20.2	20.3	20.6	18.9
High sulfur	62.3	60.3	60.7	63.1	61.5	23.8	24.7	21.9	21.9	22.4
Average sweet	57.1	56.0	56.1	55.3	55.0	22.1	20.7	19.7	20.0	20.4
σ, %	0.63		1.39			0.66		0.74		

The precision of the two methods should also be of considerable interest, and the analytical results are presented in a somewhat different fashion in Table IX to illustrate this point. The standard deviation for the fluorescent indicator adsorption total olefin results is 0.63% (16 tests), whereas that for the mass spectrometer results is 1.39% (24 tests). The standard deviation for total aromatics is 0.66% by fluorescent indicator adsorption and 0.74% by mass spectrometer. The deviation in the mass spectrometer total olefin results is perhaps somewhat larger than desired and indicates the desirability for more adequate control. This can most readily be achieved by normalizing the mass spectrometer results to agree with a fluorescent indicator adsorption analysis on the sample.

The analyses described above were made shortly after the calibration data were obtained. Therefore, in connection with the problem of the long-term applicability of the calibration data, another catalytic naphtha determination is considered, made approximately 8 months after the calibration data were obtained. In Table X it is compared directly to results obtained on this same naphtha by a method utilizing the best techniques which were available prior to the development of the low voltage mass spectrometer method. Included in the analytical scheme were distillation, percolation of numerous distillation fractions, recombination of concentrates, hydrogenation, and further distillation with subsequent mass spectrometer and infrared analyses on segregated fractions. The results of this extensive analysis are believed to be accurate and the agreement between the low voltage mass spectrometer method and the analysis obtained by the more complex procedure is considered to be satisfactory, especially in view of the relative amounts of time required by the two methods. The rather detailed analysis obtained from the low voltage mass spectrometer requires only approximately 1.5 hours, about 40 minutes of which is actual instrument scanning time, which could be appreciably reduced with improved instrumentation. At least 500 man-hours would be required to duplicate the more complicated analysis, although the summary results shown in Table X conceal a considerable amount of detailed information actually obtained.

Table X. Analysis of Catalytic Naphtha from High Sulfur Gas Oil by Low Voltage Mass Spectrometry

	Liquid Volume %		
Component	Low voltage MS		Detailed composition study
Benzene	0.3		0.4
Toluene	1.6		1.7
C_8 benzenes	3.3		2.9
C_9 benzenes	3.9		3.8
C_{10} benzenes	2.7		2.6
C_{11} benzenes	2.2		2.9[a]
Indane	0.2		0.3
Methylindanes	1.1		1.4
Dimethylindanes	0.9		...[a]
Naphthalene	...		0.2
Acyclic olefins			
C_6	15.9		12.3
C_8	10.2		10.5
C_7	7.2		7.3
C_8	5.6		5.5
C_9	3.6		
C_{10}	2.7	8.9	10.5
C_{11}	1.5		
C_{12}	1.1		
Cyclic olefins			
C_5	1.3		0.5
C_6	3.9		2.5
C_7	4.4		4.7
C_8	3.6		4.3
C_9	2.3		
C_{10}	1.2	4.3	7.2
C_{11}	0.4		
C_{12}	0.4		

[a] Includes dimethylindanes.

Table XI. High Voltage Mass Spectrometer Sensitivities of C_9 Benzenes

		Peak Height at Mass			
Compound	B.P., ° F.	120	106	92	78
Isopropylbenzene	306	426	147	9	96
n-Propylbenzene	319	464	7	233	138
m-Ethyltoluene	322	524	149	74	61
p-Ethyltoluene	324	500	144	41	58
o-Ethyltoluene	329	528	153	50	63
1,3,5-Trimethylbenzene	329	844	112	32	59
1,2,4-Trimethylbenzene	337	757	117	24	56
1,2,3-Trimethylbenzene	349	720	38	31	57
Weighted average[a]		661	117	45	61

[a] According to infrared analysis of C_9 aromatics concentrate.

Analysis of Virgin Naphthas and Hydroformates. Another application of the low voltage method is to the determination of the aromatic molecular weight distribution in hydroformer feeds and products in the higher boiling ranges (about 300° F. and higher), and particularly in distillation cuts thereof. This analysis has been made by high voltage mass spectrometry; however, this method has serious limitations, which are easily overcome by the low voltage technique. The determination of the molecular weight distribution of aromatics by high voltage mass spectrometry was originally designed to be performed on broad cut distillation fractions such as a 200° to 330° F. virgin naphtha or hydroformate. This was essential because the mass spectrometer sensitivities of the individual isomers in a given molecular weight range vary widely; weighted average sensitivities are obtained for actual analyses by preparing synthetic C_8, C_9, and C_{10} benzene mixtures corresponding to the known ratio of occurrence of the isomers. The sensitivities of the individual C_9 benzene isomers and the weighted average are given in Table XI by way of illustration. From the boiling points shown in this table it is obvious that the average sensitivities as applied to a narrow range distillation fraction would give results seriously in error, not only for the C_9 benzene content, but also for C_8, C_7, and C_6. To emphasize the point, the composition of a 315° to 321° F. fraction from the distillation of an

Table XII. Analysis of Hydroformate Distillation Fractions by Mass Spectrometer

	Sample 1			2			3			4		
	Boiling Range, ° F. 342–352			352–357			362–368			367–373		
	Liquid Volume % by											
	HV	LV1	LV2	HV	LV1	LV2	HV	LV1	LV2	HV	LV1	LV2
Benzene	0.6	...	...	0.7	...	...	0.8	...	...	0.5	...	...
Toluene	2.5	...	...	3.9	...	...	4.2	0.3	0.3	1.6	...	...
C_8 benzenes	3.1	0.4	0.2	6.6	...	...	7.5	0.3	0.3	4.1	...	...
C_9 benzenes	21.5	19.8	16.6	13.2	10.7	9.0	1.3	1.4	1.2	0.4	0.8	0.7
C_{10} benzenes	27.9	22.7	33.5	36.4	32.7	44.4	62.4	58.1	72.5	74.3	66.3	71.2
C_{11} benzenes	[a]	0.0	0.0	[a]	0.0	0.0	[a]	0.5	0.3	[a]	2.5	3.6
Indanes	[a]	4.4	4.4	[a]	5.0	5.0	[a]	4.5	4.5	[a]	7.7	7.7
Naphthalenes	[a]	0.0	0.0	[a]	0.0	0.0	[a]	0.0	0.0	[a]	0.0	0.0
Total aromatics	55.6[b]	46.9	54.7	60.8[b]	48.4	58.4	76.2[b]	65.1	79.1	81.6[b]	77.3	83.3
Total olefins	3.0[b]	3.9	3.9	3.9[b]	3.7	3.7	3.2[b]	2.2	2.2	3.2[b]	2.6	2.6
Total aromatics (FIA)		55.6			60.8			76.2			81.6	
Total olefins (FIA)		3.0			3.9			3.2			3.2	

HV. High voltage MS.
LV1. Low voltage MS, average sensitivities.
LV2. Low voltage MS, specific sensitivities for boiling range (see Figure 1).

[a] Not determined.
[b] Normalized to FIA results.

from plant test run hydroformates produced at different severities. Analyses of four distillation cuts from these hydroformates are shown in Table XII. The first column under each sample shows the analysis obtained from conventional high voltage mass spectroscopy. The second column gives the results obtained from low voltage mass spectroscopy, where the broadcut weighted average sensitivities for each molecular weight group are employed. The final column shows the results obtained from low voltage mass spectroscopy when the effect of boiling range of the sample is taken into consideration. The high voltage analysis did not take into consideration the presence of C_{11} benzenes and the appreciable concentrations of indanes. However, it is not likely that even had they been considered any significant improvement in the erroneous toluene and C_8 benzene contents would have been effected. The marked improvement in the agreement between the total aromatics by low voltage mass spectrometry and fluorescent indicator adsorption when the boiling range effect is taken into consideration demonstrates the significance of this refinement.

aromatic solvent (as determined by infrared) is compared to the composition which would be indicated by high voltage mass spectrometry.

	Actual (IR)	Calculated (HVMS)
Benzene	0	3
Toluene	0	10
C_8 benzenes	0	−4
C_9 benzenes[a]	100	74

[a] 64% *n*-propylbenzene. 36% *m*-ethyltoluene.

Two classes of errors are encountered in this case: the error due to using an average parent ion sensitivity and that due to using average interference coefficients. By carrying out the analysis at low ionizing voltages the interference coefficient error is eliminated, because no fragment masses are formed. One is faced then only with the problem introduced by variations in parent ion sensitivities. Although the sensitivity variation is actually greater in the case of low voltage analysis, the problem is more readily solved because of the absence of interferences between groups. The problem is further simplified by the fortunate fact that the sensitivity variation is a relatively smooth function of boiling range, the lowest boiling isomers having the lowest sensitivities and vice versa, as indicated in the following table.

Compound	B.P., ° F.	Observed Intensity
Isopropylbenzene	306	1300
n-Propylbenzene	319	1110
m-Ethyltoluene	322	1835
p-Ethyltoluene	324	1870
o-Ethyltoluene	329	1725
1,3,5-Trimethylbenzene	329	2510
1,2,4-Trimethylbenzene	337	2400
1,2,3-Trimethylbenzene	349	2300

By employing these data in conjunction with infrared analyses of distillation fractions from a C_9 aromatics concentrate, it was possible to calculate the points shown in the left-hand curve of Figure 1. Similarly, with pure compound data for individual C_{10} benzenes and infrared analyses of distillation cuts from a C_{10} aromatics concentrate, the points for the center curve were calculated. Data on individual C_{11} benzene isomers are not available; however, in view of the results for C_9 and C_{10} isomers the right-hand curve in Figure 1 is taken as a reasonable approximation. The lowest and highest boiling C_{11} isomers are the amylbenzenes and pentamethylbenzene, respectively, and these compounds are available for calibration purposes. One could also expect to prepare a reasonable plot for C_{12} benzenes from data on hexylbenzene and hexamethylbenzene.

This technique has been employed in the analysis of distillation cuts from a 300° to 350° F. plant hydroformer feed stock and

Analysis of Propylene Polymers. The low voltage technique has also been applied to the determination of the molecular weight distribution of olefins in polymers produced from the catalytic polymerization of propylene. As might be anticipated, once again it was necessary to produce concentrates for calibration of the mass spectrometer for each of the molecular weight groups of olefins. Concentrates of C_6, C_9, and C_{12} olefins are readily obtained from the distillation of a propylene polymer. The sensitivities of C_7, C_8, C_{10}, and C_{11} olefins were then obtained by interpolation. To check the validity of the assumption of

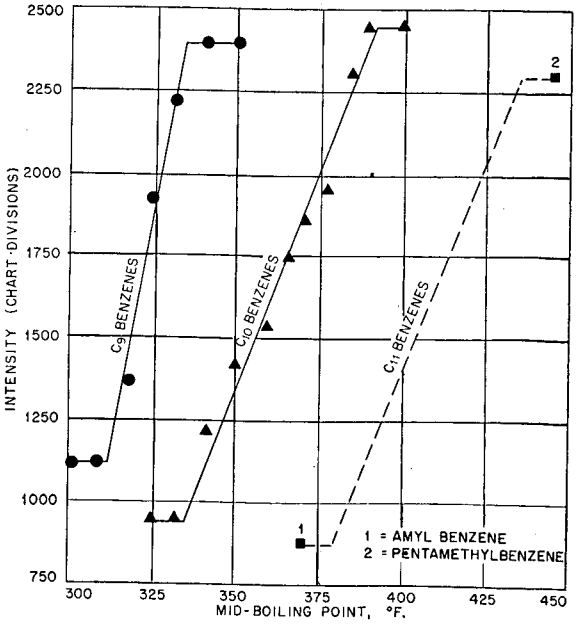

Figure 1. Low voltage mass spectrometer intensities of C_9, C_{10}, and C_{11} benzenes

linearity between sensitivity and molecular weight the sensitivities derived on the basis of the assumption were applied to cuts from the distillation falling among the C_6, C_9, and C_{12} concentrates. The analyses of these various cuts gave results totaling between 90 and 110% olefin, thus indicating not greater than a 10% error in the sensitivities. Actually, because the distillation would be expected to effect some separation of the various isomers, and average coefficients are applied, these sensitivities when applied to a propylene polymer sample of wide boiling range should give good results.

Application to High Boiling Materials. It was expected that the low voltage technique would have a particularly useful application to the quantitative determination of the compound types present in fractions obtained from the percolation of heating oil and gas oil fractions over alumina gel or similar absorbent. Preliminary investigations of this possibility have been successful, and it is anticipated that this technique will prove useful in the eventual unraveling of the composition of high boiling aromatics.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contributions of Burl L. Clark, who performed the greater portion of the experimental work. Joe Dzilsky joined the project in its later stages and his efforts, too, are greatly appreciated.

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RECEIVED for review February 10, 1956. Accepted May 3, 1956.

Quantitative Infrared Absorption Spectroscopy in Water Solution

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Quantitative infrared absorption spectroscopy can be carried out in water solution by using a very thin absorption cell with barium fluoride windows. Useful transmittance in the region from 6.5 to 10 microns is obtained on a double-beam spectrometer by insertion of a transmittance screen in the reference beam; energy is recovered by widening the spectrometer slits by a suitable amount. The method is applicable to many cases where organic materials are soluble in water.

THAT water can be used as a solvent for infrared absorption spectroscopy was shown as early as 1905 by Coblentz (*2*). More recently Gore, Barnes, and Petersen (*3*) and Blout and Lenormant (*1*) have shown that water, used in conjunction with heavy water, can have considerable use in this respect, enabling one to obtain an infrared absorption spectrum throughout almost the entire rock salt region. Their results were of a qualitative nature only, however.

Plyler and Acquista (*4*) have given quantitative absorption spectra of pure water, and have shown that there is a region from ≈6.5 to ≈10 microns where there is still enough infrared transmittance in reasonable path lengths of water to suggest its use as a solvent for quantitative analytical purposes.

The advent of barium fluoride as an optical material has made possible the construction of a permanent absorption cell. Barium fluoride seems ideally suited for this use, as it is commercially available, hard, easily polished, and essentially insoluble in water. With such a cell, water solutions can be used in much the same way, and with the same accuracy, as carbon tetrachloride or carbon disulfide solutions are used for quantitative absorption spectroscopy at present.

APPARATUS AND TECHNIQUES

The absorption cell (Figure 1) is constructed in much the same manner as the conventional rock salt cells. The spacer between the barium fluoride plates is made from 0.001-inch shim brass, and is sealed to the plates by coating the brass with mercury to form an amalgam with the brass, which sticks to the fluoride plate surface. The barium fluoride plates were obtained already cut, ground, and polished from the Perkin-Elmer Corp., Norwalk, Conn.

The cell so constructed has a path length of 0.027 mm.; this distance was determined in the usual way by a fringe pattern (shown in Figure 2, *a*) of the empty cell. The depth of the fringes and their general regularity indicate that, even with this short path length, a cell can be made with nearly perfectly parallel faces if care is used.

All spectra were obtained on a double-beam infrared spectrometer equipped with a rock salt prism. The instrument was designed and built in this laboratory; a publication describing its construction and features is in preparation.

Figure 2, *b*, is the absorption spectrum of pure water obtained in the cell just described. Comparison with Figure 2, *a*, shows that water ceases to transmit a useful amount of radiation at somewhat shorter wave length than the barium fluoride cutoff point; hence, barium fluoride is by no means the limiting factor in the use of water solutions.

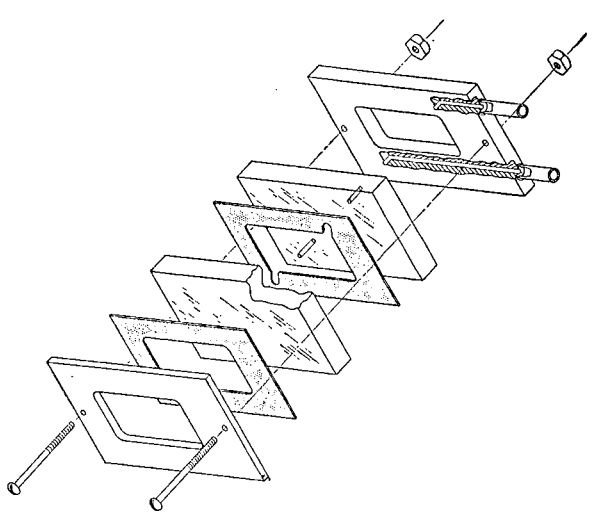

Figure 1. Barium fluoride absorption cell for use with water solutions

In order to obtain a more useful I_0 in the region from 6.5 to 10 microns, the spectrometer is stopped at 6.1 microns (absorption maximum of the water deformation vibration), and a transmittance screen is inserted in the reference beam. The absorption under these conditions is shown in Figure 2, *c*. This now results in an I_0 near the region of 100% transmittance (on the chart paper), a situation desirable in accurate photometry and convenient for qualitative study of spectra.

A useful I_0 might be obtained by placing a second absorption cell filled with water in the reference beam of the spectrometer. However, this would tend to hide the fact that the spectrometer is receiving no energy and is, therefore, useless in the regions of strong water absorption (near ≈6.1 microns and above ≈10.5 microns). Also, this would require the time and expense of a second cell, when a simple screen does just as well. Hence, this technique was not used.

The energy lost by water absorption and transmittance screen is recovered by widening the monochromator entrance and exit

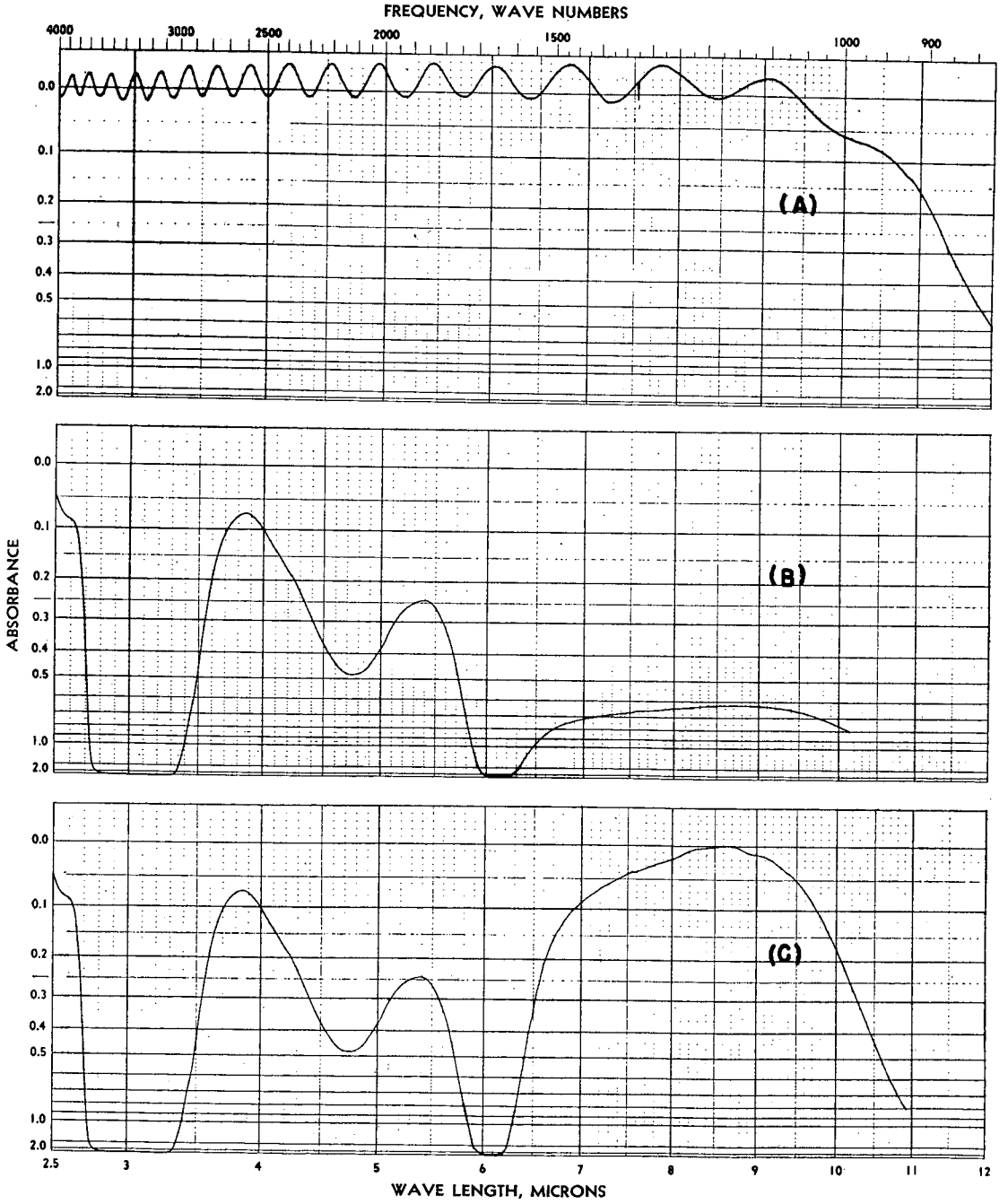

Figure 2. Absorption spectra

a. Fringe pattern of empty cell, from which is obtained optical path length of 0.027 mm.
b. Pure water
c. Pure water, with screen placed in reference beam from 6.1 to 11 microns

slits by a factor $\sqrt{\frac{1}{T}}$, where T is % light transmittance of water in the region from 6.5 to 10 microns. This gives the same signal-to-noise ratio as if neither water nor screen were in the spectrometer beams.

Of course, this widening of the spectrometer slits is done at the cost of loss of resolution, but in all cases encountered thus far this loss has not been serious. A somewhat greater departure from Beer's law might be encountered than in the case of normal slit openings, but corrections for Beer's law deviations can be made just as is usually done when effective slit widths are greater than natural band half-widths.

APPLICATIONS

Metallic Salts of Organic Acids. Solids insoluble in the usual infrared-transmitting solvents are often difficult to analyze accurately with infrared methods. Metal salts of organic acids

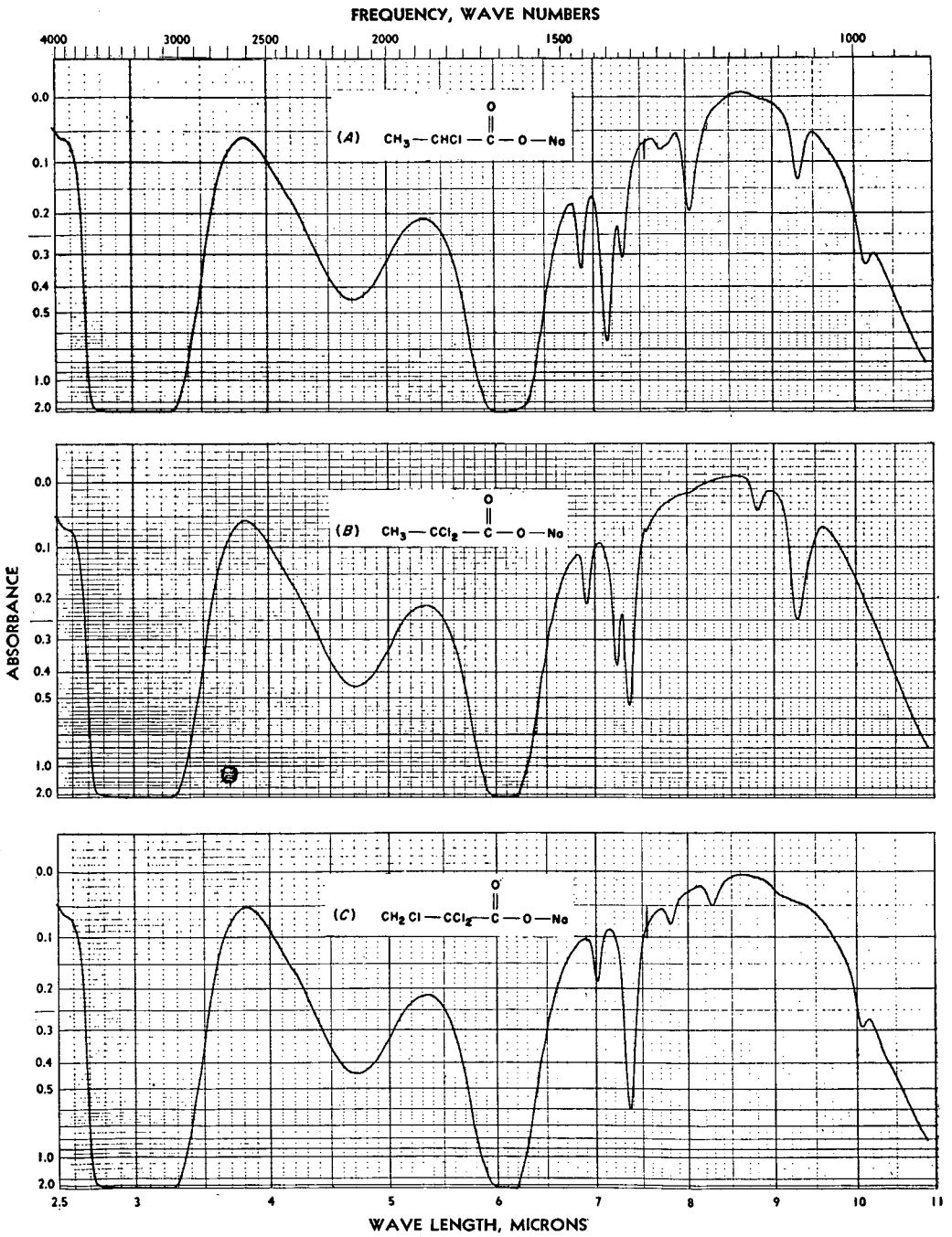

Figure 3. Absorption spectra of 10% aqueous solutions

a. Sodium 2-chloropropionate
b. Sodium 2,2-dichloropropionate
c. Sodium 2,2,3-trichloropropionate

are often determined by conversion to the acid and extraction with a suitable solvent such as carbon disulfide. The use of water solutions now gives a simpler, more direct, and generally more accurate method.

Such a problem which has been successfully solved in this laboratory with the water solution technique is the simultaneous determination of the sodium salts of 2-chloro-, 2,2-dichloro-, and 2,2,3-trichloropropionic acids. The spectra of 10% solutions in water of these three materials are shown in Figure 3. Examination of these spectra shows that they are sufficiently different to be used as the basis for the determination of these three salts in the presence of each other. Applications in this laboratory have given results generally reproducible to within 2% of the amount present for the main constituent.

Glycols. Liquids insoluble in the usual infrared solvents are also often difficult to analyze directly. The various glycols are

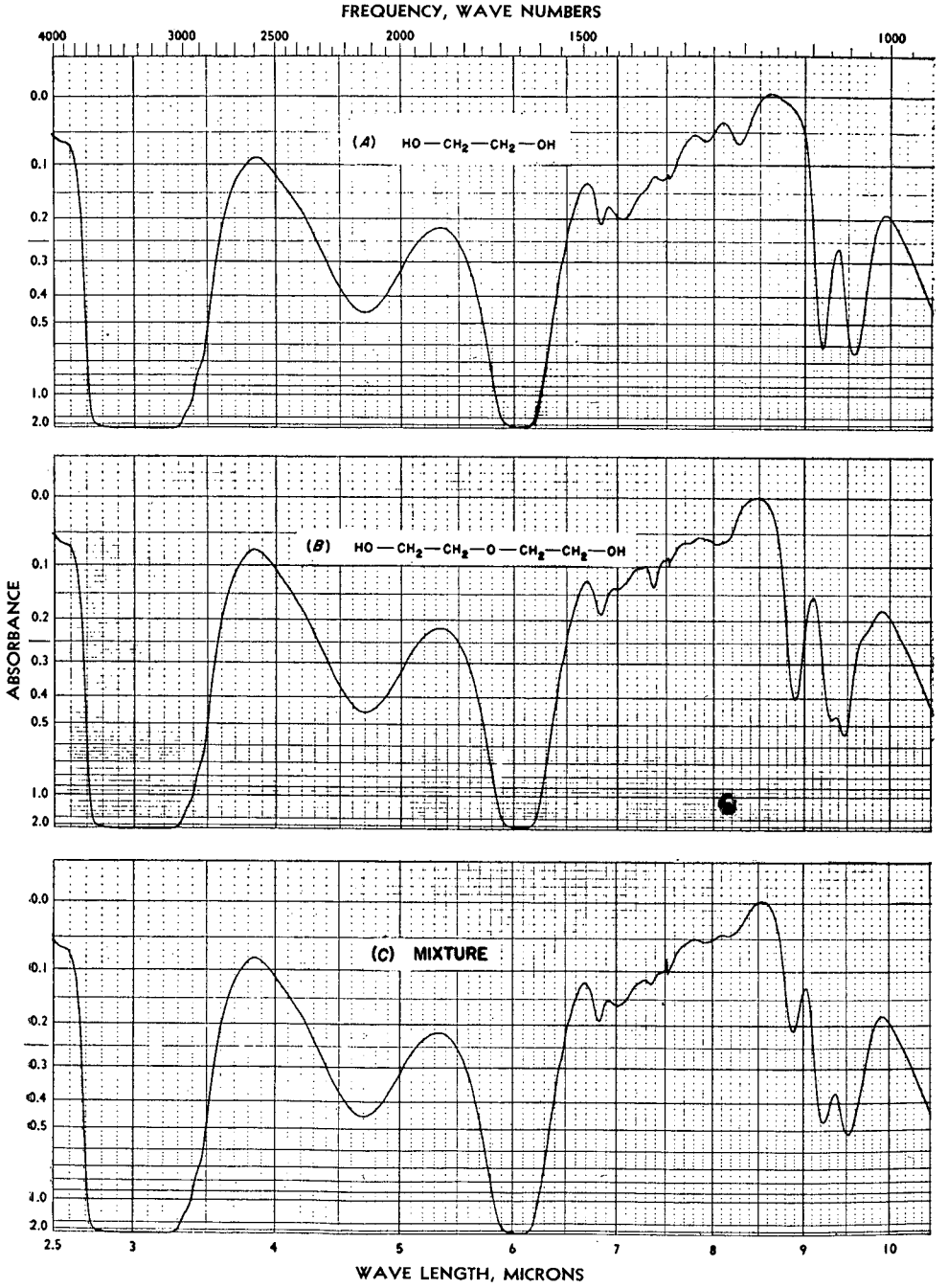

Figure 4. Absorption spectra of aqueous solutions

a. Ethylene glycol, 10%
b. Diethylene glycol, 10%
c. Ethylene glycol and diethylene glycol, 5% each

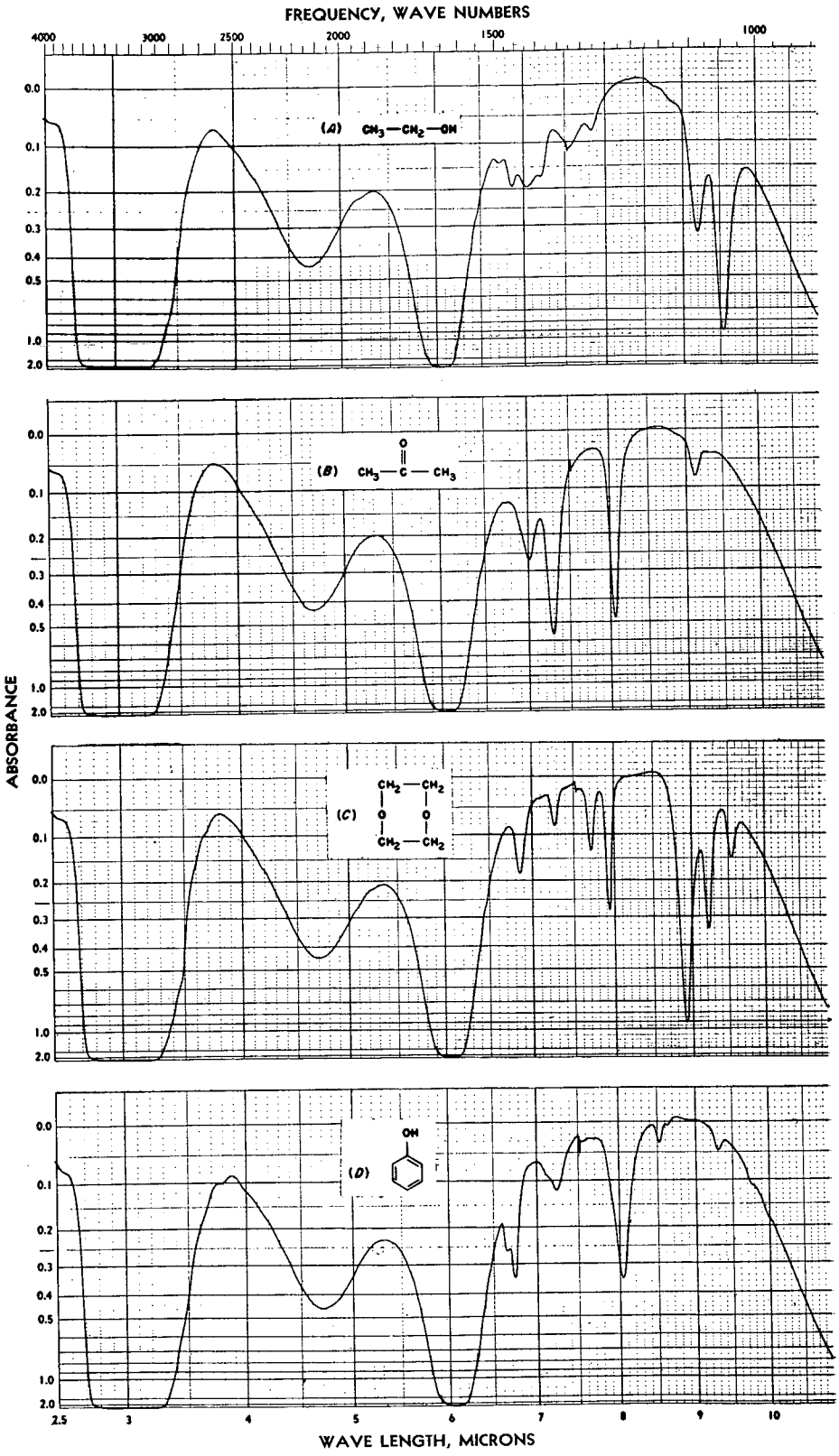

Figure 5. Absorption spectra of aqueous solutions

a. Phenol, 5%
b. Acetone, 10%
c. Dioxane, 10%
d. Ethyl alcohol, 10%

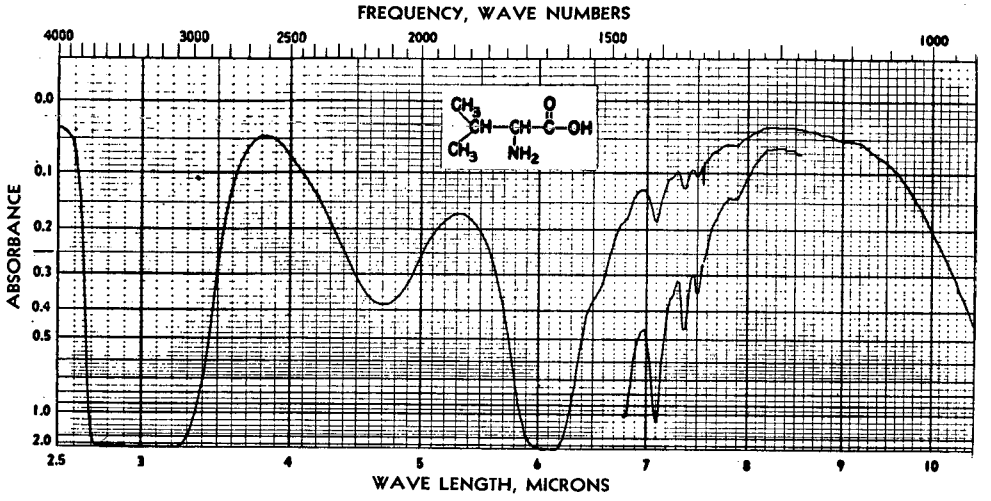

Figure 6. Absorption spectrum of 2% aqueous solution of D-valine

Obtained by use of expansion of pen motion

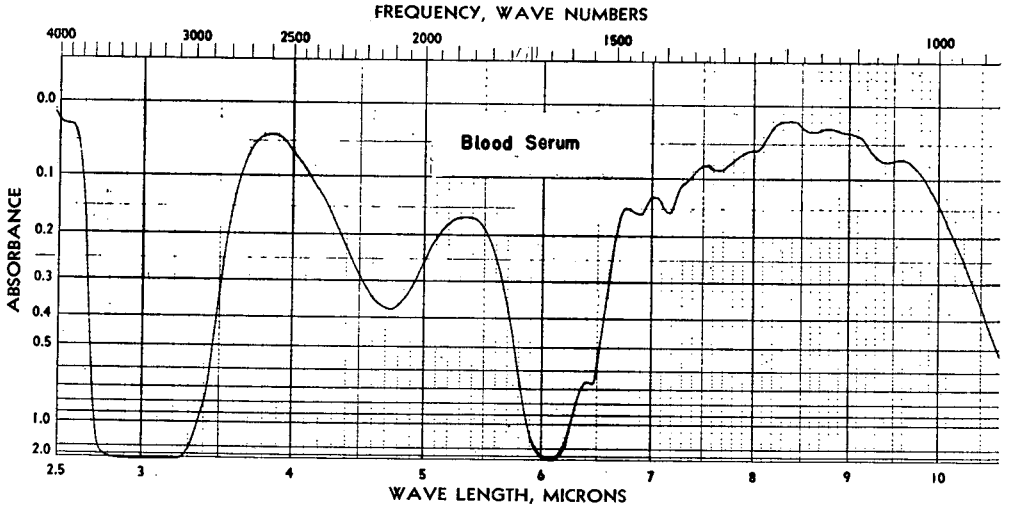

Figure 7. Spectrum of centrifuged blood plasma

Run without dilution

an example. Water solutions are shown to be helpful in certain of these problems.

Figure 4 shows spectra of 10% solutions in water of ethylene glycol and diethylene glycol, and of a water solution containing 5% of each. The absorption at ≈8.8 microns determines uniquely the amount of diethylene glycol present; ethylene glycol can be determined by the absorption at 9.2 microns after correcting for diethylene glycol absorption.

Partition between Phases. Samples for infrared analysis often consist of a water phase and an organic phase, with certain organic compounds distributed between phases. Compounds in the organic phase may be determined directly with infrared methods by solution in a suitable solvent; but for the determination of the organic materials in the water phase it is usually necessary to extract them with a suitable solvent. The water solution method performs this analysis directly, however, and without the errors attendant upon extraction techniques.

In Figure 5 are spectra of water solutions of phenol, acetone, dioxane, and ethyl alcohol. The spectra were obtained in 10% solution in water (5% in water for phenol). These are typical examples of organic materials likely to be distributed between phases.

Biological Applications. Many compounds of biological interest occur in water solution. This new technique now offers a possible method for determining and/or identifying some of these materials directly on a rapid and routine basis. The determination of amino acid is an example. Figure 6 shows the spectrum of D-valine obtained from a 2% water solution. The stronger spectrum was obtained by employing a pen-motion enlargement feature of the Dow double-beam spectrometer, because the spec-

trum of a 2% solution (near the solubility limit for amino acids) gives only small absorption bands (weaker spectrum).

This pen-motion enlargement is merely an expansion of the pen motion by a factor of three, obtained by shifting gears in the servo system of the pen. This device, of course, does not increase signal-to-noise ratio. It gives false optical densities, for the "true chart zero" is now located well below the chart paper, but it is a convenient equivalent of looking at a weak spectrum with a one-dimensional magnifying glass.

Another possible use for these techniques is direct examination of biological fluids. Figure 7 shows a spectrum of freshly centrifuged blood plasma. The absorbances are weak, and no attempt has been made at characterization. However, the relative clearness of the spectrum may be useful in that abnormalities in blood plasma might thus be detected.

At present, this technique of water solution has not been applied to any biological problems by this laboratory; hence, without more experience, it cannot be stated at this time whether or not this technique will prove useful in biological applications. But the possibility does exist, and more work along these lines should be attempted.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of L. W. Herscher of this laboratory, who designed and built the double-beam infrared spectrometer used in this study.

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RECEIVED for review December 2, 1955. Accepted February 25, 1956. Presented before Ohio State Symposium on Molecular Structure and Spectra, Columbus, Ohio, June 1955.

Rapid Determination of Metals in Organic Products with Alumina as Spectrographic Aid

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Barium, calcium, iron, lead, nickel, and zinc can be determined at concentrations ranging from a few tenths of 1 p.p.m. to a few per cent using alumina as spectrographic aid. The determination can be carried out in less than 5 hours with an accuracy within a few per cent of the amount present. The addition of concentrated nitric acid, which accelerates the ashing process, does not affect the matrix of the alumina. Alumina appears to be an excellent spectrographic aid, acting simultaneously as an ashing catalyst, an ash collector, and a spectrographic carrier. Because of the powerful retentive properties of active alumina, the ashing process can be accelerated without danger of losing metal by mechanical entrainment. The method can be applied to the determination of many metals in organic products of unknown composition.

THE coking of a sample remains a necessary step for the accurate determination of metal in organic products of unknown composition. Matrix effects related to the nature of the metallic and the organic compounds limit the analysis of this type of sample by direct analytical methods, such as the flame photometric or the porous cup and rotating electrode spectrographic methods (*4*, *6*).

McEvoy, Milliken, and Juliard have recently shown (*7*) that micro amounts of nickel and vanadium in petroleum products can be determined rapidly and accurately by ashing the sample with a large portion of silica-alumina catalyst, and by analyzing the ignited product spectrographically. Using alumina as an ashing and a spectrographic aid, the method has now been extended to other elements in organic products, at the macro as well as the micro level. Good results are obtained for nickel in crude oils, and for calcium, barium, zinc, iron, and lead in fresh and used lubricating oils and additive concentrates at concentration levels ranging from 0.001 to 7%.

The procedure is similar in some ways to the ash-aid method of Murray and Plagge (*9*), or to the magnesia buffer method of Gamble and Jones (*5*) for the determination of trace amounts of metal in oils. It differs from these two methods by the much larger proportion (from 7 to 50%) of the refractory substance added to the organic material, and also from the method of Gamble and Jones by using concentrated nitric acid instead of sulfuric acid as ashing accelerator. In the proposed method alumina acts simultaneously as an ashing catalyst, an ash collector, and a spectrographic carrier.

EXPERIMENTAL

Equipment. With the exception of the calculator, the equipment was made by Applied Research Laboratories, Glendale, Calif.

Spectrograph, 1.5-meter grating type.
Excitation source, ARL Multisource, Model 22.
Projection microphotometer.
Thermostated developing machine and film dryer.
Calculator, Dunn-Lowry calculation board.

Reagents. Reagents are ACS analytical grade.

Distilled water, demineralized to a residual salt content of less than 0.2 p.p.m., is used.

Alumina is prepared by grinding a pure activated alumina, with an internal surface of approximately 100 square meters per gram, in an agate mortar to a fineness comparable to talc. The sample contains as impurities trace amounts of calcium, silicon, nickel, and iron.

Silica-alumina is prepared in the same manner by grinding a synthetic product containing 87% silica and 13% alumina with an internal surface of approximately 200 square meters per gram.

Cobalt oxide and chromium oxide are prepared by the thermal decomposition of the nitrates, which are first dehydrated in a glass beaker, then ignited in a porcelain dish at 850° C.

The graphite powder used is spectrographically pure, suitable for briquetting.

Standard stock solutions of barium, iron, and lead are prepared by dissolving the nitrates in demineralized water containing 10 ml. of concentrated nitric acid per liter. Calcium and zinc solutions are prepared by carefully dissolving calcium carbonate and metallic zinc, respectively, in a slight excess of dilute nitric acid. The concentration of all the stock solutions is checked by conventional analytical procedures.

Standard working solutions are prepared by diluting the stock

solutions with water. The calcium, barium, and zinc working solutions contain approximately 1 mg. per milliliter, and the lead and iron solutions approximately 10 γ of metal per milliliter.

Spectrographic standards are prepared by making a slurry with 10 grams of alumina or silica-alumina and the standard working solution and evaporating the paste to dryness. The process is repeated until the required amount of metal has been added to the base material. The material adhering to the wall is then washed down with a small amount of water, the slurry is stirred, and the paste dried again. This procedure is repeated three times to ensure even distribution of the elements throughout the bulk of the standard. The standard is transfered to a mortar and thoroughly mixed with a pestle. It is then dried for 2 hours at 105° C. before using.

Spectrographic Conditions. ELECTRODES. The electrodes are 0.242-inch rods of pure graphite. The upper electrode is cut to a 15° cone, and the lower has a crater $^3/_{16} \times {}^3/_{16}$ inch.

EXCITATION CONDITIONS.

Resistance	18 ohms
Capacitance	60 μf.
Inductance	480 μh.
Initiator	Low power
Discharge point control	90°
Analytical gap	4 mm.
Current	11.5 amperes, direct current
Prearcing	0 second
Exposure	30 seconds
Slit	30 microns
Filtration	5% transmission filter in conjunction with a split field filter of 50% over 100% transmission

PHOTOGRAPHY. The emulsion is Eastman spectrum analysis No. 1, 35-mm. film, used with conventional techniques. The calibration of the film is done from a pure iron spectrum, photographed through the 50–100% split field filter, using the two-line method of Churchill (*3*).

INTERNAL STANDARD. Cobalt or chromium is used as an added internal standard. The internal standard mixtures are prepared by blending 5 grams of oxide with 1000 grams of spectrographic graphite. The prepared sample is blended with graphite–cobalt oxide mixture in the weight ratio of 1 to 1 for the determination of iron, lead, copper, calcium, and zinc; or with graphite–chromium oxide in the ratio of 1 to 2 for the determination of nickel. For the determination of barium the blend is made with the cobalt-graphite mixture, but aluminum is used as the internal standard.

Line pairs and the range of concentration of the corresponding analytical curves are given in Table I.

The analytical curves are established by plotting the intensity ratios of the analytical line pair against the concentration of the metal in the refractory carrier. Each point of the curve is the average value of 10 arcings.

Each spectrographic determination is computed from the average value of the intensity ratios corresponding to the arcing of six pellets of the same blend. The standard deviation of this ratio reaches approximately 4% of the measured value for intensity ratios close to 1.

Table I. Analytical and Reference Lines

Element	Wave Length, A.	Concentration Range, γ per Gram of Catalyst	Internal Standard for:
	Analytical Lines		
Ba	2335.3[a]	800–8000	
Ca	3179.3	400–2000	
Zn	3345.0	200–2000	
Fe	2947.9	50–1000	
Pb	2833.1[a]	25–100	
Ni	3414.8[a]	5–50	
	3446.3[a]	25–150	
Cu	3274.0	2–10	
	Reference Lines		
Cr	3445.6[a]		Ni
Co	2989.6		Fe, Pb, Cu,
	3283.5		Ca, Zn
Al	2321.6[a]		Ba

[a] Using unfiltered portion of split field.

Procedure. SPECTROGRAPHIC DETERMINATION. Two grams of alumina are weighed into a porcelain dish to the nearest 5 mg. A quantity of organic material, containing an amount of metal such that its concentration in the ignited product will fall approximately midway between the limits of the analytical curves, and weighed to the nearest 5 mg., is blended with the alumina. The weight of the material added should not be less than 2 grams to get a homogeneous impregnation of the metal on the carrier. If the sample is too concentrated, or if it is in the solid state, it must be dissolved or diluted in a metal-free organic solvent before blending. Two millimeter of concentrated nitric acid are added to speed the ashing process.

The mixture is then heated on an air bath with intermittent stirring, carefully at first until frothing ceases, and then more rapidly until fuming ceases. Finally the coke residue is air oxidized by heating the dish in an electric oven at 600° C. for 2 hours. While still warm, the metal-impregnated carrier is ground in an agate mortar to talc consistency, and then cooled in a desiccator.

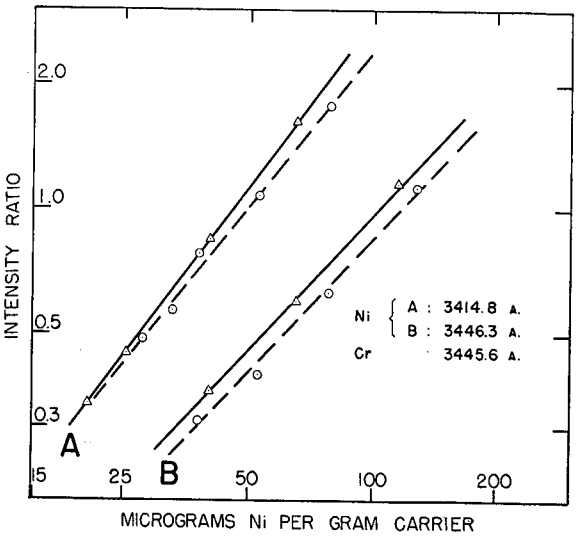

Figure 1. Matrix effect of alumina and silica-alumina on nickel spectroemissivity

– – – – – Alumina
——— Silica-alumina

The sample is weighed in the proper proportions with the chosen internal standard mixture in 1-ounce bottles. Eight stainless steel ball bearings are added to the mix, and the bottle is shaken for 1 minute to blend the sample and internal standard. The blend is pelleted at a pressure of 3000 pounds per square inch and the pellets are arced under the given conditions.

The concentration of the element sought in the sample is given by the following formula:

$$\text{Weight \% element} = a \times b/10{,}000\, w$$

where a = weight of element as obtained from the analytical curve, expressed in micrograms per gram of spectrographic aid
b = grams of spectrographic aid
w = grams of sample

CHEMICAL DETERMINATION. Lead, present as tetraethyllead in 2-methyl- or *n*-heptane, is extracted by an aqueous hydrochloric acid solution, and the extract is wet oxidized with concentrated nitric acid (*1*). The other organic compounds are coked by sulfuric acid in a platinum dish. The coke is ignited in an electric oven at 600° C. for 2 hours, the ash fused with sodium carbonate, and the melt dissolved in dilute hydrochloric acid solution.

Barium and zinc are determined gravimetrically as sulfate; calcium, volumetrically as oxalate; and iron and lead, colori-

Table II. Influence of Nature of Ashing Aid on Nickel Determination

(Determined in Mid-Continental flashed tower bottom; 2 grams of spectrographic ashing aid added in all cases)

Spectrographic Aid	Sample, Grams	Concd. HNO_3 Added, Ml.	Intensity Ratio, Ni/Cr[a]	Nickel in Oil, P.P.M.
Synthetic silica-alumina	2.287	0	0.75 ± 0.02	18.7
	11.01	0	1.15 ± 0.04	18.3
	11.46	10	1.28 ± 0.08	19.3
Activated alumina	2.053	0	1.04 ± 0.02	21.8
	11.60	0	1.29 ± 0.10	19.5
	11.20	10	1.18 ± 0.09	18.6

[a] Range is average deviation obtained by arcing six pellets of the same blend.

Table III. Effect of Acid on Alumina Matrix

(Spectrographic determination of 2.00 mg. of zinc impregnated into 2 grams of alumina, using cobalt as internal standard)

Concd. Acid	Ml. per Gram of Al_2O_3	Zn, Mg. per Gram of Al_2O_3
None		0.99 ± 0.05
Sulfuric	0.1	1.05 ± 0.02
Sulfuric	0.1	1.04 ± 0.03
Hydrochloric	0.1	1.03 ± 0.12
Nitric	1.0	1.05 ± 0.05

metrically as sulfocyanate and dithizonate (*2*, *8*), respectively according to conventional procedures.

RESULTS AND DISCUSSION

Because activated alumina has catalytic cracking and metal-retaining properties close to that of catalytic active silica-alumina, one can expect that alumina can be substituted for silica-alumina as a spectrographic acid. Activated alumina has the advantage of being more easily ground to a fine powder than silica-alumina. In addition, it is known that alumina has a favorable matrix effect on the intensity of some spectrographic lines (*10*). It has the disadvantage over silica-alumina of being more easily soluble in acids, but this fact does not seem to affect the matrix properties when it is used as a spectrographic carrier.

Analytical curves corresponding to two analytical line pairs of nickel and chromium, obtained using silica-alumina and alumina, respectively, as carriers, are given in Figure 1. The abscissa represents the amount of nickel added by impregnation plus the trace amount of nickel already present in each of the base materials. The figure shows that alumina and silica-alumina manifest approximately the same matrix effect on nickel and chromium lines. The nearness of the two pairs of lines shown in this figure indicates that the same analytical and reference lines can be used for the determination of nickel at the same concentration level in organic products, when alumina is used instead of silica-alumina as an ashing aid.

Table II gives the nickel concentration of a petroleum product, determined spectrographically using either silica-alumina or alumina as ashing and spectrographic aid. The weight of oil and the volume of concentrated nitric acid added to the blend are also shown.

The figures of Table II show that use of either activated alumina or silica-alumina as ashing and spectrographic aid gives accurate results for micro amounts of nickel. Because it has already been shown that accurate results are obtained with silica-alumina (*7*), this should enable alumina to be substituted for silica-alumina for these purposes.

The effect of the addition of different acids in the determination of zinc when alumina is used as an ashing and spectrographic aid is shown in Table III. The acid was added to the zinc-impregnated alumina in a platinum dish, and the mixture was then heated at 600° C. for 1 hour.

The data in Tables II and III show that the addition of concentrated acids for ashing accelerators does not affect the spectrographic properties of activated alumina in the determination of nickel, chromium, zinc, or cobalt. The fluctuation between the determinations made with and without the addition of 10 ml. of concentrated nitric acid to 10 grams of oil and 2 grams of alumina (Table II) is well within the limit of reproducibility of the measurements, and is in the same range as that observed with silica-alumina. With zinc, which is a more volatile metal, the results are approximately 5% higher when acid is added to the alumina prior to ignition of this material (Table III). It seems rather surprising that concentrated hot acids do not alter the spectrographic matrix affect of alumina. This unexpected fact allows the time of the ashing process to be shortened.

Table IV gives results for trace amounts of lead and iron in different organic liquids, and Table V gives the same information for barium and calcium at higher concentrations in lubricating oils. All the results are compared to the values obtained by conventional chemical methods. The results show that alumina acts as an excellent spectrographic aid in the determination of iron, lead, and zinc at the micro level, and for barium and calcium at the macro level. The difference between the spectrographic and chemical determination of the same sample is within the limit of reproducibility of the measurements, with the exception of two determinations out of 20.

CONCLUSIONS

Activated alumina acts as an excellent ashing aid and spectrographic carrier for the determination of micro or macro amounts of metal in organic products, when added in a large amount to the organic material. Accurate and rapid results can be ob-

Table IV. Iron and Lead Determinations in Organic Products

Sample	Metal Content: Photometric	Metal Content: Spectrographic
	Fe, %	
Used lubricating oil		
A	0.0080	0.008
B	0.0080	0.009
C	0.0080	0.011
D	0.0080	0.007
E	0.0150	0.011
F	0.0241	0.025
G	0.14	0.18
	Pb, P.P.M.	
Tetraethyllead		
In *n*-heptane	0.132	0.14
In 2-methylheptane	0.152	0.16
	1.14	1.18

Table V. Barium, Calcium, and Zinc Determinations in Petroleum Products

Sample	Metal, %: Chemical	Metal, %: Spectrographic
	Barium	
Lubricating oil		
A	0.34	0.35
B	0.18	0.19
C	0.74	0.73
D	0.26	0.26
Concentrated additive E	7.90	7.22
	Calcium	
Lubricating oil		
F	0.15	0.14
G	0.88	0.88
Calcium hexoate in *n*-hexane	6.35	6.02
	Zinc	
Lubricating oil		
H	0.053	0.055
F	0.097	0.104

tained for nickel, barium, calcium, zinc, lead, and iron using chromium or cobalt as internal standard, and the method can be extended to cover other metallic elements present in organic products.

The determination of one element in an organic product can be performed by the spectrographic procedure described in approximately 4 hours, an additional 15 minutes being required for the determination of each subsequent element.

ACKNOWLEDGMENT

The authors wish to express their thanks to Charles R. MacIntyre and A. Earl Creamer, Jr., for assisting in the spectrographic analysis; to Walter J. Savournin, Jr., and James A. Crielly for the chemical analyses; and to Jack Grider for coordinating the analytical work. They also thank Houdry Process Corp. for permission to publish this paper.

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RECEIVED for review October 4, 1955. Accepted March 3, 1956. Presented in part before Division of Petroleum Chemistry, 127th Meeting, ACS, Cincinnati, Ohio, March 1955.

Spectrophotometric Determination of Cerium in Carbonate Solution

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The spectrophotometric determination of cerium in potassium carbonate solution should avoid the difficulties due to flocculated colloids when determination is made in potassium carbonate solution containing hydrogen peroxide. The feasibility of the method has been shown by this investigation and conditions requisite for the determination are described. Cerium is determined in the quadrivalent state by measuring the absorbancy in 3.0*M* potassium carbonate solution having a pH in the range 11 to 12. The optimum range of cerium concentration is from 2 to 25 p.p.m. The expected standard deviation of absorbancy measurements is 0.003 and the accuracy is within ±0.2 p.p.m. over the range 6 to 18 p.p.m. The solutions are stable. The effect of diverse ions is shown.

SEVERAL colorimetric or spectrophotometric methods for the determination of cerium have been described (*2–4, 6, 8, 9, 11, 12*). In most cases where measurements are taken in the visible region of the spectrum, the methods lack desired sensitivity. Freedman and Hume (*2*) and Medalia and Byrne (*4*) have verified Sandell's (*8*) suggestion that increased sensitivity may be obtained by working in the ultraviolet region of the spectrum, where ceric ion shows an absorbancy maximum.

The term "absorbancy" is used in this paper to indicate the nature of the experimentally measured quantities—the relative intensities of the light transmitted by a potassium carbonate solution and a potassium carbonate solution containing cerium. The practical measurement is that of the ratio of transmitted light intensity of the sample to the transmitted light intensity of the reference.

Telep and Boltz (*11*) have proposed a method which utilizes the ultraviolet absorption of the ceric–hydrogen peroxide complex in concentrated potassium carbonate solution. The nature of this complex has been examined by Merritt (*5*), who found that solutions of quadrivalent cerium in concentrated potassium carbonate contain dispersed colloids in the presence of excess hydrogen peroxide. As the solutions employed for the determination of cerium must invariably contain hydrogen peroxide in excess, and the sols thus produced are easily flocculated, the conditions under which spectrophotometric measurements are made are highly critical. Furthermore, the absorption spectrum of a solution of quadrivalent cerium in concentrated potassium carbonate is nearly identical to that of a solution which also contains hydrogen peroxide (Figure 1). Although the absorbancy index of the ceric-peroxide-carbonate complex is somewhat greater than that of the ceric-carbonate complex at wave lengths longer than 380 mμ, no advantage is offered by the peroxide complex in the region of the absorbancy maximum. This study was therefore undertaken to show the feasibility of determining cerium by measurement of the absorbancy of quadrivalent cerium in potassium carbonate solutions alone.

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PREPARATION OF STANDARDS AND REAGENTS

The cerium solutions used were of three types.

Reagent grade ceric perchlorate (ca. 0.5*M* in 6*M* perchloric acid as supplied by the G. F. Smith Chemical Co.) was diluted to a concentration of approximately 0.01*M* in cerium ion and 0.6*M* in perchloric acid.

Reagent grade ceric ammonium sulfate was used to prepare stock solutions at approximately 0.1*M* cerium in 0.5*M* sulfuric acid. These solutions were subsequently diluted a thousandfold with 0.5*M* sulfuric acid and employed as the working solutions.

Stock solutions of ceric sulfate were prepared from chemically pure ceric sulfate at concentrations of approximately 0.1*M* and 0.2*M* with respect to cerium ion in ca. 0.5*M* sulfuric acid and subsequently diluted a thousandfold in ca. 0.5*M* sulfuric acid.

The cerium stock solutions were standardized against either reagent grade arsenic trioxide or reagent grade ferrous ammonium sulfate.

The carbonate solutions were prepared from reagent grade potassium carbonate and distilled water, and in all cases were filtered immediately after preparation.

Solutions of interfering ions were prepared from reagent grade chemicals when available; otherwise C.P. grade chemicals were used. If solubility permitted, concentrations of 10,000 p.p.m. were prepared with distilled water, or, wherever necessary, dilute sodium hydroxide or sulfuric acid was used as the solvent medium.

All the solutions taken for absorbancy measurement were prepared by diluting appropriate aliquots of the stock solutions to give the desired final concentration of the constituents. The absorbancy measurements were all taken with respect to reference solutions of equivalent concentrations of potassium carbonate.

APPARATUS

Continuous spectra were measured on a Cary recording spectrophotometer, Model 11, using 2.0-cm. cells. All other absorbancy measurements were made in 1.0-cm. cells on a

Beckman Model DU quartz spectrophotometer. pH measurements were made with a Beckman pH meter, Model H-2, using a calomel–glass electrode system.

RESULTS AND DISCUSSION

Each of the variables that may affect the accuracy or precision of the method was studied systematically.

In order to determine whether perchlorate, sulfate, or ammonium ions present in the various cerium stock solutions had an effect, parallel studies of the choice of solvent concentration and the effect of pH, described below, were carried out using all three sources of cerium. When it was subsequently found that identical results were obtained, only one cerium stock solution was used for the later experiments. The choice was made solely on the basis of convenience and is identified in each case in the following descriptions. Sulfate and ammonium ions do not interfere, even when present in large excess.

Choice of Solvent Concentration. A series of spectra of quadrivalent cerium in solutions of varying concentration of potassium carbonate was obtained in order to evaluate the effect of carbonate concentration upon the absorbancy index. The carbonate concentration was varied from 0.5*M* to 6*M*. All the spectra were of identical shape, with a broad maximum in the region 300 to 310 mμ. The magnitude of the absorbancy index at the maximum was found to vary only slightly with the changing carbonate concentration. The extent of this change is seen in Figure 2. Because the change in absorbancy index is negligible in the region 2.0*M* to 5.0*M*, a concentration of 3.0*M* potassium carbonate was chosen as optimum for further study. A 3.0*M* solution of potassium carbonate is somewhat less viscous than a 4.0*M* solution and is more convenient to manipulate.

Effect of pH. Because the determination of cerium would probably involve the dilution of an acidic aliquot of cerium with 3.0*M* potassium carbonate solution, the pH of the solution taken for absorbancy measurement would be somewhat less than that of a 3.0*M* carbonate solution and would vary with the size of the aliquot. It also seemed that solutions of 3.0*M* potassium carbonate previously made more alkaline with potassium hydroxide might offer some advantage, in view of the expected pH decrease upon mixture with the cerium aliquot. Accordingly, the changes in absorption caused by changes in pH were investigated by examining the spectrum of quadrivalent cerium in 3.0*M* potassium carbonate solutions, whose pH had been adjusted by the addition of various amounts of potassium hydroxide or sulfuric acid.

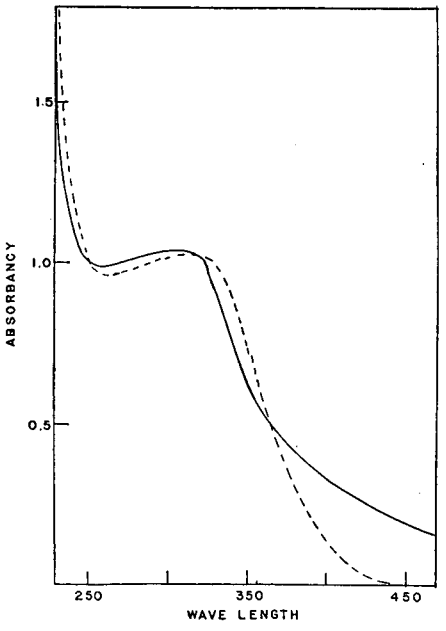

Figure 1. Absorption spectra of cerium (IV) in potassium carbonate solution

- - - - 2×10^{-4} *M* cerium(IV)
——— 2×10^{-4} *M* cerium(IV) and 4×10^{-4} *M* hydrogen peroxide

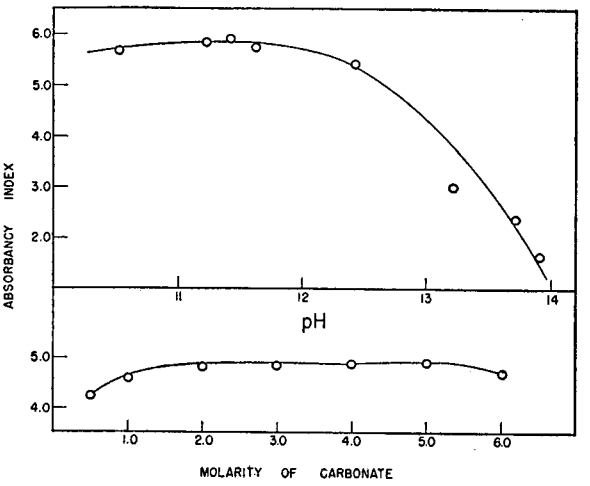

Figure 2. Effect of carbonate concentration and pH on absorbancy index of cerium(IV)

The results are shown in Figure 2, where the absorbancy index at the maximum is plotted as a function of pH. The optimum range of pH is found to be between 11 and 12. The pH of a solution of 3.0*M* potassium carbonate is about 12.4, but the addition of appropriate aliquots of an acidified cerium solution (ca. 0.5 to 0.6*M* in perchloric or sulfuric acids) usually lowers the pH to a value within the optimum range. Hence, in the usual procedure for the preparation of solutions for absorbancy measurement no adjustment of pH is necessary.

Calibration Curve. Aliquots from a solution of ceric perchlorate (0.001*M* in 0.5*M* perchloric acid) were diluted with 3.0*M* potassium carbonate to give final concentrations ranging from 0.2×10^{-4} to 2.0×10^{-4}*M* in cerium. The solutions were filtered to remove any potassium perchlorate which formed and the absorbancy was measured in 1.0-cm. silica cells at 305 mμ with a Beckman Model DU spectrophotometer at a constant slit width of 2.4 mm. A plot of absorbancy against corresponding values of concentration of cerium gave a straight line, indicating conformity to Beer's law.

In order to determine the optimum range of cerium concentration for a minimum spectrophotometric error, a Ringbom (7) plot was made from the data obtained. The optimum range found is from 2.0 to 25.0 p.p.m.

Conformity to Beer's law and the same optimum concentration range were also found when the sulfate or the ammonium sulfate double salt, each in sulfuric acid, was taken as the source of cerium.

Reproducibility. From a stock solution of ceric perchlorate (0.001*M* in ca. 0.5*M* perchloric acid) a 3-ml. aliquot was diluted to volume in a 50-ml. volumetric flask with 3.0*M* potassium carbonate. The final cerium concentration was 9.6 p.p.m. In this manner a sample was prepared each day on successive days for a total of 15 days. The absorbancy of all the solutions was measured each day at 305 mμ against a 3.0*M* potassium carbonate reference solution, giving a total of 120 absorbancy measurements (Table I).

The experimental error involved may be considered to include the normal errors due to the use of different pipets and flasks. The study was carried out at room temperature and no corrections were made for errors due to changes in temperature, since the stock solutions of cerium and carbonate and the samples were stored under the same conditions.

Table I. Absorbancy Measurements for Solutions on Consecutive Days

	Solutions														
Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.459														
2	0.463	0.459													
3	0.465	0.461	0.461												
4	0.466	0.455	0.460	0.462											
5	0.467	0.460	0.458	0.461	0.460										
6	0.468	0.465	0.460	0.465	0.463	0.462									
7	0.470	0.468	0.462	0.464	0.463	0.461	0.466								
8	0.467	0.461	0.458	0.460	0.458	0.457	0.458	0.461							
9	0.471	0.471	0.467	0.468	0.468	0.465	0.467	0.468	0.462						
10	0.470	0.474	0.465	0.467	0.467	0.463	0.467	0.469	0.460	0.463					
11	0.472	0.468	0.466	0.468	0.470	0.465	0.468	0.469	0.452	0.466	0.464				
12	0.475	0.472	0.470	0.471	0.472	0.470	0.472	0.472	0.465	0.470	0.468	0.465			
13	0.471	0.471	0.467	0.468	0.467	0.468	0.471	0.473	0.465	0.470	0.462	0.467	0.465		
14	0.468	0.467	0.463	0.467	0.467	0.462	0.468	0.472	0.462	0.468	0.464	0.462	0.465	0.453	
15	0.472	0.477	0.468	0.471	0.472	0.468	0.472	0.477	0.463	0.474	0.470	0.470	0.471	0.460	0.463

A statistical evaluation (*10*) of the data was made. The grand average, X, of all the absorbancy measurements was 0.466, having a range, R, of 0.025, a standard deviation, σ, of 0.005, a coefficient of variation of 1.07, and a per cent deviation of the mean of 0.1%.

The data were interpreted by means of control charts. The averages of measurements for each solution, when plotted around the grand average as a center point, showed fairly narrow distribution above and below the average and were found to lie within values of $\pm 2\sigma$ as limits of variation (see Figure 3). When, however, a second control chart was made, and the average of first measurements of all the solutions, the second measurements, the third, etc., was plotted around the grand average, a gradual increase was found, indicating a slight increase in absorbancy with time (Figure 4). The last conclusion was verified by analyzing the data according to the method of mean square successive differences as described by Bennett (*1*).

It was thus apparent that the precision of reading the absorbancy of different solutions would be greater if the readings were taken at a consistent time. The precision of reading the first day, second day, third day, etc., was evaluated and the standard deviation found was 0.003 absorbancy unit in each case.

Thus, if the solutions are read the same day on which they are prepared or at any fixed time interval thereafter, the expected deviation will be 0.003. In the case of random reading of the absorbancy, the deviation will not be greater than 0.005. The subsequent study of the accuracy of the method shows that reading on the day of sample preparation leads to the most accurate result.

Stability of Solution. A 3-ml. aliquot taken from a stock solution of ceric sulfate (0.001M in ca 0.5M sulfuric acid) was diluted to 50 ml. with 3M potassium carbonate, giving a final cerium concentration of 9.6 p.p.m. A series of absorbancy measurements was taken on this solution at half-hour intervals over a period of 4 hours, beyond which the data accumulated in the reproducibility study became applicable. The changes in absorbancy found are not greater than those expected from consideration of the reproducibility of setting the absorbancy dial of the spectrophotometer and hence represent a negligible change.

Effect of Interfering Ions. A number of ions whose nature would indicate either occurrence or interference with cerium were studied. Known amounts of the ion to be studied were added to aliquots of cerium(IV) in a 50-ml. volumetric flask. The contents were diluted to volume with 3M potassium carbonate and the absorbancy of the solution was determined against a 3M carbonate reference solution. Concentrations of the solutions of interfering ions were adjusted so that aliquots not greater than 5 ml. were added, in order to minimize dilution effects on the carbonate and to minimize significant change in pH. The results, expressed as the ratio of ion to cerium concentration required to produce a 3% error in absorbancy, are shown in Table II.

In addition to the expected effects of either increasing or decreasing the absorbancy measurement, in some cases the maximum or equilibrium absorbancy was found only after a lapse of time. This effect was noted with sulfate, iodide, chloride, thorium, neodymium, and vanadyl ions. The absorbancy readings were constant, however, after 0.5 hour. In each case during this study, the absorbancy was not recorded until it showed no further change with time.

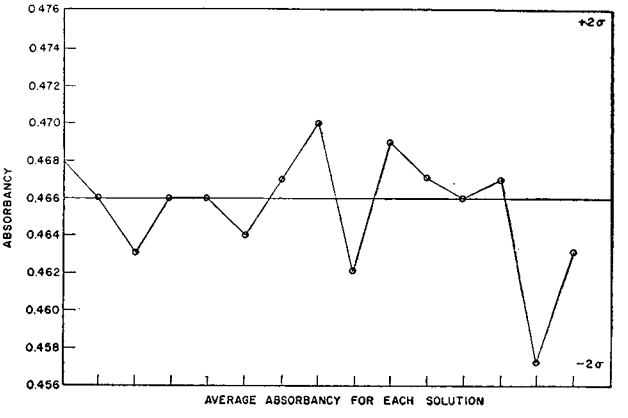

Figure 3. Control chart for absorbancy averages

Average absorbancy for each solution using $\pm 2\sigma$ as upper and lower limits

Table II shows that a number of ions interfere seriously: bromide, tartrate, nitrate, dichromate, permanganate, ferric, uranyl, cupric, yttrium, and vanadyl. In most cases, however, such interference may be conveniently eliminated prior to the determination of cerium.

Recommended Procedure. The cerium(IV) to be determined is dissolved in approximately 0.5M sulfuric acid, giving a final cerium concentration of approximately 0.001M. Aliquots of from 3 to 5 ml., containing between 0.3 and 0.9 mg. of cerium, are placed in a 50-ml. volumetric flask and diluted to volume with 3M potassium carbonate, giving a final cerium concentration between 6 and 20 p.p.m. The absorbancy of the solution is measured against a 3M potassium carbonate reference solution at 305 mμ.

Whenever necessary, cerium(III) is oxidized to cerium(IV) with potassium persulfate according to the procedure suggested by Medalia and Byrne (*4*). However, silver sulfate may be used in place of silver nitrate to catalyze the oxidation, in order to minimize the interference caused by the nitrate ion.

Evaluation of Accuracy. The accuracy of the method was evaluated as follows: A stock solution of ceric sulfate was carefully standardized by titration against primary standard grade arsenious oxide. From this solution five solutions of different cerium concentration were prepared by aliquot dilution. A 3-ml. aliquot of each of these solutions was then taken for spectrophotometric measurement in accordance with the recommended procedure. Four separate spectrophotometric determinations were made of the cerium content of each of the five solutions.

Taking one of the solutions arbitrarily as a standard for calibration, the results of the spectrophotometric determination of the remaining four were compared with the values calculated from volumetric dilution. The data are given in Table III.

The distribution of differences between volumetric and spectrophotometric results was statistically analyzed by calculating the average difference and standard deviation. For purposes of this analysis, only the absolute magnitudes of the differences were considered. The average difference, $\bar{d}$, calculated from the formula

$$\bar{d} = \frac{\Sigma|d|}{N}$$

in which d represents the difference between a volumetric and spectrophotometric result and N represents the total number of differences, was 0.088 p.p.m. The standard deviation of the differences, σ_d, calculated from the formula

$$\sigma_d = \sqrt{\frac{\Sigma(d - \bar{d})^2}{N}}$$

was 0.066 p.p.m. Assuming that the differences were random and normally distributed, the normal probability curve is applicable. From this it is calculated that the error will be not be greater than the average difference plus a given sigma value for a given probability. The limits of variation for given probabilities are

0.68	$\lvert\bar{d}\rvert + \sigma$
0.95	$\lvert\bar{d}\rvert + 2\lvert\sigma\rvert$
0.997	$\lvert\bar{d}\rvert + 3\lvert\sigma\rvert$

Hence, the probability is 0.997 that the spectrophotometric result will not be in error by more than 0.29 p.p.m., or the probability is 0.68 that the result will be within 0.15 p.p.m.

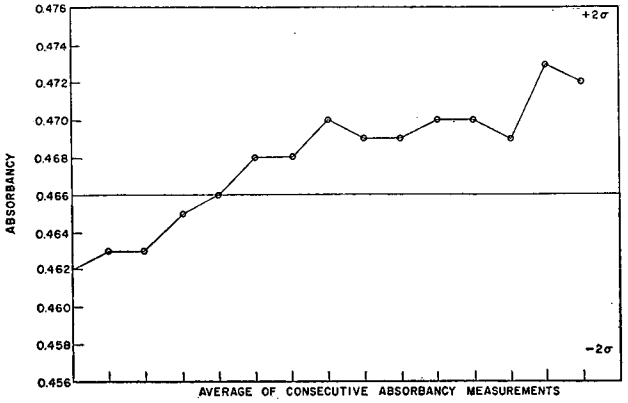

Figure 4. Control chart for absorbancy averages

Average absorbancy for 1st measurements, 2nd measurements, etc., using ±2σ as upper and lower limits

SUMMARY AND CONCLUSIONS

The spectra of cerium(IV) in carbonate and in carbonate with hydrogen peroxide are essentially the same in the ultraviolet region. Conditions requisite for the determination of cerium in carbonate were investigated.

The absorbancy index at the absorbancy maximum is essentially constant over a range of carbonate concentration from 2.0 to 5.0*M*. The optimum concentration is 3.0*M*.

The optimum range of pH of the final cerium carbonate solution taken for absorbancy measurement is 11 to 12. No adjustment is necessary when the recommended procedure is followed.

Conformity to Beer's law is shown for cerium concentrations up to 40 p.p.m. The optimum concentration range is from 2 to 25 p.p.m.

Solutions of cerium(IV) in potassium carbonate solution are stable over a 24-hour period.

The method is highly precise. A standard deviation of 0.003 absorbancy unit can be expected.

A number of ions interfere, but in most cases interference is eliminated prior to determination of cerium.

The method is accurate to approximately 0.2 p.p.m. over the range 6 to 20 p.p.m.

The study shows that the proposed method for determination of cerium in potassium carbonate solution offers several advantages over the method employing hydrogen peroxide in potassium carbonate. These include a broader range of pH and freedom from difficulty due to formation of sols or decomposition of peroxide. The precision of the method is comparable to other methods and the accuracy is established.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of Chas. Pfizer & Co., Inc., Brooklyn, N. Y., in whose laboratories a large part of this work was done. The authors also thank Henry Fairchild, supervisor of statistical quality control, Chas. Pfizer & Co., for his advice concerning the statistical treatment of the data.

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Table II. Effect of Diverse Ions

Ion	Ion-Cerium Concn. Ratio to Produce 3% Error in Absorbancy	Change in Absorbancy at 305 Mμ
SO_4^{--}	>125	None[a]
Br^-	2.85	Decrease
Cl^-	200	Decrease[a]
I^-	25	Decrease[a]
$C_4H_4O_6^{--}$	2.1	Decrease
NO_3^-	3	Decrease
PO_4^{---}	25	Decrease
$C_2H_3O_2^-$	288	Decrease
$S_2O_8^{--}$	125	Increase
$C_6H_5O_7^{---}$	59.5	Decrease
MoO_4	>100	None
$Cr_2O_7^{--}$	0.4	Increase
MnO_4^-	0.02	Increase
NH_4^+	>100	None
Fe^{+++}	0.18	Increase
UO_2^{++}	0.82	Increase
Cu^{++}	0.24	Increase
Zn^{++}	100	Decrease
VO^{++}	>0.5	Increase[a]
Th^{++++}	50	Increase[a]
Ge^{++++}	27.1	Decrease
Y^{+++}	2.1	Increase
Nd^{+++}	28	Increase[a]
La^{+++}	>14	Decrease
$(Sm\text{-}Gd)^{+++}$	>100	None
Ag^+	>10[b]	None

[a] After no further change in absorbancy with time.
[b] Limit of solubility of Ag^+ in 3.0*M* K_2CO_3.

Table III. Accuracy of Determination of Cerium

Concn. of Cerium, P.P.M. Calcd.	Concentration of Cerium, P.P.M. Found							
	Detn. 1		Detn. 2		Detn. 3		Detn. 4	
	Concn.	Diff.	Concn.	Diff.	Concn.	Diff.	Concn.	Diff.
6.4	6.5	+0.1	6.4	0.0	6.4	0.0	6.4	0.0
11.1	11.2	+0.1	11.2	+0.1	11.2	+0.1	11.0	−0.1
12.6	Used as standard for calibration							
15.9	15.9	0.0	15.7	−0.2	15.8	−0.1	15.6	−0.3
17.5	17.5	0.0	17.4	−0.1	17.5	−0.0	17.3	−0.2

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RECEIVED for review December 8, 1955. Accepted April 27, 1956.

Spectrophotometric Determination of Phenylmercuric Acetate

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With the increased use of phenylmercuric acetate as a herbicide and fungicide, an accurate and rapid method for its determination is needed. The proposed spectrophotometric method depends on the ultraviolet absorption of an aqueous phenylmercuric acetate solution that contains a small amount of added perchloric acid. Absorption measurements may be made at 256 or 262 mμ. The method is very rapid and is accurate to within about 1%.

THE titrimetric method recommended by Gran (*3*) for the determination of phenylmercuric acetate yields reliable results but is time-consuming because it involves the precipitation of a solution of phenylmercuric acetate with a measured quantity

Table I. Study of Reproducibility

(0.0425 gram taken)

Found, Gram					
At 250 mμ	Deviation from mean	At 256 mμ	Deviation from mean	At 262 mμ	Deviation from mean
0.0424	0.0000	0.0421	−0.0002	0.0419	−0.0003
0.0427	0.0003	0.0425	0.0002	0.0425	0.0003
0.0426	0.0002	0.0425	0.0002	0.0424	0.0002
0.0426	0.0002	0.0423	0.0000	0.0422	0.0000
0.0425	0.0001	0.0422	−0.0001	0.0421	−0.0001
0.0422	−0.0002	0.0423	0.0000	0.0421	−0.0001
0.0422	−0.0002	0.0426	0.0003	0.0424	0.0002
0.0422	−0.0002	0.0425	0.0002	0.0427	0.0005
0.0424	0.0000	0.0423	0.0000	0.0422	0.0000
0.0426	0.0002	0.0421	−0.0002	0.0421	−0.0001
0.0424	0.0000	0.0422	−0.0001	0.0421	−0.0001
0.0420	−0.0004	0.0420	−0.0003	0.0421	−0.0001
0.0424		0.0423		0.0422	

Table II. Study of Interference

Impurity	Mole Added to 100 Ml. of Soln. Contg. 1.26×10^{-4} Mole C_6H_5HgOAc	Molar Ratio of Impurity to C_6H_5HgOAc Present	% Error without $HClO_4$ (Negative error: experimental less than known value)			% Error with $HClO_4$ (Negative error: experimental less than known value)		
			250 mμ	256 mμ	262 mμ	250 mμ	256 mμ	262 mμ
HOAc	2.62×10^{-4}	2:1	...	...	...	0.2	0.5	0.5
	8.76×10^{-4}	7:1	5.9	5.8	6.8	...	...	...
	1.31×10^{-3}	10:1	...	...	...	0.2	0.0	−0.2
	4.38×10^{-3}	35:1	5.0	4.1	4.8	...	...	...
	8.76×10^{-2}	696:1	...	...	...	8.2	−0.5	−0.7
	2.28×10^{-1}	1810:1	...	...	...	33.6	0.9	0.9
	4.35×10^{-1}	3450:1	...	...	...	75.6	5.2	−0.7
	6.10×10^{-1}	4850:1	...	...	...	110.6	10.0	2.4
NaOAc	1.00×10^{-4}	0.8:1	−1.9	−1.9	−1.6	...	...	...
	1.26×10^{-4}	1:1	...	...	...	−1.4	−1.2	−0.9
	5.00×10^{-4}	4:1	−5.4	−2.5	−2.0	0.2	0.2	1.2
	1.00×10^{-2}	79:1	...	...	...	−0.7	−1.2	−0.2
	5.00×10^{-2}	398:1	...	...	...	−9.2	−4.0	−2.1
$NaNO_3$	1.26×10^{-4}	1.0:1	4.2	0.3	0.0	3.3	0.5	0.5
	2.00×10^{-4}	1.6:1	...	...	...	4.5	0.0	0.0
	2.52×10^{-4}	2.0:1	8.9	1.6	2.0	7.1	1.9	1.2
	6.30×10^{-4}	5.0:1	...	...	...	20.5	4.5	3.1
Na_2SO_4	3.53×10^{-3}	28:1	2.7	1.0	0.8	−0.7	0.0	0.0
	7.06×10^{-3}	56:1	5.3	2.8	3.6	0.0	0.7	0.7
	1.41×10^{-2}	112:1	...	...	...	−0.2	0.7	1.2
	2.11×10^{-2}	167:1	...	...	...	−1.2	1.2	1.9
	3.53×10^{-2}	280:1	...	...	...	1.2	2.4	4.4
Na_3PO_4	5.68×10^{-4}	4.5:1	...	...	...	0.2	0.5	0.7
	1.14×10^{-3}	9.0:1	−8.7	−3.2	−2.4	0.0	0.2	0.7
	1.70×10^{-3}	13.5:1	...	...	...	−0.7	0.7	1.2
	2.83×10^{-3}	22.5:1	3.2	3.0	6.4	−4.0	0.0	3.1
	3.40×10^{-3}	27.0:1	...	...	...	4.3	9.3	14.2
NaCl	2.52×10^{-4}	0.02:1	−0.4	−0.3	−0.4	−1.2	−0.5	0.0
	8.82×10^{-6}	0.07:1	−0.8	−0.6	−0.4	−0.2	−0.5	0.0
	1.01×10^{-5}	0.08:1	Turbid	Turbid	Turbid	−0.5	0.5	1.6
	1.14×10^{-5}	0.09:1				Turbid	Turbid	Turbid
NH_4OH	1.11×10^{-4}	0.9:1	−8.5	−5.4	−4.0	−0.7	0.0	−0.2
	8.20×10^{-4}	6.5:1	−11.5	−6.7	−4.8	−1.2	−0.9	−0.2
	8.20×10^{-3}	65:1	...	...	...	−1.2	0.0	0.5
	1.17×10^{-2}	93:1	...	...	...	−1.4	−1.7	−0.7
	1.31×10^{-2}	104:1	...	...	...	−2.4	−1.7	−1.4
NaOH	2.0×10^{-3}	16:1	−23.0	−7.0	−3.1	0.7	0.0	0.9
	1.5×10^{-2}	119:1	...	...	...	0.7	0.5	1.2
	1.8×10^{-2}	143:1	...	...	...	−0.7	0.0	−0.2
	2.0×10^{-2}	159:1	...	...	...	−23.8	−14.6	−13.2
Ethanolamine	1.49×10^{-4}	1.2:1	−9.3	−3.0	−5.3	...	...	...
	2.28×10^{-4}	1.8:1	...	...	...	0.2	0.0	0.5
	4.39×10^{-4}	3.5:1	...	...	...	1.6	0.9	0.7
	7.37×10^{-4}	5.8:1	...	...	...	1.9	0.9	0.5
	9.97×10^{-4}	7.9:1	...	...	...	4.2	3.1	3.1

of standard potassium iodide, filtration of the precipitate, and determination of the excess potassium iodide in the filtrate.

Gran suggested that for small amounts of phenylmercuric acetate (about 2 to 15 mg. per liter), an aqueous solution of phenylmercuric acetate may be extracted with dithizone in chloroform and the absorbance measured at 497 mμ. Smaller quantities (0.001 to 0.150 mg.) have been determined by a dithizone procedure in which the green color of the unreacted reagent is measured (*5–7*).

The method proposed in the present paper is suitable for the analysis of samples containing 0.01 to 0.1 gram of phenylmercuric acetate and utilizes the fact that the ultraviolet absorption curve for an acidified aqueous solution of phenylmercuric acetate is a modified benzene curve with maxima at 250, 256, and 262 mμ (Figure 1). In a recent publication Gowenlock and Trotman (*2*) studied the ultraviolet absorption spectra of a number of mercury compounds.

EXPERIMENTAL

Reagents and Apparatus. The phenylmercuric acetate was obtained from Metalsalts Corp. and was 99.5% pure.

A standard phenylmercuric acetate solution was prepared by dissolving 0.850 gram in hot water, cooling to room temperature, and diluting to 1 liter.

The pipets used to take aliquots of the standard solution were treated with Beckman Desicote (*1*) and recalibrated.

Absorption measurements were made with a Beckman Model DU quartz spectrophotometer equipped with a hydrogen lamp and 1-cm. quartz cells.

Preparation of Standard Curve. Add 2 ml. of 60% perchloric acid to aliquots of the standard phenylmercuric acetate solution, dilute to 100 ml. with distilled water, and measure the absorbance at 256 or 262 mμ (Figure 2).

As the maximum absorbance is obtained at 256 mμ, in general this wave length should be used. However, Table II should be consulted in order to determine whether a wave length of 262 mμ would be more advantageous. The choice will depend on the impurities likely to be present in the sample to be analyzed.

Procedure. Dissolve a sample containing 0.01 to 0.10 gram of phenylmercuric acetate in about 50 ml. of hot water, cool to room temperature, and transfer to a 100-ml. volumetric flask. Add 2.0 ml. of 60% perchloric acid and dilute to 100 ml. with distilled water. Measure the absorbance at either 256 or 262 mμ against a reference solution containing 2.0 ml. of 60% perchloric acid diluted to 100 ml. Use a minimum slit width.

DISCUSSION

Absorption Curves. Figure 1 is a series of absorption curves for 1.26×10^{-4} mole of phenylmercuric acetate in 100 ml. of water containing 0, 1, 2, and 3 ml. of 60% perchloric acid. The ultraviolet absorption spectrum of phenylmercuric acetate shows strong absorption bands at 250, 256, and 262 mμ. The presence of the very slightly absorbing perchloric acid shifts the maxima to slightly lower wave length values and has a marked enhancing effect on the absorbance. However, as shown in Figure 1, there is very little change in absorbance when the perchloric acid con-

Table II. Study of Interference (*Continued*)

Impurity	Mole Added to 100 Ml. of Soln. Contg. 1.26×10^{-4} Mole C_6H_5HgOAc	Molar Ratio of Impurity to C_6H_5HgOAc Present	% Error without $HClO_4$ (Negative error: experimental less than known value)			% Error with $HClO_4$ (Negative error: experimental less than known value)		
			250 mμ	256 mμ	262 mμ	250 mμ	256 mμ	262 mμ
Ag^+	4.57×10^{-5}	0.4:1	0.8	0.3	−0.4	0.0	−0.5	−0.7
	2.46×10^{-4}	2.0:1	...	...	...	0.9	−0.7	0.0
	3.07×10^{-4}	2.4:1	13.0	6.0	6.4	1.2	0.2	−0.2
	4.45×10^{-4}	3.5:1	...	...	...	4.7	1.6	1.4
Cu^{++}	2.00×10^{-5}	0.2:1	6.1	2.6	3.3	0.7	0.5	0.0
	4.01×10^{-5}	0.3:1	...	...	...	1.2	0.7	0.7
	8.02×10^{-5}	0.6:1	...	...	...	2.1	0.0	−0.2
	2.00×10^{-4}	1.6:1	...	...	...	7.3	2.8	1.2
	4.00×10^{-4}	3.2:1	...	...	...	15.5	5.2	2.6
Fe^{+++}	2.16×10^{-8}	0.00017:1	1.9	1.6	1.2	0.9	0.5	0.5
	3.46×10^{-8}	0.00026:1	...	...	...	−0.7	−0.7	−0.2
	8.95×10^{-8}	0.00071:1	...	...	...	1.0	0.3	0.4
	4.48×10^{-7}	0.0036:1	...	...	...	3.9	3.8	4.5
$Hg(OAc)_2$	1.56×10^{-5}	0.12:1	...	...	...	2.8	1.2	0.5
	3.12×10^{-5}	0.25:1	...	...	...	3.8	0.9	0.5
	7.85×10^{-5}	0.62:1	...	...	...	5.2	1.9	1.2
	1.56×10^{-4}	1.2:1	...	...	...	7.6	3.3	1.9
Ethylene glycol	4.83×10^{-2}	383:1	0.8	1.0	0.8	0.7	0.7	0.5
	6.45×10^{-2}	512:1	1.5	1.6	1.2	1.6	0.9	1.2
	9.67×10^{-2}	767:1	2.7	2.2	2.4	2.8	1.2	1.9
	1.29×10^{-1}	1024:1	...	...	...	3.5	2.4	2.6
Diethylene glycol	1.93×10^{-3}	15:1	0.8	−0.6	0.0	0.7	0.7	0.7
	3.56×10^{-3}	28:1	2.2	1.3	1.2	...	...	...
	3.81×10^{-3}	30:1	...	...	...	0.7	0.7	0.7
	4.69×10^{-3}	37:1	...	...	...	1.9	1.6	1.9
Triethylene glycol	1.00×10^{-3}	8:1	0.8	0.0	0.0	0.2	0.7	0.7
	1.68×10^{-3}	13:1	2.3	1.6	1.6	0.0	0.2	0.5
	2.51×10^{-3}	20:1	...	...	...	2.8	2.4	2.6
Hexylene glycol	1.83×10^{-3}	15:1	0.8	0.6	0.0	0.9	0.5	0.5
	2.64×10^{-3}	21:1	...	...	...	2.1	1.2	1.2
	3.41×10^{-3}	27:1	3.4	1.9	2.4	...	...	...
	3.88×10^{-3}	30:1	...	...	...	2.1	1.2	1.9
	5.10×10^{-3}	40:1	...	...	...	6.1	3.1	3.8
Dipropylene glycol	5.93×10^{-4}	5:1	0.8	−0.6	−0.4	0.0	0.0	−0.2
	1.04×10^{-3}	8:1	0.8	0.6	0.0	...	...	...
	1.15×10^{-3}	9:1	2.7	1.9	1.6	1.2	0.9	0.5
	2.52×10^{-3}	20:1	...	...	...	2.1	1.6	1.2
	3.16×10^{-3}	25:1	...	...	...	2.8	2.1	2.4
CH_3OH	1.24×10^{-1}	984:1	0.0	0.3	−0.4	0.2	−0.5	0.5
	2.48×10^{-1}	1968:1	...	...	...	1.2	0.2	0.7
	4.94×10^{-1}	3921:1	0.6	−0.4	1.2	1.0	−0.1	1.2
	1.19	9444:1	...	...	...	8.2	2.8	6.6
C_2H_5OH	3.42×10^{-2}	271:1	−0.8	−1.0	−0.4	0.0	0.0	−0.7
	5.13×10^{-2}	407:1	−0.8	−0.3	−0.4	1.2	0.2	0.5
	6.84×10^{-2}	543:1	0.0	−0.3	−0.4	1.6	0.7	0.7
	8.55×10^{-2}	679:1	3.3	1.9	4.0	2.6	1.6	1.9

centration is varied from 1 to 3 ml. of perchloric acid per 100 ml. of solution; 2 ml. per 100 ml. are used in the proposed procedure.

Reproducibility. Table I gives results that were obtained by using the proposed method, including the absolute deviation for each of the three wave lengths. The relative average deviation of a single determination is ±0.40% at 250 mμ, ±0.35% at 256 mμ, and ±0.40% at 262 mμ.

Interference. Known weights of impurities were added to 50 ml. of an aqueous solution that contained 1.26×10^{-4} mole of phenylmercuric acetate, 2 ml. of 60% perchloric acid were added, and the solution was diluted to 100 ml. The absorbance was measured at 250, 256, and 262 mμ. This was repeated without adding the perchloric acid.

Table II shows that, in general, the presence of perchloric acid considerably reduces the error caused by impurities. Table II also indicates that either 256 or 262 mμ may be used for the determination, depending upon the impurities that are present; 250 mμ is not recommended, as the absorbance at this wave length is usually influenced to a much greater extent by the presence of impurities than at either 256 or 262 mμ.

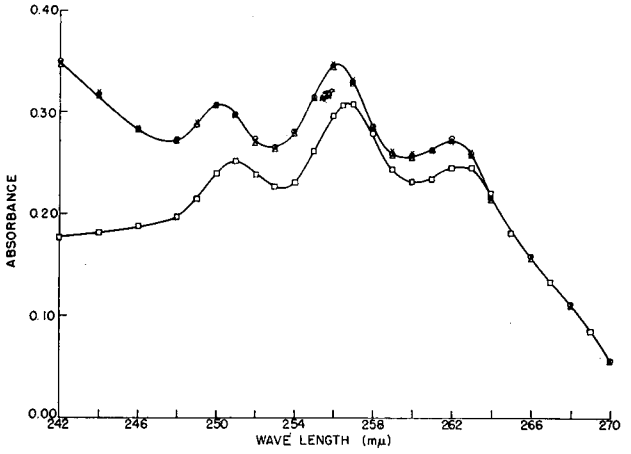

Figure 1. Absorption curves

□ 1.26 × 10^{-4} mole of phenylmercuric acetate per 100 ml.
× + 1.0 ml. of 60% $HClO_4$ per 100 ml.
○ + 2.0 ml. of 60% $HClO_4$ per 100 ml.
△ + 3.0 ml. of 60% $HClO_4$ per 100 ml.

Small amounts of chloride ion react with phenylmercuric acetate to form phenylmercuric chloride and cause the solution to become turbid.

Because the ultraviolet absorption curve of phenylmercuric acetate is a modified benzene curve, benzene interferes strongly.

Interference caused by the presence of diphenylmercury and ethylmercury chloride and the preparations Ceresan M [7.7% *N*-(ethylmercuri)*p*-toluenesulfonanilide] and Semesan [30% 2-chloro-4(hydroxymercuri)phenol] was studied.

Known weights of these materials were added to weighed quantities of phenylmercuric acetate and were heated to boiling with 50 ml. of water. After cooling, 2 ml. of perchloric acid were added and the solution was diluted to 100 ml. with water. The solutions were centrifuged and the absorbance of the clear solutions was measured. The insoluble material cannot be separated by filtration with paper, because some phenylmercuric acetate is adsorbed on the paper and low results are obtained. Interference caused by the liquid preparations MEMA (11.4% 2-methoxyethylmercury acetate) and Panogen 15 [2.2% cyano-(methylmercuri)-guanidine] was determined by the addition of known volumes of these preparations to 50 ml. of an aqueous solution that contained 0.0425 gram of phenylmercuric acetate. Two milliliters of 60% perchloric acid were added and the solution was diluted to 100 ml. with water. The slightly turbid solutions were centrifuged and the absorbance of the clear solutions was measured.

Table III. Interference of Organic Mercurials

Impurity	Impurity Taken, G.	C_6H_5HgOAc Taken, G.	% Error 250 mμ	% Error 256 mμ	% Error 262 mμ
$(C_6H_5)_2Hg$	0.0050	0.0455	0.2	0.7	0.7
	0.0050	0.0455	0.4	0.4	0.2
C_2H_5HgCl	0.0028	0.0448	1.8	−0.7	0.1
	0.0045	0.0494	−0.6	−0.4	−0.4
	0.0130	0.0470	0.6	−0.2	0.0
Ceresan M	0.0045	0.0459	12.9	8.1	7.8
	0.0578	0.0476	67.6	41.2	42.2
Semesan	0.0088	0.0487	18.9	12.1	16.6
	0.0138	0.0440	25.5	18.2	23.6
MEMA	0.01 ml.	0.0425	18.6	19.2	24.2
	0.05 ml.	0.0425	44.2	42.5	54.6
Panogen 15	0.05 ml.	0.0425	31.1	29.9	36.1
	0.25 ml.	0.0425	66.0	60.5	71.0

Table IV. Comparison of Iodide-Thiosulfate Titrimetric and Spectrophotometric Methods

	Taken, Gram	Found, Gram	Error, %
Titrimetric	0.0505	0.0499	−1.2
	0.0621	0.0618	−0.5
	0.0550	0.0547	−0.6
Spectrophotometric			
250 mμ	0.0534	0.0539	0.9
	0.0477	0.0481	0.8
	0.0524	0.0522	−0.4
256 mμ	0.0534	0.0539	0.9
	0.0477	0.0477	0.0
	0.0524	0.0528	0.8
262 mμ	0.0534	0.0538	0.8
	0.0477	0.0475	−0.4
	0.0524	0.0522	−0.4

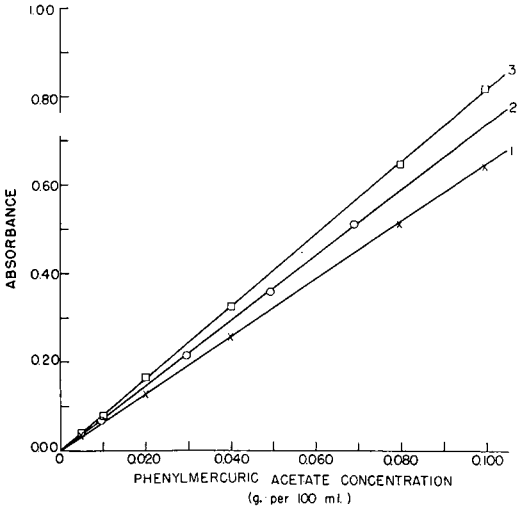

Figure 2. Concentration curves

1. 262 mμ
2. 250 mμ
3. 256 mμ

Table III gives the results of this study. The recorded interference for the Ceresan M, Semesan, MEMA, and Panogen 15 is caused by the active mercury compounds and/or other organic or inorganic substances that may be present in these commercial fungicide preparations.

Comparison of Iodide-Thiosulfate Titrimetric and Spectrophotometric Methods. A comparison of the results obtained using Gran's titrimetric method (*3*) and the spectrophotometric

Table V. Determination of Phenylmercuric Acetate by Titration with Thiocyanate

Known Weight of C_6H_5HgOAc, G.	$Hg(OAc)_2$ Added, Mole	C_6H_5HgOAc Found, G.	% Error
0.0527	...	0.0527	0.0
0.0554	...	0.0550	−0.7
0.0429	4.74×10^{-5}	0.0736	72
0.0425	7.72×10^{-5}	0.0935	120

method is shown in Table IV. Both methods are accurate to within about 1%. The proposed spectrophotometric method is much more rapid and convenient.

Comparison of Thiocyanate and Spectrophotometric Methods. Mercury compounds may be determined by direct titration with standard thiocyanate (*4*). Table V shows that for pure phenylmercuric acetate this method is as accurate as the proposed spectrophotometric method. It is also rapid and convenient. However, mercuric salts, such as mercuric acetate, interfere. This is particularly serious, as the thiocyanate and phenylmercuric acetate react in a 1 to 1 mole ratio, whereas thiocyanate and mercuric acetate react in a 2 to 1 ratio. As shown in Table II, 7.85×10^{-5} mole of mercuric acetate in 100 ml. of solution containing 1.26×10^{-4} mole (0.0425 gram) of phenylmercuric acetate causes less than 2% interference in the spectrophotometric method; this concentration causes over 120% error in the thiocyanate method, as is shown in Table V.

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RECEIVED for review August 15, 1955. Accepted May 2, 1956.

X-Ray Powder Diffraction Data of Some Molecular Complexes of TNT

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X-ray diffraction patterns are a useful means of characterizing crystalline compounds. During the course of studies of binary systems containing 2,4,6-trinitrotoluene, diffraction data have been accumulated on a number of 2,4,6-trinitrotoluene complexes and constituent compounds.

THE use of x-ray diffraction patterns as an adjunct to thermal studies of binary systems containing 2,4,6-trinitrotoluene (TNT) as one of the components has resulted in the accumulation of diffraction data on a number of 2,4,6-trinitrotoluene complexes and of the constituent compounds. Some of these data are presented here. The flat specimen technique employed in these studies may lead to line intensities which differ from those obtained by the rotated capillary technique due to orientation effects. For example, phenanthrene gives markedly different intensities by the flat specimen technique from those obtained from randomly oriented specimens (*1*).

Diffraction data on 2,4,6-trinitrotoluene have been given elsewhere (*4, 5*) but are repeated here because certain differences appear in the diffraction pattern which depend on the immediate history of the sample. Samples of 2,4,6-trinitrotoluene obtained by subliming onto a condensing surface held at temperatures close to the melting point (*2*) or by freezing melts at temperatures close to the melting point consist solely of the simple monoclinic form. Samples of 2,4,6-trinitrotoluene obtained by crystallization from solvents at room temperature or from strongly supercooled melts consist primarily of the monoclinic variant forms. Orthorhombic 2,4,6-trinitrotoluene which may be prepared at low temperatures (*3*) or, as found by Taylor (*6*), from solutions containing picryl chloride, has a diffraction pattern visually indistinguishable from that obtained from the monoclinic variant material. The crystal forms of 2,4,6-trinitrotoluene and their occurrence have been discussed by Burkardt and Bryden (*3*).

EXPERIMENTAL PROCEDURES

Diffractometer samples were prepared by grinding small amounts in an agate mortar. The sample was then pressed into

Table I. Complexes and Constituent Compounds

	Compound	Source	Molar Ratio
1	TNT	From melt	
2	TNT	From ethyl alcohol	
3	TNT	From ethyl alcohol containing 4% picryl chloride	
4	TNT-naphthalene	From melt	1–1
5	TNT-anthracene	From ethyl alcohol	1–1
6	TNT–2,4-dinitroanisole	From melt	1–1
7	TNT–2,4-dinitroanisole	From melt	8–1
8	2,4-Dinitroanisole	Eastman Kodak m.p., 94° C.	
9	TNT–2,4-dinitromesitylene	From melt	1–1
10	2,4-Dinitromesitylene	Eastman Kodak m.p., 87.5° C.	
11	TNT-phenanthrene	From melt	1–1
12	Phenanthrene	Eastman Kodak m.p., 101.2° C.	
13	TNT–2-iodo-3-nitrotoluene	From melt	1–2
14	2-Iodo-3-nitrotoluene	Eastman Kodak m.p., 64° C.	

Table II. Interplanar Spacings and Line Intensities

1. TNT (from Melt)

d, A.	I/I_1
10.28	0.04
7.14	0.45
6.61	0.03
5.70	0.15
5.47	0.06
5.03	0.60
4.96	0.04
4.32	0.18
4.19	0.03
3.88	1.00
3.76	0.14
3.70	0.15
3.56	0.11
3.47	0.09
3.35	0.11
3.30	0.11
3.16	0.03
3.07	0.26
3.03	0.17
2.94	0.06
2.89	0.18
2.80	0.06
2.75	0.15
2.69	0.53
2.62	0.04
2.56	0.03
2.54	0.03
2.51	0.03
2.46	0.08
2.43	0.12
2.36	0.06
2.33	0.06
2.24	0.02
2.14	0.05
2.10	0.02
2.01	0.09
1.97	0.02
1.94	0.03
1.88	0.03
1.71	0.02
1.67	0.02

2. TNT (from Ethyl Alcohol)

d, A.	I/I_1
10.22	0.13
7.14	0.17
5.66	0.14
5.01	1.00
4.63	0.06
4.30	0.06
4.04	0.08
3.88	0.63
3.78	0.04
3.73	0.05
3.53	0.14
3.47	0.03
3.35	0.22
3.07	0.27
3.01	0.09
2.94	0.06
2.73	0.15
2.68	0.71
2.60	0.02
2.50	0.03
2.45	0.08
2.44	0.08
2.37	0.02
2.31	0.03
2.21	0.02
2.14	0.02
2.00	0.08
1.94	0.02
1.93	0.02
1.90	0.02
1.87	0.02
1.84	0.02
1.67	0.02

3. TNT (from Ethyl Alcohol plus 4% Picryl Chloride)

d, A.	I/I_1
10.28	0.06
7.20	0.20
6.11	0.02
5.75	0.08
5.68	0.04
5.61	0.04
5.52	0.04
5.08	0.84
4.35	0.14
4.23	0.02
3.92	1.00
3.78	0.10
3.73	0.04

3. TNT (from Ethyl Alcohol plus 4% Picryl Chloride) (*Contd.*)

d, A.	I/I_1
3.56	0.18
3.46	0.10
3.35	0.18
3.29	0.25
3.06	0.29
3.02	0.16
2.93	0.10
2.88	0.20
2.74	0.25
2.68	0.43
2.61	0.04
2.56	0.04
2.54	0.04
2.51	0.04
2.46	0.14
2.33	0.08
2.24	0.02
2.23	0.02
2.15	0.04
2.01	0.04
1.94	0.04
1.88	0.04
1.85	0.02
1.84	0.02
1.79	0.02

4. TNT-Naphthalene

d, A.	I/I_1
7.66	0.54
7.02	0.56
6.66	1.00
5.87	0.06
4.90	0.13
4.37	0.45
4.29	0.45
4.19	0.37
4.18	0.02
3.97	0.04
3.85	0.26
3.79	0.28
3.63	0.04
3.52	0.22
3.35	0.63
3.27	0.43
3.21	0.35
3.15	0.06
3.01	0.26
2.97	0.11
2.95	0.11
2.90	0.26
2.85	0.06
2.75	0.09
2.70	0.09
2.65	0.04
2.42	0.06
2.36	0.02
2.31	0.02
2.21	0.09
2.18	0.09
2.05	0.04
2.03	0.04
1.99	0.06
1.86	0.04
1.80	0.02
1.71	0.02

5. TNT-Anthracene

d, A.	I/I_1
13.60	0.02
9.51	0.22
8.76	1.00
7.56	0.04
7.08	0.35
6.86	0.22
6.35	0.07
6.07	0.01
5.05	0.32
4.85	0.18
4.56	0.36
4.21	0.05
3.99	0.05
3.87	0.06
3.75	0.05
3.59	0.05
3.54	0.03
3.40	0.08
3.33	0.10

5. TNT-Anthracene (*Contd.*)

d, A.	I/I_1
3.27	0.21
3.16	0.07
3.02	0.06
2.94	0.08
2.88	0.03
2.83	0.02
2.73	0.02
2.67	0.03
2.61	0.02
2.58	0.03
2.50	0.02
2.44	0.03
2.37	0.03
2.23	0.02
2.17	0.01
2.10	0.02
2.07	0.04
2.01	0.02
1.94	0.03
1.88	0.03

6. TNT-2,4-Dinitroanisole

d, A.	I/I_1
10.40	0.15
7.83	0.30
7.08	0.70
6.92	0.35
6.33	0.10
6.07	0.05
5.72	0.07
5.49	0.05
5.18	0.35
5.05	0.27
4.87	0.20
4.40	0.15
4.31	0.12
3.94	0.55
3.88	1.00
3.77	0.25
3.71	0.20
3.56	0.15
3.45	0.40
3.38	0.30
3.28	0.20
3.16	0.07
3.06	0.22
3.02	0.32
2.89	0.15
2.79	0.05
2.75	0.20
2.68	0.20
2.54	0.10
2.44	0.10
2.32	0.07
2.25	0.05
2.14	0.05
2.01	0.02
1.88	0.05

7. TNT-2,4-Dinitroanisole

d, A.	I/I_1
10.05	0.08
7.76	0.09
7.08	0.80
6.89	0.10
6.07	0.12
5.68	0.13
5.47	0.10
5.01	0.75
4.93	0.08
4.82	0.06
4.30	0.22
4.17	0.06
3.88	1.00
3.77	0.24
3.71	0.27
3.56	0.22
3.46	0.17
3.35	0.20
3.28	0.21
3.16	0.06
3.07	0.40
3.02	0.45
2.94	0.11
2.89	0.18
2.78	0.11

7. TNT-2,4-Dinitroanisole (*Contd.*)

d, A.	I/I_1
2.75	0.37
2.69	0.49
2.61	0.07
2.54	0.06
2.51	0.05
2.46	0.13
2.44	0.18
2.37	0.05
2.35	0.05
2.32	0.07
2.14	0.06
2.01	0.04
1.94	0.04
1.88	0.06
1.83	0.04
1.79	0.02

8. 2,4-Dinitroanisole

d, A.	I/I_1
7.29	0.07
6.94	0.10
6.49	0.18
5.91	0.02
5.66	0.13
5.07	0.12
4.94	0.09
4.80	0.12
4.47	0.11
4.40	0.11
4.14	0.11
4.00	0.17
3.79	0.07
3.60	1.00
3.41	0.38
3.18	0.09
3.12	0.07
3.04	0.07
2.95	0.09
2.86	0.03
2.79	0.06
2.74	0.02
2.70	0.02
2.67	0.02
2.57	0.02
2.16	0.02
2.06	0.04
1.95	0.03

9. TNT-2,4-Dinitromesitylene

d, A.	I/I_1
11.12	0.19
8.76	0.10
7.47	0.63
7.14	0.95
6.56	0.70
6.11	0.07
5.72	0.19
5.50	0.24
5.36	0.12
5.07	0.22
4.98	0.05
4.48	0.15
4.34	0.32
4.08	0.19
3.91	1.00
3.79	0.22
3.73	0.19
3.64	0.44
3.52	0.12
3.43	0.27
3.39	0.12
3.30	0.24
3.24	0.05
3.18	0.06
3.08	0.24
3.03	0.24
2.91	0.17
2.76	0.27
2.71	0.15
2.63	0.07
2.58	0.02
2.45	0.10
2.34	0.07
2.24	0.05
2.15	0.05
2.05	0.02
2.02	0.02
1.94	0.05
1.88	0.05
1.81	0.02

10. 2,4-Dinitromesitylene

d, A.	I/I_1
11.34	0.20
8.94	0.08
7.50	0.39
7.14	1.00
6.66	0.68
5.52	0.22
5.42	0.12
4.53	0.17
4.35	0.12
4.15	0.07
3.93	0.04
3.63	0.15
3.41	0.39
3.35	0.07
3.29	0.03
3.23	0.05
3.16	0.02
3.09	0.03
2.98	0.02
2.88	0.04
2.76	0.12
2.66	0.03
2.52	0.02
2.45	0.03
2.43	0.04
2.34	0.02
2.30	0.02
2.24	0.03
2.22	0.03
2.16	0.02
1.94	0.05

11. TNT-Phenanthrene

d, A.	I/I_1
14.14	0.13
7.90	0.53
7.14	1.00
4.86	0.21
4.72	0.08
4.53	0.06
4.23	0.06
4.06	0.06
3.90	0.05
3.62	0.15
3.40	0.27
3.30	0.03
3.20	0.04
3.04	0.03
2.88	0.03
2.82	0.02
2.77	0.01
2.56	0.01
2.47	0.01
2.42	0.01
2.28	0.02
2.19	0.03
2.10	0.02
2.01	0.03
1.96	0.01
1.94	0.01
1.92	0.01

12. Phenanthrene

d, A.	I/I_1
9.72	1.00
7.69	0.02
5.95	0.01
5.04	0.06
4.74	0.27
4.60	0.04
4.42	0.03
4.28	0.02
4.08	0.05
3.65	0.01
3.60	0.01
3.47	0.02
3.41	0.04
3.16	0.04
2.97	0.01
2.95	0.01
2.78	0.01
2.55	0.01
2.37	0.06
2.07	0.02
1.94	0.02
1.89	0.02
1.82	0.01

13. TNT-2-Iodo-3-nitrotoluene

d, A.	I/I_1
7.25	0.05
7.02	0.08
6.78	0.08
6.42	1.00
5.96	0.08
5.23	0.05
5.03	0.05
4.09	0.07
4.01	0.14
3.95	0.15
3.87	0.18
3.83	0.14
3.63	0.48
3.51	0.44
3.39	0.09
3.31	0.14
3.21	0.26
3.07	0.30
3.00	0.10
2.97	0.10
2.87	0.05
2.86	0.06
2.75	0.04
2.67	0.04
2.60	0.03
2.57	0.04
2.55	0.04
2.53	0.04
2.48	0.05
2.43	0.12
2.39	0.19
2.36	0.07
2.33	0.05
2.28	0.07
2.23	0.03
2.17	0.03
2.14	0.03
2.10	0.03
2.04	0.03
1.88	0.03
1.84	0.04
1.75	0.03

14. 2-Iodo-3-nitrotoluene

d, A.	I/I_1
14.25	0.09
12.81	0.06
11.95	0.04
10.40	0.05
9.51	0.09
7.02	0.15
6.81	0.23
6.41	1.00
5.95	0.23
5.22	0.32
4.77	0.09
4.40	0.04
4.08	0.11
4.00	0.23
3.93	0.23
3.83	0.19
3.63	0.49
3.49	0.78
3.38	0.15
3.30	0.26
3.21	0.30
3.07	0.67
3.01	0.09
2.97	0.15
2.86	0.13
2.75	0.04
2.66	0.04
2.58	0.09
2.56	0.09
2.54	0.06
2.48	0.04
2.43	0.15
2.39	0.11
2.37	0.11
2.29	0.11
2.28	0.11
2.13	0.13
2.04	0.11
1.97	0.06
1.88	0.10
1.84	0.08
1.77	0.06
1.61	0.06

Table III. Interplanar Spacings and Line Intensities

d, A.	I/I_1	d, A.	I/I_1	d, A.	I/I_1
1. TNT (from Melt)		1. TNT (from Melt) (*Contd.*)		2. TNT (from Ethyl Alcohol) (*Contd.*)	
9.94	1	1.88	2	2.93	1
7.08	7	1.83	1	2.87	4
5.99	1	1.78	1	2.79	1
5.61	8	1.75	1	2.72	5
5.18	2	1.68	1	2.67	5
5.01	3	1.64	1	2.60	3
4.62	2	1.58	1	2.52	1
4.28	5	1.53	1	2.43	3
4.00	2	1.46	1	2.36	2
3.87	10			2.30	3
3.71	3			2.23	1
3.49	4	2. TNT (from Ethyl Alcohol)		2.18	1
3.31	1			2.14	3
3.16	1			2.10	1
3.06	6	9.88	2	2.04	2
3.00	3	7.02	6	2.01	2
2.92	2	6.66	1	1.97	1
2.89	1	6.02	1	1.93	1
2.72	4	5.63	5	1.88	3
2.68	4	5.44	4	1.83	1
2.59	2	5.22	1	1.81	1
2.55	1	4.97	4	1.78	1
2.44	3	4.29	7	1.76	1
2.37	2	3.87	10	1.73	1
2.30	2	3.73	2	1.70	1
2.24	1	3.53	2	1.67	1
2.20	1	3.43	3	1.64	1
2.13	3	3.34	1	1.61	1
2.06	1	3.27	4	1.58	2
2.03	1	3.16	2	1.53	2
1.99	1	3.05	4	1.43	1
1.92	1	3.01	4	1.39	1

standard flat-specimen sample holders. Patterns were recorded with a Norelco diffractometer using copper radiation filtered through nickel foil (λ = 1.5418 A.).

Powder patterns of 2,4,6-trinitrotoluene samples were also obtained using a rotated capillary in a 114.6-mm. diameter camera with copper radiation filtered through nickel foil.

EXPERIMENTAL DATA

Table I lists the compound, source, or method of preparation and molar ratio in the case of the complexes. Interplanar spacings are given in Table II for the range 22.09 to 1.54 A. Intensities are given as peak height above the background level, with the most intense reflection given a value of 1.00.

Interplanar spacings and intensities for 2,4,6-trinitrotoluene obtained from the rotated capillary samples are given in Table III. Intensities were visually estimated and are given on a basis of 10 to 1, where 10 represents the most intense line.

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RECEIVED for review June 4, 1955. Accepted February 24, 1956.

Photometric Determination of Germanium with Phenylfluorone

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In an improved photometric phenylfluorone method for the determination of microgram quantities of germanium, interference from other metals is eliminated by isolating the germanium with a carbon tetrachloride extraction previous to the photometric determination. Very rapid color development is achieved by adjusting the solution to pH 3.1 before adding the phenylfluorone. A study has been made on the use of complexing reagents to eliminate metallic interference.

A RELIABLE method for the determination of microgram quantities of germanium in the presence of milligram quantities of other metals is needed in certain phases of semiconductor research and development.

A photometric method, based on the phenylfluorone spot test of Gillis, Hoste, and Claeys (*3*), was developed by Zischkau (*9*), who was able to eliminate the interference of antimony and tin by complexing with fluoboric and phosphoric acids, but found no simple method for the elimination of interference due to molybdenum. Cluley (*1*), working independently, presented a similar method in which the germanium is first isolated from all interfering elements by distillation as chloride. This method is very satisfactory for most analyses, but is time-consuming. Schneider and Sandell (*7*) reduced the time of analysis by replacing the distillation with a carbon tetrachloride extraction of the germanium from 9*N* hydrochloric acid solution (*8*). The only metal that accompanies the germanium, in more than trace quantities, into the carbon tetrachloride layer is trivalent arsenic, which causes no interference in the phenylfluorone method.

In the photometric methods mentioned the acidity of the solution at color development is high, and as much as 30 to 60 minutes is required for full color development. Ernst (*2*) has shown that the time can be reduced to 1 or 2 minutes by adjusting the pH of the solution to 4.5 with the aid of a sodium acetate–acetic acid buffer before adding the phenylfluorone. It appeared possible to develop a very rapid photometric method for germanium based on the extraction technique of Schneider and Sandell, followed by the color-forming technique of Ernst. This paper describes the authors' work in developing such a method.

APPARATUS

A Beckman Model B spectrophotometer, with absorption cells having a light path of 1 cm., was used.

REAGENTS

SODIUM HYDROXIDE SOLUTION (5%). Dissolve 5 grams of sodium hydroxide in 100 ml. of water in a polyethylene bottle.

STANDARD GERMANIUM SOLUTION (10 γ of germanium per ml.). Transfer 0.1441 gram of pure germanium dioxide to a 100-ml. platinum dish. Add 3 ml. of (5%) sodium hyroxide solution, and stir and rub with a polyethylene rod until all the oxide has dissolved. Add about 75 ml. of water. Neutralize to Congo red paper by adding sulfuric acid (1 + 9) dropwise. Add 3 or 4 drops in excess after the paper has turned blue. Remove the Congo red paper and transfer the solution to a 1-liter volumetric flask. Dilute to the mark with water and mix. Transfer 50.0 ml. of the solution to a 500-ml. volumetric flask, dilute to the mark with water, and mix.

HYDROCHLORIC ACID (9*N*). Transfer 385 ml. of hydrochloric acid to a 500-ml. volumetric flask, dilute nearly to the mark with water, cool, dilute to the mark with water, and mix.

BUFFER SOLUTION (pH 5). Dissolve 900 grams of hydrated sodium acetate, $NaC_2H_3O_2.3H_2O$, or 540 grams of the anhydrous salt, in about 700 ml. of water by warming on a hot plate. Filter with suction through a Whatman No. 40 paper and transfer to a 2-liter volumetric flask containing 480 ml. of acetic acid. Cool, dilute to the mark with water, and mix.

GUM ARABIC SOLUTION. Dissolve 0.5 gram of powdered gum arabic (gum acacia) in 50 ml. of hot water by stirring. Filter with suction through a Whatman No. 42 paper on a platinum cone. Prepare fresh each day as needed.

PHENYLFLUORONE SOLUTION. Transfer 0.0500 gram of phenylfluorone (2,3,7-trihydroxy-9-phenyl-6-fluorone, obtainable from Jasonols Chemical Corp., Brooklyn, N. Y.) to a 100-ml. beaker. Add 50 ml. of methanol and 1 ml. of hydrochloric acid, and stir until dissolved. Transfer to a dry 500-ml. volumetric flask, dilute to the mark with methanol, and mix. This solution is stable for at least a month. Do not store in a polyethylene bottle.

PROCEDURE

Preparation of Calibration Curve. Transfer 0, 0.5, 1.0, 2.0, 3.0, and 4.0 ml. of standard germanium solution (10 γ of germanium per ml.) to 60-ml. Squibb-type separatory funnels. Add 2 ml. of sulfuric acid (1 + 1), dilute to 7 ml. with water, and add 19 ml. of hydrochloric acid. Add 20 ml. of carbon tetrachloride, stopper, and shake vigorously for 1 minute. Allow the layers to separate and run the carbon tetrachloride layer into a second 60-ml. separatory funnel which is dry or has been rinsed with 9*N* hydrochloric acid. Add 2 ml. of carbon tetrachloride to the acid solution in the first funnel, shake for 10 seconds, allow to settle, and run the lower layer into the second funnel. Wash the combined carbon tetrachloride solutions by shaking for 10 seconds with 2 ml. of 9*N* hydrochloric acid. Allow the layers to separate and transfer the lower layer to a third 60-ml. separatory funnel which has been dried in an oven or with an air jet. Add from a buret 12.0 ml. of water to the carbon tetrachloride solution in the third funnel. Stopper and shake vigorously for 1 minute. Allow the layers to separate and discard the lower layer.

Filter the aqueous layer through a small dry Whatman No. 40 filter paper and collect the filtrate in a dry 50-ml. conical flask. Pipet 10.0 ml. of the filtrate into a 50-ml. volumetric flask. Add 1.5 ml. of sulfuric acid (1 + 1), then 10 ml. of buffer solution, and then 1 ml. of gum arabic solution from measuring pipets, swirling after each addition. Add 10.0 ml. of phenylfluorone solution, stopper, and mix. Ignore any precipitation of sodium acetate, as the precipitate will dissolve upon subsequent acidification. Let stand 5 minutes. Dilute to the mark with hydrochloric acid (1 + 9) and mix. Transfer a portion of the solution to a 1-cm. absorption cell and make the photometric measurement immediately at 510 mμ, using water as the reference solution. Prepare a calibration curve.

Analysis of Sample. Dissolve the sample (containing no more than 40 γ of germanium) in a small excess of a suitable acid or alkali in a 50-ml. conical flask. Halogen acids or their salts must not be used at any stage in the preparation of the sample solution. Organic matter, if present, must be destroyed by wet oxidation. Finally add 2 ml. of sulfuric acid and evaporate on a Meker-type flame to about 1.25-ml. volume. Carry a reagent blank through all steps of the analysis. If the sample is known to contain less than about 0.25 mg. of antimony, add 5 drops of perchloric acid and evaporate on the flame to a volume of 0.75 to 1 ml. Otherwise add 2 ml. of sulfuric acid plus about 0.1 gram of hydrazine sulfate and evaporate on a flame to a volume of 0.75 to 1 ml. Cool. If a precipitate of iron or chromium sulfate is present, add 1 ml. of water and heat uncovered on a low temperature hot plate until fumes of acid just appear in the flask; if necessary, repeat the addition of water and heating just to fumes one or more times to dissolve the precipitate completely. Cool. Ignore insoluble sulfates of lead, barium, or calcium. Add 6 ml. of water, pour into a 60-ml. separatory funnel, and wash in with 19 ml. of hydrochloric acid. Add 20 ml. of carbon tetrachloride and continue as in preparation of calibration curve.

DISCUSSION

Most of the conclusions of Schneider and Sandell regarding the germanium extraction have been confirmed. Quantitative tests have shown that the recovery of germanium is only about 95% complete when a single carbon tetrachloride extraction is used. As additional extractions do not improve the recovery, it is necessary to compensate for the loss of germanium by including the extraction in the preparation of the calibration curve.

Considerable difficulty was experienced in establishing suitable conditions for rapid color development in buffered solutions. Cloudy solutions were frequently obtained upon dilution to volume after color development. However, optimum reagent concentrations were eventually found that made it possible to overcome this difficulty. It is desirable to avoid altering the absolute or relative concentration of any of the reagents used in the photometric determination, for fear of changing the intensity of the germanium color produced or of obtaining cloudy solutions.

In order to establish the optimum pH for rapid color development, 40-γ portions of germanium, in the form of aliquots of a standard germanium solution, plus various amounts of sulfuric acid were diluted to 10 ml.; a 10-ml. aliquot of pH 5 acetate buffer solution was added; the pH of the mixture was measured; and the germanium was then determined photometrically as directed above. Reagent blanks for each sample were carried through the photometric analysis (see Table I).

Table I. Optimum pH for Color Development

No.	Sulfuric Acid Present, Ml.	pH of Mixture	Absorbance		
			Reagent blank	Germanium	Difference
1	0.25	4.3	0.29	0.96	0.67
2	0.50	4.0	0.08	0.91	0.83
3	0.75	3.1	0.06	0.95	0.89
4	1.00	1.8	0.04	0.94	0.90
5	1.25	1.0	0.03	0.80	0.77

Table II. Determination of Germanium by Carbon Tetrachloride Extraction-Photometric Method

No.	Other Metals Added	Germanium Found, γ
1	Li, Na, K, Rb, Cs, B, Be, Mg, Ba, Sr, Ca	41
2	Cr, Mn, Re, Co, Ni	40
3	Pd, Pt, Al	40
4	Ir, Os, Rh	41
5	Ru	41
6	Cu, Ag, Au, Zn, Cd, Hg	40
7	As, Sb, Bi	39
8	In, Tl, Pb, Si, P	40
9	Fe, Ga, Mo, Se, Te	39
10	Ce, Nd, Sm, Pr, Sc, Y, La, Hf, Th	41
11	Ta	39
12	Nb	39
13	V, W, Zr, Ti	40
14	Sn	39
15	U	40

The optimum pH for color development is 3.1, as the sensitivity of the germanium determination is high and does not vary appreciably with change in pH. Complete color development occurs in less than 2 minutes when the solution is more alkaline than pH 1.8. Nevertheless, as a precautionary measure, the time has been extended to 5 minutes in the method described. Color development at pH 1.8 is attractive from the standpoint of decreasing the interference of other metals, but completely clear solutions and more stable colors are obtained when color development is made at pH 3.1. Even at this pH, however, there is a tendency toward a fading of the germanium-phenylfluorone color after 10 or 15 minutes of standing, following the dilution to volume with hydrochloric acid (1 + 9).

Some difficulties are presented by samples that contain appreciable amounts of chromium, antimony, or titanium. In order to prepare a sample for the carbon tetrachloride extraction it is necessary to fume it with sulfuric acid. When this is done, chromium, regardless of its original valence state, precipitates as a basic sulfate. Severe loss of germanium by occlusion occurs if this precipitate is not redissolved before proceeding. The precipitate from small amounts of chromium—i.e., less than 100 γ—can be redissolved by digesting in hot sulfuric acid (1 + 1), but solution of larger amounts is often virtually impossible. To date no completely satisfactory method for the analysis of samples containing more than traces of chromium has been found. Iron behaves in the same way as chromium, but the iron sulfate is much more easily dissolved.

If more than about 0.25 mg. of quinquevalent antimony is present after fuming with sulfuric acid, the antimony is hy-

drolyzed on dilution with water previous to the carbon tetrachloride extraction, and germanium is lost through coprecipitation. When more than traces of antimony are present in the sample to be analyzed, the antimony must be reduced to the trivalent state with hydrazine sulfate before dilution with water.

Iron and titanium cause no trouble when present separately. When present together, no difficulty is encountered if the sample is fumed with perchloric acid before the carbon tetrachloride extraction. On the other hand, if the sample is fumed with hydrazine sulfate before the extraction, a black precipitate appears which is insoluble in cool 9*N* hydrochloric acid. It dissolves if heat is applied, but the results obtained for germanium are invariably low.

Use of Complexing Reagents. It seemed probable that in certain instances, where the concentration of interfering metals is known to be low, interference might be eliminated by the use of complexing reagents rather than by preliminary isolation of the germanium by carbon tetrachloride extraction. In order to investigate the possibilities of such a method, it was necessary first to determine which metals interfere.

Phenylfluorone reacts with several metals in acid solution to form colored compounds, which vary in their stability towards acid. The compounds of germanium and tin are particularly stable and can be formed in highly acid solution. Others are produced slowly or not at all in strongly acid solution. Zischkau and Cluley have been able to minimize interference in the photometric determination of germanium by maintaining a high acidity at color development. In the method described above, where the color is developed at pH 3.1, the solution is made highly acid just before the photometric measurement in order to destroy, as completely as possible, interfering metal-phenylfluorone compounds. Some of the compounds are completely destroyed by the acidification, while others are only partially destroyed.

In order to determine which metals yield stable colored phenylfluorone compounds, specificity tests were made on 0.1-mg. portions of each of the 59 metals listed in Table II. Each metal aliquot plus 1.5 ml. of sulfuric acid (1 + 1) was diluted to 10 ml.; 10 ml. of pH 5 buffer plus 1 ml. of gum solution plus 10.0 ml. of phenylfluorone solution were added; the solutions were then allowed to stand 5 minutes, diluted to volume with hydrochloric acid (1 + 9), and measured photometrically. The only metals that produced colored compounds at pH 3.1 that were not completely destroyed upon subsequent acidification before photometric measurement were tin, antimony(III), titanium, zirconium, hafnium, vanadium(V), niobium, tantalum, molybdenum, tungsten, iron(III), and gallium. The colors were of various intensities and hues, but all absorbed to some extent at 510 mμ. Iron(II), vanadium(IV), and antimony(V) produced no color with the phenylfluorone. Several metals other than those listed would probably interfere, if more than 0.1 mg. was used—for example, 0.2 mg. of bismuth reacts with the phenylfluorone to form an orange-pink color.

Of the twelve interfering metals listed above, all but three cause high results in a germanium determination when color is developed at pH 3.1. Zirconium, gallium, and ferric iron cause low results. Apparently, when appreciable amounts of metals that react with phenylfluorone are present, full color development is prevented, because germanium has to compete with the other metals for the limited amount of phenylfluorone available. As the colors due to zirconium, gallium, and ferric iron are appreciably bleached upon dilution with hydrochloric acid (1 + 9) previous to the photometric measurement, the incompleteness of the germanium color development becomes apparent. (On the other hand, at pH 4.3, the colors due to these three metals are not sufficiently bleached on addition of the hydrochloric acid, and results for germanium are high; at pH 1.8, the reaction of the three metals with phenylfluorone is so suppressed that full color development of the germanium is permitted and the results obtained are again high.)

Investigation of various complexing reagents has shown that the interference of 0.1-mg. portions of all but three of the interfering metals mentioned above can be completely suppressed, without appreciably reducing the color due to germanium itself, by adding 2 ml. of a 10% solution of ethylenediaminetetraacetic acid (EDTA) just before addition of the phenylfluorone. The interference due to antimony(III), molybdenum, and niobium is reduced but not eliminated. However, the interference of antimony can be eliminated by oxidizing it to the quinquevalent state previous to color development. The most convenient method for performing this oxidation is to fume with perchloric acid. The oxidation is not complete (*4*), but the slight amount of antimony(III) that remains is sufficiently complexed by the EDTA to prevent interference. The interference of molybdenum and niobium can be prevented by adding 2 ml. of a 3% solution of hydrogen peroxide prior to addition of the buffer, gum, EDTA, and phenylfluorone. The perchloric acid–hydrogen peroxide–EDTA method yields very satisfactory results for germanium in the presence of 0.1 mg. of any of the metals listed in Table II. However, the method is of limited applicability, because not much more than a few tenths of a milligram of interfering metals can be completely complexed with the recommended amounts of hydrogen peroxide and EDTA.

During the present investigation, it was noted that all the metals that interfere in the photometric determination can be precipitated from strong acid solution with cupferron (*5*). Attempts to make this the basis of a new method of isolating the germanium were not successful. Removal of the interference by gravimetric separation with cupferron or by solvent extraction of precipitated cupferrates into chloroform was tested. Several of the interfering metals were incompletely removed, and in the extraction procedure, difficulty due to the insolubility of tantalum cupferrate in chloroform was encountered (*6*). It is evident that the cupferron separation method cannot compete with the distillation or carbon tetrachloride extraction methods for the isolation of the germanium. However, the cupferron extraction method has proved to be useful for removing most of the interference previous to a determination in which hydrogen peroxide and EDTA are used as complexing agents. In this manner the range of applicability of the complexing method is appreciably increased. In certain instances, where appreciable amounts of interfering metals are present in the sample to be analyzed, it may be desirable to use hydrogen peroxide and EDTA in the color development, following the separation of the germanium by carbon tetrachloride extraction.

Table III. Determination of Germanium by Carbon Tetrachloride Extraction–Photometric Method

No.	Other Metals Added	Germanium Found, γ
1	As	41
2[a]	As	40
3	Sb	18
4[a]	Sb	39
5	Sn	41
6	Se, Te	41
7	Zr, Ti, Ga	40
8	Fe, V, Hf	38
9	Mo, V	39
10	Cu, Zn, Ni, Bi, Cr	40
11	Cr	2
12	Fe, Ti	42
13[a]	Fe, Ti	19
14[a]	Fe	42
15[a]	Ti	41

[a] Sample fumed with hydrazine sulfate rather than perchloric acid before carbon tetrachloride extraction.

EXPERIMENTAL

In order to test the proposed method, synthetic sample solutions were prepared and analyzed as directed. Each sample solu-

tion was made up to contain 40 γ of germanium plus 100 γ of one or more of 59 of the more commonly encountered metals. All samples were fumed with perchloric acid before the carbon tetrachloride extraction. The results are shown in Table II.

In order to investigate the effect of larger amounts of impurities, the experiment was repeated using 40 γ of germanium, plus 10 mg. of one or more of the most commonly encountered metals (Table III). All but five of the samples were fumed with perchloric acid before the carbon tetrachloride extraction. The low results for germanium shown in Nos. 3, 11, and 13 are caused by precipitation of the antimony, chromium, or titanium. In No. 10, the precipitated chromium sulfate was successfully dissolved and germanium quantitatively recovered.

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RECEIVED for review January 14, 1956. Accepted May 9, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 1956.

Photometric Determination of Tin with Phenylfluorone

Determination of Tin in Lead and 1% Antimony-Lead Alloys

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A photometric phenylfluorone method for the determination of microgram quantities of tin in organic and inorganic samples has been developed. Interference from other metals is eliminated by separating the tin from the bulk of the sample and then isolating it by means of carbamate-chloroform extractions before making the photometric determination. The new method has been adapted to the determination of tin in lead and 1% antimony-lead cable sheath alloys.

BECAUSE available photometric methods for the determination of tin lack specificity and sensitivity (*6*), a new method was needed. The fact that tin interferes seriously in the determination of germanium with phenylfluorone (*4*) made it probable that this reagent could be used for the determination of tin. This proved to be true. Sensitivity is high, and by employing suitable separations before the photometric determination, complete specificity can be achieved. Tin can be determined in most metals and alloys, if it can be isolated sufficiently for the photometric determination. Thus, the method has been adapted to the determination of tin in lead and in 1% antimony-lead alloys used in the manufacture of telephone cables. The method should also be useful in the determination of tin in organic samples.

APPARATUS

A Beckman Model B spectrophotometer, with absorption cells having a light path of 1 cm., was used.

REAGENTS

STANDARD TIN SOLUTION (20 γ of tin per ml.). Transfer 0.2000 gram of pure tin metal to a 250-ml. Vycor conical flask, add 10 ml. of sulfuric acid, and heat on a Meker-type flame to dissolve the metal. When solution is complete, heat to copious fumes to expel sulfur dioxide. Add 30 ml. of sulfuric acid, cool, and then add about 125 ml. of water. Cool to room temperature, transfer to a 200-ml. volumetric flask, dilute to the mark, and mix. Ignore the presence of the globule of sulfur. Transfer 20.0 ml. of the solution to a 1-liter volumetric flask. Add 300 ml. of cool sulfuric acid (1 + 2), dilute to 950 ml., cool to room temperature, dilute to the mark, and mix.

CARBAMATE SOLUTION. Dissolve 2 grams of Eastman's diethylammonium diethyldithiocarbamate in 200 ml. of chloroform. Prepare fresh each day as needed.

THIOGLYCOLIC ACID SOLUTION. Dilute 20 ml. of thioglycolic acid to 200 ml. with water and mix.

POTASSIUM IODIDE–ASCORBIC ACID SOLUTION. Dissolve 6 grams of potassium iodide plus 1 gram of ascorbic acid in 40 ml. of water and mix. Prepare fresh each day as needed.

HYDROGEN PEROXIDE (3%). Dilute 5 ml. of hydrogen peroxide (30%) to 50 ml. with water.

BUFFER SOLUTION (pH 5). Dissolve 900 grams of hydrated sodium acetate, $NaC_2H_3O_2.3H_2O$, or 540 grams of the anhydrous salt in about 700 ml. of water by warming on a hot plate. Filter with suction through a Whatman No. 40 paper and transfer to a 2-liter volumetric flask containing 480 ml. of acetic acid. Cool, dilute to the mark with water, and mix.

GUM ARABIC SOLUTION. Dissolve 0.5 gram of powdered gum arabic (gum acacia) in 50 ml. of hot water by stirring. Filter with suction through a Whatman No. 42 paper on a platinum cone. Prepare fresh each day as needed.

PHENYLFLUORONE SOLUTION. Transfer 0.0500 gram of phenylfluorone (2,3,7-trihydroxy-9-phenyl-6-fluorone, obtainable from Jasonols Chemical Corp., Brooklyn, N. Y.) to a 100-ml. beaker. Add 50 ml. of methanol and 1 ml. of hydrochloric acid. Stir until dissolved. Transfer to a dry 500-ml. volumetric flask, dilute to the mark with methanol, and mix. This solution is stable for at least a month. Do not store in a polyethylene bottle.

CUPFERRON SOLUTION. Dissolve 1 gram of cupferron in 100 ml. of water. Prepare fresh each day as needed.

COPPER SULFATE SOLUTION. Dissolve 0.5 gram of copper sulfate, $CuSO_4.5H_2O$, in 100 ml. of water.

TARTARIC ACID SOLUTION. Dissolve 1 gram of tartaric acid in 100 ml. of water.

LEAD NITRATE SOLUTION. Dissolve 15 grams of lead nitrate in 500 ml. of water.

PERCHLORIC ACID–NITRIC ACID MIXTURE. Mix 50 ml. of perchloric acid (70%) with 10 ml. of nitric acid.

POTASSIUM PERMANGANATE SOLUTION. Dissolve 1 gram of potassium permanganate in 100 ml. of water.

MANGANESE NITRATE SOLUTION. Mix 5 ml. of manganese nitrate (50% solution) with 50 ml. of water.

GENERAL PROCEDURE

Preparation of Calibration Curve. Transfer 0, 1.0, 2.0, 3.0, and 4.0 ml. of standard tin solution (20 γ of tin per ml.) to 125-ml. conical flasks. Add enough sulfuric acid so that each flask contains 5 ml. of the acid, dilute to 50 ml. with water, and cool to room temperature.

Transfer the sample to a 125-ml. Squibb-type separatory funnel. Add 25 ml. of carbamate solution, shake momentarily, relieve the pressure in the funnel, and then shake vigorously for

30 seconds. Allow the layers to separate and then drain off and discard the lower layer. Pour 5 ml. of chloroform into the funnel and drain off and discard the lower layer. Then add 5 ml. more of chloroform, shake for 10 seconds, allow to settle, and drain off and discard the lower layer.

Drain the aqueous solution to a clean 125-ml. conical flask, heat to 50° C., pour back into the funnel, and add 2 ml. of thioglycolic acid solution followed by 1 ml. of potassium iodide–ascorbic acid solution. (Do not add these reducing agents until the solution has been transferred back to the funnel.) Stopper, shake once or twice to mix, and then allow to stand unstoppered for 10 minutes to reduce the tin. Stopper and cool the solution to room temperature by shaking gently in running tap water. Wash off the funnel with distilled water. Add 10 ml. of carbamate solution, shake vigorously for 30 seconds, allow to settle well, and drain the lower layer to a clean 125-ml. Vycor conical flask. Add 5 ml. of chloroform to the funnel, shake 10 seconds, allow to settle well, and drain the lower layer to the flask. Discard the aqueous solution remaining in the funnel.

Add 2 ml. of sulfuric acid plus 1 ml. of nitric acid plus 0.5 ml. of perchloric acid to the flask and evaporate on an asbestos pad on a low temperature hot plate until the chloroform and most of the brown fumes have been expelled. Remove to the bare plate and continue the heating until white fumes begin to appear in the flask. Finally, heat on a Meker-type flame to fumes of sulfuric acid to destroy the remaining organic matter and to expel perchloric acid. Cool somewhat, add about 0.25 ml. of perchloric acid, and heat on the flame until all acid has been expelled from the flask. In order to accomplish this efficiently, it is necessary to flame the sides as well as the bottom of the flask. When all but traces of the sulfuric acid have been expelled, remove from the flame and blow over the top of the flask to expel the small amount of acid that remains in the neck. Cool to room temperature. Add 5 ml. of sulfuric acid (1 + 4) from a measuring pipet. Heat to boiling on a flame until fumes of sulfuric acid just appear in the flask. Avoid expelling any of the acid. Cool, add 9 ml. of water from a measuring pipet, and then cool to room temperature. Add 1 ml. of hydrogen peroxide (3%), then 10 ml. of buffer solution, then 1 ml. of gum arabic solution, and then 10.0 ml. of phenylfluorone solution, swirling after each addition. Let stand for 5 minutes. Add about 16 ml. of hydrochloric acid (1 + 9), swirl, and pour the solution into a 50-ml. volumetric flask. Dilute to the mark with hydrochloric acid (1 + 9) and mix. Transfer a portion of the solution to a 1-cm. absorption cell and make the photometric measurement immediately at 510 mμ, using water as the reference solution. Prepare a calibration curve.

Analysis of Sample. In the analysis of organic samples, destroy the organic material completely by wet oxidation with sulfuric acid plus nitric acid. In the analysis of inorganic samples, isolate the tin from the bulk of the sample by the usual classical methods of separation. Finally, evaporate the sample solution to fumes of sulfuric acid in a 250-ml. Vycor conical flask on a flame. All organic matter must be destroyed before proceeding. Carry a reagent blank through all the steps of the analysis.

At this point if the sample to be analyzed is known to contain no more than about 0.05 mg. of germanium, 10 mg. of each of the other interfering metals, or 0.25 gram of phosphoric acid, dilute to 10-ml. volume with sulfuric acid, add 1 ml. of perchloric acid, and heat on a flame to 7-ml. volume. Cool somewhat, add about 0.3 gram of hydrazine sulfate, and heat carefully on the flame until the foaming subsides. (Once reduction of antimony has been accomplished, it is desirable to carry the analysis without unnecessary delay through the two subsequent carbamate extractions.) Finally, evaporate the sample on the flame to a volume of 5 ml. to destroy all the hydrazine sulfate and to expel sulfur dioxide. Cool, add 5 ml. of water, heat to boiling on a flame, cover, and digest on a low temperature hot plate for 1 or 2 minutes, or longer if necessary, to dissolve precipitated sulfates of iron or chromium. Then boil once more on a flame. Do not evaporate off all the water; otherwise salts may reprecipitate. Cool. If a precipitate of lead sulfate is present, add 30 ml. of water, filter through a No. 40 Whatman paper to a 125-ml. conical flask, and wash well with about 10 ml. of water. Discard the paper and precipitate. If no lead sulfate is present, add 40 ml. of water. Cool to room temperature.

Transfer the solution to a 125-ml. separatory funnel and proceed to the double carbamate separation and photometric determination as in preparation of the calibration curve. If the lower layer in the first carbamate extraction is very colored, indicating that considerable amounts of metals have been extracted, repeat the extraction with one or more 10-ml. portions of carbamate solution in order to ensure complete removal of extractable metals.

In some analyses it may be necessary to isolate the tin further by acid sulfide separation or cupferron extraction (or both), after separation of the tin from the bulk of the sample and before final isolation by carbamate extraction. If an acid sulfide separation is required, obtain the tin in 5 ml. of sulfuric acid in a 125-ml. Vycor conical flask, add 5 ml. of water, boil, digest, boil, and cool. Add 50 ml. of water, filter off lead sulfate if necessary and add 1 ml. of copper sulfate solution plus 2 ml. of tartaric acid solution. Pass into the solution a moderately rapid stream of hydrogen sulfide for 5 minutes. Filter on a Whatman No. 42 paper and wash the flask and paper thoroughly with sulfuric acid (1 + 99) which has been saturated with hydrogen sulfide. Discard the filtrate. Transfer the paper and precipitate back to the conical flask, add 10 ml. of sulfuric acid, cool, add 10 ml. of nitric acid, cover, and digest on a low temperature hot plate to destroy all the organic matter. Finally, add 1 ml. of perchloric acid and evaporate to 7-ml. volume to expel all nitric and perchloric acids. If, at this point, it is desirable to make a cupferron extraction, proceed as directed in the cupferron extraction method described below. Otherwise, add about 0.3 gram of hydrazine sulfate and proceed to the reduction, dilution, double carbamate separation, and photometric determination as directed above.

If a cupferron extraction is required after the separation of the tin from the bulk of the sample, obtain the tin in 7 ml. of sulfuric acid in a 125-ml. Vycor conical flask, add 1 ml. of perchloric acid, and evaporate on a flame to 5 ml. volume. Cool, add 5 ml. of water, boil, digest, boil, cool, add 40 ml. of water, filter off lead sulfate if necessary, and cool to room temperature. Add 10 ml. of cupferron solution, swirl to mix, allow to stand about 30 seconds, and transfer the solution to a 125-ml. separatory funnel. Add 20 ml. of chloroform, shake momentarily, relieve the pressure in the funnel, and shake vigorously for 30 seconds. Allow the layers to separate and then drain off the lower layer to a clean 125-ml. Vycor conical flask. Add 5 ml. of chloroform to the funnel, shake for 10 seconds, allow to settle, and drain the lower layer to the flask. Discard the aqueous layer. Expel the chloroform and destroy the organic matter by wet oxidation with small amounts of sulfuric, nitric, and perchloric acids as in preparation of the calibration curve. Evaporate to fumes of sulfuric acid to expel all nitric and perchloric acids. Dilute to 7-ml. volume with sulfuric acid, add about 0.3 gram of hydrazine sulfate, and proceed to the reduction, dilution, double carbamate separation, and photometric determination as directed above.

PROCEDURE FOR ANALYSIS OF LEAD AND 1% ANTIMONY-LEAD ALLOYS

Preparation of Calibration Curve. Transfer 0, 1.0, 2.0, 3.0, and 4.0 ml. of standard tin solution (20 γ of tin per ml.) to 250-ml. Vycor conical flasks and evaporate to complete dryness on a flame to expel all sulfuric acid. Cool somewhat, add 3 ml. of perchloric acid–nitric acid mixture, and heat until the acid begins to condense on the walls of the flask. Then add 50 ml. of lead nitrate solution and heat to gentle boiling. Add 1 ml. of potassium permanganate solution followed by 1 ml. of manganese nitrate solution, swirling to mix immediately after each addition. Continue to boil for 1 or 2 minutes to coagulate the precipitate. Filter through a No. 40 Whatman paper and wash the flask and paper thoroughly with water. Discard the filtrate. Transfer the paper and precipitate back to the flask. Add 10 ml. of sulfuric acid, cool, add 10 ml. of nitric acid, cover, and digest on a low temperature hot plate to destroy the organic matter. Add 1 ml. of perchloric acid and evaporate to 7-ml. volume to expel all nitric and perchloric acids. Add about 0.3 gram of hydrazine sulfate and proceed to the reduction, dilution, filtration, double carbamate separation, and photometric determination as in the general method for analysis of sample. Ignore traces of permanganic acid that may be present after evaporation to 7 ml. to expel perchloric acid. Prepare a calibration curve.

Analysis of Sample. Transfer 1 gram of the milled sample of lead or 1% antimony-lead alloy to a 250-ml. Vycor conical flask. Add 3 ml. of perchloric acid–nitric acid mixture, cover, and heat gently to dissolve the sample. When solution is complete, add 50 ml. of water, heat to boiling, add potassium permanganate and manganese nitrate, and continue as in preparation of the calibration curve. Carry a reagent blank, including the 50 ml. of lead nitrate solution, through all the steps of the procedure.

DISCUSSION

The behavior of tin with phenylfluorone closely resembles that of germanium. Optimum conditions for color development of the two metals are identical and the sensitivity of the reaction with phenylfluorone is about equal on a molar basis. No cloudy tin solutions have been observed. The tin-phenylfluorone color fades more rapidly than the corresponding germanium color.

The same metals interfere, but zirconium, gallium, and ferric iron cause high rather than low results for tin when color is developed at pH 3.1. Tantalum causes low results. Severe loss of tin is caused by coprecipitation with sulfates of chromium and iron. When appreciable amounts of phosphate are present, the tin is complexed and very low results are obtained.

During the early stages of the development of the method for tin it was considered desirable to develop the color at pH 1.8 instead of 3.1, in order to minimize possible metallic interference. Now that the excellent double carbamate method is used for the isolation of tin, there appears to be no advantage in working at pH 1.8. However, as very satisfactory results have been obtained at this higher acidity, the latter has been recommended in the method described above. At this higher acidity, the pH must be closely controlled, if reproducible results are to be obtained. Such control can be had by evaporating the tin solution to complete dryness and then redissolving the tin salt in a measured amount of sulfuric acid. If more than traces of other metals are present at the time of expulsion of the acid, results are low.

Table I. Determination of Tin in Synthetic Mixtures

No.	Other Metals and Materials Added	Mg.	Tin Found, γ
1	Nb	0.5	80
2	Ta	0.5	78
3	H_3PO_4	0.2 g.	79
4	Ta	0.5	54
	H_3PO_4	0.2 g.	
5	Nb	0.5	78
	H_3PO_4	0.2 g.	
6	Ti + Zr + Hf	0.5	80
7	Mo + V	0.5	80
8	Ga + As	0.5	79
9	Ge	0.5	126
10[a]	Ge	0.5	78
11	Sb	5	78
12	Sb	10	80
	H_3PO_4	0.2 g.	
13	Sb	10	80
14[a]	Sb	10	80
15	Fe	5	79
16	Cu + Bi	5	80
17	Pd + Te + Se + Cd + In	0.5	78
18	Cr + Ag + Hg	0.5	78
19	Fe + Mo	1	79

[a] Preliminary cupferron extraction performed before double carbamate separation of tin.

In the analysis of metal or organic samples it is necessary first to separate the tin quantitatively from the bulk of the sample. The traces of interfering metals that remain must be complexed or separated, to allow use of the phenylfluorone method. Hydrogen peroxide can be used to suppress the interference of small amounts of such metals as molybdenum, titanium, niobium, and tantalum, but, because the complexing action is not very effective and interference due to germanium and phosphate must be guarded against, separations are usually required. In some methods this final isolation of the tin is accomplished by distillation (*1*). In the present investigation it seemed desirable to find a more rapid method for isolating the tin.

A modification of the double carbamate extraction method used by Wyatt (*7*) for the isolation of arsenic, antimony, and tin provides an elegant means of isolating the tin following its separation from the bulk of the material of the sample. Such extractable metals as copper, bismuth, and mercury are separated from quinquevalent arsenic and antimony or quadrivalent tin by solvent extraction with a chloroform solution of diethylammonium diethyldithiocarbamate from sulfuric acid (1 + 9). Following this the arsenic, antimony, and tin are reduced to their lower valence states with thioglycolic acid and then separated from nonextractable metals by repeating the carbamate extraction. In order to isolate tin, any arsenic and antimony present must be selectively reduced to the trivalent state with hydrazine sulfate, so that they will be removed in the first extraction.

To establish the applicability of the double carbamate method for the isolation of tin, it was necessary to determine which metals are removed in the first carbamate extraction. To do this 0.5-mg. portions of each of 59 of the more common metals (*4*) were obtained in solution in 50 ml. of sulfuric acid (1 + 9) and extracted with 25 ml. of 1% carbamate solution. The only metals that showed appreciable extraction were arsenic(III), antimony(III), tin(II), mercury, silver, cadmium, indium, copper, bismuth, selenium, tellurium, palladium, molybdenum, chromium(VI), and iron(III). If a compatible acid such as perchloric acid is used in place of sulfuric acid, lead will be extracted. If the sulfuric acid concentration is less than 1 + 9, the number of metals that will be extracted by carbamate will increase. The addition of hydrazine sulfate and evaporation to copious fumes of sulfuric acid before dilution and extraction with carbamate do not prevent extraction of any of the metals listed except chromium. Colloidal elemental platinum or palladium tends to collect in the chloroform layer during the extraction.

Not all of the extractable metals listed can be quantitatively removed by carbamate extraction from sulfuric acid (1 + 9). The removal of ferric iron is particularly incomplete. Where extraction is incomplete, a small amount of the metal in question will accompany the tin in the second carbamate extraction. Experiments on 0.5-mg. portions of the interfering metals have shown that only molybdenum and ferric iron accompany the tin in measurable quantities in the double carbamate extraction. Fortunately, the amount of molybdenum found in the second extract is small, so that its interference in the photometric phenylfluorone determination can be prevented by the addition of hydrogen peroxide. Moreover, Wyatt has shown that extraction of iron by carbamate can be prevented by reducing it to the ferrous state by heating the solution to 50° C. and adding potassium iodide plus ascorbic acid. When not over about 0.1 mg. of iron is present, adequate reduction of the iron occurs at room temperature during the reduction of the tin with thioglycolic acid.

According to Wyatt, the recoveries of tin in the double carbamate extraction method tend to be slightly low, owing to reduction of traces of the tin by the carbamate itself in the first extraction. In order to minimize this reduction, he recommends the addition of a small amount of hydrogen peroxide before the first extraction. In the present investigation, where the amounts of tin present are small, the recoveries in the double carbamate extraction method are quantitative even in the absence of hydrogen peroxide. In view of this it should not be necessary to include the carbamate extractions in the procedure for the preparation of the calibration curve. On the other hand, as it is good practice to simulate conditions existing in an actual analysis, the carbamate separations have been included in the method recorded.

While the carbamate extraction method provides an excellent means of isolating tin from moderate quantities of interfering metals, it seemed desirable to provide additional separations for use where the impurity content is high after the separation of the tin from the bulk of the sample. It appeared that an acid sulfide precipitation or a cupferron-chloroform extraction of the tin from sulfuric acid (1 + 9) would serve the purpose. The acid sulfide precipitation, in the presence of tartrate and a small amount of copper to act as a coprecipitant, can be used to separate the tin from gallium, iron, vanadium, titanium, zirconium, hafnium, tantalum, niobium, tungsten, and many others. The cupferron separation can be used to separate the tin from germanium, arsenic, antimony(V), most of the copper, some of the bismuth and gallium, and a host of other metals. In the method recorded above, whenever necessary, either or both of the sepa-

rations are used previous to the carbamate extractions. Because of the insolubility of tantalum cupferrate in chloroform (*5*), tin is lost by occlusion when a cupferron extraction is performed on a tantalum-containing sample. In such cases it is advisable to precede the cupferron extraction by an acid sulfide separation to remove most of the tantalum.

In order to prevent antimony from accompanying tin in the cupferron separation, it is first oxidized to the quinquevalent state, by fuming with perchloric acid. As the oxidation is not quite complete (*3*), results for tin may be slightly high if no additional separation is made after the cupferron extraction, even though precautions are taken to obtain the contaminating antimony in the quinquevalent state at the time of color development. For this reason it is necessary, after a cupferron separation, to fume with hydrazine sulfate in order to remove the antimony in the first carbamate extraction. Separation of tin from large amounts of antimony by cupferron-chloroform extraction is not practical because of the tendency of antimony(V) to hydrolyze.

Of the metals that interfere in the phenylfluorone method for tin, only molybdenum and bismuth will accompany the tin in more than trace quantities, in the acid sulfide–cupferron extraction separation. Fortunately, both metals can be adequately removed subsequently by carbamate extraction.

Germanium is not extracted by carbamate, but when more than about 50 γ of germanium is present, the tin must be separated by a cupferron-chloroform extraction from dilute sulfuric acid solution before the carbamate separations are made. When the amount of germanium present is very high, two consecutive cupferron extractions are needed. Traces of tin may be determined in germanium metal by dissolving the sample in sodium hydroxide plus hydrogen peroxide (*2*), acidifying with sulfuric acid, performing two consecutive cupferron extractions, and finally isolating the tin by double carbamate extraction and determining it photometrically by the phenylfluorone method.

Phosphate complexes tin strongly in neutral or slightly acid solution. On the other hand, if the carbamate or cupferron extractions or the acid sulfide separation is performed from sulfuric acid (1 + 9) the complexing action is sufficiently attenuated so that microgram quantities of tin can be completely separated in the presence of as much as 0.25 gram of phosphoric acid.

The acid sulfide separation–cupferron extraction–carbamate extraction method for the isolation of tin fails only when the sample to be analyzed contains both tantalum and phosphorus. Neither metal causes trouble in individual or combined separations when present alone in moderate quantities, except for the difficulty due to the insolubility of tantalum cupferrate. Tin can be quantitatively separated by the carbamate or acid sulfide methods from solutions containing moderate amounts of tantalum plus phosphate, as long as the mixture has not been taken to fumes of sulfuric acid previous to the separation. If the sample has been fumed, as it must be in most cases, recoveries of tin are extremely low in the individual or combined separations, presumably because of the formation of some nonreactive tin-tantalum-phosphorus compound. Because of this and the fact that tin may be lost by coprecipitation with insoluble phosphates of zirconium, hafnium, antimony(V), and possibly titanium, if these metals plus phosphate are present in appreciable quantities, it is necessary to isolate the tin by distillation.

In order to adapt the phenylfluorone method for tin to the analysis of lead and 1% antimony-lead alloys it is necessary first to separate the tin from the bulk of the lead. Removal of the lead by precipitation as sulfate is not satisfactory, as low and variable recoveries of tin are encountered. Recovery of tin is much more complete when it is separated from the lead by coprecipitation with manganese dioxide from perchloric acid–nitric acid solution. This procdure has been recommended in the method for lead and 1% antimony-lead alloys recorded above. In order to simulate an actual analysis, lead is added to the samples used for the preparation of the calibration curve. Because tin-free lead is not usually available, pure lead nitrate is used for this purpose. Considerable amounts of the iron and bismuth that are present in the solution at the time of the manganese dioxide coprecipitation will accompany the tin. In contrast to this, copper is not appreciably coprecipitated. This suggests that the manganese dioxide separation will be useful for the separation of traces of tin from copper and its alloys.

EXPERIMENTAL

In order to determine the reliability of the general method for tin, synthetic sample solutions were prepared from aliquot portions of standard solutions of tin and other metals and then analyzed for tin as directed in the general method. The acid sulfide separation and cupferron extraction were not used. Each sample was made up to contain 80 γ of tin, 1 mg. of copper, 1 mg. of bismuth, and measured amounts of one or more other metals. The results obtained are shown in Table I.

Table II. Determination of Tin in Lead and 1% Antimony-Lead Alloys

No.	Sample	Tin Added, γ	Other Metals Added	Mg.	Tin Recovered, γ
1	Pb	20	Cu + Bi	1	20
2	Pb	40	Cu + Bi	1	41
3	Pb	80	Cu + Bi	1	81
4	Pb	80	Cu + Bi + Fe	1	81
5	Pb	80	Sb	1	80
6	Pb	80	Sb	5	80
7	Pb	80	Sb	10	82
8	Pb	80	Sb	10	80
			Fe	1	
9	1% Sb-Pb	20	None		21
10	1% Sb-Pb	40	None		42
11	1% Sb-Pb	80	Cu + Fe	1	80
12	1% Sb-Pb	80	None		80

In order to test the method for the analysis of lead and 1% antimony-lead alloys, aliquot portions of standard tin solution were evaporated to dryness in 250-ml. Vycor conical flasks to expel water and sulfuric acid. Aliquot portions of standard solutions of various metals (in perchloric acid solution) were added and evaporated to expel most of the water. One-gram portions of test lead or of a sample of Bell System 1% antimony-lead cable sheath alloy which was known to be low in tin were added. Tin was then determined by the method for lead and 1% antimony-lead alloys. In some instances an aliquot portion of a standard solution of antimony (in sulfuric acid solution) was added to the flask after the paper and manganese dioxide precipitate had been returned to the flask. The results obtained (Table II) have been corrected for small amounts of tin present in the test lead and 1% antimony-lead samples.

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RECEIVED for review January 14, 1956. Accepted May 9, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 1956.

Polarographic Analysis of Solutions of Alkyl Aryl Ketones and Benzaldehyde

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The polarographic characteristics of *tert*-butyl phenyl ketone, isopropyl phenyl ketone, *n*-propyl phenyl ketone, and benzaldehyde, and mixtures of the ketones with benzaldehyde are given. The half-wave potential of each is independent of the other carbonyl, and sufficiently separated so that mixtures can be analyzed with errors of less than 3% in the concentration of ketone. The diffusion currents are linear with concentration and independent of the presence of the other carbonyls. The errors for benzaldehyde are less than for ketone.

A SERIOUS difficulty in the study of oxidations of alkylarylcarbinols is the lack of a suitable method of analyzing the resulting oxidation mixture. If some of the constituents of this mixture are reducible at the dropping mercury electrode, there exists the possibility of analyzing the mixture polarographically.

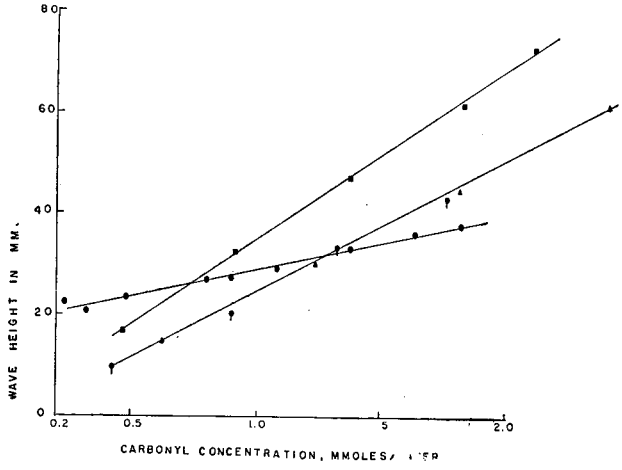

Figure 1. Wave height as a function of carbonyl

● Benzaldehyde
▲ Isopropyl phenyl ketone
♦ *n*-Propyl phenyl ketone
■ *tert*-Butyl phenyl ketone

The literature of organic polarography is voluminous (*8*), but relatively little has been done on the determination of two or more reducible organic compounds in the same solution. Adkins and coworkers (*1*, *2*) have worked with aryl aryl and alkyl aryl ketones. In some two-ketone systems the diffusion current was quantitatively proportional to the concentration of the ketones being determined and independent of other ketones present—e.g., *n*-propyl phenyl ketone and benzophenone, and isopropyl phenyl ketone and benzophenone—but this was not the case with other combinations—e. g., acetophenone and benzalacetone. Benzalacetone had a marked effect on the wave height of acetophenone.

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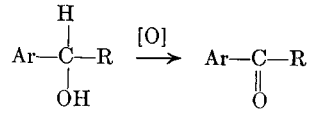
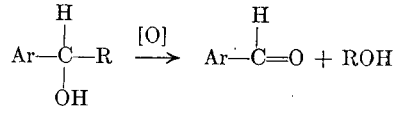
The oxidation mixture to be analyzed is a result of the normal oxidation of an alkylarylcarbinol (*4*, *7*)



with simultaneous anomalous oxidation:



The cleavage products may undergo further oxidation to give ArCOOH and R'CHO, if ROH is a primary alcohol. These secondary oxidations are minimized by properly choosing the conditions of the reaction (*4*).

The carbinols oxidized were *tert*-butylphenylcarbinol, isopropylphenylcarbinol, and *n*-propylphenylcarbinol, each of which gave the corresponding ketone and benzaldehyde as products. The information obtained by determination of the concentration of the ketone and the benzaldehyde will add considerably to the knowledge of the mechanism of normal and anomalous oxidations.

Kolthoff and Lingane (*5*) give polarographic characteristics of *n*-propyl phenyl ketone, isopropyl phenyl ketone, and benzaldehyde. This paper is concerned mainly with the determination of the characteristics of *tert*-butyl phenyl ketone, and the relationship of the diffusion current to the concentration of the ketones in solution with benzaldehyde.

Table I. Solvents and Supporting Electrolytes Studied

Solvents	Supporting Electrolytes
Isopropyl alcohol	Tetraethylammonium bromide
50% dioxane-water	Lithium chloride
80% dioxane-water	Tetra-*n*-butylammonium iodide
50% ethyl alcohol–water	Lithium hydroxide

In an attempt to find the best solvent and electrolyte to use in this work, the materials in Table I were investigated. A combination of lithium hydroxide and 50% ethyl alcohol–water gave the best results. A clearly defined wave and an electrolyte with a half-wave potential that did not interfere with the determination of the benzaldehyde or the ketones were obtained. In all the work reported in this paper 0.1*M* lithium hydroxide was used as the electrolyte and 50% ethyl alcohol–water as the solvent.

EXPERIMENTAL

Materials. The *tert*-butyl phenyl ketone was prepared in these laboratories by the oxidation of *tert*-butylphenylcarbinol (*7*). It had a refractive index ($n_D^{20.1}$) of 1.5073; literature value is 1.5086 (*6*). The isopropyl phenyl ketone was Eastman Kodak white label with the following constants: $n_D^{23} = 1.5142$, $d_4^{23°} = 0.981$; literature values are $n_D^{18.6} = 1.5192$, $d_{20°}^{20°}$ 0.985 (*6*).

The *n*-propyl phenyl ketone was also Eastman Kodak white label: $n_D^{22.8} = 1.5169$, $n_4^{23.8°}$, 0.984; literature values: $n_D^{18.3} = 1.5202$, $d_4^{18} = 0.990$.

Eastman Kodak chlorine-free benzaldehyde was distilled in a nitrogen atmosphere at reduced pressure before use. Experimental: $n_D^{20} = 1.5442$; literature value: 1.5456 (*6*).

Baker's C.P. lithium hydroxide was used as the electrolyte.

The ethyl alcohol was C.P. absolute alcohol. Originally it was distilled through a fractionating column, but as no noticeable interferences appeared, if used without this purification, the purification was discontinued.

Distilled water was used throughout.

Stock solutions (10^{-2} *M*) of the ketones and benzaldehyde in 50% ethyl alcohol–water solvent were prepared quantitatively. A lithium hydroxide stock solution, also prepared in 50% ethyl alcohol–water, had a strength such that upon final dilution a 0.1*M* solution resulted.

Apparatus. A Sargent-Heyrovský Model XII polarograph was used for this analysis. All polarograms were taken on photographic paper. A Beckman Model G pH meter was used for pH measurements. In conjunction with a saturated calomel electrode, the pH meter was used to correct the cell potential to volts *vs.* the saturated calomel electrode, by immersing the saturated calomel electrode in the solution and inserting the other lead in the form of a copper wire into the mercury arm of the cell. The correction was found to be −0.210 volt, which must be added to the electromotive force as calculated from the rotating potential divider. (No correction was made for the internal resistance-drop across the instrument.)

The capillary was a Sargent dropping mercury capillary with these characteristics: pressure, 35.9 cm. of mercury, $m = 1.886$ mg. per second, $t = 7.8$ seconds per drop, $m^{1/6}t^{2/3} = 2.150$ (open circuit in distilled water). The drop time in solution was 3.9 seconds per drop at 0.0 volt and 3.3 at 1.00 volt.

A conventional Heyrovský polarographic cell was used in all analyses.

Temperature was maintained at 25.0° ± 0.5° C. by immersing the entire cell in a water bath.

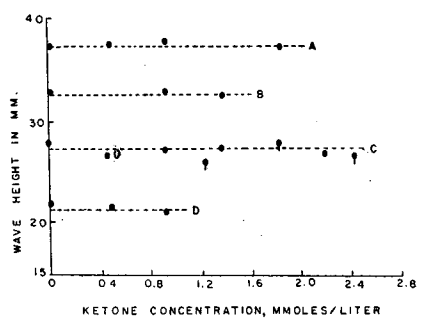


Figure 2. Dependence of wave height of benzaldehyde on ketone concentration

A. 1.83 m*M* in benzaldehyde
B. 1.37 m*M* in benzaldehyde
C. 0.916 m*M* in benzaldehyde
D. 0.46 m*M* in benzaldehyde
● *tert*-Butyl phenyl ketone
◆ Is opropyl phenyl ketone

Procedure. The solution to be analyzed was prepared in the cell from the stock solutions by dilution to give a ketone and benzaldehyde concentration in the neighborhood of $10^{-3}M$. The cell was placed in the constant temperature bath for about 8 minutes to allow it to reach temperature equilibrium.

A photographic plate was used to record the curve of current *vs.* voltage. After developing, this graph was analyzed to determine the diffusion current and the half-wave potential. The pH and the electromotive force correction were measured directly in the cell.

RESULTS AND DISCUSSION

The results of the benzaldehyde determination are given in Figure 1. The diffusion current is directly proportional to the concentration. If the curve is extrapolated to zero benzaldehyde concentration, it does not pass through the origin. This seems to be due to a slight prewave, a rise that immediately precedes the benzaldehyde wave. As this is constant and cannot be separated from the benzaldehyde diffusion current, its height was incorporated in the height of the benzaldehyde wave. Being a constant, it does not interfere with the analysis. The estimated half-wave potential of this prewave does not correspond to any impurity in ethyl alcohol. The lithium hydroxide, the ethyl alcohol, and the water were varied independently without producing a noticeable effect on the prewave. The intercept of Figure 1 corresponds to the height of the prewave. The primary concern of this work is the concentration determination of the species; as the prewave does not interfere, it was not studied further.

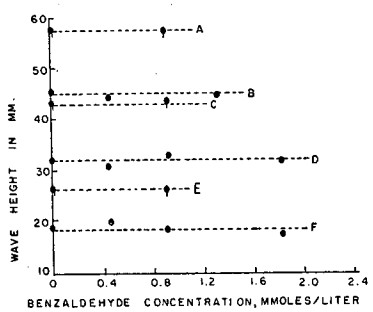


Figure 3. Dependence of wave height of ketone on benzaldehyde concentration

A. 2.44 m*M* isopropyl phenyl ketone
B. 1.37 m*M* *tert*-butyl phenyl ketone
C. 1.83 m*M* isopropyl phenyl ketone
D. 0.92 m*M* *tert*-butyl phenyl ketone
E. 1.22 m*M* isopropyl phenyl ketone
F. 0.46 m*M* *tert*-butyl phenyl ketone

Borchesdt, Meloche, and Adkins (*2*) report a constant correction factor which must be subtracted from the wave height to give a proportionality between wave height and concentration. No prewave is apparent upon inspection of their published polarograms. The intercept of Figure 1 would correspond to a mathematical factor to be subtracted from the measured wave height to give proportionality.

The prewave does affect the measured half-wave potential of the benzaldehyde, which was determined to be −1.51 ± 0.02 volts *vs.* S.C.E. at an apparent pH of 12.8. The literature reports −1.48 volts *vs.* N.C.E. at pH 11.3 (*5*).

Figure 1 also indicates the linearity of the diffusion current with the concentration of *tert*-butyl phenyl ketone, isopropyl phenyl ketone, and *n*-propyl phenyl ketone. The half-wave potentials of the ketones are −1.92 ± 0.03, −1.82 ± 0.02, and −1.75 ± 0.02 volts, respectively, all measured against a saturated calomel electrode at an apparent pH of 12.8. The literature values for the reduction potentials of isopropyl phenyl ketone and *n*-propyl phenyl ketone are −1.70 and −1.64 volts *vs.* N.C.E. (pH not given) (*3*).

Although the concentrations of the ketones and benzaldehyde are directly proportional to their diffusion current, the relationship may not hold when one of the ketones is in solution with benzaldehyde. Figure 2 gives the diffusion current of a series of benzaldehyde solutions as a function of the ketone concentration. Figure 3 gives the diffusion current of ketone solutions as a function of the benzaldehyde concentration. The slopes of each curve are 0 within experimental error, indicating that the diffusion current of the carbonyl is independent of the other component.

The half-wave potential measured for each component was the same as in the absence of the second component.

ACKNOWLEDGMENT

The authors wish to thank H. A. Neidig, Lebanon Valley College, for his encouragement and assistance on this problem.

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RECEIVED for review November 25, 1955. Accepted May 4, 1956.

X-Ray Fluorescence Determination of Barium, Titanium, and Zinc in Sediments

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X-ray fluorescence analysis has been applied to the rapid and quantitative assay of barium, titanium, and zinc in marine sediments. Internal standards were used to minimize matrix effects in the samples, which ranged in composition from nearly pure calcium carbonate to glauconite and deep-sea clays, which have compositions similar to materials encountered in ordinary silicate analysis. Arsenic was employed as the internal standard for zinc, and lanthanum served for both barium and titanium. The barium $L\alpha_1$ and titanium $K\alpha$ peaks, which had the highest usable sensitivities, overlapped and empirical corrections to compensate for this effect were ascertained. The lower limits of detectability were 0.01% for titanium and barium and 0.004% for zinc.

STUDIES on the geochemistry of marine sediments have necessitated the determination of such minor elements as barium, titanium, and zinc in clay and calcareous materials. X-ray fluorescent techniques appeared advantageous for a number of reasons. With the selection of proper internal standards (*1*), the methods can be applied to a large variety of mineral types.

Table I. Composition of Typical Marine Clay and Mock Clay Used in Preparation of Standards

	Weight % of Constituents	
	Marine clay	Synthetic clay
SiO_2	60	59
Al_2O_3	15	17
Fe_2O_3	7	8
Na_2O	5	3
K_2O	4	5
MgO	4	2
CaO	4	5
MnO	1	1

The nondestructive nature of the technique does not demand the sacrificing of the small amounts of sample available for analysis. Finally, the method is rapid and reasonably simple. This paper considers some of the problems encountered in the quantitative x-ray spectrographic assay of titanium, barium, and zinc in marine sediments of widely varying composition.

INSTRUMENTATION

A Norelco x-ray spectrograph with a 50-kv. tungsten tube (Machlett OEG-50), an argon-filled Geiger tube detector, and a lithium fluoride analyzing crystal were used. For the barium and titanium determinations a helium path was constructed from a polyethylene bag and attached to the apparatus in essentially the manner described by Davis and Van Nordstrand (*2*). Helium flow rates of at least 700 ml. per minute were used to obtain maximum reproducibility of results. A tenfold increase in sensitivity for the barium $L\alpha_1$ and the titanium $K\alpha$ radiation resulted from the substitution of a helium path for an air path. An aluminum planchet designed to hold about 1 gram of sample was machined to fit the sample holder provided with the instrument. The x-ray tube was operated at 45 kv. and 35 ma.

EXPERIMENTAL

Standards. Reagent grade chemicals were used throughout the investigation. Standard zinc samples were prepared by the addition of zinc acetate dihydrate to portions of a mock marine clay of the composition given in Table I, on a water-free basis. Barium and titanium primary standards were made by the addition of barium carbonate and titanium dioxide to calcium carbonate.

Internal Standards. The choice of an internal standard for a given element depends not only upon the desirable characteristic of having an x-ray fluorescence peak adjacent to the peak of the element in question but also upon the following criteria: (1) ready availability in an inexpensive and stable form; (2) sufficient peak separation from the element in question to make background measurements possible; (3) absence from the matrix

Table II. X-Ray Fluorescence Peak and Background Wave Lengths of Elements and Internal Standards

(Lithium fluoride used as analyzing crystal)

Element	Emission	Wave Length, A.	Degrees 2θ	
			Peak	Background
Zinc	$K\alpha$	1.437	41.81	40.96 46.00
Arsenic	$K\alpha$	1.177	33.98	33.00 35.00
Barium	$L\alpha_1$	2.776	87.16	85.00 89.00
Titanium	$K\alpha$	2.750	86.08	85.00 89.00
Lanthanum	$L\alpha_1$	2.665	82.86	81.50 85.00

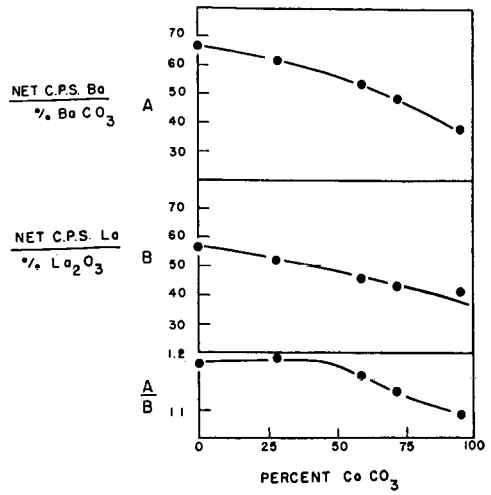

Figure 1. Effect of carbonate concentration upon fluorescent intensities of barium and lanthanum

Carbonate diluted with mock clay

under investigation as well as from the x-ray target; and (4) no absorption or enhancement effects with the element under consideration (see below). Lanthanum, the periodic table neighbor of barium, fulfilled the above requirements most completely for both barium and titanium. Arsenic was chosen as the internal standard for zinc, inasmuch as neither gallium nor germanium was available and copper was a contaminant in the target of the x-ray tube.

Preparation of Samples and Standards. The standards and sediment samples were initially ground to 300 mesh. After the addition of the internal standard to a given sample, the mixture was ground for at least 20 minutes in a machine-operated mullite mortar. Homogeneity was tested by intensity measurements of the element in question in different portions of the sample. In no case was there any indication of insufficient mixing.

The internal standard was added to the sediment or standard samples in amounts such that its peak had an intensity near that of the element in the upper concentration ranges. Arsenic trioxide addition amounted to about 0.6% of the sample weight for zinc concentrations of 0.004 to 0.6%. For titanium and barium concentration ranges of 0.01 to 2.0%, the amount of lanthanum trioxide standard amounted to approximately 2.5% of the sample weight.

Lines. The lines used in the analytical work are given in Table II. The $K\alpha$ line of barium could not be adequately excited at the 50-kv. x-ray tube operating potential and hence recourse was made to an L line of this element. Times for fixed counts of 25,600 at the fluorescence peaks and 6400 at the background positions were taken. The background was determined on both sides of the peaks and from these measurements the background at the peak was calculated by interpolation. Background measurements made on mock clays free of the element to be analyzed indicated that such interpolations were valid.

Calculations. The net counting rates per unit weight per cent of the element and of the corresponding internal standard were obtained. Linear calibration curves on log-log paper resulted from plotting the weight per cent of the element against net counts per second of element/net counts per second of internal standard per weight per cent of internal standard.

RESULTS AND DISCUSSION

Barium and Titanium Determination. Although the use of internal standards in x-ray fluorescence spectroscopy largely reduces matrix effects upon the peak intensity of an element of given concentration, enhancement or absorption of one or both of the members of a line pair must be considered. This problem has been discussed recently by Adler and Axelrod (*1*), who have divided these effects into two categories: absorption effects and excitation effects.

Preferential absorption may occur if a matrix element has an absorption edge between the internal standard line and the line of the element being determined or on the long wave-length side of these two lines. Even with the proper choice of an internal standard, the intensity ratio may show a significant difference.

Preferential excitation may be caused by a matrix element which has a fluorescence line falling between the absorption edges of the internal standard and element being measured or on the short wave-length side of both. Excitation effects are usually smaller than those due to preferential absorption but may affect the results appreciably.

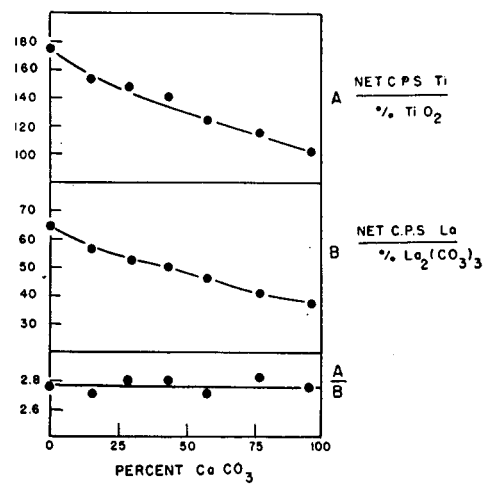

Figure 2. Effect of carbonate concentration upon fluorescent intensities of titanium and lanthanum

Carbonate diluted with mock clay

In marine sediments the calcium concentration, expressed as calcium carbonate, varies from a few to nearly 100%. As the K absorption edge of calcium is 3.070 A. and the measured peaks of barium, titanium, and lanthanum are less than this value, it was necessary to investigate the absorption effect of calcium upon the emission of the studied elements and their internal standard. The two upper curves in Figure 1 show the intensity of the barium and lanthanum lines as a function of calcium carbonate content in the mock clay. In the presence of 95% calcium carbonate, the barium intensity is only about 55% of the value in the carbonate-free clay mixture. The lanthanum intensity is diminished to a slightly lesser degree with increasing calcium content. This result is not unexpected, inasmuch as absorption by the matrix element decreases with decreasing wave length of the exciting peak. There is approximately a 10% variation in the barium-lanthanum ratio between 0 and 100% calcium carbonate. From this curve corrections are applied in analysis to the values of the barium concentration taken from the calibration curve, which was prepared in a pure calcium carbonate matrix, to correspond to the actual calcium carbonate content of the samples.

The curves in Figure 1 may reflect two effects. First, the major constituents of the clay (silicon, aluminum, potassium, sodium, and magnesium) have K absorption edges at higher wave-length values and hence absorb barium and lanthanum radiation to a smaller extent than calcium. Secondly, iron in the clay mixture has a $K\alpha$ line at 1.937 A., which may preferentially enhance the intensity of lanthanum (L_{III} absorption edge is 2.259 A.) over barium (L_{III} absorption edge is 2.363 A.) Thus, the greater

the clay content the lower the barium-lanthanum intensity ratio due to iron excitation.

Figure 2 shows the intensity of titanium and lanthanum as a function of the calcium carbonate concentration. The titanium-lanthanum ratio shown in the bottom curve is constant throughout the range of calcium carbonate concentrations in the clay. This may result from the compensation of two effects: (1) the preferential absorption of titanium over lanthanum radiation by calcium resulting in a decreasing titanium-lanthanum ratio with increasing calcium carbonate, and (2) the preferential enhancement of lanthanum $L\alpha_1$ emission over titanium $K\alpha$ emission by iron, which results in a decreasing titanium-lanthanum ratio with decreasing calcium carbonate. Hence, within experimental error the titanium-lanthanum intensity ratio is independent of calcium carbonate content.

The barium and titanium peaks used in the analysis were not completely resolved. A mutual intensity enhancement results which requires an empirical correction when both elements are present in a given sample. Titanium increases the barium intensity to a greater extent than barium affects the titanium values,

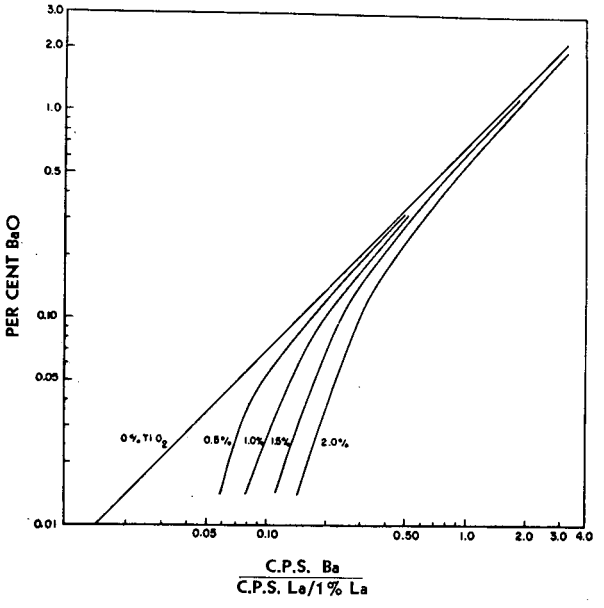

Figure 3. Barium calibration curves in presence of varying amounts of titanium

Matrix, calcium carbonate

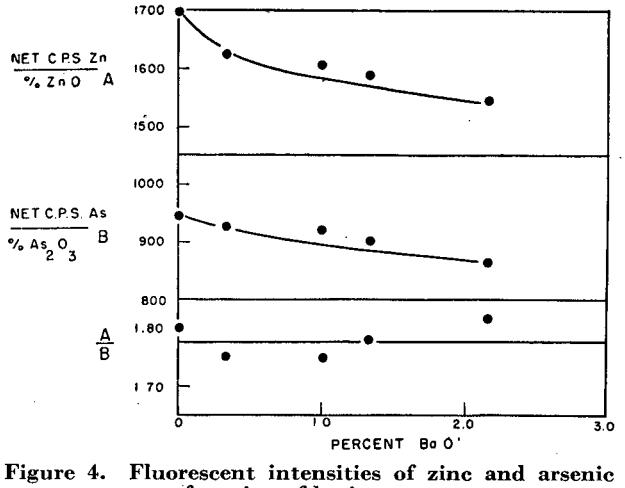

Figure 4. Fluorescent intensities of zinc and arsenic as a function of barium content

Matrix, mock clay

but for most of the sediments encountered corrections for both elements were significant. Figure 3 shows barium standard curves in the presence of varying quantities of titanium. It emphasizes the large relative error which would occur in low barium samples if the presence of moderate amounts of titanium were neglected. The correction is linear and amounts to an absolute value of -0.045% barium oxide per 1.0% titanium oxide in the range of 0.01 to 2.0% barium oxide and titanium oxide. The barium $L\gamma_1$ line, although it is in a clear part of the spectrum, was not used, as its intensity was too low for many of the samples analyzed.

Corrections for barium interference upon titanium are smaller, amounting to -0.01% titanium oxide per 1.0% barium oxide within the same ranges noted above.

Titanium was determined in five calcareous sediment samples of varying calcium carbonate content by spectrophotometry (*3*) and x-ray fluorescent techniques. A comparison of the results is given in Table III.

Zinc Determination. Zinc was determined only in the clay sediments, inasmuch as these samples always contained more than 0.004% zinc, the lower limit of assay. Davis and Van Nordstrand (*2*) had shown that the presence of 0.6% barium oxide decreases the intensity of the zinc $K\alpha$ radiation by about 30% in lubricating oils. Barium is present in marine clays in amounts up to 2.0%. Figure 4 shows the absorption effect to be slightly greater than 10% for zinc as well as arsenic. The zinc-arsenic intensity ratio is independent of the barium content. It is not surprising that barium absorbs strongly in lubricating oils but not in the sediments under consideration. The oil matrix contains low atomic number elements, which are weak absorbers, and barium becomes a major contributor to the absorption effect. In sediments, where more highly absorbing elements are present, the absorption by barium is somewhat overshadowed.

Iron with a K absorption edge of 1.744 A. should readily absorb zinc $K\alpha$ radiation (1.437 A.). The data in Table IV confirm this. However, arsenic is also absorbed to the same degree, so that the intensity ratio is not altered appreciably by wide variations in iron content.

As previously noted, arsenic was used as an internal standard in preference to the periodic table neighbors of zinc because of considerations of availability and x-ray target impurities. Arsenic $K\alpha$ radiation (1.177 A.) can be absorbed by zinc. However, by the use of a near constant weight per cent addition of the internal standard to the calibration standards, as well as the sediments, this small effect is eliminated (Figure 5).

Table III. Determination of Titanium in Sediments

Sediment Type	Number	TiO_2, % X-ray	TiO_2, % Spectrophotometry	$CaCO_3$, %
Calcareous sediments	58-5	0.09	0.08	78
	58-70	0.30	0.33	30
	58-130	0.33	0.33	25
	58-149	0.13	0.14	72
	58-471	0.33	0.30	20
Glauconite	15006B	0.18	0.19	0
	15019	0.27	0.29	0
	15030B	0.10	0.09	0

Table IV. Absorption of Zinc $K\alpha$ Radiation by Iron in Mock Clays

ZnO, %	As_2O_3, %	Zn/% ZnO, C.P.S. *A*	As/% As_2O_3, C.P.S. *B*	*A/B*	Fe_2O_3, %
0.0425	0.518	2380	1092	2.18	6.9
0.0410	0.500	2195	986	2.22	10.3
0.0396	0.483	2045	942	2.17	13.0
0.0381	0.464	1942	880	2.21	16.4
0.0368	0.449	1810	806	2.25	19.5

Table V. X-Ray and Emission Spectrographic Determination of Zinc in Pacific Ocean Pelagic Clays

Sample	Zinc Oxide, % X-ray	Emission
30 BG 24	0.014	0.011
31 BG 32	0.017	0.013
49 BP 52	0.018	0.022
49 BP 140	0.027	0.024
50 BP 16	0.018	0.017
50 BP 760	0.036	0.031

Results of zinc determinations on six red clays were assayed both by x-ray fluorescence and by the emission spectrographic method of Wedepohl (*4, 5*) in Göttingen. The results are given in Table V.

The reproducibility of an analysis on a given sample was governed by the random errors resulting from the counting techniques. For the lanthanum and barium determination, the absolute counting error varied from 10 to 4%, where the elements varied in concentration from 0.1 to 2.0%. In the case of zinc, the counting error varied from 15 to 4%, where the zinc concentration ranged from 0.004 to 0.6%.

ACKNOWLEDGMENT

This work was sponsored by the Office of Naval Research and the American Petroleum Institute, Project 51, under contracts with the University of California.

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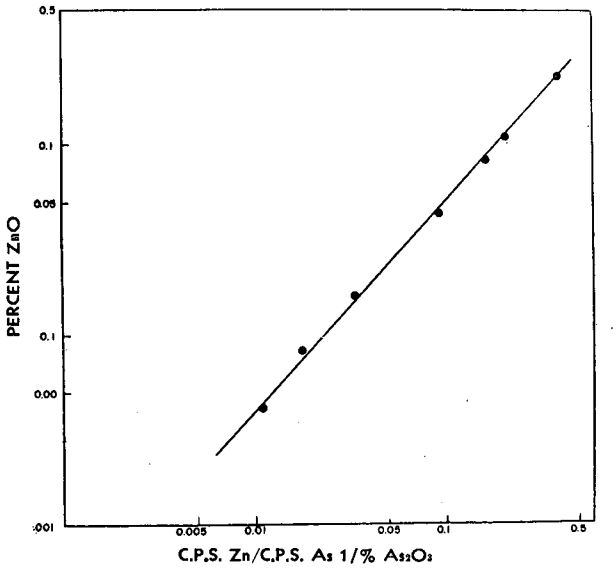

Figure 5. Calibration curve for zinc in clay sediments

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RECEIVED for review December 5, 1955. Accepted May 9, 1956. Contribution from Scripps Institution of Oceanography, No. 860.

Rapid Polarographic Determination of Uranium in Nonaqueous Solvents

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Rapid but precise polarographic methods have been developed for the determination of uranium in dissolved ores or inventory solutions, after its separation by solvent extraction from ions that interfere or from hazardous radioactivity. Optimum conditions are discussed for the separation and the subsequent polarographic analysis. Uranium is extracted with tributyl phosphate–isopropyl ether, the organic extract is diluted with a solvent, such as glacial acetic acid, and supporting electrolyte is added. By use of standard polarographic apparatus well formed uranium waves are obtained in several nonaqueous media. The simplicity of the manipulations required is advantageous in work with radioactive samples.

THE determination of the uranium content of dissolved ores or of various inventory solutions is frequently complicated by the presence of ions that interfere with the analytical method and/or by the hazardous presence of very radioactive fission products. For such analyses, the first step is usually the separation of uranium from the solution. A well-known and very effective means of separation is solvent extraction with tributyl phosphate, either alone or diluted with various solvents which tend to improve the separation of the phases. Such samples presented for analysis often contain nitric acid and may contain large amounts of aluminum nitrate.

Rapid polarographic methods for the determination of uranium in which uranium is first separated from copper and/or fission products by extraction have been developed (*1, 8, 10*). The extraction serves two purposes: decontamination of uranium from radioactivity, if present, and elimination of polarographic interferences caused by overlapping waves of cations, such as those of copper, having half-wave potentials very close to that of uranium. This method (*1, 10*) assumes prior dissolution of sample.

The procedure (*1, 10*) consists of the extraction of uranium into either tributyl phosphate–Varsol (a kerosine-type hydrocarbon) or tributyl phosphate–isopropyl ether, followed by the polarographic determination of the uranium in a nonaqueous solution of the extract or, alternatively, in a water strip (aqueous extract) of the organic extract. The isopropyl ether extraction is preferred because very satisfactory diluents were found which permit the uranium to be determined directly in the organic solution by polarographic analysis. Two procedures have been used to obtain polarograms of the uranium in the preferred extract. The tributyl phosphate–isopropyl ether extract may be diluted to

bring the uranium concentration within the polarographic range (approximately 50 γ of uranium per ml.) with a suitable solvent (glacial acetic acid or ethyl alcohol), to which a supporting electrolyte (0.25M lithium perchlorate) is added. Alternatively, the extract may be stripped with water and an aliquot of the heated strip, to which supporting electrolyte (nitric acid) has been added, analyzed polarographically for uranium.

Table I. Effect of Nitric Acid Concentration of Aqueous Phase on Extraction of Uranium(VI) into Tributyl Phosphate–Varsol (30:70 Vol. %)

Composition of aqueous phase. 1.3M $Al(NO_3)_3$
1.0 mg. of U per ml.
0.07 mg. of Cu per ml.
HNO_3, as indicated

Volume of aqueous and of organic phases. 50 ml.

HNO_3 in Aqueous Phase, N	Uranium Recovered, Mg. Run 1	Run 2
<0.01[a]	50.2	50.1
0.5	49.4	49.5
1	48.2	48.4
2	48.6	49.2
5	49.1	49.9

[a] This concentration of HNO_3 resulted from HNO_3 present in $Al(NO_3)_3$ used.

Table II. Effect of Tributyl Phosphate Content of Isopropyl Ether on Extractability of Uranium(VI) and Fission Products into Tributyl Phosphate–Isopropyl Ether

Composition of aqueous phase, as in Table I, but HNO_3 absent.
Volumes of aqueous and organic phases, 25 ml.

Sample	TBP in Isopropyl Ether Extractant, Vol. %	Uranium Found, Mg.[a]	Beta Activity of Organic Phase $\times 10^{-3}$, C.P.M./Ml.[b]	Approximate Decontamination Factor
1	1	18.9	0.99	100
2	1	19.0	0.90	100
3	5	24.95	1.1	100
4	5	24.80	1.2	100
5	5	24.82	1.2	100

[a] Total of 25.0 mg. present in original aqueous phase.
[b] Total beta activity of 1.42×10^5 c.p.m./ml. present in original aqueous phase.

Le Strange, Lerner, and Petretic (*5*) have supplemented the authors' work with a polarographic study of extraction with 100% tributyl phosphate from a uranium-containing solution that was 5N in nitric acid or from solutions slightly acid in nitric acid and highly salted with aluminum nitrate. In the method (*1*, *10*) reported here, uranium waves of excellent form were obtained when 0.25M lithium perchlorate was used as supporting electrolyte and no maxima were observed in the various recommended media.

EXTRACTION OF URANIUM INTO NONAQUEOUS SOLVENTS

The primary objective of developing the extraction procedure for uranium was to eliminate copper interference and thus to permit a precise polarographic determination of uranium. The extraction procedure was tested on a macro scale rather than a smaller scale in order to check the recovery and decontamination of uranium by use of classical analytical methods.

Tributyl Phosphate–Varsol as Separation Solvent. A sample was made by dissolving reagent grade aluminum nitrate, uranyl nitrate, and copper nitrate in distilled water to give a solution that contained 1.0 mg. of uranium and 0.07 mg. of copper per ml., and was 1.3M in aluminum nitrate. Fifty-milliliter aliquots of the sample were adjusted to various acidities with nitric acid and shaken 10 minutes with an equal volume of 30:70 volume % tributyl phosphate–Varsol mixture in a 250-ml. separatory funnel. The phases were allowed to separate and the aqueous layer was discarded. The organic layer was stripped with three separate 50-ml. volumes of distilled water. The total strip (150 ml.) was analyzed for uranium by the ferric sulfate volumetric method (*7*).

The results shown in Table I indicate that, when aluminum nitrate is present, nitric acid is not necessary to salt the uranium into the organic phase. The absence of the acid is advantageous, because the uranium is more easily stripped from the organic phase with distilled water than when acid is present.

Ether extractions without the aid of complexing agents have been used for many years to separate uranium from many elements. Furman, Mundy, and Morrison (*2*) have investigated the effects of salting out and inhibiting agents. Moore (*6*) used 20% tributyl phosphate in carbon tetrachloride to study the mechanism of extraction of uranium from nitric acid solutions. Spence and Streeton (*9*) also used tributyl phosphate in carbon tetrachloride to extract uranium in their work with a microrotary extractor.

Tributyl Phosphate–Isopropyl Ether as Separation Solvent. Some phase-separation difficulty was experienced with tributyl phosphate-Varsol mixtures because a slight emulsion formed at the interface. For this reason, a solution of tributyl phosphate and isopropyl ether (30:70 volume %) was tried as an extractant for uranium. Excellent phase separations and extraction efficiencies were obtained. Again, low acidity of the aqueous phase facilitated the stripping of uranium from the organic phase. The amounts of residual uranium in the raffinate (the original aqueous phase after the extraction of one or more components into an organic extractant) and in the stripped organic phase were checked by fluorometric (*4*) analyses. The results showed that only 0.05% of the uranium originally present was left in the raffinate and that less than 0.01% of the uranium originally present remained in the stripped organic phase. The analysis of the aqueous strip showed a recovery of 49.96 mg. of uranium from a sample that contained 50.00 mg. of uranium.

Because the samples to be analyzed by this method were expected to be highly radioactive, it was desired to determine the approximate decontamination effected by the extraction procedure.

A volume of 0.1 ml. of a uranium-free aqueous solution that contained fission products (3.55×10^7 counts per minute of beta radioactivity) was added to a 25-ml. aliquot of the sample of composition indicated above to give a solution that contained 1.42×10^5 counts per minute of beta radioactivity per milliliter. Duplicate 25-ml. samples that were made in this manner were shaken with 25-ml. of a tributyl phosphate–isopropyl ether (30:70 volume %) mixture for 10 minutes in a separatory funnel. The phases were allowed to separate and the radioactivity of each phase was determined. The duplicate samples gave counts of 6.7×10^4 and 6.6×10^4 beta counts per minute per ml. This indicated a decontamination factor of only approximately 2. M. T. Kelley suggested that decreasing the tributyl phosphate content of the extractant should increase the decontamination and still permit complete recovery of the uranium. Therefore, 25-ml. aliquots of the synthetic sample were made radioactive by the addition of 0.1 ml. of the fission product solution, and the resulting solutions were extracted with 25 ml. of isopropyl ether that contained various amounts of tributyl phosphate.

The results are shown in Table II. These above data show that a 5% solution of tributyl phosphate in isopropyl ether is an effective uranium extractant and gives decontamination factors of approximately 100.

POLAROGRAPHIC DETERMINATION OF URANIUM

In Nonaqueous Solvents. POLAROGRAPH, ACCESSORY APPARATUS, AND GENERAL POLAROGRAPHIC PROCEDURE. The polarograph used in this work is of very high sensitivity and also is well suited for work with solutions of low conductivity. It has been described in detail (*3*). The auxiliary apparatus used (cell and nitrogen gas purification train) was conventional.

For all work with organic solvents, an agar–potassium nitrate salt bridge leading to an external saturated calomel electrode was used, even though the use of special reference electrodes in nonaqueous systems is often reported in the literature. Well-defined uranium waves were obtained at an apparent half-wave potential of about −0.2 volt *vs.* the saturated calomel electrode. Although it is usual to deoxygenate organic solvent systems with purified nitrogen for protracted periods of time before polaro-

graphing them, 10 minutes was found to be satisfactory in this study. Whenever a volatile organic solvent was used, the purified nitrogen was saturated with solvent vapor by means of a gas scrubber filled with that solvent prior to use of the nitrogen as a deoxygenating agent in the polarographic cell. This prevented evaporation of the solvent in the cell, with attendant concentration changes.

In work with organic extracts, it was found necessary to dilute the extract quantitatively with a dilution solvent if the water-stripping procedure was not used. This dilution served three purposes: (1) The concentration of uranium in the solution to be polarographed was brought from about 1 mg. of uranium per ml. to within the range of the polarograph used—i.e., to about 50 γ of uranium per ml.; (2) use of polar dilution solvents of high dielectric constant enhanced the ionization and conductivity of the system; and (3) dilution of the tributyl phosphate complexing agent was achieved. Dilution with the organic extractant itself is not feasible, as a uranium wave of only very small height is obtained with this diluted solution. A supporting electrolyte was added, as is customary in the polarographic analysis of aqueous solutions.

As shown in Figure 1 and discussed below, a well-formed uranium wave without maxima is obtained in certain nonaqueous systems. These solutions are not anhydrous. The presence of small amounts of water is helpful. An alternative procedure, not requiring the polarographic analysis of nonaqueous solutions, was developed. It was found that stripping of the extract with water resulted in complete recovery of the uranium in the aqueous phase. This aqueous strip has to be heated to destroy a tributyl phosphate–uranium complex. Supporting electrolyte is added prior to the polarographic determination of uranium in the heated aqueous strip. However, this alternative is longer and less convenient than the dilution procedure.

In 30:70 Volume % Tributyl Phosphate–Varsol. The most useful polarographic medium found among those considered for this extract was a heated aqueous strip of the extract that was cooled and then adjusted to 0.1M in nitric acid. Equal volumes of extract and of copper-free distilled water were shaken together for 5 minutes in a separatory funnel. After the separation, the organic phase was discarded. The aqueous phase, or aqueous strip, apparently contained tributyl phosphate to the extent of the solubility of tributyl phosphate in water.

If an unheated strip is acidified to 0.1M in nitric acid as supporting electrolyte, it is unsatisfactory for polarographic analysis; a double wave is obtained, probably because the tributyl phosphate complexes the uranium. If the aqueous strip is heated on a hot plate for 3 minutes prior to cooling and acidification with nitric acid, a normal, well-defined uranium wave that is free of copper interference can be obtained. The heated strip is made up to a definite volume in the process of cooling and acidification, in order that the concentration of uranium found polarographically may be related to the concentration of uranium in the original sample. The heated and acidified strip is stable over a period of days. Possibly the tributyl phosphate or some impurity in the tributyl phosphate is steam distilled off by this heat treatment. An equal volume of 1M phosphoric acid was tried as a strip for the extract. No wave was obtained in this strip. Usable, though poor waves can be obtained in the tributyl phosphate–Varsol extract diluted with ethyl alcohol–water (80:20 volume %). As supporting electrolyte, 0.1M ammonium nitrate is used.

Several supporting electrolytes were tried with glacial acetic acid as extract diluent. A small poorly shaped wave was obtained with 0.25M ammonium acetate in the presence of free nitric acid. Similar results were obtained by use of 0.25M ammonium acetate alone; no maxima were observed. A poorly shaped wave was obtained when 0.25M lithium perchlorate was used; maxima were present.

An equal volume per cent solution of benzene and methanol was tried as a diluting solvent. Lithium chloride was added to make the diluted solution 0.3M. A wave of poor form was obtained.

With the following media, no uranium wave was found: 30:70 volume % tributyl phosphate–Varsol acidified with 1M nitric acid to the solubility limit; absolute ethyl alcohol; absolute ethyl alcohol acidified with 1M nitric acid to the solubility limit; absolute ethyl alcohol, 0.1M tetramethyl ammonium chloride; 80:20 volume % dioxane-water, 0.1M ammonium nitrate.

In 30:70 Volume % Tributyl Phosphate–Isopropyl Ether. ORGANIC-DILUTION PROCEDURE. Various diluents of the tributyl phosphate–isopropyl ether (30:70 vol. %) extract were tried. Of these, the two most satisfactory were glacial acetic acid and a solution of equal volumes of ethyl alcohol and water. Good uranium waves were also obtained in a heated water strip. No copper wave was found, indicating that the extraction step had separated the uranium from the copper. Dilution of the extract with the uranium extractant itself was found to be unsatisfactory, at least when either 0.1M nitric acid or 0.25M lithium perchlorate was used as supporting electrolyte.

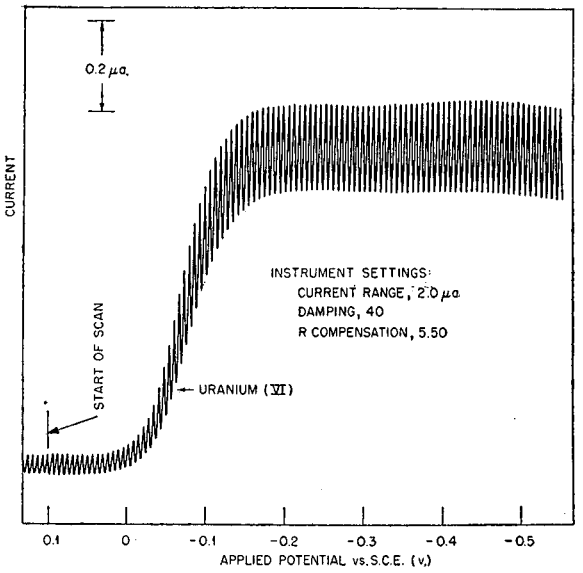

Figure 1. Polarographic wave for uranium

Extraction solvent, tributyl phosphate–isopropyl ether (30–70 vol. %)

Dilution solvent, fresh glacial acetic acid
40 γ of uranium(VI) per ml.
0.25M lithium perchlorate

When a diluent that is a mixture of equal volumes of ethyl alcohol and water is used, 0.5 to 0.6M nitric acid is a satisfactory supporting electrolyte. The concentration of nitric acid is not critical, but it must be constant, for it influences the wave height. The residual-current sections of the polarograms are not parallel to the limiting-current sections. The R compensator of the polarograph can be utilized to bring the limiting-current section parallel to the voltage coordinate axis. If a deaerated solution is allowed to stand in the polarographic cell in the presence of mercury while a series of polarograms is run over a period of time, the wave heights obtained are progressively greater. The deaerated solutions appear to be stable over several days when kept in glass in the absence of mercury. Polarograms made with a blank indicate that mercury might be dissolving. It appears that after the concentration of mercury increases beyond a certain point, its wave interferes with the uranium wave. This explanation would account for the observed dependence of wave height upon time of contact of the solution with mercury. In the cases of several other diluents, this effect renders those diluents

useless. In a solution of the extract in ethyl alcohol–water (1:1) acidified with nitric acid to about 0.6*M*, the effect is sufficiently slow to give reproducible results, if reasonable care is taken to limit the time of mercury contact. The wave height is found to be directly proportional to the concentration of uranium in the medium. Only fair shaped waves were obtained with either 0.1*M* nitric acid and 0.1*M* ammonium nitrate or 0.2*M* nitric acid and 0.1*M* ammonium nitrate as supporting electrolyte.

A diluent consisting of equal volumes of methanol and water was not successful where 0.5*M* nitric acid was used as supporting electrolyte. Low precision resulted from the fact that the observed wave height varied rapidly with time of mercury contact. Other media found unsuitable because of the dependence of wave height upon the duration of contact with mercury include: 80:20 volume % acetone-water, 0.3*M* nitric acid; 80:20 volume % 90% formic acid–water, 0.25*M* lithium perchlorate; 80:20 volume % glacial acetic acid–water, 0.25*M* lithium perchlorate; ethylene glycol monoethyl ether, 0.25*M* lithium perchlorate; and diethylene monobutyl ether, 0.25*M* lithium perchlorate.

Where glacial acetic acid is used as diluent, the amount of water present must be considered. As the water content of acetic acid is increased, the stability of the polarographic medium in the presence of mercury decreases. This instability is not experienced with the amount of water present in fresh glacial acetic acid. If anhydrous acetic acid were used, a chloranil reference electrode might be employed instead of the saturated calomel electrode used in the work reported. It is not deemed advisable to use anhydrous media, because they necessitate the inconvenience of using nonstandard polarographic apparatus. The relation of diffusion current, i_d, to uranium concentration is linear in glacial acetic acid as solvent. A typical, well-defined polarographic wave of uranium in a nonaqueous solvent is shown in Figure 1. It is a polarogram of 40 γ of uranium(VI) per ml. in glacial acetic acid with 0.25*M* lithium perchlorate as supporting electrolyte. No maxima were observed in this medium and the wave heights were large. A poorly shaped wave was obtained with 0.15*M* lithium perchlorate and 0.1*M* perchloric acid as supporting electrolyte in glacial acetic acid.

Waves of only fair shape were obtained with the following media: 80:20 volume % acetone-water, 0.25*M* lithium perchlorate; acetonitrite, 0.25*M* lithium perchlorate; 90% formic acid, 0.25*M* lithium perchlorate; and 80:20 % 90% formic acid–water.

A very small wave was obtained in diethylene glycol monoethyl ether. The supporting electrolyte was 0.25*M* lithium perchlorate.

Of the nine Cellosolves and Carbitols tested as extract diluent, Dowanol-1 (ethylene glycol monophenyl ether) gave the best results. With this solvent, 0.25*M* lithium perchlorate was used as supporting electrolyte. The relation of diffusion current, i_d, to uranium concentration was linear, the waves were well defined, and the solution was stable in the presence of mercury, but the i_d values were small compared to those found in glacial acetic acid or in equal volumes of ethyl alcohol–water. The addition of 10 volume % of water degraded the wave form.

No uranium waves were found in the following media: 80:20 volume % dioxane-water, 0.25*M* lithium perchlorate; equal volumes of ethyl alcohol–formamide, 0.25*M* lithium perchlorate; 25:25:50 volume % ethyl alcohol–formamide-water, 0.125*M* lithium perchlorate; 20:76:4 volume % ethyl alcohol–nitromethane–water, 0.25*M* lithium perchlorate; ethylene monobutyl ether, 0.25*M* lithium perchlorate; equal volumes of water–ethylene glycol monobutyl ether, 0.12*M* lithium perchlorate; ethylene glycol diethyl ether, 0.25*M* lithium perchlorate; diethylene glycol monomethyl ether, 0.25*M* lithium perchlorate; and diethyl glycol diethyl ether, 0.25*M* lithium perchlorate.

AQUEOUS-STRIP PROCEDURE. The procedure for heated aqueous strips, outlined above for tributyl phosphate–Varsol extracts, was successfully applied to tributyl phosphate–isopropyl ether extracts of uranium. As an alternative to the use of nonaqueous solutions of the tributyl phosphate–isopropyl ether organic extract, one may use a heated water strip of the extract acidified to be 0.1*M* in nitric acid, in which normal, well-formed uranium waves are obtained. Polarograms of unheated aqueous strips of tributyl phosphate–isopropyl ether extracts were unsatisfactory, as were those of tributyl phosphate–Varsol extracts. A normal uranium wave was obtained in an unheated water strip of a solution of uranyl nitrate hexahydrate in isopropyl ether. A double complex uranium wave having the same form as those obtained in unheated aqueous strips of tributyl phosphate–Varsol or tributyl phosphate–isopropyl ether extracts was obtained in an unheated aqueous strip of a solution of uranyl nitrate hexahydrate in 30 to 70 volume % tributyl phosphate–hexane. From this experimental evidence, it appears that tributyl phosphate is the cause of the double, complex wave obtained by the polarographic analysis of the unheated strip. The double, complex wave is obtained with unheated strips even after prolonged aeration with nitrogen gas. It is not caused by traces of isopropyl ether or its decomposition products.

CONCLUSIONS

The following procedure is recommended as a rapid and satisfactory method for the separation and polarographic determination of uranium.

Separate the uranium from the sample solution to be analyzed by extracting it into tributyl phosphate–isopropyl ether. If the sample solution is highly radioactive, extract the uranium by means of a solution that contains 5 volume % tributyl phosphate. In general, from 5 to 30 volume % tributyl phosphate can be used.

By either of the following methods, prepare a solution of the extracted uranium for polarographic analysis.

Shake the tributyl phosphate–isopropyl ether extract of uranium with an equal volume of copper-free distilled water, heat the separated aqueous strip for 3 minutes on a hot plate, cool it, and acidify it with nitric acid so that the final solution will be 0.1*M* in nitric acid. Dilute the strip to a convenient volume with copper-free distilled water, so that the concentration found polarographically can be related to the concentration of uranium in the original sample. Determine the uranium in the prepared strip polarographically.

Dilute the tributyl phosphate–isopropyl ether extract of uranium with glacial acetic acid to a concentration of about 50 γ per ml. and make the solution 0.25*M* with lithium perchlorate (use the solid salt) as supporting electrolyte. Analyze the prepared organic solution for uranium polarographically. This alternative is ordinarily to be preferred to stripping.

ACKNOWLEDGMENT

The authors are grateful for the many helpful suggestions by Myron T. Kelley.

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RECEIVED for review October 12, 1955. Accepted May 4, 1956.

Identification of Alkaloids and Other Basic Drugs by Paper Partition Chromatography

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A procedure is presented for the presumptive identification of microgram quantities of alkaloids and other basic drugs by means of the pattern of their R_f values at four pH's. The use of sensitive spotting agents makes possible the detection of microgram quantities. Recovery of the compounds from the paper is readily accomplished so that other confirmatory tests can be made. The R_f patterns of 44 commonly encountered basic drugs are reported and schematically arranged so that the identification of any one of these compounds is simplified. This procedure has been effectively used in the identification of microgram quantities of basic drugs in the toxicological examination of pharmaceutical preparations and biological samples.

ONE of the most difficult analytical problems in toxicology is the identification of microgram quantities of basic drugs, which may be present in the isolated extracts of a sample or in a low concentration in a limited amount of sample. Paper partition chromatography offers a simple method for the identification of micro quantities of chemicals by means of sensitive spotting agents and characteristic R_f values. Carless and Woodhead (*1*) introduced the concept of filter papers buffered at different pH's in the development of chromatograms of alkaloids. These authors and others (*2, 3*) showed that paper partition chromatography carried out at different pH levels produced marked changes in R_f values.

A method is presented here for the identification of alkaloids and other basic drugs based on the pattern produced by the R_f values at four different pH's. By the schematic arrangement of these R_f values, a tentative identification of an unknown basic drug can be made from chromatograms run simultaneously, each at a different pH. Thus, an identification is not made from a single value but from a pattern that is highly characteristic of a basic compound. To avoid the problem of reproducibility of R_f values, a control compound, codeine, is run on the chromatogram along with the unknown. The movement of codeine is compared with the movement of the basic drug to furnish codeine ratios which are more reproducible than R_f values. In this way the effect of changes in temperature, humidity, and solvent preparation are minimized.

APPARATUS AND REAGENTS

MacIlvaine's Buffers. pH 3.0: 790 ml. of 0.1*M* citric acid diluted to 1.0 liter with 0.2*M* sodium dibasic phosphate.

pH 5.0: 480 ml. of 0.1*M* citric acid diluted to 1.0 liter with 0.2*M* sodium dibasic phosphate.

Sorenson's Buffers. pH 6.5: 680 ml. of *M*/15 potassium monobasic phosphate diluted to 1.0 liter with *M*/15 sodium dibasic phosphate.

pH 7.5: 164 ml. of *M*/15 potassium monobasic phosphate diluted to 1.0 liter with *M*/15 sodium dibasic phosphate.

Developing Solution. *n*-Butyl alcohol saturated with buffers.

Paper. Whatman No. 2 filter paper, 55- to 60-cm. lengths.

Chromatocab. Model A, Research Equipment Corp., Oakland, Calif.

Mineralight. A source of short-wave ultraviolet light; Model SL 2537, Ultra-violet Products, Inc., Pasadena, Calif.

Iodoplatinic Acid Reagent. Mix 45 ml. of 10% potassium iodide, 5 ml. of 5% platinum chloride, and 100 ml. of distilled water.

Sodium Sulfite Reagent. A 4% sodium sulfite solution in half-saturated sodium borate solution.

METHOD

Four strips of Whatman No. 2 filter paper are wetted with buffers of pH 3.0, 5.0, 6.5, 7.5 (a different pH for each strip) and allowed to dry. These pH values are preferred because they produce the most characteristic differences with the largest number of compounds. An ethyl alcohol solution of the basic drug (50 to 100 γ) is spotted near one end of the paper while the control compound (50 γ of codine) is spotted alongside the unknown.

Table I. R_f Values and Codeine Ratios of Basic Drugs and Related Compounds

Key for Codeine Ratio

0 to 1.00 = *A*	2.51 to 3.00 = *E*
1.01 to 1.50 = *B*	3.01 to 3.50 = *F*
1.51 to 2.00 = *C*	3.51 to 4.00 = *G*
2.01 to 2.50 = *D*	4.00 to —— = *H*

	pH 3.0		pH 5.0		pH 6.5		pH 7.5	
	R_f	Ratio	R_f	Ratio	R_f	Ratio	R_f	Ratio
Class I		*ABC*		*ABC*		*A*		*AB*
Dihydromorphinone (Dilaudid)	0.12	0.75	0.12	0.60	0.16	0.55	0.61	0.80
Morphine	0.15	0.75	0.13	0.60	0.26	0.70	0.73	0.90
Brucine	0.15	0.80	0.15	0.85	0.21	0.70	0.57	0.75
Chloroquine	0.15	0.80	0.16	0.80	0.26	0.70	0.89	1.10
Codeine	0.19	1.00	0.20	1.00	0.34	1.00	0.78	1.00
Homatropine	0.25	1.30	0.25	1.40	0.22	0.70	0.50	0.65
Atropine	0.29	1.65	0.37	1.70	0.34	0.94	0.62	0.75
Class II		*ABC*		*AB*		*B*		*AB*
Adenine[a]	0.19	1.00	0.39	1.85	0.39	1.05	0.41	0.50
Emetine	0.21	1.10	0.27	1.50	0.45	1.50	0.89	1.20
Methyldihydromorphinone (Metopon)	0.24	1.25	0.22	1.15	0.37	1.05	0.82	1.00
Strychnine	0.25	1.50	0.23	1.40	0.31	1.00	0.74	1.00
Ethylmorphine (Dionin)	0.29	1.55	0.29	1.45	0.55	1.50	0.86	1.10
Quinacrine	0.31	1.55	0.35	1.55	0.48	1.20	0.95	1.15
Procaine	0.30	1.55	0.36	1.64	0.53	1.50	0.90	1.10
Class III		*ABCD*		*BCD*		*CD*		*B*
Pilocarpine	0.15	0.75	0.22	1.05	0.67	2.15	0.84	1.05
Scopolamine	0.22	1.15	0.26	1.30	0.58	1.65	0.86	1.10
Diacetylmorphine (Heroin)	0.31	1.70	0.33	1.75	0.56	1.85	0.86	1.15
Nicotine	0.19	0.90	0.32	1.70	0.69	2.20	0.92	1.15
n-Allylnormorphine	0.32	1.70	0.39	1.95	0.73	2.10	0.88	1.10
Gelsemine	0.41	2.00	0.41	1.95	0.49	1.60	0.87	1.10
Cocaine	0.40	2.00	0.52	2.40	0.69	1.93	0.96	1.20
Class IV		*BCD*		*EFG*		*CD*		*B*
Doxylamine	0.26	1.50	0.56	3.00	0.61	1.80	0.90	1.15
Trimeton	0.35	2.00	0.59	3.15	0.55	1.70	0.89	1.15
Methapyrilene	0.35	2.00	0.60	3.20	0.65	2.00	0.92	1.15
Pyribenzamine	0.37	1.95	0.68	3.40	0.70	2.00	0.96	1.20
Chlor-Trimeton	0.41	2.10	0.68	3.35	0.62	1.70	0.91	1.15
Quinine	0.47	2.25	0.74	3.50	0.72	2.30	0.92	1.20
Cinchonidine	0.47	2.25	0.72	3.45	0.71	2.30	0.92	1.20
Quinidine	0.46	2.30	0.79	3.70	0.75	2.25	0.95	1.20
Cinchonine	0.50	2.50	0.72	3.60	0.68	1.95	0.91	1.15
Class V		*EF*		*EF*		*CD*		*B*
Meperidine	0.50	2.65	0.61	3.05	0.72	2.05	0.90	1.20
Tetracaine	0.53	2.90	0.50	2.80	0.68	2.25	0.91	1.20
Thonzylamine	0.50	2.85	0.56	3.00	0.62	1.90	0.92	1.20
Yohimbine	0.55	3.35	0.52	3.20	0.74	2.45	0.90	1.20
Class VI		*G*		*FG*		*CD*		*B*
3-Hydroxy-*n*-methylmorphinan (Dromoran)	0.60	3.65	0.56	3.40	0.54	1.85	0.82	1.10
Diphenhydramine	0.65	3.50	0.63	3.55	0.67	2.20	0.91	1.20
Methadone	0.71	3.70	0.74	3.55	0.68	2.20	0.93	1.20
Ambodryl	0.67	3.80	0.75	3.90	0.76	2.30	0.95	1.20
Antihistine	0.66	3.85	0.71	3.90	0.60	1.85	0.78	1.05
Class VII		*EH*		*H*		*EF*		*B*
Nicotine	0.45	2.75	0.83	5.00	0.93	3.10	0.94	1.25
Chlorcyclizine	0.71	4.15	0.77	4.15	0.87	2.70	0.96	1.25
Cyclaine	0.79	4.50	0.83	4.40	0.84	2.55	0.94	1.20
Nupercaine	0.83	4.75	0.80	5.00	0.83	2.80	0.95	1.25
Coramine	0.85	4.40	0.92	4.35	0.90	2.90	0.91	1.15

[a] Compound frequently isolated from biological samples.

The end of the filter paper nearest the spotted section is immersed in the buffered butyl alcohol of corresponding pH. The system is allowed to develop, descending, for about 15 hours in a Chromatocab. The papers are removed and the solvent front is marked and air dried. To locate the compounds, the papers are first examined with a source of short-wave ultraviolet light for areas of fluorescence or absorption, then sprayed with iodoplatinate reagent to develop characteristic dark spots of the iodoplatinate complex of the basic drugs. The front of the spot is used to measure the movement and the R_f and codeine ratios

$$\left(\frac{\text{distance traversed by the compound}}{\text{distance traversed by codeine}}\right)$$

are computed for each drug.

To recover the compounds, the iodoplatinate complex is cut out of the paper, placed in a separatory funnel, and covered with 5 ml. of a 4% sodium sulfite solution in half-saturated sodium borate (pH 9.5) to destroy the complex and free the basic compound. When the paper is decolorized, a suitable solvent is added and the separatory funnel is shaken. The solvent containing the basic drug is evaporated to dryness. Chemical tests, characteristic absorption spectra, or other tests can be made to confirm the identity of the compound.

RESULTS

Table I lists the R_f values and codeine ratios of 44 pure compounds commonly encountered by toxicologists and pharmacologists. These values represent an average of at least three determinations for a particular compound. The R_f values and codeine ratios vary approximately 15% and 10%, respectively, at the lower pH values, and about 5% and 2%, respectively, at the higher pH values. An attempt was made to decrease the deviations by more careful control of such conditions as buffer saturation of the mobile phase, temperature, humidity, and the like but this was cumbersome, time-consuming, and not always successful. The compounds are grouped into seven classes according to the pattern produced by the codeine ratios at four pH's, so that the identification of any one of these compounds can be more easily made. Consideration must be given to members of the adjoining class in borderline instances. When an unknown is found to have the following R_f values and codeine ratios, it is located as follows.

pH 3.0		pH 5.0		pH 6.5		pH 7.5	
R_f	Ratio	R_f	Ratio	R_f	Ratio	R_f	Ratio
0.31	1.90	0.62	3.20	0.72	2.10	0.95	1.25

From the key in Table I, the codeine ratios are replaced by their letter equivalents: 1.90 = *C*, 3.20 = *F*, 210 = *D*, and 1.25 = *B*. The pattern *CFDB* is checked against the classes in Table I. Only Class IV compounds include this particular pattern (Class IV: pH 3.0, *BCD;* 5.0, *EFG;* 6.5, *CD;* 7.5, *B*). In this group, pyribenzamine most closely resembles the R_f values and codeine ratios of the unknown. Methapyrilene and Chlor-Trimeton are possibilities. Because Chlor-Trimeton shows a pattern that retrogresses at pH 6.5, the unknown is less likely to be this compound. The conclusive identification can be made by running another chromatogram with the unknown along with pyribenzamine and methapyrilene or other suspected compounds.

DISCUSSION

The above chromatographic procedure offers a relatively simple method for the identification of microgram quantities of an unknown drug even in the presence of other basic compounds. Along with the characteristic pattern of R_f values and codeine ratios, the procedure provides the following additional aids in the identification of an unknown.

Examination of the paper with ultraviolet light (wave length, 255 mμ) shows the presence of fluorescent compounds as well as those compounds that strongly absorb ultraviolet light of this wave length. For example, quinine or quinacrine will be seen as fluorescent areas when present on the paper in concentrations of less than 1 γ, while compounds like strychnine or adenine appear as dark areas when present in concentrations of about 10 γ.

When the filter paper is sprayed with iodoplatinic acid reagent, all the compounds listed in Table I, except adenine, coramine, and pilocarpine, appear as blue or black spots when the concentration is approximately 50 γ. As little as 10 γ of many compounds listed in Table I can be detected with this reagent. Adenine and coramine produce yellow spots at all pH values; pilocarpine produces a black spot at pH 3.0, a brown spot at pH 5.0, and a yellow spot at pH 6.5 and 7.5.

The ability to recover the compounds from the paper in a relatively pure state is also a great advantage. Confirmatory tests can be made on the recovered extract to make a conclusive identification of the unknown drug.

When sufficient amounts of the basic compounds listed in Table I are available, as in pharmaceuticals and in many toxicological samples, the use of the above procedure has proved effective.

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RECEIVED for review February 8, 1956. Accepted May 1, 1956. Preliminary report presented at American Academy of Forensic Sciences, 5th Annual Meeting, Chicago, Ill., February 1953.

Quantitative Paper Chromatography of Methylated Aldose Sugars

Improved Colorimetric Method Using Aniline Hydrogen Phthalate

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An improved procedure is presented for the colorimetric determination of methyl ethers of D-glucose which have been separated by paper chromatography. Aniline hydrogen phthalate is the chromogenic agent in this procedure, and the optimum range of sugar is 90 to 200 γ. Similar color development was observed with methyl ethers of D-mannose, D-galactose, and D-xylose, indicating the general value of this method for the determination of methyl ethers of aldose sugars.

OF CONSIDERABLE importance in structural studies of polysaccharides by the methylation technique is a reliable method for the determination of methylated reducing sugars after their separation from mixtures by means of paper chromatography. Such a method has been developed by modification of a known colorimetric procedure using aniline hydrogen phthalate as chromogenic agent.

The color-forming reaction of the simple aldose sugars with this reagent was first demonstrated by Partridge (*20*), who used

a solution of aniline and phthalic acid in butyl alcohol as a spray reagent for the qualitative detection of sugars on paper chromatograms. The same spray reagent has recently been used for determining methylated reducing sugars by a densitometric procedure (*17*). The first colorimetric method using aniline phthalate for the quantitative determination of the simple aldose sugars was proposed by Blass, Macheboeuf, and Nunez (*4*). Shortly thereafter, Gardell (*11*) reported a quantitative procedure using aniline in trichloroacetic acid. Application of the method of Blass and others to the determination of the methyl ethers of glucose then was reported by Bartlett, Hough, and Jones in a preliminary note (*2*).

Table I. Analyses of Known Mixtures

	Mixture	Spotted, γ	Recovery from Chromatogram, γ	Recovery from Chromatogram, % of original	Av. Dev. from Mean, %
A	2,3,4,6-Tetra-*O*-methyl-D-glucose	145	140	97	3.1
	2,3,4-Tri-*O*-methyl-D-glucose	150	143	95	2.2
	2,3-Di-*O*-methyl-D-glucose	152	149	98	1.2
B	2,3,4,6-Tetra-*O*-methyl-D-glucose	145	142	98	1.5
	2,4-Di-*O*-methyl-D-glucose	153	148	97	2.6
C	2,3,4,6-Tetra-*O*-methyl-D-glucose	98	97	99	1.3
	2,3,4-Tri-*O*-methyl-D-glucose	2196	2045	93	0.7
	2,4-Di-*O*-methyl-D-glucose	100	110	110	2.1

This present procedure is also based on the colorimetric method of Blass, Macheboeuf, and Nunez (*4*), with the unique modification that the color-producing reaction is carried out in a small volume of tetraethylene glycol dimethyl ether, a high-boiling solvent. This modification provides a homogeneous reaction mixture and enables better reproducibility and higher sensitivity than the original method, in which the reactants were heated in the solid state. Thus, an 84% increase in color intensity was obtained in the determination of 2,3,4,6-tetra-*O*-methyl-D-glucose.

A further modification involves the use of crystalline aniline hydrogen phthalate (*13*, *15*), instead of an equimolecular mixture of aniline and phthalic acid, for preparation of the reagent. The salt is stable indefinitely, and is easily purified by recrystallization to eliminate dark-colored oxidative impurities commonly formed in aniline. Use of the salt has the added advantage of ease of accurate weighing in making the reagent solution.

APPARATUS AND MATERIALS

The usual apparatus for descending chromatography may be used (*21*).

The Gilmont combination micropipet-buret (Emil Greiner Co., New York), a modification of the earlier ultramicroburet (*12*), proved convenient and accurate for use in applying sugar solutions to chromatograms.

The extraction apparatus, which enabled rapid and complete removal of sugars from cutout sections of paper chromatograms, was similar to that of previous workers (*4*, *10*) except that a small glass ring was fused to the drip tip of the reflux condenser. Paper to be eluted was attached to this ring by means of a small wire hook.

A constant temperature water bath, suitable for operation at 90° to 98° C., was used. The water may be covered with a layer of paraffin to minimize evaporation.

Selected borosilicate glass culture tubes were used as cuvettes in conjunction with a Coleman Junior Model 6A spectrophotometer.

The water azeotrope of methyl ethyl ketone (*5*), containing 1% by volume of aqueous concentrated ammonium hydroxide, was used for developing chromatograms. In the absence of ammonia, this solvent mixture partially resolves individual sugars into their anomeric forms, causing elongation of sugar spots. The ketone was saturated with water and treated at room temperature overnight with a slight excess (only a few crystals) of potassium permanganate in the presence of magnesium sulfate (0.25 gram per liter) prior to azeotropic distillation.

The spray reagent (*5*) was 0.4% *N,N*-dimethyl-*p*-aminoaniline hydrochloride in aqueous 2% trichloroacetic acid. This reagent is usable for 1 week when kept at 2° C.

The colorimetric reagent consisted of a 2.410% ± 0.006% (w./v.) solution of crystalline aniline hydrogen *o*-phthalate in methanol. When kept at 2° C. the reagent was stable for 3 months.

The solvent used for extracting sugars from chromatograms consisted of methyl alcohol containing 0.900% ± 0.006% (w./v.) of tetraethylene glycol dimethyl ether (2,5,8,11,14-pentoxapentadecane).

Approximately 1.5% aqueous sugar solutions were found convenient in applying known sugars to paper chromatograms. The 2,3,4,6-tetra-, 2,3,4-tri-, and 2,3-di-*O*-methyl-D-glucoses were prepared by known procedures (*6*); 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2,3,4,6-tetra-*O*-methyl-D-mannose were prepared through methylation of their methyl glycosides by a modified Muskat procedure (*19*); and 2,4-di-*O*-methyl-D-glucose (*23*) and 1,3,4,6-tetra-*O*-methyl-D-fructose (*22*) were prepared by new and improved procedures.

PROCEDURE

Separation. The mixture of sugars is applied to a paper chromatogram in 1-μl. aliquots with the micropipet-buret in an arrangement that will provide space for guide spots and in an amount that will give at least 90 γ of the smallest constituent in the mixture. In the present studies, the following arrangement of spots was adopted.

Chromatograms were prepared on 15 by 40 cm. sheets of Whatman No. 1 filter paper, having the long dimension parallel with the machine direction and the starting line 7.5 cm. from one end of the sheet. Three spots were applied on the starting line, two of which were guide spots placed 2 cm. from the edges of the paper. The third spot, containing the sugars to be eluted, was in the form of a band 2 cm. long composed of five adjacent spots placed at the center of the starting line. This arrangement allows a space of 4.5 cm. between the guide spots and the center band of spots.

After the paper has been spotted, the chromatogram is developed with a suitable partitioning solvent. Ammoniacal methyl ethyl ketone–water azeotrope provided good separation of methylated glucoses in less than 3 hours, during which time the solvent front advanced approximately 35 cm. This solvent evaporated rapidly from the developed chromatogram and left no residues which interfered with the sugar determinations if the aqueous ammonia was added immediately before use. Best resolution was obtained by avoiding equilibration of the chromatogram in a solvent-saturated atmosphere prior to development.

Strips containing the guide spots are cut from the developed chromatogram, sprayed with a suitable reagent, and heated under the proper conditions to indicate the position of sugar spots (125° C. for 1 to 2 minutes in the case of *N, N*-dimethyl-*p*-aminoaniline). With the aid of the treated guide strips, appropriate sections are then cut from the unsprayed portion of the chromatogram, and the area of each sugar-containing section is measured so that a correction can be applied to compensate for the interfering substances extracted from the paper.

Elution. Each section of paper containing a sugar component is rolled, secured with a coil of nickel wire, and extracted under reflux (*4*, *10*) for 20 minutes (*4*) with 5 ml. of 0.9% methanolic tetraethylene glycol dimethyl ether, added from a pipet.

In some cases—e.g., 2,3,4-tri-*O*-methyl-D-glucose in Table I, mixture *C*, and in Table II—the eluate must be diluted in order to obtain an absorbance within the optimum range of the spectrophotometer. In the present work, greatest accuracy was obtained in the range of 90 to 200 γ of methylated glucose. Dilutions, of course, must be made with the eluting solvent, 0.9% tetraethylene glycol dimethyl ether in methanol, in order to provide the standard amount of glycol ether in the 5-ml. aliquots upon which the determinations are performed. It is not feasible to make dilutions after color development, for such dilutions result in low values.

Determination. The eluate or a suitable aliquot of it is mixed with 1 ml. of the aniline phthalate reagent, and the methanol is removed by evaporation in vacuo at 30° C. (bath temperature). The residual liquid is then heated for 35 minutes (Figure 1) at either 98° C. in the case of aldohexose methyl ethers or 90° C. for aldopentose methyl ethers. Shorter heating periods result in lower reproducibility.

The flasks are then removed from the hot water bath and quickly cooled to room temperature. The small, red-brown pool of reaction products is dissolved in 10 ml. (from a pipet) of 95%

ethyl alcohol, and the absorbance is measured with the spectrophotometer against 95% ethyl alcohol at 415 mμ for the methylated aldohexoses and at 460 mμ for the methylated aldopentoses. The curves in Figure 2, which were obtained with a Cary recording spectrophotometer, are representative of those of other methylated aldose sugars. The curve of the reagent blank (heated 35 minutes at 98° C.) indicates little interference at the wave lengths of absorbance measurement.

Because the colors fade after development (1 to 2% during the first hour, 5 to 10% overnight), the time interval between the end of the heating step and the measurement of absorbance should be held reasonably constant. The authors found a 30-minute interval to be convenient.

The reagent and paper blanks are subtracted from the absorbance reading, giving the net absorbance due to sugar in the eluate. As will be explained later, the use of mean values of the blanks, established by multiplicate (at least 10) determinations, is preferable to evaluation of the blanks with each sugar determination. The mean values apparently are applicable over extended periods of time. In the determination of methylated hexoses, the absorbance of the reagent blank was equal to that of 40 γ of 2,3,4,6-tetra-*O*-methyl-D-glucose, and the paper blank gave an equivalent of 0.4 γ per sq. cm. of paper. For the methylated pentoses, which were heated at a lower temperature, the reagent blank was one third of the value given for hexoses.

For the determination of the paper blank, a known amount of sugar should be added to the extracting solvent to raise the absorbance to within the optimum range of the spectrophotometer. The determination is made on paper that has been chromatographically washed with the developing solvent. The reagent blank determination is made on extracting solvent plus reagent in the absence of sugar.

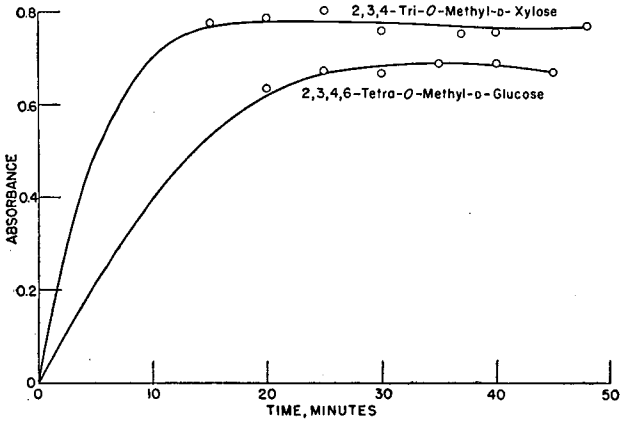

Figure 1. Intensity of color related to time of heating for two representative sugars

After the reagent and the paper blanks have been deducted, the weight of eluted sugar is determined by referring to a standard absorption curve which has been prepared previously (Figure 3). The curves for all sugars departed from Beer's law (in a reproducible manner) at quantities below 90 γ. This was possibly due to instrument error.

RESULTS

Application of the above procedure of quantitative paper chromatography to synthetic mixtures of methylated glucoses yielded the results shown in Table I. The experimental values, which represent the mean of results from triplicate chromatograms, are given with the average deviation of the individual values from their mean. Recoveries from the chromatograms averaged within 4% of the amount spotted on the starting lines. The highest variation, 10%, was encountered in the analysis of mixture *C*, which contained the most unfavorable proportion of sugars. Within the sets of triplicate determinations the average deviation of individual values from the mean for the set was 2.1%, with a maximum of 4.7%.

Table II. Analysis of Methylated Dextran Hydrolyzate

(Dextran from *L. mesenteroides* NRRL B-512)

Sugar Present[a]	Weight, γ	Mole %	Molar Ratio
2,3,4,6-Tetra-*O*-methyl-D-glucose	158	6.8	1.5
2,3,4-Tri-*O*-methyl-D-glucose	1929	88.7	19.8
2,4-Di-*O*-methyl-D-glucose	92	4.5	1.0

[a] Identified by derivative formation after separation on a cellulose column (*25*).

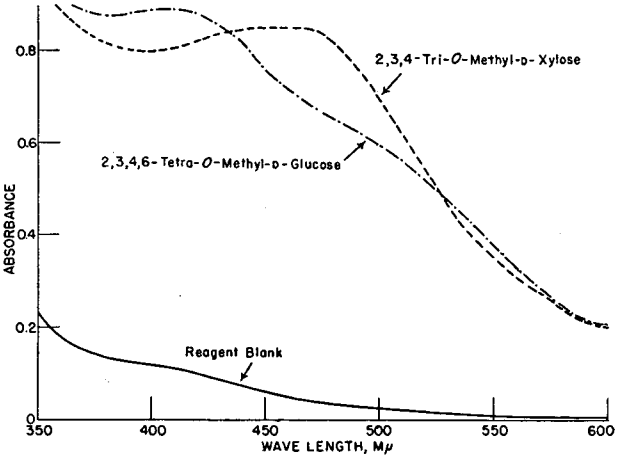

Figure 2. Spectral absorbance curves for reaction products of methylated sugars heated with aniline phthalate

The application of the method to a hydrolyzate of methylated dextran (*Leuconostoc mesenteroides* NRRL B-512) yielded the results in Table II. The data reveal that 95.5% of the anhydroglucose units in the dextran studied were linked at either the 1- or the 1- and 6- positions, in good agreement with 94.5% found by periodate oxidation studies (*16*) and 95.5 to 96% found by periodate oxidation followed by reduction and hydrolysis (*1*). Analyses of mixtures of this type, in which one component sugar was present in a high proportion, were made possible by the excellent resolution of sugar spots obtained with the ammoniacal methyl ethyl ketone–water azeotrope. The dextran studied had been methylated to 45.3% methoxyl in a 60% yield and had been hydrolyzed to constant rotation with a hydrochloric acid–glacial acetic acid mixture (*24*) similar to that of Bell (*3*).

In addition to analyses of hydrolyzates, this procedure was also applied to a study of the extent of demethylation which occurred when various partially methylated glucoses were subjected to conditions of acid hydrolysis (*24*), and to the analysis of cellulose column fractions in which overlapping of sugars occurred.

Exploratory experiments with 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3,4,6-tetra-*O*-methyl-D-mannose, as well as the work with 2,3,4-tri-*O*-methyl-D-xylose, indicated that this method is generally applicable to methylated aldose sugars. The above fully methylated sugars gave colors of somewhat greater intensity than that produced by the same weight of 2,3,4,6-tetra-*O*-methyl-D-glucose. When the color intensity of the latter was made equal to 100, the intensity of the galactose derivative was 124; that of the mannose derivative, 106; and that of the xylose derivative (heated at 90° instead of 98° C.), 121. Determinations of 1,3,4,6-tetra-*O*-methyl-D-fructose gave negative results. This was to be expected because aniline phthalate does not produce colors with ketoses.

Table III. Variations in Experimental Factors Which Produce Change of ±1% in Absorbance[a]

Variable	Standardized Value	Relative Tolerance, ±
Aniline phthalate reagent	24.1 mg.	0.3 mg.
Tetraethylene glycol dimethyl ether	45.0 mg.	1.4 mg.
Temperature of heating	98° C.	3° C.
Time of heating	35 min.	4 minutes
Wave length		
O-Methyl aldohexoses	415 mμ	10 mμ
O-Methyl aldopentoses	460 mμ	10 mμ

[a] Determined for 150 γ of 2,3,4,6-tetra-*O*-methyl-D-glucose.

DISCUSSION

The present colorimetric procedure is particularly well adapted to the determination of aldoses resolved by paper chromatography. In contrast to procedures using strong mineral acids (*7–9*), this method does not require the removal of extraneous cellulose particles, which are inevitably present in eluates from filter papers. While the present method is less sensitive than those using mineral acids, the amount of aldose required (90 to 200 γ) is readily obtained in most chromatographic separations.

Accuracy and Reproducibility. In 14 single determinations, not involving chromatographic separations, on samples of 2,3,4,6-tetra-*O*-methyl-D-glucose which contained 145 γ of sugar, the mean value obtained colorimetrically was 145 γ, with a standard deviation of 5.6 γ (3.8% of the mean). These determinations were performed over a period of 3 months and were based on the standard curve of Figure 3. The reagent used was of varying age and was obtained from a number of successive preparations. Thus, it is seen that a known solution need not be included with each set of determinations.

During the course of the work, it was observed that variations of the reagent blank were not consistent with variations of the absorbance of the corresponding sugar sample—i.e., it appeared that the absorbances of the sugar samples and of the blanks varied independently. Therefore, sugar determinations have been calculated on the basis of an established mean value for the blank. If, instead, the above 14 determinations are calculated on the basis of the individual blanks which were run simultaneously with each determination, the mean value becomes 143 γ with a standard deviation of 6.2 γ (4.4% of the mean).

A study of the effects of some selected variable factors yielded the data given in Table III. In each case the relative tolerance, determined graphically, represents the amount of change in the given variable that would produce a change of ±1% in a determination of 150 γ of 2,3,4,6-tetra-*O*-methyl-D-glucose. The tolerances are presented in this manner in order to indicate the relative influence exerted by variations in the factors encountered in this procedure.

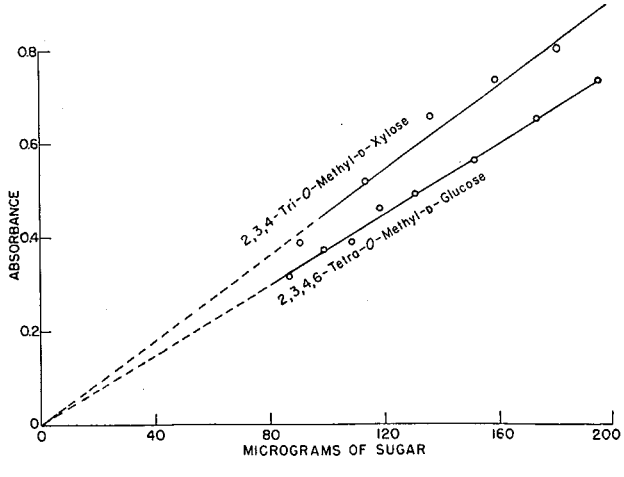

Figure 3. Typical absorbance curves for colored products obtained from methylated sugars

Solvent Media for Color Reaction. An important consideration was the choice of solvent to serve as the medium for the color-producing reaction. In the present studies, several high-boiling solvents were examined, but none proved so satisfactory as tetraethylene glycol dimethyl ether. When glycerol or tetraethylene glycol was used, the reaction mixtures became so viscous during the heating step that difficulty was encountered in dissolving the colored products in 95% ethyl alcohol to give the solution needed for spectrophotometric measurement. This increase in viscosity was possibly due to the formation of acetals (*14*) or to polymerization (*18*). Anisole was found to be too volatile, and mineral oil was eliminated because it failed to dissolve aniline hydrogen phthalate.

ACKNOWLEDGMENT

The authors are grateful to I. A. Wolff for his many valuable suggestions, both during the experimental work and in the preparation of the manuscript. They are also indebted to E. H. Melvin and T. A. Scott for spectral absorbance curves, and to J. E. Hodge for the sample of methylated NRRL B-512 dextran.

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RECEIVED for review September 21, 1955. Accepted March 29, 1956. Division of Carbohydrate Chemistry, 124th Meeting, ACS, Chicago, Ill., September 1953. The use of trade names in this paper does not necessarily constitute endorsement of these products nor of the manufacturers thereof.

Thermometric Titration Curves

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Equations are derived for the relationship between temperature change and volume of titrant added in thermometric titrations. The effects of the heat capacity of the apparatus, the change in volume during titration, and nonadiabatic conditions are derived and discussed. It is shown how heats of reaction, solution, and dilution may be estimated accurately from automatically recorded thermometric titration curves by interpretation of slopes; results obtained are compared with literature values.

THE automatic recording of thermometric titration curves was described several years ago by Linde, Rogers, and Hume (*4*). Extension of this work to titrations in nonaqueous media and various refinements in measuring techniques have made it of interest to compare theoretical and observed titration curves and to determine the best method for obtaining fundamental thermochemical data from titration curves measured under ordinary experimental conditions. The usual technique of automatic thermometric titration (*4*) involves addition of titrant, isothermal with the solution to be titrated, at a constant rate and measurement of any temperature change in the mixture by means of a rapid-response element such as a thermistor. A Dewar flask and appropriate thermal shielding are employed to make the system as nearly adiabatic as possible. The speeds of reaction and mixing are such that thermal equilibrium is established immediately in the solution, but because a titration requires only a few minutes for performance, true thermal equilibrium is not reached between the solution and its container. This and the fact that the heat capacity of the system is undergoing constant change due to the addition of titrant makes the temperature *vs.* volume plot nonlinear.

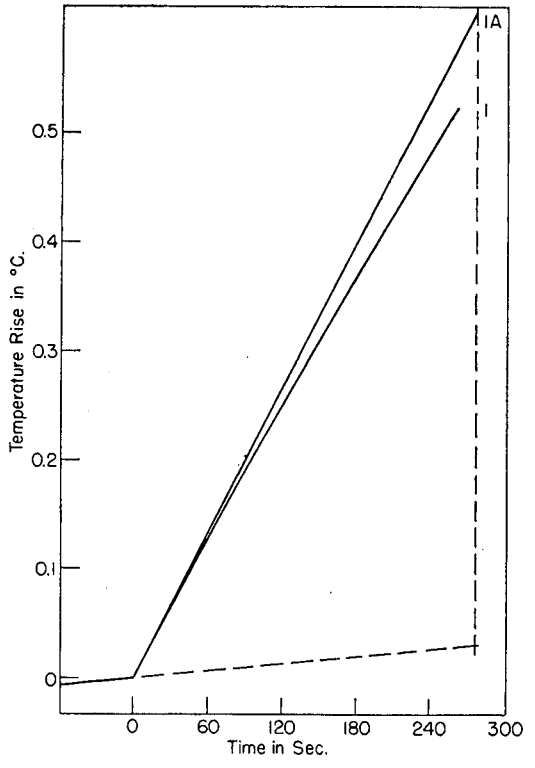

Figure 1. Comparison of curves of temperature *vs.* time

Heat generated electrically at constant rate
I. Recorded
I, *A*. Calculated

It is useful, first, to consider the effect of nonequilibrium with cell and surroundings by examining what happens when a small electric heating coil is used to deliver known amounts of heat to a solution in the titration cell. Because no titrant is being added, the heat capacity of the system is constant. The heat added, dt, during an infinitesimal interval is given by the relation

$$dH = C_p dT = i^2R(4.185)^{-1}dt \quad (1)$$

where i is current in amperes; R, resistance in ohms; t, time in seconds; H, heat in calories; T, centigrade temperature; and C_p, the heat capacity, in calories, of the titration cell and its contents. Accordingly, the relationship between temperature change and time is given by

$$\frac{dT}{dt} = \frac{i^2R}{4.185C_p} \quad (2)$$

Temperature rise is linear with time if no heat is lost to the environment. Comparison with a typical curve of T *vs.* t obtained this way under ordinary good experimental conditions reveals that the empirical plots are invariably curved because of heat loss (Figure 1). This might at first seem to be a serious deterrent to use of titration curves for measuring heats of reaction, but, as will be seen, it is an effect which can be eliminated readily by taking the initial slope to estimate a value of C_p which applies to the system at the initial temperature, T_1, in equilibrium with its surroundings.

TITRATION CURVES

Consider a simple titration in which the titrant is isothermal with the initial solution and the only process contributing to temperature change is the chemical reaction. In any practical automatic thermometric titration, the reaction must be rapid. Then,

$$-dH = \Delta H_{T_1} dn = -C_p dT \quad (3)$$

where ΔH_{T_1} is the isothermal heat of reaction at T_1 in kilocalories per mole and dn is the increment of titrant in millimoles. Because $dn = MdV$ where V is expressed in milliliters and because V is linear with time t,

$$\frac{dt}{dV} = \frac{\Delta H_{T_1} M}{C_p} \quad (4)$$

The plot of T *vs.* V should then be linear if ΔH and C_p are constant. However, the variation in C_p due to added titrant is significant, as may be seen in Figure 2. If we consider C_p to consist effectively of two terms—the heat capacity of the liquid and the heat capacity of the Dewar flask, stirrers, and the like—we have

$$C_p = (\mathrm{cd})V + C' \quad (5)$$

where c is the specific heat of the liquid (assumed constant throughout the titration), d is its density, and C' is the heat capacity of the calorimeter—i.e., the titration apparatus. Then, integrating Equation 4 between the initial and final temperatures,

T_1 and T_2, and the corresponding volumes in the Dewar, V_1 and V_2, we have for the relationship between the total temperature rise and the volume of titrant

$$\Delta T = \int_{T_1}^{T_2} dT = -\Delta H_{T_1} M \int_{V_1}^{V_2} \frac{dV}{cdV + C'}$$
$$= \frac{-2.3\Delta H_{T_1} M}{cd} \log \left[\frac{cdV_2 + C'}{cdV_1 + C'}\right] \quad (6)$$

The nonlinearity of the titration curve caused by changing heat capacity introduces no difficulty in locating end point breaks, because these are very clear and sharp for most reactions. The combined effect of nonlinearity due to variable C_p and heat loss would complicate direct calculation of ΔH from ΔT, but it is found that the initial portion (approximately 30%) of most titration curves follows very closely to exact linearity. Therefore, by using the initial slope and Equation 4, both difficulties can be circumvented. Three additional advantages are obtained from the use of the initial slope: Curvature in the vicinity of the end point caused by incompleteness of reaction does not affect the result; errors due to a difference in temperature between the titrant reservoir and the titration solution are minimized, inasmuch as the first part of the titration curve comes from reagent in the buret tip which has the greatest chance of being in thermal equilibrium with the solution; and the heat capacity of the initial system, which can be measured readily by means of Equation 2, is appropriate for the calculation.

HEATS OF REACTION

The application of this technique to measurement of heats of reaction is illustrated by the following data on the heat of neutralization of sodium acetate in glacial acetic acid by anhydrous perchloric acid in glacial acetic acid. The titrations were performed using a motor-driven syringe for titrant delivery and a thermistor in a bridge circuit, capable of detecting changes of 0.001° C. The circuit is designed so that the relationship between unbalance potential and temperature change is essentially linear for changes up to 0.50° C. Temperature changes were recorded on a 2-mv. Speedomax recorder. The effective heat capacity, C', of the Dewar flask, stirrer, and the like was measured previously under the same conditions by determining the temperature rise of a known quantity of water when a measured amount of electrical energy was delivered to it in the form of heat.

Table I. Comparison of Observed and Calculated Temperature Rises in Three Titrations of Sodium Acetate with Perchloric Acid

(Glacial acetic acid solvent)

V, Ml. of 0.5M $HClO_4$	ΔT, ° C.				
	Obsd., curve 1[a]	Calcd.[a]	Obsd., curve 2[b]	Obsd., curve 3[b]	Calcd.[b]
0.50	0.045	0.047	0.042	0.043	0.043
1.50	0.134	0.136	0.127	0.131	0.127
2.50	0.213	0.225	0.206	0.215	0.209
3.50	0.285	0.314	0.279	0.293	0.292
4.50	0.353	0.399	0.343	0.363	0.371
5.50	0.418	0.483	0.409	0.433	0.450
5.95	0.442	0.521	0.433	0.461	0.485

[a] Heat capacity C' independently measured as 2.6 cal. per degree.
[b] Heat capacity C' independently measured as 4.8 cal. per degree.

Table I shows experimental data from three separate titration curves (the data in column 2 are from the curve reproduced in Figure 2) and compares observed temperature rise with that calculated from the measured initial heat capacity of the system and the literature value (*1*) of the heat of reaction. Although deviations occur after the first 30% of the titration, the initial slopes are very close to the theoretical. Table II shows the agreement between the heat of reaction estimated from the initial slope of the titration curves and the literature value, which was obtained by standard calorimetric techniques (*1*). Under the appropriate experimental conditions (temperature rise small, titration duration short, system nearly adiabatic) an excellent estimate of the heat of the titration reaction can be obtained from the initial slope of the titration curve. However, the system is not strictly adiabatic, the solution does not reach thermal equilibrium with the container, and the titrant may not be exactly at the same temperature as the solution. Hence, the ordinary approach of measuring total temperature change, correcting for dilution by the method of mixtures, and correcting for the equilibrium heat capacity of the calorimeter, is not as attractive. When the temperature changes involved are very small—sometimes only a few hundredths of a degree—the titration method still gives a usable result although the classical method with the same apparatus is unreliable.

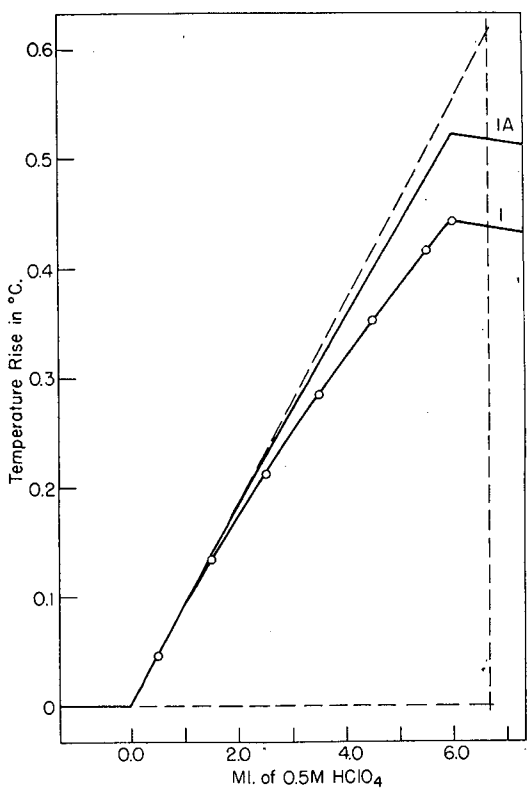

Figure 2. Titration curves

1. Experimentally determined using continuous strip-chart recorder; points indicated are taken from continuous record and presented in Table I
1, *A*. Calculated

Table II. Estimation of ΔH of Reaction Between Sodium Acetate and Perchloric Acid in Glacial Acetic Acid from Thermometric Titration Curves

Curve	Initial $\frac{dT}{dV}$		$-\Delta H$ (Kcal. per Mole)	T, ° C.	NaOAc, % Found
	Measured	Theoretical[a]			
1	0.092	0.092	5.7	22.6	99.9
2	0.085	0.086	5.6	23.2	99.3
3	0.088	0.086	5.9	22.2	99.6
			Av. 5.7		99.6
			Std. Dev. 0.16		0.30

[a] Calculated from Equation 4 with Jolly's value of 5.7 kcal. per mole for $-\Delta H$ and independently determined heat capacities of solutions and titration cells.

Titrant and Solution Not Isothermal. In practical work it is often very difficult to adjust and hold the titrant and the solution to the same initial temperature. If the difference is no more than about half the temperature rise expected in the titration, the error in initial slope, under conditions similar to those in which the sodium acetate-perchloric acid were performed, is less than 5%. In general, if all symbols have the same meaning as previously, the rate of temperature change due to mixing with titrant of a different temperature, T_3, is given by

$$\frac{dT}{dV} = \frac{(T_3 - T_1)\mathrm{cd}}{C_p} \tag{7}$$

Correspondingly,

$$\Delta T = \int_{T_1}^{T_2} dT = 2.3\,(T_3 - T_1)\log\left[\frac{\mathrm{cd}V_2 + C'}{\mathrm{cd}V_1 + C'}\right] \tag{8}$$

where it is assumed that both the specific heat and density are the same for titrant and solution. It is seen again that the plot of T *vs.* V is not linear.

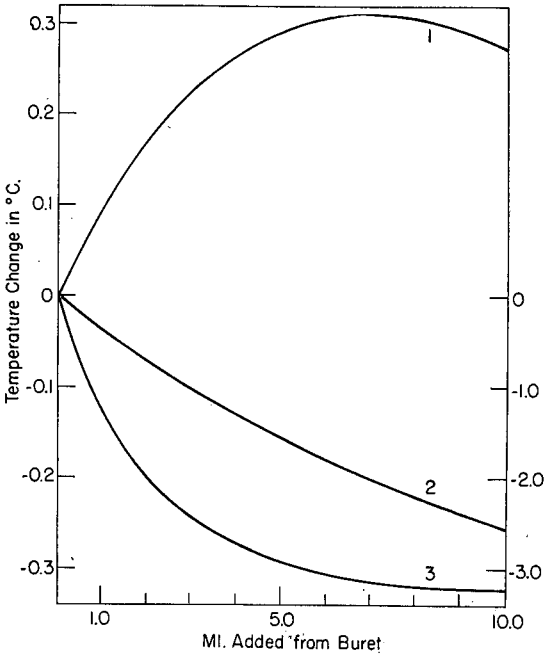

Figure 3. Recorded temperature changes on mixing acetic acid or water with their solutions

Temperature scale at right applies to curve 3 only
1. Acetic acid containing <0.01% water; added from buret to 50.00 ml. of water
2. Acetic acid containing <0.01% water; added from buret to 50.00 ml. of acetic acid initially containing 9.38% water
3. Water; added from buret to 50.00 ml. of acetic acid containing <0.01% water

HEATS OF SOLUTION

In the above discussion it has tacitly been assumed that heats of dilution and mixing are negligible. This is not necessarily the case, particularly with nonaqueous solvents. Although proper choice of concentrations and solvents usually permits evasion of mixing difficulties, rather large heat effects are sometimes observed. The thermometric titration technique permits ready estimation of heats of solution and dilution from temperature *vs.* volume curves obtained in the usual way.

Consider first the addition of n_2 moles of liquid solute, such as acetic acid, to n_1 moles of solvent—e.g., water—both initially at the same temperature. When the solute is added from the buret to a fixed quantity of solvent, $dn_1 = 0$ and

$$d\Delta H = \left(\frac{\partial \Delta H}{\partial n_2}\right) dn_2 = -C_p dT \tag{9}$$

The following symbols and terms are then used, which is essentially the terminology of Lewis and Randall (*3*), as modified by Klotz (*2*).

Subscripts: 1 = solvent; 2 = solute.

$\overline{H}$ = partial molal heat content
$\overline{H}^\circ$ = partial molal heat content in infinitely dilute solution
$H^\bullet$ = molal heat content of pure substance
$\overline{L}$ = relative partial molal heat content, $\overline{H} - \overline{H}^\circ$
$L^\bullet$ = relative molal heat content of pure substance, $\overline{H}^\bullet - \overline{H}^\circ$
$\left(\frac{\partial \Delta H}{\partial n_2}\right)_{n_1}$ = differential heat of solution = $\overline{H}_2 - H_2^\bullet = \overline{L}_2 - \overline{L}_2^\bullet$
$\left(\frac{\partial \Delta H}{\partial n_1}\right)_{n_2}$ = differential heat of dilution = $\overline{H}_1 - H_1^\bullet = \overline{L}_1$

If M is the molarity of the pure solute, $dn_2 = MdV$, and

$$\frac{dT}{dV} = -\frac{\left(\frac{\partial \Delta H}{\partial n_2}\right) M}{C_p} = \frac{-(\overline{L}_2 - L_2^\bullet)M}{C_p} \tag{10}$$

then the initial slope has again a special significance. Here, $\overline{L}_2 = \overline{H}_2 - \overline{H}^\circ = 0$ because we start with an infinitely dilute solution in the Dewar, and

$$\left(\frac{dT}{dV}\right)_{\text{initial}} = \frac{L_2^\bullet M}{C_p} \tag{11}$$

Thermometric titration thus provides a simple and direct method for measuring differential heat of solution in infinitely dilute solution.

In the reverse situation, where solvent is added gradually to pure liquid solute, $dn_2 = 0$ and

$$d\Delta H = \left(\frac{\partial \Delta H}{\partial n_1}\right) dn_1 = -C_p dT \tag{12}$$

$$\frac{dT}{dv} = -\frac{\left(\frac{\partial \Delta H}{\partial n_1}\right) M}{C_p} = \frac{-\overline{L}_1 M}{C_p} \tag{13}$$

where M is the molarity of pure solvent. The quantity $\overline{L}_1$ is not a constant but varies with composition, approaching zero as more and more solvent is added.

The use of these equations is illustrated by the estimation of the differential heats of solution and dilution of acetic acid in water from curves recorded when acetic acid or water was run into water or acetic acid solutions. In Figure 3 are shown automatically recorded plots of the temperature changes occurring when pure acetic acid was added to pure water (curve 1), when pure acetic acid was added to 90.62% acetic acid (curve 2), and when pure water was added to pure acetic acid (curve 3). From Equation 11 and the initial slope of curve 1, the value of $\overline{L}_2$ was estimated to be 320 cal. per mole of acetic acid. From Equation 10 and slopes taken at selected points on curves 1 and 2, values of $\overline{L}_2 - \overline{L}_2^\bullet$ were estimated, and the resulting values for $\overline{L}_2$ have been listed in column 3 of Table III. For comparison, the values of $\overline{L}_2$ in column 6 were calculated from the $\overline{L}_1$ data at 20° C. reported by Payn and Perman (*5*) in an investigation of the differential heats of dilution and solution of acetic acid and water. The values of $\overline{L}_2$ and $L_2^\bullet$ obtained from the use of titration apparatus agree remarkably with those from the earlier study.

As a further illustration of what might be done with the data, values of $\overline{L}_1$ (column 4) were calculated from the measured values of $\overline{L}_2$ in column 3 using the Gibbs-Duhem equation, and then compared with the directly measured values of Payn and Per-

man in column 5. In view of the fact that the actual values of $\bar{L}_1$ represent very small numbers of calories, the agreement is satisfactory.

The very large temperature change ($-3°$ C.) observed in curve 3 made quantitative estimates based on the assumption of an adiabatic system impractical. However, the curve is in excellent qualitative agreement with the observations of Payn and Perman, who likewise found a large and rapidly changing endothermic heat of dilution under these circumstances.

HEATS OF DILUTION

When a solution is diluted by addition of pure solvent, a heat effect may occur. The situation is analogous to the addition of solvent to pure liquid solute, and Equation 13 applies. Conversely, if the pure liquid solute is added to the solution, Equation 10 is appropriate. When a solution is added to pure solvent, however,

$$d\Delta H = \left[\left(\frac{\partial \Delta H}{\partial n_1}\right)^f - \left(\frac{\partial \Delta H}{\partial n_1}\right)^i\right] dn_1 + \left[\left(\frac{\partial \Delta H}{\partial n_2}\right)^f - \left(\frac{\partial \Delta H}{\partial n_2}\right)^i\right] dn_2 \quad (14)$$

By the same line of reasoning as before,

$$\frac{dT}{dV} = -\frac{(\bar{L}_1^f - \bar{L}_1^i)M_1 + (\bar{L}_2^f - \bar{L}_2^i)M_2}{C_p} \quad (15)$$

where the superscripts i and f refer to initial and final states, and M_1 and M_2 are the molar concentrations of solvent and solute, respectively, in the titrant. At the start of the addition, $\bar{L}_1^f$ and $\bar{L}_2^f$ are both zero; hence, the initial slope is

$$\frac{dT}{dV} = \frac{\bar{L}_1^i M_1 + \bar{L}_2^i M_2}{C_p} \quad (16)$$

If either $\bar{L}_1$ or $\bar{L}_2$ is known, this relationship may be used to calculate the other.

In the more usual case where one solution is added to another, Equation 15 is applicable for a two-component system.

Data from which heats of dilution can be estimated are very rarely available, and from a practical standpoint it is best to select systems in which these effects are as small as possible. In general, when nonaqueous solvent systems are used, it is advisable to have the same solvent mixture for titrant and titrated solution.

Table III. Estimation of Relative Differential Heat Contents of Acetic Acid and Water in Mixtures

HAc, % by Wt.	N_2/N_1, Moles HAc per Mole H_2O	$\bar{L}_2$, Calories per Mole HAc[a]	$-\bar{L}_1$, Calories per Mole H_2O[b]	$-\bar{L}_1$, Calories per Mole H_2O[c]	$\bar{L}_2$, Calories per Mole HAc[d]
3.23	0.010	94	0.5		
	0.020	180	1.7		
	0.030	240	3.4		
	0.031	250	3.5	2.3	
	0.040	300	5.2	(7.4)	
	0.047	330	6.7	8.7	
14.3	0.050	340	7.1	(9.2)	
90.9	3.00	380			380
92.1	3.50	360			370
		$L_2^\bullet = 320$			$L_2^\bullet = 340$

[a] Values calculated from curves 1 and 2, Figure 3, by Equation 10.
[b] Estimated from $\bar{L}_2$ by Gibbs-Duhem relationship.
[c] Experimental measurements of Payn and Perman (5); parenthetical values were interpolated.
[d] Estimated from experimental measurements of $\bar{L}_1$ at 20.0° C. given by Payn and Perman by Gibbs-Duhem relationship.

ACKNOWLEDGMENT

The authors are indebted to the United States Atomic Energy Commission for partial support of this work.

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RECEIVED for review February 27, 1956. Accepted May 10, 1956. Taken in part from the doctoral dissertation by Hubert J. Keily, Massachusetts Institute of Technology, Cambridge, Mass., May 1956.

Determination of Platinum and Palladium in Ferronickel Assay Buttons

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Because of the siderophilic nature of the platinum metals it was thought that their collection in iron and nickel would be free of the objections to which lead collection is subject. The isolation of platinum and palladium from large proportions of iron, nickel, and copper was accomplished by cation exchange; known gravimetric and colorimetric methods of analysis were adapted to the effluents. The procedures developed not only provide the basis of a method for button analysis but may find a much wider application in the direct analysis of platinum ores and concentrates and in the refining of platinum metals.

THE determination of platinum metals in ores usually involves a fire assay in which these metals are collected by molten lead. No proved alternative method has been published. There is ample evidence that for at least some of the "insoluble" platinum metals, lead is not a particularly suitable collector. Its use is largely the result of tradition.

From time to time the senior author has considered the application of molten iron or nickel for the purpose of collections. Recently this interest has been encouraged by Falconbridge Nickel Mines, Ltd., which has carried on related experiments during the past few years. A search of the literature revealed little pertinent information on the subject of iron collection.

Benedicks and Lofquist (*2*) point out that the presence of carbon in a melt consisting predominantly of iron and sulfur causes such a melt to split into two phases, of which one is metallic and the other sulfidic. Rankama and Sahama (*8*) state that "In the presence of a metal phase and a sulfide phase, the platinum metals, being siderophile, preferentially enter the former."

Only recently the special heating equipment required was made available for this study. At the same time it became necessary to develop an entirely new approach to the treatment of the buttons because cupellation is excluded. The present paper deals with the preparation and dissolution of buttons, isolation of base metals, and methods of determination of platinum and palladium.

The processes considered for the determinations were divided into four main classes: ion exchange (*3*), precipitation, solvent extraction, and acid parting. Each was examined and it was felt that ion exchange separations were the most promising.

The success of the ion exchange method depends on the fact that the platinum metals form anion complexes and the base metals form cations. The base metals in the presence of strong acids also form anions, but it was hoped that a separation could be effected by adjustment of the acidity. Cation exchange methods were preferred to anion methods in this study because of the difficulties in the quantitative elution of platinum from anion resins. However, there was the possibility that the large cation column required to adsorb the base metals might also adsorb some of the platinum metals. In the following report the efficiency of the separation is recorded.

APPARATUS AND REAGENTS

Dowex-50 cation exchange resin (40 mesh) was used in the adsorption of the base metals.

Absorbance measurements were made with a Beckman Model DU spectrophotometer.

An Ajax-Northrup 40-kw. induction furnace was used for the preparation of ferronickel buttons from ore samples. The furnace was of the 50-pound, spill-type.

A Fisher Infra-Radiator with two 250-watt reflector bulbs was used in button solution evaporations.

Solutions. PALLADIUM. Powdered palladium sponge (J. Bishop Co., Platinum Works, Malvern, Pa.,) was used in preparing a standard solution. A weighed quantity of the metal was dissolved in aqua regia and the solution was evaporated to dryness. The residue was taken up in aqua regia and the evaporation repeated. This procedure was repeated twice with hydrochloric acid and twice with water. The final residue was dissolved in a few milliliters of concentrated hydrochloric acid, diluted to 50 ml. with distilled water, filtered, and diluted to a known volume.

PLATINUM. A standard platinum solution was prepared in the same manner as the palladium solution. Platinum metal sponge was obtained from Johnson, Matthey, and Mallory, Ltd., Toronto.

SYNTHETIC BASE METAL. Preliminary experiments were made on synthetic base metal solutions because of the time and expense involved in the button preparation. Base metal analyses were made on the iron buttons prepared by the procedure described later and a synthetic base metal solution was made which simulated the dissolved constituents in the solution of the button. Twenty-five grams of iron, 3 grams of nickel, and 1 gram of copper in the form of their hydrated chloride salts were dissolved in 5 ml. of hydrochloric acid and 20 ml. of water and diluted to approximately 1.5 liters.

PROCEDURE

Initial experiments indicated that platinum and palladium passed through the column. Quantitative capacity measurements indicated that a resin bed 60 cm. deep and 4 cm. in diameter should have a capacity sufficient to adsorb 30 grams of base metals.

The column was prepared and regenerated by passing 4*N* hydrochloric acid through until the eluate solution contained no base metals. The excess acid was displaced from the column by washing with 1 liter of water.

The following procedure was used in preliminary experiments with base metal solutions. A volume of 1.5 liters, at a pH of approximately 1.5, is passed through the column at a rate of 25 ml. per minute. The column is then washed with 1 liter of water. Because of the adsorption capacity of the columns used, solutions containing more than 30 grams of base metals were divided into two portions and each portion was treated separately during the column operation.

The solution in a volume of approximately 2.5 liters, is evaporated in a 4-liter beaker to about 20 ml. It is then transferred to a 150-ml. beaker and the evaporation is continued almost to dryness. The sample is diluted to 70 ml., the pH is raised above 1.5, and 5 ml. of 2% solution of sodium nitrite is added. In order to convert the chloro compounds of platinum and palladium to the complex nitrites, the solution is boiled for about 5 minutes. The pH of the hot solution is raised to 8 to precipitate all the iron, copper, and most of the nickel. The precipitated base metals are filtered off, dissolved in 3*N* hydrochloric acid, and reprecipitated after digesting the solution of the base metals with sodium nitrite as before. The combined filtrates from the nitrite hydrolysis are acidified with hydrochloric acid and the solution is evaporated to dryness. To ensure the complete decomposition of the nitrite complexes, the salt residue is moistened twice with concentrated hydrochloric acid. The platinum metals are determined by the procedures described below.

Separation and Determination of Platinum and Palladium. Microgram quantities of platinum and palladium are commonly separated by dimethylglyoxime extraction procedures (*11*) or by bromate hydrolysis (*6*). For various reasons these methods were rejected in favor of the procedure developed by Yoe and Kirkland (*9*) which was used with some modifications. Palladium was separated from platinum by extraction of the palladium–*p*-nitrosodimethylaniline complex into chloroform. Platinum also forms a similar complex but requires an extended time at elevated temperatures, whereas the palladium complex forms almost immediately at room temperature. The separation of palladium from platinum was not quantitative in the presence of the large amounts of salt resulting from the hydrolytic precipitation. A preliminary separation of platinum and palladium from the salt was accomplished by reducing platinum to the divalent state with potassium iodide and extracting the diethyldithiocarbamate complexes of platinum and palladium with chloroform (*9*). Following the destruction of the organic extract the palladium–*p*-nitrosodimethylaniline complex was formed, extracted with chloroform, and determined according to the method of Yoe and Kirkland (*9*). After evaporation of the aqueous solution containing the platinum and destruction of the organic matter by treatment with fuming nitric acid and hydrogen peroxide, the platinum was determined by the tin(II) chloride method (*1*), which the authors found to be more satisfactory than the procedure of Kirkland and Yoe (*7*).

Where only platinum or palladium was present, as in the case of some of the solutions which were salted, the following methods were used to determine the metals.

Palladium. Milligram quantities of palladium were determined by precipitation of the dimethylglyoxime-palladium complex at pH 1, followed by direct weighing of the complex rather than ignition of the metal.

Microgram quantities of palladium were determined colorimetrically by the method of Yoe and Overholser (*10*) using *p*-nitrosodiphenylamine reagent.

In the colorimetric procedure some difficulty arose from the interference of a residual color in the sample caused by organic matter leached from the large resin bed. This interference was decreased considerably if the column was washed with water immediately before use. The organic matter in the evaporated effluent was successfully destroyed by the action of fuming nitric-perchloric or nitric-sulfuric acid mixtures. However, fuming in these mixtures resulted in the formation of a red insoluble palladium compound and subsequent analysis yielded low results; this difficulty was avoided by using perchloric-sulfuric-nitric acid mixtures.

Platinum. Thiophenol was used successfully for the gravimetric determination of platinum (*4*). Although it is a rather unpleasant reagent to work with, it is not subject to interference from any traces of base metals.

Tin(II) chloride was found to be a good reagent for the determination of microgram quantities of platinum (*1*). However, organic matter leached from the column increased the absorbance of the solution. Low results due to the precipitation of platinum were encountered when fuming nitric-sulfuric acid mixtures were used to destroy this organic matter but this difficulty was overcome by the use of perchloric and nitric acids.

DISCUSSION

The capacity of the resin column described here for adsorbing base metals was proved satisfactory by passing through the

column 2 liters of the synthetic base metal solution. Trace amounts of iron were found in the effluent. Further synthetic solutions were passed through the column at different pH values and different degrees of dilution. Only 3.5 mg. of iron escaped adsorption when 1.5 liters of base metal solution were passed through the column at pH 1.5. At pH 1.0 the separation still appeared satisfactory but at pH 0.8 the efficiency of adsorption was considerably lowered. Similarly, at pH 1.5 and a total volume of 1 liter, the separation was not as efficient (because of a high chloride ion concentration). At a pH greater than 2 and at a dilution of 1.5 liters the hydrous oxide of ferric iron started to precipitate.

The rate of flow of the base metal solution through the column was not critical, but flow rates exceeding 40 ml. per minute or less than 5 ml. per minute resulted in increasing the iron concentrations in the effluent. The low adsorption efficiency in the latter case was not expected. A possible explanation lies in assuming that if the column is operated so slowly as to approach equilibrium conditions, the hydrogen ion is preferentially adsorbed at this acid concentration.

Table I. Determination of Palladium or Platinum in Base Metal Solutions

Palladium		Platinum	
Added, Mg.	Recovered, Mg.	Added, Mg.	Recovered, Mg.
5.013	4.994	5.02	5.13
5.013	4.807	5.02	5.05
5.013	5.037	5.02	5.00
5.013	5.037	5.02	5.16
3.008	3.013	5.02	5.02
3.008	2.998	5.02	5.07
		5.02	5.17
		5.02	5.11
		5.02	5.04
500 γ	505 γ		
100	101		
100	100		
50	44	500 γ	495 γ
50	49	500	503
40	38	500	500
40	38	500	502
20	17	100	100
10	9	100	99
		100	95
		100	98
		50	50
		50	51

Table II. Determination of Platinum and Palladium in Base Metal Solution[a]

Palladium, γ		Platinum, γ	
Added	Recovered	Added	Recovered
100	98	100	102
100	102	100	99
100	98	100	102
100	99	100	100
100	98	100	98
100	97	100	98
100	101	100	101

[a] Standards for determination of palladium in presence of platinum were prepared by separating 100 γ of palladium from 1 gram of salt by solvent extraction procedures.

Salting of Base Metal Solutions. In order to facilitate the development of an analytical method, a solution containing only base metals was passed through the column and the effluent, containing the traces of base metals which had escaped adsorption, was salted with known amounts of platinum and palladium. This solution was evaporated and the small amount of base metals was separated hydrolytically prior to the determination of the precious metal content (*5*). Base metal solutions were also salted with platinum and/or palladium prior to passing through the column, and treated as described above. The results are recorded in Tables I and II.

DETERMINATION OF PLATINUM AND PALLADIUM IN BUTTONS

Preparation of Furnace. A sheet of mica was bent to form a cylinder and was placed inside the working coil. A layer of carbon black was spread at the bottom of the coil and the graphite crucible ($6^1/_4$ inches in outside diameter, $5^1/_4$ inches in inside diameter, $7^1/_2$ inches deep) was set in place. The space between the mica and the crucible was filled with carbon black, tamped firmly in place to avoid air pockets. A thin layer of refractory cement was spread over the carbon black and around the top of the crucible. This was allowed to dry overnight.

Preparation of Buttons. The ferro-alloy buttons used for this work were prepared at the Falconbridge Nickel Mines Laboratories as follows.

CHARGE. An ore sample was crushed to about 200 mesh and dried thoroughly. It was placed on a paper with sodium carbonate and borax fluxes and rolled until a mixture of uniform color was obtained.

Stoichiometric calculations were made from the following equations:

$$SiO_2 + Na_2CO_3 \rightarrow Na_2O.SiO_2 + CO_2 \quad (1)$$
$$SiO_2 + Na_2B_4O_7 \rightarrow Na_2O.SiO_2 + 2B_2O_3 \quad (2)$$

The amount of borax added was that calculated to react with one fourth of the silica present in the sample. The reaction between the oxides of the ore and the silica was neglected.

For the crucible size given above, the following charge was used:

	Grams
Ore sample	1000
Na_2CO_3	424
$Na_2B_4O_7$	270

The charge was poured into the crucible and packed down. A number of small holes were punched through the packed charge to allow escape of gases and to prevent surges of gas from ejecting part of the charge. The crucible was covered with a flat graphite lid through which a hole $^3/_4$ inch in diameter was drilled.

SMELTING. The furnace was heated slowly at first with the power control set at about 10 kw. When the crucible attained a red heat, a yellow flame appeared at the hole in the crucible lid. Power was increased slowly until the pressure of the flame decreased and the charge was molten. The power was then set at 20 kw. and the crucible lid was removed. An optical pyrometer was used to measure the temperature at the contact between the liquid surface and the crucible wall. When a temperature of 1350° C. was reached, the time was noted and the reaction was allowed to proceed for at least 2 minutes. At the end of the reaction time, the power was cut off and the melt was poured immediately into a conical graphite mold.

After the melt solidified, it was removed from the mold. The divisions between slag, matte, and the small metal button at the tip of the cone were fairly sharp. The metal button was tapped loose and any matte adhering to it was removed by wetting, after which the matte became powdery and flaked off.

It was found that a reaction time of 2 minutes at 1350° C. was required to produce a metal button weighing about 35 grams. Less metal was produced at shorter reaction times, but between 2 minutes and 15 minutes a linear relationship was noted between weight of metal and reaction time. At 15 minutes reaction time, a button weighing 85 to 90 grams was produced. Longer reaction times did not give further weight increases.

Dissolution of Buttons. The evaporation of the aqua regia solution of the metal button on a hot plate was exceedingly slow and resulted initially in the formation of hydrated basic oxides. This was avoided by evaporation with a heat lamp. After reduction to a small volume, dilution of the sample with distilled water resulted in the formation of some hydrous ferric oxide. This difficulty could not be completely eliminated by increasing the acid concentration. No adjustment of acidity was necessary prior to column operation, as the solutions prepared according to the following procedure had a pH of 1.3 ± 0.2.

Procedure. The button is treated with aqua regia for about 12 hours. The dissolving process is hastened by gentle heating on the steam bath and repeated additions of aqua regia during the course of the dissolution.

When the button is reduced to a small insoluble residue, most of which is carbon, the sample is transferred to a 14-inch evaporating dish and an infrared heat lamp is mounted 5 inches above it. The evaporation of the button solution is continued until there are no visible hydrochloric acid fumes evolved. To remove excess nitric acid, 5-ml. portions of hydrochloric acid are added three times and the evaporation is repeated each time. The base metal solution, now at a volume of approximately 60 ml., is diluted to 1.5 liters with distilled water. Any hydrous ferric

Table III. Determination of Platinum and Palladium in Salted and Unsalted Aliquots

(100 γ each of platinum and palladium added to aliquot *B*)

Button Wt., Grams	Platinum Found, γ		Palladium Found, γ	
	A	*B*	*A*	*B*
60	134	237	9	111
46	38	133	11	97

oxide formed on dilution and the insolubles remaining after the aqua regia treatment are removed from the solution by suction filtration through a Whatman No. 40 filter paper. The filter paper and residue are burned at 400° C. in a furnace and the ignited residue is leached three times with 10-ml. portions of aqua regia. The extracts are combined and the nitric acid is removed by evaporation in hydrochloric acid. After evaporation to a small volume the extract is added to the solution of the button. This solution is passed through the column and the determinations are carried out as described above.

When the ferronickel button solutions were passed through the column, the effluent solution was highly colored. This color was due to carbonaceous matter from the assay treatment and it was destroyed by fuming with perchloric and nitric acids. Fuming nitric acid and hydrogen peroxide were equally effective in the decomposition process and the latter method was adopted because the less volatile acids are difficult to remove and sometimes cause the precipitation of platinum metals.

RESULTS

Because no published method is known for the determination of platinum and palladium in ferro-alloy buttons obtained from platiniferous materials, the methods proposed here were checked in the following manner.

Buttons were dissolved in aqua regia and diluted to 3 liters. Half of this amount was salted with 100 γ each of platinum and palladium. The base metals were adsorbed and the platinum metals separated and determined as described above. The results are shown in Table III.

ACKNOWLEDGMENT

The authors are indebted to P. G. Thornhill of the University of Toronto and F. R. Archibald of Falconbridge Nickel Mines, Ltd., for advice concerning the metallurgical processes.

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RECEIVED for review November 14, 1955. Accepted May 2, 1956.

Titration of Mercury with Bis(2-hydroxyethyl)dithiocarbamate

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A direct titrimetric method permits the determination of mercury(II) in the presence of chloride, bromide, and numerous metal ions. The mercury is titrated with a standard dithiocarbamate solution in the presence of ethylenediamine tetraacetate and copper(II). Although most mercury dithiocarbamates are insoluble, formation of a precipitate is avoided by using bis(2-hydroxyethyl)dithiocarbamate as the titrant and acetone-water as the solvent for the mercury. The end point of the titration is indicated by the appearance of a yellow color caused by reaction of the first excess dithiocarbamate with copper(II). The titration may also be followed potentiometrically using a silver-dithiocarbamate indicator electrode.

SEVERAL titrimetric methods for mercury are available. Some of these are excellent for certain uses, but all are somewhat limited in their scope of application. Chloride, bromide, and some metallic ions interfere in the widely used titration of mercury(II) with thiocyanate (*12*). A fairly recent alkalimetric method permits rapid and accurate titration of mercury in chloride solutions (*3*), but a great many metallic ions might interfere. Reduction of mercury to the metal, as in the Rupp method (*8*), followed by titration with a standard oxidizing agent, is less convenient than direct titration methods, in that quantitative isolation of a precipitate is required. Titrimetric methods employing ethylenediaminetetraacetic acid (EDTA) are rather nonselective for mercury (*9*).

In the method reported here, a standard dithiocarbamate solution is used to titrate mercury(II). Sodium diethyldithiocarbamate and certain other dithiocarbamates have been used for years in the colorimetric determination of copper. Many other metallic ions form complexes with dithiocarbamate, however, and some of these are highly colored. The selectivity of this reagent is considerably improved by using it in conjunction with masking agents. For example, copper can be determined colorimetrically in the presence of most other metals by adding ethylenediaminetetraacetic acid to complex these metals while the copper dithiocarbamate is extracted into a suitable nonaqueous solvent (*2, 6, 11*). Copper is also complexed by ethylenediaminetetraacetic acid but forms an even stronger dithiocarbamate complex. It is reported that shaking the nonaqueous layer containing cupric dithiocarbamate with aqueous mercury(II) will cause the copper complex to be broken, apparently by forming more stable mercury dithiocarbamate (*7*).

In this paper three methods are proposed for the titration of mercury(II) with dithiocarbamate. The first employs Thiomichler's ketone as a visual indicator (*4*). The second is a potentiometric titration using a silver-silver dithiocarbamate indicator

electrode. The third involves titration in the presence of ethylenediaminetetraacetic acid using a small amount of copper(II) as the indicator. Here the dithiocarbamate first combines with the mercury(II), forming essentially a colorless complex. The first slight excess of titrant then reacts with the copper(II), forming a highly colored yellow complex. This third method appears to be the most convenient and widely applicable and is therefore presented in more detail than the others.

APPARATUS

For potentiometric titrations a silver–silver dithiocarbamate and calomel electrode system was used. A silver electrode (which may be either a button type or a silver wire) was immersed for about 1 minute in 1 to 1 nitric acid, then soaked for 10 to 20 minutes in a 5% aqueous solution of diethylammonium bis(2-hydroxyethyl)dithiocarbamate plus 1% ammonium acetate. This treatment was repeated before each titration. A sleeve-type calomel electrode was employed. When it was desired to exclude chloride, a potassium nitrate salt bridge was used in conjunction with the calomel electrode. A Beckman Model G pH meter was used as the titrator. All titrations were carried out using a 15-ml. buret which could be read accurately to 0.01 ml.

The ion exchange column used was made from 24-mm. glass tubing. It had a sintered-glass disk sealed in to hold the resin and a top section of wider diameter to permit expansion of the resin during backwashing. A bed of Dowex 50 × 8 resin (100 to 200 mesh) approximately 2 inches high was employed.

REAGENTS AND SOLUTIONS

Bis(2-hydroxyethyl)dithiocarbamate, 0.02*N*. Add 1.52 grams of carbon disulfide and 8.40 grams of diethanolamine to 100 ml. of isopropyl alcohol and dilute to 1 liter with isopropyl alcohol. Standardize by titration against mercury(II), using mercuric chloride as the primary standard.

Bis(2-hydroxyethyl)ammonium Bis(2-hydroxyethyl)dithiocarbamate. Add 15 ml. of carbon disulfide with stirring to a solution of 15 grams of diethanolamine in about 300 ml. of methanol. Add 5 ml. of concentrated ammonia, allow to stand for a few minutes, then add 200 ml. of ether. Filter off the crystals and wash with small portions of methanol and ether.

Copper Nitrate–Ethylenediamine Tetraacetate. Mix 5 ml. of 0.1*M* cupric nitrate with 25 ml. of 0.05*M* disodium ethylenediamine tetraacetate. Add 0.5 gram of ammonium acetate.

Mercuric Chloride, reagent grade, anhydrous. Dry 1 or 2 hours at 110° C. before using as a primary standard.

Table I. Stability of Bis(2-hydroxyethyl)dithiocarbamate Solution

Age of Titrant, Days	Titrant Required to Titrate Mercury, Ml.
0	1.24
2	1.24
3	1.25
4	1.26
9	1.29
10	1.29
16	1.29

Mercuric Nitrate, 0.02*N* (0.01*M*). Dissolve 3.42 grams of mercuric nitrate monohydrate in about 75 ml. of water which contains 1 or 2 ml. of concentrated nitric acid. Dilute with water to 1 liter.

Thiomichler's Ketone. Prepare a 0.1% solution in acetone.

PROCEDURES

1. Copper(II) Indicator. Take for analysis a sample that contains between 4 and 25 mg. of mercury. Dilute with water and acetone, so that the volume is between 50 and 100 ml. and contains 30% acetone. Neutralize to apparent pH 2 to 7 with dilute ammonia and add approximately 0.5 gram of ammonium acetate. Add sufficient ethylenediamine tetraacetate to complex all foreign metal ions present in the sample. Add 3 drops of copper nitrate–ethylenediamine tetraacetate mixture and titrate with 0.02*N* bis(2-hydroxyethyl)dithiocarbamate to the first appearance of a permanent yellow.

2. Potentiometric Titration. Use the amount of sample, solvent, and buffer recommended in Procedure 1. Add sufficient ethylenediamine tetraacetate to complex any foreign metal cations present. Using a silver–silver dithiocarbamate and a calomel electrode, titrate with 0.02*N* bis(2-hydroxyethyl)dithiocarbamate. In the immediate vicinity of the end point it is often necessary to allow a minute or two for the potential to reach a constant value. In the other regions of the curve, readings may be taken rapidly.

Table II. Effect of Cations on Titration of Mercury

Ion Added[a]	Titrant, Ml. Theory	Titrant, Ml. Actual	Difference, Ml.
Ag^{+}	2.44	Interferes	...
Al^{+++}	2.44	2.43	−0.01
Au^{+}	2.44	Interferes	...
Ba^{++}	2.44	2.44	0.00
Be^{++}	2.44	2.44	0.00
Bi^{+++}	2.44	2.47	+0.03
Ca^{++}	2.44	2.44	0.00
Cd^{++}	2.44	2.44	0.00
Ce(IV)	2.44	2.44	0.00
Co^{++}	2.44	2.44	0.00
Cr^{+++}	2.44	2.48	+0.04
Dy^{+++}	2.49	2.49	0.00
Er^{+++}	2.49	2.49	0.00
Fe^{++}	2.50	2.44	−0.06
Fe^{+++}	2.50	2.48	−0.02
La^{+++}	2.49	2.49	0.00
Mg^{++}	2.45	2.45	0.00
Mn^{++}	2.45	2.45	0.00
Ni^{++}	2.45	2.44	−0.01
Ru^{+++}	2.49	2.51	+0.02
Sn^{++}	2.45	2.45	0.00
Sn(IV)	2.45	2.46	+0.01
Th(IV)	2.45	2.45	0.00
UO_2^{++}	2.49	2.49	0.00
Zn^{++}	2.49	2.49	0.00
Zr(IV)	2.49	2.54	+0.05

[a] Mole ratio of ion added to mercury(II) was 2 to 1.

3. Thiomichler's Ketone as Indicator. Follow Procedure 1, but omit addition of cupric nitrate–ethylenediamine tetraacetate mixture. Add 1 drop of Thiomichler's ketone solution and titrate with dithiocarbamate, taking the color change from blue to clear yellow as the end point.

4. Ion Exchange Procedure. Convert the resin column to the sodium form; rinse with distilled water and lastly with 0.05*M* sodium chloride. Pass a sample, which contains a suitable quantity of mercury(II), through the column. Use three or four 15-ml. portions of 0.05*M* sodium chloride to wash the column free of sample. Titrate the mercury(II) in the effluent (or in an aliquot thereof) according to Procedure 1.

GENERAL CONDITIONS FOR TITRATION

Titrant. Most metal complexes of diethyldithiocarbamate are insoluble in water. This may cause serious coprecipitation error when one metal is titrated in the presence of others (*10*). To avoid precipitation, bis(2-hydroxyethyl)dithiocarbamate is employed as the titrating agent. Apparently the increased water solubility of the metal complexes of this reagent is due to the alcoholic groups present in the molecule. The potassium, sodium, and diethanolammonium salts of bis(2-hydroxyethyl)dithiocarbamic acid are readily soluble in water. An aqueous solution of the pure salt is unstable, and a precipitate appears within an hour or two. A titrant which remains perfectly clear for at least 2 months can be prepared very easily by mixing carbon disulfide with excess diethanolamine in isopropyl alcohol. [This is very similar to the reagent prepared by Geiger and Müller (*5*) for use as a colorimetric reagent for copper.] The stability of this titrant was demonstrated by titrating mercury at various time intervals (see Table I).

Solvent. When mercury(II) is titrated in water with 0.02*M* bis(2-hydroxyethyl)dithiocarbamate, a precipitate appears slightly before the end point, unless the mercury(II) solution is extremely dilute. Addition of an appropriate nonaqueous solvent will prevent the formation of any precipitate. Titration of mercury(II) in a mixture containing about 3 volumes of acetone to 7 volumes of water is recommended.

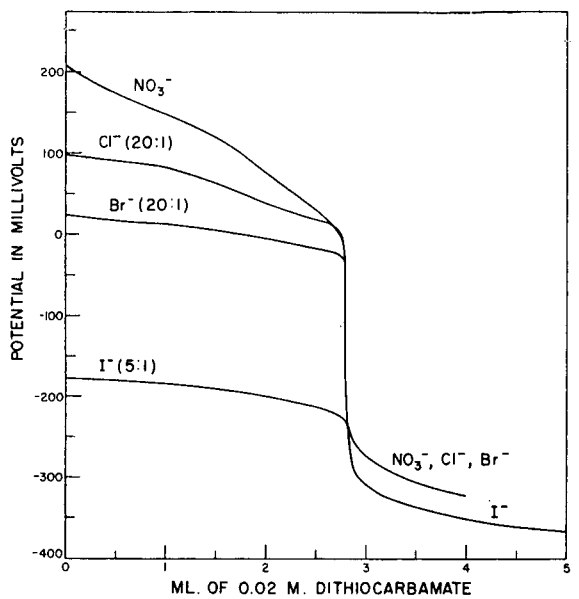

Figure 1. Potentiometric titration of mercury(II) in presence of different anions

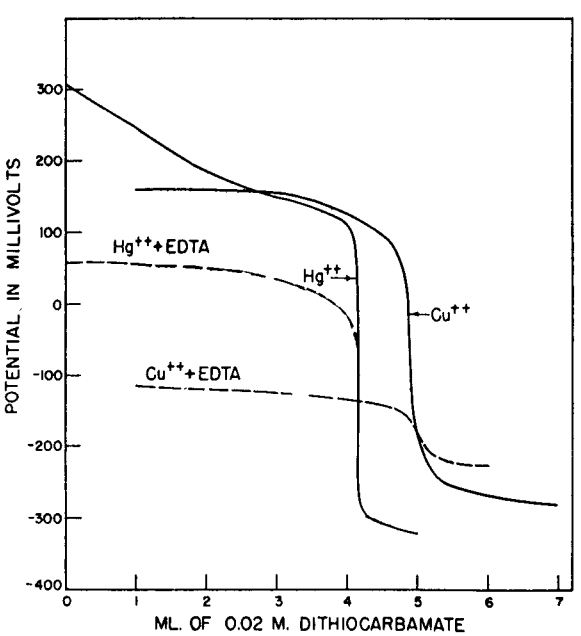

Figure 2. Effect of EDTA on titration curves of mercury(II) and copper(II)

Acidity. Dithiocarbamate and metal dithiocarbamates decompose in acid solution. For this reason a pH of 6 or above is recommended in work with dithiocarbamates (*1*). If mercury(II) is titrated with dithiocarbamate in alkaline solution, no distinct end point is obtained. The best results are obtained using ammonium acetate buffer, so that the apparent pH is about 7.

TITRATION USING THIOMICHLER'S KETONE AS INDICATOR

Thiomichler's ketone [4,4′-bis(dimethylamino)thiobenzophenone] forms a blue-violet complex with mercury(II) (*4*) and can be used as the indicator in the titration of mercury(II) with dithiocarbamate. The end point is a very sharp change from blue to yellow, corresponding to the conversion of the mercury-indicator complex to colorless mercury dithiocarbamate with the simultaneous release of the yellow free indicator. This appears to be a rather selective method for mercury, but the end point is much less sharp when chloride or ethylenediamine tetraacetate is present.

POTENTIOMETRIC TITRATIONS

Potentiometric titrations were carried out using an indicator electrode prepared by cleaning a silver wire by brief immersion in 1 to 1 nitric acid, followed by 10 to 20 minutes' soaking in 5% aqueous bis(2-hydroxyethyl)ammonium bis(2-hydroxyethyl)dithiocarbamate solution which was also 1% in ammonium acetate.

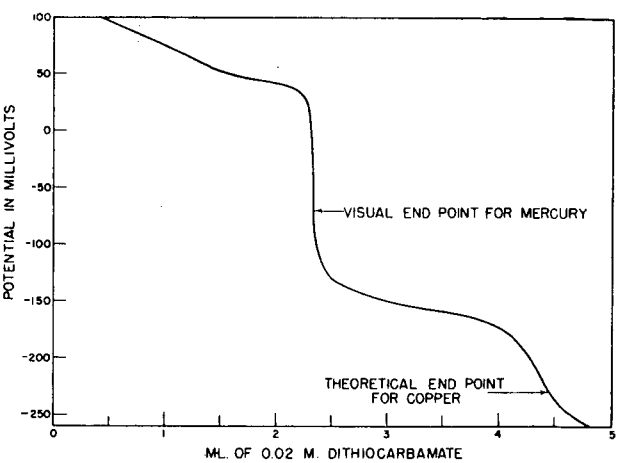

Figure 3. Potentiometric titration of mercury(II) and copper(II) in presence of EDTA

This treatment produces a thin coating on the surface of the silver electrode, which is believed to be silver bis(2-hydroxyethyl)-dithiocarbamate. After about three titrations this electrode appears to lose most of this coating and does not function well until the nitric acid–dithiocarbamate treatment is repeated. In the titration of mercury(II) with dithiocarbamate, the potential at various parts of the curve varies somewhat from one titration to another, but the general shape of the curve and the end point seem to be reproducible.

A typical potentiometric curve for titration of mercuric nitrate with dithiocarbamate is shown in Figure 1. The effect of certain anions is shown by the other curves in Figure 1. Chloride and bromide have a negligible effect on the sharpness of the end point, but cyanide and iodide make the end point ambiguous. Figure 2 shows the effect of ethylenediamine tetraacetate on the titration curves of mercury and copper. From these curves it will be seen that in the absence of complexing agents, copper forms a dithiocarbamate complex of approximately the same strength as mercury. However, copper forms a much stronger ethylenediamine

tetraacetate complex at pH 7 than does mercury; hence, in the presence of ethylenediamine tetraacetate, the formation of mercury dithiocarbamate is more favorable than the formation of copper dithiocarbamate. This constitutes the basis of a visual indicator method for titrating mercury. Mercury(II) is titrated with dithiocarbamate in the presence of ethylenediamine tetraacetate and copper. The titrant first reacts with the mercury(II).

Table III. Effect of Anions on Titration of Mercury

Ion Added	Ratio of Ion Added to Hg^{++}	Titrant, Ml. Theory	Titrant, Ml. Actual	Difference, Ml.
Fluoride	20:1	2.48	2.48	0.00
Chloride	10:1	2.48	2.48	0.00
Bromide	10:1	2.48	2.48	0.00
Iodide	12:1	2.48	2.32	−0.16
Thiocyanate	20:1	2.48	2.48	0.00
Cyanide	20:1	2.48	Interferes	...
Oxalate	20:1	2.48	2.46	−0.02
Phosphate	20:1	2.48	2.48	0.00
Citrate	20:1	2.48	Interferes	...
Titrate	20:1	2.48	2.48	0.00
Vanadate	2:1	2.48	2.49	0.00

The end point occurs when the first slight excess of dithiocarbamate reacts with copper(II) to form a yellow complex. The potentiometric curve for this titration is shown in Figure 3. The visual end point appears approximately at the steepest portion of the potentiometric curve.

TITRATION USING COPPER(II) AS INDICATOR

The effect of varying the concentration of copper(II) and ethylenediamine tetraacetate was studied. Varying the copper(II) concentration from $5 \times 10^{-4}M$ to $5 \times 10^{-5}M$ did not affect either the sharpness of the end point or the stoichiometry of the reaction. A copper(II) concentration greater than about $10^{-3}M$ reduced the sharpness of the end point because of the rather deep blue color of the cupric ethylenediamine tetraacetate complex. The concentration of ethylenediamine tetraacetate was varied from $5 \times 10^{-2}M$ to $5 \times 10^{-3}M$ without affecting the results. The prime requisite is that sufficient ethylenediamine tetraacetate be present to complex all of the copper and other metals present.

Table IV. Analysis of Synthetic Samples

(Sample dissolved in nitric acid, diluted, and suitable aliquot taken for analysis)

Metal Taken	G.	Hg Found, G.	Recovery, %
Hg	1.5897	1.579	99.3
Pb	3.0	1.582	99.5
Ni	1.0	1.582	99.5
Hg	1.915	1.920	100.3
Pb	24.0	1.913	99.9
		1.917	100.1

Data for individual titrations of mercury(II) in the presence of various ions are given in Tables II and III. Of the cations studied, silver and gold(I) interfere by forming a yellow complex with the titrant, bismuth and chromium(III) cause high results, and iron(II) causes slightly low results. Of the anions studied, chloride and bromide do not interfere even if present in large amounts. Small concentrations of iodide can be tolerated. Oxalate, phosphate, and tartrate do not interfere, but thiocyanate causes a fading end point, and cyanide, citrate, and arsenite prevent the appearance of an end point.

To check the method further, synthetic samples were prepared by taking known amounts of metallic mercury together with other metals. Data for the analysis of several such samples are presented in Table IV.

Mercury(II) can be separated from relatively large quantities of most metal ions by a simple ion exchange procedure. The principle of this separation is that mercuric chloride is highly associated and will pass through a cation exchange column.

Table V. Titration of Mercury Following Ion Exchange Separation

Ion Added	Ratio of Ion Added to Hg	Titrant, Ml. Theory	Titrant, Ml. Actual	Difference, Ml.
Cu^{++}	20:1	5.27	5.23	−0.04
	40:1	5.27	5.22	−0.05
	60:1	5.27	5.24	−0.03
	80:1	5.27	5.26	−0.01
	100:1	5.27	5.24	−0.03
UO_2^{++}	2:1	5.30	5.28	−0.02
	4:1	4.20	5.24	−0.06
	10:1	5.30	5.30	0.00
	20:1	5.30	5.30	0.00
	30:1	5.30	5.24	−0.06
Fe^{+++}	50:1	5.40	5.29	−0.11
	100:1	5.40	5.43	+0.02
	300:1	5.40	5.40	0.00
	500:1	5.40	5.40	0.00

Most other metal ions do not combine strongly with chloride and are quantitatively taken up by the resin column. To ensure excess chloride at all times, a sodium chloride solution is used to wash the sample through the column. In Table V, data are given for the titration of mercury(II) samples following ion exchange separation of the other metal ions present.

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RECEIVED for review December 12, 1955. Accepted April 30, 1956. Division of Analytical Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955. Work performed in Ames Laboratory, U. S. Atomic Energy Commission.

Microdetermination of Methyl Groups Attached to Carbon

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This study was undertaken as a first step toward a better understanding of the dichromate oxidation of aliphatic compounds containing methyl groups. Samples were digested in sealed tubes in a rocking furnace. A jacketed distillation apparatus, used in the acetic acid distillation step, eliminated carry-over of sulfuric acid spray and reduced the distillation time when the jacketing substance boiled in the range 110° to 118° C. Acetic acid is produced quantitatively from several low boiling aliphatic compounds, but not from terminal methyl groups attached to aliphatic amines of low molecular weight. Three complex substances were investigated, employing the Kuhn-Roth oxidation mixture and the increased amount of sulfuric acid recommended by Ginger. The results do not show one reaction mixture to be clearly superior, but they illustrate that the conditions of this determination cannot be as rigorous as has been previously employed.

THE Kuhn-Roth method (*3*) for the determination of terminal methyl groups is based upon the oxidation of hydrocarbon chains, $CH_3—C<$, with a chromic acid–sulfuric acid mixture. Under the conditions employed, the oxidation of acetic acid by the reaction mixture is relatively slow as compared to the oxidation of the sample to acetic acid.

Ginger (*2*) reported that the amount of sulfuric acid employed in the Kuhn-Roth procedure was not sufficient to dissolve fatty acids. He found that increasing the amount of sulfuric acid from 1 ml. to 2 ml. resulted in complete solubility of the fatty acid and a marked increase in the rate of digestion. The higher results obtained by Ginger suggest that a re-examination of the procedure and apparatus might lead to further improvements in this functional group determination, provided the rate of oxidation of acetic acid is not markedly increased.

Campbell and Morton (*1*) showed that they could not obtain quantitative yields of acetic acid when *gem*-dimethyl or *tert*-butyl groups were present in the compound studied. They also stated that low results could not be attributed to the formation of acetone in the oxidation step, because samples of acetone and mesityl oxide each produced approximately three fourths of the theoretically expected amount of acetic acid. Although Campbell and Morton demonstrated that the major portion of each of these samples was oxidized to acetic acid, the missing one fourth in each instance is unexplained. For acetone, the loss must be due either to volatility or to stability of the molecule itself. In the case of mesityl oxide the problem is complicated further by the presence of a $(CH_3)_2—C<$ group, for which Campbell and Morton obtained recoveries of the order of 75 to 85%. However, loss of sample by volatility should have been reduced by use of this higher boiling substance. The fact that the same relative amount of acetic acid is produced in the oxidation of mesityl oxide as of acetone tends to support the argument for the stability of some nonacidic fragment in the reaction mixture.

Because of the uncertainty associated with volatilization from the reaction mixture of organic compounds of low molecular weight during the refluxing step, all samples in the present work were digested in sealed tubes. Also, to ensure continuous mixing of a sparingly soluble solid, an immiscible liquid, or a gaseous organic compound with the sulfuric acid–chromic acid solution, digestions were carried out in a rocking furnace.

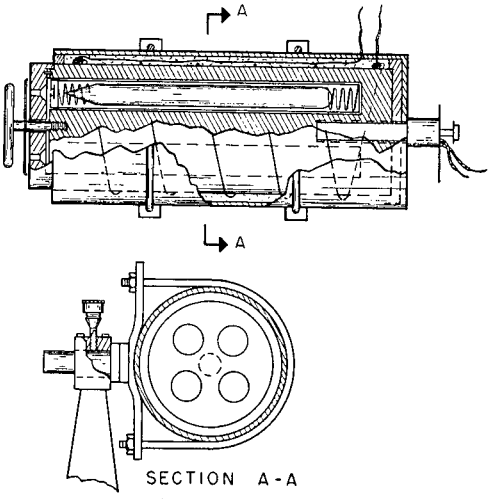

Figure 1. Rocking furnace for digestion of samples

The conventional apparatus of Kuhn and Roth for the distillation of acetic acid was not employed, for three reasons. First, too frequently, the distillate contains appreciable amounts of sulfate ion. Secondly, it is desirable to shorten the distillation time, and this is accomplished by jacketing the distilling flask at a temperature that approximates the boiling point of acetic acid. Lastly, the nuisance of collecting 5-ml. portions of distillate as well as the cumulative errors resulting from their successive titrations is avoided.

APPARATUS

Rocking Furnace. The furnace shown in Figure 1 is constructed so that it rocks through an angle of 45°. It is driven by a Bodine Electric Co. 1/6-hp., Type NSI-55RH motor which revolves at 38 revolutions per minute. The furnace is constructed from a 12-inch length of 4-inch diameter Dural. Four 10-inch holes, each $1^1/_8$ inches in diameter, are bored in the block. The cover is drilled so that each compartment has an outlet to the air, and a baffle is placed between the cover and the handle to protect the operator, should one of the tubes break. A 1/4-inch pin on the face of the furnace, which fits into a hole in the cover, centers each vent over a compartment. The opposite end of the Dural block is drilled to contain a Fenwal thermoswitch which can be controlled over the temperature range 110° to 205° C. within ±0.5° C. Two rings made from 0.5-inch Transite are used to center the Dural block in an outer steel tube. The base ring is countersunk as shown in the figure. The two rings are slipped onto the Dural block and in each case held in place by means of three countersunk screws.

The walls of the Dural block are covered with asbestos paper and wrapped with 30 feet of 22-gage Chromel wire. The wire is fastened securely to two $^1/_4$-inch Transite pins mounted in the side of the Dural block, as shown in the figure. The Chromel wire then is covered with asbestos rope and the unit is inserted into the steel tube, which is $4^3/_4$ inches in outside diameter and has a $^1/_{16}$-inch wall thickness. The steel tube is fastened to each Transite ring with screws. The furnace is attached to a rocking arm and mounted on a stand by means of a bearing as shown in Figure 1, *A-A*.

Sealed Tube Container. The sealed tube container shown in Figure 2 is made of steel tubing 1 inch in diameter, having a $^3/_{32}$-inch wall thickness, and is approximately 10 inches long. Its length is adjusted to fit into the furnace compartment, so that it will not slide when the furnace is rocking. The cap contains a baffle, as indicated in Figure 1, as a protection to the operator if a sealed tube should break. Coils of wire are inserted before and after the sealed tube to prevent breakage while the furnace is rocking and also to allow variation in the length of the tube. The sealed tubes are made from borosilicate glass tubing 18 mm. in outer diameter and may vary from 6 to 9 inches in length.

Distillation Apparatus. The distillation apparatus is shown in Figure 3. Its use completely eliminates carry-over of sulfuric acid spray during distillation and decreases the time necessary to obtain quantitative distillation of acetic acid. This apparatus permits jacketing the distilling flask at a temperature above that of the entering steam, thus increasing the rate of distillation of acetic acid.

Figure 2. Steel casing and cover containing sealed tube to be placed in rocking furnace

PROCEDURE

The oxidation mixtures all contained 4 ml. of 5*N* chromic acid. The hydrogen ion concentration was varied, however, and, in each set of experiments, the amount of acid employed is specified, together with the results. A sample sufficiently large to yield 0.05 to 0.08 mmole of acetic acid was employed for these determinations. When the sample to be analyzed was a solid, it was weighed into the borosilicate glass tube, the oxidation mixture added, and the tube sealed. When the sample was a liquid having a low partial pressure, so that it could be handled in air, it was weighed into a boat. The boat and sample were transferred to the oxidation mixture in the borosilicate glass tube, and the tube was sealed. If the sample was a liquid with a high vapor pressure, it was weighed in a capillary tube, the tube was dropped into the reaction mixture in the borosilicate tube, and the tube was sealed. If the boiling point of the sample was greater than 100° C., it was expelled from the capillary prior to the digestion period. The sealed tube was carefully tilted to leave the capillary suspended on the wall of the vessel free of the oxidation mixture and the tube was warmed gently in the immediate vicinity of the sample until the liquid was expelled. The tube was righted rapidly to draw some of the oxidation mixture into the capillary. The sealed tube was then placed in the steel container shown in Figure 2 which, in turn, was inserted into the rocking furnace at the desired temperature and the sample was heated for the required period of time. Prior to opening the sealed tube, the tip was heated to drive the liquid from it and the contents were cooled to room temperature. Finally the tube was opened, using an oxygen torch.

The contents of the tube were transferred into the distilling flask (the inner flask of Figure 3) with the aid of three small distilled water rinses. A small quantity of lubricant was applied to the ground-glass area. The outer flask shown in Figure 3 was detached from the apparatus, and the distilling flask was connected to the 24/40 joint and fixed in position by means of a spring. The outer flask containing the jacketing substance was attached to the apparatus. The complete assembly was clamped in position and the outer flask heated by means of a Glascol mantle (size for a 250-ml. boiling flask). When the jacketing substance was heated to boiling, steam generated from a boiling flask was introduced into the distilling flask through the side arm by attachment to the 12/9 ball joint. The distillate was collected in a 125-ml. Erlenmeyer flask until the acetic acid was quantitatively transferred (the volume required for quantitative transfer is shown in a subsequent section). The contents of each Erlenmeyer flask were boiled to expel carbon dioxide, stoppered, and cooled in an ice bath. The acetic acid was titrated with standard 0.01*M* sodium hydroxide.

RESULTS

Titration of Acetic Acid. The pH at the stoichiometric end point in the titration of 0.002*M* acetic acid with 0.01*M* sodium hydroxide is approximately 8. As the titration error at the phenolphthalein end point (pH = 8.5 to 8.7) is about 0.5%, phenol red is a more suitable indicator. Bromothymol blue produces a smaller titration error than does phenolphthalein, if the change in color from green to blue is selected as the end point. In this study bromothymol blue was used.

Distillation of Acetic Acid. The volume of distillate required to transfer the acetic acid quantitatively from the reaction mixture to the receiver was determined by jacketing the distilling flask at the boiling point of three compounds: water, toluene (boiling point 110.5° C.), and *n*-butyl alcohol (boiling point 117.7° C.). The rate of distillation of acetic acid at the three jacket temperatures is shown in Figure 4. When the distilling flask was jacketed with *n*-butyl alcohol, 50 ml. of distillate were required to transfer 0.05 mmole of acetic acid quantitatively, while a toluene-jacketed apparatus required 60 ml. of distillate. A water-jacketed apparatus was not satisfactory, because the jacket temperature was of the order of 5° C. less than the boiling point of the reaction mixture. As a consequence, the volume increase of the reaction mixture during distillation seriously retarded the rate of transfer of the acetic acid, as shown by the figure. The apparatus was not jacketed with a substance boiling higher than *n*-butyl alcohol because the temperature differential between the jacket and the reaction mixture (boiling point approximately 105° C.) is sufficiently large to make the initial rate of distillation very rapid, and bumping may result. However, even when *n*-butyl alcohol was used to jacket the apparatus, no samples were lost by carry-over of sulfuric acid in the course of the study.

Stability of Acetic Acid. The $\Delta F_{298°\,K.}$ per mole of acetic acid for the reaction:

$$4H^+ + 4HSO_4^- + CH_3COOH = 4SO_2(g) + 2CO_2(g) + 6H_2O$$

is -1 kcal. Consequently, it is possible that decomposition of acetic acid by sulfuric acid would be appreciable under conditions employed in the determination of terminal methyl groups. However, when 0.05 mmole of acetic acid is heated in 14*M* sulfuric acid at 180° and 120° C. in sealed tubes, the acetic acid is recovered quantitatively at each temperature after a 4-hour digestion period.

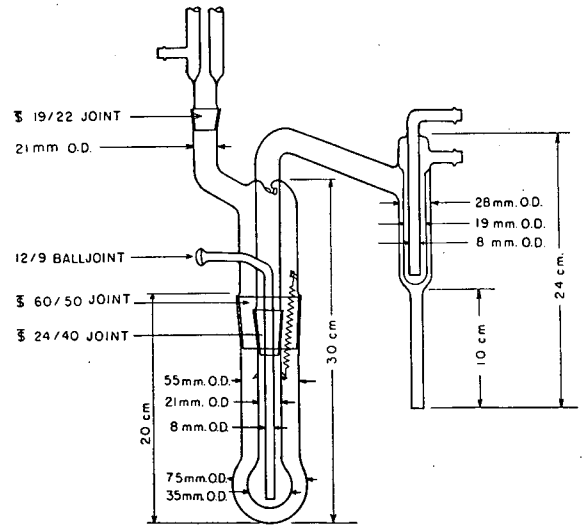

Figure 3. Jacketed apparatus for distillation of acetic acid

For the reaction:

$$32H^+ + 8HCrO_4^- + 3CH_3COOH = 8Cr^{+++} + 6CO_2(g) + 26H_2O$$

$\Delta F_{298°}$ K. per mole of acetic acid is -279 kcal. Even with this very large value for the free energy favoring the oxidation, the rate of loss of acetic acid during digestion was slow. After a 4-hour digestion period at 120° C. in 0.1*M* dichromate, 96% of the acetic acid was recovered from a solution which was 7.2*M* in sulfuric acid, 23% from a solution which was 10.8*M* in sulfuric acid, and 3% from a solution which was 14.4*M* in sulfuric acid. Throughout all of the distillations the dichromate in the reaction mixture was not reduced with hydrazine, because it was found that during the distillation the oxidation of acetic acid was negligible.

Because the sulfuric acid concentration of the reaction mixture in the first of the above three experiments approaches that employed by Ginger (*2*), the stability of acetic acid was studied using the quantity of acid employed by Kuhn and Roth and by Ginger. The results are shown at three temperatures in Figure 5. Figure 5 (left) shows that at 150° C. the rate of oxidation of acetic acid is rapid when the sulfuric acid concentration in the reaction mixture is 3.6*M* (1 ml. of concentrated sulfuric acid) and 6.0*M* (2 ml. of concentrated sulfuric acid). When 6*M* sodium hydrogen sulfate is used as the source of hydrogen ion, however, the rate of oxidation of acetic acid is reduced sufficiently so that it may be used in place of sulfuric acid, if the oxidation of the organic compound to acetic acid in the bisulfate solution is sufficiently rapid.

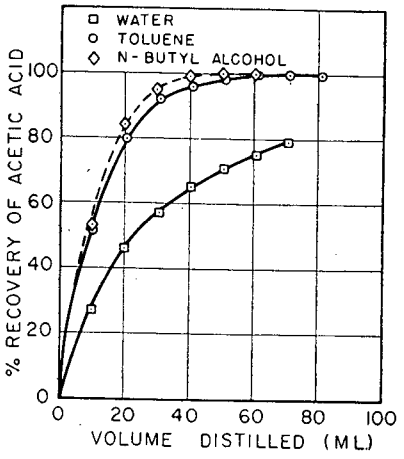

Figure 4. Effect of jacket temperature on rate of distillation of 0.05 mmole of acetic acid

From solution containing 4 ml. of 5*N* chromic acid, 1 ml. of concentrated sulfuric acid, and 5 ml. of water

Table I. Acetic Acid Recovered from Oxidation of Simple Aliphatic Compounds

(Reaction mixture contains 4 ml. of 5*N* chromic acid and 1 ml. of concentrated sulfuric acid. Digestion temp., 120° C.)

Sample	Digestion Time, Hours	HOAc, Moles/Mole of Compound Found	Present
Acetone	0.25	0.98	1
	0.5	0.99	
	4	0.99	
Mesityl oxide	0.5	1.95	2
	1	1.98	
	2	2.00	
Isovaleric acid	1	0.96	1
	2	0.99	
	4	0.98	
tert-Amyl alcohol	1	1.92	2
	2	1.96	
	4	1.96	
Trimethylacetic acid	2	0.92	1
	4	0.96	
tert-Butyl alcohol	2	0.98	1
	4	0.99	

Figure 5 (center) shows the rate of oxidation of acetic acid at the temperature usually prescribed for sealed tube digestions, 120° C., when the sulfuric acid concentration is 3.6*M* and 6*M* and when 6*M* sodium hydrogen sulfate is used as the hydrogen ion source. The oxidation is sufficiently slow at this temperature to avoid serious loss of acetic acid with digestion periods as long as 8 hours. Figure 5 (right) illustrates the rate of oxidation at 100° C. for the two reaction mixtures.

Figure 5 also shows the rate of oxidation of benzoic acid in 4*N* chromic acid and 3.6*M* sulfuric acid, at the three temperatures studied. The comparatively rapid oxidation of benzoic acid permits the determination of terminal methyl groups in the presence of benzoyl or benzoate groups, if the digestion time is extended to a period considerably longer than usually recommended for the terminal methyl determination.

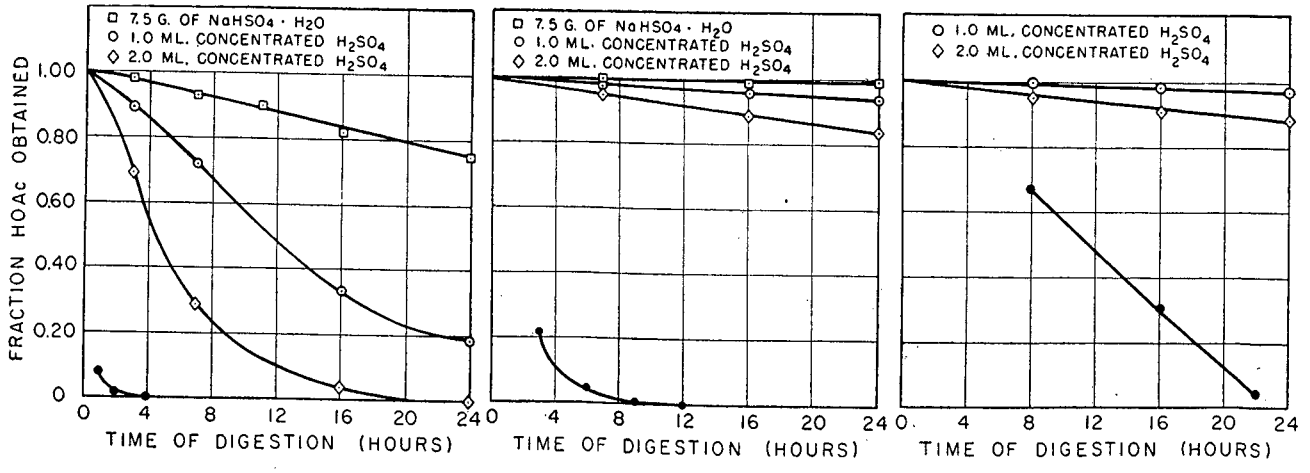

Figure 5. Rate of decomposition of 0.06 mmole of acetic acid at 150° C.

●, Rate of decomposition of 0.06 mmole of benzoic acid in reaction mixture containing 1.0 ml. of concentrated sulfuric acid and 4 ml. of 5*N* chromic acid

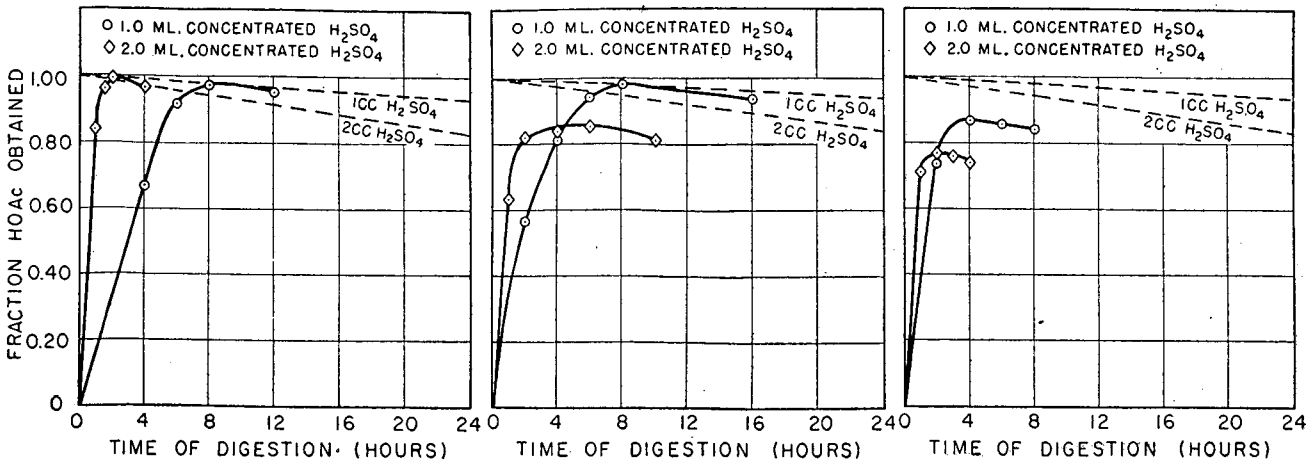

Figure 6. Rate of formation of acetic acid at 120° C.

Left. **From 0.06 mmole of stearic acid**
Center. **From 0.03 mmole of 18-*n*-propylheneicosanoic acid**
Right. **From 0.015 mmole of 2,3,4-trimethylhexadecanoic acid**
Reaction mixture contains 4 ml. of 5*N* chromic acid

Determination of Terminal Methyl Groups in Some Simple Aliphatic Compounds. Campbell and Morton (*1*) concluded, on the basis of their work, that $(CH_3)_2$—C< and $(CH_3)_3$—C— groups form acetic acid upon digestion with the chromic acid reaction mixture and that acetone is not a stable product in the oxidation step. However, this leaves unexplained the loss of 15 to 25% of the expected amount of acetic acid in the relatively simple samples they analyzed. For comparison, this study includes determinations of a number of simple compounds (tabulated in Table I).

With the exception of trimethylacetic acid, the Kuhn-Roth reaction mixture yields the theoretically expected amount of acetic acid for the simple compounds studied. Although the data are not listed, the reaction mixture containing 2 ml. of concentrated sulfuric acid used by Ginger yields comparable results.

Table II. Acetic Acid Recovered from Oxidation of Simple Aliphatic Amines

(Reaction mixture contains 4 ml. of 5*N* chromic acid and 1 ml. of concentrated sulfuric acid. Digestion temp., 120° C.)

Sample	Digestion Time, Hours	HOAc, Moles/Mole of Compound Found	Present
n-Butylamine	0.25	0.54	1
	0.5	0.66	
	1	0.73	
	2	0.72	
n-Amylamine	1	0.89	1
	2	0.89	
n-Hexylamine	1	0.69	1
	2	0.88	
Triethylamine	1	0.12	3
	2	0.06	
Tri-*n*-butylamine	4	2.22	3

Amines of low molecular weight do not yield acetic acid quantitatively when oxidized with the chromic acid reaction mixture. Table II lists the results of the digestion of several such compounds using the Kuhn-Roth reaction mixture at 120° C.

Determination of Terminal Methyl Groups in Several More Complex Aliphatic Compounds. The effect of varied digestion conditions on four compounds was studied: Three of these were the fatty acids—stearic acid, 18-*n*-propylheneicosanoic acid, and 2,3,4-trimethylhexadecanoic acid. The fourth, 5,5-dimethyl-1,3-cyclohexanedione, was selected for study because it was thought

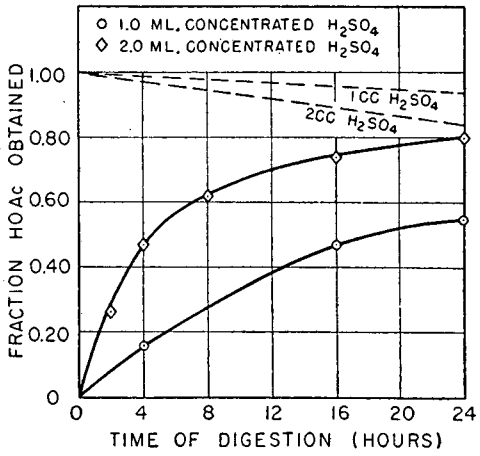

Figure 7. Rate of formation of acetic acid from 0.06 mmole of 5,5-dimethyl-1,3-cyclohexanedione at 120° C. in reaction mixture containing 4 ml. of 5*N* chromic acid

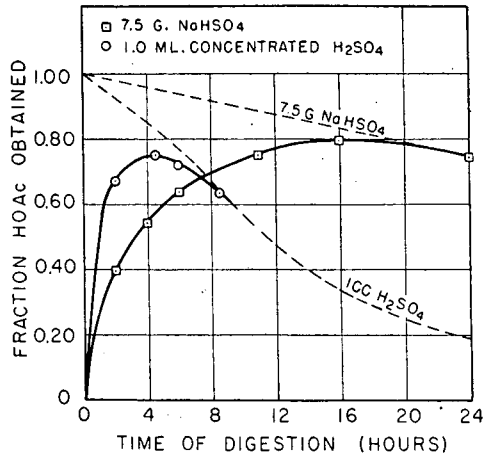

Figure 8. Rate of formation of acetic acid from 0.06 mmole of 5,5-dimethyl-1,3-cyclohexanedione at 150° C. in reaction mixture containing 4 ml. of 5*N* chromic acid

that it might be a bridge to a better understanding of the oxidation of compounds containing a benzene ring.

Figures 6 and 7 show the fractions of acetic acid obtained in the dichromate oxidation by using the Kuhn-Roth digestion mixture and the increased amount of sulfuric acid recommended by Ginger at 120° C. The dashed lines represent the rate of decomposition of acetic acid in the reaction mixture at the same temperature as shown in Figure 5 (center). All four compounds yield a greater quantity of acetic acid at the end of a 90-minute digestion period when Ginger's modification is used. Figure 6 (left) shows that the time required to produce the maximum amount of acetic acid from stearic acid is increased fourfold by using the Kuhn-Roth procedure rather than the Ginger modification, but within experimental error the same total amount of acetic acid is obtained by either procedure. The oxidation of 18-*n*-propylheneicosanoic acid by the Kuhn-Roth procedure yields 2 moles of acetic acid per mole of sample very nearly quantitatively, as shown in Figure 6 (center). For this compound the greater amount of sulfuric acid employed in the Ginger modification apparently enhances the destruction of the terminal methyl groups at a rate sufficiently rapid to result in the formation of about 13% less acetic acid than was produced by the Kuhn-Roth reaction mixture. Figure 6 (right) shows the results of the oxidation of 2,3,4-trimethylhexadecanoic acid by the two mixtures. Again the oxidation is more rapid when 2 ml. of concentrated sulfuric acid is used, but a greater yield of acetic acid is obtained from the four terminal methyl groups when the smaller amount of acid is employed. The results of the analyses of these two samples suggest that still further reduction of the sulfuric acid concentration may yield acetic acid more nearly quantitatively, if the required digestion period is not increased unreasonably.

Figure 7 shows the very slow oxidation of 5,5-dimethyl-1,3-cyclohexanedione by either reaction mixture. After a digestion period of 24 hours the oxidation is nearly complete, when the Ginger modification is used. With the Kuhn-Roth oxidation mixture, digestion of the sample is not complete at the end of 24 hours and the slow rate of formation of acetic acid illustrated in Figure 7 indicates that considerably more time is required for complete digestion.

Because of the rapid destruction of acetic acid at 150° C., few samples were digested at this temperature. The effect of increased temperature upon the digestion of 5,5-dimethyl-1,3-cyclohexanedione is interesting, however, because it indicates that a temperature greater than 120° C. may be desirable for the digestion of samples which are relatively stable to dichromate oxidation. Figure 8 shows the rate of formation of acetic acid when the Kuhn-Roth procedure is used, and also the milder oxidation mixture containing 7.5 grams of sodium hydrogen sulfate. Both curves indicate that the *gem*-dimethyl group yields one acetic acid molecule but that the relatively long time required for the oxidation destroys sufficient acetic acid, so that the maximum amount produced approximates 75 to 80% of the total possible amount.

CONCLUSIONS

The present investigation is concerned only with the determination of terminal methyl groups attached to aliphatic compounds. A relatively limited number of substances have been studied, but the information permits a number of conclusions, and points the way to further study of the procedure. The rate of distillation of acetic acid is speeded up appreciably by jacketing the distillation flask with toluene or *n*-butyl alcohol. The oxidation of acetic acid at the temperature of distillation is so slow that there is no need to reduce the chromic acid with hydrazine or hydrogen peroxide.

At 150° C. the Kuhn-Roth reaction mixture and the one containing an increased amount of acid proposed by Ginger oxidize acetic acid so rapidly that satisfactory results are not obtained. At 120° C., the rate of oxidation of acetic acid is decreased—a 20-hour digestion period is required to destroy 5% of the total acetic acid present if the Kuhn-Roth reaction mixture is used; approximately 7 hours are required to destroy a like quantity if the Ginger reaction mixture is employed. No marked decrease in oxidation occurs when the temperature is further lowered to 100° C. at either acid concentration.

Acetic acid is considerably more stable to oxidation by chromic acid than is benzoic acid. Consequently, by extending the digestion period, it is possible to remove the benzoic acid completely, while losing only a small amount of the acetic acid formed. By minimizing the digestion period and digestion temperature it is possible to estimate the sum of the two acids.

The chromic acid oxidation of *tert*-butyl and *tert*-amyl alcohols shows that one molecule of acetic acid is obtained quantitatively for the $(CH_3)_2\!-\!C<$ and $(CH_3)_3\!-\!C\!-$ groups present. These results, plus the rapid and quantitative formation of acetic acid from acetone, confirm the conclusion of Campbell and Morton that acetone is not a stable product in the oxidation of these two groups. The determination of terminal methyl groups attached to aliphatic amines requires further study.

The results of the oxidation of the more complex aliphatic compounds reported in this investigation illustrate that a general procedure for the terminal methyl determination cannot be so rigidly established as that generally employed. The results in Figure 6 (center and right) show two instances where less acetic acid is obtained by using the increased amount of sulfuric acid recommended by Ginger. However, Figure 7 indicates that for 5,5-dimethyl-1, 3-cyclohexanedione the greater sulfuric acid concentration is necessary at 120° C.

As a result of this investigation the authors have adopted a policy of determining terminal methyl groups under a single set of conditions only when it has been previously determined that the sample is easily digested or when the quantity of material available for analysis is the limiting factor. In general, the Kuhn-Roth reaction mixture is employed and the digestion periods for two samples of the substance to be analyzed are varied by a factor of 2. These two results then serve as a basis for determining whether digestion is complete and, if necessary, what the conditions should be for further study.

In each terminal methyl determination the rate of destruction of acetic acid after digestion of the sample was slightly greater than that for the digestion of acetic acid itself. Westheimer (4) states that +4 and +5 chromium intermediates are produced during the reduction of dichromate and that at least one of these species is capable of oxidizing manganous ion to manganese dioxide. In the digestion of acetic acid no appreciable reduction of dichromate occurs, whereas for a sample this is not true. It seems, therefore, that the presence of very small amounts of such a powerful oxidizing agent could be responsible for the slight difference in rate of oxidation of acetic acid in the two cases. Of course, one of these intermediates also could oxidize the terminal methyl group directly and thus be responsible for the low results shown in Figure 6.

The design of the equipment used in this study is more elaborate than that usually employed for the terminal methyl determination. However, the authors believe that the simplicity of operation, the reduced quantity of distillate required for quantitative recovery of acetic acid, and the complete elimination of sulfuric acid carry-over in the distillation step represent improvements to the method of analysis. In addition, the appreciably better results which have been obtained using sealed tubes and the rocking furnace for the digestion of samples justify their use.

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RECEIVED for review February 13, 1956. Accepted May 5, 1956.

Spot Test Procedures for Differentiation of Quinoline and Isoquinoline

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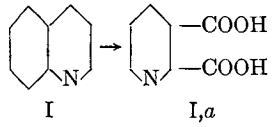
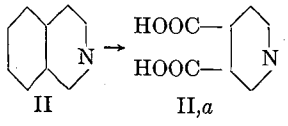
2,3- and 3,4-Pyridinedicarboxylic acids (quinolinic and cinchomeronic acids) can be differentiated by the fact that only quinolinic acid forms an insoluble blue copper salt in weak mineral acid solution. Quinoline and isoquinoline can be distinguished from each other by reduction with zinc and hydrochloric acid. On subsequent oxidation the dihydroquinoline yields a brown precipitate when treated with potassium persulfate and copper sulfate, or a red precipitate when bromine water is added. The dihydroisoquinoline gives no reaction with persulfate and only a slight coloration with bromine water. Quinaldine behaves like quinoline, but its precipitation sensitivity is much less. Solutions of eight other derivatives of quinoline with substituents in the pyridine ring do not give these reactions after they have been reduced with zinc and hydrochloric acid.

NO PURELY chemical procedure is known for differentiating between quinoline (boiling point, 240° C.) and isoquinoline (boiling point, 239° C.). Differentiation of these isomeric bases has depended on the preparation of derivatives with characteristic melting points. The picrates (melting points, 203° and 222° C.) and methiodides (133° and 159° C.) have been recommended particularly (*3*). Although the preparation of such derivatives and the determination of their melting points can be accomplished on the semimicro or micro scale, the necessary manipulations require not only special equipment but also more time, material, and experience than do spot tests based on color or precipitation reactions. The sensitive tests described here should have considerable interest and value, particularly in view of their importance with respect to clarifying the structures of alkaloids and the like, whose degradation products may include quinoline and isoquinoline. These procedures were derived through consideration of the chemistry of specific, selective, and sensitive reactions (*4*).

Oxidation with permanganate in neutral or alkaline solution converts quinoline (I) into 2,3-pyridinedicarboxylic acid (I,*a*) and isoquinoline (II) into 3,4-pyridinedicarboxylic acid (II,*a*) (*7*).



I I,*a* (1)



II II,*a* (2)

These products (quinolinic and cinchomeronic acid, respectively) might be expected to show a divergent behavior toward copper ions because of the relative position of their coordinatable nitrogen atoms with respect to the carboxyl group in the α- and β-position. In fact, when an acid solution of I, *a*, is treated with a solution of copper sulfate or acetate a blue color results, whereas II, *a*, gives no reaction. The blue color obviously is due to the formation of a chelated copper compound. In concentrated solutions, a precipitate appears; its composition conforms to $Cu(C_7H_4O_4N)_2 . H_2O$ (*1*).

If the reaction of quinolinic acid with copper ions is conducted in dilute solution, the visible precipitate of the organic copper salt may not appear for several hours. However, the product can be brought down almost immediately by adding several drops of ether and warming gently. This device is of importance in spot test procedures to accelerate sluggish precipitations. The rapid precipitation is probably caused by the marked solubility of ether in water, which therefore increases the rate of formation of the nuclei and the rate of growth of water-insoluble compounds. It seems to be effective only when crystalline precipitates are involved.

It seemed logical to base a possible differentiation of quinoline and isoquinoline on their oxidation to the respective dicarboxylic acids and the divergent behavior of these products toward copper ions. This method can be followed for the identification of macro amounts of quinoline, but the sensitivity is inadequate for spot test purposes. The same difficulty was encountered when quinoline was oxidized with hydrogen peroxide in the presence of a copper salt in the hope of thus arriving directly at the precipitation of the blue chelated product.

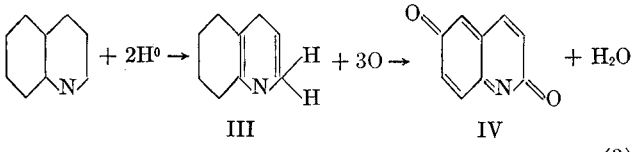
Because the oxidation of quinoline to quinolinic acid with hydrogen peroxide and copper sulfate does not proceed rapidly enough for analytical purposes, attempts were made to reach this goal after hydrogenation of the quinoline. Koenigs (*9*) found that dihydroquinoline or its dimer is formed when quinoline is dissolved in glacial acetic acid and treated with metallic zinc. The reduction might be expected to proceed more rapidly in hydrochloric acid solution; with more rapid production of dihydroquinoline a more abundant yield of the oxidation product should be detectable by precipitation of the copper salt of quinolinic acid. This expectation was not fulfilled, although the following reaction, which was characteristic, took place.

A drop of 5% quinoline containing considerable hydrochloric acid was placed in a depression of a spot plate along with several granules of zinc. As soon as the evolution of hydrogen started, a drop of 30% hydrogen peroxide and a drop of 1% copper sulfate solution were added. A violet color appeared almost at once and quickly changed to red-brown; this was followed by a precipitate of the same color. Dilute solutions of quinoline yield light to dark brown colorations. Isoquinoline solutions show the same effect but to a much smaller degree. However, when potassium persulfate was used as oxidant in place of the peroxide, isoquinoline gave no color, whereas with quinoline the color or precipitation reaction started after 1 or 2 minutes and reached its maximum in about 3 minutes. These observations served as the

foundation for developing a new method for differentiating between quinoline and isoquinoline.

Another and more rapid differentiation of the two isomeric bases is provided by their behavior toward bromine water after their solutions have been subjected to the action of zinc and hydrochloric acid. Even dilute solutions of quinoline give an immediate red precipitate, while isoquinoline yields a similar precipitate only when the concentration of its solution exceeds 10%. These colored precipitates disappear within a few minutes if they are treated with sulfosalicylic or sulfurous acid.

All attempts to isolate pure specimens of the colored precipitate obtained from dihydroquinoline by the action of persulfate and copper sulfate, or bromine water, have failed. Lacking a reliable analysis, there is no real experimental basis for conjectures regarding the chemistry of these reactions. There is little doubt that persulfate oxidizes the dihydroquinoline (III) obtained from the quinoline. Karrer (*8*) has found that quinoline is hydrogenated ortho to the nitrogen atom. Serious consideration must be given to the formation of the quinoidal oxidation product (IV) shown in the following series of reactions.



III IV

(3)

Another possibility is the formation of a quinoidlike compound intermediate between III and IV. In view of the decomposition by sulfosalicylic or sulfurous acid of the precipitate produced by bromine from acidic solutions of III, the assumption of the production of a polybromide has some probability.

As shown in Equation 3, dihydroquinoline functions as a hydrogen donor. It was shown (*6*) that organic compounds which are reductants in alkaline solution can be detected by a color test that was originally recommended by Bose (*2*) for the detection of reducing sugars. The reagent for this test is an alkaline, alcohol solution of *o*-dinitrobenzene; it turns violet when warmed with hydrogen donors because of the formation of quinoidal *o*-nitrosonitrobenzene (*10*). If the quinoline solution is reduced with zinc and hydrochloric acid and then made basic, it gives a violet color with *o*-dinitrobenzene. The same result was obtained with a reduced solution of isoquinoline; demonstrating that this base also yields a reduction product which functions as a hydrogen donor. Koenigs (*9*) also proved that, because dihydroquinoline yields a nitrosamine, it contains an NH-group. This group is also formed when isoquinoline is hydrogenated, as demonstrated by its positive response to the color reaction for secondary amines with sodium nitroprusside and acetaldehyde (*5*). Accordingly, it is highly probable that isoquinoline is converted to dihydroisoquinoline by zinc and hydrochloric acid, and that the differentiating reactions, employing persulfate or bromine, rest on the facts that in dihydroquinoline the reduction potential and the ability to form a polybromide are distinctly more marked than in the isomeric dihydroisoquinoline.

PROCEDURE

With Persulfate. A drop of the test solution containing considerable hydrochloric acid is placed in a depression of a spot plate and 4 or 5 granules of 10-mesh zinc are added. After 1 or 2 minutes, the unused zinc is removed with the aid of a thin glass rod. A drop of 1% copper sulfate is added and then about 20 mg. of solid potassium persulfate. The system is agitated by blowing on the surface. Depending on the quantity of quinoline present, a red-brown precipitate or brown to yellow color appears within 1 to 2 minutes. The color reaches its maximum intensity after 3 to 4 minutes.

The limit of identification is 20 γ of quinoline.

Isoquinoline solutions, no matter what their concentration, show no color change when subjected to this procedure.

With Bromine Water. The reduction (hydrogenation) is performed as just described, and the reduced solution is then transferred to filter paper by means of a pipet. The spot is held over strong bromine water for about 30 seconds. The moist spot turns red immediately, or pink when smaller amounts of quinoline are present. When the paper is dried in an oven at 110° C., the color becomes paler but does not disappear entirely. The full color is restored if the spot is again exposed to bromine vapors.

The limit of identification is 2.5 γ of quinoline.

Isoquinoline solutions of any concentration leave no more than a light yellow stain on the filter paper after being carried through this procedure.

Although the procedures just described function best when quinoline and isoquinoline are not both present, a definite test for quinoline is obtained from the persulfate oxidation of 1 drop of a mixture of 50 γ of quinoline and 2000 γ of isoquinoline.

The behavior of derivatives of quinoline was tested with 10% solutions of: quinoline methiodide and ethiodide; quinaldine; acridine; 6-nitroquinoline; cinchophen; *m*-bromoquinoline nitrate; 4-hydroxy-7-chloroquinoline; 4-hydroxy-7-chloroquinoline-3-carboxylic acid; and β-naphthoquinoline. Only the methiodide, ethiodide, and quinaldine behaved analogously to quinoline, but the reaction with persulfate or bromine was much weaker with quinaldine than with the parent base. The limit of identification was 50 γ of quinaldine using either procedure. Consequently, the tests described in this paper appear to be selective.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of this study by the Conselho Nacional de Pesquisas.

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RECEIVED for review March 5, 1956. Accepted April 12, 1956.

Spot Tests for Detection of *N*-Nitroso Compounds (Nitrosamines)

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In contrast to *C*-nitroso compounds, *N*-nitroso compounds can be hydrolyzed to the corresponding NH compounds by a wet method under mild conditions, and by a dry method by heating with hydrated zinc sulfate or manganese sulfate. The nitrous acid which splits off can be detected by the color reaction with Griess reagent or by the precipitation reaction with sulfamic acid and barium chloride. In the pyrolytic hydrolysis, the test with the Griess reagent is made on the vapor phase. The detection of nitrosamines can be accomplished within the technique of spot test analysis with identification limits of 0.5 to 15 γ.

A MACRO test, discovered by Rosenthaler (*5*) for acetanilide, was the starting point in the development of a spot test (*3*) for acyl derivatives of aromatic amines, arylurethanes, and monoalkylureas. It was observed during this study that, in contrast to *C*-nitroso compounds, *N*-nitroso compounds (nitrosamines) are decomposed by hydrazoic acid (NaN_3 + HCl). This effect, which occurs even at room temperature and is valuable for the differentiation of *C*- and *N*-nitroso compounds, was ascribed to a direct denitrification of nitrosamines (Equation 1). However, consideration must be given also to the two partial reactions that may be involved in the denitrification.

Most nitrosamines are very slightly but definitely soluble in water and it may be assumed that the dissolved material undergoes hydrolysis, as shown in Equation 2. The nitrous acid is completely removed from this hydrolysis equilibrium by the well-known instantaneous reaction with hydrazoic acid (*6*), as shown in Equation 3. Consequently, the denitrification can be complete, even under the most unfavorable circumstances, if both the solution equilibrium and the hydrolysis equilibrium of the nitrosamines are re-established rapidly.

$$\rangle N{-}NO + HN_3 \rightarrow \rangle NH + N_2O + N_2 \qquad (1)$$

$$\rangle N{-}NO + H_2O \rightleftarrows \rangle NH + HNO_2 \qquad (2)$$

$$HNO_2 + HN_3 \rightarrow H_2O + N_2O + N_2 \qquad (3)$$

Summation of Equations 2 and 3 obviously yields Equation 1—namely, the representation of the direct denitrification.

If the denitrification of nitrosamines by hydrazoic acid proceeds via hydrolytically split-off nitrous acid, it may be expected that nitrosamines will show the characteristic reactions of nitrous acid and should be detectable on this basis. Actually this is true. If nitrosamines are warmed with Griess reagent (an acetic acid solution of sulfanilic acid and 1-naphthylamine), in many cases there is almost immediate production of the red color because of the formation of an azo dye. In every case, this result is obtained if the mixture is warmed with a little strong hydrochloric acid.

DETECTION WITH GRIESS REAGENT

Reagent. A 1% solution of sulfanilic acid in 30% acetic acid, and a 0.1% solution of 1-naphthylamine in 30% acetic acid. Equal volumes of these two solutions are mixed to obtain the Griess reagent.

Procedure. The test is conducted in a micro test tube. One drop of the Griess reagent and 1 drop of hydrochloric acid (1 to 1) are added to 1 drop of the test solution. The mixture is warmed in a water bath. If nitrosamines are present, a more or less intense red-violet color appears at once or within several minutes, the time and depth of color depending on the amount of *N*-nitrosamine.

This procedure revealed the following:

Compound	Formula	γ
N-nitrosodibenzylamine	$C_6H_5CH_2{-}N(NO){-}CH_2C_6H_5$	10
N-nitrosodicyclohexylamine	$C_6H_{10}{-}N(NO){-}C_6H_{10}$	9
N-nitrosodiphenylamine	$C_6H_5{-}N(NO){-}C_6H_5$	1
N-nitrosomethylurea	$OC(NH_2){-}N(NO){-}CH_3$	0.4
N-dinitrosopiperazine	$ON{-}N(CH_2{-}CH_2{-}CH_2{-}CH_2){-}N{-}NO$	4
N-nitrosoacetanilide	$C_6H_5{-}N(NO){-}COCH_3$	1

A less sensitive procedure, which is adequate for many purposes, is based on the removal of the nitrous acid from the hydrolysis equilibrium by means of sulfamic acid:

$$HNO_2 + NH_2SO_3H \rightarrow H_2SO_4 + H_2O + N_2 \qquad (4)$$

Because the barium salt of sulfamic acid is soluble in water, the occurrence of Reaction 4 is signaled by the precipitation of barium sulfate if barium ions are present. This procedure, which is the basis of the Baumgarten and Marggraff (*1*) macro test for nitrite and also of its gravimetric determination, can be translated into spot test technique as follows.

DETECTION WITH SULFAMIC ACID

Reagent. Five grams of barium chloride dihydrate and 5 grams of sulfamic acid are dissolved in 100 ml. of a mixture of equal volumes of dioxane and water. Any precipitate should be removed (Pyrex filtering crucible *F*). The solution becomes cloudy on standing and must be clarified before use.

Procedure. A micro test tube is used. One drop of the test solution (aqueous or alcoholic) is treated with 1 drop of the reagent solution and, if necessary, the test tube is gently warmed in hot water. A precipitation or turbidity results if *N*-nitrosamines are present. A comparison blank is advisable if small amounts are suspected.

The procedure revealed 10 γ of *N*-nitrosodiphenylamine and 10 γ of *N*-nitrosomethylurea.

Equation 2, which represents the hydrolysis leading to nitrous acid, does not show any participation of hydrogen ions, although addition of acid is necessary when nitrosamines are to be denitrified rapidly by the wet method. Obviously, as in so many other hydrolyses, the hydrogen ions hasten the hydrolysis.

However, an extensive hydrolysis can be secured in the absence of acids if a dry mixture of a nitrosamine and hydrated zinc

sulfate or manganese sulfate is heated to 200° C. Nitrous vapors are evolved because the following reaction takes place. When these sulfates are heated to 150° to 200° C. and 154° to 300° C. respectively, they are changed into the anhydrous sulfates (*2*). Superheated steam results when the water is lost. The steam reacts, at the place of its release, with the nitrosamine with which it is in contact, and hydrolysis of the latter occurs. Nitrous acid can also be produced without the addition of any water-releasing material, if nitrosamines are heated to incipient charring with access of air. In this case, the superheated steam resulting from the decomposition of the organic compound brings about the hydrolysis of the nitrosamine. These instances are additional evidence of the analytical value of hydrolyses occasioned by superheated steam, a matter that has been discussed elsewhere (*4*).

This pyrolytic splitting off of nitrous acid when a dry mixture of a nitrosamine and hydrated zinc sulfate or manganese sulfate is heated can also be utilized as a test for these organic compounds. No nitrous acid is yielded when *C*-nitroso compounds are subjected to the pyrolytic procedure.

DETECTION BY HEATING WITH HYDRATED MANGANESE (ZINC) SULFATE

Reagents. Hydrated manganese sulfate or hydrated zinc sulfate and Griess reagent (prepared as described previously).

Procedure. The test is made in a micro test tube. One drop of the test solution or a tiny bit of the solid is mixed with several centigrams of hydrated manganese or zinc sulfate, and taken to dryness if need be. The mouth of the test tube is covered with a disk of filter paper that has been moistened with a drop of Griess reagent, and the tube is heated in the flame of a microburner. A red-violet stain appears on the colorless paper if *N*-nitroso compounds are present.

A positive response was given by 10 γ of *N*-nitrosodicyclohexylamine, 5 γ of *N*-nitrosodiphenylamine, and 15 γ of *N*-dinitrosopiperazine.

ACKNOWLEDGMENT

This study was kindly supported by the Conselho Nacional de Pesquisas.

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RECEIVED for review February 13, 1956. Accepted April 18, 1956.

Rapid Determination of Carbonyl Content in Acrylonitrile

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Low concentrations (0 to 0.2%) of aldehydes and ketones in acrylonitrile can be rapidly determined by a modified hydroxylamine hydrochloride method employing a unique nonaqueous system. Accurate acetaldehyde content is found by allowing the sample to react with an alcoholic solution of the reagent for 1 minute before titration of the liberated acid with methanolic caustic to the thymol blue end point; total carbonyls are determined by increasing the reaction time to 5 minutes. The precision of the method for acetaldehyde is within ±0.003% in the range from 0 to 0.1%. For the higher molecular weight ketones which react more slowly, or for mixtures of carbonyls, the precision is within ±0.005%.

VARIOUS carbonyl impurities are produced in the manufacture of acrylonitrile from acetylene and hydrogen cyanide. Among these are acetaldehyde (*5*, *8*, *16*), methyl vinyl ketone (*5*, *16*), and paraldehyde (*8*). Because of their chemical and physical properties some of these carbonyl compounds may not be completely removed during purification of crude acrylonitrile; hence, rapid methods for the determination of aldehydes and ketones in product acrylonitrile became necessary.

Many methods are available for the determination of carbonyl compounds, but few can be applied for trace amounts. One widely used method for the detection and determination of aldehydes (*11*, *15*) is the fuchsin procedure. However, for quantitative results the reagent must be freshly prepared and kept in an inert atmosphere (*11*); a calibration curve must also be prepared for each batch of reagent for best results. Different aldehydes give varying color intensities and hence, the method is poor for mixtures of aldehydes. The silver oxide method (*12*) for aldehydes was modified in an attempt to apply it to low concentrations, but it was also found to be unsatisfactory.

A widely used method for total carbonyl determination is the hydroxylamine hydrochloride method, which was used for formaldehyde as early as 1895 (*2*) and has been improved by using pyridine as the oximation catalyst for quantitative determination of pure compounds (*3*). A hydroxylamine hydrochloride procedure has been employed using mixed aqueous-alcoholic reagents (*1*, *10*, *14*), with the end point detected by pH determination or by use of indicators (*3*, *14*).

Table I. Determination of Acetaldehyde

Weight %	
Taken	Found
0.135	0.145
0.170	0.176
0.123	0.125
0.235	0.240
1.20	1.25
<0.005	<0.005
0.090[a]	0.085, 0.089
0.090[b]	0.910, 0.095
0.090	0.089, 0.091
0.08–0.10[c]	0.100, 0.103, 0.104
0.07–0.08[c]	0.096, 0.092, 0.087
0.0194	0.020, 0.023
0.000	0.000

[a] 0.25% lactonitrile added.
[b] 3.0% lactonitrile added.
[c] Fuchsin procedure.

Small quantities of carbonyl compounds can be determined by differential pH measurements (*4*, *13*) after reaction with aqueous hydroxylamine hydrochloride. The carbonyl content is calculated from a predetermined calibration curve of pH change *vs.* carbonyl content. The procedure (*13*) was applied to

the determination of carbonyl compounds in methanol, water, dioxane, and benzene. A 5-minute reaction time was found to be sufficient for aldehydes and methyl ketones. The method is useful for total carbonyls but will not distinguish aldehydes from ketones.

Joslyn and Comar (*9*) have evaluated the bisulfite and hydroxylamine hydrochloride methods for the determination of traces of acetaldehyde in wines. The reaction between traces of acetaldehyde and hydroxylamine hydrochloride was found to be practically instantaneous, but low recovery (83 to 96%) was given by both methods.

Fowler and others (*6, 7*) recently published a volumetric procedure for the determination of aromatic aldehydes in the presence of aromatic ketones based on the difference in reaction rates of oxime formation. The method was applied to high concentrations of aldehyde (50% or more).

On the basis of this literature search the hydroxylamine hydrochloride method appeared to have the most promise and was investigated.

REAGENTS

Methanolic hydroxylamine hydrochloride. Dissolve 40 grams of C.P. hydroxylamine hydrochloride (J. T. Baker Chemical Co.) in 2 liters of C.P. methanol. Prepare fresh weekly.

Thymol blue. Dissolve 0.04 gram of thymol blue (National Aniline & Chemical Co.) in 100 ml. of C.P. methanol. The indicator should be prepared fresh weekly or indistinct color changes may occur.

Methanolic caustic, 0.03*N*. Dissolve 2.4 grams of sodium hydroxide in 2 liters of C.P. methanol. Prevent carbon dioxide absorption by use of an Ascarite bulb. Standardize against potassium acid phthalate or standard acid.

Acetaldehyde, Eastman white label.

Methyl vinyl ketone, Monomer-Polymer Co., Leominster, Mass.

Acrylonitrile, carbonyl-free. Reflux acrylonitrile with acid 2,4-dinitrophenylhydrazine for 6 hours. After distillation save and use the heart cut.

PROCEDURE

Add 25 ml. of acrylonitrile and 8 drops of thymol blue indicator to a clean, dry (acetone-free) 250-ml. iodine flask. If necessary, neutralize traces of acids with 0.03*N* methanolic sodium hydroxide until yellow. To a second clean iodine flask add 100 ml. of hydroxylamine hydrochloride solution and 8 drops of indicator and neutralize with 0.03*N* methanolic sodium hydroxide to the yellow color. Disregard the volumes of sodium hydroxide used. Add the reagent to the sample and start timing. Mix thoroughly. An immediate red color indicates the presence of acetaldehyde. After 1 minute, titrate rapidly with 0.03*N* methanolic sodium hydroxide to a yellow color which persists 10 to 15 seconds without fading to a definite pink. For total carbonyl determination allow the solution to stand 5 minutes after mixing before titration with sodium hydroxide. Titrate to a yellow end point which persists 10 to 15 seconds.

RESULTS AND DISCUSSION

The use of the aqueous hydroxylamine hydrochloride procedure is limited by the solubility of acrylonitrile in water (approximately 7%). Water-alcohol reagent mixtures (*2, 3, 14*) eliminated the solubility problem. However, because the indicators recommended (*3, 14*)—thymol blue and bromophenol blue—gave rise to difficulty in matching the end points, a complete nonaqueous system seemed feasible. With methanolic reagents, bromophenol blue indicator still gave an end point difficult to distinguish.

Thymol blue indicator has been recommended for 80% methanol–20% water solvent for hydroxylamine hydrochloride (*14*). Previous work in this laboratory indicated that the higher the alcohol content, the sharper the end point of thymol blue. Use of C. P. methanol verified this; a very distinct color change occurred using methanolic reagents.

Because water decreases the indicator sensitivity, the upper limits of interference were investigated. Although samples containing more than 4% water gave indistinct end points and rendered the thymol blue indicator useless, it was found that sharp end points were obtained in the range of water content normally encountered (<2%) in these samples; thus thymol blue was chosen for this study.

Table II. Effect of Methyl Vinyl Ketone upon Acetaldehyde Determination

Methyl vinyl ketone, taken	Weight % Acetaldehyde		Reaction time, minutes
	Taken	Found	
0.000	0.093	0.091, 0.095	1
0.200	0.093	0.098, 0.100	1
0.220	0.095	0.110	2 (cooled)
0.220	0.095	0.125	5 (cooled)
0.220	0.095	0.085–0.100	1/2
0.220	0.095	0.125	2
0.220	0.095	0.118, 0.110, 0.118	1
0.050	0.100	0.108, 0,100, 0.095, 0.103, 0.108, 0.103	1
0.042	0.000	0.025 (as MVK)	2
0.042	0.000	0.043, 0.045 (as MVK)	5
0.000	0.000	0.000, 0.000	1, 5

Table III. Determination of Acetaldehyde and Methyl Vinyl Ketone Mixtures

Total Carbonyl, Weight %	
As methyl vinyl ketone, taken	As methyl vinyl ketone, found
0.015	0.014, 0.014
0.025	0.026, 0.024
0.035	0.038, 0.035
0.050	0.050, 0.053
0.077	0.075, 0.075, 0.068, 0.075, 0.076
0.066	0.064, 0.059
0.042	0.043, 0.045

To determine whether the reaction between traces of acetaldehyde and hydroxylamine hydrochloride is instantaneous as reported (*9*), the procedure was applied to known concentrations of acetaldehyde in acrylonitrile using various reaction times. A 1-minute reaction time was found to be sufficient. The effect of lactonitrile, which may dissociate to give acetaldehyde and hydrogen cyanide, was also investigated and found not to interfere. The data for a 1-minute reaction time are given in Table I.

A study of the interference of methyl vinyl ketone in the acetaldehyde determination proved that a slight interference occurs in a 1-minute reaction time. The data in Table II show that acetaldehyde and methyl vinyl ketone mixtures give slightly high but reproducible (+0.005%) values for acetaldehyde when the samples are allowed to react for 1 minute. The data also show that methyl vinyl ketone reacts completely in 5 minutes; therefore, it is possible to determine the total carbonyl content in acrylonitrile samples by allowing the reaction to continue for 5 minutes. The results given in Table III indicate the reproducibility and accuracy of the method for the determination of total carbonyls.

Mixtures of acetaldehyde and methyl vinyl ketone in acrylonitrile may be analyzed in this manner by the difference in their reaction rates. Acetaldehyde is determined by allowing the sample to react with the reagent for 1 minute before titration, while total carbonyls are determined by using a 5-minute reaction time. The difference in the two titrations is due to methyl vinyl ketone and its concentration can be calculated.

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RECEIVED for review January 20, 1956. Accepted April 6, 1956. Division of Analytical Chemistry, 11th Southwest Regional Meeting, ACS, Houston, Tex., December 1955.

Determination of Particle Size with a Simple Recording Sedimentation Balance

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A simple automatic recording sedimentation balance utilizes a sensitive spring to weigh the particles settled on the pan. As the weight on the pan changes, a shutter mechanism intercepts parallel light reaching a photocell. The resulting change in photocell current is recorded automatically by means of a Speedomax recorder. The sedimentation balance is simple in construction and easy to operate. Its precision is good and results compare favorably with those obtained with the Andreasen pipet.

THE use of an analytical balance for weighing particles settling out of a suspension was first described by Oden (*6*). This method is generally considered accurate, but requires constant attention and tedious weighings. A recent modified form (*2*) of the sedimentation balance utilizes a torsion wire to weigh the particles settled on the pan, but it also requires periodic manual recording of the data.

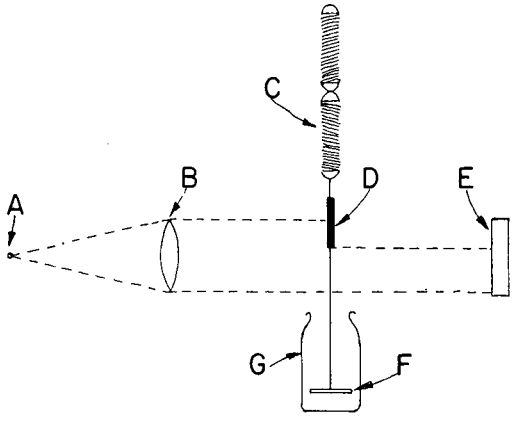

Figure 1. Illustration of principle of simplified sedimentation balance

Automatic recording sedimentation balances have been reported (*1*, *7*), and electronic recording analytical balances suitable for conversion to sedimentation balances have been described (*3*, *5*). In general, these instruments require complicated servomechanism balancing circuits. To prevent excessive oscillations of the balance arms, additional discriminator or damping circuits also must be used.

The automatic recording sedimentation balance described utilizes a sensitive spring to weigh the particles settled on the pan. As the weight on the pan changes, a shutter mechanism intercepts parallel light reaching a photocell, and the change in photocell current is recorded automatically by a Speedomax recorder. The instrument is simple in construction and easy to operate.

PRINCIPLE

The principle of operation may be illustrated by Figure 1.

A pan, *F*, is immersed in a settling vessel, *G*, which contains a suspension of particles. The pan is attached to the lower end of a shutter, *D*, by means of a stiff wire. The shutter, in turn, is attached to the lower end of a sensitive spring, *C*. Light originating at source, *A*, is collimated by lens, *B*, and is intercepted by the photocell, *E*. As particles settle on the pan, a downward extension of the spring occurs. The shutter, moving simultaneously with the pan, intercepts part of the light reaching the photocell. The resulting change in photocell current is recorded automatically by means of a Speedomax recorder.

It has been determined experimentally that the light output was uniform and the photocell response was linear over the area used for the measurements. Thus, the recorded curve is proportional to the extension of the spring. As the extension of the spring is proportional to the weight settled on the pan, the recorded data can be converted directly to data on per cent weight settled and, thus, used for particle size analyses.

CONSTRUCTION

A diagrammatic sketch of the sedimentation balance is illustrated in Figure 2. Additional details are shown in Figure 3.

Table I. Reproducibility of Sedimentation Balance on Duplicate Analyses

Diameter, μ	Weight % Greater than Diameter, Analysis 1	Analysis 2
2.5	97.0	97.0
5.0	87.5	88.0
7.5	78.5	79.5
10.0	71.0	71.5
12.5	63.0	63.0
15.0	55.0	55.0
17.5	47.0	48.0
20.0	39.0	41.0
22.5	33.0	34.5
25.0	27.5	29.5
27.5	23.0	24.5
30.0	19.0	20.5

The light source, 1, consists of a 6.3-volt lamp operating from a constant voltage transformer housed in the left-hand compartment, 15. The collimating lens, 2, is a double convex lens with a 24-cm. focal length. The sensitive spring, 5, manufactured by the Kline Spring Co., Cleveland, Ohio, consists of two sections, one having a left-hand winding, the other a right-hand winding, to prevent a twisting action of the spring as it extends. The spring is made of stainless steel wire, Type 302, 0.012 inch in diameter, and has a body of 1.3 inches. The spring is attached to a movable rod, 3, both enclosed in the spring housing, 4, to prevent oscillations of the spring due to air currents. The sedimentation pan, 13, is about 5 cm. in diameter and is constructed of either a thin sheet of aluminum or of plastic. The pan is immersed in a settling vessel, 14, which consists of a wide-mouthed jar having a cap with a center hole constructed as indicated in the diagram. The pan wire is just long enough to reach the top of the lid, so that the final dispersing procedure consists of placing a thumb over the hole and shaking the jar. The jar is placed in a water bath, 11, maintained at 25° C. by means of constant temperature water circulated through the coils, 12.

An adjustable slit, 7, is located between the shutter and the photocell for the purpose of adjusting the zero and final recorder positions. A millimeter scale located at the side of the slit is partially illuminated by the collimated light. The shadow of the shutter falls on the scale, so that the total downward extension of the spring may be determined by noting the initial and final slit readings. A lens, 8, is provided to spread the light over a greater portion of the photocell, thus improving the linear response of the photocell, 9 (GE photovoltaic cell). The voltage drop across the photocell is adjusted by means of a resistance decade, 10, located in the right-hand compartment. The light reaching the photocell can be conveniently interrupted by means of the entrance shutter, 16.

OPERATION

Complete dispersion of the suspended particles in a medium is essential for accurate particle size measurements. There is no technique applicable to all suspensions and considerable care is generally needed to ensure adequate dispersion.

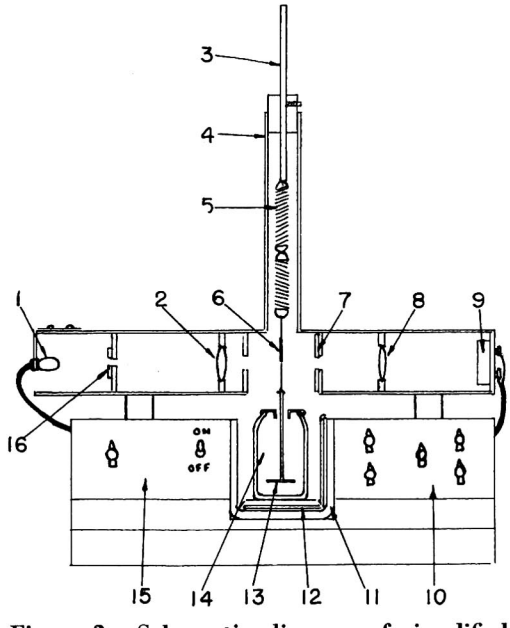

Figure 2. Schematic diagram of simplified sedimentation balance

In this laboratory, the dispersing media generally used are water containing Daxad 11 as a dispersing agent, butyl acetate, or methanol. From 1.0 to 1.5 grams of powder are used for an analysis, depending on the density of the powder and the sensitivity settings of the instrument.

After 400 ml. of suspension have been prepared, the instrument is adjusted by use of a blank consisting of 400 ml. of the proper dispersing medium. The exit slit is adjusted to a vertical opening

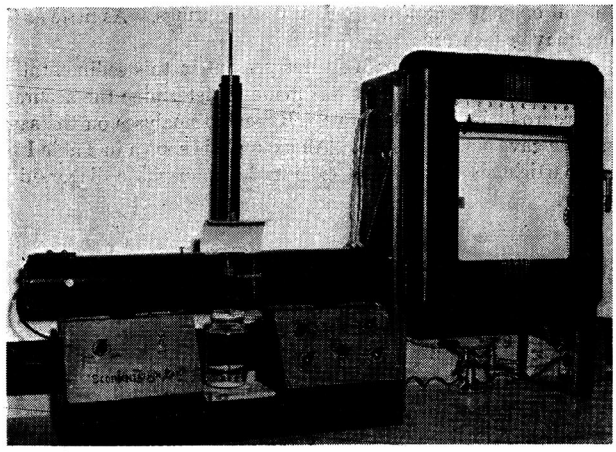

Figure 3. Simplified recording sedimentation balance

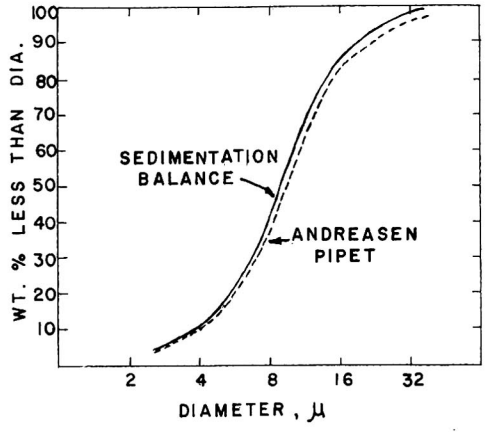

Figure 4. Cumulative weight per cent curves for zinc phosphate

of about 0.6 cm. The pan is immersed in the medium and attached to the shutter. The adjustable rod is changed until a recorder reading of about 72 divisions is reached and the slit reads about 0.2 cm. The distance from the pan to the meniscus is about 8 cm. The initial pan height, slit, and recorder values are noted. The pan is removed from the blank and placed in the suspension, which is then allowed to reach bath temperature. Next, the settling vessel is vigorously shaken in a rotating manner while a thumb is placed over the center hole. The jar is quickly placed in position and the pan wire is attached to the shutter. This should take less than 5 seconds. The entrance shutter is immediately opened and the settling recorded. At the end of the run, the final slit value is recorded. The difference between the initial and final slit readings indicates the distance the pan has settled during the run. This value is subsequently used in the calculations.

After the settling curves are obtained, the weight distribution curves may be calculated according to the procedure of Gaudin, Schumann, and Schlechter (*4*). This consists of plotting weight per cent settled, p, against ln t (seconds). The curve is differentiated by the method of tangents to give dp/d ln t (seconds), which is plotted on the same graph. The difference, p-dp/d ln t (seconds), represents the weight per cent of particles greater than a given diameter.

RESULTS AND DISCUSSION

When the adjustable slit opening is set at 0.6 cm. and the resistance decade is set at about 1500 ohms, the sensitivity of the instrument is such that one division on the Speedomax recorder paper (100 divisions full scale) corresponds to a change of 0.006 cm., which, in turn, represents about 0.0015 gram of phosphor settled on the pan. Increased sensitivity is obtained by reducing

the slit opening and using more sensitive springs. As little as 0.3 mg. may be measured.

Because of the unusual features present in this sedimentation balance, considerable effort was made to determine the accuracy and reproducibility obtainable. Repeated analyses on the same sample gave similar results. An example is shown in Table I for the particle size analyses of calcium halophosphate dispersed in water.

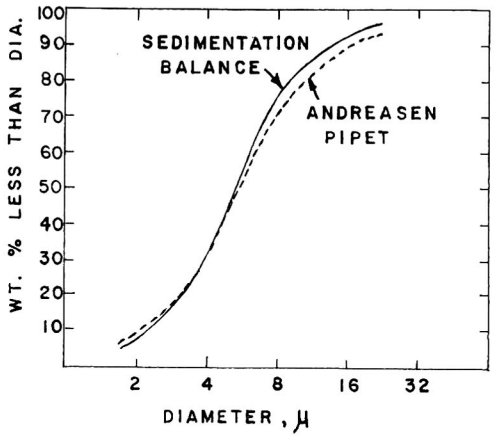

Figure 5. Cumulative weight per cent curves for zinc silicate

Good precision is obtained in the size range of 2 to 30 microns. Some hindered fall may occur because of the relatively large sample used (2.5 grams per liter). This disadvantage is balanced by the fact that use of a larger sample minimizes errors due to poor sampling. Procedural errors are present to some extent. During the final dispersing operation, about 5 seconds are required to set the settling vessel into position and to attach the pan to the shutter, and 3 to 5 seconds are required for the pan oscillations to cease. Thus, about 10 seconds elapse before undisturbed settling occurs. During this time some coarse particles will settle out. The error associated with determining percentage of particles larger than 35 microns is considerable, as these generally settle out in less than 1 minute. This type of error is present in practically all liquid settling methods and can be minimized by increasing the settling height to 20 cm. or more.

The accuracy of the instrument could be evaluated on a comparative basis only, because there are no absolute methods for determining particle size distributions. A comparison with the Andreasen pipet was considered the best approach, as both methods utilize liquid dispersions and weight distributions are obtained. Two comparisons are illustrated in Figures 4 and 5.

Good agreement was found between the two methods, further indicating that the basic principle of the simplified sedimentation balance is sound and that other possible sources of error, such as the small downward displacement of the pan, are not significant. An inherent source of error in the Oden method of analyzing the experimental curves is in the need for differentiation of the curve. However, because good agreement was obtained with the Andreasen pipet method where no differentiation is employed, it must be concluded that this error is relatively small. Another possible source of error in the sedimentation method is that small convection currents may be caused by changes in suspension density directly under the pan as particles settle out. In the present work this change in density would be from about 1.0012 to 1.0000 (based on 2 grams of powder per liter of suspension). Thus only small convection currents should occur.

Additional evaluations and comparisons are being made with other particle size instruments, such as the Sharples Micromerograph, turbidimeter, and Roller particle analyzer.

ACKNOWLEDGMENT

The authors wish to acknowledge the helpful suggestions and criticisms of Shannon Jones and Fred Geraghty.

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RECEIVED for review October 31, 1955. Accepted April 19, 1956.

Evaluation of 2-Furoyltrifluoroacetone as an Analytical Reagent

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2-Furoyltrifluoroacetone is a sensitive nonspecific spot test reagent suitable for detecting as little as 2 γ of some metals in 0.1 ml. of solution. It may be used to concentrate and isolate heavy metals by precipitation, extraction, or chromatography of the metal chelate. Light absorption characteristics of the metal chelates in organic solvents suggest application of the reagent to the spectrophotometric or fluorophotometric determination of some metals. As a precipitant for the gravimetric determination of palladium, 2-furoyltrifluoroacetone compares favorably with dimethylglyoxime.

SEVERAL β-diketones have been studied in this laboratory to determine their potential use as analytical reagents. This study concerns the analytical applications of 2-furoyltrifluoroacetone.

Probably the most interesting characteristic of the β-diketone is its ability to form stable chelates with a large number of simple metal ions. Many of these metal chelates are easily prepared, stable, intensely colored or fluorescent, insoluble in water, and soluble in common organic solvents. The analytical applications suggested for this reagent are based on the inherent characteristics of the metal chelates.

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Table I. Ions Detected by Spot Test Procedures

Ion	Color of Complex	Sensitivity, γ/0.1 Ml.	Most Useful Extracting Solvent
Fe^{++}	Purple	0.2	1-Butanol
Fe^{+++}	Red	2	Methyl isopropyl ketone
Co^{++}	Yellow	2	Methyl isopropyl ketone
Ni^{++}	Green	2	Methyl isopropyl ketone
Cu^{++}	Green	2	Methyl isopropyl ketone
Cu^{+}	Green	20	1-Butanol
Mn^{++}	Yellow	2	Methyl isopropyl ketone
Ce^{++++}	Brown	2	.....
Au^{+++}	Blue-black	1	.....
Pd^{++}	Yellow	5	1-Butanol
Ti^{++++}	Yellow	1	.....
UO_2^{++}	Yellow	1	1-Butanol
Mg^{++}	Fluorescent	<2	.....
Zn^{++}	Fluorescent	2	.....
Al^{+++}	Fluorescent	2	.....
La^{+++}	Fluorescent	2	.....
Be^{++}	Fluorescent	<2	.....

Table II. R_f Values for Metal 2-Furoyltrifluoroacetonates

Solvent Ratio, Benzene-Methanol	R_f Values				
	Fe	Cu	Ni	Co	Mn
100:0	1.0	1.0	0.00	0.00	0.00
98:2	1.0	1.0	0.90	0.00	0.28
95:5	1.0	1.0	1.00	0.00	0.32

Table III. 2-Furoyltrifluoroacetone and Dimethylglyoxime as Precipitants for Palladium

Pd Solution, Ml.	Dimethylglyoxime		2-Furoyltrifluoroacetone	
	Wt. of ppt.	Pd, p.p.m.	Wt. of ppt.	Pd, p.p.m.
30.00	0.0999	1054	0.1530	1053
	0.0999	1054	..	..
	0.0993	1047	..	..
	0.0998	1053	..	..
20.00	..	..	0.1024	1057
	..	..	0.1011	1044
	..	..	0.1020	1053

APPARATUS AND REAGENTS

2-Furoyltrifluoroacetone (Midcontinent Chemical Co.) was prepared and used as a 10 weight % solution of the reagent in 95% ethyl alcohol.

Whatman No. 1 filter paper strips.

Test solutions of metal ions were prepared from reagent grade chemicals as a 1 weight % solution of the metal. Nitrate salts were used when available.

All common organic solvents (bulk grade) were redistilled.

The chromatographic chamber was a sealed glass container with provisions for ascending chromatography.

A Beckman Model DK recording spectrophotometer was used, with matched 1.000-cm. quartz cells.

Palladium(II) chloride (1.77 grams) was dissolved in 100 ml. of concentrated hydrochloric acid and diluted to 1.000 liter with distilled water.

In spot test procedures a micro extraction pipet (*2*) was used to determine what common organic solvents would extract the metal chelates.

PROCEDURE

Standard procedures (*3*) were employed to determine the effectiveness of 2-furoyltrifluoroacetone as a spot test reagent. All test solutions were buffered to a pH of about 7.5 with sodium acetate. Successive sample dilutions were used to determine the sensitivity of the test. The test solutions were also observed under ultraviolet light.

Mixtures of the metal chelates were separated by ascending paper chromatography using a mixed organic solvent, benzene and methanol. The techniques used in developing the chromatograms and detecting the chelates after migration have been described (*1*).

Spectral absorption curves were obtained for 0.01*M* solutions of the metal chelates in absolute ethyl alcohol, using a Beckman Model DK recording spectrophotometer. The blank was absolute ethyl alcohol.

The stock palladium solution was standardized gravimetrically. Aliquots were delivered from a pipet into a beaker and diluted to about 100 ml. with distilled water. The palladium was precipitated with dimethylglyoxime and determined gravimetrically by the procedure of Hillebrand and Lundell (*4*).

The standardization was checked gravimetrically with 2-furoyltrifluoroacetone as the precipitant. Measured aliquots of the stock palladium solution were diluted to about 100 ml. with distilled water and adjusted to pH 7 with dilute sodium hydroxide solution and 3 grams of sodium acetate. An excess of the precipitant was added as a 10 weight % solution in ethyl alcohol. The solution was stirred until the precipitate coagulated and was filtered immediately on a sintered-glass crucible. The precipitate was washed with hot sodium acetate solution (30 grams of sodium acetate per liter) and finally with hot distilled water, dried at 110° C. for 1 hour, and weighed.

RESULTS AND DISCUSSION

The results of spot tests for some metal ions, including the solvent found most useful for extracting colored chelates from aqueous solution and the smallest quantity of each ion detected in 0.1 ml. of solution, are given in Table I. In each case the chelate was formed rapidly, as shown by the immediate formation of a precipitate.

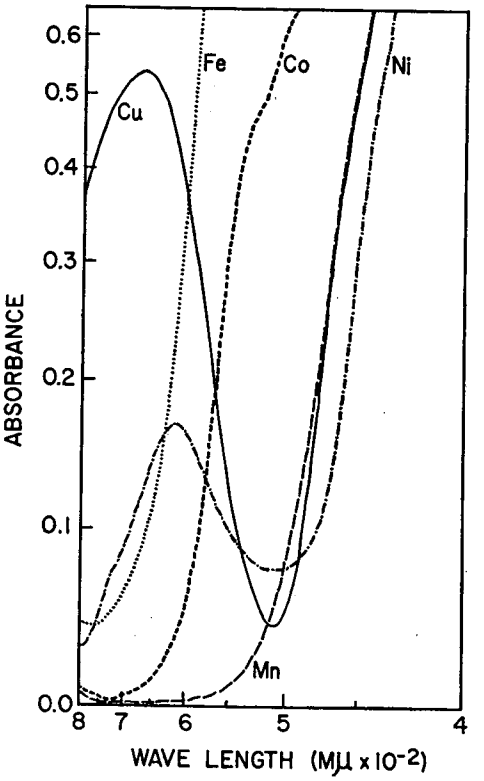

Figure 1. Spectral absorption curves for metal 2-furoyltrifluoroacetone chelates

Mercury(II), lead(II), zinc(II), cadmium(II), beryllium(II), magnesium(II), calcium(II), strontium(II), barium(II), aluminum(III), gallium(III), indium(III), lanthanum(III), thorium(IV), and zirconium(IV) formed white precipitates which were difficult to detect against a white background. A black spot plate was useful.

The other common metal ions, such as those of the alkali metals, silver(I), antimony(III), bismuth(III), mercury(I), tin(II), thallium(I), chromate, dichromate, and the chlorocomplexes of platinum(IV), rhodium(III), and iridium(IV), gave no visible reaction product with the reagent under test conditions.

The β-diketone is a good nonspecific spot test reagent capable of detecting from 2 to 5 γ of some metal ions in 0.1 ml. of solution. Because of the various colors exhibited with different metal ions, this reagent has found useful applications in this laboratory as a spot test reagent. Specificity is not always desired in a reagent.

Considerable interest has been shown recently in the chromatographic separation of metals as chelates. Small amounts of metals may be concentrated for chromatography or other uses, after chelation, in two ways. The chelate may be removed by filtration, dried, and then dissolved in an organic solvent, or it may be extracted directly into an organic solvent. In either case, the organic extract is used to spot the paper for chromatography.

Table II gives the R_f values for the paper chromatographic separation of mixtures of the iron(III), copper(II), nickel(II), cobalt(II), and manganese(II) chelates of 2-furoyltrifluoroacetone when the composition of the developing solvent was varied. A total of 1 to 2 γ of each metal was present in the mixtures.

The method may also be useful for other systems.

Investigation of the absorption spectra for several metal chelates revealed the curves shown in Figure 1. It is probable that spectrophotometric procedures, based on the light absorption of the metal chelates in organic solvents, may be devised for the quantitative determination of several metals. A procedure for the determination of copper appears most promising.

Fluorophotometric procedures for some metals may also be possible.

2-Furoyltrifluoroacetone compared favorably with dimethylglyoxime in the determination of palladium (Table III). Numerical comparison of chemical factors for palladium in the 2-furoyltrifluoroacetonate and dimethylglyoximate, 0.2064 and 0.3167, respectively, favors the new reagent greatly.

The effect of the presence of other platinum group metals was investigated. Platinum, rhodium, and iridium interfered slightly because their hydrous oxides precipitate at pH 7. A reprecipitation technique or a masking reagent may remove these interferences and make this a useful technique for the determination of palladium in ores or alloys. The reagent is excellent for the gravimetric standardization of stock palladium solutions.

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RECEIVED for review January 3, 1956. Accepted April 5, 1956.

Determination of Carboxylic Acids, Acid Chlorides, and Anhydrides by Chlorine-36—Isotope Dilution Method

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The use of chlorine-36 derivatives as a means of determining acylatable compounds by isotope dilution was found to offer so many advantages that the method has been extended to systems characterized by other functional groups. The principle has been applied to the determination of carboxylic acids, acid chlorides, and anhydrides. The compound to be analyzed is quantitatively converted to the *p*-chloroanilide, which is then determined by an ordinary isotope dilution method, using the chlorine-36-tagged *p*-chloroanilide of the acid in question.

THE principle of a chlorine-36–isotope dilution method has been presented recently for the determination of acylatable compounds (*13*). The compound to be determined is quantitatively converted to a chlorine-containing derivative, which is determined by an ordinary isotope dilution method.

The method has the following advantages: The determination is usually absolutely specific for the acid in question; with one radioactive compound [*p*-chloro(36)-aniline in this case] different carboxylic acids may be analyzed; the reference compound for the determination is a *p*-chloroanilide, which is easy to prepare in a pure state; radioactivity measurements are done with chlorine-36, and, therefore, good precision is obtainable.

Compared with an ordinary isotope dilution analysis, the disadvantage of the method is that the compound to be determined must be quantitatively converted to a *p*-chloroanilide.

The quantitative conversion of acid chlorides and anhydrides to anilides is well known (*6*, *8–10*). By preparing an anilide from a carboxylic acid, the acid is usually first converted to the acid chloride, and if the acid chloride is nonvolatile, this two-step reaction may be carried out quantitatively (*1*). A one-step reaction, by which the carboxylic acid is treated with a phosphazo compound, has been suggested by Grimmel, Guenther, and Morgan (*5*), and has further been investigated by Goldschmidt and coworkers (*2–4*).

When *p*-chlorophenylphosphazo-*p*-chloroanilide is used under the proper reaction conditions, carboxylic acids may be quantitatively converted to *p*-chloroanilides. *p*-Chloroanilides are easily purified and have sharp melting points.

METHOD

Reagents. *p*-CHLOROANILINE. A technical product was distilled under vacuum and a middle fraction was crystallized twice from 60% alcohol; the melting point was 70–71° C.

p-CHLOROPHENYLPHOSPHAZO-*p*-CHLOROANILIDE. The compound was prepared according to Grimmel's directions (*5*). To a three-necked flask equipped with an efficient stirrer were added 130 grams of *p*-chloroaniline (melting point, 70–71° C.) and 1000 ml. of toluene (reagent grade, dried over sodium); 100 ml. of toluene was distilled to remove the last traces of moisture from the flask. The solution was stirred at refluxing temperature and treated dropwise with a solution of 25 grams of phosphorus trichloride in 50 ml. of toluene. The reaction mixture was further heated for 1 hour. The hot reaction mixture was filtered to remove *p*-chloroaniline hydrochloride, then the filtrate was evaporated nearly to dryness at reduced pressure. The residue was treated with 200 ml. of absolute alcohol at room temperature and allowed to cool to 0° C.; the precipitate was filtered and washed with 50 ml. of alcohol. Finally the product was crystallized by dissolving it in 200 ml. of hot toluene and adding 400 ml. of alcohol to the clear solution. The crystals were cooled overnight at 0° C., removed by filtration, washed with a mixture of alcohol and toluene, then with petroleum ether, and finally dried at room temperature. The yield was 13 grams. The product decomposes at about 160° C. The elementary analysis indicates a content of half a mole of alcohol per mole of the monomer.

Analysis of Cl—C_6H_4—N=P—NH—C_6H_4—Cl. $^1/_2C_2H_5OH$	Calcd., %	Found, %
Carbon	50.8	51.4
Hydrogen	3.91	3.7
Nitrogen	9.14	9.3
Chlorine	23.1	23.0

p-CHLOROANILIDES. These compounds were prepared by conventional methods from the acid chloride or anhydride, and were carefully crystallized to a constant melting point.

RADIOACTIVE COMPOUNDS. *p*-Chloro(36)-aniline. A solution of 1.40 grams of acetanilide in 10 ml. of acetic acid was chlorinated at room temperature with the chlorine produced from 2.93 grams of active silver chloride (*12*). The reaction mixture was diluted with 50 ml. of water, heated to give a clear solution, and crystallized by cooling. The product was recrystallized from 50 ml. of 20% acetic acid and yielded 0.92 gram of acet-*p*-chloro(36)-anilide with a melting point of 178.5–179.0° C. An amount of 0.80 gram of acet-*p*-chloro(36)-anilide was hydrolyzed by 20 ml. of concentrated hydrochloric acid by heating on a vapor bath for $1^1/_2$ hours, followed by evaporation to dryness at reduced pressure. The residue was then dissolved in 10 ml. of water and 5 ml. of 4*N* sodium hydroxide was added. The solution was cooled to 0° C. and the crystals were removed by filtration, washed with ice water, and dried at 40° C. at 1 mm. Finally the product was distilled on a cold finger at 1 mm., the distillation flask being slowly heated in a water bath to 100° C. The distilled *p*-chloro-(36)-aniline (0.50 gram) had a melting point of 70.5–71.0° C. The product was prepared with an activity of 1 μc. per mmole.

Some chlorine-36 was regenerated as silver chloride. Yield of acet-*p*-chloro(36)-anilide on chlorine consumed was about 65%.

p-Chloro(36)-anilides. *p*-Chloro(36)-aniline was treated with an excess of acid chloride (in pyridine) or acid anhydride. The crude products were crystallized twice from alcohol or from alcohol diluted with water. The melting points differed not more than a few tenths of a degree from that of the pure compound.

In addition to these reagents, the following are also needed:
Toluene, reagent grade dried over sodium.
Acetone, reagent grade dried over Drierite.
Dioxane, reagent grade.
Alcohol, reagent grade, 99 to 99.5%.
Thionyl chloride, purified.

Measurements. DETERMINATION OF PURITY. The purity of a *p*-chloroanilide was determined from the melting point (*12*). The molar melting point depression must be determined for each compound.

RADIOACTIVITY MEASUREMENTS. The technique has been described (*14*). A ratio of two activities was determined with a statistical error corresponding to a standard deviation of 0.6 to 0.7%.

Preparation of Active Solution. Dissolve an amount of the *p*-chloro(36)-anilide corresponding to 0.7 μc. in 100 ml. of dioxane.

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

Procedure I. ACIDS. Weigh accurately an amount which is estimated to contain 1 to 2 meq. of the acid to be determined. Add 10 ml. of toluene. Add about 0.4 gram of *p*-chlorophenylphosphazo-*p*-chloroanilide and 0.8 gram of *p*-chloroaniline for each milliequivalent of acid. Reflux for $1^1/_2$ hours. Cool, then add 1.5 ml. of active solution, determining the amount accurately by weighing before and after addition. Now add 10 ml. of alcohol and boil until a practically clear solution is obtained. Remove the organic solvents with water vapor, add 10 ml. of 4*N* hydrochloric acid, cool, and remove the precipitate by filtration. After washing with water, crystallize the precipitate from absolute or diluted alcohol or from water, until the melting point differs by less than 1° C. from that of the pure *p*-chloroanilide. Determine the purity from the melting point. Measure the specific activity (activity per unit weight) of this final sample as a ratio of the specific activity of the standard sample (correct for background and self-absorption).

Procedure II. ACIDS (acid chloride nonvolatile). Weigh accurately in the reaction flask (Figure 1) about 1 meq. of the acid to be determined. Add 3 ml. of thionyl chloride and heat the mixture under reflux for half an hour with stopcocks *B* closed and *D* open. Remove excess thionyl chloride into the freezing trap by distillation first at water pump–vacuum and then at 0.1 mm. for 15 minutes. The reaction flask should not be heated during distillation, but, because of the flexible connection at *E*, it may be shaken gently by hand. Then close stopcock *D* and, without breaking the vacuum, add from the dropping funnel 2 grams of *p*-chloroaniline dissolved in 10 ml. of acetone. Heat gently until a clear solution appears. Add 1.5 ml. of active solution, determining the amount accurately as in Procedure I. Add water containing 10 ml. of 4*N* hydrochloric acid, crystallize the precipitate, and continue as described by Procedure I.

Procedure III. ACID CHLORIDES AND ANHYDRIDES. The procedure is essentially the same as Procedure II, except that the first treatment with thionyl chloride is omitted.

Calculation.

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

and

$$\text{mg. of compound} = \frac{\text{molecular weight of compound} \times B}{\text{molecular weight of } p\text{-chloroanilide}}$$

where

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

A = milligrams of active solution added
B = milligrams of *p*-chloroanilide present after conversion of compound to *p*-chloroanilide
a = milligrams of active solution used in preparing standard sample
b = milligrams of *p*-chloroanilide used in preparing standard sample
y = milligrams of *p*-chloroanilide per milligram of active solution
P = per cent purity of final sample

DETERMINATION OF ACIDS

The method has been applied to the assay of the four compounds listed.

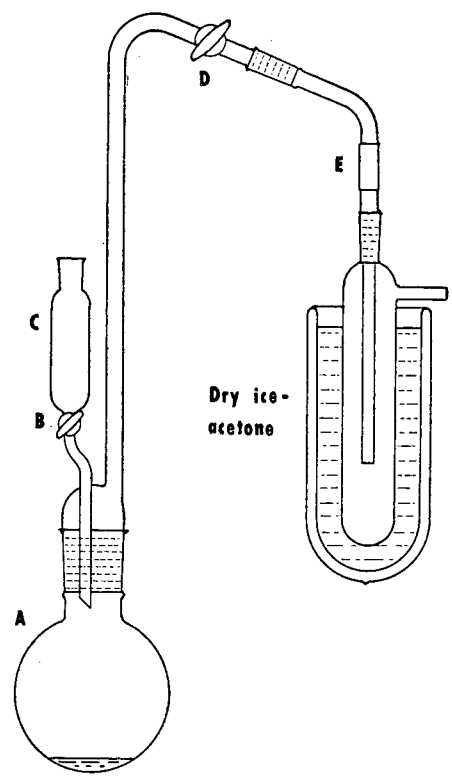

Figure 1. Apparatus for quantitative conversion of carboxylic acid to *p*-chloroanilide via acid chloride

A. 100-ml. reaction flask
B, D. Stopcocks
C. Dropping funnel
E. Flexible rubber connection

Acetic acid, analytical grade. Titration with standard sodium hydroxide showed a purity of 100.0 ± 0.2%. Procedure I was followed, except that a mixture of 5 ml. of benzene and 5 ml. of toluene was substituted for the toluene. The *p*-chloroanilide was

Table I. Determination of Carboxylic Acids and Anhydrides

Taken, Mg.	Recovered Mg.	Recovered %
	Acetic Acid	
126.6	126.8	100.2
127.8	127.3	99.6
125.0[a]	126.8	101.4
125.5[b]	127.2	101.3
122.2[b]	121.4	99.3
123.5[c]	122.7	99.3
128.2[d]	127.0	99.0
128.6[d]	130.4	101.4
128.0[d]	127.2	99.4
	Acetic Anhydride	
114.2	114.3	100.1
117.3	118.2	100.8
	Benzoic Acid	
131.2	131.1	99.9
134.0	134.3	100.2
134.7	133.5	99.1
132.0	132.3	100.2
134.9	135.3	100.3
126.0[a]	127.1	100.9
132.4[b]	131.9	99.6
130.9[b]	128.3	98.0
	Stearic Acid, Procedure I	
251.9	247	98.1
255.0	250	98.0
259.3	254	98.0
248.1	247	99.6
246.3[e]	245	99.5
251.4[e]	244	97.1
250.6[f]	248	99.0
254.7[f]	253	99.3
	Stearic Acid, Procedure II	
250.1	248	99.2
255.4	258	101.0
244.5	244	99.8
264.5	264	99.8

[a] 10 mg. of water added to sample.
[b] 25 mg. of water added to sample.
[c] 50 mg. of water added to sample.
[d] 10 mg. of formic acid and 10 mg. of propionic acid added to sample.
[e] 25 mg. of palmitic acid added to sample.
[f] 50 mg. of palmitic acid added to sample.

Table II. Effect of Varying Excess of *p*-Chlorophenylphosphazo-*p*-chloroanilide on Determination of Acetic and Benzoic Acids

Acid Taken, Mg.	*p*-Chlorophenylphosphazo-*p*-chloroanilide, Mg.[a]	Recovered Mg.	Recovered %
	Acetic Acid		
125.2	400	120.5	96.2
126.6	400	125.9	99.5
124.5	600	125.0	100.4
	Benzoic Acid		
130.9	200	131.8	100.7
134.0	300	132.5	98.9
136.3	500	135.3	99.3
134.2	600	134.6	100.3

[a] Amount of *p*-chloroanilide was twice that of *p*-chlorophenylphosphazo-*p*-chloroanilide.

crystallized from 80 ml. of water and twice from 10 ml. of 30% alcohol.

Acetic anhydride, analytical grade. Determination by the method of Kappelmeier (*6*) showed an acetic anhydride content of 100.0 ± 0.4%. Procedure III was followed and the *p*-chloroanilide was crystallized twice from 10 ml. of 30% alcohol.

Benzoic acid. This was an analytical grade product that was crystallized from toluene and then sublimed at 0.1 mm. Titration with standard sodium hydroxide showed a purity of 100.1 ± 0.2%. Procedure I was followed for this determination. The *p*-chloroanilide was crystallized from 10 ml. of absolute alcohol and twice from 10 ml. of 60% alcohol.

Stearic acid, melting point, 69–70° C. A laboratory sample with an estimated purity of 99% was furnished by The Danish Soyacake Factory, Ltd. Procedures I and II were both used. The *p*-chloroanilide was crystallized at least three times from 10 ml. of absolute alcohol.

The *p*-chloroanilides of the acids are all known (*7, 11, 15*), and the melting points of the prepared *p*-chloroanilides were as follows: acetic acid, 179.2–179.5° C.; benzoic acid, 193.2–193.5° C.; and stearic acid, 103.8–104.2° C.

In the determinations of acetic acid, acetic anhydride, and benzoic acid the samples for radioactivity measurements were purified until the melting point differed by less than 0.2° C. from that of the pure substance. The correction due to the impurity is then very small; an estimated melting point depression of 0.5° C. per per cent of impurity was used. In the determination of stearic acid the difference in melting point varied from 0.1° to 0.6° C.; the melting point depression of stearo-*p*-chloroanilide was determined to be 0.2° C. per per cent content of palmito-*p*-chloroanilide.

DISCUSSION

The results given in Tables I and II show that the acids are quantitatively converted to *p*-chloroanilides by treatment with a mixture of *p*-chlorophenyl-phosphazo-*p*-chloranilide and *p*-chloroaniline. Even moderate amounts of water are permissible. The reaction may also occur quantitatively with *p*-chlorophenylphosphazo-*p*-chloroanilide alone, but experiments showed that a purer reaction mixture was obtained when *p*-chloroaniline was added.

Grimmel suggests that the reaction should be carried out in toluene, and this solvent has also been recommended in Procedure I. However, in the analysis of acetic acid satisfactory results were obtained only with a mixture of benzene and toluene. The same mixture could also be used in the analysis of benzoic acid and stearic acid, but experiments indicated that the determinations were considerably more sensitive to water and to varying excess of *p*-chlorophenylphosphazo-*p*-chloroanilide.

Only a few experiments were performed to confirm the quantitative reaction of acid chlorides and anhydrides with *p*-chloroaniline, because other investigators have shown this type of reaction to be quantitative.

In the analysis of stearic acid via stearoyl chloride (Procedure II) the *p*-chloroanilide was a little difficult to purify. However, it was possible to obtain melting point differences as low as 0.2° C., but it is supposed that the correction for impurities does not hold in this special case. This may explain the slightly higher results obtained by Procedure II.

Dicarboxylic acids cannot usually be determined by the method proposed, because of the formation of half-anilides or imides. Experiments with succinic acid using Procedure I gave conversions of only about 60%.

ACKNOWLEDGMENT

The author wishes to express his thanks to Sadolin & Holmblad, Ltd., for permission to publish this article. The assistance of Jytte Jørn-Jensen in performing the radioactivity measurements is gratefully acknowledged. Ernst Boss kindly analyzed the phosphazo compound.

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RECEIVED for review February 4, 1956. Accepted April 20, 1956.

General Color Reaction for Nitrogen Compounds

Ehrlich's Reagent in Toluene and Ethyl Alcohol

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Previous investigations with *p*-*N*-dimethylaminobenzaldehyde have generally used aqueous systems and shown that several classes of nitrogen compounds will give a color reaction. By performing the condensation in a nonaqueous system, it has been possible to enlarge the scope of the reaction. Color reactions have been obtained with all types of heterocyclic nitrogen compounds tested as well as with aliphatic and aromatic amines.

THE reaction of *p*-*N*-dimethylaminobenzaldehyde with indoles and pyrroles has been used qualitatively and quantitatively for many years (*1*, *3*, *5*, *7*, *10*). The reaction with aromatic amines to give Schiff bases is equally well documented (*4*). Not so well known, the Wasicky reaction, utilizing 94% aqueous sulfuric acid solution, gives a color reaction with alkaloids (*12*) and purines (*8*, *9*). In 1944, Werner (*13*) reinvestigated the reaction with nitrogen compounds and found that in a more dilute aqueous system "aromatic compounds react in the presence of mineral acid, provided the $-NH_2$ group is directly attached to the benzene nucleus. . . .No reaction occurs with (*i*) aliphatic amines and amino acids, (*ii*) *N*-substituted aromatic amines, (*iii*) heterocyclic amino compounds, or (*iv*) amino derivatives of the cycloparaffins—e.g., cyclohexylamine." More recently, Burmistrov (*2*) isolated as picrates, from toluene solutions, the reaction products of secondary aromatic amines with Ehrlich's reagent.

The author has reinvestigated this reaction and extended it with some modification. In his hands, it has been possible to obtain color formation with every class of nitrogen compounds tried.

PROCEDURE

To several milligrams of sample, add an equal amount of Ehrlich's reagent (*p*-*N*-dimethylaminobenzaldehyde). Add 0.3 ml. of toluene and then 0.02 ml. of concentrated sulfuric acid. Allow to stand about 1 minute. Agitate, add 1.0 ml. of ethyl alcohol, and mix thoroughly.

On addition of sulfuric acid to the toluene suspensions of the amino acids, a yellow color was obtained. Upon addition of ethyl alcohol to these solutions, the colors noted in Table I developed. There were five exceptions to this: The color shown in Table I for cysteine, homocysteine, dihydroxyphenylalanine, djenkolic acid, and tryptophan was developed in toluene. Addition of ethyl alcohol effected no change.

Tables II and III indicate the color reaction for both steps of the test: acid-toluene solution and acid-toluene plus ethyl alcohol.

When toluene is omitted, no color development occurs with the amino acids (except tryptophan) nor with such compounds as octadecylamine, the benzo (*f*) quinolines, purines, pyrimidines, or quinine. Fleig (*6*), and later van Urk (*11*), found that when *p*-*N*-dimethylaminobenzaldehyde reacted with various non-nitrogen compounds and heterocyclics, color developed. How-

Table I. Color Reaction of Amino Acids and Related Compounds

α-Alanine	Pinkish purple	Histidine	Orange
β-Alanine	Pale orange	Hydroxyproline	Orange
α-Aminobutyric acid	Pale orange	Isoleucine	Pale orange
Arginine	Orange	Leucine	Almost colorless
Aspartic acid	Orange	Lysine	Pale purple
Citrulline	Pale orange	Methionine	Reddish
Creatine	Pale purple	Methionine sulfoxide	Orangish yellow
Creatinine	Pale orange	Norleucine	Almost colorless
Cysteine	Intense red	Norvaline	Pale orange
Cystine	Pale yellow	Ornithine	Pale orange
Dihydroxyphenylalanine	Pale purple	Phenylalanine	Pale orange
Diiodotyrosine	Pale purple	β-Phenylserine	Pale purple
Djenkolic acid	Intense red	Proline	Bright orange
Ethionine	Almost colorless	Sarcosine	Pale purple
Glutamic acid	Pale orange	Serine	Pale orange
Glutamine	Pale orange	Taurine	Pale orange
Glutathione	Bright orange	Threonine	Orange
Glycine	Orange-yellow	Allothreonine	Pale purple
Homocysteine	Intense red	Tyrosine	Pale orange
Homocystine	Orange	Tryptophan	Orange soln. and blue ppt.
Homoserine	Reddish	Valine	Pale orange

Table II. Color Reaction of Nitrogen Heterocyclics

	Acid-Toluene Only	Acid-Toluene and Ethyl Alcohol
Piperidine	Reddish	Orange-yellow
Pyridine	Light purple	Colorless
Pyridoxine	Yellow	Pale purple
Quinidine. HCl	Yellow	Light purple
Quinine	Amber	Reddish
Quinoline	Yellow	Yellow
Benzo(*f*)quinoline	Red	Pale purple and ppt.
3-Methylbenzo-(*f*)quinoline	Yellow	Pale purple and ppt.
Caffeine	Yellow	Red-orange
Adenine	Blue to lavender upon standing	Red-purple ring
Adenylic acid	Blue to brownish upon standing	Trace or no color
Guanine	Light purple	Ring
Guanylic acid	Reddish	Trace of ring
Guanosine	Reddish	No ring
Cytosine	Blue-purple	No ring
Cytidine	Dark lavender purple	Reddish ring
Cytidylic acid	Pale lavender purple	Trace or no ring
Uridine	Light lavender	Trace of ring
Xanthine	Purple color, dissipated by agitation	No ring

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ever, in both cases it was necessary to heat the reaction mixtures for varying periods of time or even evaporate and then redissolve in water.

Table III. Miscellaneous Nitrogen Compounds

	Acid-Toluene	Acid-Toluene Plus Ethyl Alcohol
Ethanolamine	Yellow	Yellow
Octadecylamine	Dark amber	Purple
Urea	Yellow	Orange-yellow
Biuret	Yellow	Pale orange
Methylurea	Yellow	Orange-yellow
Phenylurea	Orange	Orange-yellow
Hippuric acid	Yellow	Pale purple
Diphenylamine	Brownish	Yellow-green
Benzylamine	Yellowish	Reddish purple, dissipated by agitation

Twenty-one primary aromatic amines, three containing naphthalene ring systems, were also tested and all gave positive color reactions.

When the modification indicated is employed, Ehrlich's reagent will react at room temperature with every class of nitrogen compounds tried. Although this precludes its use in identification of a class of compounds, under the modified conditions used, differentiation within a class of compounds is possible. In one case, color differentiation between the stereoisomers quinine and quinidine has been shown (Table II).

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RECEIVED for review November 3, 1955. Accepted April 13, 1956.

Alkaline Solutions for pH Control

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A series of alkaline solutions useful for control of pH at any point between pH 7 and 13 can be prepared by adding standard solutions of hydrochloric acid or sodium hydroxide to portions of the following stock solutions: 0.1*M* tris(hydroxymethyl)aminomethane, 0.025-*M* borax, 0.05*M* sodium bicarbonate, 0.05*M* disodium hydrogen phosphate, and 0.2*M* potassium chloride. The compositions and buffer values of the solutions are given for intervals of 0.1 pH unit, and the dilution values and approximate temperature coefficient of pH are also indicated. The estimated accuracy is within ±0.02 pH unit. The measured pH values agree well with those computed by the mass law.

CONTROL of pH in the range from 7 to 13 is most commonly accomplished by means of buffer solutions containing phosphates, borates, barbiturates, ammonia, carbonates, or glycine (*5, 6, 7, 9, 11, 13*). Because of side reactions with proteins or carbohydrates, however, the phosphate and borate solutions intended for use in the pH range from 7 to 9 are often not well suited to studies in physiological media, while phosphates and carbonates are incompatible with calcium salts. Ammonia buffers are not highly stable, but triethanolamine buffers may be used from pH 7 to 8.5 (*4*). The low solubility of barbituric acid in cold water and the anomalous behavior of the silver–silver chloride electrode in barbiturate buffers (*12*) are sometimes of concern. Likewise, glycine is considered unsuitable in certain applications, in view of the uncertain effect of ampholytes upon the ionic strength.

It does not appear possible at the present time to find a series of solutions for the alkaline range that will be entirely free from these objections. The present paper presents the results of a determination of the pH at 25° C. of some solutions that will prove useful in many instances for pH control in the range from 7.0 to 13.0. These are divided into six series, as follows:

pH Range	System
7.0– 9.0	Tris(hydroxymethyl)aminomethane–hydrochloric acid
8.0– 9.1	Borax–hydrochloric acid
9.2–10.8	Borax–sodium hydroxide
9.6–11.0	Sodium bicarbonate–sodium hydroxide
10.9–12.0	Disodium hydrogen phosphate–sodium hydroxide
12.0–13.0	Potassium chloride–sodium hydroxide

The pH is based on the conventional activity scale defined by the NBS standards.

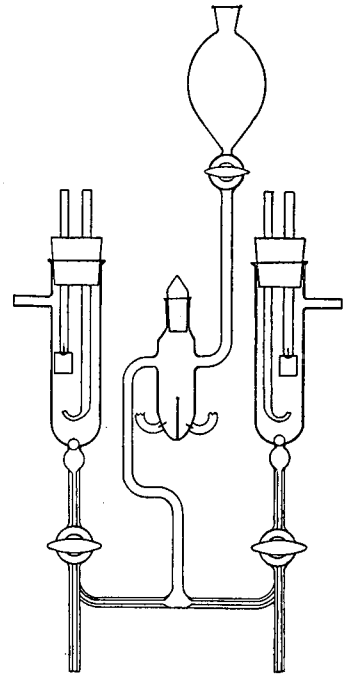

Figure 1. Cell vessel

The pH values of the six series of solutions were determined at 25° C. by measurement of the electromotive force of the cell (3)

$$\text{Pt; } H_2(\text{gas, 1 atm.}), \text{ soln. } X(pH_X) \left| \begin{matrix} \text{satd.} \\ \text{KCl} \end{matrix} \right| \text{standard}(pH_S), H_2(\text{gas, 1 atm.}); \text{ Pt}$$

in which the unknown solution is designated by X and the pH standard by S. The cell vessel is shown in Figure 1. To enhance the convenience and versatility of the assembly, the cell is provided with a saturated calomel electrode that is brought into contact with the intermediate salt bridge. The value of pH_X is related to pH_S and the electromotive force, E, of the cell (all at 25° C.) by

$$pH_X = pH_S + 16.904\,E \quad (1)$$

with Equation 1. As a consequence of the well-recognized liquid junction error near the ends of the pH scale, however, the pH of the 0.1M solution of sodium hydroxide, measured with respect to the borax or phosphate standards, appeared to be about 0.05 unit too low—that is, the measured E was too small, and a pH value of 12.83 at 25° C., instead of 12.88, was indicated for the hydroxide solution. A correction was applied for this defect in the experimental pH measurement, on the assumption that the voltage departure is a linear function of pH between the standardizing points of 9.18 and 12.88. Hence, in effect, the pH in this highly alkaline region was computed by

$$pH_X = pH_S + 17.141\,E \quad (2)$$

EXPERIMENTAL PROCEDURE

There was a slight unavoidable disturbance of the two junctions when a free connection was made between the two hydrogen electrode compartments (Figure 1). These junctions were located in the centers of the bulbs just below each compartment. Hence the potential difference between the two hydrogen electrodes was usually determined by the measurement of each electrode individually against a saturated calomel reference electrode located in the intermediate bridge solution. One hydrogen electrode was isolated by a closed stopcock during the measurement of the potential of the other, so that no disturbance of the liquid junctions resulted. The electromotive force of the cell usually remained constant within ±0.2 mv. (0.0035 pH) for one half hour or longer. The potential of the reference electrode cancels out when the difference between the two measurements is taken.

To obtain stable hydrogen electrode potentials in the carbonate buffer solutions, it was found necessary to pass the hydrogen gas through a presaturator containing a portion of the same carbonate solution; otherwise, removal of carbon dioxide by the bubbling hydrogen caused a slow drift in the direction of increasing alkalinity.

Tris(hydroxymethyl)aminomethane of high purity was furnished by Commercial Solvents Corp., and the sodium bicarbonate

Table I. Compositions, Buffer Values (β), and Dilution Values ($\Delta pH_{1/2}$) of Buffer Systems

Tris(hydroxymethyl)aminomethane

(50 ml. 0.1M tris(hydroxymethyl)aminomethane, x ml. 0.1M HCl, diluted to 100 ml.)

$dpH/dt \approx -0.028$ unit deg.$^{-1}$; $I = 0.001x$

pH	x	β	$\Delta pH_{1/2}$[b]
7.00	46.6	...	−0.02
7.10	45.7	0.010	
7.20	44.7	0.012	
7.30	43.4	0.013	
7.40	42.0	0.015	
7.50	40.3	0.017	−0.02
7.60	38.5	0.018	
7.70	36.6	0.020	
7.80	34.5	0.023	
7.90	32.0	0.027	
8.00	29.2	0.029	−0.02
8.10	26.2	0.031	
8.20	22.9	0.031	
8.30	19.9	0.029	−0.01
8.40	17.2	0.026	
8.50	14.7	0.024	
8.60	12.4	0.022	
8.70	10.3	0.020	−0.01
8.80	8.5	0.016	
8.90	7.0	0.014	
9.00	5.7	...	−0.01

Borax, pH 8.00 to 9.10

(50 ml. 0.025M borax, x ml. 0.1M HCl, diluted to 100 ml.)

$H/dt \approx -0.008$ unit deg.$^{-1}$; I 0.025

pH	x	β	$\Delta pH_{1/2}$[a]
8.00	20.5	...	
8.10	19.7	0.009	+0.07
8.20	18.8	0.010	
8.30	17.7	0.011	
8.40	16.6	0.012	
8.50	15.2	0.015	+0.05
8.60	13.5	0.018	
8.70	11.6	0.020	
8.80	9.4	0.023	+0.04
8.90	7.1	0.024	
9.00	4.6	0.026	+0.02
9.10	2.0	...	

Borax, pH 9.20 to 10.80

(50 ml. 0.025M borax, x ml. 0.1M NaOH, diluted to 100 ml.)

$dpH/dt \approx -0.008$ unit deg.$^{-1}$; $I = 0.001(25 + x)$

pH	x	β	$\Delta pH_{1/2}$
9.20	0.9	...	
9.30	3.6	0.027	+0.01[a]
9.40	6.2	0.026	+0.01[b]
9.50	8.8	0.025	
9.60	11.1	0.022	+0.01,[a] + 0.01[b]
9.70	13.1	0.020	
9.80	15.0	0.018	+0.01[b]
9.90	16.7	0.016	
10.00	18.3	0.014	
10.10	19.5	0.011	−0.01[a]
10.20	20.5	0.009	0.00[b]
10.30	21.3	0.008	
10.40	22.1	0.007	
10.50	22.7	0.006	
10.60	23.3	0.005	
10.70	23.80	0.004	
10.80	24.25	...	

Carbonate

(50 ml. 0.05M $NaHCO_3$, x ml. 0.1M NaOH, diluted to 100 ml.)

$dpH/dt \approx -0.009$ unit deg.$^{-1}$; $I = 0.001\ (25 + 2x)$

9.60	5.0	...	+0.02,[a] +0.03[b]
9.70	6.2	0.013	
9.80	7.6	0.014	
9.90	9.1	0.015	+0.03[b]
10.00	10.7	0.016	+0.04[a]
10.10	12.2	0.016	+0.04[a]
10.20	13.8	0.015	
10.30	15.2	0.014	+0.02[b]
10.40	16.5	0.013	
10.50	17.8	0.013	
10.60	19.1	0.012	+0.03[a]
10.70	20.2	0.010	0.00[b]
10.80	21.2	0.009	
10.90	22.0	0.008	
11.00	22.7	...	

Phosphate

(50 ml. 0.05M Na_2HPO_4, x ml. 0.1M NaOH, diluted to 100 ml.)

$dpH/dt \approx -0.025$ unit deg.$^{-1}$; $I = 0.001(77 + 2x)$

10.90	3.3	...	
11.00	4.1	0.009	
11.10	5.1	0.011	−0.06,[a] −0.07[b]
11.20	6.3	0.012	
11.30	7.6	0.014	
11.40	9.1	0.017	−0.09,[a] −0.10[b]
11.50	11.1	0.022	
11.60	13.5	0.026	
11.70	16.2	0.030	−0.15[b]
11.80	19.4	0.034	−0.13,[a] −0.17[b]
11.90	23.0	0.037	
12.00	26.9	...	

[a] Measured.
[b] Calculated.

Hydroxide-Chloride

(25 ml. 0.2M KCl, x ml. 0.2M NaOH, diluted to 100 ml.)

$dpH/dt \approx -0.033$ unit deg.$^{-1}$; $I = 0.001(50 + 2x)$

pH	x	β	$\Delta pH_{1/2}$[b]
12.00	6.0	0.028	−0.28
12.10	8.0	0.042	
12.20	10.2	0.048	−0.28
12.30	12.8	0.060	
12.40	16.2	0.076	
12.50	20.4	0.094	−0.28
12.60	25.6	0.12	
12.70	32.2	0.16	
12.80	41.2	0.21	−0.28
12.90	53.0	0.25	
13.00	66.0	0.30	−0.27

When pH_X exceeds pH_S the sign of E is positive (positive electrode on the right), whereas E is negative when pH_X is less than pH_S.

STANDARDS

For measurements between pH 7 and pH 13, the NBS phosphate (pH_S = 6.86 at 25° C.) and borax (pH_S = 9.18 at 25° C.) standards were supplemented with a 0.1M solution of sodium hydroxide, to which a pH of 12.88 was assigned (2). The phosphate standard was 0.025M with respect to both potassium dihydrogen phosphate and disodium hydrogen phosphate. It was used for the tris(hydroxymethyl)aminomethane series, and the 0.01M borax standard was chosen for the carbonate series and the two series of borax buffer solutions. For the highly alkaline solutions of the phosphate-hydroxide and chloride-hydroxide series, both the borax standard and the supplementary standard of sodium hydroxide were employed.

When the two cell solutions were the phosphate and borax standards, the observed value of E was completely consistent

was reagent grade. Both were found to assay 100.0% by titration with standard acid. The stock solution of disodium hydrogen phosphate was prepared from NBS Standard Sample 186II*b*.

Hydrated borax usually loses its water of hydration slowly during storage. Although this change in composition is almost without effect on the pH of the 0.01*M* standard (*2*), it might have a larger influence upon the pH of buffer solutions formed from the borax and strong acid or alkali. Accordingly, the stock solution of borax was prepared from material that had been dehydrated by drying overnight at 110° C. and then raising the temperature gradually to 400° C. The water from which the solutions were prepared was purged with air that had been freed of carbon dioxide.

RESULTS

The pH values calculated by Equations 1 and 2 were plotted on a large scale as a function of the volume of strong acid or alkali added to 50 ml. (or 25 ml.) of stock solution. Smooth curves were drawn through the points, and the volumes of reagent were read at intervals of 0.1 pH unit. The results are summarized in Table I. The estimated accuracy of the pH values is ±0.02 unit. Gomori (*8*) has determined the pH of six solutions containing tris(hydroxymethyl)aminomethane and hydrochloric acid at 23° and 37° C. His pH values, interpolated at 25° C., agree with those obtained here within ±0.02 unit.

The Van Slyke buffer value, β, defined as $db/d\text{pH}$ (*14*), where b represents a number of moles of strong alkali added to 1 liter of buffer solution, is given in the table.

Also listed are a few values of the dilution value, $\Delta\text{pH}_{1/2}$ (*1*), a quantity that expresses the change of pH resulting from dilution of a portion of the buffer solution with an equal volume of pure water. The value of $\Delta\text{pH}_{1/2}$ is positive when the pH increases on dilution and negative when it decreases. In view of the good agreement between the measured and calculated pH values for the tris(hydroxymethyl)aminomethane buffers and for the potassium chloride–sodium hydroxide solutions (see below) the dilution values of the solutions in these two series were computed from the composition, equilibrium constants, and the Debye-Hückel equation. Dilution values for several compositions in the other buffer systems were determined by measuring the pH before and after dilution. Some calculated values are also included in the table for comparison with those obtained by direct measurements.

The effect of temperature changes (in pH units per degree C. near 25°. C.) is indicated by the approximate calculated value of $d\text{pH}/dt$ (*2*) given for each buffer along with a formula for the ionic strength, I. Like that of other alkaline solutions, the pH of these buffer mixtures is rather sensitive to temperature changes. A high buffer value, a low dilution value, and a small value of $d\text{pH}/dt$ are desirable properties of buffer solutions for pH control.

The pH values given in the table were compared with the pH calculated from the dissociation constant, the compositions of the solutions, and conventional activity coefficients defined by the Debye-Hückel equation in its "second-approximation" form, which contains an ion-size parameter, a_i. For the mixtures composed of univalent ions and uncharged species alone, the pH calculated with a_i between 4 and 6 A. differed by no more than ±0.01 unit from the measured values. In view of the complexity of the equilibria in solutions containing appreciable concentrations of boric acid (*10*), no calculation for the borax–hydrochloric acid series was attempted.

When the solutions contain bivalent or multivalent ions, however, the activity-coefficient term is considerably larger than when only univalent ions are present, and the calculated pH is more sensitive to the value chosen for a_i. However, the calculated pH was again consistent with the measured pH, but only when somewhat larger values of a_i—namely 10 for the carbonate series and 8 for the phosphate series—were used. While such large values for bivalent and trivalent ions are certainly not surprising, they enhance the difficulty of interpreting measured pH values simply and accurately in terms of chemical equilibria.

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RECEIVED for review November 25, 1955. Accepted April 17, 1956.

Determination of Methylene Chloride in Aqueous Solutions

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A quantitative method for determining small quantities of methylene chloride in aqueous solutions has been developed, in which the methylene chloride is saponified with sodium hydroxide in a peroxide Parr bomb ignition-type apparatus. This method is simple and rapid and has a relative mean deviation of 2.6 parts per thousand and a relative mean error of 0.010%.

A METHOD for the quantitative determination of small quantities (0.5 to 1.5%) of methylene chloride in aqueous solutions was desired for routine control analysis. Two things were required—speed and a reasonable degree of accuracy.

The standard method of combustion (*3*) and subsequent determination of the chlorides by volumetric or gravimetric procedures did not lend itself readily to this type of sample, and a considerable amount of time was required for each determination.

A review of the literature showed several detection methods (*5–7*), but these were qualitative in nature. An infrared and mass spectrometric method was likewise noted (*1*), but this required apparatus not currently in use in this laboratory. Vogel (*4*) gives a saponification method with alcoholic potassium hydroxide solution as a characterization test for alkyl halides. As this seemed a simple procedure, that would be applicable to methylene chloride, it was tried quantitatively. Very low results were obtained, with a poor degree of accuracy, possibly because

of loss of methylene chloride when the sample was heated, or insufficient heat for complete hydrolysis (*2*). To overcome this, an apparatus was required that would be vapor-tight and also stand a considerable pressure. The peroxide Parr bomb flame ignition type of apparatus seemed to be the answer and it was subsequently used. In this way, a simple, rapid and accurate method was developed for the determination of methylene chloride in aqueous solutions.

APPARATUS

Parr peroxide bomb, 22-ml. flame ignition type, Series 2100. Gasket type, service asbestos sheet packing, Style 60-S, manufactured by Canadian Johns-Manville, Ltd.

REAGENTS

Sodium hydroxide, approximately 1.0*N*. Dissolve 40 grams of analytical reagent grade sodium hydroxide in 1000 ml. of distilled water.

Sulfuric acid, approximately 1.0*N*.

Silver nitrate, 0.1*N*. Dry some finely powdered analytical reagent grade silver nitrate at 150° C. for 2 hours and allow to cool in desiccator. Weigh out 8.5 grams, dissolve in water, and make up to 500 ml. in a volumetric flask. Standardize against primary standard analytical reagent grade sodium chloride, using potassium chromate as indicator.

Potassium chromate indicator solution. Dissolve 5 grams of analytical reagent grade potassium chromate in 100 ml. of water.

Methyl red indicator, 0.1% solution. Dissolve 1 gram of the free acid in 600 ml. of alcohol and dilute to 1000 ml. with water

PROCEDURE

Pipet 10 ml. of 1.0*N* sodium hydroxide solution into the bomb and weigh. Add 10 ml. of sample, containing up to 175 mg. of methylene chloride, and weigh again. Seal the bomb and pipet 2 ml. of distilled water onto the top. Place the bomb in the steel ignition housing and heat with a gas burner, using a medium flame, until the distilled water starts to boil; continue heating for 5 minutes, time by stop-watch, then extinguish burner.

Table I. Calculation of Relative Mean Deviation and Relative Mean Error

	CH_2Cl_2, Wt. %	Deviation, %
1	1.14	0.002
2	1.13	0.008
3	1.14	0.002
4	1.14	0.002
5	1.13	0.008
6	1.14	0.002
7	1.14	0.002
8	1.14	0.002
9	1.14	0.002
10	1.14	0.002
11	1.14	0.002
12	1.14	0.002
13	1.14	0.002
14	1.14	0.002
15	1.13	0.008
Mean	1.138	
	Mean deviation	0.003

$$\text{Relative mean deviation } \frac{0.003 \times 100}{1.138} = 0.26\% = 2.6 \text{ parts per thousand}$$

$$\text{Relative mean error } \frac{1.150 - 1.138}{1.15} = 0.010\%$$

Remove the bomb with a wire hook and quench it in cold water. Dismantle the bomb and transfer the contents quantitatively into a 500-ml. Erlenmeyer flask. Add 5 drops of methyl red indicator and neutralize the solution with 1.0*N* sulfuric acid to the red end point. Titrate with 0.1*N* silver nitrate to the potassium chromate end point.

Table II. Results on Synthetic Aqueous Methylene Chloride Samples

Sample	CH_2Cl_2 Present, Wt. %	CH_2Cl_2 Found, Wt. %	Recovery, %
A	0.26	0.26	100
		0.25	96.1
B	0.65	0.65	100
		0.64	98.4
C	1.15	1.14	99.1
		1.13	98.3
D	1.25	1.21	96.8
		1.20	96.0

DISCUSSION

Calculation. Methylene chloride, % w./w.

$$\frac{\text{Titration} \times N\ AgNO_3 \times 0.00425 \times 100}{\text{weight of sample}}$$

Chemical Reaction. Sodium hydroxide reacts with methylene chloride under the pressure and temperature developed in the bomb (*2*).

$$5NaOH + 2CH_2Cl_2 = 4NaCl + 2H_2O + CH_3OH + HCOONa$$

The sodium chloride is titrated argentometrically.

$$NaCl + AgNO_3 = AgCl + NaNO_3$$

Interferences. Inorganic chlorides and other alkyl halides will interfere, if present. The authors have found chloroform and carbon tetrachloride to react quantitatively.

Experimental. A standard sample was prepared which contained 1.15 weight % methylene chloride. Fifteen determinations of methylene chloride were made using the Parr bomb saponification method. Table I gives the results and calculations for the relative mean deviation and relative mean error.

A series of synthetic samples was prepared covering a range of 0.25 to 1.25% methylene chloride, on which duplicate determinations were made (Table II).

Before this method was adopted as a standard analytical procedure in this laboratory, the authors consulted the Parr Instrument Co. as to its safety. The reply stated that if all precautions for the operation of the bomb were strictly adhered to, no danger was involved.

The Parr Co. supplied several types of gaskets; No. 27AC Hycar type was very good, but the type reported in the method was considered slightly better.

ACKNOWLEDGMENT

The authors thank the Parr Instrument Co. for the interest and information supplied and Canadian Celanese, Ltd., for permission to publish this paper.

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RECEIVED for review November 29, 1955. Accepted April 27, 1956.

Ion Exchange–Spectrophotometric Determination of Aluminum

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An effort has been made to eliminate the large number of separations of interfering metals usually required in the determination of aluminum by the aluminum-aluminon method. Aluminum was separated from nearly all interfering metal ions by a strongly basic ion exchange resin. Aluminum in the effluent from the column was determined as the ammonium aurintricarboxylate lake at a wave length of 525 mμ with a relative standard deviation of 1.5% in the range from 5 to 50 γ of aluminum. The method is applicable generally to copper-aluminum, uranium-aluminum, and tin-lead alloys, stainless steel, and iron alloys.

ALUMINON reagent, the ammonium salt of aurintricarboxylic acid, has been used for many years to determine small amounts of aluminum. When prepared as an aluminon buffer solution by the method described by Craft and Makepeace (*1*) and later by Luke and Braun (*6*), it has been shown to be a realiable reagent for aluminum that is free from interfering ions. These authors have adequately established the optimum amount of reagent and the pH required to obtain maximum sensitivity.

The principal problem in the use of aluminon to determine aluminum is the removal of interfering ions. In the removal of interfering ions in alloys of aluminum with copper, tin, and lead, Luke (*5*) makes use of a mercury cathode separation, a cupferron extraction, an oxine extraction, and finally the aluminon-aluminum procedure. Craft and Makepeace (*1*) have used perchloric acid to remove silica and tungsten from steels, and an isopropyl ether extraction to remove iron. Teicher and Gordon (*8*) separated small amounts of iron(III) from large amounts of aluminum by complexing iron with thiocyanate and passing the sample through a chloride-form anion exchange column at pH 1.0. This method is impractical when several different metal contaminants are present.

Table I. Adsorption by Dowex 1 Chloride-Form Resin of Metals Found in Alloys of Aluminum with Iron, Copper, Uranium, Tin, and Lead

Ions Adsorbed from 9*M* HCl Solutions	Distribution Coefficient (D_v)	Ions Not Adsorbed from 9*M* HCl Solutions ($D_v < 2$)
Mn(VII)	High	Al(III)
Cr(VI)	High	Mn(II)
V(V)	High	Cr(III)
Mo(VI)	90	Ni(II)
Cu(II)	8	V(IV)
Zn(II)	60	Ti(III and IV)
Cd(II)	40	Mg(II)
Fe(II)	4	Th(IV)
Fe(III)	3×10^4	Rare earths(III)
Zr(IV)	100	Be(II)
U(VI)	500	Pb(II)
Sn(II)	50	
Sn(IV)	5×10^3	
Co(II)	40	
Sb(III)	50	
Sb(V)	2×10^5	
W(VI)	40	
Tc(VII)	High	

In the present investigation, the purification of aluminum has been simplified so that a single operation is sufficient to separate aluminum from nearly all interfering ions that will be encountered in copper-aluminum, uranium-aluminum, and in tin-lead alloys, iron, and stainless steel.

The method is made possible by the fact that Dowex 1 anion exchange resin in the chloride form adsorbs from a 9*M* hydrochloric acid solution of the sample nearly all ions that interfere in the aluminon method for the determination of aluminum, whereas aluminum is not adsorbed in this medium (*7*). The amount of an ion adsorbed by the resin is dependent upon the hydrochloric acid concentration of the solution containing the ion. Although 9*M* hydrochloric acid is not optimum for the adsorption of all metal impurities in aluminum sample solutions, the adsorption coefficient of most metals found in the common alloys is large enough to provide complete separation of aluminum from these metals. Adsorption data reported by Kraus and Nelson (*3*) for metals found in the alloys under investigation are shown in Table I. The distribution coefficient,

$$D_v = \frac{\text{amt. of metal adsorbed per cc. of dry resin + voids}}{\text{amt. of metal per cc. remaining in solution phase}}$$

is sufficiently large for most of the metals to permit their separation from aluminum in a single pass through the resin column. Lead(II), which is not adsorbed from 9*M* hydrochloric acid, is strongly adsorbed from 2*M* hydrochloric acid. This element may be separated by adjusting the effluent from the 9*M* column to 2*M* in hydrochloric acid and passing it through a second ion exchange column.

Interferences. Of those ions that pass through the resin column, the trivalent rare earths, manganese(II), vanadium(IV), chromium(III), nickel(II), titanium(III and IV), beryllium(II), and thorium(IV) interfere in the determination of aluminum. Interference by nickel(II) can be masked by pyridine. Chromium(III) is slightly adsorbed by the resin; if it is present in amounts greater than the aluminum present, the sample should be treated with perchloric and hydrochloric acids (*2*) to remove all but traces of chromium. Titanium(III and IV) will not interfere if present in amounts equal to or less than the aluminum. Manganese(II) can be tolerated in an amount 25 times that of aluminum (*1*). Beryllium, thorium, and the rare earths are uncommon components of the copper-aluminum, uranium-aluminum and tin-lead alloys, iron, and stainless steels. Interference by anions has been discussed by Craft and Makepeace (*1*).

APPARATUS

Spectrophotometer. The Beckman Model B spectrophotometer with cells of 1-cm. cross section was used to determine the absorbance of the aluminum-aluminon lake.

Ion Exchange Column. A portion of a 10-ml. graduated pipet was used for the ion exchange column. The tip was drawn out to fit into the neck of a 50-ml. volumetric flask.

REAGENTS

Standard Aluminum Solution (50 γ of aluminum per ml.). Prepared from spectrographically pure aluminum metal dissolved in hydrochloric acid.

Ion Exchange Resin. Dowex 1 chloride-form anion exchange resin, 50 to 100 mesh, with 10% divinylbenzene (DVB), supplied by Dow Chemical Co., Midland, Mich.

Aluminon-Buffer Composite Solution. Reagent grade chemicals were used. The solution was prepared by the procedure of Luke and Braun (*6*) except that all weights and volumes were halved to produce 1.5 liters of finished reagent instead of 3. Aurintricarboxylic acid–ammonium salt, Eastman Chemical

Co., produced a highly reproducible calibration curve. The reagent has been in use for more than 6 months and is still good.

EXPERIMENTAL

Adsorption Characteristics of Resin. In the analysis of most alloys, there is one major constituent that will be adsorbed by the resin. The amount of this constituent to be adsorbed depends, of course, upon the ratio of the major constituent to aluminum in the sample. Preliminary tests should be made to determine that none of this constituent passes through the column when the procedure is followed. If any passes through, the resin volume must be increased.

It was found, for example, that Dowex 1 (10% DVB) ion exchange resin can adsorb from a 9*M* hydrochloric acid solution about 1.5 meq. of uranyl ion per gram of dry resin and about 0.75 meq. of cupric ion per gram of dry resin. One gram of Dowex 1 (10% DVB) resin saturated with 9*M* hydrochloric acid occupies a volume of about 2 ml. A resin volume allowance of plus 100% should be made to ensure complete adsorption of the major constituent plus other metal ions in the sample. Allowance must be made also for tailing ions—that is, ions whose coefficient of adsorption in 9*M* hydrochloric acid is low.

A rinse consisting of at least two column volumes (resin volumes) of 9*M* hydrochloric acid is required to ensure complete separation of the aluminum from interfering metals. This must be taken into account in determining a practical size for the column. The column should not be too small in diameter, or the flow of solution will be too slow. A flow of about 1 ml. per minute is satisfactory. If the flow is too fast, the tailing ions may pass through with the aluminum. The resin should be kept saturated at all times to prevent channeling.

The regeneration of the ion exchange resin—that is, the elution of adsorbed metals from the resin—presents few problems. Most of the metals can be removed from the resin by elution with dilute hydrochloric acid.

The elements technetium, tin, and molybdenum are difficult to remove from the resin (*3*). When the removal of these metals is incomplete, their adsorption bands are spread the entire length of the column, with the result that some of the ions pass into the next sample that is placed on the column. This difficulty, caused by the presence of molybdenum, was encountered in the separation of aluminum from the National Bureau of Standards steel sample, 111*B*. The cost of the Dowex 1 chloride-form resin is less than 5 cents for the amount specified in this method and it is suggested that a large volume of resin be treated with 9*M* hydrochloric acid, so that fresh resin may be used for each determination.

Pyridine as Complexing Agent. Some of the sample solutions in which aluminum was to be determined were known to contain nickel(II), which is not adsorbed by Dowex 1 resin (*3*). Craft and Makepeace (*1*) have shown that this ion interferes in the determination of aluminum.

Pyridinium chloride (*4*) has been used to complex nickel(II) ion. The following experiments were carried out to show whether or not pyridine interferes with the formation of the aluminum-aluminon lake.

An aliquot of a solution that contained 10 γ of aluminum was pipetted into a 50-ml. volumetric flask and 25 mg. of nickel(II) was added. To the flask were added 5.0 ml. of concentrated hydrochloric acid, 5.15 ml. of pyridine (11.9*M*), and 15 ml. of aluminon-buffer solution. The flask was placed in boiling water for 5 minutes. The solution was cooled, diluted to the mark, and the absorbance was determined at 525 mμ *vs.* distilled water. This experiment was repeated for varying amounts of nickel with proportional amounts of pyridine and hydrochloric acid. When 0.25 ml. of concentrated hydrochloric acid and 0.4 ml. of pyridine were added to 10 γ of aluminum and the sample treated according to the above procedure, the absorbance was identical to that obtained when nickel was present. Additional tests were made to determine the range of nickel covered by each combination of pyridine and hydrochloric acid.

The results are shown in Table II. Pyridine is an effective masking agent for nickel in the presence of aluminon reagent. The amounts of pyridine and hydrochloric acid probably will vary slightly for different bottles of these reagents. The amount of free pyridine is not too critical, but too much will upset the buffering action of the aluminon-buffer solution.

PROCEDURE

Preparation of Column. Prepare a slurry with Dowex 1 (10% DVB) resin and 9*M* hydrochloric acid. Insert a plug of glass wool in the bottom of the column and add the slurry in increments, allowing the resin to drain after each increment, until the column is filled to the desired level. (A volume of 4.5 ml. of wet resin was used in this work.) Place a plug of glass wool on top of the resin in the column to prevent disturbance of the resin when adding solution to the column. Connect a 125-ml. separatory funnel to the column with Tygon tubing after addition of the sample to provide 9*M* hydrochloric acid for washing aluminum through the column. Connect a separatory funnel that contains 0.2*M* hydrochloric acid to the column when it is desired to remove adsorbed impurities. All of this equipment is attached to a ring stand or other convenient support. If the resin has not been washed previously with 9*M* hydrochloric acid, pass 100 ml. of the acid through the column, after which it is ready for use.

Table II. Amounts of Pyridine and Hydrochloric Acid Required to Complex Varying Amounts of Nickel

[Pyridine, 11.9*M* (approx.); hydrochloric acid, 11.7*M* (approx.); nickel present as nickel sulfate; 10 γ of Al(III) present]

Ni(II) Added, Mg.	Concd. HCl Added, Ml.	Pyridine Added, Ml.	Al(III) Found, γ	Range of Nickel(II) Covered, Mg.
0	0.25	0.40	10.0	0 to 0.75
1	0.50	0.65	9.4	0.75 to 2.5
5	1.00	1.15	10.1	2.5 to 7.5
10	2.00	2.15	10.0	7.5 to 12.5
15	3.00	3.15	10.2	12.5 to 17.5
20	4.00	4.15	9.6	17.5 to 22.5
25	5.00	5.15	10.0	22.5 to 27.5

Calibration Curve. Pipet 1 ml. of standard aluminum solution (50 γ of aluminum per ml.) into a 50-ml. volumetric flask. Add 0.25 ml. of concentrated hydrochloric acid and 0.4 ml. of pyridine. Add 15 ml. of aluminon-buffer solution and place the flask in briskly boiling water for 5 minutes. Cool and dilute to volume. Determine the spectral absorbance in cells of 1 cm. cross section at a wave length of 525 mμ with distilled water for a reference. Repeat the procedure for 0.1, 0.2, 0.4, 0.6, and 0.8 ml. of the standard aluminum solution. Prepare a new calibration curve for each fresh batch of aluminon-buffer solution.

Determination of Aluminum. Samples That Do Not Contain Lead. Dissolve solid samples in hydrochloric or perchloric acid. Evaporate to dryness and dilute to volume with 9*M* hydrochloric acid. If the sample is a solution, evaporate it to dryness and dilute to volume with 9*M* hydrochloric acid. If chromium is present in an amount greater than the aluminum, it should be removed by the method of Hoffman and Lundell (*2*). Evaporate to dryness and dilute to the desired volume with 9*M* hydrochloric acid. Place the tip of the column in a 50-ml. volumetric flask. Pipet an aliquot of the sample onto the Dowex 1 resin. Allow to drain almost completely. Attach the separatory funnel that contains 9*M* hydrochloric acid to the column and allow 15 ml. to pass through. (The volume is not critical, but it should not be smaller than 15 ml.). Place the 50-ml. volumetric flask on a hot plate and evaporate the effluent to dryness, being careful not to allow the residue to bake on the glass. Cool the flask and add concentrated hydrochloric acid and pyridine according to the amounts listed in Table II. Add 15 ml. of aluminon-buffer solution and place the flask in briskly boiling water for 5 minutes. Cool and dilute to volume. Determine the spectral absorbance in 1-cm. cells at a wave length of 525 mμ, using distilled water as a reference.

Samples That Contain Lead. Prepare the samples as just described. Then, place the tip of the column in a graduated cylinder and pipet an aliquot of the sample onto the ion exchange column. Allow to drain almost completely, then add 15 ml. of 9*M* hydrochloric acid. When the column has drained, adjust the 9*M* hydrochloric acid effluent to 2*M* by adding distilled water. Prepare a 2*M* hydrochloric acid resin column and pass the ad-

Table III. Composition of NBS Samples and Aluminum Content Determined by Proposed Method

Sample No.	Composition[a] % C	Mn	P	S	Si	Cu	Ni	Cr	V	Mo	Al	Zn	Sn	Pb	Fe
Ni-Mo steel 111*B*	0.193	0.706	0.012	0.014	0.302	0.028	1.81	0.070	0.003	0.255	0.043	...	..	..	Balance
Mn-Al bronze 164	...	4.68	...	...	0.038	63.76	0.046	...	...	...	6.21	21.89	0.63	0.22	2.52

	Aluminum, % Present	Found	Diff., %
111*B*[b]	0.043	0.044	+0.001
		0.042	−0.001
		0.042	−0.001
		0.045	+0.002
164[c]	6.21	6.10	−0.11
		6.19	−0.02
		6.29	+0.08
		6.30	+0.09

[a] These values are NBS averages in per cent.
[b] Dissolved 1 gram in perchloric and hydrochloric acids, evaporated to dryness, and dissolved residue in 9*M* hydrochloric acid. Diluted to 50 ml. with 9*M* hydrochloric acid.
[c] Dissolved 0.1 gram in concentrated hydrochloric acid, evaporated to dryness, and dissolved residue in 9*M* hydrochloric acid. Diluted to 100 ml. with 9*M* hydrochloric acid.

justed effluent through the column, this time collecting the effluent in a beaker. Rinse the 2*M* effluent through the column with 15 ml. of 2*M* hydrochloric acid. Evaporate the final effluent to dryness, dissolve the residue in hydrochloric acid (Table II), and transfer the contents of the beaker to a 50-ml. volumetric flask. Add pyridine according to the amounts listed in Table II, and continue the determination as described above. If lead is present in an amount that is much smaller than the amount of aluminum in the sample, use the procedure for samples without lead.

RESULTS

Table III shows the Bureau of Standards average values for percentages of the metals and impurities in alloys 164 and 111*B*, as well as results of the aluminum determination on these samples.

Table IV shows the results obtained from the determination of aluminum in a synthetic sample which represents the corrosion of stainless steel by uranyl sulfate solution under high temperature and pressure.

Table IV. Determination of Aluminum in Synthetic Corrosion Sample of Uranyl Sulfate Solution in Contact with Stainless Steel

Solution Composition, Grams per Liter

Uranium(IV)	10
Copper(II)	2
Nickel(II)	0.1
Chromium(III)	0.1
Iron(III)	0.1
Aluminum(III)	0.05

Aluminum, γ Present	Found	Diff., γ
50	49.5	−0.5
30	30.4	+0.4
20	19.8	−0.2
10	9.4	−0.6

ACKNOWLEDGMENT

The research on the adsorption characteristics of Dowex 1 ion exchange resin by K. A. Kraus and Frederick Nelson of Oak Ridge National Laboratory, Chemistry Division, has been very valuable to the authors in the development of this method.

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RECEIVED for review December 27, 1955. Accepted April 30, 1956.

C—H Vibrations in Aldehydes

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The infrared spectra of four monodeuterated aldehydes, in which the deuterium was on the aldehyde group, were measured. Comparison of any one of these spectra with that of its protium homolog shows many bands which are unchanged in frequency or intensity; other bands are shifted by large amounts. The method thus enables one immediately to determine whether or not the aldehyde hydrogen is involved in any band of any aldehyde. Assignments in aldehyde spectra are discussed.

CHARACTERISTIC regions in the infrared spectrum have been listed for aldehydes (*2*). While the C=O and C—H stretching regions are well established, some of the other correlations have been questioned (*1*). Four monodeuterated aldehydes recently became available to this laboratory, and an investigation of their infrared spectra was carried out.

EXPERIMENTAL

The four compounds studied were benzaldehyde, *p*-chlorobenzaldehyde, piperonaldehyde, and butyraldehyde. The light

compounds were commercial samples, purified by distillation immediately before recording their spectra. The heavy compounds were prepared by a lithium aluminum deuteride reduction (*7, 8*) which replaces only the aldehyde hydrogen by deuterium. The infrared spectra also showed the absence of deuterium directly substituted on the ring, because all of the C—D stretching vibrations appeared well below 2200 cm.$^{-1}$ [or 2200 *K* (kaysers), which is the same unit as wave numbers]. In the deuterated benzenes and naphthalene, practically all of the C—D stretching vibrations appear above 2200 cm.$^{-1}$ (*3–5*). The spectra were all recorded with a Perkin-Elmer Model 21 spectrometer, using a sodium chloride prism. The benzaldehydes were measured in the pure liquid state, as solutions in carbon disulfide and in carbon tetrachloride, and at low temperature in the solid state. The other compounds were measured in carbon tetrachloride solution, with partial compensation by a cell filled with pure carbon tetrachloride in the standard beam. The butyraldehyde was not examined in so great detail as the others; thus, only the stronger bands in its spectrum were measured.

RESULTS AND DISCUSSION

2700- and 1400-Cm.$^{-1}$ Regions. All of the compounds examined showed two strong bands in the higher region, and one band in the lower region which disappeared upon deuteration, showing that they are due to vibrations involving the aldehyde hydrogen. The bands which disappeared upon deuteration in these regions were: for benzaldehyde, 2780, 2700, and 1397; for *p*-chlorobenzaldehyde, 2790, 2710, and 1415; for piperonaldehyde, 2750, 2715, 2650 (weak), and 1391; and for butyraldehyde, 2785, 2690, and 1385. The origin of two bands rather than one, in the 2700 region, is thus established as due to Fermi resonance with the overtone of the C—H bending vibration at about one half the frequency (*6*). It also confirms the 1400 region as involving a bending of the aldehyde C—H bond (*2*).

It is interesting to note that all the heavy aromatic aldehydes show two intense peaks in the C—D stretching region, whereas the butyraldehyde shows a strong and a weak peak in this region. In some of the heavy aldehydes, the displaced C—D bending vibration is clearly evident, while in others it is possibly overlapped by other strong bands of the molecule. The shifted frequencies for the monodeuterated aldehydes are: for benzaldehyde, 2082, 2035, and 1040 (weak?); for *p*-chlorobenzaldehyde, 2095, 2045, and 1080 (shoulder); for piperonaldehyde, 2070, 2025, and 1029 (shoulder); and for butyraldehyde, 2180 (weak), 2060, and 1095. Evidently, there is still Fermi resonance between the overtone of the C—D bending fundamental and the C—D stretching fundamental; the weakness of the 2180 band in butyraldehyde can probably be laid to the higher value of the C—D bending fundamental frequency.

Remainder of Spectrum. Other effects were observed, such as disappearance and appearance of weak bands, and small shifts in positions of other bands. While the effects are not as clear-cut as in the other regions, they do help to substantiate some earlier assignments in aldehyde spectra. Only those additional bands which shift appreciably in position or intensity are listed; the others are identical in both spectra and are not listed here. Approximate intensities of the bands are denoted by the abbreviations w, mw, m, s, and vs for weak, medium weak, medium, strong, and very strong, respectively.

On deuteration of light benzaldehyde, bands at 1289 mw, 1170 m, 1008 mw, 1205 s, 922 w, 830 s, and 753 vs disappear and appear in heavy benzaldehyde at 1232 s, 1215 s, 1254 (shoulder), 939 mw, 790 s, and 733 vs. On deuteration of light *p*-chlorobenzaldehyde, bands at 1297 ms, 1202 s, 1007 w (shoulder), 835 s, and 698 m disappear and appear in heavy *p*-chlorobenzaldehyde at 1220 s, 1238 w (shoulder), 1008 w (shoulder), 880 s, and 685 s. On deuteration of light piperonaldehyde, bands at 1120 w, 1083 w (shoulder), 943 w (shoulder), 934 s, 924 m, 876 m, and 860 m disappear, and appear in heavy piperonaldehyde at 940 s, 923 s, 912 w, 894 ms, and 888 w (shoulder).

The shifts observed in this region are more complex and difficult to interpret. If the concept of group frequencies were rigidly obeyed, there should be a total of only four bands shifting: the C—H stretching, the C—H in-plane bending, the C—H out-of-plane bending, and the torsion of the aldehyde group against the remainder of the molecule. The last is probably outside the range of observation. The two kinds of C—H bending can be distinguished by analogy with formaldehyde, ethylene, and benzene (*3*), where the out-of-plane bending vibrations are found at much lower frequencies. In the present investigation the number of bands shifting is far more than three or four. This must mean that the actual vibrations are mixtures of group frequencies and are shifted, more or less, upon isotopic substitution. It is a bit premature, therefore, to designate one of these as the lower C—H bending frequency (*6*). However, these bands which shift may still be used as an indication of the presence of an aromatic aldehyde; the benzaldehyde and *p*-chlorobenzaldehyde shifts confirm the assignments of Colthup for this class at 1260 to 1320, 1160 to 1230, and 825 to 970. However, the piperonaldehyde shifts show nothing in the first two ranges, probably because of the ring substitution, suggesting caution in the use of these correlations for highly substituted aromatic aldehydes.

ACKNOWLEDGMENT

The authors wish to thank K. B. Wiberg for the synthesis of the deuterated compounds. The lithium aluminum deuteride was obtained from Metal Hydrides, Inc., under consignment from the Atomic Energy Commission.

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RECEIVED for review September 17, 1955. Accepted April 17, 1956.

Extraction Method for Colorimetric Determination of Phosphorus in Microgram Quantities

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Extraction of phosphorus as molybdophosphoric acid and subsequent development of the molybdenum blue color complex, either in the aqueous or the alcoholic phase, eliminate significant interference from the major inhibitory ions and, because of the lower acidity employed, prevent hydrolysis of the labile phosphates. The use of hydroquinone as reductant is shown to be superior in producing greater color stability, in permitting the use of a low acid concentration, and in obviating the need for heating. Mixtures of inorganic and organic phosphates can be determined separately with precision. The lower limit of sensitivity is about 0.5 γ of phosphorus per ml. with an accuracy within ±2%.

THREE procedures for the colorimetric determination of phosphorus have been extensively described in the literature: The molybdenum blue complex is developed in the aqueous phase without extraction (*2, 3, 5, 7, 9–13, 15–17, 19*); the phosphate is first precipitated as magnesium ammonium phosphate, and then the usual procedure is applied with modifications (*1, 8*); the phosphorus is converted to molybdophosphoric acid which is extracted with various alcohols, and the blue complex is developed in the alcoholic phase (*4, 14, 18*).

The greatest disadvantage of the first procedure is interference from inhibitory ions (*4, 10*). The second procedure is time-consuming and requires a boiling water bath for development of the color. The third procedure requires a high acidity (1*N*) for reducing the molybdophosphoric acid to the blue complex, which can be measured only in the alcoholic phase (*4, 14, 18*).

The purpose of this investigation was (1) to develop a more accurate procedure for the determination of inorganic orthophosphates in the presence of labile phosphates, pyrophosphates, and the major inhibitory ions, (2) to increase the stability of the color complex, and (3) to eliminate the nonspecific blue color (*4, 13*) which tends to develop in strong acid solutions with certain reducing agents.

Five reducing agents have been employed in the biological field to develop the molybdenum blue complex—namely, hydroquinone (*2, 3, 5, 7, 10*), 1-amino-2-naphthol-4-sulfonic acid (*1, 6, 8, 9, 16*), stannous chloride (*4, 11, 12, 14, 18*), ferrous sulfate (*18, 19*), and ascorbic acid (*13*).

Stannous chloride and ferrous sulfate require high final acid concentrations (1.1*N* and 0.76*N*, respectively) in the methods of Kuttner and Cohen (*11*) and Rockstein and Herron (*17*). Three of these five reducing agents (hydroquinone, aminonaphtholsulfonic acid, and ascorbic acid) are relatively weak and can be used at a lower acidity. This suggested their use for determination of the inorganic phosphate in the presence of labile phosphate.

The color stability produced by these reducing agents was tested at about pH 2.0. The observed data are plotted in Figure 1, where curves 5 and 6 clearly show that color intensity was much more stable with hydroquinone than with the other reducing agents shown in curves 1, 2, 3, and 4. Curves 7 and 8, Figure 2, show that with hydroquinone, when the digestion time is doubled between the addition of sulfite mixture and the addition of hydroquinone, there is little change in color intensity and that the color is stable for many hours. Curves 9 to 12, Figure 3, show that, when hydroquinone is used, color intensity increases only slightly with acid concentration. The acid concentration used to obtain these curves was 2 to 20 times as much as used in Method II (which follows).

APPARATUS AND REAGENTS

A Beckman Model DU spectrophotometer and Cary recording spectrophotometer, Model 11 MS, were used with 1.0-cm. cells for both models. The same amount of reagent was used in the reference cell as in the sample cell for each experiment.

The following reagents were employed: approximately 10*N* sulfuric acid (280 ml. of concentrated sulfuric acid diluted to 1 liter), 2% hydroquinone (Coleman & Bell, C.P.), 15% sodium hydrogen sulfite, 20% sodium sulfite, sodium phosphate heptahydrate (dibasic), sodium phosphate (tribasic), 5% ammonium molybdate, *n*-butyl alcohol, 1-amino-2-naphthol-4-sulfonic acid (Eimer and Amend, C.P.), ascorbic acid, glucose-6-phosphate (Sigma Chemical), sodium β-glycerophosphate (Mallinckrodt), fructose-1,6-diphosphate magnesium salt and glucose-1-phosphate dipotassium salt (Schwarz Laboratories, Inc., New York), disodium phenyl phosphate for testing phosphatase activity

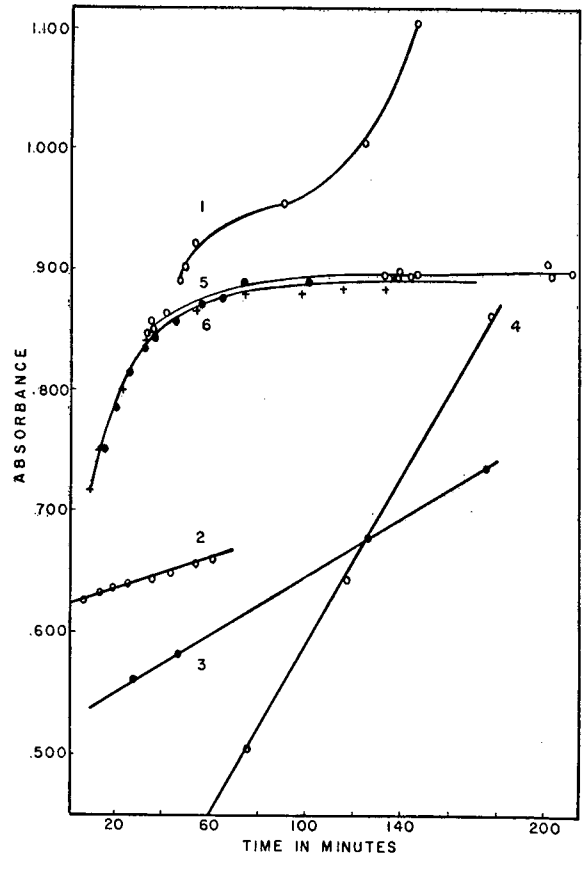

Figure 1. Stability of blue color complex produced by three reducing agents

1. 1-Amino-2-naphthol-4-sulfonic acid; 165 γ of phosphorus per 25 ml.
2. 1-Amino-2-naphthol-4-sulfonic acid; 124 γ of phosphorus per 25 ml.
3. 1-Amino-2-naphthol-4-sulfonic acid; 103 γ of phosphorus per 25 ml.
4. Ascorbic acid; 62 γ of phosphorus per 25 ml.
5, 6. Hydroquinone; 165 γ of phosphorus per 25 ml.

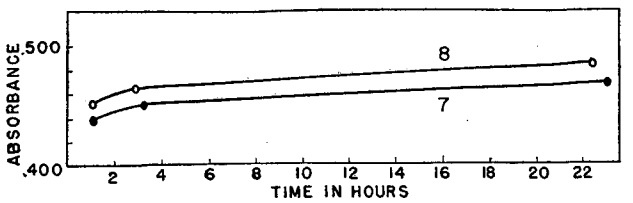

Fig. 2. Effect of digestion time on color intensity and stability

7. 15-minute digestion
8. 30-minute digestion

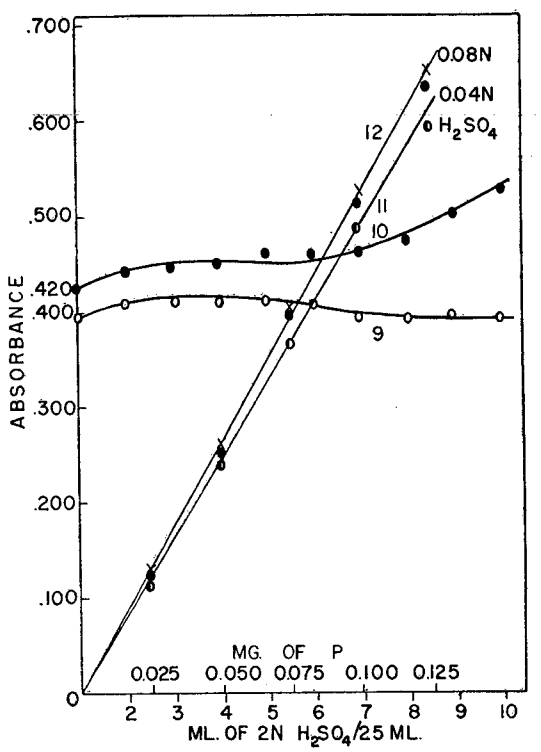

Figure 3. Comparison of color intensities produced at various acid concentrations with hydroquinone

9. 3¾ hours after hydroquinone addition; phosphorus, 0.075 mg. per 25 ml.
10. 18 hours after hydroquinone addition; phosphorus, 0.075 mg. per 25 ml.
11, 12. Standards containing indicated mg. of phosphorus per 25 ml.

(Paul-Lewis Laboratories, Milwaukee, Wis.), sodium arsenate, ammonium oxalate, sodium acetate, sodium sulfate, sodium fluoride, sodium citrate, and sodium silicate.

PROCEDURES

After the appropriate reducing agent and the role of acid concentration had been determined, an extraction method was used to eliminate the effect of the inhibitory ions and the labile phosphates.

Method I. In Presence of Labile Phosphates. About 10 ml. of distilled water is transferred to a separatory funnel (50 to 100 ml.), and the following are added in the order given: a standard solution containing 0.025 to 0.125 mg. of phosphorus as orthophosphate (equivalent to 1 to 5 γ of phosphorus per ml. in the final dilution), 5 ml. of 0.2*N* sulfuric acid, and 2 ml. of 5% ammonium molybdate solution. This solution is mixed well by shaking a few seconds and allowed to stand at room temperature for 1 minute. To extract the yellow molybdophosphoric acid which forms, 7 to 8 ml. of *n*-butyl alcohol is added and the mixture is shaken vigorously for 30 to 60 seconds. After the alcoholic and aqueous layers have distinctly separated (1 to 2 minutes), the aqueous layer is drained off and discarded. Fifteen milliliters of water is added to the alcoholic extract remaining, and the mixture is shaken for a few seconds. At this stage a fairly stable emulsion, lasting 5 to 10 minutes, is observed. The formation of this emulsion is desirable because it serves as an indicator of low acidity. To the emulsion 1 ml. of 2% hydroquinone is added and the solution is again shaken and allowed to stand 5 to 6 minutes. A light green emulsion is now observed. Finally 1 ml. of sodium sulfite–sodium hydrogen sulfite solution (95 ml. of 15% sodium hydrogen sulfite plus 5 ml. of 20% sodium sulfite) is added, and the mixture is shaken until the yellow complex and the emulsion have disappeared. The solution is allowed to stand for 5 to 6 minutes. Then the blue aqueous layer is transferred to a 25-ml. volumetric flask and diluted to the mark with water. The absorbance is measured at 720 mμ in a 1-cm. cell. A calibration curve is shown in Figure 4. The unknown sample and the blank are prepared in the same manner as the standards, except that no phosphate is added.

In Presence of Major Inhibitory Ions. If the major inhibitory ion in the sample is beyond the normal range shown in Table I, the concentration of sulfuric acid is increased 5 to 10 times (5 ml. of 0.2*N* sulfuric acid is replaced by 5 ml. of 1*N* or 2*N*), and the volume of molybdate is increased from 2 to 5 ml. In this case it is convenient to remove excess acid and molybdate from the alcoholic extract by washing once with 5 ml. of water and then adding 15 ml. of water and shaking until the emulsion forms. The procedure then follows that just described.

The blue complex can also be measured in the alcoholic phase by drawing off the blue aqueous layer before diluting to volume and adding approximately 8 ml. of *n*-butyl alcohol, followed by 5 ml. of 1*N* sulfuric acid. When the solution is shaken, the molybdenum blue complex is extracted quantitatively into the alcoholic phase. At this point there is no visible color observed in the aqueous phase and it can be discarded. The alcoholic phase is transferred to a 25-ml. volumetric flask. The funnel is washed twice with alcohol and the washings are also transferred. A perfectly clear solution is obtained. A calibration curve for this procedure is also shown in Figure 4.

Method II. In Absence of Either Major Inhibitory Ions or Labile Phosphorus. A standard solution of phosphorus is

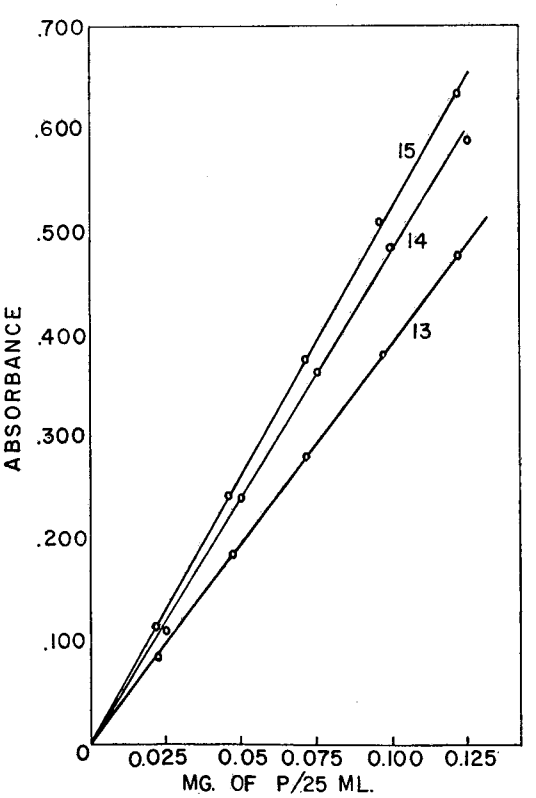

Figure 4. Calibration curves for Methods I and II

13. Method I, color developed in aqueous phase
14. Method II
15. Method I, color developed in alcoholic phase

Table I. Effect of Major Inhibitory Ions on Determination of Orthophosphate

(Each solution contains 10 ml. of 1*N* sulfuric acid, 2 ml. of 5% ammonium molybdate, and 125 γ of orthophosphorus per 25 ml.)

Inhibitor	Absorbance[a]	Ratio, Inhib. Anion to Orthophosphate
None	0.551	...
Sodium arsenate	0.553	2.8
	0.592	8.3
	0.663	10.1
Sodium fluoride	0.538	15.2
Ammonium oxalate	0.530	70.0
Sodium citrate	0.547	151
Sodium silicate	0.570	2.2

[a] Measured 20 to 30 minutes after final mixing.

Table II. Effect of Labile Phosphates on Determination of Orthophosphate

(Each solution contains 5 ml. of 0.2*N* sulfuric acid, 2 ml. of 5% ammonium molybdate, and 62 γ of orthophosphorus per 25 ml.)

Labile Phosphate	Absorbance	*T*[a]	*F*[b]	% Hydrolyzed or Impurity	Ratio, Labile to Ortho
None	0.286	...	...	...	...
Sodium β-glycerophosphate	0.322[c]	2100	23	1.1	34
Glucose-1-phosphate	0.360[c]	1150	16	1.4	18.5
	0.293	987	Negligible	..	16.0
Disodium phenyl phosphate	0.630[c]	2480	75	3.0	40
	0.525	3860	57	1.5	62
Glucose-6-phosphate	0.326[c]	774	9	1.1	12.5
	0.286	..	Negligible	..	..

[a] *T*, phosphorus concentration (γ/25 ml.) added in addition to orthophosphorus.
[b] *F*, orthophosphorus determined in excess of that initially added.
[c] Aqueous solutions remained at room temperature for several days before measurements were made.

pipetted into a 25-ml. volumetric flask and washed to the lower portion of the flask with about 10 ml. of distilled water. This is followed in succession by 5 ml. of 0.2*N* sulfuric acid, 2 ml. of 5% ammonium molybdate, and 1 ml. of 2% hydroquinone. The solution is mixed and allowed to stand at room temperature for 15 to 20 minutes. Then 1 ml. of the sodium sulfite–sodium hydrogen sulfite solution is added and mixed, and the solution is diluted to 25 ml. It is allowed to stand for 10 to 15 minutes before the absorbance is measured at 720 mμ. The unknown sample and the blank are prepared in the same manner as the standards, except that no phosphate is added. A calibration curve is given in Figure 4. The same amounts of phosphorus were used to prepare this curve as were used for the curves prepared by both modifications of Method I.

DISCUSSION

All data in the tables and points on the curves are the average of two runs with the exception of curves 5 and 6, Figure 1, which are from a single determination.

Table I shows that, when arsenate is present, the ratio of inhibitory anion to phosphorus can be raised to 3 before inhibition occurs; for the other anions considerably larger ratios may be tolerated (15 to 150). Higher ratios—oxalate = 1060, fluoride = 220, citrate = 2300—of anions to phosphorus have also been tested. No significant error was introduced by any of these except arsenate and silicate.

Data on the effect of labile phosphates on this determination are given in Table II.

The applicability of this method to the analysis of biological fluids is demonstrated by curves 16 and 17, Figure 5, which were obtained by diluting urine to the proper strength.

This extraction method presents several advantages. The molybdenum blue complex is developed in the aqueous phase after extraction of the molybdophosphoric acid. Any appreciable amount of molybdate or molybdic acid is removed, thereby eliminating the nonspecific blue color and the catalytic effect of molybdate on the labile phosphates. pH is increased to 4 (measured after full color development and dilution), which prevents the interference of acid-labile phosphate. This method also provides for removal of excess reductant, which is much more soluble in alcohol than in water. Thus, reduction due to the nonspecific types can further be prevented. Lastly, the advantages of the fundamental procedures are retained, such as validity of Beer's law, stability of color, sensitivity, accuracy, high pH, and elimination of the major inhibitory ions.

If only very small samples are available, the volume and the concentrations of different reagents, of course, can be proportionally reduced and the addition of distilled water, molybdate, and acid also combined as one step. A series of samples can be run almost simultaneously if a mechanical shaker is used. Hydroquinone solution was found to be much more stable in *n*-butyl alcohol than in aqueous solution.

Method II is essentially the same as that of Ging and Sturtevant (*7*) with a few modifications (change in molybdate added from 5 ml. to 2 ml., increase in hydroquinone concentration, and a change in the wave length from 810 to 720 mμ). The reasons for these changes are to permit measurement by both the Cary recording and the Beckman spectrophotometers and to enable samples to be run at pH 2. At this pH other published methods (*6, 11–13*) were found to be unsatisfactory, because a nonspecific blue color develops either immediately or fairly rapidly in the absence of orthophosphate.

The final acid concentration of Method II is 0.04 *N*, which is from 7 to 27 times lower than other authors have reported (*3–6, 8, 11, 12, 17–19*). At such low acidity (final pH after dilution is approximately 2), no significant hydrolysis of the labile phosphates is observed, but when the inhibitory ions, arsenate, oxalate, acetate, and fluoride, reach the concentrations given in Method I or, in fact, are even lower, satisfactory results cannot be obtained. The following concentrations of added ion cause only negligible change in color development by Method II.

	γ per 25 ml.
Sulfate as sodium sulfate	116,500
Nitrate as potassium nitrate	3,900
Ferric as ferric chloride	540
Citrate as sodium citrate	94,500

In the presence of major inhibitory ions Method II and all of the other published methods are found to be unsatisfactory

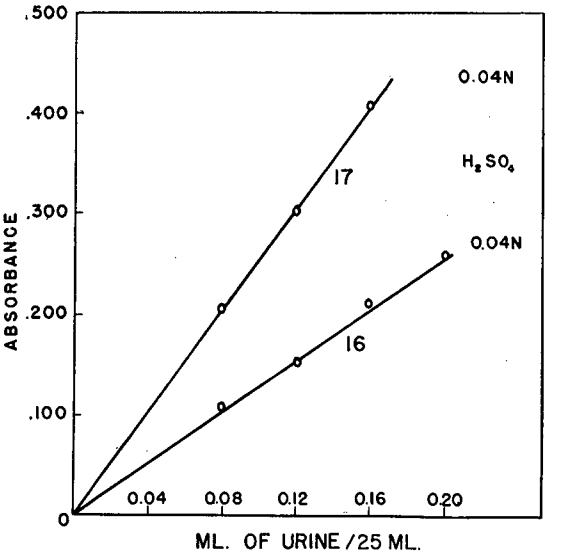

Figure 5. Dilutions of urine

when the blue complex is developed in the aqueous phase without prior extraction. This point has been thoroughly discussed by several authors (*4, 10, 18*).

If total phosphorus in the biological materials is desired, the organic matter is first destroyed with various oxidizing agents (*2, 9*). Then the described procedure is followed with proper strength of acid and molybdate reagent.

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RECEIVED for review November 5, 1955. Accepted March 19, 1956. Supported in part by an Institutional Grant from the American Cancer Society and in part by Grant No. CS 9051 from the National Cancer Institute National Institutes of Health, Public Health Service, Bethesda, Md.

Analysis of Mixtures of D-Glucose and D-Mannose by Paper Electrophoresis

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A simple method is proposed for analyzing mixtures of D-glucose and D-mannose in which there is a high ratio of glucose to mannose. The sugars are separated by paper electrophoresis and determined by the phenol-sulfuric acid method. The construction of a simple inexpensive apparatus for carrying out paper electrophoresis is described.

IN STUDIES on certain wood pulps and polysaccharides containing glucose and mannose, the problem of analyzing a mixture of these two sugars arises. Although a separation of glucose and mannose can be effected using paper partition chromatography there are in general two disadvantages which, depending upon the solvent system used, render this technique impractical for quantitative work. Either the difference in R_f values is not great enough or the R_f values are so small that an undesirable length of time is required for the separation of the sugars. This paper describes a simple apparatus (*1, 4*) and a relatively rapid method for the analysis of mixtures of D-glucose and D-mannose by paper electrophoresis.

EXPERIMENTAL

Whatman No. 1 filter paper (11 × 22.5 inches) was used in all experiments as the supporting medium.

Reagents. Sodium tetraborate (borax), 0.1*M* buffer solution, pH = 9.2.

p-Anisidine trichloroacetate. Dissolve 0.1 gram of recrystallized *p*-anisidine in 20 ml. of water containing 3.0 grams of trichloroacetic acid (*5*).

Methanolic hydrogen chloride. Prepare a 1% solution of anhydrous hydrogen chloride in anhydrous C.P. methanol.

Phenol. Saturate freshly distilled phenol with water. The solution contains 80% phenol (w./w.).

Sulfuric acid, concentrated C.P. reagent grade.

Paper Electrophoretic Apparatus. The apparatus is a simple sandwich type made from inexpensive materials (Figure 1). A filter paper (11 × 22.5 inches), enclosed between two sheets of thin polyvinyl plastic sheet to prevent evaporation and to isolate it from the other components, rests on a piece of foam rubber, $^1/_4$ × 12 × 18 inches. These components are placed between two sheets of plate glass, $^3/_8$ × 12 × 18 inches, which are held in a simple wooden clamp. The foam rubber sheet is employed to ensure an even pressure on the paper (Figure 2). The filter paper, extending from the ends of the sandwich, dips into wide borosilicate glass trays containing the buffer solution. These trays are isolated from the platinum wire electrodes by a buffer bridge. The potential was generated by a well-filtered direct current power supply having an output of 0 to 1500 volts at 0 to 300 ma.

Procedure. A known volume of a solution containing a mixture of D-glucose and D-mannose is put on the starting line, which is always equidistant from the end compartments containing the buffer. Only the middle 6 inches of the 11-inch wide

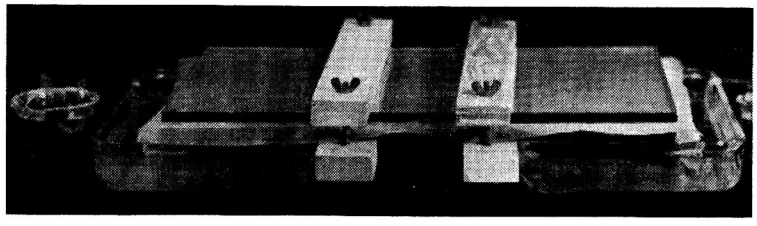

Figure 1. Apparatus for paper electrophoresis

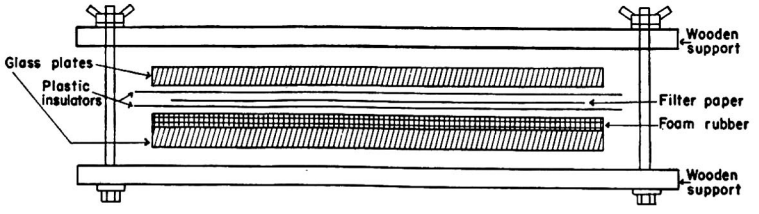

Figure 2. End view showing assembly of components for paper electrophoresis

paper are used for the analysis; identification spots of the sugar mixture are placed on the starting line in the margins, 1.5 inches from the outer edges of the filter paper. After the paper is allowed to dry in air, it is wetted by dipping one end in the buffer solution to within 0.5 inch of the starting line and then freed from the excess of the buffer by blotting between two sheets of Whatman No. 3 filter paper. The dipping and blotting procedure is then repeated with the other end of the paper. The paper is inserted between the two plastic sheets and placed on the foam rubber between the two glass plates; pressure is applied by the clamps. The ends of the paper are allowed to dip into the buffer in the end compartments and approximately 10 minutes is allowed for equilibration after the two liquid fronts have met at or near the starting line. The potential is applied for about 3.5 hours and no attempt is made to cool the system (*4*). The paper is removed and dried in air.

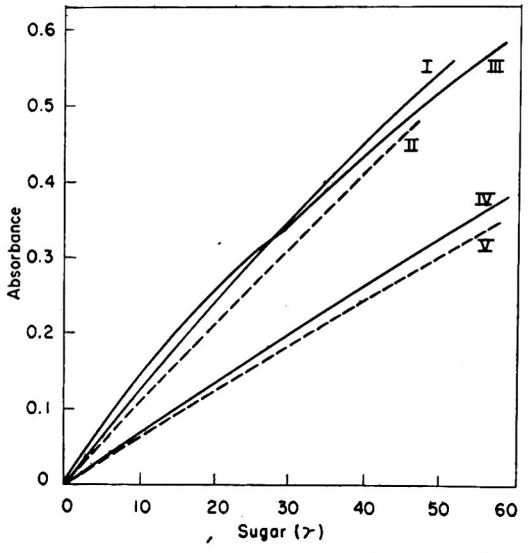

Figure 3. Standard curves for D-glucose and D-mannose at 490 mμ

I. D-Glucose
II. D-Mannose
III. D-Glucose and D-mannose after treatment with methanolic hydrogen chloride
IV. D-Glucose in presence of borate
V. D-Mannose in presence of borate

The marginal strips containing the zone identification spots are then cut from the chromatogram and sprayed with *p*-anisidine trichloroacetate. These strips are heated at 120° to 130° C. for 3 to 5 minutes to develop the yellow-brown color. Zones of equal area containing the separated components are cut from the paper and eluted with a suitable volume of water (*2, 3*). A section of the chromatogram of the same size as those containing the sugars is cut out and extracted with water in the same way to serve as the blank. These solutions are filtered through a glass wool plug to remove any cellulose fibers and an aliquot of each is evaporated to dryness in vacuo at room temperature. To the residue is added 10 ml. of methanolic hydrogen chloride, and the solution evaporated to dryness in vacuo at room temperature to remove borate. The residue is dissolved in water and the volume adjusted so that the amount of sugar in the solution is 10 to 50 γ per 2 ml. The solutions are again filtered through glass wool and 2-ml. aliquots are analyzed for sugar content by the phenol–sulfuric acid method (*2, 3*) as follows.

The 2-ml. aliquot is treated with 0.1 ml. of 80% phenol followed by 5 ml. of sulfuric acid, the latter being added rapidly from a pipet or glass syringe. After mixing and cooling at room temperature the absorbance is determined with a Coleman Junior spectrophotometer (*2, 3*). The amount of each component in the original mixture is then calculated from a standard curve.

The standard curve for each sugar is prepared by evaporating to dryness a solution containing a known amount of the sugar plus an amount of borate approximately equal to that on the sections of the chromatogram which contained the component sugars after separation. The residue is treated with methanolic hydrogen chloride as previously described and dissolved in a suitable volume of water. Aliquots are removed and diluted with water so that the concentration of sugar varies from 10 to 60 γ per 2 ml. A 2-ml. aliquot of the diluted sugar solution is then treated with the phenol reagent and sulfuric acid, and the color is determined spectrophotometrically as previously described. The standard curve for each sugar is obtained by plotting the absorbance *vs.* concentration. Both D-glucose and D-mannose gave the same standard curve (Figure 3).

RESULTS AND DISCUSSION

While the separation of D-glucose and D-mannose can be effected by paper partition chromatography in 50 to 70 hours using methyl ethyl ketone–water azeotrope (*8*) and in about 20 hours with pyridine–ethyl acetate–water (1 to 2.5 to 3.5) (*6, 7*), a better separation was obtained by the paper electrophoretic method in only 3 to 4 hours. In a typical experiment of 3 hours' duration at 600 volts, D-glucose moved a distance of 11 cm. while D-mannose moved 7.5 cm. On the other hand, paper partition chromatography and irrigation with methyl ethyl ketone–water azeotrope for 102 hours produced a chromatogram in which D-glucose traveled 9 cm. and D-mannose 14 cm. It was also found that the spots of the sugars diffused much less during electrophoretic separation than they did with the partition method. In paper electrophoresis it was also noted that D-glucose moved more rapidly than D-mannose, while the reverse was true with partition chromatography.

When potentials are such that heat is generated, cooling of the paper is usually recommended (*4*). When the apparatus proposed herein was used with an applied voltage of 600 volts, it was found to be advantageous not to cool the paper because cooling reduced the rate of migration of the sugars. In taking advantage of the heat generated by the current in this manner, it is essential that the components to be separated should be applied to the central uniformly heated zone and not at or near the outer edges of the glass plates, where there is a temperature gradient.

Table I. Analysis of Mixtures of D-Glucose and D-Mannose by Paper Electrophoresis

(Paper, Whatman No. 1; 0.1*M* borate buffer, pH 9.2; 600 volts)

Mixture	Sugar	Amount Put on Paper, Mg.	Ratio, Glucose : Mannose	Recovery Mg.	Recovery %
1	D-glucose	3.077	31 to 1	3.132	101.8
	D-mannose	0.099		0.100	100.5
2	D-glucose	3.234	31 to 1	3.217	99.5
	D-mannose	0.104		0.105	101.2
3	D-glucose	3.948	40 to 1	3.861	97.8
	D-mannose	0.098		0.096	98.3
4	D-glucose	0.293	2.6 to 1	0.291	99.3
	D-mannose	0.114		0.113	99.1

When other substances such as proteins are being separated and cooling is required, the upper glass plate is replaced by one of the same size to which is cemented, by an Epon polyamide resin, a borosilicate glass dish (9 × 14 inches) with inlet tubes through which water can be circulated. With this modified apparatus, the weight of the upper plate plus the dish full of water eliminates the necessity of the wooden pressure clamps.

When the separation of D-glucose from D-mannose by paper electrophoresis was examined quantitatively, it was found that only 70 to 75% of the sugars could be recovered. Further investigation showed that the borate eluted from the paper with the sugars interfered with the phenol–sulfuric acid method of color development. The recovery was roughly inversely proportional to the borate concentration and reached a constant value at approximately 70% when the borate concentration was sufficiently high. In an attempt to overcome this difficulty an excess of borate was added to the eluted sugars in order to produce a constant decrease in the recovery. This was found to be unsatisfactory, however, because the sensitivity of the method was

reduced to a point where it was useless for microanalytical work. The alternative procedure of removing the borate proved to be successful. This was accomplished by evaporating the sugar solutions containing borate to dryness and treating the dry residue with 1% methanolic hydrogen chloride. The effects of this procedure are shown in Figure 3 together with the effect of borate on the sensitivity of the method. After this treatment, the standard curves for D-glucose and D-mannose are identical.

The results of the method when applied to mixtures having high ratios of D-glucose to D-mannose are presented in Table I. Results are given (mixture 4) which show that the method can also be used for analyzing mixtures containing more nearly equal amounts of D-glucose and D-mannose. Although the method is particularly suited for the analysis of these two sugars, it is possible that other sugars could interfere (*1*). This difficulty might be overcome either by adjusting the pH of the buffer or by making a preliminary separation by partition chromatography.

This method can be adapted for the analysis of any carbohydrate compounds that can be separated by paper electrophoresis. The apparatus has also been found useful for the separation of sugar alcohols, methylated sugars, amino acids, and proteins.

ACKNOWLEDGMENT

The authors thank General Mills, Inc., Minneapolis, Minn., for the Epon polyamide resin; E. F. Garner thanks E. I. du Pont de Nemours and Co., Inc., for financial support.

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RECEIVED for review December 27, 1955. Accepted April 27, 1956. Division of Carbohydrate Chemistry, 128th meeting, ACS, Minneapolis, Minn., September 1955. This paper (No. 3486, Scientific Journal Series, Minnesota Agricultural Experiment Station) will form part of a thesis to be submitted by E. F. Garner to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy.

Densities, Refractive Indices, and Rotations of Mixtures of Active Amyl and Isoamyl Alcohols

Application to Analysis

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The nature of the dependence of density, refractive index, and observed rotation on composition for mixtures of active amyl and isoamyl alcohols is presented. To facilitate laboratory analyses of mixtures of these two alcohols, a plot of observed rotation *vs.* composition is given. The departures from linearity in the density-composition and the refractive index-composition functions support the conclusion that active amyl and isoamyl alcohol do not form ideal solutions on mixing. Deviations from ideality are largest for mixtures containing more than 70% active amyl alcohol.

GRAPE brandy fusel oils have been found to consist mainly of alcohols with boiling points higher than that of ethyl alcohol (*7*). Analyses by careful fractional distillations of rigorously dried samples have been generally successful in separating all of the components, with the exception of the two isomeric five-carbon alcohols, isoamyl (3-methyl-1-butanol) and active amyl (2-methyl-1-butanol) alcohols (*7*, *10*). Mixtures of these two alcohols, when distilled through fractionating columns with an efficiency of from 100 to 150 theoretical plates, give only small proportions of the pure substances; the bulk of the material is collected as break fractions containing both isomers. Difficulties in separating these two alcohols by distillation have been pointed out in many publications (*1-6*, *11*, *12*).

In these investigations active amyl alcohol of from 95 to 99% purity was obtained by repeated fractionations of the amyl alcohol cut from fusel oils on columns of high efficiency. Marckwald and McKenzie (*8*) isolated active amyl alcohol from fusel oil and purified it further through repeated recrystallizations of the 3-nitrophthalate ester, subsequent saponification, and isolation of the alcohol. The use of the measurement of optical activity as a means of analyzing mixtures containing isoamyl, and active amyl alcohols was also suggested. To check the variation of optical activity with composition, mixtures that contained active amyl and isoamyl alcohols in proportions of 1 to 3, 1 to 1, and 3 to 1 were prepared. From the analysis of these mixtures it was concluded that optical activity was directly proportional to the active amyl alcohol content of the mixture.

Hafslund and Lovell (*6*) isolated active amyl alcohol of 99.34 mole % purity [calculated on the basis that the physical constants given by Marckwald and McKenzie (*8*) are those of 100% pure active amyl alcohol] by repeated fractionation of fusel oil in an efficient column. A plot of density as a function of composition was presented, which was represented as a straight line. In addition, the rotations of eight mixtures of isoamyl and active amyl alcohol were measured, from which data it was calculated that the specific rotation of active amyl alcohol was a linear function of composition.

This paper reports further information concerning the rotations, the refractive indices, and the densities of isoamyl–active amyl alcohol mixtures.

REAGENTS AND EQUIPMENT

Synthetic isoamyl alcohol was dried and carefully fractionated through an efficient column to give material boiling constantly at 132.0° C. at 1 atm. pressure, n_D^{25} 1.4046, d^{25} 0.8056.

Active amyl alcohol was isolated from grape brandy fusel oil by repeated fractionations through a Podbielniak Heligrid column 48 inches long and 8 mm. in diameter to give constant boiling material with constant physical properties: α_D^{25} −9.49 ± 0.01° (2-dm. tube), n_D^{25} 1.4088, d^{25} 0.8079. The purified material gave a 3,5-dinitrobenzoate derivative, melting point 84–5° C. [literature value (*11*) 83–4° C.], which chromatographed as a single zone.

Mixtures for the experimental measurements were prepared by

weighing the proper amounts of each of the purified alcohols into weighing bottles on a standard analytical balance.

The optical rotation measurements were made on a Model 8 Rudolph polarimeter equipped with a sodium light source and apparatus to control the temperature at 25° C. and using 2-dm. sample tubes.

The refractive index measurements were made on a Spencer refractometer with the temperature controlled at 25° C. by means of circulating water from a thermostat.

Density measurements were made with a 2-ml. pycnometer suspended in a thermostat controlled at 25° C., which permitted the determination of densities with an accuracy within ±0.0002 gram per ml.

RESULTS AND DISCUSSION

The isoamyl and active amyl alcohol samples used in this investigation were of high purity, as evidenced by the observed physical constants and comparisons with values available in the literature. The observed values for the refractive index and the density of the isoamyl alcohol sample agreed well with values interpolated from the data of Timmermans and Hennaut-Roland (*9*) for this temperature, 1.4046 *vs.* 1.4047 and 0.8056 *vs.* 0.80551, respectively. A mass spectrometric analysis of the isoamyl alcohol indicated that the sample was free of any impurities with molecular weights greater than that of isoamyl alcohol, as no peaks for higher molecular weights were observed. The purity of the active amyl alcohol sample is best indicated by the fact that the observed optical rotation reached a constant maximum value which could not be increased by further fractional distillation and was comparable to the highest literature values (*8*).

The compositions of the mixtures of isoamyl and active amyl alcohols used for the experimental measurements, and the observed values of the rotations, densities, and refractive indices are presented in Table I.

Table I. Properties of Mixtures of Active and Isoamyl Alcohols

Active Amyl Alcohol, Wt. %	α_D Obsd., 25° C., 2-Dm. Tube	n_D^{25}	d^{25}
100.00	−9.49	1.4088	0.8150
90.04	−8.60	1.4080	0.8145
79.98	−7.70	1.4075	0.8139
70.14	−6.79	1.4073	0.8125
60.04	−5.87	1.4070	0.8115
50.04	−4.86	1.4066	0.8105
40.02	−3.95	1.4062	0.8096
30.44	−3.00	1.4058	0.8085
20.05	−1.97	1.4053	0.8073
10.16	−1.00	1.4050	0.8063
0.00	0.00	1.4046	0.8056

The observed rotation, refractive index, and density data are plotted as functions of the composition of the active amyl–isoamyl alcohol mixtures in Figure 1.

The conclusion of Marckwald and McKenzie (*8*), that optical activity is directly proportional to the amount of active amyl alcohol in mixtures of the two alcohols, is nearly substantiated by the data presented in Table I. Examination of the plot of observed rotation *vs.* composition in Figure 1 shows that the departure from a straight line is very small. Calculations from the data of Hafslund and Lovell (*6*) also give a linear plot of observed rotation *vs.* composition, as they must, because these workers assumed that both the specific rotation–composition function and the density-composition function were linear.

For the purpose of analysis of mixtures of active amyl and isoamyl alcohols it would seem desirable to use a plot of observed

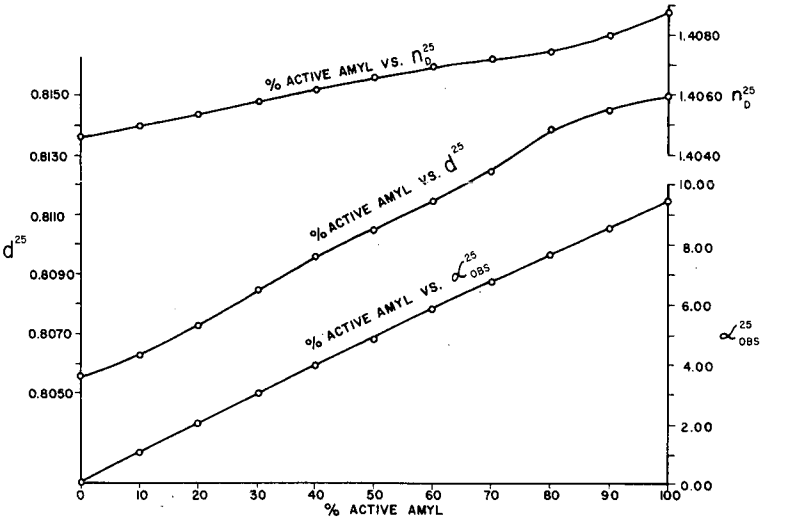

Figure 1. Relation of concentration of active amyl alcohol in isoamyl alcohol to refractive index, density, and observed optical rotation

rotation *vs.* composition rather than the specific rotation function which involves the two assumptions of linearity. Indeed, the data presented above indicate that neither the density-composition function nor the observed rotation–composition function is strictly linear.

Examination of the plot of density *vs.* composition in Figure 1 indicates definite deviations from linearity, particularly in the mixtures containing more than 60 mole % active amyl alcohol. These deviations are believed to be real, because of the number of measurements which were made on independently prepared mixtures and the fact that the over-all error in the density determinations is estimated to be not more than ±0.0002 gram per ml. Careful consideration of the density-composition data of Hafslund and Lovell (*6*) indicates a possible variation from linearity in the same region as indicated in Figure 1, although the number of points included in that region was not sufficient to permit the conclusion of anything other than a linear relationship. The plot of refractive index *vs.* composition in Figure 1 also shows departure from linearity in the mixtures containing more than 70 mole % active amyl alcohol. The data presented in this paper demonstrate the fact that mixtures of active amyl alcohol and isoamyl alcohol form solutions which definitely deviate from ideality, particularly in those solutions containing high mole percentages of active amyl alcohol.

ACKNOWLEDGMENT

The authors acknowledge with gratitude the gift of synthetic isoamyl alcohol from the laboratories of Sharples Chemicals, Inc.

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RECEIVED for review February 10, 1956. Accepted May 4, 1956.

Spectrophotometric Determination of Acetone by the Salicylaldehyde Method

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A spectrophotometric method for determining acetone in aqueous solutions by means of salicylaldehyde is described. The color conforms to Beer's law in the range of 0.00015 to 0.0028 mmole and after the initial 2 hours it is stable for several hours.

CSONKA (*3*) proposed the salicylaldehyde method for determining acetone. The method of color development, which has been revised by several authors, involves the reaction of acetone with a solution of salicylaldehyde in ethyl alcohol in alkaline solution at an elevated temperature. The results are not reproducible and the color is not stable.

The present author, when studying the determination of pyruvic acid by the salicylaldehyde method (*1*), observed that the use of pure salicylaldehyde instead of an ethyl alcohol solution of this reagent gave much better results. The technique has been applied to the determination of acetone in aqueous solutions. The results are in agreement with Beer's law and the color intensity after the initial 2 hours remains constant for several hours.

Braunstein (*2*) reported that all compounds containing a CH_3CO group linked directly to a hydrogen or carbon atom give a positive salicylaldehyde reaction. Thomson (*4, 5*) reported that the specificity range of the reaction is not as narrow as reported by Braunstein. According to Thomson, the mechanism of the salicylaldehyde color reaction involves condensation of the salicylaldehyde with a methylene group in the α-position to an unsaturated group such as carbonyl (CO). Among substances that interfere with the determination of acetone by the salicylaldehyde method are acetaldehyde, diacetyl, acetophenone, benzoylacetone, pyruvic acid, levulinic acid, and acetoacetic acid. According to experiments by the present author, bisulfite ions and formaldehyde in large amounts prevent to some extent the development of color.

APPARATUS, REAGENTS, AND SOLUTIONS

Beckman Model DU spectrophotometer.

Acetone, "pour analyse, No. 1001," Union Chimique Belge.

Salicylaldehyde, from bisulfite compound, No. 225, Eastman organic chemicals.

Sodium hydroxide solution, 425 grams per liter.

RECOMMENDED PROCEDURE

In a 50-ml. volumetric flask 2 ml. of sodium hydroxide solution is added to the aqueous sample containing 0.00015 to 0.0028 mmole of acetone. The solution is diluted with water to about 25 ml. and 0.6 ml. of salicylaldehyde is added from a microburet. The flask is shaken, 20 ml. of sodium hydroxide solution is added, and the volume is adjusted to 50 ml. with water. The flask is allowed to stand for at least 2 hours before the extinction is read against a reagent blank at 474 mμ. The amount of acetone in the sample is then evaluated from a calibration curve.

EXPERIMENTAL

The conditions that affect the development of color were investigated systematically, using about 0.00065 mmole of acetone. The effects of time, sodium hydroxide concentration, and salicylaldehyde concentration on the color development were investigated (Table I). To determine the influence of temperature on color development, the reaction mixture was treated for half an hour at various temperatures (Table II). As can be seen, the best color development is obtained at room temperature with 2 + 20 ml. of sodium hydroxide solution and 0.6 ml. of salicylaldehyde. More than 2 + 20 ml. of sodium hydroxide solution cannot be used because of decreasing solubility of salicylaldehyde with increasing sodium hydroxide concentration. The color is stable for several hours after the initial 2 hours.

Table I. Effect of Time and Reagent Concentration

Added, Ml.	Hours 0.5	1	2	3	5	24
	Absorbance					
Sodium hydroxide						
2 + 5	0.015	0.019	0.032	0.048	0.066	0.148
2 + 10	0.097	0.090	0.120	0.155	0.188	0.208
2 + 15	0.149	0.172	0.231	0.240	0.235	0.223
2 + 20	0.230	0.241	0.253	0.249	0.250	0.240
Salicylaldehyde						
0.4	0.197	0.243	0.239	0.247	0.236	...
0.5	0.225	0.253	0.260	0.257	0.261	...
0.6	0.267	0.273	0.272	0.274	0.272	...
0.7	0.253	0.261	0.263	0.262	0.261	...

Table II. Effect of Temperature

Temp., ° C.	Absorbance
20	0.271
50	0.249
75	0.228
100	0.212

The curves of extinction *vs.* wave length at five different concentrations of acetone (0.0002 to 0.0020 mmole) were determined. The curves had a maximum at 474 mμ, independent of the acetone concentration. As the reagent blank had a considerable light absorption at 474 mμ, the readings were made against a reagent blank.

Experiments with 0.0001 to 0.0050 mmole of acetone were carried out to get a calibration curve. Beer's law was found to be valid up to an absorbance of 1.0, allowing a maximum amount of 0.0028 mmole of acetone to be determined according to the calibration curve. The relative error in the range of 0.00015 to 0.0028 mmole was less than 2%.

ACKNOWLEDGMENT

The managing director of AB Pripp & Lyckholm has kindly given permission for this work to be published.

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RECEIVED for review November 30, 1955. Accepted April 27, 1956.

Determination of Cerium and Chromium in Cerium-Chromium-Uranium Mixtures

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A procedure is described for the determination of cerium and chromium in cerium-uranium-chromium alloys. Chromium is determined (on an aliquot of the sample) titrimetrically after it has been preferentially oxidized to chromium(VI) with fuming perchloric acid. Cerium is not oxidized by fuming perchloric acid under the conditions used. Cerium and chromium are determined on another aliquot of the sample by titrating with iron(II) after complete oxidation of cerium and chromium with peroxydisulfuric acid in the presence of silver(I). A reason is suggested for the low results often obtained for chromium following the perchloric acid oxidation, and conditions are described for the quantitative oxidation of chromium with perchloric acid.

CERIUM and chromium are commonly determined titrimetrically by first oxidizing them to cerium(IV) and chromium(VI), then titrating either directly or indirectly with a standard reducing agent. The oxidation methods usually employed, such as a sodium peroxide fusion or oxidation in acid solution with sodium bismuthate or ammonium peroxydisulfate in the presence of silver nitrate (*3*), result in the oxidation of both cerium and chromium. Perchloric acid has been used to oxidize chromium, but does not oxidize cerium unless mixed with sulfuric or phosphoric acid (*12*). It would seem possible then to use the peroxydisulfate-silver nitrate oxidation to determine the total amount of cerium and chromium, and the perchloric acid oxidation to determine the chromium alone. Cerium could be determined by difference.

The original Von Knorre (*15*) peroxydisulfate method for the oxidation of cerium was not completely satisfactory, but since the discovery of the catalytic effect of silver(I) by Barbieri (*1*), it has given excellent results (*19*). Chromium is also quantitatively oxidized by ammonium peroxydisulfate and silver(I) under the same conditions (*9*).

Willard (*16*) first reported the oxidation of chromium with perchloric acid, and Lichtin (*6*) and James (*5*) later recommended perchloric acid oxidation in the determination of chromium. Willard and Gibson (*17*) studied this oxidation, observing that manganese was not oxidized unless phosphoric acid was present. These workers reported that decomposition of the perchloric acid gave chlorine and oxygen

$$4\,HClO_4 \longrightarrow 2Cl_2 + 7O_2 + 2H_2O$$

rather than oxides of chlorine as reported by Lichtin (*6*), and that low results were obtained for chromium if the oxidized chromium solution stood for a while before the chromium was titrated. Lundell, Hoffman, and Bright (*7*) in a study of perchloric acid oxidation of chromium reported that results were invariably low for chromium, but with special precautions the amount of chromium oxidized could be increased. Smith (*12*) attributed the apparently incomplete oxidation of chromium to the simultaneous oxidizing and reducing properties of hot concentrated perchloric acid:

$$4HClO_4 \longrightarrow 2Cl_2 + 7O_2 + 2H_2O$$

$$2HClO_4 \longrightarrow Cl_2 + 3O_2 + H_2O_2$$

Smith (*12*) reported that the first reaction was by far the more important, but that the second occurred simultaneously and the hydrogen peroxide formed by the reaction reduced part of the chromium.

Hoffman and Lundell (*4*) reported that chromium is volatilized as chromyl chloride, even if no chloride is present in the solution initially, and that the loss of chromyl chloride is probably the chief cause of the low results found for chromium after a perchloric acid oxidation. Schuldiner and Clardy (*11*) and Ewing and Banks (*2*) published similar procedures; the chromium was oxidized in a flask equipped with a distillation head and any chromyl chloride volatilized was returned to the original solution before titration. They claimed quantitative recovery of chromium using this procedure. Roger (*10*) reported that, in experiments with the perchloric acid method, chromium was not lost as chromyl chloride and the results for chromium were not influenced by the formation of oxygenated compounds. Roger (*10*) claimed the method is rapid and gives reproducible results. Chemists of the U. S. Steel Corp. (*14*) described a procedure for the oxidation of chromium with perchloric acid, which they claimed would give reproducible results. They made no provision for the loss of chromyl chloride, but cooled the solution immediately after oxidation as recommended by Smith and added silver nitrate to precipitate any chloride in the solution. Recently Lynn and Mason (*8*) published a procedure for the oxidation of small amounts of chromium with a mixed perchloric acid–sulfuric acid–silver nitrate reagent.

In this investigation conditions were found with which small amounts of chromium could be quantitatively oxidized with hot concentrated perchloric acid in the presence of uranium and cerium. Cerium was not oxidized. The loss of chromium as chromyl chloride was negligible when the recommended procedure was followed. The low results often found for chromium after a perchloric acid oxidation may be due to reduction of chromium by the chloride ion.

REAGENTS

Ferroin Indicator, $Fe(C_{12}H_8N_2)_3SO_4$, 0.025*M*. The ferroin indicator solution was prepared by dissolving 3.714 grams of 1,10-phenanthroline 1-hydrate (G. Frederick Smith reagent grade) and 1.737 grams of reagent grade iron(II) sulfate 7-hydrate in water and diluting to 250 ml. A 0.001*M* solution was prepared by diluting a 10-ml. aliquot to 250 ml.

Iron(II) Sulfate, 0.10*N*. An iron(II) sulfate solution was prepared by dissolving 39.5 grams of reagent grade iron(II) ammonium sulfate 7-hydrate and 10 ml. of concentrated reagent grade sulfuric acid in water and diluting to 1 liter. The solution was standardized each day against standard ceric sulfate.

Potassium Dichromate Solution. A solution was prepared by dissolving 18.9132 grams of primary standard grade potassium dichromate (National Bureau of Standards, sample 136) in a small amount of nitric acid, transferring to a tared 2-liter volumetric flask, and diluting to approximately 2 liters. The weight of the solution was 2028.29 grams and it contained 3.2977 mg. of chromium per gram of solution.

Cerium(IV) Sulfate Solution, 0.1*N*. A stock solution of cerium(IV) sulfate was prepared by dissolving 548.26 grams of reagent grade ammonium hexanitratocerate(IV) in 560 ml. of concentrated sulfuric acid and slowly diluting with vigorous stirring to 10 liters. The solution was standardized against electrolytic iron wire and arsenious oxide (Mallinckrodt primary standard) and found to be 0.1090*N*.

Cerium(IV) Nitrate Solution. A solution was prepared by dissolving 37.834 grams of reagent grade ammonium hexanitrato-

Table I. Oxidation of Chromium with Perchloric Acid

Procedure Used	Cr Added, Mg.	Cr Found, Mg.	Error, Mg.
Willard and Gibson (*17*)	8.115	7.56	−0.56
	8.260	7.23	−1.03
Ewing and Banks (*2*)	7.670	7.43	−0.24
	8.600	8.19	−0.41
Proposed procedure	8.280	8.29	+0.01
	16.96	16.94	−0.02
	38.66	38.64	−0.02
	30.76	30.80	+0.04

Table II. Effect of Cerium and Uranium on Perchloric Acid Oxidation of Chromium

U Added, Mg.	Ce Added, Mg.	Cr Added, Mg.	Cr Found, Mg.	Error, Mg.
94.0	5.0	1.50	1.49	−0.01
5.0	94.0	1.64	1.64	0.00
94.0	1.0	4.32	4.31	−0.01
0.0	0.0	8.28	8.29	0.01
5.0	80.0	12.23	12.27	0.04
0.0	0.0	16.96	16.94	−0.02
0.0	0.0	38.66	38.64	−0.02
94.0	40.0	83.07	82.97	−0.10

cerate(IV) (G. Frederick Smith Chemical Co.) to 2 liters with 1*M* nitric acid. This solution was compared with the standard cerium(IV) sulfate solution and found to contain 4.257 mg. of cerium per ml.

Perchloric Acid, reagent grade, 70 to 72% $HClO_4$.

Uranium(VI) Nitrate Solution, $UO_2(NO_3)_2$. A stock solution was prepared by dissolving 10.0069 grams of primary standard grade uranium oxide, U_3O_8, in concentrated nitric acid and diluting to approximately 2 liters in a tared flask. The weight of the solution was found to be 2000.38 grams. The uranium solution was compared with the standard cerium(IV) sulfate and was found to contain 4.242 mg. of uranium per gram of solution. This agrees well with the calculated value of 4.242 mg. of uranium per gram of solution.

Sodium bicarbonate, reagent grade.

Sulfuric acid, reagent grade, specific gravity 1.84, 98% H_2SO_4.

Nitric acid, reagent grade, specific gravity 1.42, 70% HNO_3.

EXPERIMENTAL

Samples of the standard chromium solution were weighed into 250-ml. Erlenmeyer flasks using weight burets. The chromium(VI) was reduced by adding 1 to 2 drops of 30% hydrogen peroxide. The excess peroxide was destroyed by boiling a few minutes. The chromium samples were oxidized using the procedures recommended by Willard and Gibson (*17*), Ewing and Banks (*2*), and the proposed procedure. In all cases the chromium(VI) present after oxidation was determined by adding a known excess of iron(II) sulfate and back-titrating with standard cerium(IV) sulfate to the ferroin end point.

The results obtained are tabulated in Table I.

The effects of cerium and uranium on the perchloric acid oxidation of chromium were studied by adding known amounts of standard cerium and uranium solutions to the chromium samples before oxidation. The cerium was added as cerium(IV), but was reduced to a cerium(III) by the hydrogen peroxide used to reduce the chromium(VI).

The results obtained are given in Table II.

The total cerium and chromium in the presence of uranium was found by oxidizing cerium and chromium to cerium(IV) and chromium(VI) with potassium peroxydisulfate and silver. This procedure has been used in this laboratory for some time for the determination of cerium and chromium in uranium with excellent results. The amount of cerium was calculated for two known samples by correcting the experimentally determined total for the amount of chromium added (Table III).

DETERMINATION OF CHROMIUM

A sample of the uranium-chromium-cerium alloy is weighed into a 400-ml. beaker and dissolved with dilute hydrochloric or nitric acid. When dissolution is complete, the solution is transferred to a volumetric flask and diluted to volume. An aliquot containing 2 to 50 mg. of chromium is taken for analysis. If the sample was dissolved in hydrochloric acid, the sample is taken down several times with nitric to remove the hydrochloric acid. This may be accomplished more easily if the solution is contained in a beaker. For the oxidation the solution is transferred to a 500-ml. Erlenmeyer flask, 10 ml. of 72% perchloric acid are added, and the solution is placed on the hot plate and heated until the perchloric acid begins to fume. A refluxing still head is placed on the neck of the Erlenmeyer flask and the solution is fumed 3 to 5 minutes after the conversion of chromium(III), green, to chromium(VI), red, is complete.

The solution is removed from the hot plate, immediately placed under a hot water tap to start the cooling, and cooled under the cold water tap. [If Vycor glassware is used, the hot flasks may be immediately plunged into an ice-water mixture for even more rapid cooling (*13*).] The cool sample is diluted as quickly as possible to 100 to 150 ml. and 2 to 3 grams of sodium bicarbonate are added. The solution is again placed on the hot plate and boiled for 10 to 15 minutes until moist starch-iodide paper held over the flask no longer gives a chlorine test. The samples are cooled and 5 ml. of concentrated sulfuric acid are added. The chromium(VI) is determined by adding a known excess of standard iron(II) sulfate and 1 ml. of 0.001*M* ferroin indicator. The excess iron(II) sulfate is back-titrated with standard cerium (IV) sulfate.

DETERMINATION OF CERIUM

An aliquot of the uranium-chromium-cerium sample is pipetted into a 600-ml. beaker and 5 to 10 ml. of concentrated sulfuric acid are added. The solution is evaporated until fumes of sulfur trioxide are evolved to remove all of the hydrochloric acid. The solution is cooled and diluted to 250 ml. with water. About 0.05 gram of silver nitrate and 2 grams of potassium peroxydisulfate are added. The solution is boiled vigorously for about 20 minutes and the solution is removed from the hot plate and cooled. A known excess of standard iron(II) sulfate and 1 ml. of 0.001*M* ferroin indicator are added. The excess iron(II) sulfate is back-titrated with standard cerium(IV) sulfate. The amount of iron(II) sulfate used is corrected for the amount required to titrate the chromium. The amount of chromium present is found by the perchloric acid oxidation procedure. The cerium is then calculated by difference.

DISCUSSION

The results obtained by the recommended procedure indicate that chromium can be quantitatively oxidized by perchloric acid. The necessary conditions, as found by the authors and reported by others (*12, 14, 18*), seem to be the rapid cooling of the sample after oxidation, and the immediate dilution of the sample when cooled sufficiently.

Table III. Determination of Cerium by Difference after Peroxydisulfate-Silver Oxidation of Cerium-Chromium Total

Cr Added, Mg.	U Added, Mg.	Ce Added, Mg.	Ce Found, Mg.	Error, Mg.
5.02	5.0	239.9	239.9	0.0
17.22	94.0	195.4	195.1	0.3

The authors believe the low results often obtained for chromium following a perchloric acid oxidation are caused by reduction of chromium(VI) by chloride ion in the concentrated perchloric acid medium. It can be readily demonstrated that chloride ion will reduce chromium(VI) in concentrated perchloric acid. Furthermore, the presence of the chloride ion in chromium samples after a perchloric acid oxidation has been observed by the authors and others (*8, 14*).

The hydrogen peroxide mechanism postulated by Smith (*12*) does not seem to explain the slow reduction of chromium(VI) in concentrated perchloric acid medium after the sample is cooled. Hydrogen peroxide should not form in the cool sample, and, if any formed during the oxidation of the chromium, it would be expected to react with the chromium(VI) immediately.

In the procedure recommended, the sample is immediately cooled and diluted after oxidation. Sodium bicarbonate is added, which reduces the acidity and helps sweep any chlorine out of the solution by liberation of large quantities of carbon dioxide. This tends to reduce the amount of chloride formed

through disproportionation of chlorine and, by reducing the hydrogen ion concentration, prevents oxidation of any chloride already formed by shifting the chromium(VI) to chromium(III) reduction potential to a more negative value than the chlorine to chloride ion reduction potential.

APPLICATIONS

The procedure described was found to be generally useful for the determination of chromium and cerium in uranium-chromium-cerium alloys, giving equally good results for both uranium-rich and cerium-rich alloys. As cerium is determined by difference, it is desirable that the cerium-chromium ratio be large.

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RECEIVED for review March 7, 1956. Accepted May 17, 1956. Contribution 447. Work performed in the Ames Laboratory of the U. S. Atomic Energy Commission.

Determination of Traces of Gallium and Indium in Germanium and Germanium Dioxide

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In the photometric determination of 1 to 10 p.p.m. of gallium or indium in germanium and germanium dioxide germanium is removed by distillation as chloride. Gallium in the residue is isolated by ether extraction from 6*N* hydrochloric acid, followed by oxine-chloroform extraction from alkaline cyanide solution, and is determined by photometric measurement of the yellow oxine-chloroform extract. Indium in the residue from the germanium distillation is isolated by dithizone-chloroform extraction from alkaline cyanide solution, followed by oxine-chloroform extraction from aqueous solution at pH 3.5, and is determined by photometric measurement of the yellow oxine-chloroform extract.

THE present investigation represents a continuation of the authors' work on the development of photometric methods of determining traces of Group III and Group V metals in semiconductor materials (*3*, *4*). The methods described provide for the determination of 1 to 10 p.p.m. of gallium and indium in germanium and germanium dioxide. Suitable separations are included for the elimination of possible interference from any of 59 commonly encountered metals. The apparatus recommended previously (*4*) was used in the present investigation, except that a Beckman Model B spectrophotometer was used.

DETERMINATION OF GALLIUM

Moeller and Cohen (*5*) have shown that small amounts of gallium can be determined by extraction with a chloroform solution of oxine (8-quinolinol), at pH 3.5, followed by photometric measurement of the yellow extract. Because this method is by no means specific for gallium, a preliminary isolation of the gallium by ether extraction will be required in most work. Of the metals that accompany gallium into the ether layer, only molybdenum, thallium, and iron interfere in the oxine extraction at pH 3.5. The interference due to molybdenum can be prevented by adding a small amount of lead nitrate to the solution just before the oxine extraction; the precipitated lead molybdate remains in the aqueous phase. Interference due to thallium can be eliminated by reducing this metal to the monovalent state. On the other hand, no method of preventing interference of iron was found. For this reason the oxine extraction at pH 3.5 was abandoned in favor of extraction from alkaline cyanide solution (*2*). None of the metals that accompany the gallium in the ether extraction will accompany it in an oxine extraction from alkaline cyanide solution. Thus by using these two extractions in proper sequence the gallium can be readily isolated and determined.

Reagents. DISTILLED WATER. In the development of the methods for gallium and indium ordinary distilled water was passed through a mixed cation-anion exchange resin mixture before use.

STANDARD GALLIUM SOLUTION (5 γ of gallium per ml.). Transfer 0.0134 gram of gallium oxide, Ga_2O_3, to a 125-ml. Vycor conical flask. Add 5 ml. of hydrochloric acid, cover, and warm gently to dissolve. Cool, transfer to a 200-ml. volumetric flask, dilute to the mark with cool hydrochloric acid (1 + 1), and mix. Pipet 50.0 ml. of this solution to a 500-ml. volumetric flask, dilute to the mark with cool hydrochloric acid (1 + 1), and mix.

ACID-WASHED ETHER. Transfer 200 ml. of anhydrous reagent grade ether to a 500-ml. separatory funnel. Add 30 ml. of hydrochloric acid (1 + 1) in which about 50 mg. of sodium sulfite has been dissolved. Shake gently, opening the stopcock frequently, until excess pressure ceases to build up. Then shake vigorously for 30 seconds. Drain off and discard the acid layer. Repeat the washing with two more 30-ml. portions of hydrochloric acid (1 + 1) plus sodium sulfite.

m-CRESOL PURPLE INDICATOR SOLUTION (0.1%). Dissolve 0.1 gram of *m*-cresol purple in 10 ml. of water containing 1 pellet of sodium hydroxide, by warming gently. Cool and dilute to 100 ml. with water.

SODIUM CYANIDE SOLUTION. Transfer 20 grams of sodium cyanide to a 500-ml. polyethylene bottle, add 200 ml. of water, and swirl to dissolve. Keep stoppered when not in use.

OXINE SOLUTION. Transfer 2.000 grams of 8-quinolinol to a clean dry 200-ml. volumetric flask. Dilute to the mark with chloroform and mix. Prepare fresh just before use.

Preparation of Calibration Curve. Transfer 0-, 1.0-, 3.0-, 5.0-, and 7.0-ml. portions of standard gallium solution (5 γ of gallium

per ml.) to 125-ml. Squibb-type separatory funnels. Add sufficient hydrochloric acid (1 + 1) to bring the volume in each funnel to 25 ml. Working individually, add 25 ml. of acid-washed ether to the funnel. Stopper and shake gently, releasing pressure from time to time by means of the stopcock. After excess pressure ceases to build up, shake vigorously for 30 seconds. Drain off and discard the lower layer. Add 2 ml. of hydrochloric acid (1 + 1) and shake 10 seconds. Drain off and discard the lower layer. Repeat this wash-extraction once more with 2 ml. of hydrochloric acid (1 + 1). Add 15 ml. of water. Stopper and shake gently, releasing the pressure from time to time by means of the stopcock. After pressure ceases to build up, shake vigorously for 30 seconds. Run the lower layer into a 125-ml. Vycor conical flask. Repeat the extraction with a 10-ml. portion of water and drain the aqueous extract to the flask. Place the flask on a low temperature hot plate and heat for a few minutes until the solution no longer smells of ether. Cool. Transfer the solution with a minimum of washing to a 125-ml. Squibb-type separatory funnel.

Add one drop of *m*-cresol purple indicator solution and add ammonium hydroxide dropwise until the indicator just turns yellow. Immediately add 5 ml. of sodium cyanide solution and swirl to mix. Add 20.0 ml. of oxine solution, stopper, shake for a few seconds, release the pressure, and shake vigorously for 30 seconds. Allow to settle and then drain all but about 2 ml. of the chloroform layer through a dry 5-cm. Whatman No. 41 filter paper in a small dry funnel to a dry 50-ml. conical flask. Allow all but about 1 ml. of the solution to drain through the paper to the flask. Without delay swirl the solution in the 50-ml. conical flask to mix well, transfer to a 5-cm. absorption cell, and measure photometrically at 400 mμ, using chloroform as the reference solution. Prepare a calibration curve.

Procedure. Dissolve and distill the sample of germanium or germanium dioxide as directed in the method for phosphorus (*4*), but omit the distillation with hydrobromic acid. Carry a reagent blank through all the steps of the procedure. When the germanium has been completely removed, evaporate to dryness on a flame to expel all perchloric acid. Cool, add 25 ml. of hydrochloric acid (1 + 1), heat almost to boiling, cool, and proceed to the extractions and photometric determination as in preparation of the calibration curve for gallium. With the aid of the calibration curve, determine the weight of gallium present in the sample and in the reagent blank.

Discussion. Maximum recovery of gallium in the ether extraction is obtained when the hydrochloric acid concentration is 6 to 6.5*N*. Perchloric acid tends to cause lower recoveries and should therefore be expelled before the extraction. Even with optimum conditions, only about 95% of the gallium is transferred to the ether layer with a single extraction. Additional extractions do now, however, improve the recovery. Compensation for this loss of gallium is made by including the ether extraction in the procedure for the preparation of the calibration curve.

Because most of the metals that are present in trace amounts in reagent grade chloroform will be transferred to the aqueous layer in an oxine extraction from alkaline cyanide solution, there is usually no need to use redistilled chloroform in the preparation of the oxine-chloroform solution.

DETERMINATION OF INDIUM

Indium can be determined photometrically by the dithizone-chloroform or the oxine-chloroform extraction method (*6*). Neither is specific for indium, but by separating the indium first by dithizone extraction and then determining it by the oxine extraction method the desired specificity is obtained. Dithizone extraction from alkaline cyanide solution isolates the indium from all other elements except bismuth, divalent tin, lead, and monovalent or trivalent thallium. Bismuth can be removed by a dithizone extraction from acid solution previous to the extraction from alkaline cyanide solution (*1*). The extraction of tin from alkaline cyanide solution with dithizone can be eliminated by converting it to the quadrivalent state. Lead will not interfere in the indium determination, as it is not extracted by oxine at pH 3.5. The same is true of thallium, provided it is obtained in the monovalent state before the oxine extraction.

Reagents. Standard Indium Solution (5 γ of indium per ml.). Dissolve 0.1000 gram of indium metal in 5 ml. of nitric acid by warming. Boil to expel brown fumes. Cool, transfer to a 1-liter volumetric flask, dilute to the mark, and mix. Transfer 50.0 ml. of this solution to a 1-liter volumetric flask, dilute to the mark, and mix.

Buffer Solution (pH 3.5). Transfer successively 40 ml. of nitric acid, plus 40 ml. of ammonium hydroxide, plus 4 grams of potassium acid phthalate to 2 liters of water in a 4-liter borosilicate glass-stoppered bottle. Swirl to dissolve the phthalate. Cool to room temperature and then dilute to 4 liters with water. Neutralize carefully with ammonium hydroxide until the pH of the solution is 3.5 as measured on a pH meter. Discard the small portions of the buffer solution used in the pH measurements.

Redistilled Chloroform Solution. Transfer a few grains of 20- to 30-mesh silicon carbide plus about 0.1 gram of calcium oxide plus 800 ml. of reagent grade chloroform to a 24/40 standard-taper 1-liter borosilicate glass round-bottomed flask. Connect to a 24/40 standard-taper water-cooled borosilicate glass condenser. Heat the flask on a water bath until the chloroform boils gently. Discard the first 25-ml. portion of the distillate and then catch the next 700 ml. in a dark bottle to which has been added 7 ml. of ethyl alcohol to act as a preservative. When the distillation is complete, swirl to mix the distillate. Use this redistilled chloroform to prepare the oxine solution or wherever washing with chloroform is called for in the procedure for indium recorded below.

Oxine Solution. Transfer 1.000 gram of 8-quinolinol to a dry 200-ml. volumetric flask, dilute to the mark with redistilled chloroform, and mix. Prepare fresh just before use.

Ammonium Citrate Solution. Dissolve 10 grams of ammonium citrate in 100 ml. of water.

Dithizone Solution (0.1%). Transfer 0.5 gram of diphenylthiocarbazone—i.e., dithizone—to a dry 500-ml. volumetric flask and dilute to the mark with chloroform. Store in a refrigerator when not in use.

Dithizone Solution (0.01%). Transfer 50 ml. of 0.1% dithizone solution to a dry 500-ml. volumetric flask and dilute to the mark with chloroform. Store in a refrigerator when not in use.

Ammonium Hydroxide Wash Solution. Add 1 drop of ammonium hydroxide to 500 ml. of water and mix. Prepare fresh each day as required.

Sodium Metabisulfite Solution. Dissolve 1 gram of sodium metabisulfite, $Na_2S_2O_5$, in 100 ml. of water. Prepare fresh every 3 days as required.

Preparation of Calibration Curve. Transfer 0-, 2.0-, 4.0-, 6.0-, 8.0-, and 10.0-ml. of standard indium solution (5 γ of indium per ml.) to 125-ml. conical flasks and add 2 ml. of perchloric acid (1 + 9) to each. Dilute to 20 ml., and add 1 drop of *m*-cresol purple indicator solution plus a small piece of Congo red paper. Neutralize carefully by dropwise addition of ammonium hydroxide until the solution just starts to turn yellow. Continue the careful neutralization with ammonium hydroxide (1 + 9) until the edges of the Congo red paper just start to turn red. Pour the solution into a 125-ml. Squibb-type separatory funnel. Wash down the walls of the flask with 25 ml. of pH 3.5 buffer solution and pour into the funnel. Add 20.0 ml. of oxine solution and proceed to the extraction and photometric determination of the indium as in preparation of the calibration curve for gallium.

Procedure. Dissolve and distill the germanium as directed for gallium. When the germanium has been completely removed, expel all but about 0.2 ml. of the perchloric acid by heating on a flame. Cool, wash down the sides of the flask with 10 ml. of water, and pour into a 125-ml. Squibb-type separatory funnel. Repeat the wash-transfer with two additional 5-ml. portions of water. Add 2 ml. of ammonium citrate solution and 1 drop of *m*-cresol purple indicator solution. Neutralize by dropwise addition of ammonium hydroxide until the solution just turns yellow. Add 5 ml. of perchloric acid (1 + 9). Add 10 ml. of dithizone solution (0.1%), stopper and shake a few seconds, open the stopcock momentarily to relieve the pressure, shake vigorously for 1 minute, and then allow the two layers to separate. Drain off and discard the chloroform layer. Rinse down the inside walls of the funnel with about 2 ml. of chloroform from a wash bottle and again drain off and discard the chloroform layer. Repeat the extraction with a second 10-ml. portion of dithizone solution (0.1%) to ensure complete removal of bismuth. Rinse down the inside walls with 2-ml. of chloroform, drain off, and discard. Add 5 ml. of chloroform, shake for 15 seconds, allow to settle, and drain off and discard the chloroform layer. If the aqueous solution is very pink as a result of the presence of chromium, add 1 or 2 drops of *m*-cresol purple indicator solution in order to make a more pronounced color change in the subsequent neutralization.

Neutralize by dropwise addition of ammonium hydroxide until

the solution turns yellow and then, as the neutralization is continued, just purple. Add 5 ml. of 10% sodium cyanide solution. Add 10 ml. of 0.01% dithizone solution, shake momentarily, relieve the pressure, shake vigorously for 1 minute, and then allow the layers to separate. Drain off the lower layer to a second 125-ml. Squibb-type separatory funnel. Wash down the inside walls of the funnel with about 2 ml. of chloroform from a wash bottle and again drain the lower layer to the second 125-ml. funnel. Repeat this extraction and chloroform wash twice more with 10-ml. portions of dithizone solution (0.01%), catching the chloroform extracts and washes in the same 125-ml. funnel. After the last chloroform wash, discard the aqueous solution.

Add 50 ml. of ammonium hydroxide wash solution, shake vigorously for 15 seconds, and allow the layers to separate. Drain the lower layer to a 125-ml. Vycor conical flask. Wash down the inside walls of the funnel with about 2 ml. of chloroform and drain to the Vycor flask. Discard the aqueous solution. Add 0.5 ml. of nitric acid to the chloroform solution and place on an asbestos pad on a low-temperature hot plate. Boil gently until the chloroform is completely removed. Add 1 ml. of perchloric acid and move to a somewhat warmer portion of the plate. Allow the solution to evaporate until copious fumes of perchloric acid begin to appear. Do not rush this evaporation; otherwise the oxidation of the organic matter may not be complete and high values for indium will be obtained in the oxine extraction. Finally, when the oxidation is complete, swirl over an open flame to expel all but about 0.2 ml. of the acid. Cool and then wash down the inside walls of the flask with 20 ml. of water. The solution should be colorless. Add 1 ml. of sodium metabisulfite solution plus a small piece of Congo red paper plus 1 drop of *m*-cresol purple indicator solution and proceed to the neutralization, extraction, and photometric determination as in preparation of the calibration curve for indium. With the aid of the calibration curve determine the weight of indium present in the sample and in the reagent blank.

Discussion. Specificity tests of the dithizone-oxine method show that of the 59 commonly encountered metals only rhodium and iridium interfere. Extremely low results for indium are obtained when 0.1-mg. portions of either of these metals are present. Apparently they react in some way to prevent the extraction of the indium by the dithizone. Tests indicate that beryllium, if present in amounts greater than about 0.1 mg., will cause slightly low results for indium. This is probably due to loss by coprecipitation as a result of hydrolysis of the beryllium (*4*).

Attempts to simplify the method by removing the indium from the dithizone-chloroform solution by extraction into nitric acid (1 + 99), instead of using the evaporation-oxidation technique, were abandoned when it was found that an appreciable amount of some colored organic material was accompanying the indium into the nitric acid solution and subsequently into the oxine-chloroform layer.

Table I. Determination of Gallium in Synthetic Mixtures

No.	Metals Added	Gallium Found, γ
1	None	24
2	Mg, B, Al, Th, Se, Ag	25
3	Ti, Zr, Hf, Co, Ni, Zn, As(V), Bi	26
4	Be, Re, Fe(III), Pt, Pb, P, Te	25
5	Sc, Nb, Os, In, U(VI), Cu, Ge	25
6	Y, Ta, Ir, Au, Tl(III), Sb(V)	25
7	La, Mo(VI), Mn, Rh, Cd, Sn(IV)	25
8	Li, K, Na, Rb, Cs, Cr(III), Ru, Pd, Si, Hg(II)	25
9	Ba, Ca, Sr, Pr, Sm, Ce, V(V), W, Al	25

Attempts were made to eliminate the use of perchloric acid in the method, so that potassium cyanide could be used in place of sodium cyanide. It was found, however, that when the solution was acidified with 5 ml. of hydrochloric acid (1 + 9) instead of 5 ml. of perchloric acid (1 + 9) previous to the first dithizone extraction the removal of bismuth was not complete.

CONFIRMATORY TESTS

Synthetic sample mixtures were prepared by evaporating 25 γ of gallium or 30 γ of indium plus 100 γ of each of one or more other metals, all in the form of aliquots of standard solutions, plus 2 ml. of perchloric acid, to dryness or to 0.2-ml. volume on a flame. Gallium or indium was then determined by the appropriate procedure, beginning with the ether extraction or the dithizone extraction from acid solution. The results obtained are shown in Tables I and II.

Table II. Determination of Indium in Synthetic Mixtures

No.	Metals Added	Indium Found, γ
1	Cu, Zn, Cd	29
2	Bi, Fe(III), Co	30
3	Tl(III), Mn, Sn(IV), Pd	29
4	Pt, Pb, Au, Ni	29
5	Hg(II), Be, Al, Zr	30
6	Ag, B, Ce, Te(IV)	30
7	Sb(III), U(VI), Sm, V(V), Mg, Hf	31
8	Cr(III), Na, K, Li, Rb, Cs, Sc, Y	28
9	Se(IV), As(III), Nd, Th	29
10	Mo(VI), Ga, Re	28
11	Ru, Si, P	30
12	Os, Ge, Nb, Ti(IV)	29
13	W, La, Pr, Pb, Pd	29
14	Ta, Ca, Ba, Sr, Ru, Au	30
15	Bi, Tl(III)	30
16	Rh, W, La, Pr	8
17	Rh	2
18	Ir, Ta, Cu, Ba, Sr	16
19	Ir	12

Aliquot portions of standard gallium solution or standard indium solution plus 0.5 ml. of perchloric acid were evaporated to 0.2-ml. volume. Two-gram samples of very pure powdered germanium or 3-gram samples of very pure germanium dioxide were added and gallium or indium was determined by the appropriate procedure. The results obtained are shown in Table III.

Table III. Determination of Gallium or Indium in Germanium and Germanium Dioxide

No.	Sample	Gallium Added, γ	Gallium Found, γ
1	Ge	10	10
2	Ge	25	26
3	GeO_2	10	11
4	GeO_2	25	26
		Indium Added, γ	**Indium Found, γ**
5	Ge	10	10
6	Ge	30	29
7	Ge	50	49
8	GeO_2	10	10
9	GeO_2	30	30

ACKNOWLEDGMENT

The authors are indebted to Clarence Zischkau and W. G. Wagner, American Smelting and Refining Co., South Plainfield, N. J., for suggesting the method used for the indium determination and for helpful advice during the development of the method.

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RECEIVED for review January 14, 1956. Accepted April 28, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 1956.

Polarographic Determination of Elemental Sulfur in Liquefied Petroleum Gases

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A method has been developed for the polarographic determination of elemental sulfur present in liquefied petroleum gases in concentrations as low as 0.01 p.p.m. Samples of liquefied petroleum gases are collected in specially fitted stainless steel bombs. After volatilization of the sample, elemental sulfur remaining in the sample bomb is dissolved in a pyridine-methanol-hydrochloric acid solvent and the resulting solution electrolyzed polarographically.

ELEMENTAL sulfur in petroleum products presents an important problem in the industry because of the ease with which it is formed and its corrosive nature. In contact with most metals, elemental sulfur reacts to form the metal sulfides.

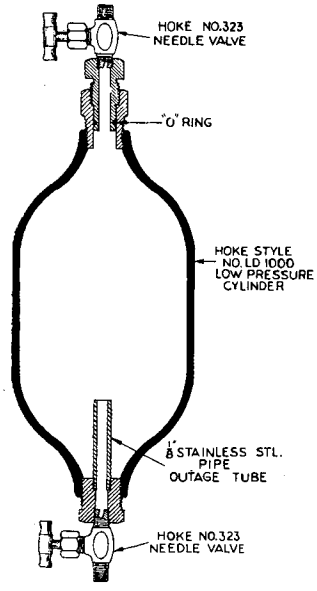

Figure 1. Sample container

The corrosive action of elemental sulfur on mercury, copper, and silver has been used for some time as a semiquantitative test for sulfur in petroleum products (*1, 6, 9*). During recent years more accurate quantitative methods have become increasingly important in the development and evaluation of new refinery processes. Levin and Stehr (*8*) determined elemental sulfur in petroleum oils by shaking them with copper gauze and determining iodometrically the copper sulfide produced. To increase the sensitivity and decrease the analysis time of the sulfur test, Uhrig and Levin (*15*) shook gasoline with mercury and compared the mercuric sulfide turbidity produced with the turbidity of standard suspensions. The sensitivity of this latter test was reported to be less than 1 p.p.m. of free sulfur in gasoline.

Mitchell (*10*) improved on the comparision technique by measuring the turbidity photometrically. The mercuric sulfide method was extended by Shively and Levin (*13*) to the determination of elemental sulfur in liquefied petroleum gases (LPG) by weathering off the liquid sample, dissolving the residue in a hydrocarbon, and determining the amount of sulfur present by the procedure of Uhrig and Levin (*15*).

Another property of elemental sulfur which has been utilized for its determination is its reaction with alkali metal cyanide to form thiocyanate (*12*). Recently, Bartlett and Skoog (*3*) developed a rapid, accurate method for the determination of as little as 2 p.p.m. of elemental sulfur in hydrocarbons based on this reaction, combined with a colorimetric determination of the thiocyanate with ferric iron. This technique has so far not been extended to the analysis of liquefied petroleum gas samples.

The polarographic reduction of elemental sulfur has been used for its determination in hydrocarbon liquids (*4, 7, 11*). In the authors' laboratories this method has been found to be the simplest and most sensitive for the determination of elemental sulfur in crude oils, gasolines, and intermediate distillates. Less than 1 p.p.m. of elemental sulfur in gasoline can be determined accurately.

The present study was undertaken to extend the use of the polarographic method to the quantitative determination of elemental sulfur present in liquefied petroleum gases at concentration levels normally associated with the sensitivity limit of the mercury corrosion test (0.1 p.p.m.). Investigation has shown that it is possible to determine polarographically a few hundredths of 1 p.p.m. of elemental sulfur in liquefied petroleum gases.

APPARATUS AND REAGENTS

Polarographic measurements were made with a Sargent Model XXI polarograph. H-type polarographic cells containing a saturated calomel reference electrode separated from the solution compartment by a 1-cm. fine sintered-glass disk and a potassium chloride–agar plug were employed.

Oxygen was removed from the analyzed solutions by bubbling with prepurified nitrogen (99.9+%) previously saturated with pyridine-methanol solvent. Polarograms were run with the solutions maintained at a constant temperature of 26.0° C. in a thermostatically controlled water bath.

All chemicals used were of reagent grade.

Sampling containers used were 1-liter, two-valved, stainless steel bombs (Hoke Style No. LD 1000) fitted as shown in Figure 1 with an outage tube at one end and an O-ring closure at the other to facilitate rinsing of the bomb.

RECOMMENDED PROCEDURE

Sampling. Obtain a sample of liquefied petroleum gas in the prescribed sample bomb, using the procedure outlined by the American Society for Testing Materials (*2*).

Analysis. Weigh the sample bomb and contents to the nearest gram. Clamp the bomb in a vertical position with the O-ring up and vent the gas slowly through the upper needle valve. (Because of the flammability of liquefied petroleum gas, this operation should be carried out in a well-ventilated hood in the absence of fire.) Upon initial evaporation the bomb cools, the vapor pressure in the bomb approaches 1 atm., and the needle valve may gradually be opened fully. To hasten the volatilization of the sample at this point, the bomb may be placed in a tepid water bath.

After complete volatilization of the sample, reweigh the bomb to ascertain the weight of the sample. Remove the O-ring fitting and pipet 5 ml. of pyridine into the bomb. Rotate the bomb so that the pyridine comes completely in contact with the inside walls, avoiding loss of the liquid. Transfer the pyridine quantitatively to the 50-ml. volumetric flask, rinsing the last few drops into the flask with a stream of methanol. Repeat the rinsing

Table I. Elemental Sulfur in Commercial Butane

Sample	Wt. of Sample, Grams	Elemental Sulfur, P.P.M. Added	Found
	Bomb Evaporation Method		
Blank	...	...	<0.005[a]
1	151	0	0.03
	150	0	0.05
	150	0	0.06
	150	0	0.06
	137	0	0.04
	997	0	0.048
	973	4.1	4.3
2	588	0	0.04
	426	0.32	0.32
	473	0.11	0.15
3	474	0	0.02
	540	0.01	0.05
	Low Temperature Method		
4	158	0	<0.02
	210	0	<0.02
	199	0.68	0.68
	193	0.35	0.35
5	114	0	<0.02
	165	0	<0.02
	202	0.03	0.03
	200	0.03	0.03

[a] On basis of 400-gram sample.

procedure with another 5-ml. portion of pyridine, adding the wash liquid to the first portion. Rinse the bomb thoroughly with several portions of methanol, transferring each portion to the 50-ml. volumetric flask.

Add 1 ml. of concentrated hydrochloric acid to the volumetric flask and dilute the solution to the volume mark with methanol. Mix the solution thoroughly and transfer a portion to the polarographic cell. Bubble the solution with nitrogen for 10 minutes to remove oxygen and record a polarogram from −0.1 to −0.6 volt (*vs.* S.C.E.). Measure the diffusion current and calculate the amount of elemental sulfur present by comparing the diffusion current with that obtained for the standard solution described below.

Standardization. Prepare a standard solution of sulfur in pyridine to contain 10 mg. of sulfur per liter. Pipet 10 ml. of this solution into a 50-ml. volumetric flask, add 1 ml. of hydrochloric acid, and dilute to the volume mark with methanol. Record a polarogram for this solution as outlined in the procedure above and measure the diffusion current. The solution contains 100 γ of sulfur in the 50 ml. prepared.

EXPERIMENTAL

Samples of commercial butane were analyzed by the recommended procedure before and after the addition of known amounts of elemental sulfur (Table I). The elemental sulfur was added as a solution in *n*-heptane.

To be successful, the weathering procedure requires that the elemental sulfur present in the sample be deposited quantitatively when the liquefied petroleum gas is volatilized. The vapor pressure of orthorhombic sulfur between 20° and 80° C. was determined by Fouretier (*5*) and Taillade (*14*). On the basis of their data and the established vapor pressures of butane and propane at room temperature, the amount of elemental sulfur in liquefied petroleum gas vapor was calculated to be less than 0.001 p.p.m.

To prove this experimentally, several samples of liquefied petroleum gas were obtained in bombs to which elemental sulfur had been added. The sample was vented as a gas through a gas bubbler containing pyridine. No sulfur was found in the pyridine after over 1000 grams of sample had been bubbled through it. In terms of the volatilized liquefied petroleum gas it may, therefore, be expected that less than 0.002 p.p.m. of elemental sulfur will be lost during the prescribed evaporation step.

An alternative method of weathering off the sample, paralleling that recommended by Shively and Levin (*13*), was explored.

A sample bomb filled with liquefied petroleum gas was weighed and approximately 400 ml. of the material transferred into a beaker chilled in a dry ice–acetone bath. The weight of the sample taken was determined by reweighing the partially emptied bomb. The sample was allowed to evaporate from the beaker at room temperature, after which the residue was dissolved in 10 ml. of pyridine and rinsed quantitatively with methanol into a 50-ml. volumetric flask. One milliliter of concentrated hydrochloric acid was added and the solution was diluted to the volume mark with methanol. The solution was electrolyzed polarographically as described above.

Results of elemental sulfur determinations performed in this manner are presented in Table I. In several cases, elemental sulfur dissolved in heptane was added to the liquefied petroleum gas sample in the beaker and mixed in thoroughly.

Comparative results by the two methods described, obtained by the analysis of identical materials, are shown in Table II.

Table II. Elemental Sulfur in Commercial Butane

Sample	Wt. of Sample, Grams	Elemental Sulfur Found, P.P.M. Bomb method	Low temp. method
6A	531	0.03	...
6B	296	...	0.06
	304	...	0.06
7A	459	0.02	...
	491	0.02	...
7B	300	...	0.01
	311	...	0.01
8	336	0.03	...
	201	...	0.02
9	276	0.02	...
	210	...	0.02
10	301	0.02	...
	186	...	0.02
11	293	0.03	...
	205	...	0.02
12	248	0.12	...
	207	...	0.09
13	127	0.37	...
	127	...	0.36

DISCUSSION

Great care must be exercised in obtaining representative samples of liquefied petroleum gases for the determination of elemental sulfur. Some of the important factors which must be considered in the sampling procedure are as follows:

Air Oxidation of Hydrogen Sulfide. Hydrogen sulfide in hydrocarbon samples is readily oxidized to elemental sulfur by air and mild oxidizing agents. It is possible that this is the source of much of the elemental sulfur present in low-boiling hydrocarbon fractions. If the liquefied petroleum gas sample contains hydrogen sulfide, it is essential that all air be expelled from the bomb before it is filled. The sampling procedure outlined by the American Society for Testing Materials (*2*) makes allowance for flushing out the bomb with liquefied petroleum gas vapor. Air in the bomb may be replaced by nitrogen or helium gas before the sample is taken, if desired.

Nonvolatile Nature of Elemental Sulfur. As shown above, elemental sulfur is essentially nonvolatile at room temperature.

Table III. Elemental Sulfur in Pentane Stocks

Weight of Sample, Grams	Elemental Sulfur, P.P.M. Added	Found
549	0	0.01
540	0	0.01
552	0.07	0.06
560	0.71	0.70
609	1.1	1.2
599	1.1	1.3
567	1.2	1.4

Upon evaporation liquefied petroleum gas deposits the elemental sulfur which it contains. As sampling cocks in the field often have a deposit of sulfur around them, sampling cocks and tubes must be flushed out thoroughly before the bomb is attached. It is likewise important that the sample be taken from the liquid and not the gas phase. In venting the bomb before completely filling it, it is important that only a part of the liquefied petroleum gas in the bomb be vented as a gas and most of the material be vented as a liquid.

Corrosive Nature of Sulfur on Metals. Because elemental sulfur reacts with many metals, the bomb must be made of a material resistant to sulfur corrosion. Stainless steel bombs were found suitable for taking liquefied petroleum gas samples for the determination of elemental sulfur.

Results of analysis by the recommended procedure and the low temperature batch evaporation from an open beaker agree reasonably well. Slightly lower results by the latter technique may indicate loss of sulfur in the valve of the sample bomb or in the sampling tube during transfer of the material. Volatilization of the entire sample from the bomb is to be preferred.

By varying the proportions of methanol, pyridine, and hydrochloric acid, it was found that the most readily interpretable polarographic curves are obtained when the recommended solvent is employed. Normal sensitivity of the method is 0.01 p.p.m. of elemental sulfur on the basis of a 400-gram sample of liquefied petroleum gas. Below a concentration of 1 p.p.m. of elemental sulfur the precision and accuracy of the method are within 0.02 p.p.m.

From 3 to 4 hours are required for a single determination, of which some 30 minutes is actual operator time.

The procedure was extended to higher boiling light hydrocarbon fractions. To volatilize these materials it was necessary to place the sample container in warm water. Results of analyses of pentane stocks are shown in Table III.

When the procedure was applied to straight-run gasolines it was necessary to heat to a higher temperature to effect volatilization in a reasonable time, and it was observed that sulfur is lost thereby. On the basis of the vapor pressure equations of Fouretier (*5*) and Taillade (*14*) this loss is to be expected. In evaporating higher boiling hydrocarbons at atmospheric pressure, losses of elemental sulfur shown in Table IV should be anticipated.

Table IV. Vapor Pressure of Sulfur

Temp., ° C.	Vapor Pressure of Sulfur, Atm. × 10^6 (≈P.P.M. S° Lost)
0	0.00007
10	0.003
20	0.0013
30	0.0054
40	0.019
50	0.063
60	0.19
70	0.55
80	1.5
90	4.0
100	9.2
110	21.0

ACKNOWLEDGMENT

The authors wish to express their appreciation to W. W. Howland and G. W. Brown, who supplied the necessary samples for this study, and to F. O. Bartella, who performed the analyses. Permission of the Union Oil Co. of California to present and publish this paper is gratefully acknowledged.

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RECEIVED for review March 1, 1956. Accepted April 30, 1956. Symposium on Methods for Testing Liquefied Petroleum Gases, St. Louis, Mo., September 27 and 28, 1954.

Dry Combustion and Volumetric Determination of Isotopic Carbon and Hydrogen in Organic Compounds

Removal of Nitrogen Dioxide, and Gas Temperature Correction Factors

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Small amounts of nitrogen dioxide in the presence of excess oxygen will pass through a dry ice-cooled radiator trap at low pressures, making possible the freezing out of water before the removal of nitrogen dioxide by external means during the dry combustion of organic compounds. In applying a temperature correction to the gas pressure measurements of carbon dioxide and water by means of a two-liquid manometer, an empirical correction of greater magnitude (about 0.5% per degree) than that calculated by means of the gas law gives the most consistent results.

WHILE the nitrogen oxide is successfully removed by manganese dioxide from the gas stream during the dry combustion of nitrogen-containing organic compounds (*2*), nitrogen dioxide (the oxide normally present below 150° C.) (*6*) would not be expected to pass cleanly through a radiator trap at dry ice temperature. Such a trap is used in the present system to hold the water formed during the combustion, placed before the manganese dioxide trap for the removal of nitrogen dioxide. A question was raised as to whether the nitrogen oxide present is actually nitrogen dioxide, which has a vapor pressure of 0.03 mm. at −80° C. (*4*), or is nitric oxide, which does not freeze at −80° but should

not be removed by manganese dioxide (*1, 5*). Nitric oxide is the oxide present at the temperature of the combustion furnace (*6*).

Further investigation has shown the following facts to be true under these conditions. Manganese dioxide does not remove nitric oxide appreciably, if at all, in agreement with previous observations (*1, 5*); manganese dioxide does remove nitrogen dioxide from the gas stream whether or not oxygen is present, if it is not passed through the trap too rapidly; nitrogen dioxide alone or with relatively low proportions of oxygen will freeze to a large extent in a radiator trap at −80° C.; and nitrogen dioxide in the presence of a large excess of oxygen (the actual conditions during a combustion) will largely pass through the dry ice trap and be subsequently removed by manganese dioxide.

Table I. Removal of Nitrogen Dioxide from Combustion Gases

Run	Sample Gas	Sample Cc.	Rise, Cm. In dry ice trap[a]	Rise, Cm. In liquid N_2 trap[a]	Rise, Cm. Expected from condensable gas introduced[a]
1	Oxygen (blank)	50	0.03	0.12	...
2	Nitric oxide	6.0	0.09	12.17	15.8
3	Nitrogen dioxide	2.3	3.06	0.28	6.0
4	Oxygen (blank)	50	0.10	0.17[b]	...
5	Nitrogen dioxide Oxygen	0.7 40	0.25	0.41[b]	1.9
6	Nitrogen dioxide Oxygen	0.7 40	0.17	0.38	1.9
7	Oxygen (blank)	50	0.09	0.44[b]	...
8	Nitrogen dioxide Oxygen	10.8 10	11.91	15.22[c]	28.5
9	Nitrogen dioxide Oxygen	1.6 40	0.41	3.29[c]	4.2
10	Nitrogen dioxide Oxygen	1.6 40	0.36	4.00[c]	4.2
11	Nitrogen dioxide Oxygen	0.4 40	0.10	0.53	0.95
12	Water Oxygen	25 mg. 40	>30	0.48	72
13	Oxygen (blank)	50	0.04	0.45	...

[a] 1-cm. rise equals 0.38 cc. of gas for carbon dioxide or nitrogen dioxide; for water, 0.42 cc. of gas.
[b] Rise in blank between runs 4 and 5 is due to fact that several runs intervened in which large amounts of nitrogen dioxide were inadvertently passed through too rapidly. This seemed to raise the blank in the liquid nitrogen trap permanently (but reproducibly).
[c] Not passed through manganese dioxide.

The results of these experiments are summarized in Table I. It would appear that the dry ice–cooled radiator trap does not efficiently remove nitrogen dioxide from the gas stream under the conditions that prevail during a normal combustion. A small amount of the gas (about 1- to 3-mm. rise on the manometer) may be trapped in the dry ice trap during a normal combustion, but this represents a maximum of about 2% of the water in a typical analysis and would not unduly affect the hydrogen value obtained. Compounds containing 30% nitrogen have been analyzed by this system, with acceptable hydrogen values.

Introducing a large proportion of nitrogen dioxide to the mixture (run 8), or using nitrogen dioxide alone, increased considerably the amount of gas trapped by the dry ice trap. That the trap was efficient in removing water was clearly demonstrated by run 12, where no water appeared in the following liquid nitrogen trap (the amount of water present in the dry ice trap was too great to be measured directly on the available volume system). In the cases involving nitric oxide, some of the oxide introduced was not trapped by the liquid nitrogen trap. This might be expected, as vapor pressure of nitric oxide is 0.08 mm. at −196° C. (*4*), and the system was eventually pumped down to a pressure of 0.05 to 0.1 mm. The amounts trapped in other cases were within the experimental error of measurement of the amount of condensable gas introduced.

For the runs listed in Table I, samples of the two oxides (Matheson Co., Inc., East Rutherford, N. J.), either alone or mixed with oxygen, were introduced into 50-cc. bulbs and passed through the trap system at flow rates approximating as nearly as possible those existing during the normal combustions (about 25 cc. per minute). Blanks were taken periodically by passing 50 cc. of dry oxygen through the system, and these amounts should be taken into account in noting the amounts of gas trapped. The blanks, which were higher than those normally encountered in the combustions, were mainly due to the fact that the oxygen used was not run through so rigorous a purification system as that used with the combustion train itself.

In summary, it is apparent that nitrogen dioxide is the oxide produced during these combustions (as would be expected), that it does pass through a dry ice–cooled radiator trap in the presence of a large excess of oxygen, and that it is subsequently removed by the manganese dioxide employed for the purpose, as previously described (*2*).

GAS TEMPERATURE CORRECTION FACTORS

It was previously pointed out (*2, 3*) that a temperature correction should be made in the gas pressures measured by the system in question. However, it has been found that a correction greater than that calculated by the gas law should be applied, mainly because of expansion of the mercury and oil in the manometer as the temperature rises. Expansion of the glass is a negligible factor in this case.

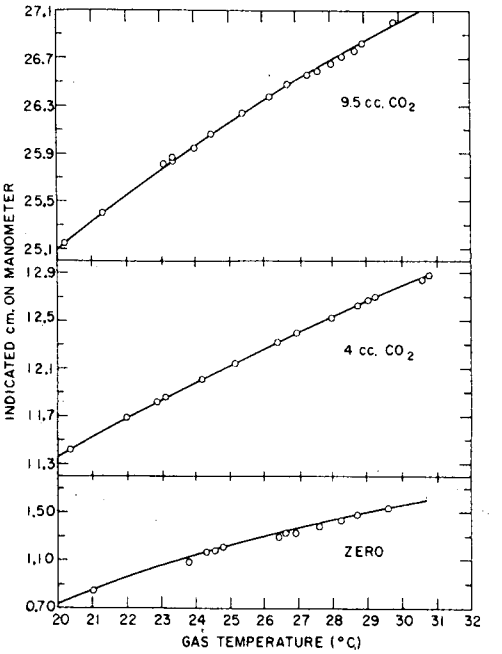

Figure 1. Variation of manometer readings with temperature

The correction calculated by the ideal gas law ($P_1/T_1 = P_2/T_2$) amounts to approximately 0.33% per degree Centrigrade, assuming constant volume (approximately true). Empirically it is found that an additional correction of about 0.2% per degree Centigrade should be employed for the other causes mentioned above. This is strictly true only with a manometer of the construction previously described (*2*), and using dibutoxytetraethylene glycol as the oil phase. The correction factors listed in Table II are used to correct the pressure readings to 20° C., and the standard rise on the manometer for both carbon dioxide and water is also related to that temperature for each measurement system. Temperatures are read on thermometers in the wells in

the large volumes on the measuring system. The manometer reading may take as much as 15 minutes to come to its equilibrium value after an ambient temperature change of 1° C.

Table II. Gas Pressure Correction Factors at Various Temperatures[a]

Temp., ° C.	Correction Factor	Temp., ° C.	Correction Factor
20	1.000	27	0.965
21	0.995	28	0.961
22	0.990	29	0.956
23	0.985	30	0.951
24	0.980	31	0.946
25	0.975	32	0.941
26	0.970		

[a] Total correction factor is 0.5%/degree.

The empirical additional correction is an average of several observations of this factor with amounts of tank carbon dioxide in the system giving about 10- and 25-cm. difference in readings on the two-liquid manometer. Figure 1 shows the change in these pressure readings, and in the zero reading, at various temperatures. The differential readings at each temperature are corrected by the gas law to 20° C.; then the additional correction necessary is noted to be 0.2 ± 0.05%. The total correction used to calculate the values in Table I is 0.50% per degree. There is some variation in this value with the amount of carbon dioxide present, but an average value gives sufficiently good results and is much more convenient to use. For extreme accuracy, a sliding scale of factors might be used, to vary with the actual amount of carbon present.

The use of these correction factors rather than those calculated by the gas law alone has led to greater precision, both in carbon and hydrogen percentages and in carbon-14 activity results (because of the greater accuracy in measuring the amount of carbon dioxide placed in a counting tube). Carbon values determined by this method are regularly within 0.5% of the correct value. The average deviation of carbon results previously reported (*2*) was 0.33% (only the gas law correction being used), while a comparable set of analyses using the present correction factors showed an average deviation of 0.24% from the theoretical value. In the case of the carbon-14 results, the error introduced from uncertainty in the amount of gas in the counting tube is calculated to be less than 0.1% when these correction factors are used, a percentage which is negligible compared to the statistical and other systematic errors affecting the activity determinations (*3*).

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RECEIVED for review February 6, 1956. Accepted May 8, 1956. Research performed under auspices of the U. S. Atomic Energy Commission.

Improved Rapid Colorimetric Microdetermination of Dissolved Oxygen

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Comparison of a colorimetric method for determining dissolved oxygen in 0.5-ml. samples with the standard Winkler method revealed that the calibration curve did not pass accurately through the origin and was slightly concave downward. This paper describes three modifications of the method, whereby a strictly linear curve passing accurately through the origin can be obtained; other improvements make possible its use as a rapid micro alternative to the standard Winkler method.

A COLORIMETRIC determination of dissolved oxygen in 0.5-ml. samples (*2*), which could be completed in about 1 minute, consisted of measuring the red color produced by partially oxidizing reduced indigo carmine with the oxygen dissolved in the test sample of water. Atmospheric oxygen was excluded by carrying out the reaction in an airtight syringe that could be placed directly in a Beckman spectrophotometer.

In a subsequent comparison of this method and the standard Winkler method (*1*, *6*), it was found that the resulting calibration curve did not pass accurately through the origin and was not strictly linear but slightly concave downward. Consequently, three modifications of the original method were devised, whereby a strictly linear curve passing through the origin was obtained. This paper describes these modifications, together with various other improvements developed while using this method as a rapid micro alternative to the standard Winkler method. The coefficient of variation between the two methods was 2.5% (Table I).

APPARATUS

A 1-ml. Tuberculin syringe, graduated in 0.01 ml., with No. 22 hypodermic needle (A. H. Thomas Co., 9404) is used. A small steel ball (or nail head) is inserted into the barrel of the syringe, so that shaking the syringe effectively mixes its contents. A 1-cm. piece of tape is wound around the barrel of the syringe between the 0.75 and 0.90 markings, as illustrated in Figure 1. This bushing of tape serves to hold the syringe snugly inside the borosilicate glass absorption cell (10-mm. light path) of a Beckman spectrophotometer. If the tape is wound around four small pieces of wire set equidistantly around the barrel of the syringe, the resulting cross section is approximately square and the syringe may be locked into position with a slight twist. The syringe should always be placed in one predetermined position, with the graduations at one side, so as to give as clear a light path as possible. If the plunger descends during a determination, the spring clip at the end of the barrel should be tightened.

A Beckman spectrophotometer is adapted to receive the syringe, with its needle still attached, by drilling a 1-cm. hole in the floor of the sliding absorption cell carrier immediately beneath the fourth square compartment near the back of the instrument; and drilling a second 3-mm. hole through the main housing of the sample compartment. This second hole is centered with the first, so that the needle of the syringe can pierce the housing and project beneath the spectrophotometer (Figure 1). These two holes may be drilled with a drill press in a few minutes; they do not interfere with the normal operation of the spectrophotometer. Only under exceptional conditions of illumination is it necessary to place a small piece of black tape over the 3-mm. hole to make it completely lighttight.

Table I. Variation of Absorbance at 586 Mμ of Present Method and Concentration of Dissolved Oxygen as Determined by Winkler Method

Winkler Method, Concn. of Dissolved Oxygen, Mg./L. O_2	Present Method, Absorbance at 586 Mμ	Ratio[a]
0.97	0.189	5.15
1.30	0.241	5.4
1.94	0.330	5.9
2.74	0.479	5.75
3.28	0.564	5.8
3.45	0.590	5.85
4.00	0.721	5.55
4.68	0.807	5.8
4.96	0.865	5.75
5.10	0.913	5.6
5.72	0.995	5.75
6.50	1.138	5.75
7.40	1.252	5.9
7.58	1.352	5.6
8.80	1.541	5.7
9.21	1.543	5.95
9.80	1.670	5.85
10.50	1.813	5.75

[a] Mean of ratios of two methods (omitting two at top of list) is 5.85. Standard deviation is 0.15 and coefficient of variation is 2.5%.

The bottom plate of a borosilicate glass absorption cell (10-mm. light path) is removed by tapping it with a small metal rod. This allows the syringe and needle to be placed directly in the bottomless absorption cell and lowered until the butt of the needle rests on the bottom plate of the compartment housing.

As the height of the syringe precludes closing the sample compartment with its usual cover, a substitute is made by stapling 2 inches of black cloth to the edges of a 4 × 5 × 5 inch light-tight cardboard box, which is placed over the sample compartment during a determination.

A 16-ounce narrow-mouthed acid bottle (Fisher Scientific Co., 2-922), fitted with a rubber cap (3-225), is used to store the reagent. Hypodermic needles may be inserted repeatedly through such rubber caps without leakage of air. Small 60-ml. serum bottles (3-220) may also be used; they make convenient waste bottles, into which to eject the dye following a determination.

Oxygen-free nitrogen, "prepurified nitrogen" (Matheson), may be used. Commercial nitrogen must be purified before use by bubbling it through alkaline pyrogallol.

REAGENTS

Two grams of indigo carmine (National), glucose, and anhydrous potassium carbonate are placed in a 16-ounce reagent bottle and 200 ml. of water are added. The rubber cap is wired in place. The air space within the bottle is flushed with oxygen-free nitrogen for 10 minutes through two No. 22 hypodermic needles, after which the bottle is left under 5-pound pressure of nitrogen. The reagent is reduced in about an hour in an 80° C. water bath, or in about a week at room temperature. Additional purified nitrogen is introduced through a needle from time to time to maintain the internal pressure at about 5 pounds.

PROCEDURE

The spectrophotometer is first adjusted to zero with water in the syringe. Following this, the syringe is rinsed twice with reagent and filled to about the 1.00 mark, the needle pointing upward during all such operations. The syringe is wiped with a piece of Kleenex and shaken about 50 times, following which the blank absorbance of the reagent alone is measured at 586 mμ.

The plunger of the syringe is advanced to exactly the 0.40 mark. A strong light behind the syringe considerably increases the accuracy of this placement. The syringe is then lowered into the sample of water to be tested and the barrel is filled to exactly the 1.00 mark. After wiping and shaking, the experimental absorbance of the sample is determined at 586 mμ without delay.

CALCULATION

The net absorbance of the sample is obtained by subtracting 50% of the blank absorbance from the experimental value. The concentration of dissolved oxygen in milligrams per liter is then obtained by dividing the net absorbance by a calibration factor obtained as described below. Results in either milliliters of oxygen per liter or per cent saturation may be obtained graphically, if desired, with the aid of Rawson's nomogram (*3–5*).

Example

Experimental absorbance 1.630

Blank absorbance 0.176

Net absorbance $1.630 - \frac{0.176}{2} = 1.542$

$$\text{Oxygen concentration} = \frac{\text{net absorbance}}{\text{calibration factor}} = \frac{1.542}{0.171} = 9.0 \text{ mg. per liter } O_2$$

DETERMINATION OF CALIBRATION FACTOR

As the net absorbance of the colorimetric method varies linearly with the oxygen content of the sample, the desired calibration factor may be obtained by determining the net absorbance of any sample of water, if the oxygen content is already known. Although the standard Winkler method was used to determine oxygen content in this work, it is usually more convenient to standardize the reaction against a sample of water, when the oxygen content can be calculated from the temperature at which it was equilibrated with air.

A bottle is half-filled with water and shaken vigorously for several minutes to ensure complete equilibration of the water with the air in the bottle. The temperature of the water is then determined to the nearest 0.5° C. and its oxygen content is obtained from the standard tables published by the American Public Health Association (*1*).

The net absorbance of the sample of water is then determined by the method described in this paper. The required calibration factor is obtained by dividing the net absorbance of the sample by its oxygen content in milligrams of oxygen per liter, as obtained from the standard tables. Thus, for example, if the temperature of a shaken sample of water is found to be 21° C., its oxygen content is 8.99 according to the tables. If its net absorbance is now determined and found to be 1.537, the required calibration factor is $\frac{1.537}{8.99} = 0.171$. Duplicate determinations, as well as determinations of samples equilibrated at different temperatures, should check each other within ±1%.

DISCUSSION

As originally described, this method was calibrated against a graded series of samples obtained by mixing different proportions of reagent (0% oxygen) and fully saturated (100% oxygen) water whose oxygen content could be calculated from the temperature at which it had previously been equilibrated with air (*1*). This simple and rapid method appeared adequate for most purposes, especially in view of the practical difficulties involved in preparing a graduated series of water samples of known oxygen content, protected from the air, and in sufficient quantity for determination by the Winkler method (*2*).

Subsequent work has shown that such a graduated series of water samples may be prepared by siphoning oxygen-free water into a series of B.O.D. bottles (Fisher Scientific Co. 2-926)

containing increasing amounts of fully saturated water; the resulting mixtures are protected from the air before use by the ground-glass stoppers of the especially designed B.O.D. bottles.

A large supply of completely oxygen-free water can be obtained conveniently as follows:

A 2-gallon, wide-mouthed bottle is filled two thirds full with water and its remaining air space is flushed with purified nitrogen. A 50-ml. beaker is then hung with copper wire from the bottom of a large size rubber stopper and lowered into the bottle so that it hangs freely in the air space without touching the water. One gram of pyrogallic acid dissolved in 5 ml. of hot water and 20 ml. of 80% potassium hydroxide are then pipetted carefully into the beaker, the rubber stopper is lowered into place, and the bottle is stirred with a magnetic stirrer for about a week.

Within this time, all the oxygen dissolved in the water distilled into the beaker of alkaline pyrogallol. This method was used, because the direct addition of reducing agents interferes subsequently with the Winkler method, while boiling under vacuum does not completely remove dissolved oxygen unless continued for longer than overnight with consequent excessive evaporation.

When the graduated series of B.O.D. bottles prepared in this way were analyzed simultaneously by both the Winkler and colorimetric methods, the resulting calibration curve did not pass accurately through the origin and was slightly concave downward, instead of being strictly linear. Consequently, three modifications of the original method were instituted so that the linear curve passed accurately through the origin.

Modification. The spectrophotometer was adapted to receive the syringe even when its needle was still attached (Figure 1). This seemingly small change, besides making the method more convenient and rapid in practice, eliminated an error of between 1 and 2 mg. per liter of oxygen by preventing contamination of the sample with about 1 to 2 γ of atmospheric oxygen during the removal and subsequent replacement of the needle on the syringe.

The volume of reagent within the bore of the needle itself was taken into account. It was found that the absorbance of a sample of oxygen-free water was exactly 50% of the blank only when the syringe was filled with reagent to the 0.40-ml. mark before it was filled with the sample, and not to the 0.50-ml. mark as previously described.

The operative wave length was raised from 580 to 586 mμ. This corrected the slight downward concavity in the calibration curve present in the original method and, together with the other changes, yielded a strictly linear curve that passed accurately through the origin.

The general reliability, ease of reduction, and stability (several months) of the reagent were markedly improved, when it was made with oxygen-free rather than commercial nitrogen gas. Furthermore, slow reduction at room temperature yielded a reagent whose color did not fade the 1 to 2% per minute observed with reagent reduced rapidly at 80° C. In practice, either method of reduction may be used, as it is usually practical to measure the absorbance within 30 seconds after mixing.

Water samples containing above 10 mg. of oxygen per liter may be analyzed by the present method by reducing the ratio of sample to reagent, so that the absorbance of the mixture falls within the limits of the linear part of the curve. In such cases, the percentage of the blank to be subtracted from the experimental value is determined by taking a sample of the desired size of oxygen-free water into the syringe and finding the percentage of the blank that remains. A convenient and stable source of oxygen-free water for such purposes may be obtained by making up two bottles of reagent in the usual manner, but omitting the dye from one of them. The color of the reagent bottle then may be used as an indicator of the degree of reduction within the water bottle. It is preferable to reduce such bottles slowly at room temperature, as alkaline glucose solutions turn brown on being heated to high temperature. Because reduction in the size of the sample changes the slope of the calibration curve, a new calibration factor must be determined for every different size of sample used.

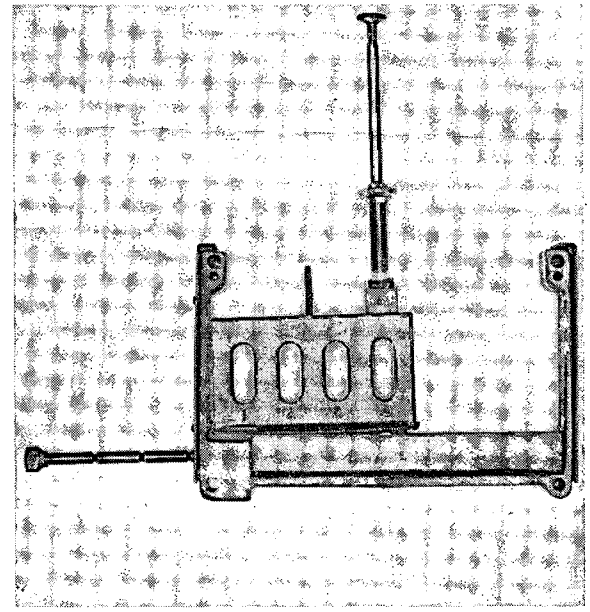

Figure 1. Final assembly of apparatus

As the improved method is calibrated directly against Winkler-determined standards, it is free from errors induced by changes in the blank absorbance of reduced and oxidized samples. As with the Winkler method, however, compounds capable of oxidizing or reducing indigo carmine, such as nitrates, chlorates, nitrites, iron salts, or sulfites, will interfere unless previously removed by appropriate methods (*1*).

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RECEIVED for review November 18, 1955. Accepted April 27, 1956.

Fluorescent X-Ray Spectrographic Determination of Uranium in Waters and Brines

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In a search for a method sensitive to small concentrations of uranium in water and brine, a modification of a fluorometric method was adopted. The uranium is precipitated from the water or brine by the method of Smith and Grimaldi, and the ashed precipitate is analyzed by fluorescent x-ray spectrography. When an internal standard is required, yttrium nitrate is added. The sample holder of the Norelco x-ray spectrograph was modified to place the small precipitate samples in the most intense part of the beam from the molybdenum target x-ray tube. As little as 0.01 mg. of uranium, equivalent to 0.01 p.p.m. in a 1-liter water sample, can readily be measured, when the matrix contains no appreciable concentration of highly absorbing elements. The time for analysis is about 10 minutes per sample, when no internal standard is used, and about 15 minutes when the internal standard is used, exclusive of the precipitation process.

THE recent public interest in the potentialities of oil-field waters as a source of uranium led to an investigation of methods for the analysis of waters and brines containing very small concentrations of this element. None of the methods described in the literature was entirely satisfactory, particularly for use in a study of a possible economical process for recovering uranium from water and brine solutions.

Table I. Results Obtained from Repeated Analyses of Three Synthetic Samples

(No internal standard used)

Sample No.	1	2	3
Uranium added, mg.	0.70	0.50	0.10
Uranium found, mg.	0.72	0.50	0.10
	0.70	0.51	0.09
	0.69	0.51	0.09
	0.70	0.49	0.09
	0.70	0.52	0.11
	0.68	0.50	0.10
	0.68	0.49	
		0.51	
		0.48	
		0.48	
Mean	0.70	0.50	0.10

Birks and Brooks (*1*) describe a fluorescent x-ray spectrographic method in which a 1-ml. sample of a water solution of uranium was evaporated in a shallow cup and the residue was analyzed. This method was not directly applicable to brine samples and its sensitivity was inadequate for the purposes of the investigation. The optical fluorometric method described by Smith and Grimaldi (*3*), while accurate for the quantities of uranium found in field waters and brines, was not convenient in this laboratory. However, this method suggested the possibility of following their procedure for precipitating the uranium from solution, and then using fluorescent x-ray spectrography to analyze the ashed precipitate. In practice, this was found to be a convenient and very satisfactory method.

Synthetic solutions prepared in the laboratory for use in the experiments on recovery methods were analyzed without the use of an internal standard. Actual field samples, however, required an internal standard, because of the widely varying nature of the dissolved materials which were carried down with the uranium in the precipitation process.

SAMPLE PREPARATION

The process of precipitating the uranium from solution follows exactly the first few steps outlined in the procedure of Smith and Grimaldi. Briefly summarized, this procedure is as follows for samples in which no internal standard is used.

A 500-ml. aliquot of sample is filtered through glass wool and acidified with nitric acid. A solution containing 20 mg. of alumina is then added, followed by the addition of 60 mg. of diammonium phosphate in solution. The aluminum ion is added to act as a carrier for the precipitation of uranium, while the diammonium phosphate ensures the precipitation of the aluminum and uranyl ions as the phosphates, which are easier to filter than the hydroxides. The solution is boiled for about 1 minute to remove any carbon dioxide, and the pH is adjusted to the color transition point of methyl red by adding ammonium hydroxide. After a 10-minute waiting period, the mixture is filtered through ashless filter paper, and the precipitate is washed with dilute ammonium nitrate, and ashed. This ash is then analyzed by the fluorescent x-ray method.

In the case of field samples, a solution containing 0.28 mg. of yttrium is added to the 500-ml. sample aliquot to provide the internal standard. The precipitation process is then carried out exactly as described, and the yttrium, which behaves like uranium in this process, is completely recovered in the ash.

INSTRUMENTATION

Norelco spectrographic equipment was used, with both tungsten and molybdenum target tubes for sample excitation. A lithium fluoride analyzing crystal was used exclusively, and line intensities were determined by the automatic scanning-recording method, with a scanning speed of 0.25° (2θ) per minute.

The volume of the ash samples obtained for analysis was frequently as low as 0.1 to 0.2 cc., and a special sample cup was used which ensured that the sample was reproducibly positioned in the area of most intense radiation from the x-ray tube. To accomplish this, the distribution of x-ray intensity from the tube was roughly determined by irradiating a piece of soft glass in the sample position and noting the intensity of discoloration induced. A cup with the dimensions 15 × 20 × 0.5 mm. was then formed in the top of an aluminum block in a position which corresponded to the area of most intense discoloration of the glass, when the block was placed in the sample holder of the spectrograph.

PROCEDURE AND RESULTS

The precipitates obtained from the synthetic solutions used in the laboratory studies mentioned previously were well suited for analysis without the use of an internal standard. No interfering elements of significance were present, and the absorption of the UL_{α_1} line in the aluminum phosphate matrix was relatively low and essentially constant from sample to sample. Standard samples were prepared by following the regular chemical precipitation procedure with 500-ml. aliquots of water to which 0.10, 0.25, 0.50, 0.70, and 1.0 mg. of uranium had been added as uranyl acetate. Duplicate preparations of each of these were checked and found to be identical, within the limits of reproducibility of the x-ray measurements.

The intensity of the UL_{α_1} line from all of the standard samples was measured at least three times and the peak heights obtained for each uranium concentration were averaged. A straight-line

calibration curve was obtained when these average values were plotted as a function of uranium content. The sensitivity, as deduced from this curve, is such that 0.01 mg. of uranium can be measured. This corresponds to 0.02 p.p.m. in the original 500-ml. water sample, but could be altered to almost any desired value by a proper choice of the volume of the sample aliquot used for analysis.

Two synthetic samples to which 0.70 and 0.50 mg. of uranium had been added were prepared and analyzed repeatedly to test the method. The results are shown in Table I, which also includes measurements made on the 0.10-mg. standard sample, to illustrate the reproducibility obtained in this concentration range. It was found, generally, that all the results obtained for synthetic samples fell within ±3% of the mean in the 1.0-mg. range and within ±15% of the mean in the 0.10-mg. range.

The method, as adapted for the analysis of field samples, is based on the ratio of the intensity of the $UL_{\alpha 1}$ line to that of the K_α line from the known amount of added yttrium. The use of yttrium as an internal standard for uranium analyses has been reported by Cope and Lingard (*2*), who were interested in the determination of uranium in mineral-bearing rocks. The YK_α line, with a wave length λ = 0.831 A., is absorbed by most elements to about the same extent as the UL_{α_1} line, with λ = 0.911 A. However, gold, platinum, mercury, and tellurium have absorption edges which occur between these two wave lengths, so that an error in the uranium analysis will be introduced by the presence of any of these elements in the precipitate. An error of smaller magnitude is also introduced by lead and bromine, which have absorption edges on the near long wave-length side of the UL_{α_1} line.

Of all the interfering elements mentioned, lead and tellurium are thought to be the most likely to occur in solution in field waters. The effect of lead on the ratio of the UL_{α_1} to YK_α line intensities was investigated and found to be negligible for concentrations that might normally be expected, although a large effect was observed when the lead content of the precipitate was increased to several hundred milligrams. It is rather improbable that any of the other interfering elements will be found in brine samples in sufficient concentration to be troublesome. However, it is advisable to check this, at least for the first few samples from a new area or field.

Obviously, if yttrium is present in the sample as received, this method cannot be used unless the concentration is determined and a correction applied to the measured total YK_α intensity.

Samples for calibration were prepared by adding 0.28 mg. of yttrium and various amounts of uranium, from 0.08 to 1.25 mg., to 500-ml. water samples, which were then processed as described. The intensities $I_{UL\alpha_1}$ and $I_{YK\alpha}$ from the precipitates were measured by the scanning-recording method, and a smooth calibration curve was obtained by plotting the ratio $I_{UL\alpha_1}/I_{YK\alpha}$ as a function of the uranium content.

A synthetic sample containing 0.31 mg. of added uranium was analyzed five times to check the method. Another synthetic sample which contained 0.31 mg. of added uranium as well as approximately 100 mg. each of added iron and vanadium was analyzed twice to determine how well the yttrium was functioning as an internal standard. The results are shown in Table II. All the analytical values fall within ±7% of the mean.

Two field samples were analyzed both by a chemical method employing spectrophotometric absorbance and by fluorescent x-ray spectrography. Both samples showed high activity when placed near a Geiger or scintillation counter, but the observed intensity of the UL_{α_1} line was near zero for both samples. The sensitivity of the method for these particular samples was then determined by adding 0.1- and 0.3-mg. quantities of uranium to two other 500-ml. aliquots of each of the brines, which were then processed and the precipitates analyzed. On the basis of these observed UL_{α_1} line intensities, the minimum measurable uranium content was determined to be 0.05 mg., so that the concentration in the original samples was determined to be less than 0.1 p.p.m. These results were confirmed by the chemical analyses, which showed a concentration of approximately 0.03 p.p.m. in both samples.

Table II. Analyses of Synthetic Samples

(Showing effect of approximately 100 mg. each of added iron and vanadium. Yttrium added to both samples as an internal standard)

Sample	A	B	
Uranium added, mg.	0.31	0.31	plus Fe and V
Uranium found, mg.	0.34 0.31 0.33 0.31 0.30	0.33 0.29	
Mean	0.32	0.31	

These findings illustrate an observation that has frequently been made—namely, that the activity often found in field waters by Geiger or scintillation counter surveys is usually due not to the presence of uranium but to radium salts or other water-soluble decay products.

DISCUSSION

The sensitivity of the method for samples which do not contain any significant quantity of absorbing material other than the added aluminum phosphate is such that 0.01 mg. of uranium can be measured. This can be made to correspond to almost any concentration in the original solution by properly choosing the volume of sample to be analyzed. On the other hand, in the case of field samples the sensitivity is reduced by absorption in the extra materials that are found in the precipitates from these solutions. Because the quantity of these materials is variable from sample to sample, the sensitivity will also vary. In the examples cited, the minimum measurable uranium content was 0.05 mg.

The procedure for obtaining the precipitates from the water or brine solutions requires about 1 hour per sample, but four samples can conveniently be handled simultaneously by one person. These precipitates can be analyzed by one person at the rate of four per hour when the internal standard method is used, and six samples per hour when no internal standard is used. This includes time for loading the specimen holder and reduction of data, both of which can usually be done during the automatic scanning-recording cycle for another sample. These rates would generally be substantially reduced if manual counting techniques were used, but some gain in the reproducibility of the results would be realized. If this greater precision is not required, the method as described can be recommended as being very convenient, rapid, and in general, satisfactory.

ACKNOWLEDGMENT

The authors wish to acknowledge their gratitude to Blaine B. Wescott, executive vice president, Gulf Research & Development Co., for permission to publish this manuscript, and to B. B. Osthaus for the analyses of the two field samples by a chemical method employing spectrophotometric absorbance.

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RECEIVED for review March 28, 1956. Accepted May 15, 1956. Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 29, 1956.

Removal of Silicates from Solutions of Sugars Such as Isomaltose and Isomaltotriose

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An adaptation of a hydrofluoric acid method is used for the simultaneous removal of silica and ionic material from preparations of the reducing saccharides isomaltose and isomaltotriose.

CELITE (diatomaceous earth filter aid) frequently has been mixed with the carbon employed in column chromatographic adsorption analysis of sugars to provide adequate rates of flow (*1, 8, 10, 16*). However, when Celite-501 was thus employed in separating isomaltose and isomaltotriose from enzymic hydrolyzates of dextran (*8*), the aqueous ethyl alcohol used for column development leached objectionable quantities of silicious material from the Celite. Other diatomaceous earth filter aids also discharge such material into the effluent (*6, 15*). Preliminary washing of the Celite did not prevent further leaching from the column. In addition, the effluents from the carbon-Celite columns contained appreciable quantities of other inorganic salts.

Table I. Typical Results

Wt. in Original Solution			Wt. in Final Effluent, Mg.	
Sugar, grams[a]	SiO_2, mg.	Ionic salts[b], mg.	SiO_2	Ionic salts[b]
0	7.0	...	0.1	...
13	0.6	60	0.02	0.5
11	1.6	>100	0.0	2.5
11	1.9	...	0.2	...
15	3.1	...	0.1	...
16	...	...	0.3	...
8	...	...	0.3	...

[a] Recovery of sugar was quantitative.
[b] By conductivity, as sodium chloride equivalent.

Analysis of typical eluates (Table I) showed that 1 gram of isomaltose contained as high as 10 mg. of ionic material (as sodium chloride) and 0.5 mg. of silica. These impurities, although small, interfered in attempts to crystallize and characterize the products. Even evaporating the solution to dryness, dissolving in water, and filtering failed to prevent precipitation of more inorganic material when the solution was reconcentrated. Therefore, it was necessary to remove the foreign matter without loss or alteration of the saccharides. As normally employed, those anion exchange resins which can be used to deionize sugars without degradation would fail to remove silica, because silicic acid is such a weak acid. The removal of silicic acid could be effected by the use of a very basic resin, such as Amberlite IRA-400 or Dowex 2 (*9*). Such a resin, however, cannot be used with reducing sugars because, as noted by the authors as well as others (*7, 11–14*), the sugars are retained on the resin and/or destroyed. Resins of lower basic strength have been used to remove silicate from boiler feed water (*2, 4*) by adding hydrofluoric acid to the water. The resulting highly ionized fluosilicic acid ($6HF + SiO_2 \rightarrow H_2SiF_6 + 2H_2O$) then was retained by the anion exchanger.

The procedure reported here is an adaptation of this hydrofluoric acid method. It has been used for the simultaneous removal of silica and ionic material from preparations of the reducing saccharides, isomaltose and isomaltotriose. The absence of hydrolysis or other alteration of these oligosaccharides by contact with the resins or acid was demonstrated by the quantitative recovery and paper chromatographic examination of the sugars after ion exchange treatment. The effect of the procedure on sugars which are more susceptible to acid hydrolysis (furanosides such as sucrose) has not been tested under the conditions of the method. Typical data on removal of silica and ionic material are given in Table I.

PROCEDURE

Materials Used. Dowex 50 cation exchanger (hydrochloric acid regenerated) and Duolite A-4 anion exchanger (ammonium hydroxide regenerated) were used. The resins were soaked in water overnight before being placed in glass columns 49 cm. long and 4.5 cm. in diameter. The columns were fitted with a sintered-glass plate at the bottom and with connections for downwashing and backwashing. About 400 ml. of Dowex 50 (break-through capacity, 700 meq. of sodium chloride) and 300 ml. of Duolite A-4 resin (break-through capacity, 280 meq. of hydrochloric acid) were used. Because margin of safety was the main concern, no attempt was made to establish the minimum size of column which could be used. Smaller columns, especially of Dowex 50, probably would be equally effective.

Preparation of Columns. Condition the Dowex 50 column by exhaustion with 6% sodium chloride and regeneration with 4*N* hydrochloric acid until no color is discharged into the effluent. Exhaust the Duolite A-4 with 4*N* hydrochloric acid and regenerate with 10% sodium hydroxide, repeating the cycle several times. Perform the last regeneration with ammonium hydroxide (approximately 4% ammonia) made by diluting 1 volume of concentrated ammonium hydroxide (15*M*, 28% ammonium by weight) with 6 volumes of water.

Use of Ion Exchange Columns. When reagent or sugar solution is being added to a column the rate of flow should be 50 ml. per minute, but during water washing it should be 100 ml. per minute. Backwash Dowex 50, regenerate with 400 to 500 ml. of 4*N* hydrochloric acid, and downwash with 4 to 5 liters of distilled water to pH of about 7 (1 to 2 p.p.m. of ionic material). After washing is complete, collect and save 4 liters of effluent to be used as Duolite wash for removal of carbohydrate from the column.

Backwash Duolite A-4 and regenerate with 750 ml. of ammonium hydroxide (4% ammonia). When the resin is completely regenerated, the pH should be about 11. The color of the resin is the best indicator. Wash well with distilled water and let it stand overnight.

Add the sugar solution (about 50 ml.) to the Dowex 50 column. Wash the column with distilled water until the effluent is free of carbohydrate as shown by qualitative anthrone tests (*5*). About 2 liters of wash are required. Add 10 ml. of 1% hydrofluoric acid to this effluent.

Wash Duolite immediately before use to pH of about 8 (1 to 2 p.p.m. of ionic material). About 4 liters of water are needed. Add 40 ml. of 1% hydrogen fluoride to the column and downwash to pH 6 (1 to 2 p.p.m. of ionic material). Add the carbohydrate-containing effluent from the Dowex 50 column. Add 20 ml. of 1% hydrogen fluoride to the 4 liters of Dowex wash that has been saved, and use this to wash the column to a negative anthrone test (about 2 liters required). The hydrofluoric acid present must be kept at a slight excess because the formation of fluorosilicic acid is a reversible reaction. If silica precipitates on the Duolite column, it is very difficult to remove.

The Duolite should be put through an exhaustion-regeneration cycle with sodium hydroxide regeneration, followed by a cycle using ammonium hydroxide regeneration after each run to remove

silica. Ammonium hydroxide has been used instead of sodium hydroxide as a final regenerate because of the difficulty encountered in removing sodium hydroxide completely from the column.

DETERMINATION OF SILICATE AND IONIC MATERIAL

The analytical procedure for the determination of silicate is essentially that of Bunting (*3*), modified slightly to decrease the volume of sugar solution lost through testing. Sugars do not interfere with the test.

Prepare the stock silica solution by dissolving about 7 ml. of sodium silicate solution (40 to 42 Bé.) in 2 liters of distilled water and filtering through a fine sintered-glass funnel. Determine the concentration of silica in the resulting clear solution gravimetrically and dilute as required to prepare standard solution having a concentration of about 0.004 mg. of silica per ml.—e.g., stock solution containing 2.1 mg. of silica per ml., diluted 2 to 1000. Prepare other reagents as described by Bunting.

For analytical determinations, use 10 ml. of sample (diluted if necessary to give a silica content of about 1 to 5 γ per ml.). Proceed as described by Bunting with two exceptions: Use 0.1 as much of each reagent and read the absorbance at 610 mμ. Present studies showed that the spectrophotometric curve has a shoulder at 600 to 650 mμ and a peak at 815 mμ.

Calculate the concentration of silica from the relation $C = KD$, where C is the concentration of silica in milligrams per milliliter, D the observed absorbance, and K a constant determined from a standard silica solution run at the same time.

As the volumes of reductant and molybdate reagents used were only 0.1 ml., experiments were conducted to determine the effect of varying the amounts of reagent. These experiments indicated that these volumes need not be measured with high accuracy. The use of four times as much of the molybdate reagent as specified had no effect, although using half as much as specified lowered the absorbance about 40%.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Allene Jeanes for her interest and assistance in planning and carrying out this study.

They also are indebted to T. A. McGuire for gravimetric determination of silica, and M. O. Bogard and E. H. Melvin for preparation of the spectrophotometric curve.

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CRYSTALLOGRAPHIC DATA

132. Uranyl Oxalate Trihydrate, $UO_2C_2O_4 \cdot 3H_2O$

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URANYL oxalate trihydrate was prepared by adding excess of oxalic acid to a dilute solution of uranyl nitrate in 1*M* nitric acid and digesting at 80° C.

CRYSTAL MORPHOLOGY

System and Class. Monoclinic, prismatic.
Axial Elements. $a:b:c = 0.329:1:0.552$; $\beta = 98.2°$ (calculated from the unit cell dimensions).
Habit. Tabular {010}, bounded by {011}, {111}, {$\bar{1}$13}.
Interzonal Angle. {100} ∧ {10$\bar{1}$} = 53.2°.

X-RAY DIFFRACTION DATA

Space Group. $P2_1/c$ (C_{2h}^5).
Cell Dimensions. $a_0 = 5.61$ A.; $b_0 = 17.04$ A.; $c_0 = 9.41$ A.; $\beta = 98.2°$; cell volume 890 A.^{3}.
Formula Weights per Cell. 4.
Formula Weight. 412.14.
Density. 3.07 grams per cc. (x-ray); 3.076 (flotation).

OPTICAL PROPERTIES

Refractive Indices (5893 A.). $n_X = 1.476$; $n_Y = 1.486$; $n_Z =$

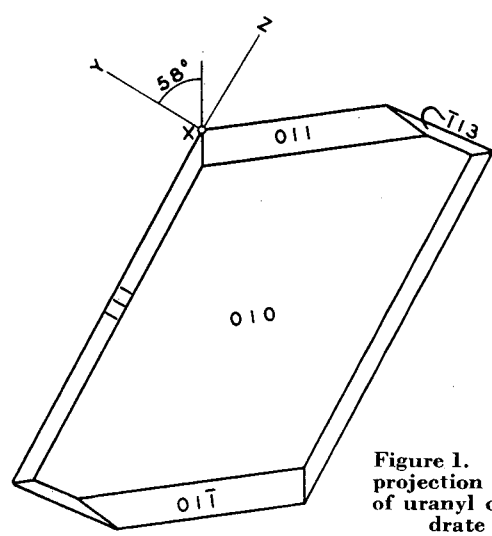

Figure 1. Orthographic projection of crystal of uranyl oxalate trihydrate on (010)

Partial Powder X-Ray Diffraction Pattern of Uranyl Oxalate

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]	*hkl*	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
020	8.52	8.47	85	$\bar{1}31$	3.80		
011	8.17	..	..	$\bar{1}12$	3.75	3.75	20
021	6.29	6.25	80	032	3.60	..	..
100	5.55	..	..	131	3.52	3.51	5
110	5.28	5.23	10	$\bar{1}22$	3.51		
$\bar{1}11$	4.89	4.87	55	140	3.38	..	..
031	4.85			102	3.34	3.34	10
002	4.66	4.62	35	$\bar{1}12$	3.28	3.28	35
$\bar{1}20$	4.65			141	3.27		
012	4.49	..	..			2.90	20
$\bar{1}21$	4.38	..	..			2.85	45
111	4.35	4.30	100			2.82	15
040	4.26	..	..			2.76	10
022	4.09	4.07	15			2.70	10
121	3.98	..	..			2.63	15
130	3.97	3.94	50			2.55	30
041	3.87					2.50	20
$\bar{1}02$	3.85	3.85	55				

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background, from densitometer measurements.

1.634; geometric mean 1.530_3. Molecular refraction 41.4 cc.
Optic Orientation. $X = b$; $Y \wedge c = 58°$.
Optic Axial Angle (5893 A.). $2V_Z = 31°$ with strong dispersion $r > v$.

Color. Yellow with absorption $Z > Y \backsimeq X$.
Fluorescence. Strong, excited by mercury vapor lamp.

Work done under the auspices of the Atomic Energy Commission.

133. Hendecahydrogen Diuranyl Pentaphosphate, $H_{11}(UO_2)_2(PO_4)_5$

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Hendecahydrogen diuranyl pentaphosphate was crystallized from a hot solution 7*M* in phosphoric acid and 1*M* in uranium dioxide. Chemical analysis. 54.1% UO_2, 45.8% PO_4, calculated 52.64% UO_2, 46.28% PO_4.

Crystal Morphology

System and Class. Monoclinic, prismatic.
Axial Elements. $a:b:c = 0.8143:1:0.5397$; $\beta = 113°1'$.

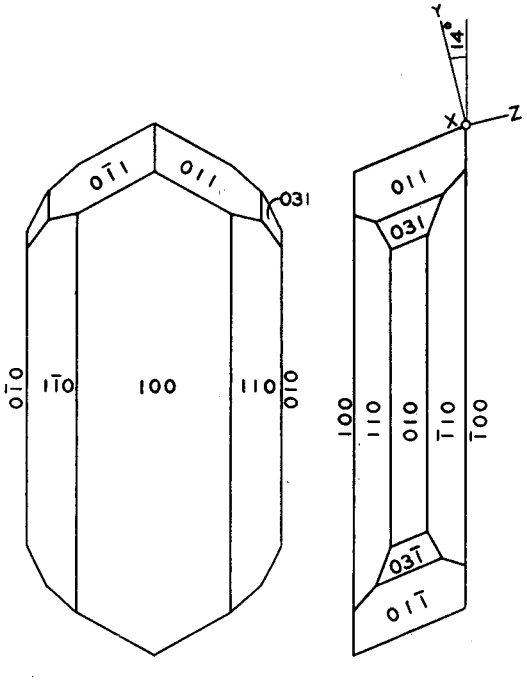

Figure 1. Orthographic projections on (100) and (010) of crystal of hendecahydrogen diuranyl pentaphosphate

Partial Powder X-Ray Diffraction Pattern of $H_{11}(UO_2)_2(PO_4)_5$

hkl	*d*, A., Calcd.	*d*, A., Meas.[a]	I/I_1[b]
110	8.35	8.32	25
020	6.97	6.91	20
011	6.15	6.14	100
200	5.21	5.20	15
$\bar{1}21$	5.00	4.97	50
021	4.89	4.89	<5
210	4.88		
030	4.65	4.67	<5
130	4.244	4.23	30
220	4.175	4.15	<5
121	4.007	4.00	20
$\bar{1}12$	3.598	3.59	15
$\bar{2}02$	3.595		
$\bar{2}31$	3.490		
040	3.485	3.49	10
$\bar{2}12$	3.481		
002	3.425	3.42	30
211	3.418		
310	3.372	3.36	10
131	3.371		
$\bar{3}21$	3.312	3.31	45
140	3.305		
$\bar{2}22$	3.195	3.19	<5
$\bar{1}41$	3.136	3.13	20
022	3.074	3.08	10
$\bar{2}41$	2.910	2.90	10
$\bar{1}32$	2.906		
$\bar{2}32$	2.843		
401	2.842	2.83	10
141	2.840		

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

Habit. Tabular {100}, elongated [001], bounded by {010}, {110}, {011}, {031}.
Polar Angles. (100) ∧ (110) = 36° 51′; (011) ∧ (0$\bar{1}$1) = 52° 50′; (100) ∧ (011) = 69° 30′.

X-Ray Diffraction Data

Space Group. $P\,2_1/c$ (C_{2h}^5).
Cell Dimensions. $a_0 = 11.37$ A.; $b_0 = 13.94$ A.; $c_0 = 7.47$ A.; $\beta = 113.5°$; $a_0:b_0:c_0 = 0.816:1:0.536$. Cell volume 1086 A.^{3}.
Formula Weights per Cell. 2.

Formula Weight. 1026.13.
Density. 3.14 grams per cc. (x-ray); 3.165 (flotation).

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.533$; $n_Y = 1.553$; $n_Z = 1.583$; geometric mean 1.5562. Molecular refraction 104.2 cc. (based on measured density).
Optic Orientation. $X = b$; $Y \wedge c = 14°$.

Optic Axial Angle (5893 A.). $2V_z = 76°$ with strong dispersion $r > v$.
Color. Yellow with moderate absorption $Z > Y > X$.
Fluorescence. Strong, excited by a mercury vapor lamp.
Thermal Data. Heated in air, crystals decompose at 84° C. This is evidenced by irregular interference colors, mottled extinctions and, eventually, clouding of crystals.

Work done under the auspices of the Atomic Energy Commission.

134. Pentapotassium Diuranyl Enneafluoride, $K_5(UO_2)_2F_9$

EUGENE STARITZKY, DON T. CROMER, and D. I. WALKER[1]
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Pentapotassium diuranyl enneafluoride was prepared by mixing dilute aqueous solutions of uranyl nitrate and potassium fluoride. Uranium by chemical analysis was 52.2%, calculated 52.52%.

Crystal Morphology

System and Class. Monoclinic, prismatic.
Axial Elements. $a:b:c = 3.215:1:1.877$; $\beta = 101° 14'$. Baker (*1*) described the crystals of this compound as triclinic with axial elements $a:b:c = 0.6222:1:0.568$; $\alpha = 72° 38'$; $\beta = 116° 23'$; $\gamma = 111° 57'$. Morphological, x-ray, and optical measurements show the symmetry to be monoclinic. Baker's measurements can also be reconciled with requirements of monoclinic symmetry by the transformation $(02\bar{1}/001/201)$ which converts the "triclinic" poles (010), (100), $(1\bar{1}0)$, (001), $(\bar{1}\bar{2}2)$, $(1\bar{1}\bar{1})$ to monoclinic poles (100), (001), $(\bar{1}01)$, $(\bar{1}11)$, $(\bar{3}10)$, $(\bar{1}11)$, respectively.

Partial Powder X-Ray Diffraction Pattern of $K_5(UO_2)_2F_9$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
200	9.71	..	..
110	5.85	5.74	<1
002	5.68	5.63	2
$\bar{2}02$	5.38	5.36	<1
$\bar{1}11$	5.33	5.27	4
111	5.08	5.03	1
400	4.85	4.80	2
202	4.54	4.48	<1
310	4.45	..	..
$\bar{3}11$	4.35	4.31	<1
$\bar{1}12$	4.20	..	..
$\bar{4}02$	4.10	4.07	3
311	3.97	3.96	4
112	3.96		
$\bar{3}12$	3.76	..	..
402	3.38	3.35	5
$\bar{5}11$	3.30	3.27	10
312	3.30		
510	3.28	3.25	3
$\bar{1}13$	3.27		
		3.04	<1
		3.00	4
		2.87	<1
		2.70	3
		2.68	1
		2.64	1
		2.58	2

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

Habit. Tabular {100} with {001}, {$\bar{1}$01}, {$\bar{1}$11}, {310}.
Polar Angles. (001) $\wedge$ (100) = 78° 46′; (001) $\wedge$ ($\bar{1}$01) = 32° 52′; (010) $\wedge$ ($\bar{1}$11) = 29° 49′.

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X-Ray Diffraction Data

Diffraction Symbol. $12/m1C\text{-}c\text{-}$. Observed development of crystal forms and failure to detect a piezoelectric effect make it probable that the space group is $C2/c$ (C_{2h}^6).

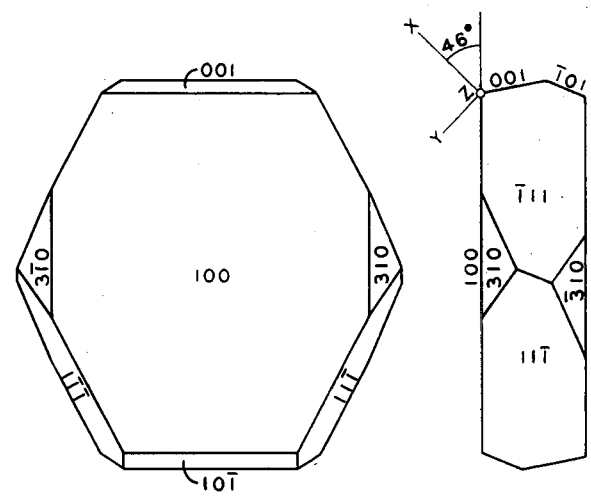

Figure 1. Orthographic projections on (100) and parallel to *b* of crystal of pentapotassium diuranyl enneafluoride

Cell Dimensions. $a_0 = 19.79$ A.; $b_0 = 6.13$ A.; $c_0 = 11.59$ A.; $\beta = 101.2°$; $a_0:b_0:c_0 = 3.23:1:1.89$. Cell volume 1379 A.^{3}.
Formula Weights per Cell. 4.
Formula Weight. 906.62.
Density. 4.37 grams per cc. (x-ray); 4.379 measured by Baker (*1*).

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.479$; $n_Y = 1.491$; $n_Z = 1.536$; geometric mean 1.5018. Molecular refraction 61.1 cc. (based on measured density).
Optic Orientation. $Z = b$; $X \wedge c = 46°$.
Optic Axial Angle (5893 A.). $2V_z = 61°$.
Color. Yellow without perceptible pleochroism.
Fluorescence. Strong, excited by mercury vapor lamp.

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Work done under the auspices of the Atomic Energy Commission.

Solubilities of Tetraphenylstibonium Salts of Inorganic Anions

Procedure for Solvent Extraction of Fluoride Ion from Aqueous Medium

SIR: Studies on tetraphenylstibonium salts (*2–4*) reveal solubility behavior which indicates possible usefulness of these compounds as reagents for inorganic analysis, particularly for the determination of fluoride. The starting material for the preparation of the salts was tetraphenylstibonium hydroxide (*4*), a compound which precipitates when ammonium hydroxide is added to a saturated aqueous tetraphenylstibonium bromide (*1, 6*) solution. Most of the salts were readily obtained from the hydroxide by treating it with the appropriate acids. The less soluble salts were also prepared by precipitation from a concentrated tetraphenylstibonium sulfate solution. Composition of the compounds listed in Table I was established by chemical analysis (*2, 4*). Solubilities were determined by shaking the compounds with pure solvent at constant temperature until saturation was achieved (*2, 4*).

As can be seen by reference to Table I, tetraphenylstibonium sulfate is highly soluble in water. Nitrate, chloride, bromide, iodide, fluoride, and perchlorate ions, however, form relatively insoluble tetraphenylstibonium salts and may on this account be more or less quantitatively precipitated from aqueous solution by addition of tetraphenylstibonium sulfate. Tetraphenylstibonium ion thus offers a possible advantage over some other reagents commonly used for precipitation of fluoride, in that it may be used in solutions containing sulfate ion. The solubility of tetraphenylstibonium fluoride in water, however, is still too high to allow good quantitative recovery of fluoride by precipitation.

Table I. Solubilities of Tetraphenylstibonium Compounds

(Grams of solute per 100 grams of solvent)

Compound	Solvent	
	H_2O (29°)	CCl_4 (31°)
$(C_6H_5)_4SbF$	0.064	10.3
$(C_6H_5)_4SbCl$ (*5, 6*)	1.68	2.4
$(C_6H_5)_4SbBr$ (*1, 6*)	1.20	1.08
$(C_6H_5)_4SbClO_4$ (*5*)	0.008	<0.001
$(C_6H_5)_4SbNO_3$	1.10	0.14
$(C_6H_5)_4SbOH$	0.005	...
$[(C_6H_5)_4Sb]_2O$[a]	...	0.84
$[(C_6H_5)_4Sb]_2SO_4$	>60	0.16

[a] Prepared by heating hydroxide at 110°.

The possibility of separating fluoride from sulfate by solvent partition was accordingly investigated. A saturated, aqueous tetraphenylstibonium fluoride solution was shaken with carbon tetrachloride (at 31° C.) until equilibrium was attained. The ratio of concentration of tetraphenylstibonium fluoride in the carbon tetrachloride phase to concentration of tetraphenylstibonium fluoride in the water phase, under the conditions specified, was found to be 16.5. That sulfate is not extracted to any appreciable extent under these conditions was demonstrated by experiments in which sodium sulfate alone and sodium sulfate–sodium fluoride mixtures containing up to 500 times as much sulfate as fluoride were carried through the extraction procedure. In these experiments the sodium sulfate was dissolved in water. A few drops of dilute sulfuric acid, 0 to 0.200 mmole of fluoride (as NaF) and 0.25 mmole of $(C_6H_5)_4Sb^+$ (as tetraphenylstibonium sulfate) were added. The final volume in each case was 19 ml. Extraction was made by shaking the aqueous solution with three 5-ml. portions of carbon tetrachloride. The combined extracts were evaporated. The residue was weighed, and the fluoride equivalent was calculated on the assumption that the residue was $(C_6H_5)_4SbF$. Fluoride recoveries ranged from 97 to 98%. The blank on 1000 mg. of sodium sulfate, treated as above but with no fluoride added, was 0.02 mg. of fluoride.

The effect of a number of foreign ions on the fluoride extraction was investigated. Iron(III) and aluminum(III) when present in excessive amount inhibited the extraction. Chloride and bromide, which are themselves extracted to some extent, led to high results. It was shown, however, that 99.2% recovery of fluoride could be obtained if chloride was first removed by precipitation with excess silver.

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RECEIVED for review October 10, 1955. Accepted May 7, 1956.

CORRESPONDENCE

"Standard Addition" of Method Polarographic Analysis

An Alternate Interpretation

SIR: In the January 1956 issue of ANALYTICAL CHEMISTRY there appears a communication by L. Meites (*4*), in which is derived an equation relating the error in the standard addition method of polarographic analysis to the diffusion currents. From this equation it is concluded that "maximum precision is attained when the diffusion current of the unknown is negligibly small compared to that obtained after adding the standard." Here an alternative derivative is proposed which leads to somewhat different conclusions.

Meites assumes that the error in determining the sample con-

centration is due entirely to errors in i_1 and i_2, the diffusion currents before and after the standard addition, respectively. It is apparent, however, that a noticeable contribution is due to the error in the residual current i_0. This is so because in this type of analysis there is no way to measure i_0 directly and it must be assumed equal to zero, extrapolated from the residual before the diffusion plateau, or estimated from the residual of solutions similar to the sample. By retaining this i_0 term and assuming, as did Meites, that the volume of the standard is negligible compared to that of the sample, it can be shown easily that

$$C_s = K\left(\frac{i_1 - i_0}{i_2 - i_1}\right) \tag{1}$$

where C_s is the concentration of the sample and k is a constant which includes the concentration of the standard and the volumes of sample and standard.

The standard deviation of C_s can be expressed approximately by an equation of the following form (*1*)

$$S_s^2 \approx \left(\frac{\partial C_s}{\partial i_0}\right)^2 S_0^2 + \left(\frac{\partial C_s}{\partial i_1}\right) S_1^2 + \left(\frac{\partial C_s}{\partial i_2}\right)^2 S_2^2 \tag{2}$$

where S_s is the standard deviation of C_s, and S_0, S_1, and S_2 are the standard deviations of i_0, i_1, and i_2, respectively.

The relative error of C_s, denoted by E_s, is obtained by substituting Equation 1 into 2 and dividing both sides of the result by C_s^2.

$$E_s^2 \approx \frac{(i_2 - i_0)^2 S_1^2}{(i_2 - i_1)^2 (i_1 - i_0)^2} + \frac{S_2^2}{(i_2 - i_1)^2} + \frac{S_0^2}{(i_1 - i_0)^2} \tag{3}$$

To simplify this equation, i_0 can be assumed to be negligible compared to i_1 and i_2 and can be dropped.

In order to determine the conditions under which E_s is a minimum, it is necessary to make some assumptions concerning the relationships of S_0, S_1, and S_2 to i_0, i_1, and i_2. Previous authors (*2, 3, 5*) evidently assume, although the assumption is never made explicitly, that S_1 and S_2 are independent of i_1 and i_2 and conclude that the ratio of i_2 to i_1 should be about 2. This assumption attributes the error in i_1 and i_2 to such factors as electroactive impurities and charging current, and neglects the appreciable contribution to the error from such factors as temperature and fluctuation of mercury height. Considering the latter factors of primary importance leads to the assumption that not S_1 and S_2 but the relative errors, S_1/i_1 and S_2/i_2, are independent of i_1 and i_2. This assumption was made by Meites. Both approaches can be combined by assuming that S_0, S_1/i_1, and S_2/i_2 are independent of i_0, i_1, and i_2. Because S_1/i_1 and S_2/i_2 are measures of the same quantity, affected in both cases by the same factors, they are indistinguishable and can be represented by a common symbol, E_i. From these considerations the following simplification of Equation 3 results.

$$E_s^2 \approx \frac{2i_2^2 E_i^2}{(i_2 - i_1)^2} + \frac{S_0^2}{i_1^2} \tag{4}$$

From this expression it is apparent that the optimum values of i_1 and i_2 depend on the relative values of E_i and S_0. There are two cases of practical interest. The first is the case in which i_1 is fixed; the second is that in which i_2 is fixed. In all cases the accuracy can be increased without bound by simply increasing i_1 and $(i_2 - i_1)$ until one or both of these limits is encountered. Hence all cases reduce to one of these two. While in theory it might be argued that i_1 can always be varied, in practice it is often not feasible for a variety of reasons. If it is not feasible to vary i_1, then E_s can be minimized only by increasing i_2. However, if i_1 can be adjusted, the optimum ratio of i_2 to i_1 can be estimated in the following manner. Obviously i_2 will be made as large as possible, in order to minimize the first term in Equation 4. In general, however, there is some natural limit to the size of i_2, due either to deviations from the Ilkovič equation or to the limitations of the current detector. Designating this limit as i_m and setting i_2 equal to it, and further substituting α for the ratio i_2/i_1, Equation 4 becomes

$$E_s^2 \approx 2E_i^2\left(\frac{\alpha}{\alpha - 1}\right)^2 + \frac{\alpha^2 S_0^2}{i^2} \tag{5}$$

The conditions for minimum error can be determined by differentiating Equation 5 with respect to α and setting the result equal to zero, bearing in mind the earlier assumptions that E_i and S_0 are independent of i_1 and i_2 and hence of α. The following equation for optimum α results.

$$\alpha_0 \approx 1 + 1.26\left(\frac{E_i\, i_m}{S_0}\right)^{2/3} \tag{6}$$

In Table I a comparison is given of the relative errors calculated from Equation 5 using $\alpha = 10$ as recommended by Meites, $\alpha = 2$ as recommended by the earlier workers, and α_6 calculated from Equation 6. E_i was set equal to 1% in cases I and II and 0.1% of i_m in case I and 1% in cases II and III.

Table I. Relative Error of Sample Concentration under Several Representative Conditions Using α's Recommended by Various Authors

	% Error		
α	I	II	III
10	1.8	10.1	10.0
2	2.8	3.5	2.0
α_0	1.8	3.4	1.4

The analyst in the practical situation is probably inclined to accept i_1 at face value and to make his standard addition sufficient to make $i_2 = i_m$. Whether the accuracy gained in the optimization of α outweighs the bother of the manipulations involved depends on the specific case. The analyst can readily determine α_0 from Equation 6 and its associated error from Equation 5. Comparing this error to that obtained with the α of the ordinary procedure should decide the question.

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MEETING REPORTS

Society for Analytical Chemistry

THE Scottish Section of the society met May 11 in Edinburgh to hear a talk by R. E. Stuckey, British Drug Houses, Ltd., London, on recent developments in complexones.

Following the introduction into analysis of a number of complexing agents by Schwarzenbach, ethylenediaminetetraacetic acid (EDTA) in particular has become increasingly used. Some methods available for the determination of metal cations using EDTA were discussed and the methods of end point determination were reviewed.

The use of a new complexing agent, 1,2-diaminocyclohexanetetraacetic acid, which forms more stable chelates than EDTA, has further extended the analytical applications of complexones, and new indicators such as pyrocatechol violet and metalphthalein have been introduced.

End point determination in EDTA titrations often presents difficulty and the applications of high frequency titration methods is of great value. The principles of high frequency methods were briefly described and simple types of apparatus were shown. Conditions necessary for EDTA titrations were described and results obtained by different methods were discussed.

At a joint meeting of the Food Group of the Society of Chemical Industry and the Society for Analytical Chemistry, held May 23 in London, two papers were presented and discussed.

Some New Factors in Pectin Gel Strength. MAMIE OLLIVER, P. WADE, AND KATHLEEN P. DENT, Chivers & Sons, Ltd., Cambridge.

Gels prepared from rapid setting powdered pectins by boiling in the presence of sugar and acid to pH 3.1 and total soluble solids content of 70% have shown increased strength in the presence of small quantities of some surface-active agents, and larger quantities of depectinized fruit juices. The degree of strength increase appears to be related to the setting temperature of the pectin, there being little change in strength with slow setting samples. In the case of gels made from concentrated extract of pomace the pattern is not constant but, in general, no marked change in strength is found even with rapid setting extracts. These results have been used for a critical examination of the British (acid in boil) and American (acid in glass) methods for the strength grading of pectins. Various anomalies between the two procedures have been resolved and a modified strength grading method was proposed which gives results more in line with practical jam manufacture.

Binding of Ions and Detergents to Pectin, Protein, and Other Colloid Systems. B. A. PETHICA, Department of Colloid Science, University of Cambridge.

The binding of ions to lyophilic colloids has been studied by electrophoresis (particularly with the colloids adsorbed on microscopic particles), equilibrium dialysis, solubility, and viscosity methods, and calorimetry. The bulk of the data was for protein and polyelectrolyte systems, but sufficient was available for pectin colloids to allow interpretation of the gel properties of pectins in relation to charge and ionic additives, including detergents. The relevance of hydrogen bonding and van der Waals' interaction may also be suggested from work on these forces in related systems such as aluminum soap gels.

A joint meeting of the Microchemistry Group and the North of England Section of the Society for Analytical Chemistry and the Bradford Chemical Society was held May 25 at Bradford, when three papers on microvolumetric analyses were presented and discussed.

Apparatus and Technique. D. W. WILSON, Department of Chemistry, Sir John Cass College, London.

Some types of apparatus for the delivery and titration of small volumes of liquids were described. Their manipulation, accuracy, and the kinds of error to which they were subject were discussed.

Primary Standards. R. BELCHER, Department of Chemistry, University of Birmingham.

The requirements of primary standards for use in microanalysis were discussed. New observations were made concerning old-established standards with special reference to those used in standardizing solutions for completing determinations in organic microanalysis. A brief survey was given of new primary standards, which appear to be of promise in both organic and inorganic microanalysis.

End Point Location. E. BISHOP, Department of Chemistry, University of Exeter.

Methods of locating the end point of a titrimetric process were enumerated and briefly examined: use of principal and ancillary reagents (color, adsorption, fluorescent, and precipitation indicators) observed visually or photoelectrically, electrical methods such as potentiometry, amperometry, and low and high frequency conductometry, and miscellaneous techniques based on properties of electrode systems.

Such methods may be applied on any scale, but physical difficulties of observation of color change, etc., or of accommodation of electrodes in the available solution volume, render the choice less wide as the operating scale is progressively reduced. Problems arising in small scale work were mentioned, and application of the differential electrolytic potentiometric technique to the ultramicro scale was briefly reported.

The Physical Methods Group met with the Photoelectric Spectrometry Group at Oxford on May 25 to discuss nuclear and paramagnetic resonance.

Analytical Applications of Nuclear Resonance Spectroscopy. R. RICHARDS, Lincoln College, Oxford.

A short description was given of what happens when a nuclear resonance spectrum is excited. Some of the factors which determine adsorption line shape, width, and intensity were discussed with particular reference to the analytical applications of the method. The nature of the "chemical shift and "multiplet interactions" which are observable under conditions of high resolution was described. The scope and limitations of the method for various types of analysis, as they appear at present, were discussed and illustrated with representative examples.

Techniques of Magnetic Resonance Spectroscopy. E. E. SCHNEIDER, King's College, University of Durham.

Electron spin magnetic resonance (usually described simply as "paramagnetic resonance") is observed with paramagnetic atoms, ions, or molecules and is associated with unpaired electron spins. Nuclear magnetic resonance is observed in diamagnetic materials and is entirely due to the magnetism of the atomic nuclei. These phenomena can be understood most easily by considering the Larmor precession of classical magnetic gyroscopes in a constant magnetic field. A resonant absorption of magnetic energy occurs if a high frequency magnetic field applied normal to the constant field is in resonance with the precession frequency. Because of the different order of magnitude of the magnetic moments of electrons and nuclei, electron spin resonance at a constant magnetic field of some kilogauss occurs at microwave frequencies, while nuclear resonance frequencies lie in the radio-frequency range. The aspects of magnetic resonance studies which are of interest to the chemist were surveyed and the relevant experimental techniques were described. A number of special experimental problems were discussed in detail: observation of paramagnetic resonance in aqueous solutions, quantitative determination of the concentration of unpaired electron spins and magnetic nuclei from the intensity of paramagnetic and nuclear resonance absorption, respectively, and accurate measurement and analysis of complex paramagnetic resonance spectra of organic radicals arising from the hyperfine interaction of the unpaired electrons with neighboring nuclei.

Detection of Photochemically Formed Radicals by Magnetic Resonance. D. J. E. INGRAM, University of Southampton.

The technique of electron resonance has been employed for some time not only to observe the state and presence of normal paramagnetic atoms but also to study organic free radicals. Until recently the work on free radicals had been confined to known stable compounds. Measurements were outlined which show that the technique can be extended to cover many different types of radicals formed by breakage of bonds with ultraviolet irradiation. This method can be applied generally to any system in which the resultant radicals can be trapped and observed after their production, or alternatively can be observed during irradiation. In this way OH radicals trapped in frozen water-peroxide solutions have been studied, as well as several different organic radicals formed from ethyl iodide, benzyl chloride, and similar compounds.

Trapped radicals can also be formed by x- and γ-ray irradiation and in both this and the ultraviolet experiments, a marked hyperfine structure is often obtained. The occurrence of such a hyperfine structure is one of the powerful analytical tools of electron resonance, as it gives immediate identification of the particular atoms involved, even if they are present in concentrations of only $10^{-9}M$ or lower.

Absorption-Titration Flask for Determination of Sulfur in Steel

Milton Roth and Seymour Lader, General Laboratory Section, Picatinny Arsenal, Dover, N. J.

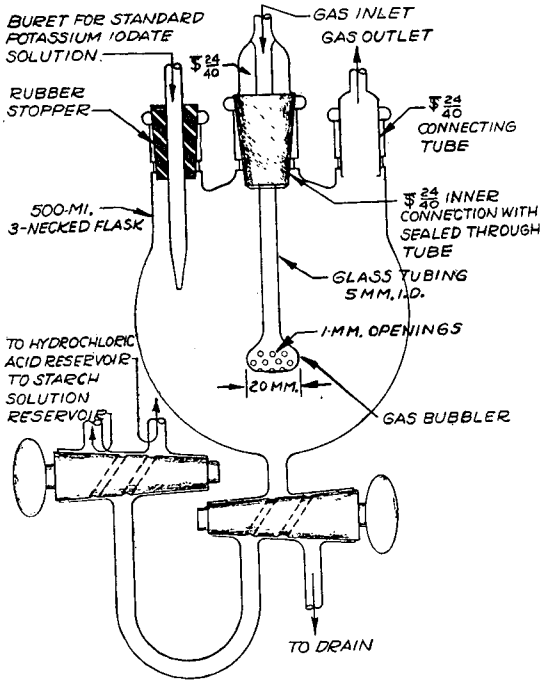
RECENTLY [Holler, A., Klinkenberg, R., Friedman, C., Aites, W. K., ANAL. CHEM., **26**, 1658 (1954)] there was described a combined method for the determination of sulfur and carbon in steel. The gases from the combustion of the ferrous metals in oxygen are passed through a suitable train, so that sulfur dioxide is dissolved in an acid solution contained in an absorption-titration flask, and the carbon dioxide is absorbed by Ascarite. Although an absorption-titration flask assembly is commercially available, it is relatively expensive and the source of supply is limited. A suitable flask can readily be fabricated from the following pieces of glassware.

Quantity	Description
1	Three-necked flask, 24/40 necks, 500-ml. capacity
2	Three-way stopcock with double oblique bore
1	Inner connection with sealed-through tube, 24/40
1	Connecting tube with 24/40 inner joint

By relatively simple glass blowing, these items are assembled as shown in the diagram.

The bubbling tube is made from a piece of glass tubing and is attached to the inner connection as shown. The bubbling tube serves to diffuse the combustion gases throughout the sulfur dioxide–absorbing solution. A conventional fritted-glass diffusion tube was found unsatisfactory for obtaining a suitable gaseous flow rate.

The operation of the flask is simple and efficient. The required reagents for the iodometric determination of sulfur are hydrochloric acid for absorption of sulfur dioxide, starch indicator, and standard potassium iodate solution (Am. Soc. Testing Materials, "Methods of Chemical Analysis of Metals," p. 129, 1950). The acid and indicator are admitted to the flask from a reservoir by means of the double stopcock arrangement. The tip of the buret is inserted into the flask and the sample is titrated while the gas is absorbed in the hydrochloric acid solution. Three determinations can be conducted in the same solution and then the vessel drained by means of a stopcock provided for that purpose.



This apparatus has been in use for the past 3 years and has been found convenient and time-saving.

Results Obtained with Apparatus

NBS Standard Sample No.	Carbon, %		Sulfur, %	
	Nominal	Found	Nominal	Found
9d	0.20	0.21	0.037	0.036
15d	0.10	0.12	0.034	0.032
16c	1.01	1.03	0.042	0.041
20d	0.41	0.41	0.093	0.093

Tris(hydroxymethyl)aminomethane as Standard Alkali in Acidimetric Combustion Method for Determining Sulfur

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THE acidimetric combustion method for determining sulfur (*2*, *3*) suffers from the disadvantage that a 0.01*N* sodium hydroxide solution is used in the titration of the sulfuric acid. Standard 0.01*N* sodium hydroxide is tedious to prepare. It has poor storage life, even though the storage bottle is equipped with a soda-lime tube to keep out the carbon dioxide of the air.

A literature survey indicated that tris(hydroxymethyl)-aminomethane (*1*) would be ideally suited for use as the standard alkali in the acidimetric combustion method for sulfur. It is a crystalline solid that can be prepared in high purity. Its hygroscopicity is comparable to that of common primary standards. Neither the pure compound nor its solutions absorb carbon dioxide from the air. The pH of its equivalence point is 4.7, which compares favorably with the end point of pH 5.3 taken in the acidimetric combustion method. Standard solutions of tris(hydroxymethyl)aminomethane are stable and easy to prepare. They have been used in this laboratory for 2 years and have given consistent results.

Tris(hydroxymethyl)aminomethane, known also as 2-hydroxy-2-amino-1,3-propanediol or trimethylolaminomethane, can be obtained from Matheson, Coleman and Bell, East Rutherford, N. J. (Catalog No. 7060).

LITERATURE CITED

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Stirrer Assembly for Use under Pressure

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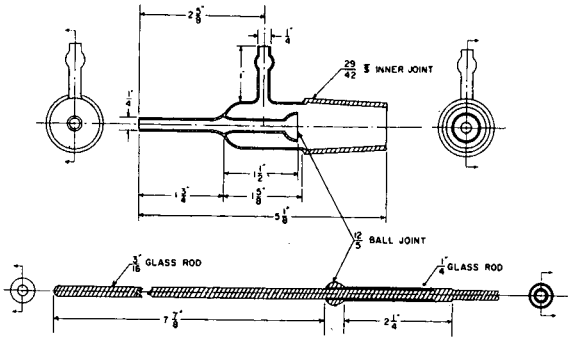
WHILE studying certain chemical reactions under slight positive pressure, up to about 20 pounds per square inch, difficulty was encountered in finding a satisfactory stirrer assembly. Several excellent assemblies designed for use with

high pressure autoclave equipment are available (*1*), but pressure stirrer seals for use with laboratory glassware apparatus are both scarce and unsatisfactory (*3*).

This note describes an adaptation of the semiball joint stirrer seal described by Patton (*2*) for use under pressure.

The seal assembly, shown in the diagram, consists of two pieces, the stirrer shaft and the housing. The ball portion of a 12/5 semiball joint is attached to the stirrer shaft, while the socket portion of the semiball joint is fastened within the housing as shown. The shaft may conveniently be attached to the ball portion of the joint by slipping the shaft through the joint and sealing as shown in the diagram. The housing contains a gas inlet tube and a standard-taper joint to fit the desired reaction vessel.



When the apparatus is assembled for use, the pressure seal is made at the union between the ball and socket portion of the semiball joint. As the pressure on the system is increased, the joint is firmly pressed together, forming a leakproof seal. This union is lubricated with a few drops of silicone oil. Adequate power for stirring is furnished by laboratory-type stirrer motors.

This seal was successfully used under pressures up to 20 pounds per square inch; higher pressures were not investigated. In several experiments a constant pressure of about 65 cm. of mercury was maintained for 24 hours.

ACKNOWLEDGMENT

The authors are indebted to W. N. DeWolff for the glass blowing and to E. E. Beals for preparation of the drawing.

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Short-Light-Path Absorption Cell for Routine Colorimetry

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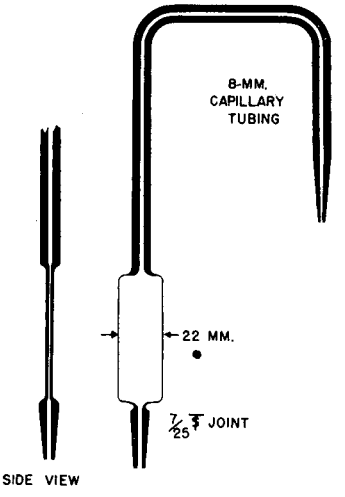
When routine dilutions are required in colorimetric analysis, faster and more accurate measurements can be made with a suitable short-light-path absorption cell. Such a cell, designed and constructed for use in an Evelyn photoelectric colorimeter [as used in the author's method of estimating protein (*Cereal Chem.*, in press)], is described here. Modifications in certain dimensions will permit its use in other photoelectric colorimeters as well as for several other lengths of light path.

The schematic drawing illustrates the features of the cell.

Two flat plates of borosilicate glass (2 mm. in thickness) were cut to the desired dimensions and ground along their long edges to about a 45° angle. Rolled strips of copper wire (0.007 inch in thickness) were placed between the glass plates to act as spacers. In order to secure the position of the plates and spacers, each end of the assembly was wrapped with nickel wire. A strip of glass rod, drawn out to approximately 0.5-mm. diameter, was fused along the gap at each side. The copper wire spacers were then removed with nitric acid. A piece of capillary tubing was attached to one end, and a standard-taper joint was sealed to the other. Space at the ends was kept at a minimum, so that only a small sample of liquid (slightly less than 1 ml.) was needed to fill the cell.



By securing a 7/25 standard-taper receptacle joint in the lower end of the tube holder (by means of a conveniently adapted rubber stopper), the absorption cell may be placed in or taken out of the tube holder easily. A length of capillary tubing was used to connect the receptacle joint to a stopcock and water trap. The trap was connected to a water aspirator, which provided a partial vacuum for pulling the sample liquid through the absorption cell.

Irregularities in the cell spacing are of no consequence, as the cell can easily be returned to the same position. This was facilitated by replacing one of the small screws in front of the tube holder by a longer screw to provide a fixed position for the horizontal capillary arm of the cell.

Formation of small air bubbles in the cell presented a major problem, until it was learned that flushing the cell with 1 or 2 ml. of a solution containing a small amount of *n*-capryl alcohol in acetone will prevent the formation of bubbles. A 0.5% solution is ample. Apparently a trace of alcohol is left on the glass surfaces, sufficient to prevent the formation of any bubbles when water is drawn through the cell.

In practice, only one flushing at the start of a series of measurements is required. Cleaning between samples is unnecessary, and the cell is kept full of liquid by closing the stopcock after a few milliliters of a new undiluted sample is run through the cell.

ACKNOWLEDGMENT

The author is indebted to George E. Harris, glassblower at the State College of Washington, for making the cell.