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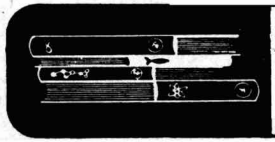

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Briefs

Note: In Vol. 12, No. 1, an incorrect brief was printed for the article, **Methods for the Isolation and Characterization of Constituents of Natural Products, Part II,** by D. P. Schwartz and C. R. Brewington, pp. 1–6. The correct brief should read

Methods for the Isolation and Characterization of Constituents of Natural Products. II. Separation of Homologous Series of Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone by Thin-Layer Chromatography. D. P. Schwartz and C. R. Brewington, *Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Washington, D. C. 20250.*

Esters of homologous series of primary, secondary, and tertiary alcohols with pyruvic acid 2,6-dinitrophenylhydrazone have been separated by thin-layer partition chromatography. Approximately 1.0×10^{-4} μmole of an ester can be detected.

Microchem. J. 12, 1 (1967).

Chelatometric Determination of Chromium(III). L. Szekeres, E. Kardos, and G. L. Szekeres, *Chemical Institute of Veterinary University, Budapest, Hungary.*

A neutral or weakly acid solution of Cr(III) is treated with acetate buffer and freshly filtered $KMnO_4$ solution. Equivalent amounts of soluble chromate and insoluble MnO_2 are formed in a short time. The MnO_2 is quantitatively collected on a fritted glass filter and washed. After dissolution with ascorbic acid the Mn^{2+} ions are titrated with EDTA. Large relative amounts of Zn, Cd, or Cu do not interfere since remaining traces in the titrate may be easily masked.

Microchem. J. 12, 147 (1967).

Schematic Qualitative Analysis of Cations by the Ring Oven Technique. R. Chatterjee and Arun K. Dey, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

The schematic separation of 20 common cations, viz., silver, mercury, lead, copper, bismuth, cadmium, arsenic, antimony, tin, iron, chromium, aluminum, zinc, cobalt, nickel, manganese, barium, strontium, calcium, and magnesium by the ring oven method with 8-hydroxyquinoline and sodium diethyldithiocarbamate used as group reagents, has been described. The cations are identified by spot test using organic reagents.

Microchem. J. 12, 151 (1967).

Separation and Microdetermination of Aluminium, Gallium, Indium, and Thallium in a Drop of Mixture. ANIMESH K. GHOSE AND ARUN K. DEY, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

The ring oven method, combined with solvent extraction technique, has been used to separate aluminium, gallium, indium, and thallium on filter papers circles. Thallium was first transferred to the ring zone by washing with chloroform-acetone mixture. Indium was made to migrate to the ring zone as the pyridine complex, while gallium was washed as acetylacetonate to the ring zone. Aluminium acetylacetonate remained fixed at the center and was lastly eluted to the ring zone by washing with a mixture of 60% ethanol and 3*N* acetic acid.

Microchem. J. **12**, 157 (1967).

4-(2-Pyridylazo) Resorcinol as a Sensitive Reagent for Vanadium, Niobium and Tantalum. BADRI VISHAL AGARWALA, AND ARUN K. DEY, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

The formation of red colored chelates between vanadium, niobium and tantalum with 4-(2-pyridylazo)resorcinol (PAR) has been reported. Adherence to Beer's law is observed over a wide range of metal concentrations; the color intensities are also stable within a wide range of pH, particularly in case of Nb-PAR chelate. The reagent is highly sensitive, especially for vanadium.

Microchem. J. **12**, 162 (1967).

Composition and Stability of the Rare Earth Chelates of Ammonium Aurintricarboxylate (Aluminon) in Aqueous Solution. SATENDRA P. SANGAL AND ARUN K. DEY, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

The characteristics of the colored chelates of rare earth metals with Aluminon have been described in detail. The studies include the determination of molar ratio by three different methods, the range of pH for the stability of the chelate and the conditional stability constants by three methods.

Microchem. J. **12**, 168 (1967).

Hot-Flask Determination of Chlorine. WOLFGANG J. KIRSTEN, BJÖRN DANIELSON, AND ELFI ÖHRÉN, *Department of Chemistry, Royal Agricultural College of Sweden, Uppsala, Sweden; and Department of Medical Chemistry, University of Uppsala, Uppsala, Sweden.*

The hot-flask combustion-diffusive absorption method for the decimilligram determination of halogen has been improved by the addition of 1 μl of orthophosphoric acid to the sample before the combustion. Alkali- and other ash-containing as well as inorganic compounds can now be easily analyzed. It was applied to the determination of chlorine with automatic differential electrolytic potentiometric titration.

Microchem. J. **12**, 177 (1967).

Methods for the Isolation and Characterization of Constituents of Natural Products. III. Separation of Alcohol Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone into Classes by Column and Thin-Layer Chromatography. D. P. SCHWARTZ, C. R. BREWINGTON, AND JENNIE SHAMEY, *Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, US. Department of Agriculture, Washington, D.C. 20250.*

Quantitative column and qualitative thin-layer chromatographic procedures are described for separating a mixture of the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of primary, secondary, and tertiary aliphatic alcohols into classes. Magnesium oxide is used as the adsorbent in both procedures and separation of the classes follows a similar pattern. The derivatives change color from yellow to violet or blue on the adsorbent. Tertiary alcohol derivatives above butyl show a blue color whereas tertiary butyl and the primary and secondary alcohol derivatives are violet. Isomeric derivatives can also be separated by thin-layer chromatography on aluminum oxide G by using a solvent system containing a strong organic base.

Microchem. J. **12**, 186 (1967).

Methods for the Isolation and Characterization of Constituents of Natural Products. IV. Amide Derivatives of Amines with Pyruvyl Chloride 2,6-Dinitrophenylhydrazone. D. P. SCHWARTZ AND C. R. BREWINGTON, *Dairy Products Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C. 20250.*

Preparation of amide derivatives of primary and secondary aliphatic amines with pyruvyl chloride 2,6-dinitrophenylhydrazone is described. The derivatives form within 5 minutes at room temperature in benzene solution. Primary amine derivatives are yellow, and all members investigated (normal C_1-C_{18}) are solids. The primary amine derivatives have an absorption maximum at 407 mμ in benzene and have a molar absorptivity near 5941. Secondary amine derivatives are orange, and all members investigated (symmetrical unbranched C_2-C_{10}) are also solids. The secondary amine derivatives have an absorption maximum at 412 mμ in benzene and have a molar absorptivity near 5367. Both classes of amine derivatives have properties which facilitate their isolation directly from lipids.

Microchem. J. **12**, 192 (1967).

Relationship between Accuracy and Sensitivity in Atomic-Absorption Flame Photometry. JUAN RAMÍREZ-MUÑOZ, *Beckman Instruments, Inc. Fullerton, California 92634.*

Instrumental limitations of accuracy based on the absorptimetric nature of atomic-absorption methods are discussed. A summary of other factors influencing accuracy in practical work is given, and some comments are included on the limitations on accuracy caused by sensitivity limitations.

Microchem. J. **12**, 196 (1967).

Lead(IV) and Thallium(III) Acetates in Trifluoroacetic Acid as Spot Test Reagents for Aromatic Compounds. HARVEY W. YUROW AND SAMUEL SASS, *Analytical Chemistry Department, Chemical Research Laboratory, Research Laboratories, U.S. Army Edgewood Arsenal, Maryland.*

Reagents consisting of lead(IV) or thallium(III) acetate in trifluoroacetic acid give intense colors with a number of aromatic compounds. The former is useful for identification of many polynuclear and polyphenyl hydrocarbons and their derivatives as well as aryl amines and methoxy ethers. The latter reagent gives positive tests with a smaller number of polynuclear hydrocarbons and with most phenols.

Microchem. J. **12**, 207 (1967).

Microdetermination of Organic Oxygen by Means of Optical Integration of Liberated Iodine in Vapor Phase. KEIICHIRO HOZUMI, *Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.*

An automatic apparatus for the microdetermination of oxygen in organic compounds is described, which introduces the instrumental technique of optical integration of the iodine, in the vapor phase, liberated from iodine pentoxide. The light absorption at 530 mμ is measured by means of a photoelectric system. Fifteen minutes are required for an analysis.

Microchem. J. **12**, 218 (1967).

Systematic Applications of Gas-Liquid Chromatography in Toxicology. I. Extraction Procedure and the Alkaloids. NARESH C. JAIN AND PAUL L. KIRK, *School of Criminology, University of California, Berkeley, California.*

A rapid method is described for the extraction of a number of alkaloids from blood after ingestion of lethal or near-lethal doses, and their subsequent gas chromatographic identification.

Microchem. J. **12**, 229 (1967).

Systematic Applications of Gas-Liquid Chromatography in Toxicology. II. The Antihistamines. NARESH C. JAIN AND PAUL L. KIRK, *School of Criminology, University of California, Berkeley, California.*

This is the second paper of this series and deals with the application of the techniques previously described to antihistamines.

Microchem. J. **12**, 242 (1967).

Systematic Application of Gas-Liquid Chromatography in Toxicology. III. The Barbiturates. NARESH C. JAIN AND PAUL L. KIRK, *School of Criminology, University of California, Berkeley, California.*

This paper of the series deals with the application of the techniques previously described to barbiturates.

Microchem. J. **12**, 249 (1967).

Systematic Applications of Gas-Liquid Chromatography in Toxicology. IV. The Tranquilizers. NARESH C. JAIN AND PAUL L. KIRK, *School of Criminology, University of California, Berkeley, California.*

This is the fourth paper of this series dealing with the application of the techniques previously described to tranquilizers.

Microchem. J. **12**, 256 (1967).

Systematic Applications of Gas-Liquid Chromatography in Toxicology. V. The Pesticides and Evaluation of Toxic Levels. NARESH C. JAIN AND PAUL L. KIRK, *School of Criminology, University of California, Berkeley, California.*

This, the fifth paper of the series, applies the techniques previously described to pesticides.

Microchem. J. **12**, 265 (1967).

A Semiautomated Method and Apparatus for Micromethoxyl Determination. N. N. SHAH, M. F. GOBEILLE, AND D. F. MOWERY, JR., *Department of Chemistry, Southeastern Massachusetts Technological Institute, North Dartmouth, Massachusetts.*

The apparatus and procedure of Steyermark for determination of alkoxyl groups on a micro scale has been modified and semiautomated so as to yield results of research accuracy in student hands. The major modifications consist of redesign of the equipment to provide greater durability, easier construction and repair, and better scrubbing action without increasing the internal gas volume appreciably. Also, the time cycle has been automatically controlled and reduced from 1½ hours to 1 hour.

Microchem. J. **12**, 273 (1967).

MICROCHEMICAL JOURNAL **12**, 147-150 (1967)

Chelatometric Determination of Chromium(III)

L. SZEKERES, E. KARDOS, AND G. L. SZEKERES

Chemical Institute of Veterinary University, Budapest, Hungary

Received July 13, 1966

Chromium(III) ions react slowly with EDTA to form a stable complex (*7, 15*). For this reason, all chromium(III) determinations to date were run indirectly (*1-3, 6-12, 14-17*). The indirect method consists essentially of boiling the sample with excess EDTA to accelerate complexation, then titration of excess EDTA.

Chromium(III) ions are easily oxidized to chromate with manganate or permanganate and determined as chromate (*4, 13*).

Oxidation in neutral or acetate-buffered solution is described by reaction [I]; manganese dioxide is formed (*13*).

$$Cr^{3+} + MnO_4^- + 4\ OH^- \longrightarrow CrO_4^{2-} + MnO_2 + 2\ H_2O. \qquad [I]$$

The stoichiometric relationship between Cr(III) and MnO_2 allow an indirect quantitative determination of Cr(III) via a manganese determination.

A microdetermination of Cr(III) was worked out based upon this principle as follows:

A solution containing 0.5-3 mg of chromium is buffered with acetic-sodium acetate buffer and reacted with a freshly filtered potassium permanganate solution (sintered glass filter). Oxidation proceeds quickly to chromate with precipitation of MnO_2. The advantage of this procedure over other indirect chelatometric methods is that larger amounts of interfering cations are removed during precipitation and washing. The remaining traces of cations are easier to mask. Another advantage is that the highly colored Cr(III)—EDTA complex is absent during the titration (*1-3, 6-12, 14-17*). In fact, a colorless solution is titrated in this procedure. Not more than 20 mg of Cr(III) could be determined with known indirect chelatometric methods due to the strong color involved. The present method allows larger amounts of Cr(III) to be determined.

Table 1 shows that adequate results are obtained with the described method.

TABLE 1

DETERMINATION OF CHROMIUM(III) IN THE PRESENCE OF METAL IMPURITIES[a]

No.	Taken (mg)				Cr Found (mg)	Difference (mg)
	Cr	Zn	Cd	Cu		
1	2,730	—	—	—	2,730	—
2	2,730	325	—	—	2,720	— 0,010
3	2,730	325	—	—	2,734	+ 0,004
4	2,730	325	—	—	2,730	—
5	2,676	650	—	—	2,695	+ 0,019
6	2,676	650	112	—	2,751	+ 0,075
7	2,676	—	—	—	2,691	+ 0,015
8	2,676	—	—	63	2,688	+ 0,012
9	2,676	—	—	—	2,671	— 0,005
10	2,676	650	—	126	2,642	— 0,034
11	2,676	—	—	—	2,667	— 0,009
12	2,676	—	—	—	2,663	— 0,013
13	1,070	—	—	—	1,123	+ 0,053
14	0,535	—	—	—	0,544	+ 0,090
15[b]	2,676	—	—	—	2,670	— 0,006

[a] The results are calculated from the average of 7-10 measurements.

[b] Values calculated from iodometric determinations.

A potassium chromium sulfate solution, gravimetrically checked, was used as standard in this investigation. Additionally, the micro-iodometric method was also used for standardization of chromium solutions.

Details of the determination are given below.

EXPERIMENTAL

Reagents

Potassium permanganate, 0.05*N*

Acetate buffer: 60 g acetic acid and 136 g sodium acetate trihydrate per 1000 ml of solution

Ascorbic acid, crystals

Ammonia, concentrated aqueous

Buffer, pH 10: 50 g ammonium chloride and 400 ml of concentrated ammonia per liter

Potassium cyanide, crystal

Eriochrome Black-T indicator: 1:100 mixture with potassium nitrate, ground up.

EDTA, 0.005*M* solution

Hydrogen peroxide, 3%
Sodium hydroxide, 1*N*
Nickel(II) sulfate, 1*M*
Hydrochloric acid, concentrated
Sodium bicarbonate, crystal
Potassium iodide, crystal
Sodium thiosulfate, 0.001*N* solution

Procedure A: Chelatometric Cr(III) Determination

Five to twenty milliliters of neutral or weakly acid solution containing 0.5-3 mg of Cr(III) and possibly 600 mg of zinc, 100 mg of cadmium, and 120 mg of copper, are treated with 20 ml of freshly filtered 0.05*N* $KMnO_4$ and 1 to 2 ml of acetate buffer. Turbidity ensues due to MnO_2 precipitation, which takes 10 minutes for completion. Solutions containing less than 0.5 mg of Cr(III) take at most 15 minutes for complete precipitation. The precipitate is filtered with suction through a fine fritted glass filter and washed thoroughly with distilled water in order to remove excess $KMnO_4$. The precipitate is dissolved off the filter by means of some ascorbic acid crystals and a few milliliters of water. The MnO_2 dissolves easily. The filter is washed thoroughly. The resulting 100 to 120 ml of solution is treated with 1-2 ml conc. ammonia and 5 ml of pH 10 buffer. Quickly warmed to about 60°C and about 0.2 g of KCN and some Eriochrome Black-T added to the warm solution. Titrate as usual with 0.005*M* EDTA standard solution.

Procedure B: Iodometric Cr(III) Determination

Five to 20 milliliters of a neutral to weakly acid solution containing 0.5-3 mg of Cr(III) is treated with 5 ml 3% H_2O_2 and 10 ml of *N* NaOH and then boiled for 10 minutes. The solution turns yellow due to chromate ions. Then add 1-2 drops *M* $NiSO_4$ and boil another 10 minutes in order to decompose excess H_2O_2. After gas evolution has ceased, dilute with 100 ml of water and add 5 ml of conc. HCl and 1 g of $NaHCO_3$. After 5 minutes reaction, the solution is air free. Add 1 g of KI and titrate the iodine with 0.001*N* sodium thiosulfate.

SUMMARY

A neutral or weakly acid solution of Cr(III) is treated with acetate buffer and freshly filtered $KMnO_4$ solution. Equivalent amounts of soluble chromate and insoluble MnO_2 are formed in a short time. The MnO_2 is quantitatively collected on a fritted glass filter and washed. After dissolution with ascorbic acid the Mn^{2+} ions are titrated

with EDTA. Large relative amounts of Zn, Cd, or Cu do not interfere since remaining traces in the titrate may be easily masked.

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MICROCHEMICAL JOURNAL **12**, 151-156 (1967)

Schematic Qualitative Analysis of Cations by the Ring Oven Technique

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Received July 25, 1966

In spite of the excellent properties of 8-hydroxyquinoline (*1, 3*) and sodium diethyldithiocarbamate (*2, 7*) as precipitants for cations, no attempt appears to have been made to study their use as group reagents in systematic qualitative analysis. In the present work attempts have been made towards this end for the qualitative separation by the ring oven method of 20 common cations when present together in a drop of solution. The method is advantageous over the method described by Weisz (*10*) and is less time consuming. Moreover, in this method it is possible to separate all the common cations in a single drop of the test solution.

EXPERIMENTAL

Apparatus. A Weisz ring oven (National Appliance Co., Portland, Oregon, U.S.A.) with its accessories was used as described earlier (*8*).

Metal solution. The metal solutions (1*M*) of nitrates of silver(I), lead(II), mercury(II), copper(II), bismuth(III), cadmium(II), ferric(III), chromium(III) aluminium(III), nickel(II), cobalt(II), zinc(II), manganese(II), barium(II), strontium(II), calcium(II), magnesium(II), sodium arsenite, potassium antimony tartrate, and stannous oxide in nitric acid were prepared by using reagent grade chemicals. Equal volumes of each of these solutions were mixed and the mixture was used for the separation. The method was also checked for its suitability by the analysis of a number of unknowns.

Reagents. 8-Hydroxyquinoline: 2.0 g of 8-hydroxyquinoline (B.D.H.) was directly weighed and dissolved in 6 ml of glacial acetic acid (B.D.H.), and 10 ml of water were added. Six milliliters of ammonia solution (B.D.H., sp. gr. 0.91) were then added and the volume was raised to 100 ml with water. The pH of this oxine solution was 5.1. Sodium diethyldithiocarbamate: 1.0 g of sodium diethyldithiocarbamate (B.D.H. AnalaR)

was weighed directly and 100 ml of an aqueous solution was prepared.

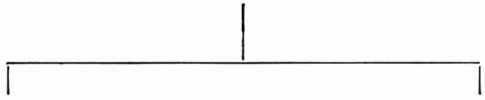
All other chemicals used were of reagent grade.

Filter paper. Circles of Whatman No. 1 filter paper (55 mm in diameter) were used throughout and are referred to as paper in the procedures, which are described below.

CHART 1

Separation of Cations into Two Groups

The mixture solution was spotted on the marked center of the paper plus six drops of oxine solution, dried, and the spot (Disc I) punched out. Disc I was kept on paper 2 and washed four times with water to a ring about 4 mm larger in diameter than Disc I. Disc I was then removed, paper 2 was dried, and Disc I, containing the soluble ions, was punched out.

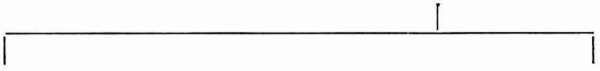


Disc I First group	Disc II Second group
Oxinates of: Cu, Ag, Bi, Hg, Cd, Zn, Ni, Co, Al, Fe as sparingly soluble	Pb, Sn, Sb, As, Cr, Mn, Ba, Sr, Ca, Mg as soluble ions

CHART 2

Separation of First Group Cations

Disc I was kept in the disc holder and exposed to bromine vapors for 1 minute. The excess bromine was removed by heating on the ring oven. The unprecipitated metal ions were washed three times with water to the ring zone A on the paper 3.



Ring A paper 3	Disc I
Al, Ni, Zn, Cd, Co	Precipitates of Cu, Ag, Fe, Bi, Hg. Disc I was taken in the disc holder, three drops of 3*N* acetic acid were added, heated in the ring oven, washed with water on paper 4 to the ring B

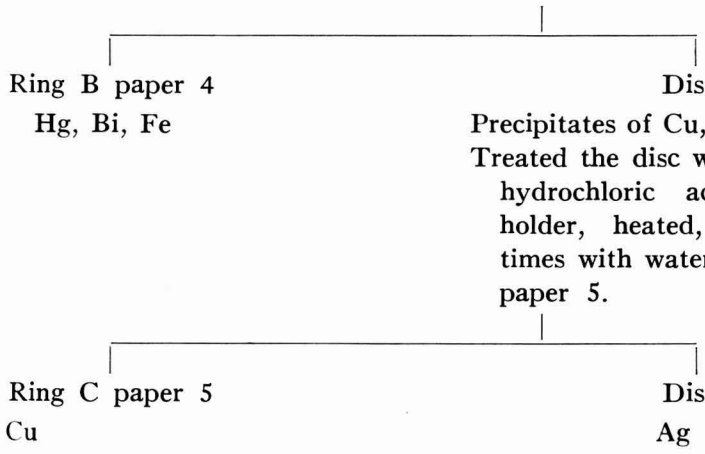
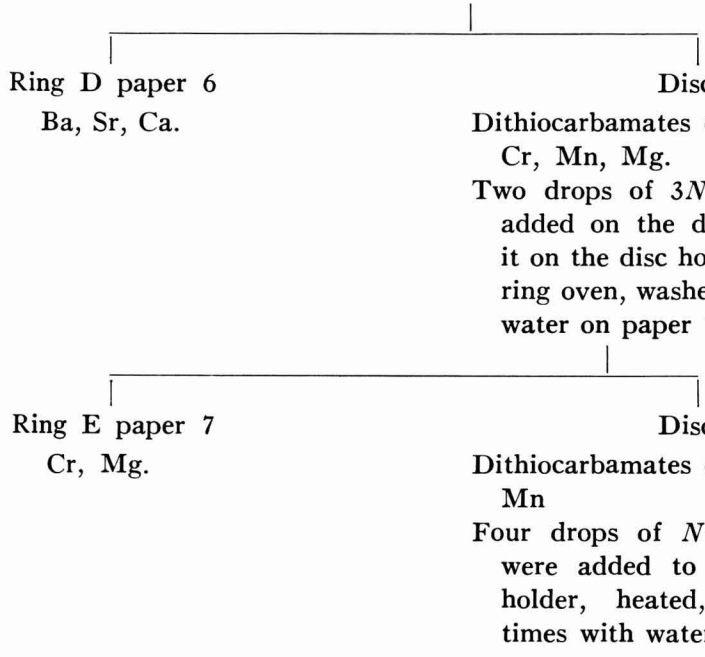
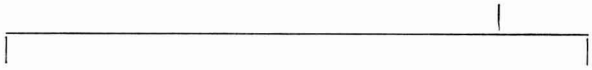


CHART 3

Separation of Second Group Cations

The Disc II was taken on the disc holder and treated with 6 drops of 1% aqueous sodium diethyldithiocarbamate, dried, and washed with water four times on paper 6.





Ring F paper 8 — Disc II

Pb, Sn, Sb, As, Mn. — Rejected

Identification. The papers containing the metal ions in the form of a ring were cut into sections according to the number of the metal ions present in the ring. Then each fragment was taken and tested for the particular ion with the help of the reagents mentioned in Table 1.

TABLE 1
REAGENTS FOR METAL IONS

Metal ion	Pretreatment	Reagent	Color	Reference
		Ring A		
Al(III)	—	Aqueous aluminon containing CH_3COONH_4	Bright red	(*10*)
Ni(II)	—	Alcoholic dimethylglyoxime and fume over ammonia	Red	(*10*)
Zn(II)	—	Dithizone in CCl_4	Purple red	(*10*)
Cd(II)	0.5N-NaOH solution	Alcoholic cadion 2B	Purple red	(*10*)
Co(II)	—	Alcoholic α-nitroso β-naphthol	Brown	(*10*)
		Ring B		
Hg(II)	Dil. HNO_3	Dimethylamino-benzylidene-rhodamine in acetone	Violet	(*10*)
Bi(III)	KI solution	Brucine citrate solution	Deep orange	(*11*)
Fe(III)	—	$K_4Fe(CN)_6$ solution	Blue	(*10*)
		Ring C		
Cu(II)	—	Alcoholic rubeanic acid and fume over ammonia	Olive green	(*10*)
		Ring D		
Ba(II)	—	Aqueous sodium rhodizonate	Red	(*10*)
Sr(II)	—	Aqueous sodium rhodizonate	Red: soluble in 0.1N HCl	(*10*)

TABLE 1 (*Continued*)

Metal ion	Pretreatment	Reagent	Color	Reference
Ca(II)	—	Zirconium-fluoride-alizarin	Red-Violet	(*10*)
		Ring E		
Cr(III)	H_2O_2 + NH_4OH heat on ring oven	Alcoholic diphenylcarbazide and dil. H_2SO_4	Violet	(*10*)
Mg(II)	—	Alcoholic titan yellow and 0.5*N* NaOH	Red	(*4, 5, 6*)
		Ring F		
Pb(II)	—	Aqueous sodium rhodizonate	Reddish violet	(*10*)
Sn(II)	—	Aqueous potassium tellurite	Black	(*9*)
Sb(III)	Treated with (1:1) HCl + a few crystals of $NaNO_2$	Aqueous rhodamine B	Violet	(*10*)
As(III)	—	Gutzeit test	Yellow: Black	(*10*)
Mn(II)	0.5N NaOH solution	Acetic acid solution of benzidine	Blue	(*10*)
		Disc I		
Ag(I)	—	H_2S water	Black	(*10*)

For spotting the metal solution on the filter paper, it is necessary to mention that four drops of the solution taken with the aid of self-filling pipette (each drop about 1.5 μl) is very suitable. To keep the diameter of the spot within reasonable limits it is necessary that each drop should be dried before introducing a second drop. This has also to be done when more than one drop of the reagent has to be added.

During the course of the work it has been noted that test for tin(II) by the potassium tellurite method is not always responded in the ring F. This is probably because a majority of tin(II) gets oxidized in tin(IV) during the treatment. This, however, offers no special difficulties since it is possible to test tin(II) in a drop of the original solution. It has also been noted that analysis is possible when the amount of a metal present in a drop (6 μl) is 12 μg. Total time required for the complete analysis of an unknown mixture is about 70 minutes.

ACKNOWLEDGMENT

The authors are thankful to the Council of Scientific and Industrial Research, New Delhi, for financial support.

SUMMARY

The schematic separation of 20 common cations, viz., silver, mercury, lead, copper, bismuth, cadmium, arsenic, antimony, tin, iron, chromium, aluminium, zinc, cobalt, nickel, manganese, barium, strontium, calcium, and magnesium by the ring oven method with 8-hydroxyquinoline and sodium diethyldithiocarbamate used as group reagents, has been described. The cations are identified by spot test in which organic reagents were used.

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MICROCHEMICAL JOURNAL 12, 157-161 (1967)

Separation and Microdetermination of Aluminium, Gallium, Indium, and Thallium in a Drop of Mixture

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Aluminium(III), gallium(III), indium(III) and thallium(III) resemble closely in properties and their separation and determination in the presence of one another offers difficulties. In connection with the work on the analytical chemistry of less familiar elements, the analysis of these four closely allied elements has been attempted by a combination of solvent extraction and ring oven methods. The organic solvents employed are: (i) a mixture of chloroform and acetone (*6*) for thallium, (ii) pyridine for indium, (iii) acetylacetone for gallium (*7*) and (iv) a mixture of acetic acid and ethanol for aluminium. After separation, the rings have been developed with organic reagents (*4*) and determined by ring colorimetry (*9*).

EXPERIMENTAL

Apparatus. A Weisz ring oven (National Appliance Co., Portland, Oregon, U.S.A.) was used with a suitable power unit yielding 25 volts from 220 V/50 cycles a.c. mains. A self-filling capillary pipette (about 1.5 μl) was employed in each case.

Filter paper. Circles of Whatman filter paper No. 1 of diameter 55 mm were employed.

Organic solvents. (i) A 5:2 mixture of chloroform and acetone (both B.D.H. AnalaR); (ii) pyridine (B.D.H. AnalaR); (iii) acetylacetone (B.D.H. AnalaR); (iv) a 1:10 mixture of 3*N* acetic acid and 60% ethanol.

Metal solutions. Solutions of aluminium chloride (B.D.H. AnalaR) and

of trichlorides of gallium, indium, and thallium (Johnson, Matthey & Co., Hatton Garden, London E. c. 1 England) were prepared and standardized by the usual methods. Solutions of aluminium chloride (0.1*M*), gallium trichloride (0.01*M*), indium trichloride (0.02*M*) and thallium trichloride (0.002*M*) were obtained by dilution and mixtures of desired concentrations were obtained from these.

Developing reagents. Aluminon (AAC): 0.1% aqueous w/v solution + 1% w/v aqueous ammonium acetate. 4-(2-Pyridylazo)resorcinol (PAR): 0.1% aqueous w/v solution.

Procedure. The mixture was spotted by the capillary pipette (1.5 µl) on the marked center of a filter paper 1, dried over ring oven and the spot (disc) was punched out. The disc was kept on another paper 2 on the ring oven, one drop of 1*N* HCl was added and the moist disc was washed five to six times with a 5:2 chloroform acetone mixture. Thallium(III) was thus transferred to ring zone (A). The disc containing the remaining metal ions was taken in the glass holder and dried in the ring oven and then fumed over strong ammonia. The disc thus treated was kept on paper 3 and washed with pyridine about ten times for complete washing. Thus indium(III) was washed to ring zone (B). The disc then containing Ga(III) and Al(III) was dried in the ring oven using the glass holder and was placed on paper 4, one drop of 2*N* HCl was then added and the moist disc was washed five to six times with acetylacetone. Thus Ga(III) was transferred to ring zone (C). The disc now contained only Al(III). It was then placed on the paper 5 and washed ten times with the acetic acid-ethanol mixture for complete washing to ring zone (D). The disc was then rejected. Filter paper 2, 3, 4, and 5, containing respectively, thallium, indium, gallium, and aluminium, were developed in the ring zones with the reagents described in Table 1.

TABLE 1
REAGENTS EMPLOYED

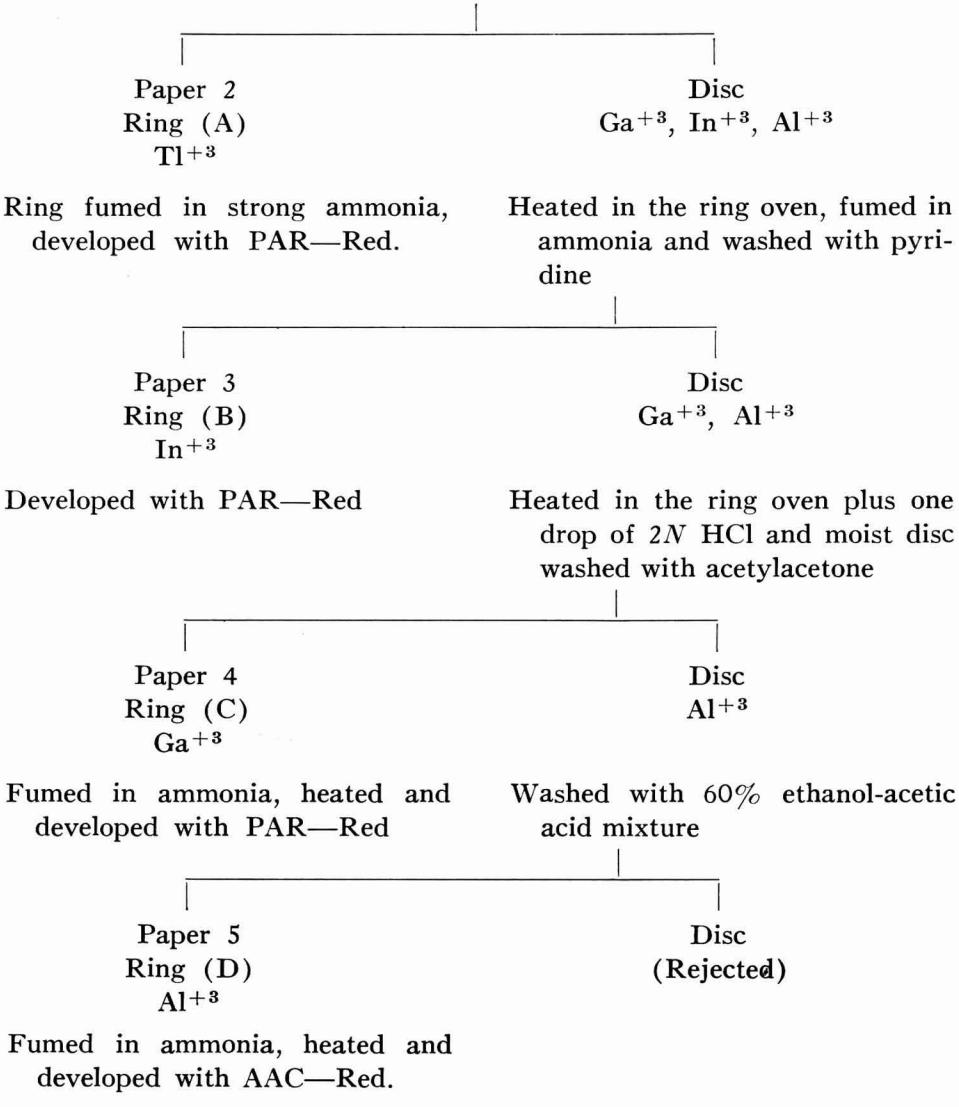
Ion	Reagent	Color	Reference
Aluminium	AAC	Bright red	(*8*)
Gallium	PAR	Red	(*5*)
Indium	PAR	Red	(*1*)
Thallium	PAR	Red	(*3*)

The scheme of separation is summarized in the chart

CHART 1

Scheme of Separation

The mixture was spotted, dried, and the disc punched out. The disc was kept in filter paper 2 plus one drop of 1*N* HCl. The moist disc was washed with a 5:2 chloroform acetone mixture.



The intensities of the color of the rings were compared with the rings of the standard scales of these ions, obtained separately with the reagents on the ring oven, and the concentrations were computed (*2, 9*). The number of drops employed were 2, 3 and 5 for the test solutions and 2, 4, 6, 8, 10, and 12 for the standard scales, which were prepared with the diluted standard solutions.

A large number of artificial mixtures were analyzed. It has been found that determinations are possible down to the following limits when present in a drop of the solution: Al, 0.02 μg; Ga, 0.006 μg; In, 0.03 μg; and Tl, 0.003 μg. Some of the typical results are shown in Table 2.

TABLE 2
RESULTS OF THE DETERMINATIONS

Taken (μg/ml)				Found (μg/ml)				% Error			
Al	Ga	In	Tl	Al	Ga	In	Tl	Al	Ga	In	Tl
13	4.2	23	2	12	4.1	22.9	2.05	— 7.69	— 2.38	— 0.43	+ 2.50
27	6.9	46	4	24	6.9	45.9	4.10	— 3.70	— 0.00	— 0.21	+ 5.00
40	10.4	67	6	39	10.3	64.0	5.70	— 2.50	— 0.96	— 4.47	— 5.00

SUMMARY

The ring oven method combined with solvent extraction technique has been used to separate aluminium, gallium, indium and thallium on filter paper circles. Thallium was first transferred to the ring zone by washing with a chloroform-acetone mixture. Indium was made to migrate to the ring zone as the pyridine complex, while gallium was washed as acetylacetonate to the ring zone. Aluminium acetylacetonate remained fixed at the center and was lastly eluted to the ring zone by washing with a mixture of 60% ethanol and 3*N* acetic acid.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL **12**, 162-167 (1967)

4-(2-Pyridylazo) Resorcinol as a Sensitive Reagent for Vanadium, Niobium, and Tantalum

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The analytical chemistry of niobium and tantalum is drawing considerable attention in recent years. This is evident from the appearance of two well-known documented reviews by Elwell and Wood (*1*) and Cockbill (*2*) and the publication of a monograph by Moshier (*3*). Among the various photometric procedures available, a variety of chromogenic reagents have been employed. The work with such reagents has been carried out with special reference to optimum concentration range, effect of temperature and pH and particularly interferences by diverse ions.

Work has been in progress in these laboratories on new reagents for the less common metal ions and in this paper the chromogenic reactions of vanadium, niobium, and tantalum with 4-(2-pyridylazo)resorcinol (PAR) have been reported. Belcher *et al.* (*4*) have earlier reported the formation of a complex of niobium with PAR, and it has been considered to be of such interest as to extend the studies with the other members of the family as well.

EXPERIMENTAL

Materials. A standard solution of vanadium was prepared by dissolving ammonium vanadate (B.D.H. AnalaR) in hot water. Standard solutions of niobium and tantalum were prepared by fusing niobium and tantalum pentoxides (Johnson, Matthey & Co.) with ten-fold excess of potassium hydrogen sulfate (E. Merck). The melt was extracted in hot 3% tartaric acid solution, and the metal contents were estimated gravimetrically by precipitation with cupferron and subsequent ignition to the pentoxides.

A stock solution of PAR (0.005*M*) was prepared by dissolving a B.D.H. Reagent sample in water.

Stock solutions containing 1 mg/ml of the various cations and anions

were prepared from their nitrates or chlorides by using analytical grade reagents.

Instruments. A Unicam SP 500 spectrophotometer with 1 cm glass cells was employed to study the absorption spectra of various solutions. A Leeds and Northrup pH indicator was used for pH measurements.

Conditions of Study. Measurements were carried out at 25°C. The total volume of all the mixtures prepared for the measurement was kept at 25 ml.

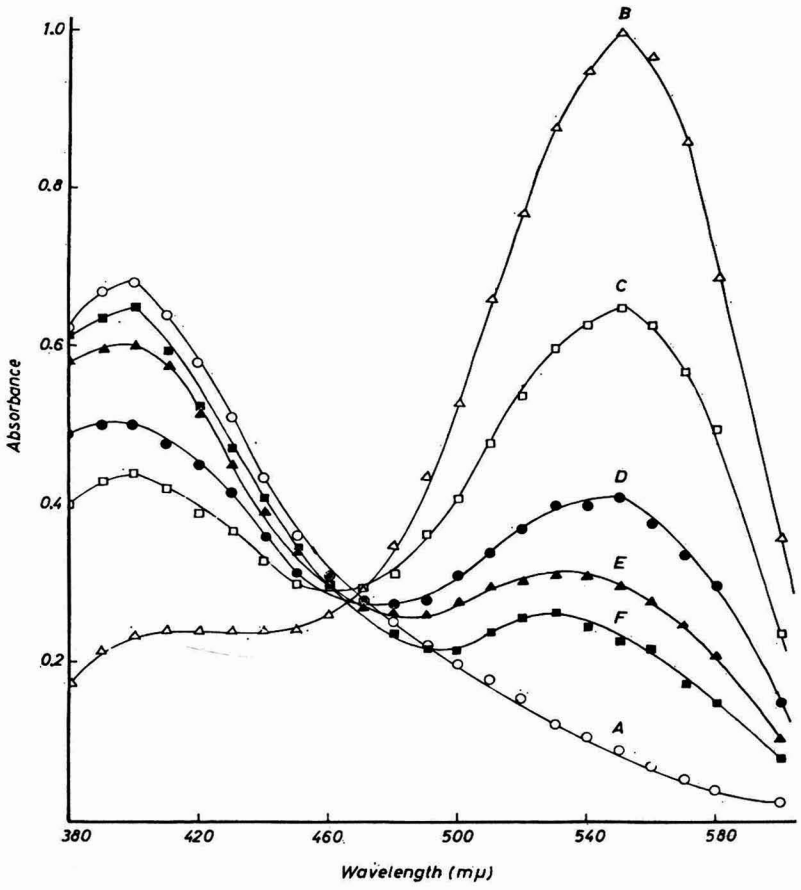

FIG. 1. Absorption spectra of vanadium-PAR chelate, pH 5.0. Curve A: Reagent, $c = 4.0 \times 10^{-5}M$; Curve B: $c = 8.0 \times 10^{-5}M$, $p = 0.5$; Curve C: $c = 4.0 \times 10^{-5}M$, $p = 1.0$; Curve D: $c = 2.0 \times 10^{-5}M$, $p = 2.0$; Curve E: $c = 1.33 \times 10^{-5}M$, $p = 3.0$; Curve F: $c = 1.0 \times 10^{-5}M$, (c = conc. of metal ion and c' = conc. of reagent, $p = c'/c$).

Description of the Chelates

Color. The color of vanadium, niobium, and tantalum chelate is red, having maximum absorbance at 550, 540, and 500 mμ, respectively. The results are shown in Figs. 1-3. The absorbance values of mixtures in various ratios of the reactants show that only one complex is formed each under the conditions of study with λ_{max} as mentioned above.

Effect of pH. The λ_{max} of V-PAR chelate holds good between pH 3.0 to

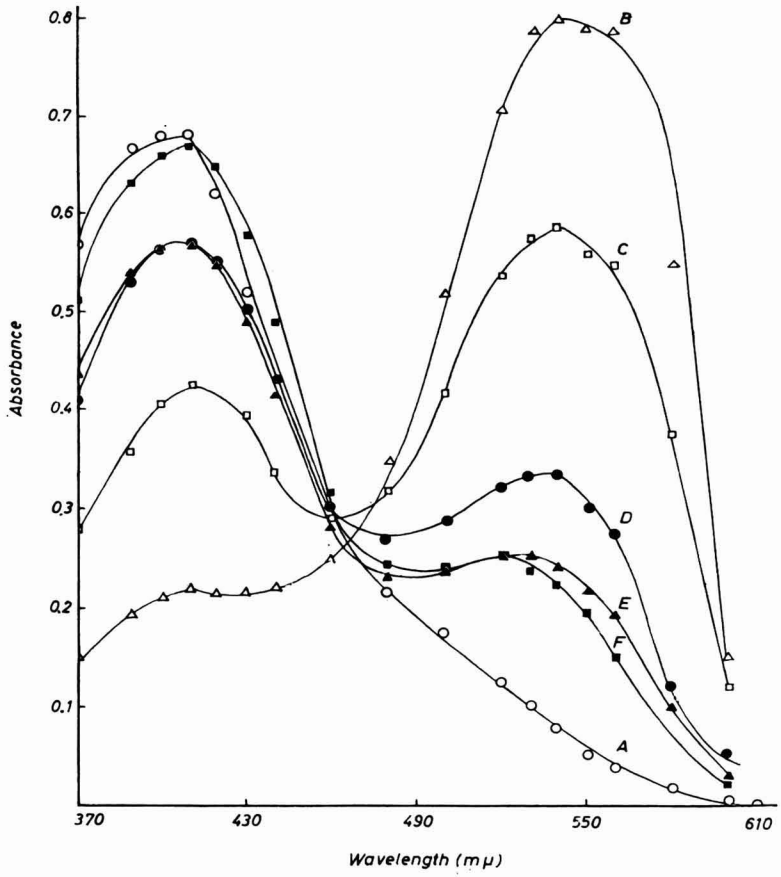

FIG. 2. Absorption spectra of niobium-PAR chelate, pH 6.0. Curve A: Reagent, $c = 4.0 \times 10^{-5}M$; Curve B: $c = 8.0 \times 10^{-5}M$, $p = 0.5$; Curve C: $c = 4.0 \times 10^{-5}M$, $p = 1.0$; Curve D: $c = 2.0 \times 10^{-5}M$, $p = 2.0$; Curve E: $c = 1.33 \times 10^{-5}M$, $p = 3.0$; Curve F: $c = 1.0 \times 10^{-5}M$; $(p = c'/c)$.

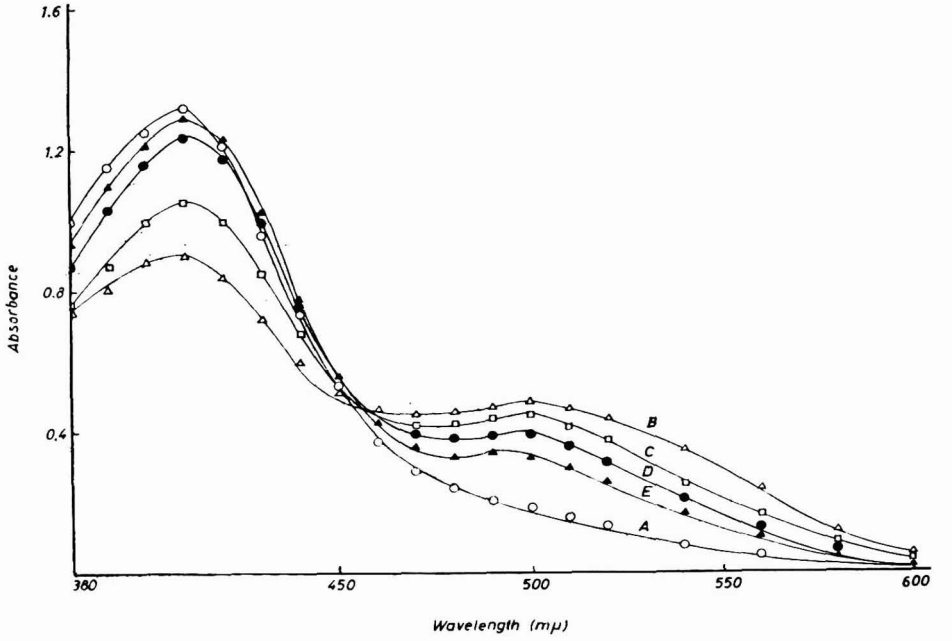

FIG. 3. Absorption spectra of tantalum-PAR chelate, pH 6.0. Curve A: Reagent $c = 8.0 \times 10^{-5}M$; Curve B: $c = 16.0 \times 10^{-5}M$, $p = 0.5$; Curve C: $c = 8.0 \times 10^{-5}M$, $p = 1.0$; Curve D: $c = 4.0 \times 10^{-5}M$, $p = 2.0$; Curve E: $c = 2.67 \times 10^{-5}M$, $p = 3.0$ $(p = c'/c)$.

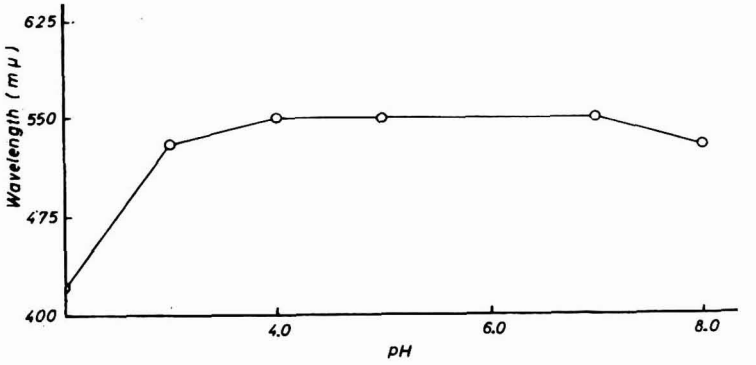

FIG. 4. Variation of λ_{max} with pH of vanadium-PAR system. Conc. of vanadium = PAR = $4.0 \times 10^{-5}M$.

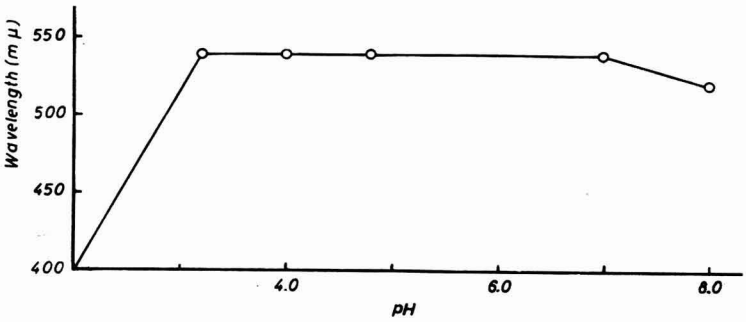

FIG. 5. Variation of λ_{max} with pH. Niobium-PAR system. Conc. of niobium = PAR = $4.0 \times 10^{-5}M$.

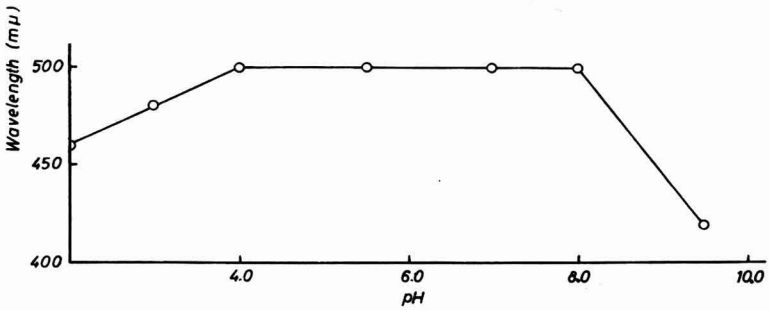

FIG. 6. Variation of λ_{max} with pH of tantalum-PAR system. Conc. of tantalum = PAR = $8.0 \times 10^{-5}M$.

7.0, while that of Nb-PAR chelate and Ta-PAR between pH 3.0 to 7.0 and pH 4.0 to 8.0, respectively (Figs. 4-6). This indicates that the chelates are stable within their range of pH as indicated. The maximum absorbance is noted at pH 5.0, for vanadium, pH 6.0 for niobium and tantalum.

Effect of Time and Order of Addition of the Reagent

It is observed that the color is developed within 15 minutes, but the mixtures were kept for an hour to attain equilibrium. The order of the addition of reagent has no effect on the absorbance values of the complexes.

Adherence to Beer's Law. The validity of Beer's law was confirmed by measuring the color intensity of the chelates with varying concentration of metal solution in the presence of a four-fold excess of the reagent. Beer's law is seen to be adhered to over the range of 0.10-7.34 ppm of vanadium, 0.25-6.69 ppm of niobium, and 0.36-6.51 ppm of tantalum.

Sensitivity. According to Sandell (based on an absorbance value of 0.001 unit), the sensitivity is 0.0125 γ/cm^2 of vanadium at 560 mμ, 0.093 γ/cm^2 of niobium at 540 mμ and 0.181 γ/cm^2 of tantalum at 510 mμ, showing the color reactions are sensitive.

Effect of diverse ions. The effect of various added cations and anions was studied spectrophotometrically: the tolerance limits were also determined. Bi(III), Pb(II), Cd(II), Zn(II), Ca(II), Hg(II), Mn(II), Ni(II), Al(III), Ga(III), In(III), Co(III), U(VI), Pd(II), Fe(III), Sc(III), Y(III), rare earths(III), and phosphate interfere at all concentrations whereas oxalate, citrate, and tartrate do not interfere.

SUMMARY

The formation of red colored chelates between vanadium, niobium and tantalum with 4-(2-pyridylazo)resorcinol (PAR) has been reported. The λ_{max} of the chelates are respectively 550 mμ (ph 5.0); 540 mμ (pH 6.0) and 500 mμ (pH 6.0). Adherence to Beer's law is observed over a wide range of metal concentrations, the color intensities are also stable within a wide range of pH particularly in case of Nb-PAR chelate. The reagent is highly sensitive specially for vanadium. The effect of various conditions like pH, time, order of the addition of the reactants and interference due to various ions have been studied.

ACKNOWLEDGMENT

An acknowledgment is made of the support received from the Council of Scientific and Industrial Research, New Delhi and for the award of research fellowship to one of them (B.V.A.).

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MICROCHEMICAL JOURNAL 12, 168-176 (1967)

Composition and Stability of the Rare Earth Chelates of Ammonium Aurintricarboxylate (Aluminon) in Aqueous Solution

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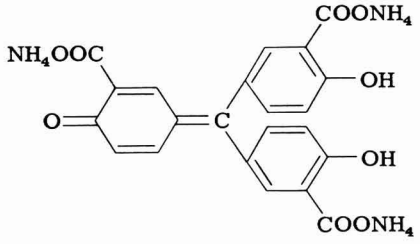
A number of hydroxytriphenyl methane dyes have been studied for their chromogenic reactions with metal ions. Recently Dey *et al.* (*1-3*) have studied the metal chelates of number of such dyes. The most important dyes studied from this point of view are Chrome Azurol S (*1*), Aluminon (*1*), Xylenol Orange (*2*) and Eriochrome Cyanine R.C. (*3*). These reagents have been observed to yield colored complexes with the lanthanoids. The chromogenic reactions are obviously due to the tendency of these organic compounds to form metal chelates, the chelating properties being due to the presence of donor groups like —COOH, —OH and =O. In the present communication the composition and stability of the chelates formed between the rare earths and ammonium aurintricarboxylate (abbrv. AAC) have been reported. The reagent can be represented by the following structure



From the structural formula of AAC it is obvious that chelation can take place either between the adjacent carboxylic and hydroxyl oxygen or between the adjacent carboxylic and quinoid oxygen. In the former case due to the liberation of hydrogen ions a fall in pH occurs, while in the latter case the pH remains the same as no protons are liberated. In the case

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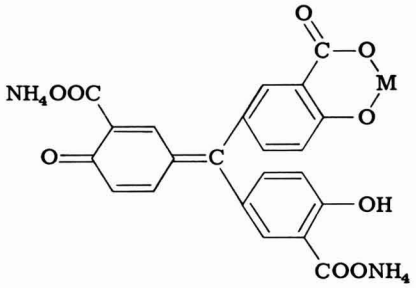
of the metal chelates of rare earths reported in the present paper, the fall in pH is observed. Thus the possible structure of the metal chelates of AAC with rare earths may be designated by the following structure:



M Stands for rare earths

where M stands for rare earths.

EXPERIMENTAL

Instruments. A Unicam SP 500 spectrophotometer was employed for the measurement of absorbance. All measurements were noted against distilled water blanks by means of 10-mm glass cells. At wavelengths 625 mμ or below, the ultrasensitive phototube was used, above this wavelength red sensitive phototube was employed. The phototube circuits were kept at maximum sensitivity. The pH of the solutions was measured with a Leeds and Northrup direct reading pH indicator with glass calomel electrode system.

Materials. Standard solutions of rare earths were obtained by dissolving the respective chlorides in water containing HCl or oxides (Johnson Matthey samples) in concentrated HCl. These were then standardized by the usual method. A stock solution ($1.0 \times 10^{-3}M$) of ammonium aurintricarboxylate (B.D.H.) was prepared in distilled water. Solutions of different concentration were obtained by suitable dilution.

Conditions of study. All experiments were carried out in an air conditioned room maintained at 25° ± 1°C. Five milliliters of 0.50*M* mannitol were added in each case to check precipitation of the lakes. The total volume was kept constant at 25 ml after adjusting the pH to 6.0 ± 0.2 by adding NaOH or HCl.

RESULTS AND DISCUSSION

Absorption spectra of the chelates. Several mixtures containing the metal ions (rare earths) and the ligand in the ratios 0:1, 2:1, 1:1, and 1:2 were

prepared and their absorbance measured from 400 to 650 mμ. The wavelength of maximum absorbance of AAC was found to be at 520 mμ, while those of the chelates of rare earths at 540 mμ in each case (Fig. 1).

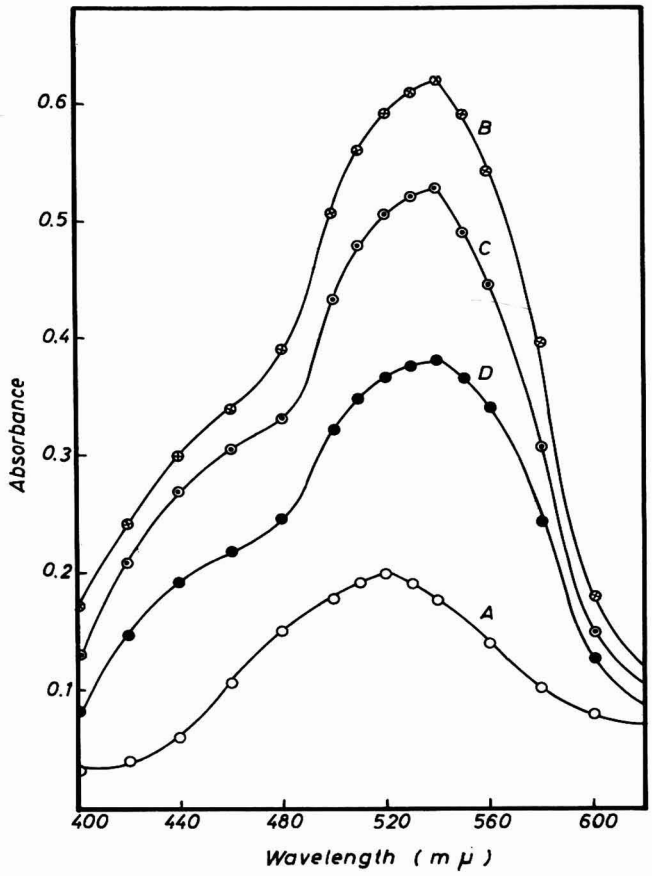


FIG. 1. Absorption spectra of neodymium-AAC chelate. Curve A, AAC $= 1.6 \times 10^{-4}M$. Curve B, AAC $= 1.6 \times 10^{-4}M$; $NdCl_3 = 3.2 \times 10^{-4}M$. Curve C, AAC $= 1.6 \times 10^{-4}M$; $NdCl_3 = 1.6 \times 10^{-4}M$. Curve D, AAC $= 1.6 \times 10^{-4}M$; $NdCl_3 = 8.0 \times 10^{-5}M$.

Effect of pH on the stability of the chelates. Various mixtures of individual rare earths and AAC were prepared in the 1:1 ratio and their absorbance measured at different pH from 400 to 650 mμ. It was found that λ_{max} of the chelates are constant only within a definite pH range, as is

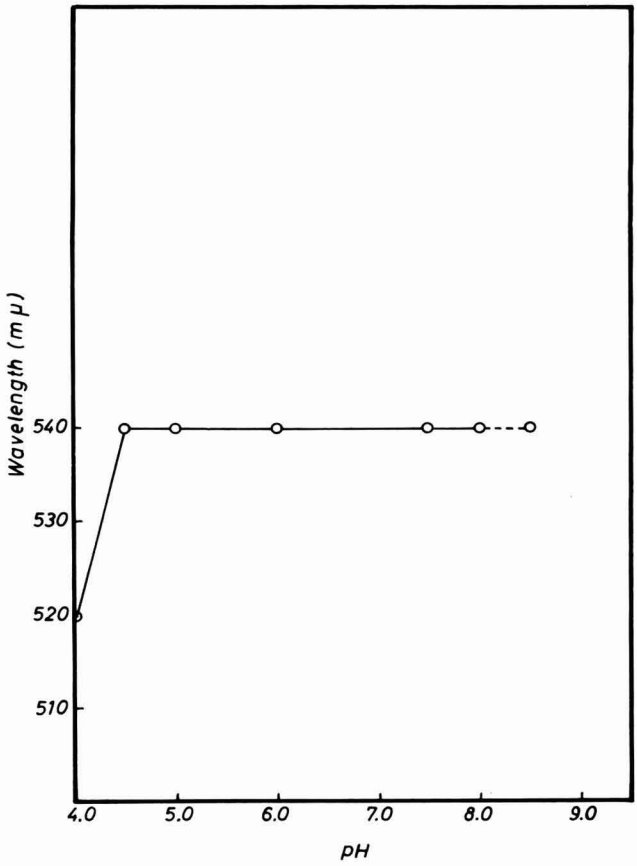


FIG. 2. Variation of λ_{max} of neodimium-AAC chelate with pH. Final concentration of AAC $= 8.0 \times 10^{-5}M$. Final concentration of $NdCl_3 = 8.0 \times 10^{-5}M$.

shown by Fig. 2 (neodymium-AAC chelate). Table 1 shows the range of pH within which a particular chelate is stable.

Composition of the chelates. Composition of the chelates has been determined by absorptiometric measurements in which three different methods were used, i.e., the method of continuous variations, mole ratio method, and the slope ratio method. The results obtained by all the three methods are in agreement with each other and show that each rare earth metal forms a 1:1 chelate with AAC (Figs. 3-5 for neodymium-AAC chelate).

Evaluation of the conditional stability constants. The evaluation of the

TABLE 1

CHARACTERISTICS OF AAC CHELATES AND THEIR STABILITY CONSTANTS (LOG, K)
pH 6.0; TEMP. 25°; COMPOSITION 1:1; λ_{max} 540 mμ

Chelate	pH range of stability	log K		
		(a)	(b)	(c)
Pr(III)-AAC	4.5–8.5	4.2 ± 0.1	4.3 ± 0.1	4.4 ± 0.1
Nd(III)-AAC	4.5–8.0	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
Sm(III)-AAC	4.5–8.5	4.5 ± 0.1	4.5 ± 0.1	4.5 ± 0.1
Eu(III)-AAC	4.5–8.0	4.6 ± 0.2	4.5 ± 0.1	4.6 ± 0.1
Gd(III)-AAC	4.5–8.0	4.8 ± 0.2	4.6 ± 0.2	4.6 ± 0.1
Tb(III)-AAC	4.5–8.5	4.8 ± 0.2	4.7 ± 0.1	4.6 ± 0.1
Dy(III)-AAC	4.5–8.0	4.9 ± 0.1	4.9 ± 0.1	4.7 ± 0.1
Ho(III)-AAC	4.5–8.0	5.0 ± 0.4	4.9 ± 0.1	4.9 ± 0.1
Er(III)-AAC	4.5–8.0	5.1 ± 0.2	5.0 ± 0.2	4.9 ± 0.1
Tm(III)-AAC	4.5–8.0	5.1 ± 0.1	5.2 ± 0.1	5.0 ± 0.1
Yb(III)-AAC	4.5–8.5	5.2 ± 0.2	5.3 ± 0.1	5.1 ± 0.1
Lu(III)-AAC	4.5–8.5	5.3 ± 0.4	5.5 ± 0.2	5.2 ± 0.2

Note. (a) Method of Dey (4). (b) Method of continuous variations. (c) Mole ratio method.

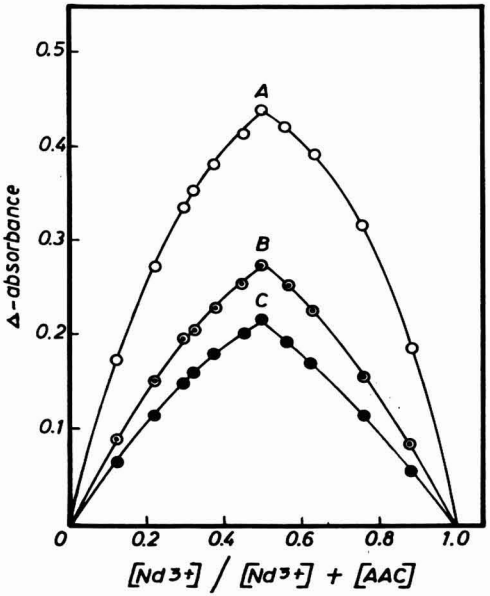


FIG. 3. Determination of the composition of neodymium-AAC chelate by the method of continuous variations using equimolecular solutions at pH 6.0 at 550 mμ. Concentrations in Curve A = $4.0 \times 10^{-4}M$; Curve B = $2.0 \times 10^{-4}M$; Curve C = $1.33 \times 10^{-4}M$. P = 1.

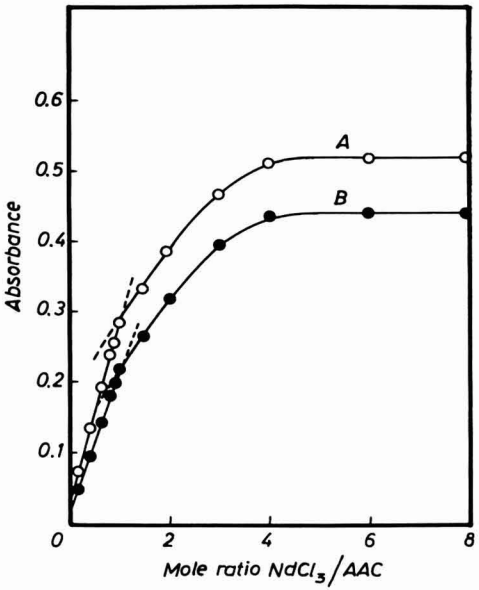


FIG. 4. Determination of the composition of neodymium-AAC chelate using mole ratio method at pH 6.0 at 550 mμ. Final concentration of AAC Curve A = $1.0 \times 10^{-4}M$. Curve B = $8.0 \times 10^{-5}M$.

stability constant offer a better understanding of chelate-forming reactions. The stability constant is concerned with the reaction between different solvated reactants and products. From a precise knowledge of the stability constants, thermodynamic constants may be evaluated. However, the method is accompanied by many difficulties and it is doubtful whether the thermodynamic quantities of chelation can be determined except in very simple cases. Rossotti and Rossotti (*5*) concluded, while discussing methods for determining stability constants, "It would, therefore, seem better to obtain reliable values of the stoichiometric constants (which describe the stability of a species relative to the corresponding complexes with solvent molecules and medium ions) than less certain values of the thermodynamic constants which do not give absolute stability either, but only stability relative to the solvated species." The values of the stoichiometric constants are reliable under a given set of experimental conditions and are useful for practical purposes. In the present study the constants determined are those obtained at fixed temperature and pH as mentioned. This constant has been termed as conditional stability constant in the

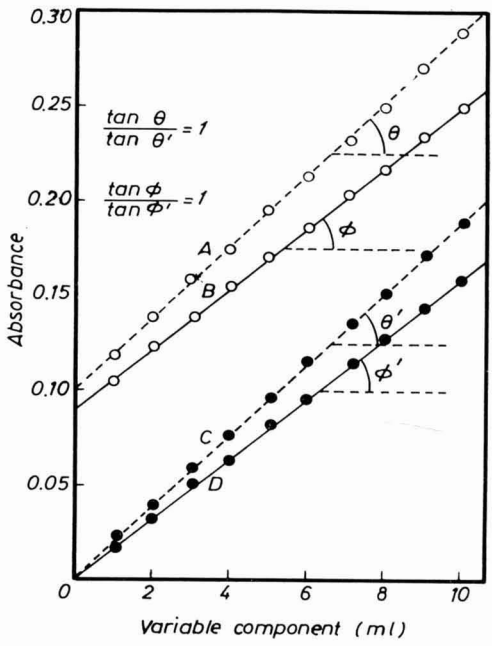


FIG. 5. Determination of the composition of AAC chelate of neodymium by the slope ratio method at pH 6.0. 10.0 ml ($5.0 \times 10^{-4}M$) excess component + x ml ($1.67 \times 10^{-4}M$) variable component + 5 ml ($0.5M$) mannitol + (10 — x) ml H_2O. Curve AB = AAC in excess, $NdCl_3$ varying. Curve CD = $NdCl_3$ in excess, AAC varying. Broken lines = 550 mμ. Solid lines = 560 mμ.

present work. The values have been determined by three different methods, i.e., the method of Mukherji and Dey (*4*), the method of continuous variations using nonequimolecular solutions, and the mole ratio method. Table 1 shows the results on stability constants (log K) obtained by all the three methods. Figures 6 and 7 show the graphs for neodymium-AAC chelate.

ACKNOWLEDGMENT

The authors are thankful to the Council of Scientific and Industrial Research, New Delhi, for financial assistance.

SUMMARY

The characteristics of the colored chelates of rare earth metals with Aluminon have been described in detail. The studies include the determination of molar ratio by three different methods, the range of pH for the stability of the chelate and the conditional stability constants by three methods.

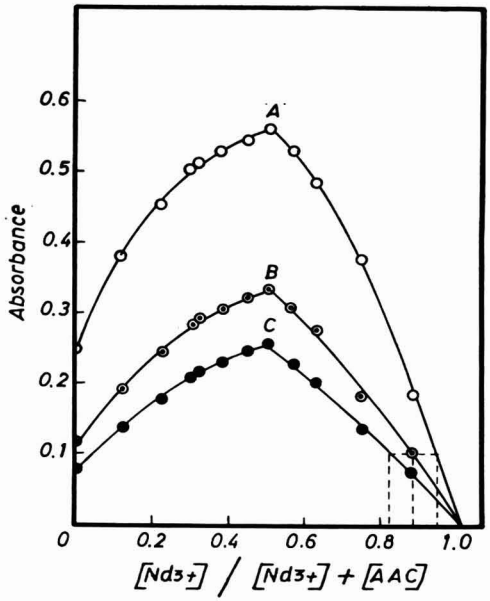


FIG. 6. Evaluation of log K of AAC chelate with neodymium by the method of Dey (4) at pH 6.0 at 550 mμ. Concentrations as in Fig. 3.

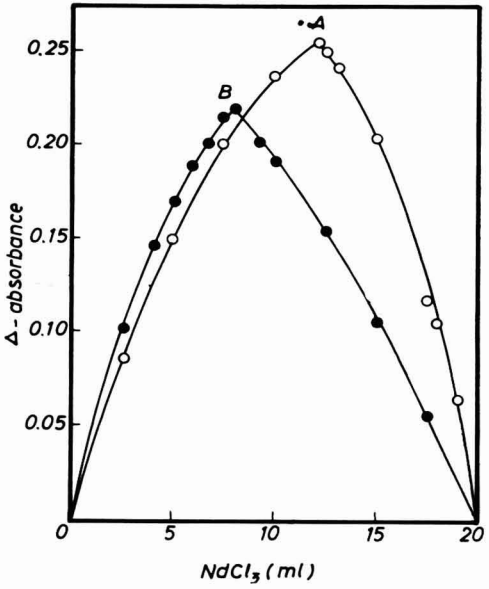


FIG. 7. Evaluation of stability constants by the method of continuous variations using nonequimolecular solutions at p H6.0 at 550 mμ. Curve A AAC $= 4.0 \times 10^{-4}M$; $NdCl_3 = 2.0 \times 10^{-4}M$. Curve B AAC $= 2.0 \times 10^{-4}M$; $NdCl_3 = 4.0 \times 10^{-4}M$.

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Hot-Flask Determination of Chlorine

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Recently two hot-flask combustion methods for the determination of halogen were described in this *Journal* (*2*). Modification II, the hot-flask combustion-diffusive absorption method was extremely simple and fast, but it could not be used for the analysis of ash-containing compounds.

In a method for the determination of sulfur (*3*), orthophosphoric acid is used for the expulsion of sulfur from the ashes of the samples. Experiments were now carried out to expel chlorine in the same manner in the hot-flask combustion method. For the titration the differential electrolytic potentiometric method (*1*) was used. The following method was developed.

COMBUSTION METHOD

Apparatus. The apparatus shown in Fig. 1 is used. It is convenient to have rods E and platinum boats which fit into E for a whole day's analysis, and to clean them all simultaneously at the end of the working day. At least two vessels K are needed for efficient work.

Reagents. Orthophosphoric acid, A.R., spec. grav. 1.7. Sodium hydroxide, A.R., 0.2*M*.

Procedure. Weigh the sample containing up to 75 μg of chlorine and not more than 300 μg of organic material into a platinum boat. Add 1 μl of phosphoric acid taking care to wet the sample as much as possible. Place the boat into the holder E. Pipet 0.3 ml of sodium hydroxide into vessel K taking care not to wet the joint. Dry joint F with filter paper and lubricate its upper part with a small amount of silicone grease with a glass rod. Blow oxygen into vessel A with capillary W at a good rate (~ 100 ml/min). Put the capillary back into test tube U, place rod E into vessel K and introduce it quickly into the hot-flask A, tightening the joint immediately. Hold vessel K firmly to the joint with the left hand and raise

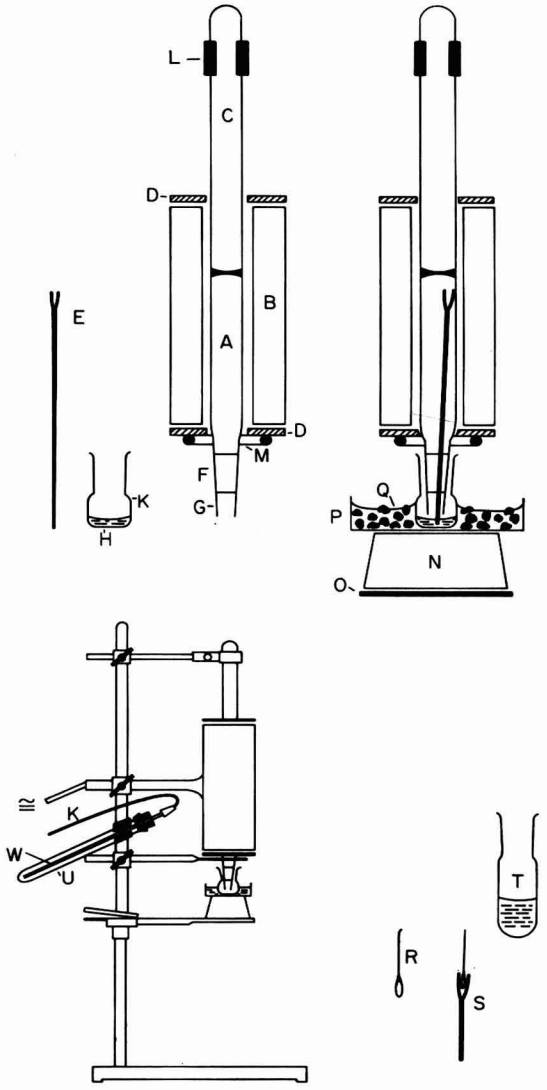

FIG. 1. Hot-flask combustion-diffusive absorption method (II). (Apparatus available from Nicroma, Tulegatan 19, Stockholm, Sweden.) A = combustion vessel of quartz, inner diameter approximately 14 mm, wall thickness about 1.5 mm, length of part in furnace about 60 mm; B = tube furnace, temperature 850°C; C = handle of opaque quartz tubing sealed upon combustion vessel A; D = asbestos sheet; E = sample holder. Quartz tubing with inner diameter about 3 mm is sealed upon quartz rod with diameter of about 2 mm. Length of container about 6 mm. Total length

table O with stopper N and vessel P with the right hand and press them firmly against vessel K. Fill vessel P with ice and ice-water Q. Allow to stand for 30 minutes.

Titrate and calculate in the meantime the preceding sample, weigh out the next sample, add phosphoric acid to it and pipet sodium hydroxide into the next vessel K.

After 30 minutes, lower table O and remove vessel K with rod E standing in it for titration. Blow oxygen once more into the hot-flask and introduce the next sample as described before.

Volatile liquids are weighed out into Supremax capillaries R, which are filled and closed in the usual manner and placed into holder E as shown in inset S, Fig. 1. If the titration method described below is used, the phosphoric acid is placed into holder E at the side of the capillary. The thin end of the capillary is then crushed when it meets the upper wall of flask A.

Overnight and over periods of waiting during the working day joint F is closed with vessel T, which is partially filled with sodium hydroxide. No alkali should touch G or joint F!

The apparatus once assembled can be used for weeks without cleaning, even if many ash-containing samples are analyzed.

Boats and rods E are cleaned with hot sulfuric acid and water at the end of the working day.

TITRATION METHOD

Apparatus. Set up according to Fig. 2. The electrodes are made of silver wire, diameter 0.6 mm, length 100-200 mm. One of them is introduced into a polyethylene tube which fits as snugly as possible. The two wires are then twisted and introduced into a snugly fitting wider polyethylene tube. Liquid polyethylene is then allowed to drop upon their lower ends from

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about 95 mm; F = joint B 12 with extension G, which ends about 2 mm above absorbing solution H, when sample has been inserted; K = vessel with joint B 12. Diameter like A, length of part below joint about 18 mm; L = support clamp which holds combustion vessel with handle C; M = ring support which holds D; O = adjustable support table; and N = soft rubber stopper securing steady press upon O from below. P = metal vessel; Q = ice and ice-water; R = sample of volatile liquid weighed out in capillary of Supremax glass; S = holder E with capillary R inserted; T = glass tube containing sodium hydroxide; U = test tube clamped to support; V = stainless steel capillary tubing which connects quartz capillary W to oxygen tank.

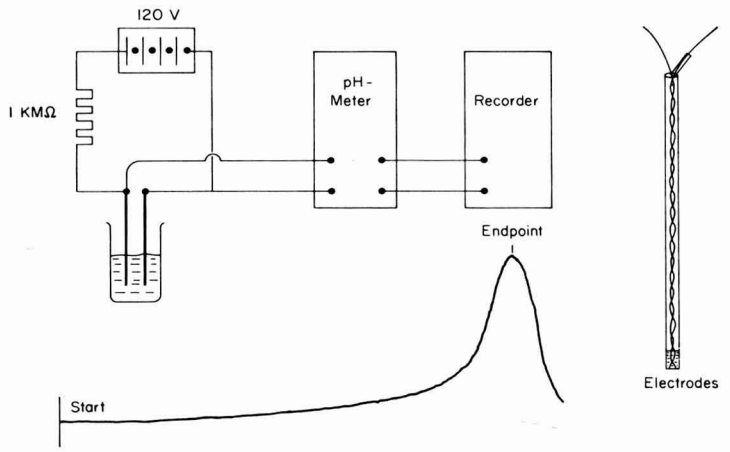

FIG. 2. Layout of titration setup, electrodes and titration curve. Even sharper end points were recently obtained with a 40-V battery instead of that with 120-V tension.

a heated polyethylene rod and is shaped to a tight seal. After cooling the end is shaped with a razor blade and a fine, sharp file so that the clean, flat ends of the silver wire lie in the same plane as the surrounding polyethylene. There should be no fissures between the metal and the plastic, because entering liquid would vitiate the measured potentials. Compare also (*1*).

Magnetic stirrer with stirring bars made of iron wire sealed into snugly fitting thin-walled glass tubing. The wire is about 0.6 mm thick and 4-5 mm long. Violent stirring is essential for good titration curves!

An Agla syringe burette mounted with a home-made motor drive for ten constant speeds and a Sargent SRL recorder connected to a Metrohm pH-meter E 300 B were used in our work, but any other arrangement for automatic recording potentiometric microtitration would be suitable. The burette is adjusted to deliver 50 μl/min and the recorder to a chart speed of 1 inch/min. A recorder sensitivity of 50 mv is suitable with the pH-meter used.

Reagents: Acetic acid, A.R., 1*M*. Silver nitrate, A.R., 0.005 N, accurately standardized. Absolute alcohol, A.R.

Procedure. Wash the lower end of rod E and the joint of the titration vessel K with 0.1 ml of acetic acid and 0.75 ml of absolute alcohol. Add a magnetic stirring bar and turn on the stirrer. Introduce electrode and

burette-tip—avoid getting grease on the electrode—and begin the titration. After the titration lower vessel K and wash electrode and burette-tip with water and replace K with a beaker with water, in which the end of the electrode is immersed. Wash vessel K with water and dry off the grease from the joint with paper. After one further washing with water it is ready for the next analysis.

It is suitable to change the polarity of the electrodes at regular intervals in order to keep the surfaces of the two silver wires equal. If the curves become irregular after some use, the end of the electrode can be reshaped by a very slight filing.

RESULTS

A series of analyses of pure organic compounds is reported in Table 1. Analyses 1-25 were run in direct succession, except that between analyses

TABLE 1
ANALYSES OF ORGANIC COMPOUNDS

No.	Substance	Sample wt. (μg)	Results		Deviation %
			Found %	Calculated %	
1	1-Chloro-2,4-dinitrobenzene	424.4	17.2	17.5	— 0.3
2	Chloranilic acid	142.5	33.7	33.9	— 0.2
3	Hexachlorobenzene	103.8	74.7	74.7	± 0.0
4	S-Benzylthiuronium chloride	225.3	17.8	17.5	+ 0.3
5	D(+)+Glucosamine hydrochloride	316.9	16.4	16.4	± 0.0
6	Benzidine dihydrochloride	179.7	27.4	27.6	— 0.2
7	1-Chloro-2,4-dinitrobenzene	319.1	17.3	17.5	— 0.2
8	Chloranilic acid	80.0	33.9	33.9	± 0.0
9	Hexachlorobenzene	84.4	74.7	74.7	± 0.0
10	S-Benzylthiuronium chloride	191.1	17.0	17.5	— 0.5
11	D(+)+Glucosamine hydrochloride	193.8	16.8	16.4	+ 0.4
12	Benzidine dihydrochloride	155.1	27.7	27.6	+ 0.1

TABLE 1 (*Continued*)

No.	Substance	Sample wt. (μg)	Results Found %	Results Calculated %	Deviation %
13	1-Chloro-2,4-di-nitrobenzene	277.6	17.1	17.5	— 0.4
14	Benzidine dihydro-chloride	175.4	27.8	27.6	+ 0.2
15	Chloranilic acid	157.9	34.5	33.9	+ 0.6
16	Hexachlorobenzene	82.5	74.7	74.7	± 0.0
17	S-Benzylthiuronium chloride	371.7	17.9	17.5	+ 0.4
18	D(+)+Glucosamine hydrochloride	297.9	16.6	16.4	+ 0.2
19	Benzidine dihydro-chloride	189.0	27.6	27.6	± 0.0
20	1-Chloro-2,4-di-nitrobenzene	268.8	17.6	17.5	+ 0.1
21	Chloranilic acid	124.9	34.1	33.9	+ 0.2
22	Hexachlorobenzene	90.5	75.3	74.7	+ 0.6
23	D(+)+Glucosamine hydrochloride	283.0	16.7	16.4	+ 0.3
24	Benzidine dihydro-chloride	204.4	27.6	27.6	± 0.0
25	S-Benzylthiuronium chloride	241.8	17.9	17.5	+ 0.4
		Analyses of Volatile Liquid			
1	Chlorobenzene	253.8	31.1	31.5	— 0.4
2	Chlorobenzene	156.3	32.2	31.5	+ 0.7
3	Chlorobenzene	345.9	31.5	31.5	± 0.0

13 and 14 four analyses were left out, which had given quite faulty results with errors between — 70% and + 5%. We could not find out what had happened.

Chlorobenzene was weighed out in capillaries of Supremax glass as described above.

A series in which an organic compound was analyzed alternating with sodium chloride, potassium chloride, silver chloride, and finally with mercuric chloride is given in Table 2. It shows, that sodium and potassium and

TABLE 2

ANALYSES OF ORGANIC AND INORGANIC COMPOUNDS

No.	Substance	Sample wt. (μg)	Results		Deviation %
			Found %	Calculated %	
1	Sodium chloride	78.7	60.4	60.7	— 0.3
2	1-Chloro-2,4-di-nitrobenzene	254.3	17.8	17.5	+ 0.3
3	Potassium chloride	118.0	48.1	47.6	+ 0.5
4	1-Chloro-2,4-di-nitrobenzene	328.7	17.7	17.5	+ 0.2
5	Silver chloride	245.7	24.9	24.7	+ 0.2
6	1-Chloro-2,4-di-nitrobenzene	339.4	17.8	17.5	+ 0.3
7	Sodium chloride	55.4	61.4	60.7	+ 0.7
8	1-Chloro-2,4-di-nitrobenzene	193.6	17.6	17.5	+ 0.1
9	Potassium chloride	131.9	47.7	47.6	+ 0.1
10	1-Chloro-2,4-di-nitrobenzene	381.6	17.5	17.5	— 0.0
11	Silver chloride	341.9	24.8	24.7	+ 0.1
12	1-Chloro-2,4-di-nitrobenzene	297.4	17.7	17.5	+ 0.2
13	Sodium chloride	70.3	63.1	60.7	+ 2.4
14	1-Chloro-2,4-di-nitrobenzene	364.3	17.6	17.5	+ 0.1
15	Potassium chloride	128.2	47.9	47.6	+ 0.3
16	1-Chloro-2,4-di-nitrobenzene	360.0	17.5	17.5	— 0.0
17	Silver chloride	285.0	25.0	24.7	+ 0.3
18	1-Chloro-2,4-di-nitrobenzene	277.2	17.7	17.5	+ 0.2
19	1-Chloro-2,4-di-nitrobenzene	394.8	17.6	17.5	+ 0.1
20	Mercuric chloride	331.5	17.3	26.1	— 8.8
21	1-Chloro-2,4-di-nitrobenzene	131.2	14.7	17.5	— 2.8
22	Mercuric chloride	173.9	20.1	26.1	— 6.0
23	1-Chloro-2,4-di-nitrobenzene	210.5	16.4	17.5	— 1.1

TABLE 2—*Continued*

No.	Substance	Sample wt. (μg)	Results		Deviation %
			Found %	Calculated %	
24	Mercuric chloride	198.8	19.2	26.1	— 6.9
25	1-Chloro-2,4-dinitrobenzene	226.6	16.4	17.5	— 1.1
26	1-Chloro-2,4-dinitrobenzene	195.7	16.3	17.5	— 1.2
27	1-Chloro-2,4-dinitrobenzene	235.7	17.5	17.5	± 0.0
28	1-Chloro-2,4-dinitrobenzene	212.1	17.3	17.5	— 0.2

even silver-containing compounds can be easily analyzed. Mercury interferes not only in the analysis of the sample which contains it, but also in at least two of the following analyses. The analyses of all three tables were run without cleaning combustion flask A.

DISCUSSION

Combustion. Several compounds have been recommended for the expulsion of acidic elements from ash-containing compounds. In an investigation of the determination of sulfur (*3*), orthophosphoric acid was found to be the only suitable and reliable compound for the expulsion of sulfate in an atmosphere of oxygen. It seemed interesting to try this compound also for the hot-flask combustion of halogen. In the first method (*2*) 0.1*M* sodium hydroxide was used for the absorption, which was completed in 20 minutes. Low results were, however, frequently obtained, when phosphoric acid was added to the samples. Obviously the phosphoric acid delayed the absorption of the chlorine. Correct results were obtained when the time for absorption was increased to 30 minutes and 0.2*M* sodium hydroxide was used for the absorption. The phosphoric acid used with the sample appears to neutralize any alkaline spots or contaminations in the hot-flask, which makes it possible to use the flask for weeks without cleaning, even when sodium chloride, potassium chloride, and silver chloride have been analyzed.

Titration: The concentration of electrolyte in the final solution is rather high, and it appeared therefore advisable to use argentometric titration with an electrometric indication of the end-point. The differential electro-

lytic potentiometric method (*1*) appeared very attractive because of its high sensitivity and simple electrodes, which need no attention, and which can easily be shaped to fit also into small vessels. The results obtained in the titration of pure sodium chloride solutions were as good as could be expected from the reading of the end-point on the recorder chart, and the application to the titration of chlorine in the absorption solution used presented no difficulties.

CONCLUSIONS

As described above the hot-flask combustion method is extremely simple. It needs no complicated apparatus, there are no transfers of solution, no washings of absorption apparatus and no intermediate boilings or other operations. It is widely applicable—sodium chloride, potassium chloride, silver chloride, and probably many other metal-containing compounds can be accurately analyzed alternately with pure organic compounds, allowing for the interference of mercury.

SUMMARY

The hot-flask combustion-diffusive absorption method for the decimilligram determination of halogen has been improved by the addition of 1 µl of orthophosphoric acid to the sample before the combustion. Alkali- and other ash-containing as well as inorganic compounds can now be easily analyzed. It was applied to the determination of chlorine with automatic differential electrolytic potentiometric titration.

ACKNOWLEDGMENT

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Methods for the Isolation and Characterization of Constituents of Natural Products

III. Separation of Alcohol Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone into Classes by Column and Thin-Layer Chromatography

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In the initial report of this series (*2*) methods were described for the preparation of a new acid chloride, namely, pyruvyl chloride 2,6-dinitrophenylhydrazone and esters prepared from it with primary, secondary, and tertiary aliphatic alcohols. Part II described normal- and reversed-phase partition systems for separating homologous series of members of the classes of alcohols by thin-layer chromatography (TLC) (*3*). The present report is concerned with the quantitative separation of a mixture of the ester derivatives into classes (namely, those derived from primary, secondary, and tertiary saturated alcohols) by column chromatography on magnesium oxide. The column procedure has been adapted qualitatively to TLC, and this technique is also described. The use of TLC on aluminum oxide to separate isomeric ester derivatives is also presented.

APPARATUS AND MATERIALS[1]

Magnesium oxide (catalog no. 2477) suitable for chromatographic use was obtained from the J. T. Baker Co., Phillipsburg, N.J. The powder had an adsorption index (Food and Drug yellow No. 4) of 12-13 and was used without further treatment. Celite 545 and Analytical Grade Celite were obtained from the Johns-Manville Co., Baltimore, Maryland. Chloroform, benzene, and methanol were ACS grade; diethylamine (Baker) was

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

redistilled; methyl cyclohexane was obtained from Distillation Industries, Rochester, N.Y., Aluminum oxide G was obtained from Brinkmann Instruments, Inc., Westbury, N.Y.

The thin-layer chromatography equipment was the same as previously described (*3*). A borosilicate glass column 15.5 × $1\frac{3}{8}$ inches I.D. containing a coarse fritted glass disc was employed for the column chromatography.

EXPERIMENTAL

Separation of classes by column chromatography. Magnesium oxide (7.5 g) and Celite 545 (30 g) are slurried together in approximately 90 ml of $CHCl_3$. The slurry is poured through a long-stemmed funnel into the chromatography tube and packed immediately by moderate air pressure until a few milliliters of solvent remain above the bed. The esters, dissolved in the minimum amount of $CHCl_3$, are quantitatively transferred to the column taking care not to disturb the top of the bed. After all of the solution has percolated into the bed, the sides of the column are washed down with two 1-ml portions of $CHCl_3$, and when these have penetrated a plug of glass wool is placed just above the bed and development of the chromatogram is begun. The esters, adsorbed as a deep violet or bluish-violet band at the top of the column, are separated using the following sequence of solvents: 50 ml $CHCl_3$, 100 ml of 0.1% MeOH in $CHCl_3$, 100 ml of 0.2% MeOH in $CHCl_3$, 100 ml of 0.5% MeOH in $CHCl_3$, 50 ml of 2% MeOH in $CHCl_3$ and, finally, 5% MeOH in $CHCl_3$ until the last band is removed from the column.

Separation of classes by TLC. Magnesium oxide (3 g) and Analytical Grade Celite (7 g) are slurried in 50 ml of distilled water and spread in the usual manner over five 8 × 8-inch plates. The plates are dried at 100°C for at least 2 hours. The esters are spotted from benzene solution and turn violet or blue as the benzene evaporates. The plate is developed in an equilibrated tank using benzene: $CHCl_3$ (3:1) as solvent. At the end of approximately 1 hour, the plate is removed and the spots marked as the solvent evaporates.

Separation of isomeric alcohol derivatives by TLC on alumina. Aluminum oxide G (25 g) is shaken with 35 ml of distilled water and the slurry is spread over four 8 × 10-inch plates. The plates are activated at 100°C for at least 2 hours, then spotted with benzene solutions of the derivatives and developed in an equilibrated tank with diethylamine:methyl cyclohexane (35:65). At the end of the development (about 1 hour) the plate

is removed and the purple spots are marked. As the diethylamine evaporates from the plate, the purple spots revert to their original yellow color.

RESULTS AND DISCUSSION

Figure 1 depicts the type of separation of the classes achieved in the system described for column chromatography. The effluent from the column was monitored continuously at 400 mμ by running it through a 0.2-ml flow-through cell in an Hitachi-Perkin Elmer model 139 spectrophotometer and recording the readings with a Honeywell-Brown 5-mv recorder. For the attempted separation of the classes, the extremes in each class (of the derivatives prepared in this laboratory) were selected. Thus the C_8 and C_4 tertiary, the C_{18} and C_3 secondary and the C_{18} and C_1 primary alcohol derivatives were employed. The extremes were selected because preliminary work indicated that the most difficult resolution would be between a short-chain compound in the faster-moving class and a long-chain compound in the class immediately following it on the column. This is also the case in the separation of 2,4-dinitrophenylhydrazones into classes (*4*). The mixture selected also ostensibly represents a more difficult situation than will occur in an unknown consisting solely of saturated aliphatic alcohol derivatives.

All of the members lying between the extremes in chain length in a given class move the same or between the extremes in that class on the chromatogram. The tertiary alcohol derivatives move fastest, and primary alcohol derivatives slowest, on the chromatogram. All tertiary alcohol derivatives above tert-butanol move as blue bands on the adsorbent. The tert-butanol is violet as are all of the primary and secondary alcohol derivatives which have been prepared thus far.

The chromatogram shown in Fig. 1 took 3 hours to complete. However, the time to complete the separation in practice can be shortened considerably since the primary alcohols can be eluted as one band, if desired, once the last of the secondary alcohols has been eluted.

Only two lots of the magnesium oxide were available for evaluation. Both lots gave highly satisfactory separations, but the chromatograms were not identical. Each band was checked for purity and was identified by thin-layer partition chromatography as described in Part II of this series (*3*).

Stability and recovery of the esters were checked by running each compound on individual columns. Recoveries ranged from 95% to 104% when

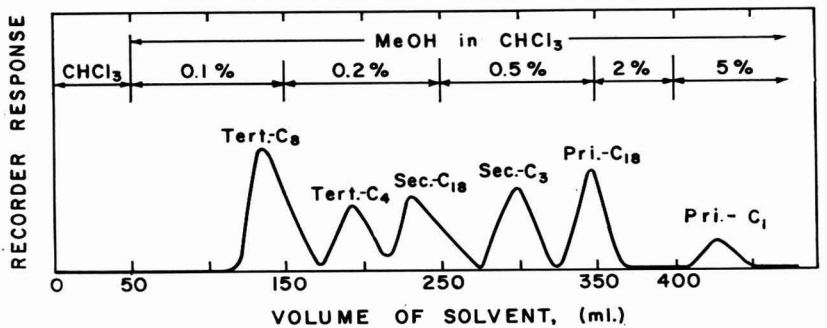

FIG. 1. Separation of a mixture of primary (pri), secondary (sec), and tertiary (tert) alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazone into classes by column chromatography on magnesium oxide.

0.5 to 1.0 μmole quantities were run. Similar amounts of esters were used in the mixtures. A 0.5 μmole of an ester can be readily seen on the column, although the intensity of the band decreases as the band nears the bottom of the column.

Figure 2 shows the separation of the classes by TLC on a magnesium oxide plate. Separation is very good and the colors displayed on the magnesium oxide column are also manifested on the plate. Both lots of adsorbent gave equally good, but not identical separations. The same mixture which was chromatographed on the column was run on the plate, but, in addition, 2-butanol, *n*-propanol, and ethanol derivatives were also included. Approximately 5×10^{-4} μmole of a derivative is still visible on the plate.

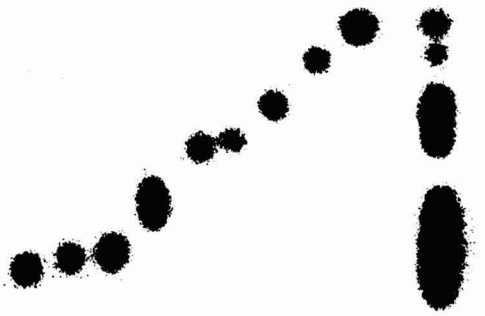

FIG. 2. Separation of a mixture of primary, secondary, and tertiary alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazone into classes by TLC on magnesium oxide. Solvent, benzene: $CHCl_3$ (3:1). Diagonally from top to bottom: tert C_8, tert C_4, tert C_5, sec C_{18}, sec C_4, sec C_3, pri C_{18}, pri C_2, pri C_1. Column on right represents a mixture of all of the compounds.

Separation is best achieved when approximately equimolar amounts of the derivatives are spotted. However, when there is a gross disproportionality of constituents, class separation is not as clear-cut and some confusion might arise concerning the proper classification of a derivative. In view of this shortcoming, it is recommended that class separation by TLC be used mainly as a check on the identity of a band obtained from column chromatograms since one can then spot on the plate approximately equimolar amounts of authentic derivatives and the unknown band.

It is also recommended that the prepared magnesium oxide plates be kept a maximum of 48 hours since there appears to be a weakening of the adsorbent when at oven temperature or at room temperature in a desiccator for longer periods.

Figure 3 shows the separation of isomeric alcohol derivatives. All primary and secondary alcohols from C_3 through C_{18} and the C_4 through the C_8 tertiary alcohols were examined. All isomeric alcohol derivatives separated in a similar fashion as the derivatives shown in Fig. 3. Under nonbasic or even weakly basic conditions, isomer separation is not accomplished. Only strong bases with a pK_b less than 3.0 were effective. Isomer separation on a neutral or acidic (silica gel G works equally as well as aluminum oxide) adsorbent by using a strongly basic solvent system is probably comparable to the class separation described above on magnesium oxide. However, in attempting the class separation on aluminum oxide G or on silica gel G, no clean-cut separation was achieved. The short-chain components of the faster-moving class and the long-chain constituents of the class immediately following overlapped considerably and precluded the use of this system for executing separation of the classes. There is also no difference between the color of the spots of tertiary alcohols and that of the other alcohols, all spots being deep purple.

FIG. 3. Separation of isomeric alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazone on aluminum oxide G. Solvent, methyl cyclohexane:diethylamine (65:35). (A) Top to bottom: tert C_6, sec C_6, pri C_6; (B) pri C_6; (C) sec C_6; (D) tert C_6; (E) Top to bottom: tert C_7, sec C_7, pri C_7; (F) pri C_7; (G) sec C_7; (H) tert C_7; (I) Top to bottom: tert C_8, sec C_8, pri C_8; (J) pri C_8; (K) sec C_8; (L) tert C_8.

Separation of the 2,6-dinitrophenylhydrazone derivative of the pyruvate esters of primary, secondary, and tertiary alcohols into classes constitutes an important step in simplifying the analysis of complex mixtures of alcohols such as might be encountered in the analysis of a natural product. The value of having a method for class separation is exemplified by the progress made in carbonyl analysis in which the 2,4-dinitrophenylhydrazones are separated into classes (*1*, *4*, *5*).

SUMMARY

Quantitative column and qualitative thin-layer chromatographic procedures are described for separating a mixture of the 2,6-dinitrophenylhydrazone derivatives of pyruvic acid esters of primary, secondary, and tertiary aliphatic alcohols into classes. Magnesium oxide is used as the adsorbent in both procedures and separation of the classes follows a similar pattern. The derivatives change color from yellow to violet or blue on the adsorbent. Tertiary alcohol derivatives above butyl show a blue color whereas tertiary butyl and the primary and secondary alcohol derivatives are violet. Isomeric derivatives can also be separated by thin-layer chromatography on aluminum oxide G by using a solvent system containing a strong organic base.

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MICROCHEMICAL JOURNAL 12, 192-195 (1967)

Methods for the Isolation and Characterization of Constituents of Natural Products

IV. Amide Derivatives of Amines with Pyruvyl Chloride 2,6-Dinitrophenylhydrazone

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In the first report of this series (*2*) preparation of a new acid chloride, pyruvyl chloride 2,6-dinitrophenylhydrazone and esters resulting from the condensation of it with primary, secondary, and tertiary aliphatic alcohols was described. The potential utility of the reagent and resultant derivatives was discussed from the standpoint of the ease with which the derivatives can be formed in and fractionated from a complex lipid mixture, and their subsequent isolation free of contamination. As a continuation of a program to develop new methods for the isolation and characterization of functional constituents in natural products, the present paper describes the preparation of a homologous series of the pyruvic acid 2,6-dinitrophenylhydrazone derivatives of primary amines and a partial series of secondary amine derivatives. These derivatives exhibit properties much like their ester analogues not only in their physical characteristics but also in the ease with which they are formed and isolated, especially at the micromole or submicromole level. These characteristics should make them particularly useful in the analyses of natural products.

APPARATUS AND REAGENTS[1]

The apparatus and reagents used to prepare the amides were identical to those described for the preparation of the esters (*2*).

All primary amines were straight-chain. All secondary amines were

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straight-chain and symmetrical. All amines were purchased from Lachat Chemical Inc., Chicago, Illinois, except for the following: methyl-, ethyl-, and dimethylamine were purchased as compressed gases from the J. T. Baker Company, Phillipsburg, New Jersey; diethylamine and di-*n*-propylamine were obtained from the Matheson Company, East Rutherford, New Jersey. All of the amines were of the highest purity available from the specified sources and were used without further purification.

EXPERIMENTAL

Amides of pyruvic acid 2,6-dinitrophenylhydrazone. The procedure for the preparation of the amides differed slightly from the preparation of esters (*2*). A stock solution of pyruvyl chloride 2,6-dinitrophenylhydrazone is made by dissolving 23.5 mg (0.08 mmole) of the acid chloride per milliliter of purified benzene. Ten milliliters of this solution are pipetted into a dry flask and the amine is added. Four or five drops of *n*-propyl through *n*-hexyl, diethyl- and di-*n*-propylamines are used. For longer-chain amines, 0.4 mmole is weighed out and transferred to the flask using a minimum of purified benzene to effect transfer when necessary. Five milliliters of a solution of triethylenediamine (containing 19.4 mg/ml of purified benzene) are added in 1-ml portions, shaking briefly between additions and the mixture allowed to stand for 5 minutes or more at room temperature. For preparation of the derivatives of methyl-, ethyl-, and dimethylamine, the gas is bubbled briefly through 10 ml of the acid chloride solution until an orange-red color is observed. Triethylenediamine is not added to these solutions.

The amide derivatives are isolated on weakened, acidic alumina in the same manner as was described for the esters (*2*) except that the secondary amine and short-chain primary amine derivatives are eluted from the column with chloroform. A green color may develop on the alumina in the case of methyl-, ethyl-, and dimethylamine derivatives. This is due to the presence of a large excess of amine. No adverse effect is evident from this, however, and the excess base is removed in the evaporation step.

For all of the derivatives, the solvent is removed on the steam bath under a gentle stream of air or nitrogen, and the residue is recrystallized from absolute ethanol. The yield is approximately 90%. The amides were recrystallized to a constant molar extinction coefficient. This was found to be a more reliable index of purity for the individual compounds than was a constant melting point. Most of the primary amines gave an

analytically pure sample after one recrystallization. The shorter-chain amines and most of the secondary amines required two recrystallizations.

RESULTS AND DISCUSSION

The physical constants determined on the amides are given in Tables 1 and 2. All of the derivatives are solid, highly crystalline materials that melt sharply without decomposition. Primary amine derivatives are yellow, and exhibit an absorption maximum at 407 mμ in benzene; derivatives of secondary amines are orange with an absorption maximum in benzene near 412 mμ. Both classes of derivatives color more or less violet in alcoholic base, a phenomenon displayed by many nitro- and polynitro compounds (*1*). This feature is associated with chemical changes which facilitate their subsequent isolation from a natural product since it enables them to adsorb strongly onto alkaline adsorbents, to ion-exchange under the proper conditions, and to extract into alkaline solution. Schwartz

TABLE 1

PHYSICAL PROPERTIES OF AMIDES OF PRIMARY AMINES WITH PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE

Primary amines	M.P.[a] (°C)	E[b]	C (calc)	C (found)	H (calc)	H (found)
C_1	208 — 209	5788	42.7	42.9	3.91	4.41
C_2	165.5 — 167	6008	44.7	45.2	4.41	4.84
C_3	127 — 128	5907	46.6	46.7	4.85	5.00
C_4	76 — 77	5924	48.3	48.1	5.26	5.18
C_5	58 — 59	5976	49.8	49.8	5.63	6.31
C_6	77 — 78	6038	51.3	50.9	5.98	6.04
C_7	67	5953	52.6	52.6	6.31	6.06
C_8	55	5963	53.8	54.8	6.59	6.74
C_9	59 — 60	5976	55.0	55.3	6.88	7.19
C_{10}	66 — 66.5	5986	55.9	55.9	7.12	7.13
C_{11}	67.5 — 68	5919	57.0	57.8	7.37	7.52
C_{12}	77	5902	57.9	57.8	7.59	7.76
C_{13}	74 — 74.5	5996	58.8	58.0	7.78	5.04
C_{14}	78 — 78.5	6059	59.7	59.4	7.96	7.88
C_{15}	78 — 79	6162	60.3	60.3	8.17	8.29
C_{16}	85.5 — 86	6136	61.0	61.0	8.35	8.44
C_{17}	82.5 — 83	6094	61.8	60.1	8.51	8.64
C_{18}	89.5 — 90.5	5938	62.5	63.0	8.67	8.92
	Average	5941				

[a] Determined with the Fisher-Jones apparatus and uncorrected.

[b] Determined in benzene in a 1-cm^2 cell at 407 mμ.

TABLE 2

PHYSICAL PROPERTIES OF AMIDES OF SYMMETRICAL SECONDARY AMINES WITH PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE

Secondary amines	M.P.[a] (°C)	E[b]	C (calc)	C (found)	H (calc)	H (found)
C_2	133.5 — 136	5323	44.7	45.8	4.40	4.63
C_4	115.5 — 117	5408	48.3	48.1	5.88	5.39
C_6	150 — 151	5368	51.3	51.2	6.55	6.33
C_8	93 — 93.5	5403				
C_{10}	46 — 48	5335	56.0	56.1	7.61	7.37
	Average	5367				

[a] Determined with the Fisher-Jones apparatus and uncorrected.

[b] Determined in benzene in a 1-cm^2 cell at 412 mμ.

et al. (*3*) have utilized these properties in 2,4-dinitrophenylhydrazones to isolate and fractionate the derivatives formed directly in lipids.

Preliminary work indicates that both primary and secondary amines can be derivatized at the submicromole level under very mild conditions. The quantitative aspects of this as well as the separation of primary and secondary derivatives into classes and subsequent resolution of the individual members is also being investigated.

SUMMARY

Preparation of amide derivatives of primary and secondary aliphatic amines with pyruvyl chloride 2,6-dinitrophenylhydrazone is described. The derivatives form within 5 minutes at room temperature in benzene solution. Primary amine derivatives are yellow, and all members investigated (normal C_1-C_{18}) are solids. The primary amine derivatives have an absorption maximum at 407 mμ in benzene and have a molar absorptivity near 5941. Secondary amine derivatives are orange, and all members investigated (symmetrical, unbranched C_2-C_{10}) are also solids. The secondary amine derivatives have an absorption maximum at 412 mμ in benzene and have a molar absorptivity near 5367. Both classes of amine derivatives have properties which facilitate their isolation directly from lipids.

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MICROCHEMICAL JOURNAL 12, 196-206 (1967)

Relationship between Accuracy and Sensitivity in Atomic-Absorption Flame Photometry

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The applicability of atomic-absorption flame photometry to the solution of an analytical problem depends on the accuracy and precision attainable relative to the practical sensitivity (fixed under a set of operating conditions, or variable at the operator's will) that can be achieved for the analyte involved in the determination.

The relationship that exists in general between accuracy and precision is well-known. Also the relationships between precision and sensitivity have been mentioned in another paper (*7*) for the particular case of atomic-absorption methods. However, it is now necessary to discuss the relationship between *sensitivity* and *accuracy*, as sensitivity can act as a limiting factor to the accuracy and sensitivity will complete the picture of the analytical characteristics of atomic-absorption flame photometry as an analytical method studied by the author.

In this paper some attention is first paid to the instrumental limitations of accuracy based on the absorptimetric nature of atomic-absorption methods, next a brief summary of other factors influencing accuracy in practical work is given, and, finally, some comments are included on the limitations on accuracy caused by sensitivity limitations. The reader will find complete details on sensitivity in atomic-absorption work in the Refs. *5-7*.

EXPERIMENTAL

Instruments

For this study, the following equipment was used:

Spectrophotometers. Beckman DU®-2 Spectrophotometer, with A.C. power supply, SERA, and Beckman 5″ Recorder, mounted with a Beckman Atomic-Absorption Accessory Model 1300. Beckman DB® Spectrophotometer with a Beckman 5″ Recorder, mounted with a Beckman Atomic-Absorption Accessory Model 1301.

Burners. Beckman Turbulent Flow Burner (TFB), air-hydrogen flame. Beckman Laminar Flow Burner (LFB), air-acetylene flame.

Experimental Conditions and Concentration Limits

Details on experimental conditions and percentual qualitative concentration limits obtained with the above mentioned equipment were given in a recent communication (*3*).

Accuracy in Atomic-Absorption Flame Photometry

Accuracy in atomic-absorption work can be considered with the same concepts used to define and to express accuracy in any analytical method (instrumental or non-instrumental). It is determined by absolute or relative errors calculated from experimental values and values considered as theoretically correct, as for example known concentrations or weights.

The above-mentioned errors can derive from a series of factors that can be grouped under the following headings:

FACTORS DEPENDING ON SAMPLE COMPOSITION

Physical factors. Among them, viscosity and surface tension can cause errors if the comparison standards are not corrected in order to match them with the solutions under determination.

Chemical factors. These are considered as one of the main sources of error in atomic-absorption work, as other components present in the solution can cause interelement effects and compound formation. Typical examples are the presence of excessive amounts of a metallic component (sodium-rich systems, iron-rich systems) and the presence of anions (phosphates, sulfates). The author has observed in his studies on sodium-rich systems that there are not only effects due to the metallic component present, but also other effects caused by the anion accompanying the concomitant metallic component. The former cases may be compensated for by adding similar concentrations of the accompanying components to the standards. The later cases require separation, compensation of the standards, and/or buffering in both solutions and standards. It is also possible to use releasers and protectors. The use of releasers and protectors are based on equilibrium phenomena. Therefore an excess of them is necessary in order to sufficiently unbalance the releasing or protecting equilibrium.

FACTORS DEPENDING ON SAMPLE PREPARATION

These types of factors are not peculiar to the atomic-absorption field. They are commonly found in all types of analytical methods. They in-

volve chemical losses, contaminations, lack of recovery in extraction methods (due to equilibria in phase transference), accidental insolubilization, dilution and weighing errors, and many others.

Factors Depending on the Instrumental System

Accidental and operational factors. Many of them are common to other instrumental methods: drifts, malfunctioning of recorders or other readout systems, operational maladjustments, etc.

Fixed inherent factors. In addition to those factors deriving from production defects, and from some limiting specifications, perhaps the most important aspect to be considered is the limitation of reading the scale between 0% absorption and 100% absorption that is the basis of the applicability of the atomic-absorption method as a typically absorptimetric instrumental method. This circumstance will be referred to later when dealing with the calculation of the most convenient ranges of concentration.

Experimentally variable factors. This is another important aspect, as it depends in most cases on the operator's decisions. Among them the use of scale expansion and variation of instrumental sensitivity are of special importance. These are also discussed later in detail.

Factors Depending on the Operator

As these factors depend on the skill of the personnel involved, they are not discussed here. Obviously, potentially good accuracy, predictable from instrumental performance and analyte characteristics, can be spoiled by careless handling of both instrumental and chemical systems.

Convenient Concentration Range for Accurate Analytical Determinations

As atomic-absorption flame photometry follows similar measurement patterns as conventional spectrophotometry (both are absorptimetric methods), the same criteria may be applied to determine theoretically the most suitable concentration ranges for best accuracy in analytical determinations. See parallel discussions for spectrophotometric analysis in Refs. *1*, *2*, *4*, and *9*.

All atomic-absorption measurements are taken within a scale that ranges between 0% absorption (no absorption, or blank adjusted to read 0% absorption) and 100% absorption (no light reaching the detector, and if necessary compensating for emission from the flame when the source is shut off).

Any discussion should be based, then, on this essential and inherent limitation of the reading scale.

The error of a determination made by atomic-absorption flame photometry can be calculated starting with the definition of absorbance

$$A = \log I_B/I_S = abc,$$

where

A = Absorbance
I_B = Intensity of source light transmitted when spraying the blank solution
I_S = Intensity of source light transmitted when spraying the test solution
a = Absorptivity
b = Path length of the absorption cell
c = Concentration of absorbing atoms in the absorption cell

Doing the necessary arrangements

$$R = \frac{-0.4343\ e^{2.303\ abc}}{A} = \frac{dc/c}{dI_S/I_B} = \frac{\text{Relative concentration error}}{\text{Relative photometric error}}.$$

It is desirable to find when the ratio R attains a minimum with respect to the value of the concentration, because this allows calculation of the concentration at which a given relative error in the reading produces the minimum error in the measured concentration.

Then for minimum error, setting

$$\frac{dR}{dc} = 0,$$
$$A = 0.4343.$$

This value of absorbance corresponds to a transmission of 36.8% (i.e., 63.2% absorption).

The optimum concentration then is that producing 63.2% absorption. Errors found for concentrations giving percentages of absorption between 30 and 80% are generally acceptable. See in Fig. 1 the curves corresponding to readings made to an accuracy of ± 0.1, ± 0.2, ± 0.5, ± 1.0, and ± 2.0 scale divisions.

This range of absorption percentages corresponds to the steepest region of the Ringbom plot (% absorption vs. logarithm of the concentration). Originally the Ringbom plot was *1-transmittance* (in %) vs. log C (*8*).

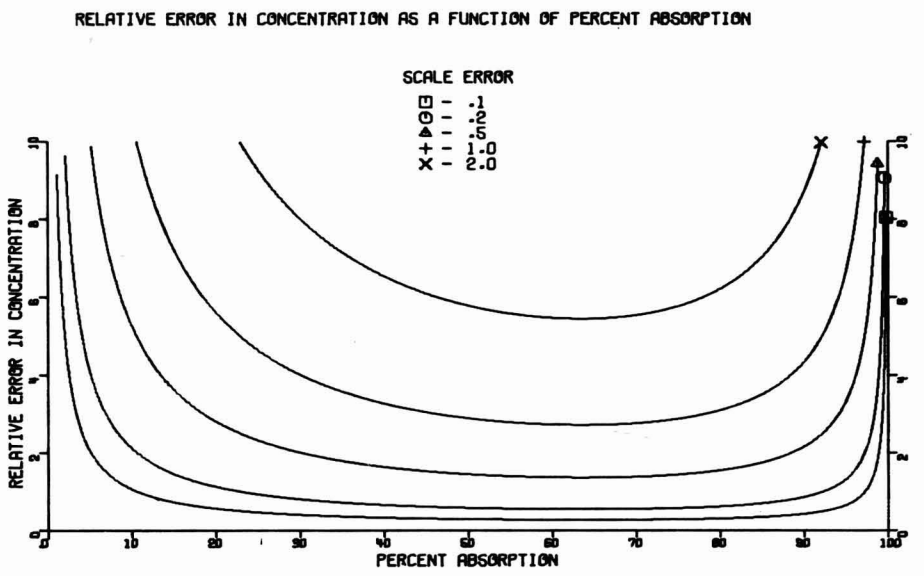

FIG. 1. Relative error in concentration as a function of percent absorption at different values of scale error. This figure is a direct reproduction of the automatic plotting obtained from computer data.

In this Ringbom plot the maximum slope is theoretically obtained for that concentration producing 63.2% absorption.

All the preceding holds when the analytical system follows Beer's Law, i.e., $A = a\,b\,c$, along a reasonable range of c (linear response) even at absorbances > 0.4343, and assuming there is a proportionality between the light intensity received in the instrument (transmitted light to be measured) and the response to the read-out system. The absorbance A is related to the solution concentration C by means of the equation $A = m\,C$. If there is a lack of proportionality in either relationship, the attainable accuracy is limited, in part or along the concentration range used. There are frequent deviations from Beer's Law (calibration graphs show curvature) due to collateral instrumental or sample factors: lack of resolution, low intensity lamps, interfering components in the test solution, etc.

In fact, if experimental data are plotted on ordinary absorbance and concentration scale, like those shown in Fig. 2, this type of graph helps to determine an upper end of the advisable dynamic range.

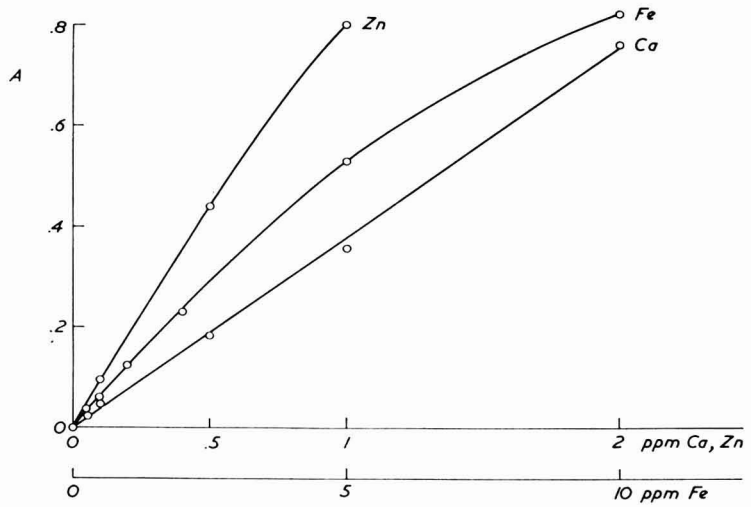

FIG. 2. Working curves, absorbance vs. concentration for Zn, DU®-2, and turbulent flow burner (3 Burners/3 Passes); Fe, DU®-2, and turbulent flow burner (3 Burners/3 Passes); Ca, DB® and laminar flow burner (1 Pass).

This end coincides with the point at which the curve begins to exhibit severe curvature, with a subsequent decrease of slope and of percentual qualitative sensitivity, as a function of concentration.

The lower end of the recommended dynamic range may be determined from the fluctuational quantitative concentration limit (*7*) or its multiples (*5*).

That fluctuational quantitative concentration limit is calculated in terms of a *precision* desired, not an accuracy. If a multiple of it is considered however, this helps to determine the lower limit corresponding to the concentration that gives sufficient signal to obtain accurate results as well. In some cases, dealing with very low analyte concentration (the analyte might also be a low sensitivity element), and even operating the instrument at maximum sensitivity, the signals recorded are very small. If other means are not provided to increase the signals, the analyst should be aware that poor precision and also poor accuracy will be obtained. This is another reason why so much effort is expanded to yield high instrumental sensitivity with atomic-absorption equipment.

If the values are plotted on a Ringbom plot (Fig. 3) it is possible to distinguish three regions: (a) a curved lower end at low concentrations;

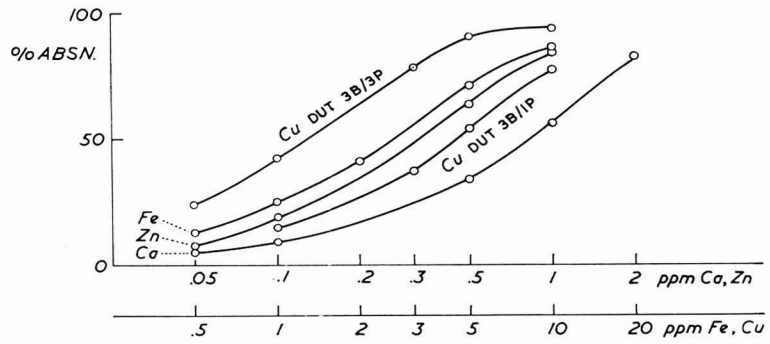

FIG. 3. Curves percent absorption vs. log concentration for Cu, DU®-2, and turbulent flow burner (3 Burners/3 Passes); Cu, DU®-2, and turbulent flow burner (3 Burners/1 Pass); Zn, Fe, Ca, same conditions as shown in Fig. 2.

In this figure DUT = DU®-2, and turbulent flow burner; 3B/3P = 3 Burners/3 Passes; 3B/1P = 3 Burners/1 Pass.

(b) a maximum slope section in which the slope is nearly constant, near the middle of the concentration range; (c) a curved upper end at high concentrations.

The best region to obtain accurate results is that mentioned under (b), and coincides with the reading range previously discussed. In the curved sections small variations in the readings produce large deviations on the concentration scale.

By extension of the data published for conventional spectrophotometry (*9*), in atomic-absorption methods it may be assumed that when readings are obtained with an accuracy of $\pm$ 0.2%[1] (practical limit for photometric accuracy) a relative error of about 0.5% will result when working in close proximity to the optimum percentage of absorption. The relative concentration error can be theoretically smaller if more accurate read-out systems are used instead of direct visual observation of the scale.

Table 1 (see p. 203) shows data obtained by computer techniques during the process of obtaining Fig. 1.

Scale Expansion

Scale expansion is an easy and convenient way to increase readability in any instrument, but it is necessary to remember that both the signal and the noise are expanded when this device is used. In spite of the fact that

[1] Most reading scales permit visual observation to 0.5 to 0.1% full scale, i.e., down to 0.5 to 0.1 division.

TABLE 1
MINIMUM VALUE OF RELATIVE ERROR IN CONCENTRATION: 63.2% ABSORPTION

Scale error	Relative error
.1	.2718
.2	.5437
.5	1.3594
1.0	2.7188
2.0	5.4376

precision can be slightly improved because of the better readability, it is hard to imagine that accuracy can be improved too much beyond the limits that the better readability allows. The magnitude of the absorption itself is not expanded, just the reading on the scale.

Scale expansion techniques can be improved by the use of auxiliary electrical condensers to decrease noise. But while this practice helps to obtain better fluctuational sensitivity *relative* (qualitative and quantitative) it does not aid much in improving the fluctuational *absolute* sensitivity (also qualitative and quantitative).

This is true because the increase in time constant requires sample-feeding for a longer time, which, in turn, increases the volume of solution consumed, and therefore the amount of analyte involved in each determination (absolute amount—weight—measured in grams).

Variations of Sensitivity

Versatile instruments which permit variation of sensitivity may increase accuracy by allowing adjustments of readings to the best range of the absorption scale. Change of sensitivity range may be desirable in either of two cases: (a) decreasing the sensitivity, in order to lower high absorption readings in those cases in which a dilution of the sample solution is not advisable or convenient; (b) increasing the sensitivity, in order to raise or increase very low absorption readings, especially in those cases in which no further preparation is possible for the solution (concentration techniques) or it is not possible to use more concentrated solutions (limited sample volume or limited concentration, danger of interferences in concentrated solutions, etc.).[2]

[2] In this second case, when increasing sensitivity, the precision can also be improved. Besides permitting work in a better region of the scale, the readability ratio is smaller:

$$\text{Readability Ratio} = \frac{\text{Limit of readability (in scale divisions)}}{\text{Signal value (in scale divisions)}}.$$

There is a way to *apparently* increase sensitivity by means of sample preparation. Nonaqueous solvent extraction, concentration by precipitation or electrodeposition, and other similar techniques are means which increase the concentration of the analyte in the final prepared solutions.

This is the reason that we frequently see in the literature that concentration techniques help to increase both precision and accuracy in analytical determinations. Really, a higher concentration in the measured solution produces a higher signal with all the above-mentioned advantages.

Good sensitivity in atomic-absorption flame photometry not only permits detection and determination of an analyte at very low concentration level, but it also produces the advantages—as previously mentioned—of higher signals for a given analyte concentration.

Then, increases of sensitivity provide advantages to the flame photometrist:

1. An increase in the percentual sensitivity means greater slope in the working curve ($A = m\,C$ type). It is easy to understand that a given error in the absorption reading will have less effects on the concentration scale if the slope is greater. In other words, the accuracy should then be better. Higher percentual sensitivity permits the obtaining of higher fluctuational sensitivity. If both sensitivities are improved, the dynamic concentration range is extended.

2. Increases in percentual sensitivity mean a horizontal translation of the curves in the log — log plots ($\log A = k \log C + K$ type) and in the Ringbom plots (% Absn. $= f$ (log C) type). Note in Fig. 3 the increase of sensitivity obtained in the data found for copper, when switching from three Burners/1 Pass to three Burners/ three Passes. This is useful when dealing with low concentrations that give signals only at the curved lower end of the curve. A translation of the curve toward the left allows determination of these concentrations in a better region of the new curve for better sensitivity. See Fig. 3 of Ref. *7*. The same applies—according to recent experience at the author's laboratory—when switching from 1 Pass to 3 Passes by using the Beckman Atomic-Absorption Spectrophotometer No. 97900 with a laminar flow burner.

The operator has at his disposal varying the number of passes, the number of burners, fuel pressure, support gas pressure (or both), and other instrumental variables. For example note in Fig. 3 how by decreasing the number of passes only, the dynamic range can be extended toward the higher copper concentrations. The dynamic ranges for copper, under standard conditions, are referred in Ref. *3*.

CONCLUSIONS

From the above discussion the following conclusions can be derived:

(a) It is possible to extend the considerations used for other absorptimetric methods to atomic-absorption flame photometry to establish the best reading conditions for best accuracy.

(b) Accuracy is closely related to instrumental sensitivity, and any means to improve percentual and fluctuational sensitivities will also help in obtaining better accuracy.

(c) Equipment which permits variations of sensitivity at the operator's discretion provides greater opportunities of taking full advantage of the instrument's potential.

(d) Appropriate chemical preparation including preliminary concentration, and use of scale expansion (for low noise readings) can also provide more accurate results.

SUMMARY

Instrumental limitations of accuracy based on the absorptimetric nature of atomic-absorption methods are discussed. A summary of other factors influencing accuracy in practical work is given, and some comments are included on the limitations on accuracy caused by sensitivity limitations.

ACKNOWLEDGMENTS

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Lead(IV) and Thallium(III) Acetates in Trifluoroacetic Acid as Spot Test Reagents for Aromatic Compounds

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The most commonly used exploratory test for aromatic compounds is the Le Rosen test (*2*), where the substance is reacted with formaldehyde in concentrated sulfuric acid to give the assumed *p*-quinoidal colored products. This test has several disadvantages: (a) a number of the compounds are colored by sulfuric acid alone, thus obscuring the test; (b) there is an appreciable lack of color differentiation for classes of compounds, e.g., red colors, with benzene and various substituted benzenes, green colors with many polynuclear hydrocarbons, etc.; (c) the test fails with aminobenzenes.

These objections have been overcome to a considerable extent by the use of a reagent consisting of lead(IV) or thallium(III) acetate in trifluoroacetic acid. Because of its relative inertness, few compounds give colors in trifluoroacetic acid alone. With the suggested reagent, polynuclear hydrocarbons give a variety of colors and aniline and its derivatives give positive tests.

MATERIALS AND METHODS

Reagents

Lead tetraacetate. 1.0% in trifluoroacetic acid (Distillation Products Industries White Label). The reagent is stable for extended periods in tightly stoppered flasks. The lead tetraacetate is prepared from red lead according to the directions of Fieser (*3*).

Thallium triacetate. 1.0% in trifluoroacetic acid. The compound is prepared from thallic oxide by the method of Hecker (*4*).

Aromatic Compounds. Commercially available compounds from Distilla-

tion Products Industries, White Label or Practical Grades, the Aldrich Chemical Company or from K&K Laboratories. Most of the methoxy compounds were prepared from the corresponding phenols and dimethyl sulfate (*5*). Stock solutions (1.0 mg/ml) of the aromatic compounds were made up in reagent grade benzene.

Procedure

A drop of the benzene test solution is treated in a test tube with 1-3 drops of the reagent and the color noted after the addition of each drop and upon standing for several minutes.

RESULTS

The results for lead tetraacetate and for thallium triacetate are shown in Table 1. By way of comparison the literature results with the Le Rosen reagent are also given (*2*). For compounds not reported in the literature, tests were made in this laboratory according to the directions of Feigl (*2*).

DISCUSSION

Effect of Substituent Group on Color Formation

A comparison of results for aromatic compounds with lead(IV) and thallium(III) acetates in trifluoroacetic acid (TFA) with those for Le Rosen's formaldehyde in concentrated sulfuric acid allows the following generalizations to be made. Color formation depends upon whether the substituents on the aromatic rings are ortho, para, or meta directing. With the TFA reagents, colors usually result only for those derivatives having an ortho, para orienting group e.g., hydroxyl, amino, alkoxy (halogen being an exception in giving a negative test); while negative results are given by the meta orienting groups, e.g., nitro, keto, carboxy, ester, and cyano. With the Le Rosen reagent the pattern is similar with the exception of colors with halogen and aldehyde derivatives and negative tests with amines. A comparison between the two TFA reagents discloses important differences with respect to color formation with aromatics. The thallium(III) reagent is a general one for the phenols while the lead(IV) compound gives colors with aniline and anisole derivatives. The effect on color formation of introducing a meta orienting group into these compounds is the following. For the phenols and thallium(III), color production is inhibited, e.g., salicylic acid, *p*-hydroxybenzonitrile and

TABLE 1

Comparison of Spot Test Results for Aromatic Compounds with Lead Tetraacetate or Thallium Triacetate in Trifluoroacetic Acid with Those for the Le Rosen Formaldehyde - Sulfuric Acid Reagent

		$PbAc_4$		$TlAc_3$		
Class	Compound	Color	Sensitivity[a]	Color	Sensitivity[a]	Le Rosen
Aromatic Hydrocarbons	Acenaphthene	pink→blue	15	violet	1	black
	Anthracene	blue	6	violet→blue	90	yellow-green
	1,2-Benzanthracene	magenta[b]	3	magenta	3	violet
	Benzene	NR[c]	-	NR	-	red
	2,3-Benzofluorene	magenta[b]	6	magenta	6	violet
	Biphenyl	yellow→violet	6	NR	-	blue-green
	2,2′-Bitolyl	orange-yellow	15	NR	-	magenta
	3,3′-Bitolyl	yellow→violet	3	NR	-	blue
	4,4′-Bitolyl	cyan	1	NR	-	NR[d]
	Chrysene	pink	6	pink	6	pink
	Chrysofluorene	green	3	green	6	blue-violet
	Fluoranthene	violet→yellow	3	blue	15	green
	Fluorene	yellow	1	orange	90	green
	Indene	pink	30	pink	6	red
	Naphthalene	pink	6	NR	-	green

	Phenanthrene	yellow-green[b]	6	blue	90	green
	Pyrene	blue→orange	1	blue	1	magenta
	Stilbene	NR	-	NR	-	brown
	Terphenyl	yellow	1	NR	-	pale blue
	Toluene	NR	-	NR	-	red
	Triphenylene	blue	1	NR	-	blue
Alcohols	Acetophenone	NR	-	NR	-	NR
Aldehydes &	Anisaldehyde	NR	-	NR	-	NR
Ketones	Benzaldehyde	NR	-	NR	-	red
	Benzyl alcohol	blue	90	NR	-	red
	9-Fluorenol	orange	3	NR	-	cyan
	o-Methoxyacetophenone	NR	-	NR	-	NR
	Methyl 1-naphthyl ketone	NR	-	NR	-	orange
	Methyl 2-naphthyl ketone	NR	-	NR	-	orange
	1-Naphthaldehyde	NR	-	NR	-	brown
	Salicylaldehyde	NR	-	NR	-	NR
	Vanillin	yellow	6	NR	-	NR

Amines	o-Aminobenzoic acid	purplish brown	3	NR	-	NR
	m-Aminobenzoic acid	orange-pink	6	NR	-	NR
	p-Aminobenzoic acid	orange	3	NR	-	NR
	p-Aminobenzophenone	orange	15	NR	-	NR
	9-Aminofluorene	NR	-	NR	-	NR
	o-Aminophenol	orange	15	orange-pink	6	NR
	m-Aminophenol	orange	15	orange	6	NR
	p-Aminophenol	yellow	6	yellow	6	NR
	Aniline	orange-pink	6	NR	-	NR
	o-Anisidine	violet	6	blue	6	NR
	m-Anisidine	orange	3	yellow	6	NR
	p-Anisidine	blue→magenta	6	violet	15	NR
	Benzidine	orange	3	yellow	15	NR
	o-Chloroaniline	violet	3	orange	90	NR
	m-Chloroaniline	magenta	15	NR	-	NR
	p-Chloroaniline	magenta	6	pink	90	NR
	p-Dimethylamino-benzaldehyde	magenta→orange	6	NR	-	NR
	1-Naphthylamine	magenta	3	violet	15	NR
	2-Naphthylamine	orange→gray	6	blue	6	NR

	o-Phenylenediamine	pink	15	orange-red	15	NR
	m-Phenylenediamine	orange	3	NR	3	NR
	p-Phenylenediamine	orange	3	orange	5	NR
	N-Phenyl-1-naphthylamine	cyan	3	cyan	15	NR
	N-Phenyl-2-naphthylamine	violet-gray	6	green	15	NR
	Acetanilide	NR	-	NR	-	NR
Carboxylic acids, Acid halides, Esters and Amides	Anisic Acid	NR	-	NR	-	NR
	Benzoic Acid	NR	-	NR	-	NR
	Benzoyl chloride	NR	-	NR	-	NR
	2-Biphenylcarboxylic acid	NR	-	NR	-	NR
	Cinnamic Acid	NR	-	NR	-	brick-red
	Cinnamyl chloride	NR	-	NR	-	orange
	9-Fluorene carboxylic acid	pink	3	NR	-	cyan
	m-Hydroxybenzoic acid	NR	-	NR	-	NR
	p-Hydroxybenzoic acid	NR	-	NR	-	NR
	Methyl cinnamate	NR	-	NR	-	NR
	Methyl benzoate	NR	-	NR	-	NR
	1-Naphthoic acid	NR	-	NR	-	green
	2-Naphthoic acid	NR	-	NR	-	pink
	Salicylic acid	NR	-	NR	-	red

Ethers	Anethole	orange	90	NR	-	orange
	Anisole	cyan[b]	6	pink	90	red-violet
	o-Chloroanisole	cyan→yellow	6	yellow green	90	magenta
	m-Chloroanisole	green→yellow	90	blue	90	violet
	p-Chloroanisole	yellow-green[b]	15	yellow	90	NR
	1,2-Dimethoxybenzene	violet→yellow	6	violet	30	violet
	1,3-Dimethoxybenzene	green	3	pink	30	red
	1,4-Dimethoxybenzene	orange	3	orange	6	green
	2,2 -Dimethoxybipheny1	orange	15	gray	15	magenta
	4,4 -Dimethoxybipheny1	orange	1	orange	1	pale cyan
	Isosafrole	purple	6	violet	15	magenta
	2-Isopropy1-5-methylanisole	cyan[b]	15	NR	-	red
	2-Methoxybipheny1	blue→yellow	6	blue	15	violet
	4-Methoxybipheny1	cyan[b]	3	cyan	3	pale blue
	o-Methylanisole	blue[b]	6	violet	90	red
	m-Methylanisole	blue	15	NR	-	magenta
	p-Methylanisole	magenta	6	pink	90	red-brown
	2-Methyl-5-isopropylanisole	yellow-green→orange-pink	30	NR	-	orange-red
	1-Naphthyl methyl ether	blue	3	red→green	3	blue

	2-Naphthyl methyl ether	yellow-green	15	orange-pink	15	yellow-green
	Phenetole	cyan→orange	6	blue→pink	90	magenta
Halogen	Chlorobenzene	NR	-	NR	-	red
	1-Chloronaphthalene	pink[b]	15	NR	-	NR
	4,4 -Dibromobiphenyl	green[b]	90	NR	-	NR
	o-Dichlorobenzene	NR	-	NR	-	NR
	Iodobenzene	NR	-	NR	-	NR
Nitriles and	Benzonitrile	NR	-	NR	-	NR
Nitro	o-Dinitrobenzene	NR	-	NR	-	NR
	p-Hydroxybenzonitrile	NR	-	NR	-	NR
	Nitrobenzene	NR	-	NR	-	NR
	2-Nitrobiphenyl	NR	-	NR	-	brown
	4-Nitrobiphenyl	NR	-	NR	-	orange
	Phenylacetonitrile	NR	-	NR	-	NR
Phenols	Carvacrol	violet	30	pink	90	brown
	Catechol	orange	6	orange	6	violet-red
	o-Chlorophenol	yellow	30	yellow	15	red
	m-Chlorophenol	pale blue	90	blue	60	orange-red
	p-Chlorophenol	yellow-green	30	cyan	30	greenish gray
	o-Cresol	yellow	15	green→yellow	15	magenta

m-Cresol	NR	-	magenta	30	magenta
p-Cresol	NR	-	magenta	15	reddish black
2,2-Dihydroxybiphenyl	yellow	30	yellow	6	magenta
4,4-Dihydroxybiphenyl	yellow	6	yellow	6	pale green
Eugenol	orange	30	magenta→gray	90	brown
2-Fluorenol	magenta→orange	6	magenta→olive	6	blue black
3-Fluorenol	orange	6	cyan→yellow	6	green black
Guaiacol	yellow	6	yellow	6	purple
Hydroquinone	yellow	90	yellow	90	black
2-Hydroxybiphenyl	orange	15	orange	15	red
4-Hydroxybiphenyl	orange→gray	15	violet-black	15	green
m-Methoxyphenol	yellow	15	cyan	15	orange-red
p-Methoxyphenol	orange	25	yellow	30	brown
1-Naphthol	orange	15	blue	30	greenish black
2-Naphthol	orange	3	orange-pink	90	brown
1,1-Bi-2-Naphthol	orange	15	orange red	3	olive
Phenol	NR	-	red,blue dichroic	6	red-violet
Resorcinol	green-yellow	30	red,blue dichroic	15	red
Thymol	NR	-	green-yellow	90	maroon

NR= a. Sensitivity in µg/drop (0.03ml) b. Rapid fading c. No reaction at 90 µg/drop d. No reaction ca 100µg
(Minimum quantity visually detectable)

salicylaldehyde. With the aniline compounds and lead(IV), color formation is not inhibited, e.g., the aminobenzoic acids, *p*-dimethylaminobenzaldehyde, and *p*-aminobenzophenone. By contrast, anisole compounds having meta-orienting groups give no color, e.g., anisaldehyde, *o*-methoxyacetophenone, and anisic acid.

The major difference in reaction of the polynuclear hydrocarbons with lead(IV) and thallium(III) reagents is that the latter gives colors with fewer compounds, probably because of its lower oxidation potential, combined with variations in reactivity of the various hydrocarbons.

The effect of steric factors of substituent groups on test results has not been studied in any detail, but by contrast to the Le Rosen test, para-position blocking does not seem to inhibit color production, e.g., *p*-chlorophenol and 4,4′-bitolyl, which would indicate other than para quinoid formation. In this connection, formation of triphenylmethane type dyes with para blocked phenols should not be overlooked (*6*).

Mechanism of Reaction

Only a brief discussion of the mechanism of reaction of thallium(III) and lead(IV) acetates in TFA with the aromatic compounds is feasible. This is so because the mechanism is probably as complex as it is with the Le Rosen test, involving as it does, oxidations and condensations (*2*). In the TFA test the inertness of the supporting solvent is important, with benzene, toluene, and chloroform being suitable, in contrast to ethyl ether and dioxane in which color formation is inhibited. It is interesting to note that with one compound tested, fluorene, a blue color was obtained on the solid material as compared to a yellow color in benzene. However, because of the slight solubility of most of the polynuclear hydrocarbons in trifluoroacetic acid, benzene solutions are preferable to solid samples. The acidity of the trifluoroacetic acid is as important as its dehydrating power since substitution of glacial acetic acid gives no colors, and of trifluoroacetic anhydride gives comparatively weaker intensities.

The function of the lead or thallium acetate is undoubtedly as an oxidant, although their modes of attack are uncertain. In glacial acetic acid, lead tetraacetate is known to acetylate polynuclear hydrocarbons, oxidize phenols to quinones, or methylate quinones, reactions which involve acetoxy or methyl free radicals (*1, 3, 7*). It is quite possible that these reactions also occur in trifluoroacetic acid.

SUMMARY

Reagents consisting of lead(IV) or thallium(III) acetate in trifluoroacetic acid give intense colors with a number of aromatic compounds. The former is useful for identification of many polynuclear and polyphenyl hydrocarbons and their derivatives as well as aryl amines and methoxy ethers. The latter reagent gives positive tests with a smaller number of polynuclear hydrocarbons and with most phenols. While the reagents probably function similarly to Le Rosen's formaldehyde in concentrated sulfuric acid in giving condensations and oxidations, they have the advantage of being appreciably more selective with respect to classes of compounds giving colors. Used in conjunction with the Le Rosen reagent they allow considerable differentiation among and within groups of aromatic compounds.

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Microdetermination of Organic Oxygen by Means of Optical Integration of Liberated Iodine in Vapor Phase

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The determination of organic oxygen has been currently carried out by the thermal decomposition in an inert gas over carbon granules at high temperature, and is followed by the oxidation of the resultant carbon monoxide by heated iodine pentoxide. Carbon dioxide or iodine produced at the latter reaction has been precisely measured gravimetrically or volumetrically (*1, 2, 4, 5, 7, 8, 10-13*).

The volumetric finish for the liberated iodine has been reputed to be the most reliable and accurate method for the determination of oxygen, probably due to the use of highly sensitive iodometry; on the other hand, the gravimetric finishes for either of the two components are sometimes recommended due to the simplicity in operation. In fact, the cause of the analytical error is not condensed at the measurement of the final products but is widely distributed at the sampling, the thermal decomposition, the unstable blank value, and the other interfering fractions, which have been formed during the pyrolysis and will be successively oxidized by the iodine pentoxide. Therefore the varieties of the final processes might not be the essential factor for the overall accuracy of the determination.

Recently a modification of the final process using a new instrumental technique has been introduced in the author's laboratory, and involves an optical integration of light absorption caused by the liberated iodine in the vapor phase and an automatic recording of out-put signal from an attached photoelectric system. A strong light absorption of vapor phase iodine takes place in a long gas cell kept at 120°C at the wavelength of 530 mμ (*9*) without overlapping from other fractions passing through the cell. The internal volume and the length of the cell have been empirically defined so that the total iodine resulting from the sample is temporarily

retained in the cell. The integration of the light absorption due to the total iodine is therefore attained at the maximum change of the out-put signal on the recorder chart. The analysis with the new apparatus can be simply and automatically carried out within 15 minutes, except sampling and calculation. The optimum sample size for this apparatus is lowered to 1-3 mg from the conventional microscale because of high sensitivity of the photometric system.

MATERIALS AND METHODS

Apparatus

A flow diagram of the apparatus with additional photometric equipment is illustrated in Fig. 1. A reagent-grade argon is supplied to a thermal

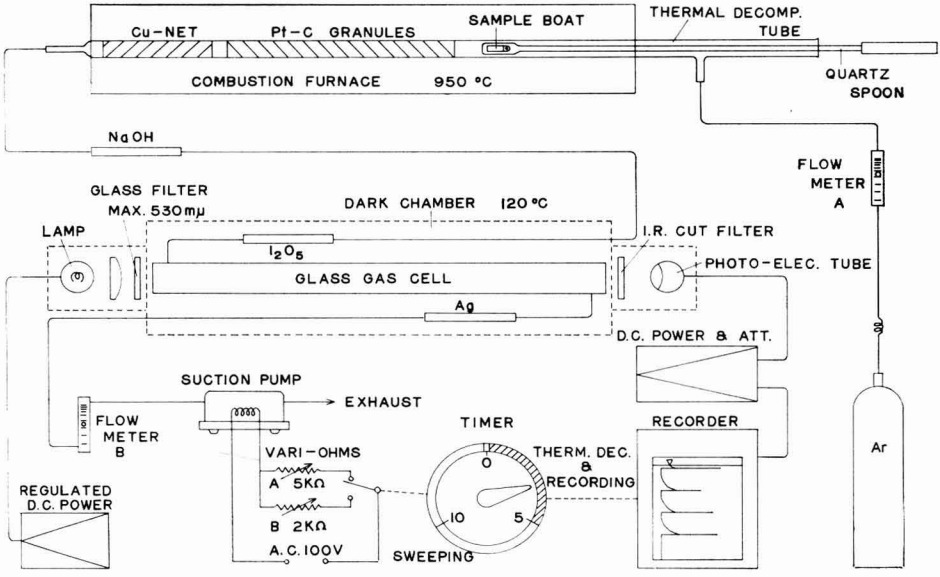

FIG. 1. Schematic diagram of oxygen analyzer.

decomposition tube via a flow meter A with a flow rate of approximately 120 ml/minute that is consistently overflowing from the mouth of the tube to prevent an inward diffusion of air. Platinized carbon granules and reduced copper net are filled in the tube as ordinarily (*7*). The tube is heated at 950°C with a conventional combustion furnace covering the tube fillings and an empty space for sample decomposition, as shown in

the figure. The tail end of the decomposition tube is piped by a stainless steel capillary to an iodine pentoxide tube via a small scrubber of sodium hydroxide granules, where some interfering acidic gases may be removed.

A dark chamber is provided to install the iodine pentoxide tube together with a borosilicate-glass gas cell, which has an outside diameter of 30 mm and a length of 38 cm, and finally a granular silver tube (*4, 6*). As the dark chamber is kept constant at the temperature of 120°C by means of a proportional temperature regulator, the iodine liberated at the iodine pentoxide tube can be introduced into the gas cell in vapor phase and afterward it is absorbed in the granular silver tube. The end of the granular silver tube is further piped to a suction pump, which is actually a modified aquarium pump operated by a.c. power, via a flow meter B. Different flow rates of 20-25 ml/minute and 60-70 ml/minute through the apparatus are obtained by changing series resistances of vari-ohms A and B, respectively. The thermal decomposition of the sample and the measurement of the carbon monoxide may be carried out during the lower flow rate to ensure the complete chemical reactions and the retention of the total iodine in the gas cell. The higher flow rate is required for quick sweeping of the iodine from the gas cell.

A combination of a small light source and a glass filter of the maximum transparency at 530 mμ is mounted at one side of the dark chamber, and the light transmission through the cell is further passed through an infrared cut filter at the opposite side of the chamber. The final light intensity is then detected by a Ce-Sb photoelectric tube which has been wired to a recorder via circuits involving a d.c. power supplier and an attenuator. The recorder chart is temporarily stopped during the thermal decomposition of the sample and the measurement of the iodine so that the recorder pen marks a bar gram which responds to the maximum change of the light transmission. A timer is mounted in the apparatus for programming the switching of the vari-ohms A and B, and also the chart advancement automatically. A photographic illustration of the whole assembly is shown in Fig. 2. The model has been recently designed and constructed by courtesy of a factory of scientific instruments, Yanagimoto Co. Ltd., Kyoto, Japan.

Thermal decomposition system. The quartz tube for the thermal decomposition has a length of 55 cm with an outside diameter of 13 mm and is filled with the platinized carbon granules and the reduced copper net (Fig. 3). The tube is introduced in the cylindrical combustion furnace

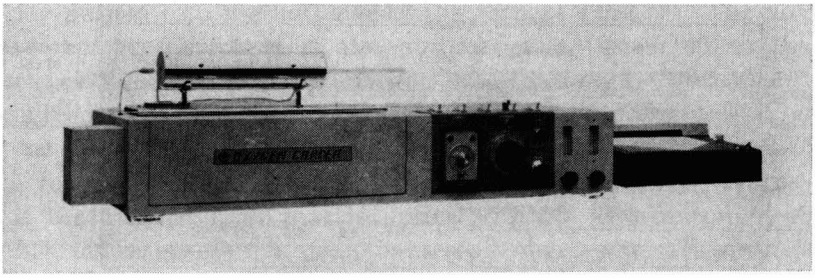

FIG. 2. Assembled oxygen analyzer.

which can be controlled at 950°C by means of a thermocouple incorporated with an on-off switching regulator. As the temperature is controlled at the middle of the furnace, the copper filling is situated at the temperature range of 400-800°C and avoids a risk of the reaction:

$$CO + 2\,Cu \rightarrow C + Cu_2O.$$

An empty space of 13 cm is provided at the other end of the furnace in which a quartz spoon (*3*) may be inserted carrying a sample boat, as shown in Fig. 1. A side arm is situated at 15 cm from the opening mouth where the argon is overflowing all the time.

Carbon monoxide measuring system. The dark chamber consists of double layer metal cases between which glass fiber is packed for heat isolation (Fig. 4). The inner case is made by heavy wall aluminum plates in order to be heated uniformly by a resistance wire tightly fixed to its back surface. The temperature of the case is controlled by the proportional temperature regulator with the maximum out-put power of 300 watts.

The borosilicate-glass gas cell is clamped in the case, and either the

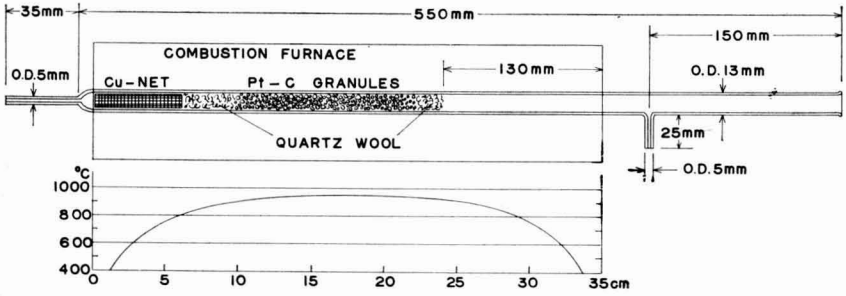

FIG. 3. Thermal decomposition tube.

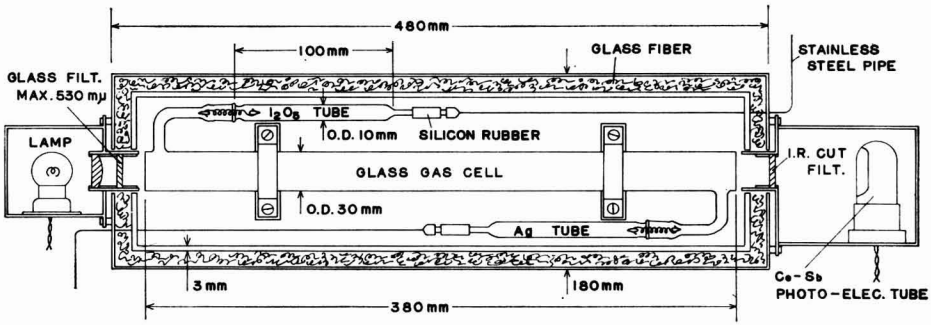

FIG. 4. Carbon monoxide measuring system.

iodine pentoxide tube or the granular silver tube is connected to the cell by ground-glass joints without lubrication, as shown in the figure. The light source is a 6V-1A tungsten filament lamp which is excited by a solid state d.c. power supplier under a stability of $\pm 0.02\%$ of the supplied voltage. A d.c. power of 90 V is applied to the Ce-Sb photoelectric tube which gives around 5 mV of the maximum out-put voltage from a loading resistance of 1 kilo-ohms without amplification (Fig. 5). A sensitivity of 2 mV with a full span of 25 cm is therefore conveniently employed for the recorder.

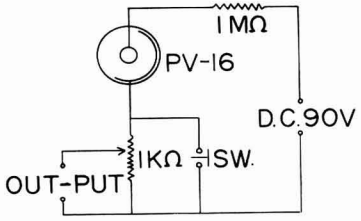

FIG. 5. Photoelectric circuit.

Procedure

After approximately 90 minutes of a conditioning run of the apparatus, a sample of 1-3 mg is weighed in platinum boat and is inserted into the thermal decomposition tube with the quartz spoon until the sample boat reaches the position just before the side arm. The overflowing argon sweeps all the air from the sample boat and the spoon during 20-30 seconds. As the timer has passed the starting zero position and at the same time the flow rate has decreased to 20-25 ml/minute, the spoon is instan-

taneously pushed into the hot zone of the furnace (Fig. 1). The advancement of the recorder chart is also automatically turned off so that the recorder pen marks a straight line until the maximum absorbance due to the resulted iodine introduced into the gas cell can be obtained. When the timer has indicated 5 minutes and the flow rate has recovered 60-70 ml/minute for the quick sweeping, the recorder pen slowly returns back towards the original level that is optically 100% transmittance. As the timer has indicated 10 minutes, the record chart starts to move again. The sweeping is continued for 10 minutes.

Figure 6 shows a sample of the recorded chart. A little decrease of

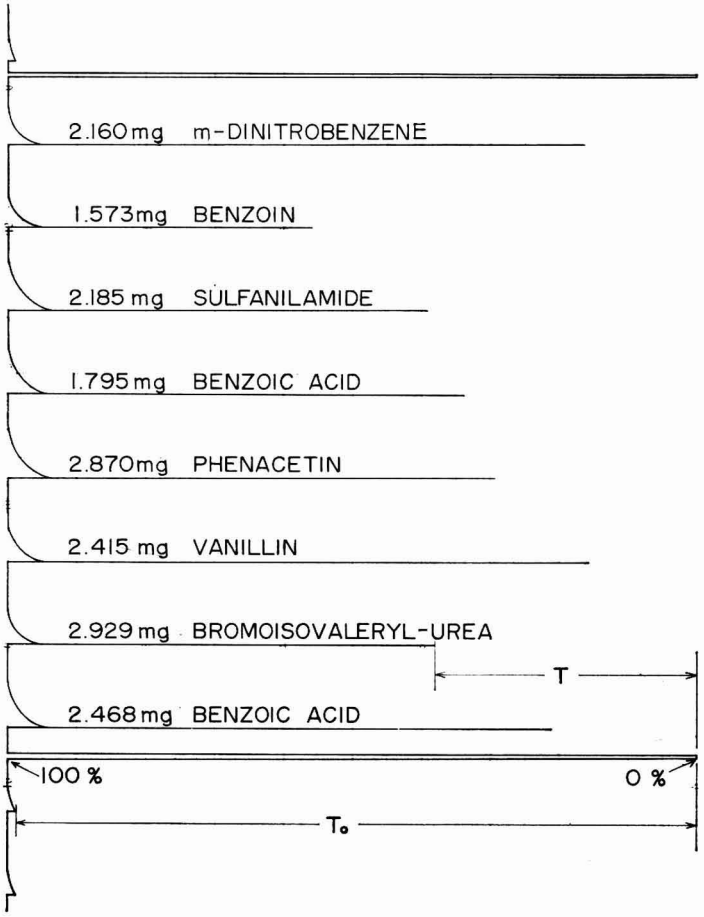

FIG. 6. Estimation of transmittance on recorder chart.

transmittance is normally observed in the blank run without charging sample because of the slower flow rate of the carrier gas than the flow rate at 100% transmittance. Base signals are therefore obtained as shown in the figure. The zero % level should be sometimes ascertained by closing a contact switch in Fig. 5 that means the zero point of the recorder itself. Actually the zero point of the recorder coincided exactly with the optical zero % transmittance obtained by turning off the lamp, probably because the dark current through the photoelectric tube might have been negligible. Therefore, a light transmittance T which responds to an oxygen containing sample is measured in millimeters with an accuracy of ± 0.1 mm, while T_0 is estimated as the transmittance at the blank tests.

Several determinations of known compounds at different sample sizes were preliminarily carried out with the above-mentioned procedure, and the relationships between the relative percentage transmittance 100 T/T_0 and microgram quantities of the oxygen in the samples were plotted on a logarithmic section paper. A straight line, which has the best approximation to the plots, is used for the calibration of the given apparatus. A sample of the calibration is shown in Fig. 7. In the determination of unknown compounds, the measurement of 100 T/T_0 directly answers the quantity of oxygen in the sample from which the percentage composition of oxygen can be calculated.

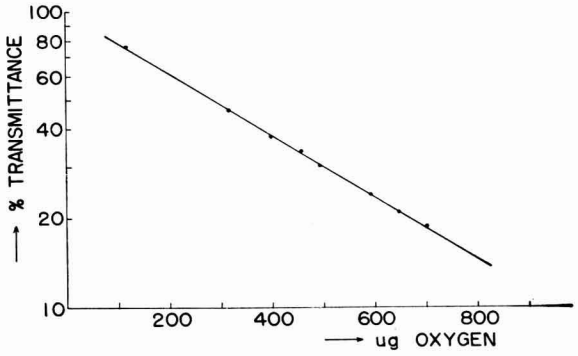

FIG. 7. Calibration line.

RESULTS

The series of analyses shown in Fig. 6 has been calculated using the calibration line of Fig. 7, and the results are tabulated in Table 1. The analytical errors in the table are thought to be almost comparative to

that of the gravimetric finish which has been hitherto carried out in the author's laboratory.

TABLE 1

MICRODETERMINATION OF ORGANIC OXYGEN WITH OPTICAL INTEGRATION METHOD

Sample	Sample wt. (mg)	Transmit. $100T/T_0$ (%)	Oxygen (μg)	Oxygen (%) Found	Calc.	Div.
Benzoic acid	2.468	20.99	651	26.37	26.20	+0.17
Bromoisovaleryl-urea	2.929	38.00	412	14.07	14.34	—0.27
Vanillin	2.415	15.76	768	31.80	31.55	+0.25
Phenacetin	2.870	28.26	518	18.04	17.85	+0.19
Benzoic acid	1.795	33.76	470	26.18	26.20	—0.02
Sulfanilamide	2.185	38.04	405	18.53	18.58	—0.05
Benzoin	1.573	55.95	236	14.88	15.08	—0.20
m-Dinitrobenzene	2.160	16.35	816	37.77	38.07	—0.30

DISCUSSION

Thermal decomposition. There are some refractory substances which need a long time and high temperature for the perfect decomposition in the Dumas nitrogen determination. The similar behavior will definitely interfere considerably with the new oxygen determination because the total iodine resulting from the oxygen in the sample must be introduced into the gas cell within a limited time. Therefore, in the case of very slow decomposition, the recorder pen will mark a differential signal instead of the integral one. Fortunately, the oxygen in the organic sample can be very quickly extracted by the carrier gas, and the given dimensions of the gas cell are empirically sufficient for the temporary retention of total iodine resulting from the sample.

Optical integration. The theory of the optical integration is based upon three hypotheses that the gas cell is a straight pipe with a uniform diameter, the iodine vapor has diffused homogeneously in the rectangular section against the tube axis, and the total iodine resulting from the sample exists in the gas cell. In a hypothetical gas cell, as illustrated in Fig. 8, in which the iodine vapor distributes nonuniformly along the tube

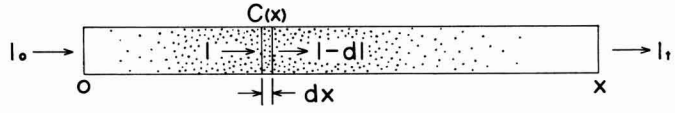

FIG. 8. Optical integration of nonuniformly distributed iodine.

axis but in which the above hypotheses are satisfied, the total iodine U existing in the cell is the sum of iodine in a thin layer dx; therefore

$$U = \int_0^x \pi r^2 C(x)\, dx = \pi r^2 \int_0^x C(x)\, dx, \tag{1}$$

where x is the length of the gas cell, r is the inside radius, and $C(x)$ is the local concentration of iodine being functional to the location between zero and x.

On the other hand, the light absorption dI in the thin layer is represented by Lambert-Beer's law as the following equation:

$$dI = -k\, I\, C(x)\, dx,$$

where I is the intensity of incident light and k is the light absorption coefficient. Total light absorption through the cell is therefore

$$\int_{I_0}^{I_t} \frac{dI}{I} = -k \int_0^x C(x)dx, \tag{2}$$

where I_0 and I_t are the intensities of incident and transmission light, respectively. From Eqs. (1) and (2), the necessary relationship between the total iodine in the cell and the light transmission is obtained:

$$\ln \frac{I_t}{I_0} = -k \frac{U}{\pi r^2}. \tag{3}$$

In practice, Eq. (3) is converted to the relation between the relative percentage transmittance $100\ T/T_0$ and the microgram quantity of oxygen in the sample Q using the overall sensitivity K of the given apparatus:

$$\log 100\ T/T_0 = 2 - K\, Q.$$

Flow rate of carrier gas. The conversion of organic oxygen to carbon monoxide is not momentarily completed, even at the suggested high temperature, and the flow rate of the carrier gas must be controlled under a certain limit. At more than 40 ml/minute significantly low results were observed and worse, the iodine vapor was transported through the gas cell so quickly that the vapor front reached to the exit end of the cell before the last vapor reached to the cell. On the other hand, at less than 10 ml/minute, the diffusion of the vapor along the cell axis was comparatively faster than the introduction of the resulting iodine into the cell so that low results again appeared. The suggestion of 20-25 ml/minute is therefore made to achieve the best reproducible results.

The vapor front of iodine in the gas cell normally reaches the exit end at 4-5 minutes from the introduction of test sample. Since it takes much time to sweep out all the iodine from the cell at the flow rate of 20-25 ml/minute, a higher flow rate of 60-70 ml/minute has been used for the quick sweeping. Therefore the timer of the apparatus has been programmed with 5 minutes for the thermal decomposition with the successive optical integration, and the following 10 minutes for the sweeping and changing the flow rates of the carrier gas automatically at the suggested levels.

SUMMARY

An automatic recording analyzer for organic oxygen has been established that introduces a new instrumental technique of optical integration of liberated iodine in vapor phase. The organic oxygen is rapidly converted to carbon monoxide and is further reacted with iodine pentoxide to liberate free iodine as usual. The iodine vapor is introduced into a long gas cell kept at 120°C, and the light absorption at 530 mμ through the gas cell is recorded by means of a photoelectric system. The optical integration is then attained as the maximum deflection of the recorder pen during the time that the total iodine resulting from the sample is temporarily retained in the gas cell. The apparatus is automatically operated with a timer, programming 5 minutes for the sample decomposition with the signal recording and the following 10 minutes for sweeping the iodine in the gas cell; a total of 15 minutes is then used in an analysis.

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Systematic Applications of Gas-Liquid Chromatography in Toxicology[1]

I. Extraction Procedure and the Alkaloids

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The isolation and identification of an unknown drug or poison found associated with such biological materials as viscera, blood, or urine, is a formidable task. The problem becomes more complicated when minute quantities of toxic materials must be isolated, as these might go undetected by conventional means. Since the days of Orfila (*12*), Stas (*15*), Otto (*13*), and Dragendorff (*7*), the common methods of extraction have essentially remained the same, i.e., extracting nonvolatile organic poisons from acidic or basic aqueous solutions by organic solvents immiscible with water. The enormous increase in the number of synthetic drugs during the last few decades has often made the use of the Stas-Otto method impractical (*1-3*). Various modifications of this process have been proposed. Newer methods, such as the ammonium sulfate method of Daubney and Nickolls (*5, 6*) further modified by Nickolls (*11*), and the continuous extraction methods of Umberger *et al.* (*16*), Feldstein and Klendshoj (*8, 9*), and Curry and Phang (*4*) were made available, but they too were tedious, cumbersome, and time consuming. Also, because the extraction procedures were so elaborate, there was the constant risk of losing the toxic material when it was present in minute quantities only.

In this study, blood was chosen as the material from which to attempt extraction of poisons, because it contains minimal amounts of impurities compared with tissue. Also, blood, urine, or stomach washings are the

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materials most likely to be available for toxicological study in cases where there has been a near-fatal overdosage of a drug. In such cases, successful treatment depends upon prompt action and hence the need for fast, simple, and reliable extraction methods.

The authors have attempted to present in this study a rapid, simple, and systematic procedure for extracting and isolating most of the non-volatile organic poisons from blood. The method here described does not require any adjustment of the pH of blood, or any special equipment, and is therefore suitable for emergency use as well as for routine laboratory procedure. The extraction is performed with a mixture of acetone and ethyl ether in equal volumes and may easily be accomplished from 0.5 ml of blood or less. While this amount of blood may not be sufficient to demonstrate a therapeutic amount of a drug from a single administration, it is sufficient to identify a toxic or lethal dose of the particular drug.

Also presented in this work is the identification of the extracted compounds by gas-liquid chromatography. For this purpose, the residue from the extraction solvents obtained above is dissolved in a suitable solvent, and an aliquot is injected directly into the gas chromatograph without subjecting it to any clean-up procedure. A flame ionization detector is used for analysis of the barbiturates, alkaloids, tranquilizers, and antihistamines, and an electron capture detector is used for the organophosphate and chlorinated hydrocarbon pesticides. Although many column substrates, such as SE 30 and SE 52 were tried, it was found that the polyester Hi-Eff-8B could be used with greater ease and versatility for the variety of drugs chromatographed under this scheme.

EXPERIMENTAL PROCEDURE

A. *Equipment.* The Hy-Fi gas chromatograph, Aerograph model 600 (Wilkens Instrument and Research, Inc., Walnut Creek, California) was used. It was equipped with a flame ionization detector and a Leeds and Northrup Speedomax H, 0-1 mV recorder, model S. The column was a spiral Pyrex glass tube of 0.125 inch o.d., 0.070 inch i.d., and 3 feet in length. It was packed with 1% (expressed as a percentage by weight of the solid support) Hi-Eff-8B (cyclohexanedimethanol succinate) on 100/120 mesh silanized Gas Chrom P.[3]

[3] Silanized Gas Chrom P is now commercially available as Gas Chrom Q. One per cent coated Hi-Eff-8B on Gas Chrom Q is available from Applied Science Laboratories, Inc., State College, Pennsylvania.

B. *Standard solutions.* Standard alkaloidal solutions were prepared by dissolving the known quantities of their recrystallized free bases, as listed in Table 1, in absolute methanol, 95% ethanol, or chloroform of A.C.S. specifications. All the stock solutions so prepared were in the concentration of 10 μg/μl, and were diluted further as needed; e.g., caffeine, cocaine, and atropine were prepared in the range of 50 ng or less per microliter. These solutions were subjected to gas chromatography to determine their retention times.

Solutions for oral administration to experimental animals (Table 2)

TABLE 1
RELATIVE RETENTION TIMES AND RECOVERY OF ALKALOIDS[a]

Alkaloid	Relative retention times				Approximate recovery from blood (%)[b]
	220°C	230°C	240°C	250°C	
Atropine	0.46	0.45	0.45	0.45	95
Caffeine	0.25	0.25	With solvent	With solvent	102
Cinchonine	3.47	3.2	3.0	2.67	85
Cocaine	0.31	0.31	0.32	0.33	95
Codeine	1.0	1.0	1.0	1.0	90
Colchicine	5.1	5.1	5.2	5.25	—[d]
Dihydrocodeinone	1.44	1.46	1.35	1.25	94
Dihydrohydroxy-codeinone	1.84	1.8	1.7	1.67	90
Heroin	2.31	2.2	2.0	1.83	90
Hydrastine	—[c]	—[c]	14.0	13.8	—[d]
Morphine	4.03	3.8	3.4	3.0	92
Narcotine	—[c]	—[c]	12.5	11.8	—[d]
Papavarine	8.59	8.0	7.1	6.5	—[d]
Piperine	8.12	7.4	6.3	5.5	—[d]
Quinine	8.06	7.7	6.8	5.42	—[d]
Strychnine	19.4	18.3	16.0	12.8	—[d]
Thebaine	1.75	1.6	1.5	1.42	100
Theobromine	0.56	0.57	0.56	0.55	100

[a] Conditions: 3-foot glass column (1/8-inch o.d.) packed with Hi-Eff-8B 1%, on 100/120 mesh silanized Gas Chrom P. Flow rate of carrier gas at 220°C, 60 ml/minute; flow rate of carrier gas at 250°C, 54.5 ml/minute; retention time of codeine at 220°C, 3.2 minutes; retention time of codeine at 250°C, 1.2 minutes.

[b] These calculations are based on adding 25 γ of respective drugs to ½ ml of whole blood.

[c] Did not emerge.

[d] Not done.

TABLE 2

DETAILS OF ANIMAL EXPERIMENTS WITH ALKALOIDS

Drug	Dose (mg/kg)	Mode of administration	Weight of rat (g)[a]	Time between dosing and drawing blood	ppm of drug found in whole blood	Observed effects on rat after administration of drug
Cocaine HCl	1000	Oral	182	10 min	40	Nearly dead.
Cocaine HCl	244 (MLD)	s.c.	174	3 hours	2	Looked nervous at the time of drawing blood.
Cocaine HCl	500	Oral	121	3 hours	—	—
Codeine SO_4	220 ($2 \times LD_{50}$)	i.p.	151	5 min	105	Suddenly collapsed within 3 min.
Codeine SO_4	2000	Oral	158	15 min	100	Twitchings and tremors within 10 min.
Codeine SO_4	1000	Oral	147	27 min	110	Severe convulsions or tremors after 20 min; becoming stiff; jumping and kicking; twitchings; died convulsing.
Codeine SO_4	1000	Oral	159	35 min	95	Severe convulsions and/or tremors after half hour.
Codeine SO_4	672 ($2 \times LD_{75}$)	s.c.	172	75 min	85	Convulsions and severe tremors after half hour; died convulsing in 75 min.
Dihydrohydroxy-codeinone HCl	2000	Oral	166	15 min	10	Stiff and was dying in 15 min.
Dihydrohydroxy-codeinone HCl	45[b]	i.v.	135	30 min	20	Convulsing and stiff soon after administration; couldn't have survived 30 min.

TABLE 2 (*Continued*)

Drug	Dose (mg/kg)	Mode of administration	Weight of rat (g)[a]	Time between dosing and drawing blood	ppm of drug found in whole blood	Observed effects on rat after administration of drug
Dihydrohydroxy-codeinone HCl	1000	Oral	180	3 hours	15	Sleeping in 2 hours; slightly stiff in next 30 minutes; muscles relaxed at time of drawing blood but almost dying.
Heroin HCl	279	s.c.	189[c]	30 min	Detected as monoacetyl morphine	Became stiff after 15 min; otherwise quiet.
Heroin HCl	279	i.v.	190	30 min	Detected as monoacetyl morphine	Stiff first, becoming depressed after 5 min; difficulty in breathing after 15 min; was stiff at time of drawing blood.
Morphine	2000[d] ($2 \times LD_{50}$)	Oral	143	2 hours	Trace	Lying quietly with head buried in the wooden shavings; slightly stiff and eyes wide open, difficulty in breathing.
Morphine	2000[d] ($2 \times LD_{50}$)	Oral	139	3 hours	5	Was stiff but quiet; seemed to have difficulty in breathing.
Quinine HCl	1000 ($2 \times$ MLD)	Oral	117	3 hours	9	—

[a] All female rats of Long-Evans strain, 2 months old.
[b] Only part of dose could be administered.
[c] Rat not fasted for 24 hours.
[d] Drug administered in suspension form.

were prepared by dissolving the weighed dose of the appropriate alkaloidal salt in 1-1.5 ml of water. Where the compound was not soluble in water, it was administered as a suspension. For intraperitoneal, intravenous, and subcutaneous injections, the required doses were dissolved in not more than 0.5 ml of normal physiological saline.

C. *Extraction solvent and procedure.* The extraction solvent was composed of equal volumes of acetone and ethyl ether. All human bloods contained EDTA as anticoagulant, but heparin was used in animal bloods. The following samples were used: (a) blood alone; (b) blood to which known quantities of drugs (or poisons) were added for recovery data; and (c) blood which was drawn from rats after administering drugs. All the samples were extracted and gas chromatographed to determine the normal background response of blood, detection and recovery of drugs (poisons), and parts per million of drug found in the whole blood, respectively.

For convenience, 500 μl of each blood was ordinarily taken in a 0.5-dram shell vial. One ml of mixed solvent (acetone-ether) was added, and the mixture was stirred for a few seconds with a melting point capillary with a sealed end, after which the clear supernatant solution was transferred to another 2-dram shell vial with a Pasteur pipet or by centrifuging the shell vial for a few seconds and decanting the supernatant solution, or both. This process was repeated three times, fresh solvent being used each time and all supernatant solutions being combined in the second shell vial. The combined extracts were evaporated to dryness at room temperature with a fine jet of air impinging on the surface of the sample. This was achieved by using a bell jar with a side-arm connected to a vacuum line. A glass capillary was mounted through the top opening of the jar to provide the air flow. The residues of blood extracts so obtained were dissolved in 500 μl or less of 95% ethanol. The suspended material was allowed to settle for a few seconds, and 1 μl of the clear supernatant solution was injected into the gas chromatograph.

D. *Gas chromatographic conditions.* The operating conditions of the gas chromatograph were: oven temperature ranging from 220° to 250°C (Table 1); injector temperature in all cases was 275°C; input impedance, 10^9 ohms; and the output sensitivity, 1 ×. Conditions of flow rate are given in Table 1.

E. *Administration of drugs to animals.* Female rats of the Long-Evans strain were used. They were 1-2 months old, weighed between 98 and

204 g, and were separately caged. The rats had been reared on White Diet and were in good health. The drugs were normally given in water solution or suspension and were administered by stomach tube. The stomach tube was connected to a hypodermic syringe containing the measured dose of the drug. All animals were fasted for 24 hours prior to each experiment and a sedative dose of ethyl ether was used to facilitate handling of the animals. In addition to being fed orally, certain alkaloidal drugs were also administered intraperitoneally, intravenously, and subcutaneously. For example, codeine was given orally, subcutaneously and intraperitoneally; heroin was given both subcutaneously and intravenously but not orally. Details of the animal experiments are listed in Table 2.

The amounts of drugs and/or poisons administered either orally or by other means was calculated closely to either LD_{50} or $2 \times LD_{50}$ doses of the respective compounds. Due to the conflict in the lethal doses as reported in the literature, and in some instances the unavailability of this information, arbitrary quantities based on simple calculations from figures of the nearest compounds of the same group and type were taken. The exact amounts of dose given to each rat are also shown in Table 2.

After administration of the drug, its effects on the animal were observed. At varying periods blood was collected from the descending aorta while the rats were under ether anesthesia. The time at which the blood was collected from the animal was predicated by the absorption of the drug into the circulatory system and the onset of pharmacological action. In most cases the time chosen was from one-half to 3 hours after drug administration. After as much blood as possible was withdrawn into a heparinized syringe, the rats were bled to death by severing the aorta. The samples were transferred to clean bottles and stored under refrigeration until extracted.

RESULTS

Table 1 shows the relative retention times of the alkaloids gas chromatographed at four different temperatures, along with percentage recoveries of added drugs to blood. The details of animal experiments showing the dose administered, mode of administration, duration of dosing, parts per million of the drug found, and the observed effects of drug on animals are given in Table 2. Figures 1 and 2 are the tracings of the gas chromatographic charts showing typical separations of some of the common alkaloids.

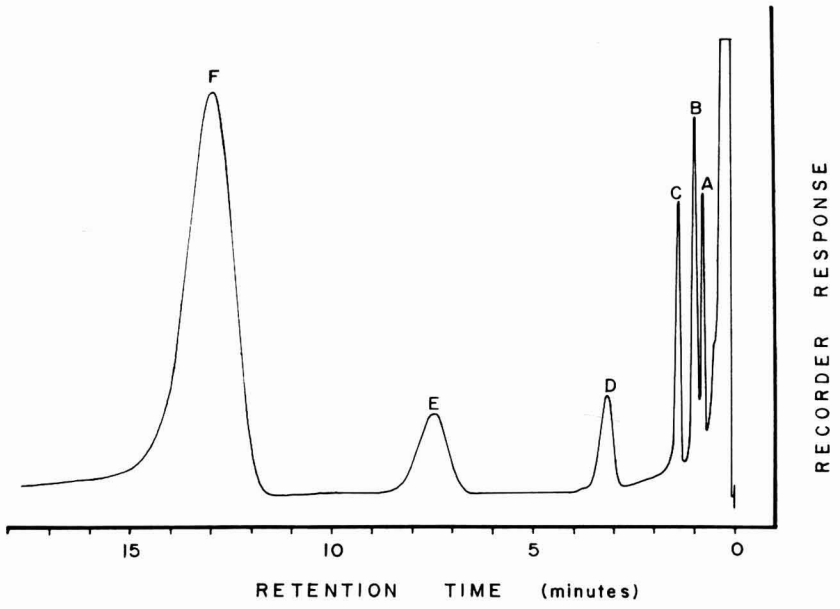

FIG. 1. Separation of alkaloids. A, Caffeine, 50 ng; B, cocaine, 50 ng; C, atropine, 50 ng; D, codeine, 0.1 γ; E, heroin, 1 γ; F, morphine, 5 γ. Conditions: 3-foot glass column (1/8 inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 220°C; injector 275°C. Flow rate of carrier gas (nitrogen) 60 ml/minute. Input impedance 10^9 ohms; output sensitivity 1 ×; attenuation 4 ×.

DISCUSSION

A. *Extraction.* Recently, Kazyak and Knoblock (*10*) proposed a simplified method of gas chromatographic applications in analytical toxicology in which the extractions from biological specimens were accomplished by adjusting the pH between 4 and 7.5 for the isolation of barbiturates, neutral compounds, and a few of the less basic drugs. The extraction method presented here does not require any pH adjustment, shaking, or samples of blood larger than 0.5 ml. This amount of blood was found to be a sufficient quantity to yield detectable quantities of drugs given in toxic doses to rats. This quantity of blood should also be sufficient to recover and identify most drugs when given to man in toxic and lethal doses. No attempt has been made to universally extend this method to the detection of therapeutic doses given to man, although the therapeutic levels of a number of drugs may in some instances be detected by this

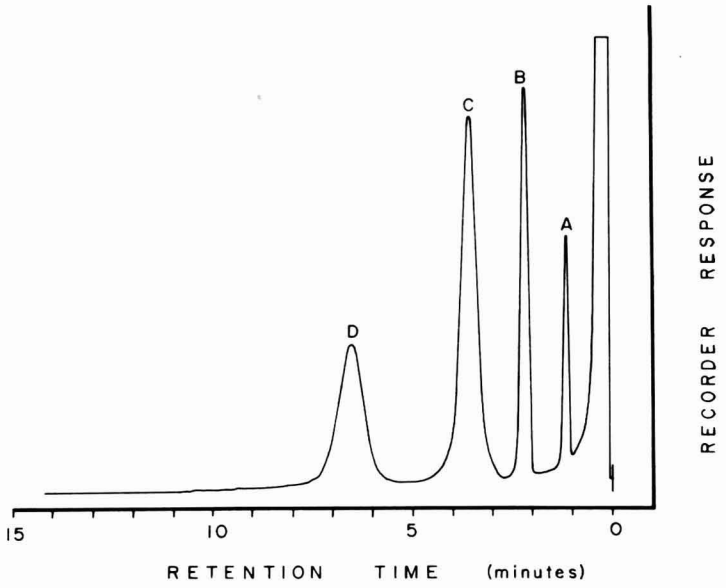

FIG. 2. Separation of alkaloids. A, Codeine, 0.1 γ; B, heroin, 1 γ; C, morphine, 2 γ; D, quinine, 2 γ. Conditions: Same as in Fig. 1, except oven temperature 250°C. Flow rate of carrier gas (nitrogen) 54.4 ml/minute.

scheme. It should be noted, however, that the efficiency of this method is directly related to the minimal amount of impurities in the blood, and no claim is made that it will work equally well with other biological material.

In an extraction with acetone-ether mixture, avoidance of a pH change is one of the vital factors in the success of this method. When a sample is acidified and extracted, the organic extracts become contaminated with hemoglobin and probably with its decomposition products. A feature of the acetone-ether extraction method was that after evaporating the solvent extracts, and redissolving the residue in 95% ethanol, some materials did not dissolve and remained attached to the side of the vial. This then serves as a purification step and eliminates any further purification before subjecting the sample to gas chromatographic analysis.

B. *Animal experiments.* The basis for the success of this work depends upon the fact that in acute poisoning cases the ingested drug is found in the blood. Drugs that rapidly disappear from the blood will go undetected. To discuss the actual fate of the drug in the body is outside the scope of this research. It should be stated, however, that after absorption the

drug circulates in the blood stream and reaches every organ and tissue of the body. The toxic action of a drug is presumed to depend on the establishment of a toxic level at the site of its toxic effect and rarely on the toxic concentration in the blood. When a drug is given orally, the concentration attained in the blood depends on its rate of absorption. After absorption, the action of the drug is determined by its rate of excretion, its fixation by tissues, and its metabolism and detoxication by such processes as oxidation, reduction, or synthesis to some less (or more) toxic material.

As was stated earlier, the purpose of the animal experiments was to test the direct applicability of this method to cases of acute poisoning. Female rats of Long-Evans strain were chosen as laboratory animals as these or mice are the most commonly used for determining the toxic levels of drugs on animals. However, it should be made clear that it is not always uniformly reliable to predict human responses, since the laboratory animals may be resistant or susceptible to certain types of chemicals. This would also vary with the strain, age, weight, and sex of animals used. A number of studies have already shown female rats to be much more sensitive than males to certain compounds (*14*).

In this study, however, this variation is not a very critical factor. Here we are not determining the toxicity or toxic levels of a drug; rather, whether or not a drug in a toxic dosage after ingestion could be detected in blood by the method proposed. A few alkaloidal drugs were administered to the laboratory animals. Table 2 shows their effects on the rats and their concentration found in blood after a known interval. Some of the observations which are worthy of note are discussed below.

These experiments were done on a very small number of animals; in the majority of cases only one rat for each drug has been used. It is therefore imperative that these figures and the observed effects noted here be taken as only approximations that indicate very roughly what might be expected under these conditions. If the purpose of this study were to evaluate factors other than the identification of toxic materials in cases of acute poisoning, more work would have to be done, using a larger number of animals, to obtain more precise information, particularly with regard to the concentrations of drugs in the whole blood. The failure to obtain the expected mortality with LD_{50} doses or a higher death rate with $2 \times LD_{50}$ doses may also relate to the species used, the small number of animals, inaccuracies in the literature, and to the fact that in some cases technical rather than purified grades of drugs were employed.

C. *The drugs.* Table 1 shows the retention values of alkaloids relative to codeine at 220°, 230°, 240°, and 250°C. It will be noted that all the alkaloids could be chromatographed, and by their different retention times distinguished from each other. Even the compounds such as morphine, quinine, and strychnine have retention volumes (or, times) short enough to be observed easily under the operating conditions. Also shown in this table are the approximate percentage recoveries of many of the alkaloids which were added to human blood (25 μg alkaloid per 0.5 ml blood), extracted, and quantitatively gas chromatographed. The lowest percentage recovery recorded is for cinchonine (85%) and the highest for caffeine (102%).

Table 2 shows the details of the animal experiments with alkaloids. As can be seen, codeine was administered to rats orally, subcutaneously, and intraperitoneally in varying doses. The interesting point of the experiment is that with an oral dose of 2000 mg/kg, the whole blood concentration of codeine in 15 minutes is almost the same (100 ppm) as that observed when a dose of 1000 mg/kg was administered. This indicates that about 100 ppm of codeine in whole blood is a maximum concentration at which the particular strain of female rat may live, irrespective of whether the dose was administered at the 1000 or 2000 mg/kg level. This is confirmed by subcutaneous and intraperitoneal administrations of the same drug in 672 and 220 mg/kg doses, respectively. The whole blood concentration with subcutaneous dose was 85 ppm and that of intraperitoneal, 105 ppm.

Cocaine was administered to rats both orally and subcutaneously, and in both cases it was possible to detect the drug in whole blood. However, when the oral dose of cocaine was cut in half, from 1000 mg/kg to 500 mg/kg, the drug could not be detected in the whole blood drawn 3 hours after feeding.

Heroin (diacetylmorphine), although no longer an official drug listed in the U.S.P., is of interest primarily as an illegal, dangerous addictive, still quite widely used. It was given to rats both subcutaneously and intravenously, and in both cases it was quickly deacetylated, being detected only as monoacetyl morphine in the whole blood.

Morphine is essentially a depressant of the central nervous and respiratory systems. It is rapidly absorbed in the body after any type of administration (other than skin), reaches the blood rapidly, and then is temporarily stored in the tissues. It is highly addictive but not so much as heroin. The experiments with rats show that on oral administration

of 2000 mg/kg of morphine, in one case 5 ppm was found in the whole blood after 3 hours, and in another only a trace after a period of 2 hours. This confirms the fact that it is very rapidly removed from the blood after absorption.

Percodan (dihydrohydroxycodeinone) is also a depressant to the respiratory system, and is an addicting drug. It is, however, less toxic than codeine. An oral dose of 1000 mg/kg gave a whole blood concentration of 15 ppm in 3 hours, and an intravenous dose of 45 mg/kg gave a concentration of 20 ppm in one-half hour, at which time the rat was about to expire, indicating that this is roughly the maximum blood concentration which can be obtained in rats on acute doses.

Quinine, the chief alkaloid of cinchona bark, is commonly used therapeutically as an antimalarial. It has no addicting action. With an oral dose of 1000 mg/kg the whole blood concentration of quinine was only 9 ppm after a period of 3 hours. This low concentration of such a high dose may be in part due to its slow absorption and partly due to its rapid fixation in tissue. Other work done in the field indicates that at 3-6 hours after ingestion the maximal amount of quinine concentration is obtained in the blood.

Figures 1 and 2 show two typical chromatograms obtained by gas chromatographing mixtures of a number of common alkaloidal drugs. All the peaks, including those of heroin and morphine, are symmetrical, and the chromatograms show high efficiency and sensitivity of the operating conditions.

SUMMARY

A rapid method for (a) the extraction of a number of alkaloids from blood after ingestion of lethal or near-lethal doses, and (b) their subsequent gas chromatographic identification is described. Extraction is done at the pH of blood by a mixture of equal volumes of acetone and ether. A 3-foot long glass column packed with 1% Hi-Eff-8B on 100/120 mesh silanized Gas Chrom P (Gas Chrom Q) is used in conjunction with a flame ionization detector, and the relative retention times of the 18 alkaloids at four different temperatures are recorded. To test the direct applicability of the method in forensic cases, animal experiments were carried out. Toxic or lethal doses of the six common alkaloidal drugs were administered; bloods were drawn at varying intervals and the concentration of the drug found in blood was recorded. Recovery data obtained by this method on adding known quantities of drugs to blood are also described.

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MICROCHEMICAL JOURNAL 12, 242-248 (1967)

Systematic Applications of Gas-Liquid Chromatography in Toxicology[1]

II. The Antihistamines

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In the preceding article (*1*) we discussed the importance of a simple extraction method which should be applicable to a wide variety of drugs. Also discussed was a gas chromatographic procedure able to separate and tentatively identify a majority of drugs. Alkaloids were thus successfully extracted from blood and gas chromatographed by the proposed method. The same scheme is now extended to extract and identify some of the commonly used antihistamines using the same extraction procedure and the same gas chromatographic column. Blood is again chosen as the biological fluid from which to do the extractions.

EXPERIMENTAL PROCEDURE

A. *Equipment.* The Hy-Fi gas chromatograph, Aerograph model 600 (Wilkens Instrument and Research, Inc., Walnut Creek, California) was used and was equipped with a flame ionization detector and a Leeds and Northrup Speedomax H, 0-1 mV recorder, model S. The column was a spiral Pyrex glass tube of 0.125 inch o.d., 0.070 inch i.d., and 3 feet in length. It was packed with 1% (expressed as a percentage by weight of the solid support) Hi-Eff-8B (cyclohexanedimethanol succinate) on 100/120 mesh silanized Gas Chrom P.[3]

1 This investigation was supported by a grant from the United States Public Health Services, AC-8-66, Division of Accident Prevention, and by a research grant from the Committee on Research, University of California.

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3 Silanized Gas Chrom P is now commercially available as Gas Chrom Q. One per cent coated Hi-Eff-8B on Gas Chrom Q is available from Applied Science Laboratories, Inc., State College, Pennsylvania.

B. *Standard Solutions.* The standard solutions of the antihistamines were made by dissolving their appropriate salts in 95% ethanol. The stock solutions were made in concentrations of 10 μg/μl. All the stock solutions were further diluted for gas chromatographic injections.

Measured doses of these compounds were dissolved in water and administered orally to laboratory animals in the same manner as discussed in Part I (*1*).

TABLE 1

RELATIVE RETENTION TIMES AND RECOVERY OF ANTIHISTAMINES[a]

Antihistamine (salt)	Relative retention times (min)		Approximate recovery from blood (%)[b]
	160°C	190°C	
Antazoline	0.51	0.58	95
Bromodiphenhydramine	2.00	1.75	96
Carbinoxamine	1.57	1.42	75
Chlorcyclizine	2.46	2.25	102
Chlorothen	2.14	1.96	80
Chlorpheniramine	0.94	0.92	88
Clemizole	—[d]	31.35	—[e]
Diphenylpyraline	1.29	1.29	90
Diphenhydramine	0.43	0.46	85
Doxylamine	0.51	0.50	100
Dramamine	0.43	0.46	100
Meclizine	—[d]	50[c]	—[e]
Methapyrilene	1.0	1.0	100
Methdilazine	11.4	8.67	—[e]
Parabromdylamine	1.57	1.50	90
Phenyltoloxamine	0.66	0.66	98
Pyrathiazine	14.00	12.16	—
Pyrilamine	3.83	3.04	80
Pyrrobutamine	5.94	4.67	82
Thonzylamine	3.14	2.50	89
Tripelennamine	0.83	0.83	105
Tripolidine	3.14	2.50	94
Methapyrilene (R.T.)	3.5	1.2	

[a] Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1%, on 100/120 mesh silanized Gas Chrom P. Flow rate of carrier gas at 160°C, 85 ml/minute; flow rate of carrier gas at 190°C, 60 ml/minute.

[b] These calculations are based on 20 γ of respective drugs ½ ml of whole blood.

[c] Broad diffused peak.

[d] Did not emerge.

[e] Not done.

TABLE 2

DETAILS OF ANIMAL EXPERIMENTS WITH ANTIHISTAMINES

Drug	Dose (mg/kg)[a]	Weight of rat (g)[b]	Time between dosing and drawing blood	ppm of drug found in the whole blood	Observed effects on rat after administration of drug
Carbinoxamine maleate	1000	159	3 hours	1.3	—
Chlorcyclizine HCl	1000	204	10 min	7	Severe convulsions soon after administration; was dying in 10 min.
Chlorcyclizine HCl	500	128	3 hours	—	—
Chlorpheniramine maleate	1000	166	3 hours	5	—
Diphenhydramine HCl	1000	150	10 min	10	Violent convulsions and/or tremors immediately after administration.
Diphenhydramine HCl	500	179	3 hours	—	In the beginning was excited and was kicking legs, but became normal after an hour.
Methapyrilene HCl	500	157	15 min	12	Excitement, jumping, and kicking soon after administering. Became quiet in 10 min; dying when blood was drawn.
Methapyrilene HCl	1000	175	45 min	20	Violent convulsions preceding the drawing of blood.
Parabromdylamine	1000	163	3 hours	3	—
Tripelennamine HCl	1000	162	10 min	20	Convulsing within 5 min.
Tripelennamine HCl	500	165	15 min	20	Convulsing, jumping, and shaking within 5 min; nearly dead at the time of drawing blood.

[a] All doses administered orally.

[b] All female rats of Long-Evans strain, 2 months old.

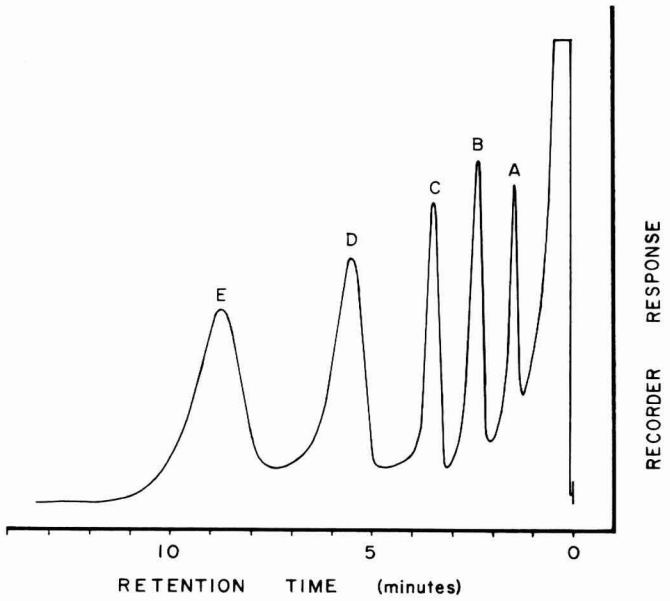

FIG. 1. Separation of antihistamines. A, Diphenhydramine, 0.5 γ; B, phenyltoloxamine, 1 γ; C, methapyrilene, 1 γ; D, carbinoxamine, 2 γ; E, chlorcyclizine, 5 γ. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 160°C; injector 275°C. Flow rate of carrier gas (nitrogen) 85 ml/minute. Input impedance 10^9 ohms; output sensitivity, 1 ×; attenuation, 4 ×.

RESULTS AND DISCUSSION

The antihistaminic drugs act specifically by preventing the effects of liberated histamine. They are basic in nature, chiefly aromatic derivatives of dialkylethylamine, and usually not very toxic. They generally produce drowsiness (thonzylamine is an exception) due to depression of the central nervous system, and because of this depressant effect they are used in the treatment of motion sickness. However, their greatest therapeutic use is on seasonal hay fever.

Table 1 gives the relative retention values of 22 antihistamines at 160°C and 190°C relative to methapyrilene, as well as the percentage recoveries for 18 of the compounds chromatographed. Here again each substance could be differentiated from others on the basis of retention times, and the recoveries of added drugs from the blood are good. The lowest recovery is 75% for carbinoxamine and 80% for chlorothen and pyrilamine. The remaining compounds gave much higher recoveries.

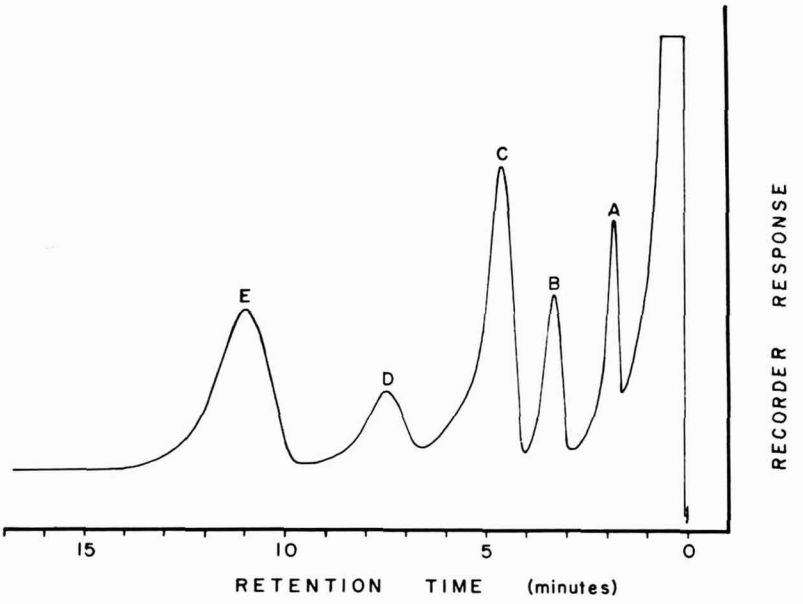

FIG. 2. Separation of antihistamines. A, Doxylamine, 0.5 γ; B, chlorpheniramine, 1 γ; C, diphenylpyraline, 3 γ; D, chlorothen, 3 γ; E, thonzylamine, 5 γ. Conditions: 3-foot glass column ($\frac{1}{8}$ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 160°C; injector 275°C. Flow rate of carrier gas (nitrogen) 85 ml/minute. Input impedance 10^9 ohms; output sensitivity, 1 ×; attenuation, 4 ×.

Table 2 shows the results of animal experiments with antihistamines. The drugs chosen for this purpose were carbinoxamine, chlorcyclizine, chlorpheniramine, diphenhydramine, methapyrilene, parabromdylamine, and tripelennamine. All doses were administered orally. In all cases the drugs could be detected in whole blood after the rats had received doses in concentrations of 1000 mg/kg. A rat which was given a dose of 1000 mg/kg of chlorcyclizine gave a whole blood concentration of 7 ppm in 10 minutes, whereas another rat receiving half this dose did not show any concentrations in the blood after 3 hours. A similar observation was noted with diphenhydramine: 10 ppm was detected in 10 minutes with a dose of 1000 mg/kg, but none on feeding 500 mg/kg after 3 hours. Two rats were given tripelennamine, one a dose of 1000 mg/kg and the other 500 mg/kg. In both the concentration of the drug was 20 ppm in whole blood 10 and 15 minutes after ingestion. This figure indicates the maxi-

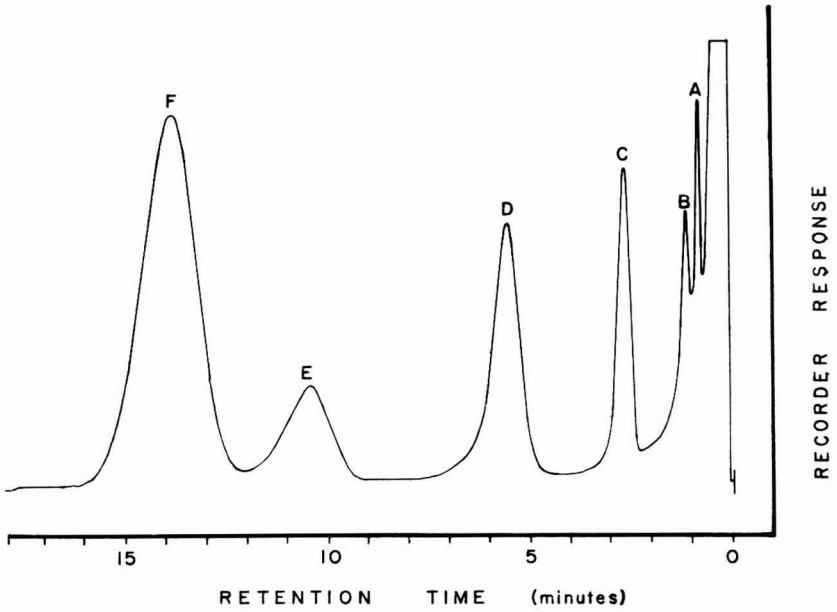

FIG. 3. Separation of antihistamines. A, Phenyltoloxamine, 0.1 γ; B, methapyrilene, 0.1 γ; C, chlorcyclizine, 0.5 γ; D, pyrrobutamine, 1 γ; E, methdilazine, 2 γ; F, pyrathiazine (Pyrrolozote), 5 γ. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mest silanized Gas Chrom P. Oven temperature 190°C; injector 275°C. Flow rate of carrier gas (nitrogen) 60 ml/minute. Input impedance 10^9 ohms; output sensitivity, 1 ×; attenuation, 4 ×.

mum level of the drug in whole blood that is to be found in rats in acute poisoning with tripelennamine, and confirms the findings of Way and Dailey (2) that tripelennamine leaves the blood rapidly, being distributed through all soft tissues, especially the lungs. Figure 1 is a chromatogram from a mixture of five antihistamines: diphenhydramine, phenyltoloxamine, methapyrilene, carbinoxamine and chlorcyclizine. Figure 2 illustrates the separation of five more antihistamines: doxylamine, chlorpheniramine, diphenylpyraline, chlorothen and thonzylamine, Figs. 1 and 2 being obtained at 160°C. Figure 3 is yet another chromatogram from a mixture of six antihistamines: phenyltoloxamine, methapyrilene, chlorcyclizine, pyrrobutamine, methdilazine, and pyrathiazine at 190°C. All the chromatograms demonstrate that these compounds could also be separated and detected in microgram or submicrogram quantities.

SUMMARY

The relative retention times of 22 antihistaminic drugs obtained at 160° and 190°C on a 3-foot glass column packed with 1% Hi-Eff-8B on 100/120 mesh silanized Gas Chrom P are recorded. Also described are the results of animal experiments, particularly the concentrations of drugs found in the bloods of rats after they were given toxic doses of seven of the common antihistaminic drugs. The simple acetone-ether extraction method (*1*) as described in Part I of this series was used to extract drugs from bloods. This procedure was also used in determining recovery data obtained on adding known quantities of drugs to bloods.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Terry L. Coddington and Jacqueline M. Ehlert in this work.

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Systematic Applications of Gas-Liquid Chromatography in Toxicology[1]

III. The Barbiturates

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The barbiturates are a very important group of central nervous system depressants which are frequently used by the public. They are all derivatives of barbituric acid (malonylurea), and differ in their action according to the stability of the substituted group. Because of their great effectiveness and the safety of their use in therapeutic doses, they have almost replaced the old hypnotics and sedatives. Although no deaths or severe side effects result when these are used in therapeutic quantities, additional doses have caused many deaths. Today, barbiturates have in fact become one of the most common suicidal poisons, hence their toxicological importance.

In a previous publication (*1*) the authors described a method using acetone-ether as the extracting solvent of barbiturates from blood, and their subsequent gas chromatographic identification by gas-liquid chromatography employing an improved column (*4*) of 1.5% SE 30 and 2% Carbowax 20M on acid washed 100/120 mesh firebrick. This study has now been incorporated in this series on the systematic applications of GLC in toxicology using a 1% Hi-Eff-8B column on Gas Chrom Q, and also extended to include the work on laboratory animals.

EXPERIMENTAL PROCEDURE

A. *Equipment.* The Hy-Fi gas chromatograph, Aerograph model 600 (Wilkens Instrument and Research, Inc., Walnut Creek, California) was

[1] This investigation was supported by a grant from the United States Public Health Services, AC-8-66, Division of Accident Prevention, and by a research grant from the Committee on Research, University of California.

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used and was equipped with a flame ionization detector and a Leeds and Northrup Speedomax H, 0-1 mV recorder, model S. The column was a spiral Pyrex glass tube of 0.125 inch o.d., 0.07 i.d., and 3-feet in length. It was packed with 1% (expressed as a percentage by weight of the solid support) Hi-Eff-8B (cyclohexanedimethanol succinate) on 100/120 mesh silanized Gas Chrom P.[3]

TABLE 1

RELATIVE RETENTION TIMES AND RECOVERY OF BARBITURATES[c]

Barbiturate	Relative retention times		Approximate recovery from blood (%)[a]
	195°C	220°C	
Alphenal	8.0	6.17	—[b]
Amobarbital (Amytal)	0.89	0.83	88
Aprobarbital (Isopral)	0.75	0.74	90
Barbital (Veronal)	0.5	0.5	90
Butabarbital (Noctinal)	0.82	0.78	—[b]
Butabital (Talbutal)	1.00	0.96	—[b]
Butethal (Neonal)	0.83	0.83	85
Cyclopal (Cyclopen)	2.43	2.17	—[b]
Dial	0.79	0.78	110
Heptabarbital	4.71	4.0	—[b]
Hexethal (Ortal)	1.54	1.30	100
Hexobarbital (Evipal)	0.86	0.83	—[b]
Mephobarbital (Mebaral)	1.29	1.22	—[b]
Methallatal (Mosidal)	0.95	1.00	93
Metharbital (Gemonil)	With solvent	With solvent	—[b]
Pentobarbital (Nembutal)	1.0	1.0	92
Pentothal (Thiopental)	1.29	1.26	85
Phenobarbital (Luminal)	5.8	4.7	80
Probarbital (Ipral)	0.6	0.65	92
Seconal	1.25	1.13	95
Thiamylal (Surital)	1.57	1.39	—[b]
Vinbarbital (Delvinal)	1.18	1.09	—[b]
Pentobarbital (R.T.)	2.8	2.3	

[a] These calculations are based on adding 20 γ of respective drugs to ½ ml of whole blood.

[b] Not done.

[c] Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1%, on 100/120 mesh silanized Gas Chrom P. Flow rate of carrier gas at 190°C, 44.4 ml/minute; flow rate of carrier gas at 220°C, 15.8 ml/minute.

[3] Silanized Gas Chrom P is now commercially available as Gas Chrom Q. One per cent coated Hi-Eff-8B on Gas Chrom Q is available from Applied Science Laboratories, Inc., State College, Pennsylvania.

B. *Standard solutions.* The recrystallized free acids of the barbiturates as listed in Table 1 were dissolved in 95% ethanol to give a concentration of 5 μg/μl. These were also diluted in the usual manner where needed. All the barbiturates were administered orally to the laboratory animals, their sodium salts (Table 2) being dissolved in water so that the required doses for rats were administered in 1-1.5 ml of solution.

The extracting solvent was a mixture of equal volumes of acetone and ether. The extraction procedure was the same as described in Part I of this work (*2*).

RESULTS

Table 1 shows the relative retention times of 23 barbiturates, gas chromatographed at 195° and 220°C. Also included in this table are the results of recoveries of some of the common drugs of this group which were added in known quantities to blood, extracted, and gas chromatographed to determine the percentage recoveries. Table 2 shows the details of animal experiments obtained from administering orally some of the commonly used barbiturates. The quantities of doses given to rats are also listed in Table 2. Figure 1 is a typical separation of the five barbiturates on the column used.

DISCUSSION

As was mentioned earlier, the method of extraction has been successfully tested by extracting barbiturates from blood (*1*). We have now extended this under this general scheme of toxicology using a Hi-Eff-8B column. The relative retention times of 23 barbiturates in relation to pentobarbital are recorded in Table 1. Also included is the recovery data for some of the widely used barbiturates. The temperatures chosen for determining the relative retention times are 195° and 220°C. As can be seen, phenobarbital has a relative retention value of 5.8 and 4.7 at 195° and 220°C, respectively. These are much smaller values in comparison to those obtained by other authors (*3*).

Table 2 shows the animal experiments done with amobarbital (Amytal), phenobarbital (Luminal), pentobarbital (Nembutal), thiopental (Pentothal), and secobarbital (Seconal). Out of these, amobarbital, pentobarbital, and secobarbital are short acting, pentothal is ultrashort, and phenobarbital is a long acting barbiturate.

Some interesting observations are noted with the administrations of amobarbital and pentobarbital to rats. Amobarbital, when given in an

TABLE 2

DETAILS OF ANIMAL EXPERIMENTS WITH BARBITURATES

Drug	Dose (mg/kg)[a]	Weight of rat (g)[b]	Time between dosing and drawing blood	ppm of drug found in whole blood	Observed effects on rat after administration of drug
Amobarbital Na	2 × MLD 640	171	10 min	250	Quiet; died after 10 min.
Amobarbital Na	MLD 320	163	10 min	250	Nearly dead in 10 min.
Amobarbital Na	160	182	3 hours	111	Sleeping at the time of drawing blood.
Pentobarbital Na	2 × MLD 280	166	23 min	60	Sleepy; almost dead in 23 min.
Pentobarbital Na	MLD 140	155	1 hour	62	Was having convulsions at the time of drawing blood.
Pentothal Na	2 × MLD[c] 304	136	15 min	11	—
Pentothal Na	MLD[c] 152	155	3 hours	12 (as Pentobarb.)	Shaky after 75 min; then slept until drawing of blood.
Phenobarbital Na	2 × LD_{50} 1056	106	1 hour	100	Died in sleep.
Secobarbital Na	2 × MLD 200	148	3 hours	60	Was cold but alive at the time of drawing blood.

[a] All doses administered orally.

[b] All female rats of Long-Evans strain, 2 months old.

[c] Incomplete solution of drug in water.

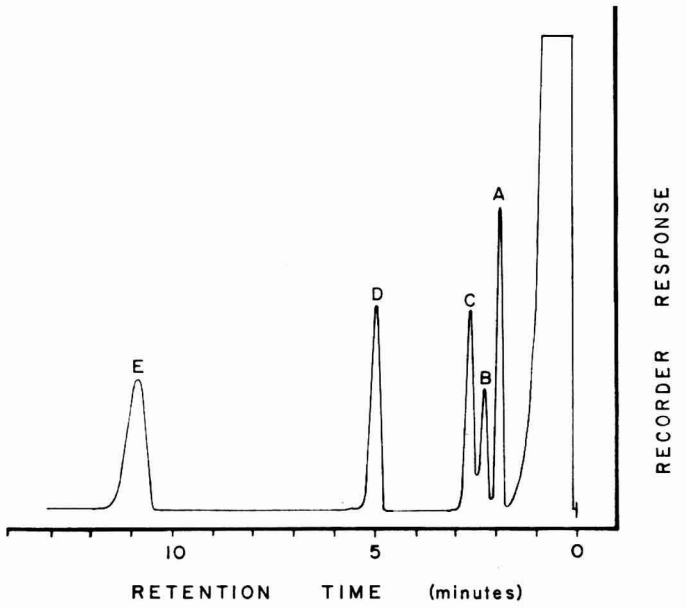

FIG. 1. Separation of barbiturates. A, Amobarbital, 0.1 γ; B, pentobarbital, 0.1 γ; C, secobarbital, 0.1 γ; D, cyclopal, 0.5 γ; E, phenobarbital, 1 γ. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 220°C; injector 275°C. Flow rate of carrier gas (nitrogen), 15.8 ml/minute. Input impedance 10^9 ohms; output sensitivity 1· ×; attenuation 4 ×.

oral dosage of 640 mg/kg, gave a whole blood concentration of 250 ppm of this drug in 10 minutes, and the same blood concentration was reached in the same period with half this dose. This indicates that 250 ppm is probably the maximum concentration of amobarbital in the whole blood of rats after ingestion of acute dosages. Any concentration higher than this may prove fatal. Similar results are obtained with pentobarbital. An oral dose of 280 mg/kg gave a whole blood concentration of 60 ppm in 23 minutes, and a similar concentration (62 ppm) was obtained with half the dose in one hour.

The rat which was given an oral dose of 1056 mg/kg of phenobarbital expired, and its whole blood concentration after one hour was 100 ppm. The fate of pentothal in the body has been a matter of controversy, but evidence indicates that, like the others, it is destroyed in the liver. It is rapidly removed from the blood and is to some extent metabolized by

oxidation to pentobarbital before further detoxication. These findings are confirmed by this work in which an oral dose of 304 mg/kg gave a whole blood concentration of pentothal of 11 ppm 15 minutes after ingestion, whereas with half the above dose and after a 3-hour interval pentothal was present in blood only as pentobarbital in a concentration of 12 ppm. There was no trace of pentothal.

It is also interesting to note that after administration, the barbiturates occur in blood in appreciable quantities which may be detected gas chromatographically. This is true with the therapeutic dosage, as out of about 35 different specimens received from a hospital, in all but seven cases, the particular barbiturate could be detected. In the absence of detailed information such as the dosage levels and the period during which these drugs were administered, no comprehensive results in a tabular form can be given. However, it is worth noting that these blood samples contained the commonly used drugs such as phenobarbital, amobarbital, pentobarbital, secobarbital, and pentothal.

Figure 1 shows the chromatogram of five of the commonly used sedatives. They are amobarbital, pentobarbital, secobarbital, cyclopal, and phenobarbital, and the separation was carried at 220°C. As is evident from the chromatogram, all the peaks are sharp, clear, and symmetrical, including that of phenobarbital.

SUMMARY

In this work are listed the relative retention times of 23 barbiturates obtained at 195° and 220°C on a 3-foot glass column packed with 1% Hi-Eff-8B on 100/120 mesh silanized Gas Chrom P. Also recorded are the results of recoveries of twelve drugs of this group which were added in known quantities to blood, extracted by the acetone-ether method (*2*), and their recoveries determined gas chromatographically on the above column. Details of animal experiments show that in all cases the administered drugs could be detected in the bloods of the rats. This was also true in the majority of blood specimens received from patients who were given therapeutic levels of the commonly used barbiturates.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Terry L. Coddington and Jacqueline M. Ehlert in this work.

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Systematic Applications of Gas-Liquid Chromatography in Toxicology[1]

IV. The Tranquilizers

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The use of tranquilizing drugs in psychopharmacology began in the last decade with the introduction of chlorpromazine and the Rauwolfia alkaloids in this country. Since then, the consumption of psychotherapeutic drugs has soared steadily. Gerard (*2*) reports that three out of every ten prescriptions written in the United States are for psychotherapeutic agents.

The phenothiazines, the major group of tranquilizers, produce their effect by depressing the central nervous system, an action differing from the depression produced by barbiturates and other sedative-hypnotics. When used therapeutically, they often reduce aggressive and destructive behavior, and also help improve certain schizophrenic states. The minor tranquilizers which are actually hypnotic sedatives, such as meprobamate, suppress the less severe forms of anxiety and tension. Even though tranquilizing drugs are not as harmful as the barbiturates, it has been said (*1*), "They constitute a definite threat to the health and even the life of patients." They may cause enough depression for people to commit suicide or, when taken in combination with alcohol, their combined effect may prove very harmful, and hence their toxicological importance.

EXPERIMENTAL PROCEDURE

A. *Equipment*. The Hy-Fi gas chromatograph, Aerograph model 600 (Wilkens Instrument and Research, Inc., Walnut Creek, California) was

[1] This investigation was supported by a grant from the United States Public Health Services, AC-8-66, Division of Accident Prevention, and by a research grant from the Committee on Research, University of California.

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used. It was equipped with a flame ionization detector and a Leeds and Northrup Speedomax H, 0-1 mV recorder, model S. The column was a spiral Pyrex glass tube of 0.125 inch o.d., 0.07 inch i.d., and 3 feet in length. It was packed with 1% (expressed as a percentage by weight of the solid support) Hi-Eff-8B (cyclohexanedimethanol succinate) on 100/120 mesh silanized Gas Chrom P.[3]

B. *Standard solutions.* The standard solutions of these drugs were made by dissolving their appropriate salts in 95% ethanol. Those salts which were not soluble in ethanol were dissolved in other solvents such as methanol, water, or chloroform. The stock solutions were made to give concentrations in the range of 5-50 μg/μl. All these solutions were further diluted for gas chromatographic injections. The vials containing light-sensitive solutions, particularly those containing phenothiazines, were wrapped with aluminum foil to avoid exposure to light. All solutions were stored under refrigeration, and whenever a discoloration of a solution was noted, either because of the action of light or by long standing, the material was rejected and a fresh solution was made.

C. *Extraction.* The same extraction procedure and the same system of extracting solvents were used to extract drugs from blood as in the case of alkaloids (*3*), antihistamines (*4*), and the barbiturates (*5*).

D. *Extraction of tranquilizers from phosphate buffer (pH 7.4).* Known quantities of the salts of tranquilizers were dissolved in a phosphate buffer made by dissolving (*A*) 0.07 *M* monopotassium phosphate (9.08 g KH_2PO_4 per liter); and (*B*) 0.07 *M* disodium phosphate (11.88 g $Na_2HPO_4 \cdot 2H_2O$ per liter) in the following proportion:

$$19.6 \text{ ml } A + 80.4 \text{ ml } B$$

to give a pH of 7.4. Because of the aqueous buffer system, the extraction was done three times with ether. The evaporated residue of the extract was dissolved in a known quantity of 95% ethanol, as were most of the standard solutions described earlier, and a 1 μl aliquot was gas chromatographed to check the recovery data (Table 1).

RESULTS AND DISCUSSION

Table 1 lists the retention values of 23 tranquilizers relative to meprobamate. These were determined at 220° and 250°C. Also included in the

[3] Silanized Gas Chrom P is now commercially available as Gas Chrom Q. One per cent coated Hi-Eff-8B on Gas Chrom Q is available from Applied Science Laboratories, Inc., State College, Pennsylvania.

TABLE 1

RELATIVE RETENTION TIMES AND RECOVERY OF TRANQUILIZERS[a]

Tranquilizers drugs[a]	Relative retention times (min)		Approximate recovery[b]	
	220°C	250°C	From blood (%)	From buffer (%)[c]
Carisoprodol	0.53	0.60	100	100
Chlormethazanone	2.3	2.17	95	85
Chlorpromazine	2.21	2.09	85	100
Ectylurea	0.09	With solvent	97	90
Ethopropazine	1.80	1.73	89	89
Fluphenazine	1.35	1.35	100	82
Frenquel	1.26	1.59	92	95
Librium	6.88	5.91	—[d]	—[d]
Mepazine	2.65	2.55	95	100
Meprobamate	1.00	1.00	96	93
Methoxypromazine	3.47	3.18	110	98
Perphenazine	4.18	3.91	78	95
Phenaglycodol	0.18	With solvent	—[d]	—[d]
Prochlorperazine	4.24	4.00	—[d]	—[d]
Promazine	1.24	1.68	85	85
Promethazine	1.73	0.97	90	100
Pyrathiazine	2.79	2.73	92	120
Reserpine	1.8	1.64	—[d]	—[d]
Thiopropazate	4.18	3.91	—[d]	—[d]
Thioridazine	20.0	17.0	98	80
Trifluoperazine	3.21	1.41	95	100
Triflupromazine	0.68	0.62	90	100
Trimeprazine	0.95	0.91	100	115
Meprobamate (R.T.)	3.4	1.1		

[a] Conditions: 3-foot glass column ($\frac{1}{8}$ inch o.d.) packed with Hi-Eff-8B 1%, on 100/120 mesh silanized Gas Chrom P. Flow rate of carrier gas (Nitrogen) at 220°C, 15.8 ml/minute; flow rate of carrier gas (Nitrogen) at 250°C, 14.6 ml/minute.

[b] These calculations are based on adding 250 γ of respective drugs to $\frac{1}{2}$ ml of whole blood.

[c] Phosphate buffer made by mixing (*A*) 0.07 *M* monopotassium phosphate and (*B*) 0.07 *M* disodium phosphate, according to 19.6 ml *A* + 80.4 ml *B*.

[d] Not done.

same table are the recovery data obtained from samples of blood and phosphate buffer (pH 7.4) to which were added known quantities of tranquilizers. Additional experiments with the phosphate buffer were performed to determine how much recovery is obtained from fluids at the

TABLE 2

DETAILS OF ANIMAL EXPERIMENTS WITH TRANQUILIZERS: HALF-HOUR PERIOD BETWEEN DOSING AND DRAWING BLOOD

Tranquilizing drugs[a]	Dose ($2 \times LD_{50}$) (mg/kg)	Weight of rat (g)[b]	ppm of drug found in the whole blood
Chlorpromazine HCl	200	119	None
Fluphenazine dihydrochloride	500	117	None
Mepazine hydrochloride[c]	550	131	None
Promazine HCl	440	119	72
Promethazine HCl	1200	99	10
Trimeprazine	1200	137	None

[a] All drugs given orally in water solution or suspension.

[b] All female rats of Long-Evans strain, one and one-half months old.

[c] In suspension form.

same pH as that of blood. As was expected, the recovery results were not significantly different, other than what could be expected within experimental error.

Tables 2 and 4 show the details of animal experiments in which twice the LD_{50} doses of chlorpromazine, fluphenazine, mepazine, promazine, promethazine, and trimeprazine were administered at one-half and 2 hour intervals, respectively. Table 3 gives details of 1-hour intervals with the

TABLE 3

DETAILS OF ANIMAL EXPERIMENTS WITH TRANQUILIZERS: ONE-HOUR PERIOD BETWEEN DOSING AND DRAWING BLOOD

Tranquilizing drug[a]	Dose $(LD)_{50}$ (mg/kg)	Weight of rat (g)[b]	ppm of drug found in whole blood
Chlorpromazine HCl	100	110	None
Fluphenazine dihydrochloride	250	101	40
Mepazine HCl[c]	275	131	None
Promazine HCl	220	98	4.8
Promethazine HCl	600	122	16
Trimeprazine tartarate	600	103	Trace

[a] All drugs given orally in water solution or suspension.

[b] All female rats of Long-Evans strain, one and one-half months old.

[c] In suspension form.

above tranquilizers in LD_{50} doses. Several interesting observations are noted. Except for promazine and promethazine, which alone could be found at the whole blood concentrations of 72 and 10 ppm, respectively, the remaining drugs could not be detected at a half-hour interval. How-

TABLE 4

DETAILS OF ANIMAL EXPERIMENTS WITH TRANQUILIZERS: TWO-HOUR PERIOD BETWEEN DOSING AND DRAWING BLOOD

Tranquilizing drug[a]	Dose ($2 \times LD_{50}$) (mg/kg)	Weight of rat (g)[b]	ppm of drug found in whole blood
Chlorpromazine HCl	200	112	8
Fluphenazine dihydrochloride	500	124	93
Mepazine HCl[c]	550	130	None
Meprobamate[c]	2500	102	Present
Promazine HCl	440	124	11
Promethazine HCl	1200	111	40
Trimeprazine tartrate	1200	140	20

[a] All drugs given orally in water solution or suspension.

[b] All female rats of Long-Evans strain, one and one-half months old.

[c] In suspension form.

[d] Because of very thick suspension of drug, only part of drug could be administered.

ever, after a 2-hour interval (Table 4), all the drugs except mepazine could be detected in the whole blood of rats in appreciable quantities. By no means are these the maximum concentrations which may be expected, but they do indicate that these drugs do not hydrolyze so rapidly that they cannot be detected in cases of acute poisoning in rats.

Table 5 shows the results of analysis of blood samples from a psychiatric clinic where the patients had been treated with the tranquilizing drugs, either alone or in combination with other drugs such as the barbiturates. Their importance is from the standpoint of detecting them in

FIG. 1. Separation of tranquilizers. A, Triflupromazine, 1 γ; B, meprobamate, 0.5 γ; C, promazine, 0.5 γ; D, fluphenazine, 1 γ; E, chlorpromazine, 0.5 γ; F, methoxypromazine, 1 γ; G, perphenazine, 2 γ. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 220°C; injector 275°C. Flow rate of carrier gas (nitrogen) 15.8 ml/minute. Input impedance 10^9 ohms; output sensitivity 1 ×; attenuation 4 ×.

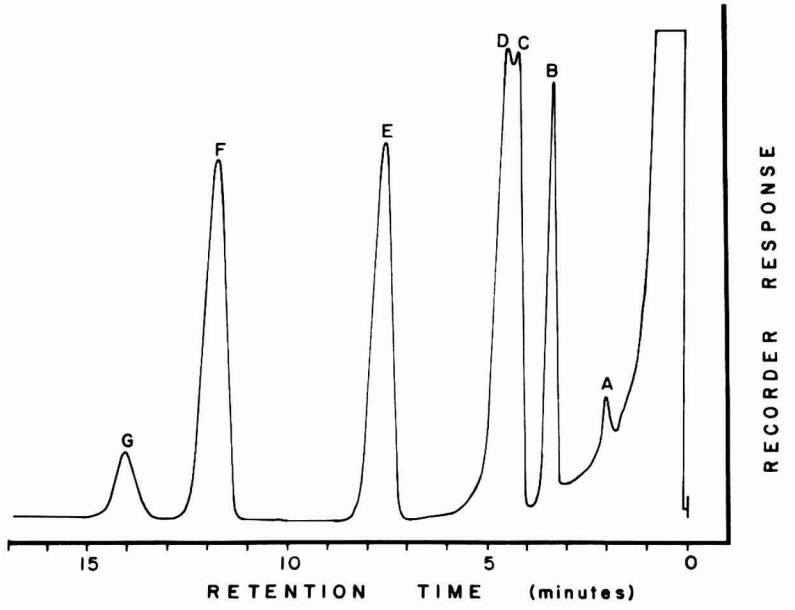
TABLE 5

ANALYSIS OF BLOOD SAMPLES FROM A PSYCHIATRIC CLINIC: PATIENTS ON TREATMENT WITH TRANQUILIZERS[a]

Sample No.		Therapeutic dose	Drug found in blood
1	Promazine	50 mg 4 × a day	+[b]
2	Chlorpromazine	25 mg 3 × a day	—
	Seconal	1½ g bed time	+
3	Reserpine	0.25 mg 2 × a day	—
4	Chlorpromazine	50 mg 4 × a day	+
5	Thioridazine	50 mg 4 × a day	—
	Amytal	No details available	—
6	Perphenazine	16 mg 4 × a day	—
7	Promethazine	12½ mg when needed	+
8	Chlorpromazine	75 mg 2 × a day	+
9	Chlorpromazine	75 mg 2 × a day	+
10	Meprobamate	400 mg 2 × a day	+
11	Chlorpromazine	50 mg 4 × a day	—
12	Meprobamate	400 mg 3 × a day	+

[a] It is not known how long these patients have been on treatment with these drugs, but it is believed to be a week or longer.

[b] + Denotes drug could be detected; — denotes drug could not be detected.



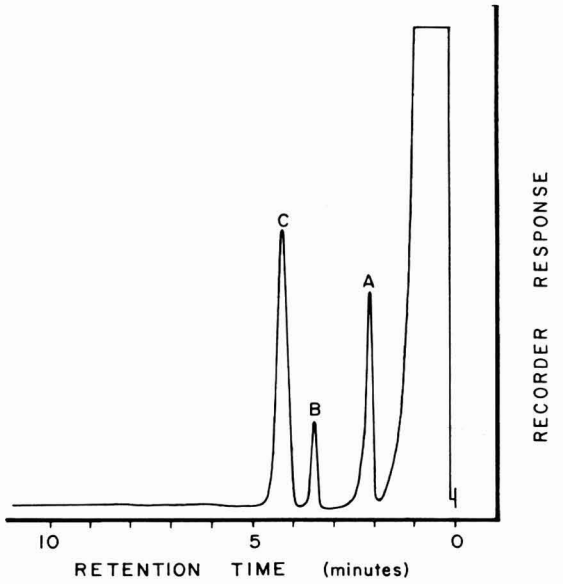


FIG. 2. Separation of tranquilizers. A, Triflupromazine, 2 γ; B, meprobamate, 0.1 γ; C, frenquel 1 γ. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 220°C; injector 275°C. Flow rate of carrier gas (nitrogen) 15.8 ml/minute. Input impedance 10^9 ohms; output sensitivity 1 ×; attenuation 4 ×.

therapeutic doses. While meprobamate could be detected in all instances, the results obtained with others were not consistent. It also depended upon the dose level and the duration of treatment, as both varied widely from one patient to another. Information as to the actual duration of treatment was not available. As a rule, as can be seen from the table, higher doses were easier to detect.

Figure 1 is an actual chromatogram at 220°C from a mixture of seven tranquilizers: fluphenazine, meprobamate, promazine, triflupromazine, chlorpromazine, methoxypromazine, and perphenazine. Figure 2 shows the separation of triflupromazine, meprobamate, and frenquel at 220°C, and Figure 3 distinguishes a mixture of meprobamate, reserpine, and librium at 250°C.

SUMMARY

The relative retention times of 23 tranquilizers determined at 220° and 250°C on the 1% Hi-Eff-8B column are listed. The recovery results obtained from samples of

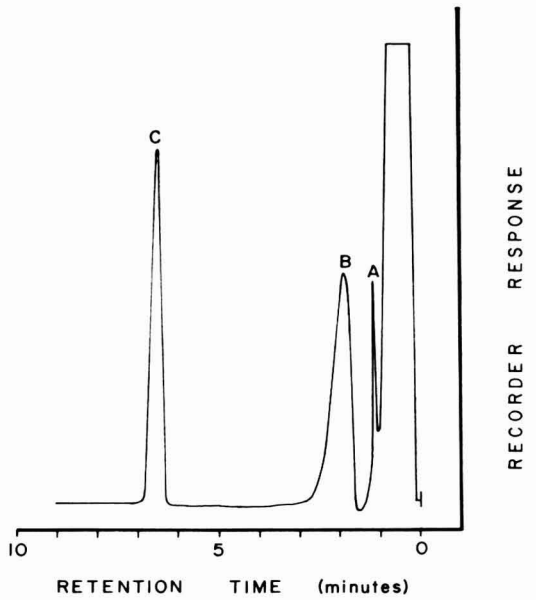


FIG. 3. Separation of tranquilizers. A, Meprobamate, 0.1 γ; B, reserpine, 5 γ; C, librium, 2 γ. Conditions: 3-foot glass column ($\frac{1}{8}$ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 250°C; injector 275°C. Flow rate of carrier gas (nitrogen) 14.6 ml/minute. Input impedance 10^9 ohms; output sensitivity 1 ×; attenuation 4 ×.

bloods and phosphate buffer (pH 7.4) on adding known quantities of tranquilizers and extracting them with the organic solvents are also recorded in this work. It is demonstrated that at varying intervals the LD_{50} and $2 \times LD_{50}$ doses of some of the common tranquilizers could be gas chromatographically detected in the bloods of rats. Analyses done on samples of bloods received from patients in a psychiatric clinic indicate that some of the administered drugs could also be detected, but this would depend further on the type of tranquilizer, dose level, and the duration of treatment.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Terry L. Coddington and Jacqueline M. Ehlert in this work.

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Systematic Applications of Gas-Liquid Chromatography in Toxicology[1]

V. The Pesticides and Evaluation of Toxic Levels

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PESTICIDES

The pesticides are not necessarily chemically related to each other, except for the organo-phosphorus compounds which could be characterized by (a) being derivatives of phosphoric acid, and (b) by their action as irreversible inhibitors of the enzyme cholinesterase. The chlorinated hydrocarbon insecticides, on the other hand, act on the central nervous system and vary widely in their chemical structure and activity. The use of pesticides to maintain food supply is extensive, and often they are responsible for illness and deaths due to excessive contamination or accidental ingestion.

In an earlier study (*2*) the pesticides were chromatographed using an SE-52 column on chromosorb W. To include this under the systematic scheme, they are now chromatographed on a 1% Hi-Eff-8B column on 100/120 mesh silanized Gas Chrom P (Gas Chrom Q).[3] This is the same column used earlier in the studies of alkaloids (*3*), antihistamines (*4*), barbiturates (*5*), and the tranquilizers (*6*). One difference, however, was the use of an electron capture detector with the gas chromatography of pesticides, whereas the rest of the drugs were chromatographed on a

[1] This investigation was supported by a grant from the United States Public Health Services, AC-8-66, Division of Accident Prevention, and by a research grant from the Committee on Research, University of California.

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[3] Silanized Gas Chrom P is now commercially available as Gas Chrom Q. One per cent coated Hi-Eff-8B on Gas Chrom Q is available from Applied Science Laboratories, Inc., State College, Pennsylvania.

flame ionization detector. The gas chromatographic equipment, results of animal experiments, and other experimental details are adequately described in the previous work (*2*). Table 1 gives the retention times

TABLE 1
RELATIVE RETENTION TIMES OF PESTICIDES[a]

Pesticides	Relative retention times (min)	
	190°C (1, 2)[b]	220°C (1, 3)
Aldrin	0.64	0.67
Chlordane	0.64, 1.14, 1.93	0.67, 1.0, 1.67
DDD (TDE)	4.14, 7.7	3.0, 5.0
DDT	2.57, 3.4, 6.64	2.0, 2.3, 4.7
p-p'-DDT	6.64	4.7
Diazinon	0.36	0.33
Dibrom	With solvent	With solvent
Dieldrin	2.29	2.0
Di-Syston	0.54	0.5
Endrin	2.79	2.3
EPN	16.7	10.0
Ethion	3.7	2.7
Heptachlor.	0.57, 1.79	0.5, 1.6
Heptachlor. epoxide	1.54	1.3
Lindane	1.0	1.0
Malathion	1.29	1.0
Methoxychlor.	6.54	6.0
Methyl trithion	0.21	0.33
Morocide	4.25	3.00
Parathion	2.00	1.6
PCNB tetrachlor.	0.54	0.58
Perthane	0.96, 3.4	0.83, 2.5
Thimet	0.5	0.5
Trithion	0.21	0.33
Lindane (R.T.)	1.4	0.6

[a] Electron capture detector was used.

[b] Conditions: (1) 3-foot glass column, Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. (2) Flow rate of carrier gas at 190°C, 15 ml/minute. (3) Flow rate of carrier gas at 220°C, 16.5 ml/minute.

relative to lindane of 23 chlorinated hydrocarbon and organo-phosphorus pesticides and one nitro compound (morocide) obtained at 190°C and

220°C on the Hi-Eff-8B column and in conjunction with an electron capture detector. Figures 1 and 2 are the tracings of typical chromatograms showing the separation of some of the commonly used pesticides.

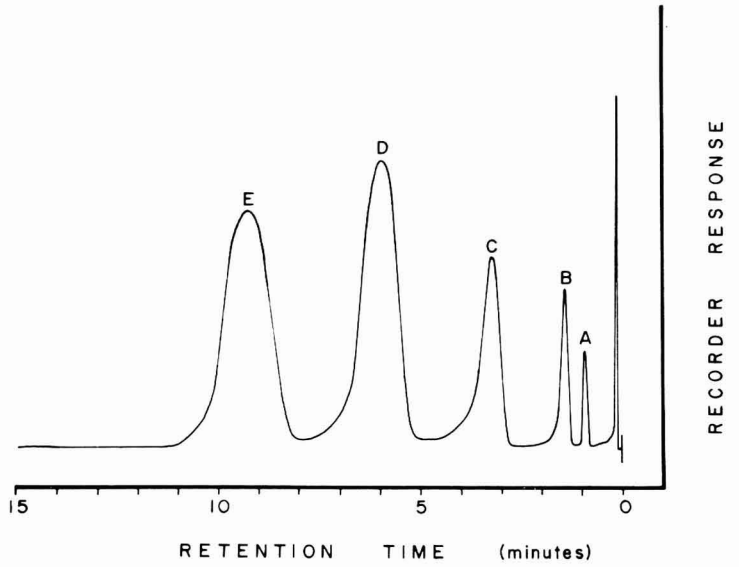

FIG. 1. Separation of pesticides. A, Aldrin, 1 ng; B, Lindane, 0.2 ng; C, Dieldrin, 5 ng; D, Morocide, 20 ng; E, *p-p'*-DDT 25 ng. Conditions: 3-foot glass column ($\frac{1}{8}$ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 190°C; injector 275°C. Detector: Electron capture. Flow rate of carrier gas (nitrogen) 40 ml/minute. Input impedance 10^7 ohms; output sensitivity 1 ×; attenuation 4 ×.

EVALUATION OF TOXIC LEVELS

The animal work done in this study was performed on rats, administering to them LD_{50}, $2 \times LD_{50}$, or other lethal doses which are considerably higher than expected in cases of acute poisoning in man. However, these levels were used only to confirm that in such high doses in animals the poison could be successfully isolated and identified gas chromatographically. It is not implied that such high doses are to be expected in man. Neither is it asserted that observations made on animal experiments could be universally or uniformly applied to man, since laboratory rats may offer resistance or susceptibility to certain drugs and show entirely different pharmacological behavior. But as far as the

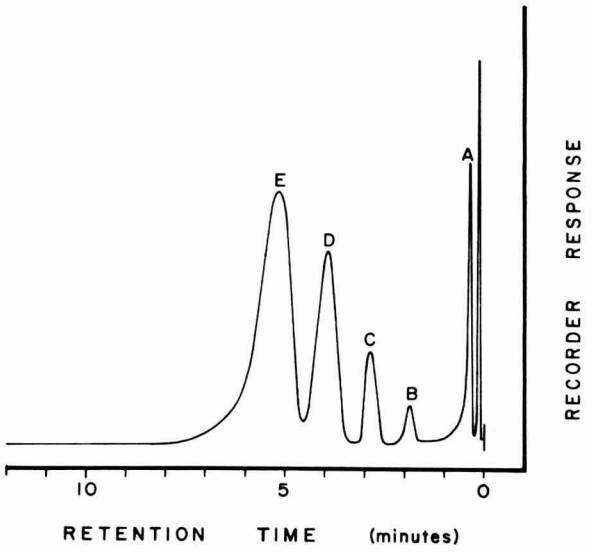

FIG. 2. Separation of pesticides. A, Trithion, 10 ng; B, Malathion, 10 ng; C, Parathion, 20 ng; D, Endrin, 10 ng; E, Ethion, 25 ng. Conditions: 3-foot glass column (⅛ inch o.d.) packed with Hi-Eff-8B 1% on 100/120 mesh silanized Gas Chrom P. Oven temperature 190°C; injector 275°C. Detector: Electron capture. Flow rate of carrier gas (nitrogen) 40 ml/minute. Input impedance 10^7 ohms; output sensitivity 1 ×; atenuation 4 ×.

fate of the drug is concerned, it is assumed that in cases of acute poisoning in man, as with rats, the drug will first pass through the blood before reaching the primary sites of toxic action. Not truly relevant to this study is the actual fate of the drug or its rate of absorption, metabolism, excretion, or detoxication. These factors will vary widely, not only from man to animals, but also from species to species, with strain, age, sex, weight, and various other factors. The emphasis here is purely analytical, from the standpoint of recovery of the drug from blood.

Table 2 is prepared (*1*) to show the expected therapeutic, toxic, and lethal concentrations which may be expected in man by some of the commonly used drugs when ingested at one time. As a matter of convenience, 10 kg is taken as the representative weight of an average child and 70 kg for an average man. As a rough approximation, ten times the higher therapeutic dose is considered as toxic and 25 times as lethal. When calculating the milligrams per kilogram (or, micrograms per milli-

TABLE 2
EXPECTED THERAPEUTIC, TOXIC, AND LETHAL CONCENTRATIONS

Drug	Therapeutic dose (mg)[a]	Toxic dose[b] (10 × therapeutic dose) (mg)	Lethal dose[b] (25 × therapeutic dose) (mg)	mg/kg of body weight, or γ/ml of blood[d]: Conc. in body if therapeutic dose ingested by a 10-kg child[c]	Conc. in body if therapeutic dose ingested by a 70-kg man[c]	Conc. in body if adult toxic dose ingested by a 10-kg child[c]	Conc. in body if adult toxic dose ingested by a 70-kg man[c]	Conc. in body if adult lethal dose ingested by a 10-kg child[c]	Conc. in body if adult lethal dose ingested by a 70-kg man[c]	Approx. detection limits attainable by gas chromatography under conditions employed
ALKALOIDS										
Atropine SO_4	0.3- 1.2	12	30	0.12	0.017	1.2	0.17	3	0.43	5 - 10 ng
Caffeine	100 -500	5000	12500	50	7.14	500	71.4	1250	178.57	5 - 10 ng
Codeine SO_4	15 - 60	600	1500	6	0.86	60	8.6	150	21.43	10 - 25 ng
Morphine	8 - 20	200	500	2	0.286	20	2.86	50	7.14	0.5 - 1 γ
ANTIHISTAMINES										
Carbinoxamine	4	40	100	0.4	0.057	4	0.57	10	1.43	0.25- 1 γ
Chlorpheniramine	2 - 8	80	200	0.8	0.114	8	1.14	20	2.86	0.25- 0.5 γ
Diphenhydramine	25 - 50	500	1250	5	0.714	50	7.14	125	17.86	0.1 - 0.25 γ
Dramamine	50 -100	1000	2500	10	1.43	100	14.3	250	35.71	0.25- 0.5 γ
Tripelennamine	50 -150	1500	3750	15	2.14	150	21.4	375	53.58	0.25- 0.5 γ
BARBITURATES										
Amobarbital	20 -500	5000	12000	50	7.14	500	71.4	1250	178.57	10 - 25 ng
Pentobarbital	30 -500	5000	12000	50	7.14	500	71.4	1250	178.57	50 -100 ng
Phenobarbital	15 -100	1000	2500	10	1.43	100	14.3	250	35.71	0.25- 0.5 γ
Secobarbital	100 -200	2000	5000	20	2.86	200	28.6	500	71.43	10 - 50 ng
TRANQUILIZERS										
Librium	10 - 20	200	500	2	0.286	20	2.86	50	7.14	0.2 - 0.5 γ
Meprobamate	200 -800	8000	20,000	80	11.43	800	114.3	2000	285.71	25 - 50 ng
Thioridazine	30 -800	8000	20,000	80	11.43	800	114.3	2000	285.71	0.25- 1 γ
SYMPATHOMIMETIC AMINES										
Amphetamine SO_4	2.5- 40	400	1000	4	0.57	40	5.7	100	14.3	0.25- 1 γ
Ephedrine	25 - 50	500	1250	5	0.714	50	7.14	125	17.86	0.5 - 2 γ
Metamphetamine	2.5- 10	100	250	1	0.143	10	1.43	25	3.57	0.25- 1 γ

[a] Obtained from the Merck Index, 6th edition, 1960. All oral doses.
[b] These are rough approximations calculated on higher therapeutic dosage.
[c] Assuming the drug is uniformly distributed throughout the body.
[d] Assuming that 1 ml of blood weighs 1 g.

liter of blood) of the drug found, it is assumed that the drug is uniformly distributed throughout the body. As can be seen from Table 2, all drugs reported are within the limits of gas chromatographic detection if ingested by a 10-kg child at the higher therapeutic levels. Obviously, toxic and lethal doses would be much easier to detect, as they would be present from 10 to 25 times the therapeutic quantity. Out of the four alkaloidal drugs reported—atropine, caffeine, codeine, and morphine—on administration of one adult therapeutic dose, atropine will be present in concentration of 0.12 mg/kg (or, 0.12 γ/ml of blood, assuming 1 ml of blood weighs 1 g), caffeine 50 mg/kg, codeine 6 mg/kg, and morphine 2 mg/kg in a 10-kg child. All of these quantities could easily be detected from 0.5 ml of blood by the method described here. Similar considerations apply to other compounds reported in Table 2. It would be easily possible to detect the drugs listed here, as the therapeutic doses are all higher than that of atropine, as high as 500 mg for amobarbital and pentobarbital, and 800 mg for meprobamate and thioridazine (Mellaril). With reference to the drugs having lower therapeutic levels, carbinoxamine has a therapeutic dose of 4 mg, amphetamine 40 mg, and metamphetamine 10 mg.

Considering these doses are ingested by a 70-kg man, the therapeutic, toxic, and lethal concentrations will be reduced to one seventh of that of a 10-kg child. Here again the toxic and lethal concentrations will be within the limits of detection on the gas chromatographic column employed. Some of the doses at therapeutic levels could also be detected in 0.5 ml of blood, except for compounds such as morphine and those others which have longer retention times and require larger samples to be gas chromatographed. Also undetected at therapeutic levels will be those drugs which would either not be present due to their early removal from blood, or present in minute quantities falling outside the detection limits of this procedure.

CONCLUSION

For a systematic toxicological analysis of a general unknown, there are several factors which are to be taken into consideration. When one is dealing with cases of acute poisoning and is concerned with the systematic applications of gas chromatography in toxicology, the subject of this work, the following points are of great importance:

(a) The choice of the material should be such that it will have in it the toxic substance which is being investigated.

(b) The extraction scheme should be able to extract the toxic material free from biological contamination. Hence the desirability of a universally applicable method which could extract a wide variety of compounds.

(c) A single-column, single-detector gas chromatographic system which could separate and tentatively identify all the possible toxic compounds on the basis of different retention values, preferably obtained under one set of operating conditions.

(d) The entire analysis is to be done in minimal time, with the least amount of material and manipulation.

As discussed earlier (*3*), blood is an ideal biological fluid, especially in cases of acute poisoning, from which to extract the poison. It is readily available from the dead body as well as from patients who have ingested acute doses of poison. It also has fewer impurities as compared with most other biological material. The extraction procedure used here is generally applicable, as it is simple and rapid, and is capable of extracting a wide variety of substances from blood. No purification of the acetone-ether extract is necessary.

Gas chromatography has been used as the analytical tool for separation and identification of these compounds. It has many advantages over other procedures because of its speed, sensitivity, and versatility. It has been shown here that the alkaloids, antihistamines, barbiturates, pesticides, and the tranquilizers could be chromatographed and identified on a single 3-foot long glass column packed with 1% Hi-Eff-8B on 100/120 mesh silanized Gas Chrom P. The only exception was with sympathomimetic amines, for which a different column was needed. A flame ionization detector was used with all the compounds except the pesticides, where an electron capture detector was found more useful.

SUMMARY

The relative retention times of 24 pesticides obtained at 190° and 220°C on the 1% Hi-Eff-8B column are recorded. An evaluation of toxic levels and how they can be detected under this systematic scheme is described. By arbitrarily taking ten times the higher therapeutic dose as toxic and twenty-five times as lethal, it is shown that most of the drugs and poisons studied under this scheme could be detected from 0.5 ml of blood when they are ingested in toxic or lethal concentrations. It is demonstrated that under the gas chromatographic conditions used, this quantity of blood will also be sufficient to detect therapeutic levels of many drugs. All the compounds are gas chromatographed on one single column: the alkaloids,

antihistamines, barbiturates, and tranquilizers in conjunction with a flame ionization detector, and the pesticides with an electron capture detector. A different column was used for the sympathomimetic amines.

ACKNOWLEDGMENT

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A Semiautomated Method and Apparatus for Micromethoxyl Determination

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Although the apparatus and procedure of Steyermark (*6, 7*) for determination of alkoxyl groups on a micro scale give good results in experienced hands, considerable difficulty has been encountered with this analysis in student hands. The major sources of trouble are (1) the great fragility and difficulty of repair of the equipment; (2) difficulty of adhering to an exact time cycle; (3) loss of nonvolatile liquids due to a tendency to creep up the sides of the platinum weighing boat; (4) difficulty of cleaning the scrubber; and (5) some tendency for absorption of alkyl iodide by the cadmium sulfate thiosulfate scrubber solution. During work to minimize sources of trouble, another change was found possible which simplified the procedure somewhat, namely conditioning the HI by heating overnight in the reaction flasks in a slow stream of carbon dioxide. The flasks so treated can be used for 10 to 12 alkoxyl analyses.

EXPERIMENTAL PROCEDURE

Fabrication of the apparatus. The reaction flasks are standard 7-ml Elek alkoxyl flasks obtainable from A. H. Thomas or Scientific Glass Apparatus Co. The second has a male 7/15 joint sealed on its gas inlet tube and bent down so as to connect to the mouth of the first. The scrubber consists of a micro condenser with a jacket approximately 75 mm long sealed to a 20-cm length of heavy wall capillary tubing of 3 mm i.d. and 9 mm o.d. A 12/30 female joint having a 10-mm o.d. tubing 75 mm long is sealed on the other end, and the capillary tube is bent into an S shape as shown in Fig. 1. The spiral is easily made by sealing a 2-mm rod to the end of a 4-mm rod and then coiling the small rod around the

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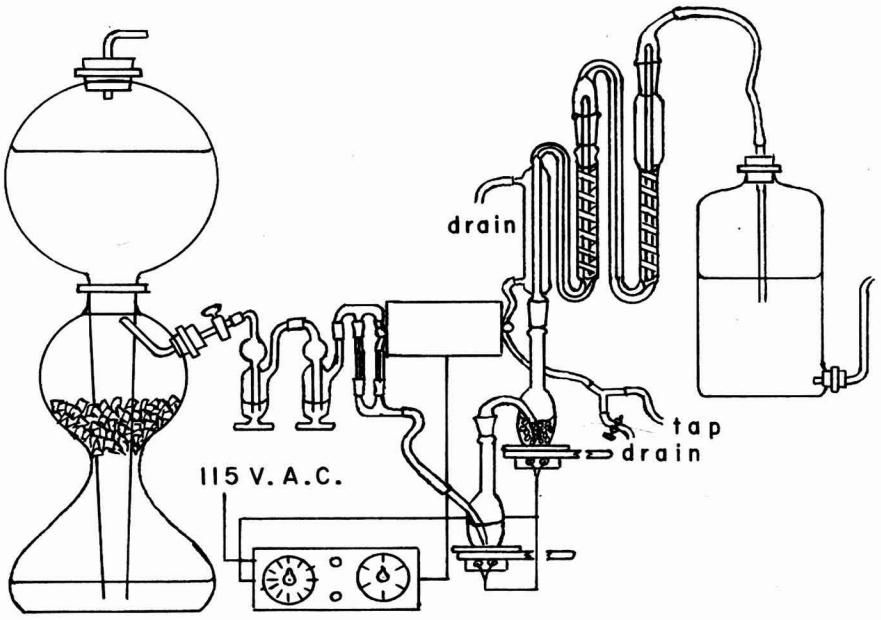

FIG. 1. Semiautomated alkoxyl apparatus.

larger one while heating the former in an oxygen flame. For the absorber a 12/30 male joint is sealed to one end of a 30-cm length of heavy wall capillary tubing and a 70-mm length of 10 mm o.d. tubing to the other end. A 12/18 female joint having a 16-mm o.d. tubing 60 mm long is then sealed to the 10-mm tube. The spiral is the same as that used in the scrubber except that the center rod is longer so that it reaches slightly above the top joint. The cap is a 12/18 male joint reduced to a 5-mm tube for connection by rubber tubing to the Mariotte bottle. Micro heaters operating on 115 V a.c. are commercially available (*3, 7*). It was found possible, however, to make satisfactory heaters by flanging the top edge of a 2-inch aluminum moisture dish over a 2-inch iron ring, insulating the inside with heavy asbestos paper and $\frac{1}{8}$-inch asbestos board, and connecting a coil of 4 feet of No. 26 Nichrome wire to two insulated binding posts. These heaters are powered by an RT-204 Stancor rectifier transformer tapped at 23 V to produce approximately 30 W. The transformer is enclosed in the timer box but is not shown in the diagram. The main disadvantage of these open coil heaters is the frequent coil replacement necessitated by attack of HI fumes. For the timer, two

Mark-Time mechanical time switches, models 74702 for a 30-minute electrocock interval and 74703 for a 60-minute heater interval, are mounted in the 5 × 6-inch face of a 4 × 5 × 6-inch box. They are wired to their respective outlets as shown in the wiring diagram of Fig. 2. The 115 V a.c. supply enters through a 5-A fuse and then powers a

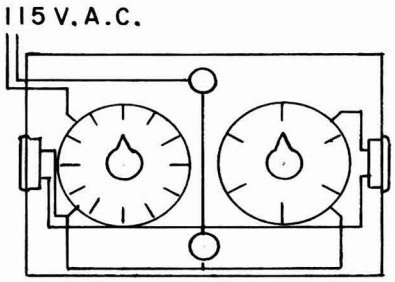

FIG. 2. Timer.

pilot light and the two receptacles. The other side of the circuit passes through the 60-minute timer, which is wired so as to power the heater outlet, the second timer, and the pilot light for 60 minutes while it counts down to zero. It then switches them off and can therefore be used as the on-off switch for the equipment. The second timer is wired to the receptacle for the electrocock operation so that the latter is off for 30 minutes while the timer counts to zero and then is switched on. The electrocock consists of a 115-V a.c. solenoid with an approximately 2 × 2-inch frame and 26-ohm coil mounted in a 3 × 4 × 5-inch box well ventilated with 8 half-inch holes in both 4 × 5-inch top and bottom. Figure 3

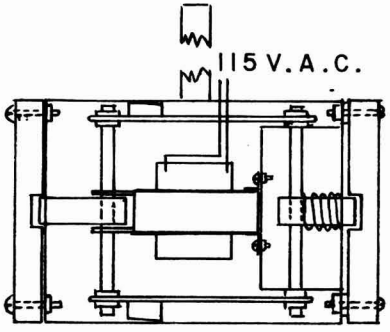

FIG. 3. Electrocock.

shows the construction. The solenoid core moves a rectangular frame consisting of two $\frac{3}{16}$-inch brass rods bolted to two aluminum side pieces with edges bent at right angles (not shown) to provide stiffness and also hold two rubber stoppers wedged between the frame and the case to reduce chatter. The $\frac{3}{16}$-inch rods carry two $\frac{1}{2}$-inch round aluminum rods with V-shaped outer ends to pinch off the rubber tubing. A half-inch aluminum rod with a $\frac{5}{8}$-inch notch filed to about the middle is bolted to each end of the box to serve as the outer jaw for the tubing closure. A compression spring is placed around the aluminum rod inner jaw on the right-hand end to keep the right-hand tubing (condenser water) normally open and the left-hand tubing (carbon dioxide) normally closed. Spacers are placed between the box and the right-hand notched aluminum rod so that adjustment can be made for the thickness of rubber tubing. A $\frac{3}{16}$-inch i.d. tubing of $\frac{3}{64}$-inch wall thickness has been found most satisfactory. For minimum hum and heating the spacers should be adjusted to allow the solenoid core to seat completely when the rubber tubing is just closed off. A half-inch aluminum rod attached to the bottom of the box provides a convenient means of support.

Standard procedure. Samples were taken so as to require about 40 ml of 0.01 *N* thiosulfate in the titration and were weighed to the nearest 0.001 mg on an M-5 Mettler microbalance. They were weighed into the smaller cup of a No. 5 gelatin capsule and capped with the larger cup, thus reducing the possibility of the liquid creeping in between the cups by capillary action. With very volatile liquids such as ethyl acetate a slow loss of weight from the capsule was observed, but this could be reduced and allowed for by removing the capsule from the balance, quickly dropping it into the reaction flask, and immediately replacing the flask in the set-up. Eastman pure materials were used without purification, and the methyl cellulose was kindly supplied by the Southern Utilization Research Branch of the Department of Agriculture at New Orleans.

The apparatus is shown in Fig. 1 with an enlargement of the timer in Fig. 2 and the electrocock in Fig. 3. Carbon dioxide is used to sweep the alkyl iodide through the scrubber and absorption tubes. It is generated from limestone and 20% HCl in a Kipp generator and is purified by passage through a 10% silver nitrate solution followed by concentrated sulfuric acid. It then passes through two capillary tubes consisting of sections from broken thermometers carefully adjusted as to length so

that the first, which is permanently in the line of gas flow, allows passage of about one bubble of gas in 2 seconds through the apparatus with all solutions in place and the Mariotte tube lowered to a horizontal position. The second capillary is of a length which will allow two bubbles of gas per second to pass through the equipment when both capillaries are placed in the line of gas flow upon activation of the electrocock. The first reaction flask contains 3 ml of a solution made by dissolving 25 g of C.P. phenol in 100 ml of 57% HI (Fisher methoxyl grade), and the second contains 2 ml of 57% HI and 3 mm solid glass beads to the liquid level. The purpose of the beads is to slow down the rise of gas bubbles in the flask and increase their contact with the hot HI solution. Steyermark uses glass wool for this purpose, but beads serve just as well and are easier to remove for cleaning. Various scrubber solutions were tried, but distilled water (*4, 5, 8*) was found satisfactory with all compounds analyzed. Two ml fills the scrubber to the top of the spiral, and it is replaced with each analysis. The absorber contains 5 ml of a solution made by dissolving 50 g of anhydrous C.P. sodium acetate in 450 ml of glacial C.P. acetic acid. Seven drops of C.P. bromine are added to the absorber immediately before use.

For very volatile liquids the two reaction flasks (*2, 6*) shown in Fig. 1 are necessary. For nonvolatile liquids and solids the second reaction flask can be eliminated and the first connected directly to the condenser. Before any analyses are run the HI solutions must be conditioned by heating overnight with a slow stream of carbon dioxide passing through. This is accomplished by plugging the heater cord into a 115 V a.c. outlet or inserting pins into holes drilled in the dials of the two timers to hold them off zero if low voltage heaters are operated from a transformer in the timer. Before introducing a capsule for a determination the heaters should be warmed either from a previous determination or a 15-minute warm-up period with slow carbon dioxide flow. The ground joint of each reaction flask is sealed with a drop of the HI solution removed from the flask on a clean stirring rod, and the other ground joints are sealed with drops of distilled water. The two timers are set for their maximum times, one hour for the heater timer and one-half hour for the electrocock timer, and the carbon dioxide flow is started. From this point the cycle is automatic. At the end of half an hour the electrocock is activated, which stops the condenser water and increases the carbon dioxide flow

for the sweep out; and at the end of one hour the heaters are turned off and the electrocock is deactivated, which reduces the carbon dioxide flow and turns on the condenser water.

At the end of the sweep-out the cap is removed from the absorber and the spiral is carefully lifted out and rinsed with distilled water into an iodine flask containing 5 ml of a solution made by dissolving 58 g of C.P. sodium acetate trihydrate in water and diluting to 200 ml. The absorber is then removed, emptied into the iodine flask, and then rinsed with distilled water and emptied several times, finally running water through from the male joint to rinse out the capillary tube thoroughly. Seven drops of formic acid are now added and the flask is stoppered and swirled for several minutes after all bromine color has disappeared. The stopper and walls of the flask are next rinsed and 3 ml of 10% H_2SO_4 and 2 ml of 10% KI (rendered colorless by dropwise addition of 0.01 *N* thiosulfate) are added, and the iodine is titrated with standardized 0.01 *N* thiosulfate using starch, added near the end of the titration, as the indicator. The thiosulfate is standardized once a month against potassium biiodate and kept over chloroform as a preservative.

DISCUSSION AND RESULTS

Initially, samples of nonvolatile liquids were weighed in platinum combustion boats, but low and erratic results were often obtained, probably due to the tendency of the liquids to creep up the sides of the boat. Solids gave correct results. The use of gelatin capsules was finally standardized upon for solids and volatile as well as nonvolatile liquids. A number 5 size capsule was of sufficient size for the samples used and had the advantage of a lower blank and introducing less gelatin into the reaction solution than larger capsules (*1*). This was important if the same HI-phenol solution was to be reused for a number of analyses. Upon use of gelatin capsules for the sample, the first half hour of the Steyermark $1\frac{1}{2}$-hour cycle, in which the sample reacts with HI-phenol at room temperature, is ineffective since the capsule floats on top of the HI and does not dissolve until heat is applied. This half hour was therefore eliminated and warm HI-phenol was used at the start and heated for one hour. Instead of conditioning the HI in bulk by boiling in a slow stream of carbon dioxide (*7*), it was found more convenient for intermittent analyses to condition the HI or a stock solution of phenol in HI overnight directly in the reaction flask and to reuse the same solution for 10-12

analyses before it became too gummy for further use. It can be calculated that dilution of the HI by this reuse is negligible. The HI so obtained usually gives a perfect blank, and the No. 5 capsules, weighing approximately 0.3 g, a blank of about 0.6 ml of 0.01 *N* thiosulfate, which is proportional to the blanks obtained on larger gelatin capsules by others (*1*). An unexplained variation of gelatin blanks is being investigated.

Table 1 gives the results obtained on less volatile liquids with the standard apparatus using a single reaction flask, a one-hour time cycle, and distilled water in the scrubber. Errors generally run below 1%.

TABLE 1
NONVOLATILE LIQUIDS ANALYZED WITH ONE REACTION FLASK

Compound	% Alkoxyl		% Error
	Theoretical	Experimental	
Methyl benzoate	22.80	22.6	0.9
		22.8	0.0
		22.9	0.4
p-Methyl anisole	25.40	25.2	0.8
		25.1	1.2
		25.6	0.8
Dimethyl phthalate	31.96	32.0	0.1
		31.9	0.2
		31.8	0.5
Ethyl benzoate	30.01	29.7	1.0
		30.0	0.0
		29.8	0.7
p-Dimethoxy benzene[a]	44.92	45.0	0.2
		45.4	1.2
		44.5	0.9

[a] Solid compound.

Table 2 shows the effect of various scrubber solutions. With the compounds analyzed most of the scrubber solutions tested appear satisfactory, and distilled water was chosen as the most convenient. With 5% cadmium sulfate-5% sodium thiosulfate low and erratic results were sometimes obtained.

Table 3 illustrates the behavior of the equipment with very volatile liquids and with methoxyl compounds which are difficult to cleave. With ethyl acetate and ethyl ether a single reaction flask gave low results. With two flasks but no glass beads in the second the results improved

TABLE 2
EFFECT OF VARIOUS SCRUBBER SOLUTIONS

Compound	Theor. % alkoxyl	2% thiosulfate Satd. NaCl	2% thiosulfate Satd. $CaCl_2$	25 % NaAc	15 % Red P	Water
o-Methoxy benzoic acid	20.38		20.3	20.4		20.3
			20.3	20.2		20.6
			20.5	20.4		20.3
Vanillin	20.39	19.1	20.4		20.4	20.3
		20.1	20.4		20.3	20.1
		20.5	20.1		20.3	20.1
Anisic acid	20.40		20.5	20.4		
			20.4	21.0		

slightly, and the addition of glass beads to the second flask to improve liquid vapor contact produced correct results using 57% HI. Methyl cellulose gave low results with 1-hour heating but results that agreed with those of the Southern Regional Research Laboratory upon heating for 2 hours in 57% HI-phenol.

TABLE 3
MODIFICATION OF REACTION CYCLE

Compound	Theor. % alkoxyl	Single reaction flask 1 hour	Single reaction flask 2 hours	Two reaction flasks No beads	Two reaction flasks Beads
Ethyl acetate	51.15	46.4	44.5	48.1	50.6
		47.0		48.1	51.2
Methyl cellulose	44.20[a]	40.9	44.5		

[a] Southern Regional Research Laboratory analysis.

CONCLUSION

The apparatus and procedure developed in this work provides a rapid and uniform method for analysis of most nonvolatile liquid or solid compounds containing alkoxy groups. It allows easy modification for volatile liquids or compounds difficult to cleave with HI, and in extreme cases a more concentrated HI solution could be used. The time required is usually 1 hour, but a maximum of 2 hours should be sufficient for the most difficult compounds, especially if HI of 1.95 specific gravity is used.

SUMMARY

The apparatus and procedure of Steyermark for determination of alkoxyl groups on a micro scale has been modified and semiautomated so as to yield results of research accuracy in student hands. The major modifications consist of redesign of the equipment to provide greater durability, easier construction and repair and better scrubbing action without increasing the internal gas volume appreciably. Also the time cycle has been automatically controlled and reduced from an hour and a half to one hour.

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Book Reviews

Encyclopedia of Industrial Chemical Analysis. Vol. 2: General Techniques, F–O (xi + 769 pp.); **Vol. 3: General Techniques, P–Z** (xi + 845 pp.). Wiley (Interscience), New York, 1966. Each volume, $45.00 ($35.00 by subscription).

The second and third volumes of the projected fifteen-volume set—ambitious in concept, meritorious in performance—are now available. This encyclopedia, concerned with the industrial applications of analytical techniques in contrast to others stressing purely scientific interest, is filling a gap that has long existed. Like the first volume, these, concluding the sections of general techniques are written clearly and concisely by well-known authorities and are amply documented. Tables of data are plentiful and complete. In Volume 2, the section on flame photometry, for example, contains tables of the detection limits for the elements excited in various diffusion flames and also a listing of prism and grating instruments. Another excellent innovation is a comprehensive discussion of laboratory safety including suggested forms for accident reports and investigations.

It is difficult to review a set of this type. No one individual can have an equal interest in every section. In volume two, that on micro- and semi-microanalysis deserves special mention. The section on microscopy is an excellent text book per se, and the other chapters are equally well done. In volume three, paper and thin-layer chromatography are particularly well handled. The section on temperature measurements and control is notable for its profusion of photographs.

Both books are amply illustrated with well-reproduced photographs and line-drawings. There is a remarkable freedom from misprints and errors in facts especially in these second and third volumes. As additions to the Encyclopedia, both books fully live up to the standard of the first and augur well for the future.

DAVID B. SABINE, *U.S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Methods in Chemical and Mineral Microscopy. By ESSAM E. EL-HINNAWI. Elsevier, Amsterdam, 1966. ix + 222 pp. $16.50.

Books on microscopy range from treatises on the theory of optics to manuals on the use of the microscope. The present volume is one of the practical ones. It assumes some acquantance with the theory of light and the optical properties of crystals, and the reader is expected to be familiar with the microscope. However, the level of

these prerequisites is not high, and very explicit instructions are given for numerous applications of the microscope.

The first chapter gives directions for preparing samples, in the form of thin sections, crushed mineral grains, or crystals grown on slides. Chapter 2 treats the determination and description of particle shape and size. Five chapters are allotted to the use of the polarizing microscope in studying such properties as refractive index, absorption, pleochroism, extinction, birefringence, interference figures, optic angle, and dispersion. Auxiliary equipment is described in successive chapters on the spindle stage, the universal stage, and the hot stage. The final chapter outlines the principles and applications of phase contrast microscopy.

This book is especially recommended to the inexperienced microscopist for the highly detailed instructions given in several sections. The writing shifts occasionally to the imperative mood, but the cookbook style may actually enhance its usefulness. An especially valuable addition is a list of 267 publications, with complete titles. There are some instances of careless notation, such as confusion between $\Sigma(nd/n)$ and $\Sigma nd/\Sigma n$. The reviewer's copy contained a curious mixture of glossy and flat-finish pages.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky*

Electron Microscopy of Thin Crystals. By P. B. HIRSCH, A. HOWIE, R. B. NICHOLSON, D. W. PASHLEY, AND M. J. WHELAN. Butterworth, Washington, D.C., 1965. 560 pp. $29.50.

This book is based on a series of twenty lectures and numerous examples and demonstrations on the electron microscope given at a Summer School on the electron microscopy of thin crystals in Cambridge, England in July 1963. A knowledge of basic electron microscopy, crystallography, classical optics, and quantum mechanics is assumed so that the brief treatment accorded the principles of the electron microscope in the first chapter is understandable. In this chapter, emphasis is placed on the use of the double condenser lens and accuracy of selected area diffraction.

The second chapter, dealing with specimen preparation techniques, considers only those methods involved in preparing thin foils either by reducing the thickness of bulk material or by deposition of thin foils. Methods for surface replication are not referred to at all. A variety of electropolishing techniques are described in reasonably good detail, with less emphasis on chemical, mechanical, and ion bombardment methods for preparing thin foils from bulk material. The discussion of techniques for the deposition of thin foils by vacuum evaporation and other methods is also quite brief. However, the short treatment accorded specimen preparation technology in this chapter is entirely compensated for in Appendix 1, which lists details of 234 conventional and jet electropolishing techniques, 10 chemical reaction techniques, 16 cleavage techniques, 53 deposition techniques, and five miscellaneous techniques as well as 272 references on methodology.

Major emphasis of the book as a whole is placed on the interpretation of transmission electron micrographs and electron diffraction patterns based on a thorough

understanding of the factors which determine the intensities of Bragg diffracted beams. Thus the geometry of electron diffraction patterns discussed in Chapters 5 and 6 is preceded by an exposition of the kinematical theory of electron diffraction in Chapter 4, presented primarily in the mathematical shorthand of the physicist and liberally diagrammed for clarity of presentation. In Chapter 5, methods for indexing the sharp spot diffraction patterns of infinite crystals, based on the reciprocal lattice construction developed in Chapter 4, are presented with appropriate examples, and, in Chapter 6, the causes of deviations from a sharp spot pattern are examined. The material in the chapters on electron diffraction is augmented by tables of atomic scattering amplitudes for electrons, electron wavelength data, and extinction distances in the appendix. Examples of indexed diffraction spot patterns and Kikuchi lines are also given.

Both the kinematical and dynamic theories of image contrast are presented in detail as an introduction to the interpretation of transmitted images. The former deals with the contribution of diffracted electrons to image contrast, and the latter is concerned with the contribution of electron scattering and the dynamic interaction of the incident and scattered waves to image contrast. The theories are applied to the interpretation of images produced by dislocations, stacking faults, slip traces, particles of a second phase and other crystal defects, illustrated with numerous excellent electron micrographs. A chapter is devoted to Lorentz microscopy of magnetic domains in ferromagnetic materials, a relatively new application of the electron microscope. A chapter on miscellaneous applications goes into the measurement of foil thickness, dislocation density, particle size and density, and stacking fault energy. The final chapter discusses the phenomenon of inelastic scattering of electrons by crystals and its contribution to image contrast.

In general, this book has achieved its stated purpose of being selective rather than comprehensive in the detailed subject matter. It is designed for the advanced student or professional who is already immersed in the electron microscopy of thin crystals.

EMIL BORYSKO, *Research Division, Ethicon, Inc., Somerville, New Jersey*

Techniques in Protein Chemistry. Second Edition. By J. LEGGETT BAILEY. Elsevier Publishing Co., Amsterdam, London, New York, 1967. xiv + 406 pp. $20.00.

Microanalytical techniques are of value in many disciplines. This is particularly true of biochemistry where available material is frequently limited. Bailey's "Techniques in Protein Chemistry" is a good example. The subject is biochemical but most of the treatment is micro. This book is a revised, enlarged edition of a well-known work. Gel filtration in various media and electrophoresis in polyacrylamide gel are given detailed treatment. Ion exchange and paper chromatography, and high voltage electrophoresis for the determination of amino acids and peptides are discussed extensively. Cleavage of disulfide bonds, selective cleavage and N- and C- terminal sequence are covered in detail along with zone electrophoresis and column chromatography of proteins. There is also a chapter devoted to miscellaneous analytical methods, primarily micro, including Goa's microbiuret method for the quantitative determination

of protein. The last chapter is a fascinating account of peptide synthesis. This handbook, designed for bench-top use, will be of value to both biochemist and microanalyst.

DAVID B. SABINE, *U.S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Applied Capillary Microscopy; The Role of Microorganisms in the Formation of Iron-Manganese Deposits. By B. V. PERFIL'EV, D. R. GABE, A. M. GAL'PERINA, V. A. RABINOVICH, A. A. SAPOTNITSKII, E. E. SHERMAN, AND E. P. TROSHANOV. Translated from the Russian by F. L. SINCLAIR, Consultants Bureau, New York, 1965. 122 pp. $22.50.

Industrial microbiology as applied to foods and medicines has developed very well during the last few decades; however, geomicrobiology has not developed as well, primarily because the investigative techniques were not available. The capillary microscopical method developed by B.V. Perfil'ev and D.R. Gabe has done a great deal to overcome this problem, and the inventors were awarded a Lenin Prize for this accomplishment. The technique involves setting up of a "microbial landscape" which permits the researcher to observe microflora under conditions which resemble their environment in the pores and fissures of rocks. One such "landscape," for instance, duplicates sedimentation and diagenesis in fresh-water lakes. Using this method, Perfil'ev has discovered new orders, families, and genera of microorganisms including *Metallogenium* and *Gaulococcus,* which are able to concentrate iron and manganese in mud. More specifically, the method calls for the construction of a capillary peloscope, a set of flat glass capillaries each with five or ten channels running their length. A slit peloscope may be constructed from a pair of broad thin glass strips at 0.1 mm apart. The peloscope is fastened to a graduated support and inserted vertically into the mud. The inventors have also developed micro methods for the determination of pH, redox potentials, and dissolved oxygen of the various "landscapes." Separate bands of iron, manganese, and other elements are deposited in the "landscape," by oxidizing bacteria at pH values different from pure chemical methods. The bacterial depositions are seen as the first stage of sedimentary ore formation in which the bacteria act as biological catalysts, first accelerating the oxidative process of elements and then converting them to the reduced state.

This special research report was originally published by Nauka in Moscow (1964) for the Savarenskii Laboratory of Hydrogeological Problems of the Academy of Sciences (USSR). It consists of nine collected papers on the capillary method and related techniques as applied to the role of microorganisms in the formation of ore deposits. There are 106 Russian and 39 non-Russian literature references cited.

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Trace Analysis: Physical Methods. Edited by GEORGE H. MORRISON. Wiley (Interscience) Publishers, New York, 1965. xx + 582 pp. $16.00.

Within the last few years the world of analytical chemistry has been substantially shaken by the emergence of a relatively new and fascinating field of endeavor. The realm of trace analysis and the importance placed on it by government, industry, and the academia have motivated several scholars to precipitate their thoughts and ideas into a compendium of theory and knowledge. One such treatise, "Trace Analysis: Physical Methods," edited by George H. Morrison, has truly been an outgrowth of this interest.

Basically, this volume comprises articles written by noted authors in the field, covering the various aspects of the physical methods of trace analysis. The format used is initially proposed by the editor in the introduction. The various chapters proceed to detail the "General Aspects of Trace Analysis," "The Role of Trace Impurities in the Physical Sciences," "Trace Elements in Biological Systems," and "Separation and Preconcentration." These are followed by chapters on the use of instrumentation in trace analysis: "Spectrophotometry and Fluorometry," "Emission Spectroscopy," "Flame Emission and Absorption Spectroscopy," "X-Ray Emission Spectrography," neutron activation or "Nuclear Methods," "Electrochemical Methods," "Mass Spectrometry," and "Nonspecific Methods for the Analysis of Solids."

Actually, this work can be divided into two basic sections. The first section covering chapters one through four are concerned primarily with a discussion of the essential importance of trace analysis, the problems involving the resolution of accuracy and precision and the importance of selectivity of technique. The emergence and potential significance of trace impurities in the physical and biological sciences are discussed in detail. Finally, Chapter 4 discusses the physical handling of samples in preparation for determining trace impurities and elements. Included in this chapter is an expanded discussion of the problems and pitfalls encountered by the analyst such as loss of trace elements, contamination of the matrices and complexity of techniques.

The second section, Chapters 5-12, detail the theory, apparatus, and scope of the method in question. Several of the chapters are more on a survey level rather than an in-depth approach, but this lends more to the understanding of the particular area involved. The chapters on "Nuclear Methods," "Electrochemical Methods," and "Nonspecific Methods for the Analysis of Solids," are examples of this type. Each author's approach tends to be personally oriented, but in general, the discussions are brimming with authoritative practical experience.

Any volume whose scope is universally inclusive tends to sacrifice depth communication. This book is no exception. However, the authors possibly feel that with the rise of this intriguingly new field depth communication must be sacrificed in order to arouse interest and insight. Truly, Dr. Morrison has achieved this. The compilation of this volume may well bring together those scientists and students whose indifference may arise from ignorance to the realization of the significance and scope of trace analysis.

RONALD E. SMITH, *Shell Chemical Company, Princeton, New Jersey*

Methods of Biochemical Analysis. Volume 14. Edited by DAVID GLICK. Wiley (Interscience), New York, 1966. ix + 562 pp. $15.00.

Volume 14 of "Methods of Biochemical Analyses" lives up to the standards of the previous publications in this series, fulfilling a need which has become well established over the years. The topics covered in this book are: Methods for Estimating Magnesium in Biological Material, Microbiological Assay of Vitamin B_{12}, Fluorimetric Analysis of Corticoids, Preparation and Analysis of Basic Proteins, Determination of Nucleic Acids, Determination of Amino Acids by Ion Exchange Chromatography, Separation and Determination of Amino Acids and Peptides by Gas-Liquid Chromatography, Newer Developments in Determination of Bile Acids and Steroids by Gas Chromatography, and Gel Electrophoresis in Buffers Containing Urea. The authors are recognized authorities in their various fields and each chapter is followed by an extensive bibliography.

The organization of each section follows a general plan which covers a background discussion and a critical evaluation of the methods of approach. Experimental details of the method (or methods) recommended are given in details sufficient for the performance of the analysis.

The editor's stated policy has been "to encourage individual expression in these presentations," so there is a variety of style and point of view, but they are uniformly well written. Typographically, the book is excellent, and the illustrations, tables, charts, and line-drawings are well reproduced. A complete index together with an admirable cumulative index for the previous volumes, covering both author and subject, add greatly to its utility.

DAVID B. SABINE, *U.S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Pratique D'Analyse Organique Colorimetrique (The Practice of Colorimetric Organic Analysis). By M. PESEZ, P. POIRIER, AND J. BARTOS. Mason et Cie, Paris, 1966. 351 pp. 70 Francs (in French).

This book provides a good guide for the colorimetric determination of many organic functional groups. Several methods are given for each group, and when interferences may be encountered, this is stated, but no methods given for eliminating the interferences.

In many instances a value "μg for $D = 0.3$" is given, this value being the sample size necessary to obtain an optical density of 0.3. For some reactions the wavelength for maximum adsorption is also given.

Alcohols, amines, nitro- and nitrile compounds, aldehydes and ketones, and steroids are among the groups covered.

Colorimetric determinations of intermediate derivatives are not given.

A number of tables are provided giving physical constants, buffer solutions, pH indicators, depression of temperatures for compounds in ice and water, and a good subject index as well as an alphabetical index. A valuable addition is an appendix describing the preparation of 26 reagents not readily available commercially.

The format is pleasing and, for the bilinguist, easily read.

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Treatise on Analytical Chemistry. Part II: Analytical Chemistry of Inorganic and Organic Compounds. Vol. 13, Edited by I. M. KOLTHOFF, *University of Minnesota* and PHILIP J. ELVING, *University of Michigan.* Wiley (Interscience), New York, 1966. xxi + 528 pp. $20.00.

Volume 13 is one of four volumes in Section B (Organic Analysis) of Part II of the Treatise and is devoted to functional groups. It is preceded by Volumes 11 and 12, and the four volumes were written with the cooperation of E. W. D. Huffman and John Mitchell, Jr., who are recognized for their broad knowledge in the area of organic analysis.

Eight authors or co-authors contributed to Volume 13, each an authority in his field. There are seven chapters, the first is by John Mitchell, Jr. (31 pp., 65 refs.) and begins with a good introduction on functional groups, including tables on their classifications, rate studies on saponification of esters, analytical data on esters, and enzyme analytical reactions. Then follows a discussion on instrumental methods with sections devoted to ultraviolet-visible spectroscopy, infrared and Raman spectroscopy, nuclear magnetic resonance spectroscopy, and X-ray diffraction. The chapter concludes with a reference table to succeeding chapters, in this volume and in Volume 14, which provide more detail on the determination of selected functional groups. The remaining six chapters in Volume 13 deal with the determination of the following types of functional groups: Active Hydrogen in Organic Compounds by F. T. Weiss (62 pp., 113 refs.); The C-Methyl Group by K. G. Stone (deceased) (36 pp., 56 refs.); Carbonyl and Derived Functions by J. G. Hanna and S. Siggia (92 pp., 358 refs.); Carboxyl and Derived Functions by S. Veibel (77 pp., 214 refs.); Phosphorus-Based Functions by D. N. Bernhart (35 pp., 27 refs.); and Divalent Sulfur-Based Functions by J. H. Karchmer (181 pp., 314 refs.). Each chapter is well written and well organized, and is headed with a detailed outline of its contents and concludes with an extensive list of references, totaling about eleven hundred and fifty in the seven chapters. The book contains many tables of useful data, figures, and graphs, and concludes with a subject index. Printing, paper, and cloth binding are of good quality.

The book maintains the high standard set by volumes in the Treatise which have appeared to date and will be welcomed by analytical chemists who have occasion to make organic analyses.

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The Theory of the Microscope. By L. C. MARTIN. American Elsevier, New York, 1966. xiv + 488 pp. $19.50.

This is a volume for users of the optical microscope who want to understand the theoretical basis of the instrument. It is neither a manual of microscope design and use nor a treatise on the physics of light, as the omission of the Maxwell equations will attest. It is instead devoted to the quantitative explanation of image formation and associated phenomena by means of classical optical theory. Elementary geometrical optics is covered in two chapters on optical systems. A chapter on the periodic nature

of light introduces wave properties and the principles of interference and diffraction. The theories of coherence, incoherence, relative coherence, and partial coherence, as applied especially to image formation, are allotted four chapters. A chapter on phase microscopy includes descriptions of various interferometers and interference microscopes.

The treatment is necessarily mathematical, but only scalar equations are used, and thorough explanations of the mathematics are given. Some mathematical tools, such as Fourier series and transforms, are developed in appendices. A moderate degree of mathematical sophistication is nevertheless essential, and the reader who needs to be told that "the combination of two minus signs means a plus" will not get far beyond page 7.

This book is well written and well organized, and it will be extremely valuable for the microscopist who wants to base his interpretations on understanding. Since much of the theory is applicable to optical systems in general, the book may serve also as an excellent introduction to classical optics.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky*

Encyclopedia of Industrial Chemical Analysis. Vol. I: General Techniques, A-E. Edited by F.D. SNELL AND C.L. HILTON. Wiley (Interscience), New York, 1966. xv + 763 pp. $45.00 ($35.00 by subscription).

This is the first volume of an ambitious undertaking expected to run into fifteen volumes. This first one is devoted exclusively to instrumentation, techniques, and procedures on subjects alphabetically covered from A to E. It does a good job. The subjects are presented by recognized authorities in their respective fields and are all concisely but adequately treated. It cannot be said that one subject is handled better than another, but the sections on chromatography and complexation in aqueous media stand out in this reviewer's opinion (perhaps because of a greater familiarity with these fields). Every chapter, of which there are 32 (Absorption and Emission Spectroscopy to Extraction), is followed by an ample bibliography. The next two volumes will cover the rest of the alphabet, and the succeeding twelve will involve the detailed listing of specific elements, compounds, products, and materials.

The purist will be annoyed by an occasional dangling participle or incomplete sentence, but these are inconsequential and will not detract from the usefulness of the book.

In the words of the editors, the Encyclopedia is intended "to give an overall view of industrial analytical chemistry" and anticipates presenting "a comprehensive coverage of the methods and techniques used in industrial laboratories throughout the world for the analysis and evaluation of chemical products." This is a tremendous project, but if the succeeding volumes live up to the first, the promise will be fulfilled. The name of Foster Dee Snell is automatically a guarantee of quality. As these books are published and assembled on the library shelf, the analyst will have at his fingertips just about everything he needs to know.

DAVID B. SABINE, *U.S. Vitamin & Pharmaceutical Corporation, Yonkers, New York*

Specimen Preparation for Electron Metallography. By I.S. BRAMMAR AND M.A.P. DEWEY. Elsevier, New York, 1966. 110 pp. $6.00.

The increased utilization of the electron microscope in the study of metals brought on by the introduction of the double condenser lens microscope in 1954 has resulted in the development of many new techniques for the preparation of specimens for direct transmission microscopy to augment the older indirect surface replication techniques. This book, designed as a laboratory handbook, presents the general principles of replication techniques and methods for thin-film preparation with excellent clarity in the beginning chapters. The balance of the book goes into detail on the selection of methods to be used for particular investigations and the specifics of specimen preparation techniques for many of the pure metals and alloys that one is apt to encounter in the metallurgical laboratory. The techniques are presented in tabular form, listed alphabetically by metals. An appendix of useful information includes many references, a listing of suppliers of materials and equipment, and tables of basic electron diffraction and crystallographic data. The book is devoid of theoretical discussions of the principles of either the electron microscope or the various specimen preparation techniques. Its value, to professionals and technicians alike, lies in its clear presentation of practical laboratory methodology.

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Announcement

The Austrian Society for Microchemistry and Analytical Chemistry will conduct the Sixth International Symposium on Microchemistry from September 7–11, 1970, in Graz, Austria. Details will be announced at a future date.

Committee: A. Holasek, H. Malissa, and H. Spitzy.