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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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CONTENTS

V. Fano and M. Scalvini. Determination by Anodic Stripping Voltammetry of Some Trace Elements in Te, Pb, and PbTe 1

S. Chandra and K. L. Yadava. Oxidation of Some Organic and Inorganic Compounds with Cu(III) 4

M. H. Hashmi, Tehseen Qureshi, and Farhat Rafique Chughtai. Spectrophotometric Determination of Palladium 18

K. Visweswariah, S. K. Majumder, and M. Jayaram. A Colorimetric Method of Estimating Hydrolyzable Chlorine in Lindane (γ-Hexachlorocyclohexane) 26

David E. Chasan and George Norwitz. Qualitative Analysis of Primers, Tracers, Igniters, Incendiaries, Boosters, and Delay Compositions on a Microscale by Use of Infrared Spectroscopy 31

A. K. Saxena. Microdetermination of Benzoic and Salicylic Acids with Guanidine Carbonate as a Titrant 61

D. P. Schwartz and C. R. Brewington. Methods for the Isolation and Characterization of Constituents of Natural Products. XIII. Regeneration of 2,4-Dinitrophenylhydrazones on a Periodic Acid Column 63

O. C. Saxena. Titrimetric Microdetermination of Yttrium and Scandium: Disodium Salt of Rhodizonic Acid as Complexing Agent 68

R. A. Lalancette and K. G. Koubek. Simple Modification of the Cary 14 Recording Spectrophotometer for Visible Photometric Titrations 72

A. K. Saxena. Microdetermination of Sulfanilic Acid with Guanidine Carbonate as a Titrant 75

Catherine O'Neil and Alice Laughlin. The Introduction of Microtechniques in Elementary Science Courses. II. Serum Cholesterol and Cholesterol Ester Determination 77

A. K. Saxena. Microdetermination of Aspartic and Glutamic Acids with Guanidine Carbonate as a Titrant 91

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TADAYOSHI IEKI AND KOJI DAIKATSU. Application of Differential Thermal Analysis to Organic Elemental Microanalysis. I. Combination with Carbon and Hydrogen Determination Apparatus 93

R. S. IYER AND P. R. KAMATH. Separation of $BiPO_4$ Carried Actinides by BiOCl Precipitation 105

ARNOLD L. SCHULTZ AND SAMUEL NATELSON. Studies on the Distribution and Concentration of Thiamine in Blood and Urine 109

SHARAD M. SHAH AND J. PAUL. Simultaneous Determination of Copper and Manganese with Sodium Diethyl Dithiocarbamate 119

BOOK REVIEWS 125

Briefs

Determination by Anodic Stripping Voltammetry of Some Trace Elements in Te, Pb, and PbTe. V. FANO AND M. SCALVINI, *Laboratorio Maspec del C.N.R., Parma, Italy.*

The best conditions for the determinations were studied. The temperature at which coevaporation and coprecipitation occur varies with the concentration.

Microchem. J. **17**, 1 (1972).

Oxidation of Some Organic and Inorganic Compounds with Copper(III). S. CHANDRA AND K. L. YADAVA, *Department of Chemistry, University of Allahabad, Allahabad, India.*

The previous work of the authors has been extended to include additional organic compounds and a number of inorganic compounds.

Microchem. J. **17**, 4 (1972).

Spectrophotometric Determination of Palladium. M. H. HASHMI, TEHSEEN QURESHI, AND FARHAT RAFIQUE CHUGHTAI, *Pakistan Council of Scientific and Industrial Research Laboratories, Lahore, West Pakistan.*

Palladium reacts with melamine in an alkaline medium to give a green color having maximum absorbance at 620–630 nm. The color reaction is specific for palladium and has a visual limit for identification of 1 μg per ml.

Microchem. J. **17**, 18 (1972).

A Colorimetric Method of Estimating Hydrolyzable Chlorine in Lindane (γ-Hexachlorocyclohexane). K. VISWESWARIAH, S. K. MAJUMDER, AND M. JAYARAM, *Central Food Technological Research Institute, Mysore-2A, India.*

Beer's law is obeyed in the range of 80–320 μg/ml of solution. The method was compared to that of Volhard and is believed to be superior.

Microchem. J. **17**, 26 (1972).

Qualitative Analysis of Primers, Tracers, Igniters, Incendiaries, Boosters, and Delay Compositions on a Microscale by Use of Infrared Spectroscopy. DAVID E. CHASAN AND GEORGE NORWITZ, *Frankford Arsenal, Philadelphia, Pennsylvania 19137.*

Spectra were made on the compounds in potassium bromide pellets. The grinding and pressing operation was found to be completely safe even with these sensitive explosives. The infrared spectra of 43 of the most common ingredients of tracers, igniters, incendiaries, boosters, and delay compositions are given over the range 2.5 to 50 μ.

Microchem. J. **17**, 31 (1972).

Microdetermination of Benzoic and Salicylic Acids with Guanidine Carbonate as a Titrant. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

The acids were titrated with guanidine carbonate using bromcresol purple as indicator.

Microchem. J. **17**, 61 (1972).

Methods for the Isolation and Characterization of Constituents of Natural Products. XIII. Regeneration of 2,4-Dinitrophenylhydrazones on a Periodic Acid Column. D. P. SCHWARTZ AND C. R. BREWINGTON, *Dairy Products Laboratory, Eastern Marketing and Nutrition Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D. C. 20250.*

A method is described for regenerating carbonyl compounds at room temperature from 2,4-dinitrophenylhydrazones. A column of $MgSO_4$ impregnated with periodic and sulfuric acids is used as the regenerant and CCl_4 is used as the solvent for the 2,4-dinitrophenylhydrazone. Examples are given for saturated aliphatic and aromatic ketones, saturated aliphatic and aromatic aldehydes, keto- and aldehydoesters, and a ketosteroid. Some oxidation of the double bond in unsaturated 2,4-dinitrophenylhydrazones occurs.

Microchem. J. **17**, 63 (1972).

Titrimetric Microdetermination of Yttrium and Scandium: Disodium Salt of Rhodizonic Acid as Complexing Agent. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad, India.*

Yttrium and scandium are determined separately by forming a complex of the metal with the disodium salt of rhodizonic acid. The complex is then decomposed with mineral acid followed by ceric sulfate titration using *N*-phenyl anthranilic acid as the indicator.

Microchem. J. **17**, 68 (1972).

Simple Modification of the Cary 14 Recording Spectrophotometer for Visible Photometric Titrations. R. A. LALANCETTE AND K. G. KOUBEK, *Department of Chemistry, Rutgers University, Newark, New Jersey 07102.*

A simple modification is described which allows photometric titrations with the Cary 14 recording spectrophotometer. An air-driven magnetic stirrer, sinter-fused glass cells, and a drawn-out buret, entering the cell compartment through the cover, make up the necessary changes.

Microchem. J. **17**, 72 (1972).

Microdetermination of Sulfanilic Acid with Guanidine Carbonate as a Titrant. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Sulfanilic acid, in the range of 0.956–0.096 mg, was determined by titration with guanidine carbonate using bromocresol purple as indicator.

Microchem. J. **17**, 75 (1972).

The Introduction of Microtechniques in Elementary Science Courses. II. Serum Cholesterol and Cholesterol Ester Determination. CATHERINE O'NEILL AND ALICE LAUGHLIN, *Division of Science, Jersey City State College, Jersey City, New Jersey 07305.*

This is the second in a series of papers to show that microanalytical techniques can be successfully taught to beginners in science. Measurements were made with a photoelectric colorimeter, a spectrophotometer and a color-comparitor block.

Microchem. J. **17**, 77 (1972).

Microdetermination of Aspartic and Glutamic Acids with Guanidine Carbonate as a Titrant. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Titrations were made using bromocresol purple as indicator. Estimations were made in the range of 0.666–0.067 mg of aspartic acid and 0.735–0.067 mg of glutamic aciid. The same procedure was used as with other types of acids, previously published by the author.

Microchem. J. **17**, 91 (1972).

Application of Differential Thermal Analysis to Organic Elemental Microanalysis. I. Combination with Carbon and Hydrogen Determination Apparatus. TADAYOSHI IEKI AND KOJI DAIKATSU, *Shionogi Research Laboratory, Shionogi and Co., Ltd., Fukushima-ku, Osaka, Japan.*

Application of differential thermal analysis to the microdetermination of carbon and hydrogen is described. The sample is combusted slowly on a thermocouple detector in a stream of air and the carbon and hydrogen values are determined gravimetrically. During the heating process, the thermal characteristics of the sample, such as, dehydration, fusion, boiling, vaporization, sublimation, explosion, decomposition, and oxidation, are indicated as the exothermic peak or endothermic negative peak in a differential thermogram. The differential thermocouples are installed in the layer of oxidizing catalyst and additional thermocouples are placed in both the water and carbon dioxide absorption tubes to monitor the combustion process of organic vapor in the effluent gas. Anomalous analytical results may be reasonably interpreted by referring to data concerning the type of thermal decomposition of the sample.

Microchem. J. **17**, 93 (1972).

Separation of $BiPO_4$ Carried Actinides by BiOCl Precipitation. R. S. IYER AND P. R. KAMATH, *Bhabha Atomic Research Centre, Health Physics Division, Bombay-85 (AS), India.*

The study was carried out to develop a rapid method for the determination of uranium, thorium, and americium in a single sample of urine.

Microchem. J. **17**, 105 (1972).

Studies on the Distribution and Concentration of Thiamine in Blood and Urine. ARNOLD L. SCHULTZ AND SAMUEL NATELSON, *Department of Biochemistry, Michael Reese Hospital and Medical Center, Chicago, Illinois 60616.*

A reliable procedure for estimating the thiamine content in human blood and urine is described. Heparinized blood is hemolyzed with hydrochloric acid. Cocarboxylase is then converted to free thiamine by means of wheat germ acid phosphatase. The liberated thiamine is adsorbed to a carboxylic acid ion exchange acrylic resin column, eluted, and oxidized to thiochrome. Readout is by fluorometry at an excitation wavelength of 371 nm and an emission wavelength of 425 nm.

The same procedure is applicable to the determination of thiamine in urine. Conversion of cocarboxylase to free thiamine is not necessary, since it was found that practically all of the thiamine found in urine is not phosphorylated.

Microchem. J. **17**, 109 (1972).

Simultaneous Determination of Copper and Manganese with Sodium Diethyl Dithiocarbamate. SHARAD M. SHAH AND J. PAUL, *Chemistry Department, University of Bridgeport, Bridgeport, Connecticut 06602.*

Copper and manganese are determined simultaneously by the selective formation of copper diethyl dithiocarbamate complex in perchloric acid solution followed by the formation of manganese diethyl dithiocarbamate complex in ammoniacal solution in the presence of the perchlorate ions.

Microchem. J. **17**, 119 (1972).

MICROCHEMICAL JOURNAL 17, 1–3 (1972)

Determination by Anodic Stripping Voltammetry of Some Trace Elements in Te, Pb, and PbTe

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Lead and tellurium, besides the interest for properties that they present in the elementary state (for instance Pb is a superconductor and Te is a semiconductor with complex valence band), have been used as components of a wide series of complex semiconductors. Both elements compose a typical semiconductor (PbTe) with considerable thermoelectrical properties greatly varying in the presence of doping elements (*4, 3*). For most of the doping elements, the process of action has not been studied yet. Some typical doping elements can be determinated by polarography HMDE up to a concentration $\simeq 10^{-6}\%$ and with a precision of the order of 10%. At this level of concentration and with this precision, a method for the analysis of Cu, Cd, and Zn in Te, Pb, and PbTe has been developed. The possibility of determination of such a microimpurity in samples of some tens of milligrams or more provides a way of controlling the samples that are commonly subject to measurements.

Among the methods of separating the matrix from impurities, we chose to precipitate Te and Pb from a nitric solution in the form of $TeO_2 \cdot xH_2O$ and $PbSO_4$ respectively. Therefore, we used reagents (HNO_3, H_2SO_4) available in a satisfactory state of purity without further purification and made a control test with a blank solution. Similar techniques may also be applicable to the analysis of Ga and In microtraces. The polarograms were made with the polarograph described in (2). The peak heights later reported refer to polarograms obtained according to the following procedure: for weight of 0.5 g sample, only one tenth of the residue is put into the cell. For weight of 0.05 g all the residue is put into the cell. In all cases the medium is 40 ml of 0.1 *N* NaCl solution.

PROCEDURE

a. A fine powder of the sample is dissolved in nitric acid (HNO_3: H_2O = 1 : 1); 8 ml of nitric solution are enough for samples of 0.5 g.

TABLE 1

DETERMINATION OF Cu, Cd, AND Zn IN Pb, Te, AND PbTe [a]

Wt and sample	Peak ht (cm; av for 5 detn)		
	Cu	Cd	Zn
Blank solution [b]	0	0	0
Blank solution + 5 μg of Cu, Cd, Zn [b]	1.1	3.8	2.5
Pb, 0.05 g	0	1.0	8.0
0.5 g	0	1.1	8.1
Te, 0.05 g	0	0.8	4.0
0.5 g	0	0.8	4.1
PbTe, 0.5 g	0	0.9	6.8
0.05 g	0	0.9	6.6
0.05 g PbTe + 5 μg of Cu, Cd, Zn	1.0	4.8	9.7
+ 10 μg of Cu, Cd, Zn	2.1	8.6	11.8
+ 15 μg of Cu, Cd, Zn	3.2	12.3	14.1

[a] Pre-electrolysis time, 5 minutes at −1.30 V vs SCE. The scanning rate is 0.4 V/minute. The temperature of the electrolytic cell is maintained at 25 ± 0.1°C.

[b] The peak heights refer to analysis of sample of 0.5 g.

A blank precipitate ($TeO_2 \cdot xH_2O$) is formed that may be soluble when heated. The nitric acid is slowly evaporated and diluted with H_2O. Such an operation is repeated until a neutral solution is obtained. When the nitric acid is diluted with H_2O, a white precipitate of $TeO_2 \cdot xH_2O$ is formed and it is removed by centrifugation after a few hours (the time necessary to reach the equilibrium).

At neutral pH, most of the Te is precipitated; however, the small quantity left in the solution would disturb the polarogram. Therefore, the Te left in the solution is removed with high purity Pb added to the neutral solution. As explained below, an analysis of the impurities of the Pb added as reductant may provide a check for spurious elements in the solution. After the reduction, the solid residue is removed by centrifugation.

b. In the obtained solution (4 ml for weight of 0.5 g) Pb is precipitated in the form $PbSO_4$ (add 0.4 ml of H_2SO_4 and 1.0 ml of H_2O). After a few hours when all Pb is precipitated, the solution is centrifugated, and H_2SO_4 is evaporated slowly. At this point the residue is diluted with H_2O and the polarogram is performed. In the case of the analysis of the elements Pb and Te, the Te separation is obtained as described in (a) and the Pb separation is obtained as described in (b) after dissolving the sample in HNO_3 ($HNO_3 : H_2O = 1 : 1$) and evaporation of the excess of HNO_3. In order to check the absence of copre-

TABLE 2

EFFECT OF TEMPERATURE ON COEVAPORATION OF MICROTRACES

Temp of evaporation (°C)	Peak ht [a] (cm; av for 5 detn)		
	Cu	Cd	Zn
120	2.0	8.5	11.8
125	2.1	8.6	11.7
130	1.3	8.5	11.7
135	1.0	8.4	11.6

[a] The peak heights refer to analysis of 0.5 g of PbTe + 10 μg of Cu, Cd, Zn.

cipitation of microtraces together with $TeO_2 \cdot xH_2O$ and $PbSO_4$ and the absence of coevaporation in the process of slow evaporation, known quantities of Cu, Cd, and Zn were added to PbTe. The results of the analysis (see Table 1) show the absence of coprecipitation and coevaporation. It has been noted (1) that the temperature at which coevaporation appears, varies in function of the element microconcentration. In the present study, the temperature of evaporation of HNO_3 and H_2SO_4 were no more than 125°C. At higher temperature, the time of analysis is reduced but the determination of Cu is not made with high precision (see Table 2).

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MICROCHEMICAL JOURNAL 17, 4–17 (1972)

Oxidation of Some Organic and Inorganic Compounds with Cu(III)

S. CHANDRA AND K. L. YADAVA

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Received October 20, 1969

INTRODUCTION

In our previous papers the estimation of some sugars (*1*), carbonyl compounds (*2*), alcohols and organic acids (*3*) and some phenolic compounds (*4*) with Cu(III) has been described. In the present paper the titrimetric determinations of two sugars, lactose and maltose; three carbonyl compounds, benzaldehyde, methyl ethyl ketone, and diethyl ketone; two esters, methyl formate and ethyl formate; some nitrogenous compounds, thiourea, urea, ammonia, and potassium cyanide; and some inorganic compounds, hydrogen peroxide, sodium sulfide, sodium thiosulfate, sodium sulfite, potassium ferrocyanide, Cr(III), potassium tellurite, and potassium nitrite using the same oxidant are described.

EXPERIMENTAL METHODS

Reagents. Lactose, maltose, thiourea, urea, ammonia, sodium sulfide, sodium thiosulfate, potassium ferrocyanide, chrome alum, and potassium tellurite were of A.R. B.D.H. grade. The standard solutions of lactose, maltose, urea, thiosulfate, chrome alum, and potassium ferrocyanide were prepared directly by weight. The solution of thiourea was standardized iodometrically (*5*). Ammonia solution was standardized against standard sulfuric acid using methyl orange as indicator. Solutions of potassium tellurite and sodium sulfide have been standardized by potassium dichromate (*6*) and potassium iodate (*7*) methods, respectively. Redistilled (C.P. B.D.H.) benzaldehyde, diethyl ketone and ethyl methyl ketone were used for the preparation of solutions. The solution of benzaldehyde has been standardized by Donnally's method (*8*). The standard solutions of diethyl ketone and ethyl methyl ketone were prepared directly by weight. Ethyl and methyl formates (C.P. B.D.H.) were purified by redistillation. The solutions of the above two esters were standardized by saponification method. Potassium cyanide, hydrogen

peroxide, sodium sulfite, and sodium nitrite used were of C.P. B.D.H. grade. The solution of recrystallized cyanide was standardized iodometrically (*9*). The solution of prepared hydrogen peroxide was standardized iodometrically using molybdate as catalyst (*10*). The solution of sodium sulfite was standardized by iodometric method. Prepared solution of potassium nitrite was standardized by Migray's method (*11*).

Preparation of Cu(III). Method of preparation and standardization of Cu(III) was described in our previous paper (*1*).

Procedure. For the estimation of these organic and inorganic compounds, an aliquot (see tables) is added to an excess of Cu(III) solution. Cu(III) and substrate mixtures were allowed to stand for (a) 0, (b) 15, and (c) 30 minutes, and in the case of (d) the reaction mixtures were boiled on plate heater and then cooled, while in (e) where nitrogenous compounds were estimated, the reaction mixture was refluxed for 30 minutes in a conical flask fitted with upright condenser by ground glass joints. The inner tube of the condenser was filled with Pyrex glass beads over which double distilled water was dropped from the upper end of the condenser at the rate of 10 drops/minute to prevent the escape of ammonia or any other oxides of nitrogen formed in the reaction mixture.

The unused Cu(III) was estimated with the help of arsenite method, previously described (*1*). Blanks were also run.

DISCUSSION

It is clear from Tables 1–19 that Cu(III) oxidizes on boiling or refluxing lactose, maltose, benzaldehyde, ethyl methyl ketone, diethyl ketone, ethyl formate, and methyl formate to CO_2 and H_2O; nitrogenous compounds to nitrate; hydrogen peroxide to water and oxygen; sodium sulfite, thiosulfate, and sulfite to sulfate; potassium ferrocyanide to ferricyanide; Cr(III) to Cr(VI); and potassium tellurite to tellurate.

The reactions for the complete oxidations of the compounds are given below:

$$C_x(H_2O)_{11} + (2x - 11)\,H_2O \longrightarrow xCO_2 + 4xH^+ + 4xe^-.$$

where x = 12 for lacote and maltose.

$$C_6H_5CHO + 13H_2O \longrightarrow 7CO_2 + 32H^+ + 32e^-.$$

Benzaldehyde

$$CH_3CH_2COCH_3 + 7H_2O \longrightarrow 4CO_2 + 22H^+ + 22e^-.$$

Methyl ethyl ketone

$$C_2H_5COC_2H_5 + 9H_2O \longrightarrow 5CO_2 + 28H^+ + 28e^-.$$

Diethyl ketone

$$HCOOCH_3 + 2H_2O \longrightarrow 2CO_2 + 8H^+ + 8e^-.$$

Methyl formate

$$HCOOC_2H_5 + 4H_2O \longrightarrow 3CO_2 + 14H^+ + 14e^-.$$

Ethyl formate

$$NH_2{-}\underset{\underset{S}{\|}}{C}{-}NH_2 + 12H_2O \longrightarrow H_2SO_4 + 2HNO_3 + CO_2 + 24H^+ + 24e^-.$$

Thiourea

$$NH_2{-}\underset{\underset{O}{\|}}{C}{-}NH_2 + 7H_2O \longrightarrow 2HNO_3 + CO_2 + 16H^+ + 16e^-.$$

Urea

$$NH_3 + 3H_2O \longrightarrow HNO_3 + 8H^+ + 8e^-.$$

Ammonia

$$CN^- + 5H_2O \longrightarrow NO_3^- + CO_2 + 10H^+ + 10e^-.$$

Cyanide

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-.$$

Hydrogen peroxide

$$Na_2S + 4H_2O \longrightarrow Na_2SO_4 + 8H^+ + 8e^-.$$

Sodium sulfide

$$Na_2S_2O_3 + 5H_2O \longrightarrow Na_2SO_4 + H_2SO_4 + 8H^+ + 8e^-.$$

Sodium thiosulfate

$$Na_2SO_3 + H_2O \longrightarrow Na_2SO_4 + 2H^+ + 2e^-.$$

Sodium sulfite

$$Fe(CN)_6^{4-} \longrightarrow Fe(CN)_6^{3-} + e^-.$$

Ferrocyanide

$$Cr(III) \longrightarrow Cr(VI) + 3e^-.$$

Chromium(III)

$$TeO_3^{2-} + H_2O \longrightarrow TeO_4^{2-} + 2H^+ + 2e^-.$$

Tellurite

$$NO_2^- + H_2O \longrightarrow NO_3^- + 2H^+ + 2e^-.$$

Nitrite

At room temperature the oxidation of benzaldehyde goes to acid, however, this fact can not be utilized for the estimation because the oxidation of formed acid also proceeds through slowly. In the case of ethyl methyl ketone and diethyl ketone the oxidation with Cu(III) at room temperature is appreciable though partial. No conclusions as to what extent the oxidation of the ketones, sugars, and esters at room temperature goes can be inferred from the observations. Thiourea is oxidized with Cu(III) at ordinary temperature in about 30 minutes to urea and sulfate requiring 8 equivalents. Beck (*12*) showed that CN^- is oxidized to nitrate state in hot condition, whereas, Keyworth and Stone (*13*) showed the oxidation proceeding to CNO^- at ordinary tem-

TABLE 1

DETERMINATION OF LACTOSE

1.60×10^{-4} *M* lactose (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of lactose	Error (%)
1.0	10.0	19.9 (a)	−58.5
	10.0	25.8 (b)	−46.2
	10.0	47.9 (d)	−0.20
2.0	10.0	48.0 (d)	0.00
3.0	10.0	48.0 (d)	0.00
5.0	10.0	47.9 (d)	−0.20
	15.0	48.0 (d)	0.00
10.0	15.0	48.0 (d)	0.00
12.0	15.0	48.0 (d)	0.00

TABLE 2

DETERMINATION OF MALTOSE

2.60×10^{-4} *M* maltose (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of maltose	Error (%)
1.0	10.0	16.8 (a)	−65.0
	10.0	23.6 (b)	−50.8
	10.0	47.9 (d)	−0.20
2.0	10.0	47.9 (d)	−0.20
3.0	10.0	48.0 (d)	0.00
	15.0	48.0 (d)	0.00
	20.0	48.0 (d)	0.00
5.0	20.0	48.1 (d)	0.20
10.0	20.0	48.0 (d)	0.00

TABLE 3

DETERMINATION OF BENZALDEHYDE

1.00×10^{-3} *M* benzaldehyde (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of benzaldehyde	Error (%)
2.0	3.0	1.61 (a)	−94.9
	3.0	2.90 (c)	−90.9
	3.0	31.86 (e)	−0.44
3.0	3.0	31.90 (e)	−0.31
	6.0	31.85 (e)	−0.47
4.0	6.0	32.00 (e)	0.00
	8.0	32.00 (e)	0.00
5.0	8.0	31.90 (e)	−0.31
6.0	8.0	31.85 (e)	−0.47

TABLE 4

DETERMINATION OF METHYL ETHYL KETONE

1.20×10^{-4} *M* methyl ethyl ketone (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of methyl ethyl ketone	Error (%)
2.0	3.0	6.90 (a)	−68.63
	3.0	8.60 (c)	−60.90
	3.0	21.92 (e)	−0.36
3.0	3.0	22.00 (e)	0.00
4.0	3.0	22.04 (e)	0.13
5.0	5.0	22.06 (e)	0.27
8.0	5.0	22.00 (e)	0.00
10.0	5.0	22.10 (e)	0.45
12.0	10.0	22.02 (e)	0.09

TABLE 5

DETERMINATION OF DIETHYL KETONE

1.10×10^{-4} *M* diethyl ketone (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of diethyl ketone	Error (%)
1.0	3.0	5.80 (a)	−79.29
	3.0	6.40 (c)	−77.14
	3.0	27.90 (e)	−0.35
2.0	3.0	28.00 (e)	0.00
3.0	3.0	28.00 (e)	0.00
	6.0	27.94 (e)	−0.21
4.0	6.0	27.92 (e)	−0.29
5.0	6.0	27.90 (e)	−0.35
6.0	12.0	27.96 (e)	−0.14

TABLE 6

DETERMINATION OF METHYL FORMATE

1.10×10^{-3} *M* methyl formate (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of methyl formate	Error (%)
2.0	3.0	2.10 (a)	−73.75
	3.0	2.60 (c)	−55.00
	3.0	8.02 (e)	0.25
3.0	3.0	8.03 (e)	0.37
4.0	4.0	8.02 (e)	0.25
	4.0	8.04 (e)	0.50
	5.0	8.03 (e)	0.37
5.0	8.0	8.02 (e)	0.25
6.0	8.0	8.00 (e)	0.00

TABLE 7

DETERMINATION OF ETHYL FORMATE

1.00×10^{-3} *M* ethyl formate (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of ethyl formate	Error (%)
2.0	3.0	2.02 (a)	−85.6
	3.0	3.40 (c)	−75.7
	3.0	14.00 (e)	0.00
3.0	3.0	13.95 (e)	−0.36
	6.0	14.03 (e)	0.21
	8.0	13.97 (e)	−0.21
4.0	8.0	13.96 (e)	−0.28
5.0	8.0	13.95 (e)	−0.36
6.0	8.0	13.98 (e)	−0.14

TABLE 8

DETERMINATION OF THIOUREA

4.04×10^{-4} *M* thiourea (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of thiourea	Error (%)
1.0	5.0	7.20 (a)	−70.00
	5.0	8.02 (c)	−66.56
	5.0	24.04 (e)	0.17
2.0	5.0	24.06 (e)	0.25
3.0	6.0	24.03 (e)	0.12
4.0	6.0	23.97 (e)	−0.12
	8.0	23.94 (e)	−0.25
5.0	8.0	24.10 (e)	0.42
8.0	8.0	23.96 (e)	−0.17

TABLE 9

DETERMINATION OF UREA

3.00×10^{-4} *M* urea (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of urea	Error (%)
1.0	5.0	0.00 (a)	−100.00
	5.0	0.01 (c)	−99.92
	5.0	16.00 (e)	0.00
2.0	5.0	15.98 (e)	−0.12
3.0	6.0	16.02 (e)	0.12
4.0	6.0	15.99 (e)	−0.06
5.0	8.0	16.00 (e)	0.00
6.0	9.0	15.97 (e)	−0.19
8.0	10.0	15.98 (e)	−0.12

TABLE 10

DETERMINATION OF AMMONIA

1.30×10^{-3} *M* ammonia (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of ammonia	Error (%)
2.0	5.0	0.54 (a)	−93.25
	5.0	2.80 (c)	−65.00
	5.0	7.98 (e)	−0.25
3.0	5.0	7.99 (e)	−0.12
4.0	6.0	7.96 (e)	−0.50
5.0	6.0	7.97 (e)	−0.37
6.0	7.0	7.96 (e)	−0.50
7.0	8.0	7.97 (e)	−0.37
8.0	10.0	7.98 (e)	−0.25

TABLE 11

DETERMINATION OF POTASSIUM CYANIDE

4.00×10^{-3} *M* cyanide (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of cyanide	Error (%)
1.0	6.0	2.01 (a)	−79.9
	6.0	5.15 (c)	−48.5
	6.0	9.98 (e)	−0.20
2.0	6.0	9.99 (e)	−0.10
3.0	6.0	9.96 (e)	−0.40
4.0	6.0	9.97 (e)	−0.30
5.0	8.0	9.97 (e)	−0.30
6.0	10.0	9.98 (e)	−0.20
7.0	15.0	9.99 (e)	−0.10

TABLE 12

DETERMINATION OF HYDROGEN PEROXIDE

3.10×10^{-3} *M* hydrogen peroxide (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of hydrogen peroxide	Error (%)
1.0	3.0	0.91 (a)	−54.50
	3.0	1.13 (b)	−3.50
	3.0	1.99 (d)	−0.50
2.0	3.0	1.99 (d)	−0.50
4.0	4.0	2.00 (d)	0.00
5.0	4.0	2.00 (d)	0.00
	6.0	2.01 (d)	0.50
6.0	8.0	2.01 (d)	0.50
	10.0	2.00 (d)	0.00

TABLE 13

DETERMINATION OF SODIUM SULFIDE

4.0×10^{-3} *M* sodium sulfide (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of sodium sulfide	Error (%)
1.0	3.0	5.94 (a)	−25.75
	3.0	7.94 (b)	−0.75
	3.0	7.98 (d)	−0.25
1.5	3.0	7.98 (d)	−0.25
	5.0	8.01 (d)	0.12
2.0	8.0	8.01 (d)	0.12
	10.0	8.01 (d)	0.12
3.0	10.0	8.00 (d)	0.00
	12.0	8.00 (d)	0.00

TABLE 14

DETERMINATION OF SODIUM SULFITE

4.0×10^{-3} *M* sodium sulfite (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of sodium sulfite	Error (%)
1.0	3.0	0.26 (a)	−87.0
	3.0	0.96 (b)	−52.0
	3.0	1.99 (d)	−0.50
1.5	3.0	1.99 (d)	−0.50
	5.0	1.99 (d)	−0.50
2.0	5.0	2.01 (d)	0.50
4.0	5.0	2.01 (d)	0.50
5.0	6.0	1.99 (d)	−0.50
	8.0	1.99 (d)	−0.50

TABLE 15

DETERMINATION OF SODIUM THIOSULFATE

4.20×10^{-4} *M* sodium thiosulfate (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of sodium thiosulfate	Error (%)
1.0	4.0	5.94 (a)	−25.75
	4.0	7.98 (c)	−0.25
	4.0	7.99 (d)	−0.12
	5.0	7.99 (d)	−0.12
2.0	5.0	8.00 (d)	0.00
	6.0	8.00 (d)	0.00
3.0	8.0	8.01 (d)	0.12
4.0	10.0	8.02 (d)	0.25
5.0	10.0	8.03 (d)	0.37

TABLE 16

DETERMINATION OF POTASSIUM FERROCYANIDE

5.00×10^{-3} *M* ferrocyanide (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of potassium ferrocyanide	Error (%)
1.0	3.0	1.00 (a)	0.00
	3.0	1.00 (b)	0.00
	3.0	1.00 (d)	0.00
3.0	3.0	1.00 (d)	0.00
	6.0	1.00 (d)	0.00
4.0	6.0	1.00 (d)	0.00
5.0	8.0	1.00 (d)	0.00
6.0	10.0	1.00 (d)	0.00
8.0	10.0	1.00 (d)	0.00

TABLE 17

DETERMINATION OF Cr(III)

4.00×10^{-3} *M* Cr(III) (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of Cr(III)	Error (%)
1.0	4.0	2.25 (a)	−25.00
	4.0	3.00 (b)	0.00
	4.0	3.00 (d)	0.00
	6.0	3.00 (d)	0.00
2.0	6.0	2.99 (d)	−0.33
3.0	6.0	2.99 (d)	−0.33
4.0	8.0	3.01 (d)	0.33
5.0	10.0	3.00 (d)	0.00
6.0	10.0	3.01 (d)	0.33

TABLE 18

DETERMINATION OF POTASSIUM TELLURITE

5.00×10^{-3} *M* tellurite (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of tellurite	Error (%)
1.0	3.0	0.91 (a)	−54.50
	3.0	1.13 (b)	−43.50
	3.0	1.99 (d)	−0.50
	5.0	1.99 (d)	−0.50
2.0	5.0	2.01 (d)	0.50
	8.0	2.01 (d)	0.50
4.0	8.0	2.00 (d)	0.00
5.0	10.0	2.00 (d)	0.00
6.0	10.0	1.99 (d)	−0.50

TABLE 19

DETERMINATION OF POTASSIUM NITRITE

4.00×10^{-3} *M* potassium nitrite (ml)	3.50×10^{-2} *M* Cu(III) (ml)	Equivalents of Cu(III) consumed per mole of tellurite	Error (%)
1.0	5.0	0.26 (a)	−87.00
	5.0	1.28 (b)	−36.00
	5.0	2.00 (d)	0.00
	6.0	1.99 (d)	−0.50
2.0	8.0	1.99 (d)	−0.50
3.0	8.0	2.01 (d)	0.50
	10.0	2.00 (d)	0.00
4.0	10.0	2.01 (d)	0.50
5.0	10.0	2.01 (d)	0.50

perature. Ammonia and urea at ordinary temperature are only very slightly oxidized by Cu(III). It may be mentioned that catalytic utility of basic surfaces of silver, copper, and nickel has been signified by a number of workers (*14, 15*) for the conversion of ammonia and other nitrogenous compounds to nitrate. It is no wonder in this context that Cu(III) solution which contains large amount of alkali and also $Cu(OH)_2$ as a result of reduction of Cu(III) by the substrate can bring about quantitative conversion of the nitrogenous compounds to nitrate state. The reaction of sulfide, thiosulfate, and hydrogen peroxide with Cu(III) is fast. The oxidation can be completed at ordinary temperature in the case of ferrocyanide. It may be mentioned that Cu(III) solution was standardized by Jensovsky (*16*) and Keyworth and Stone (*13*) potentiometrically using ferrocyanide. It is surprising that the latter workers found 4 to 4.5 equivalents of Cu(III) being consumed per gram ion of ferrocyanide. In contrast, our finding is that only 1 equivalent of Cu(III) is consumed in the reaction. Reaction with sodium sulfite, nitrite, and tellurite with Cu(III) is slow at ordinary temperatures.

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Spectrophotometric Determination of Palladium

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Many methods are known for the colorimetric and spectrophotometric determination of palladium. In the mercapto derivatives method (*1, 6, 7*) cyanide interferes strongly. When chrome azurol S is employed as a color producing reagent, the interferences from alkaline earth metals, cations, and anions is pronounced. The color reaction depends largely upon the pH control as well (*15, 24*). Pyridyl azo resorcinol also produces a green color with palladium, but niobium, silver, gold, and mercury seriously interfere (*11*). The azo derivatives method (*3*) has an error of $\pm$ 2% while iron, platinum, and silver interfere (*2, 8*).

3-Nitroso-2,6-pyridinediol (*9*), nitroso-R-salt (*14*) and 2-thio-4-amino-5-nitroso-6-hydroxypyrimidine (*19*) are also used for the colorimetric determination of palladium but ruthenium, platinum, and iron interfere. The color reaction with the latter reagent also requires a protective colloid to prevent coagulation at higher concentration (*19*). In the α-benzoyl-β-carbethoxymethyl-selenourea method, Beer's law is obeyed at from 4 to 16 μg/ml, while gold, osmium, platinum, copper, and silver interfere (*4*). With phthalimide-dioxime (1,2-dihydroxyiminosoindoline) method, the color develops after 45 to 60 minutes and a number of cations and anions interfere (*5*). The interference of cobalt, nickel, manganese, rhodium, and EDTA cannot be avoided in the yellow color produced with 2-thenoyltrifluoroacetone; also a long procedure is required for the extraction of palladium (*10*). When *N*, *N*′-bis(2-sulfoethyl)dithiooxamide is employed as a color producing reagent for palladium, the color is measured after 30 minutes while a large number of cations interfere and the Beer's law range is limited (*13, 16*). The methods based on the use of picolinealdehyde-2-quinolylhydrazone (*17*) thiooxine (*18*), thiocyanate (*21*) reduction of nickel salts (*22*), α-(*N*-methylanabasinazo-1-naphthalene-5-sulfonic acid) (MAAH-S-1,5) (*22*), *p*-nitrosoaniline (*12, 20, 25*), *p*-nitrosodimethylaniline (*12, 20, 25*), and furildioxime (*12, 20, 25*) involve long procedures, error ranges

in certain cases up to 10% (*22*); and interference from cations and anions is pronounced. Ruthenium, rhodium, osmium, iridium, iron, cobalt, nickel, copper, gold, manganese, zinc, vanadium, chromium, aluminum, platinum, chloride, thiosulfate, cyanides, bromides, and iodides generally interfere (*12, 17, 18, 20–23, 25*).

Studies of the reaction of organic reagents on inorganic compounds showed that palladium gives a green color in the alkaline medium with melamine, having 1 μg/ml as the visual limit of identification. The color reaction obeys Beer's law with maximum absorbance at 620–630 nm. Reference to the literature (*12, 20, 25*) indicates that this color reaction has not been reported previously.

The present method is based on this new but sensitive color reaction for the spectrophotometric determination of minute quantities of palladium. The quantitative assessment of tolerable amounts of other cations and anions is reported. The mechanism of the color reaction is not clear.

EXPERIMENTAL METHODS

Reagents

All reagents were of analytical grade or comparable purity.

Palladium solution was prepared by weight in distilled water by dissolving palladium chloride (from BDH). About 1 ml of conc HCl/100 ml of the solution was added to ensure complete dissolution.

Melamine, 0.2% (by wt) aqueous solution, was prepared by dissolving it in boiling water. This solution was used as a color producing reagent. The solution must be stored above 25° otherwise recrystallization occurs.

Apparatus

All absorbance measurements were made with SP 600 Unicam spectrophotometer using 1-cm cells.

The pH meter was a Pye Dynacap; and graduated pipettes, accurate to ± 0.005 ml were used.

Procedure

To 2 ml of the solution containing 1 to 120 μg of palladium, 2 ml of color producing reagent is added followed by 3 ml of 1 *N* sodium hydroxide and the pH is adjusted between 12.6 and 12.68. The volume of the solution is made to 10 ml with distilled water. The solution is shaken and then heated for 10 minutes at 95–100° in a water bath, when a green color is produced. The contents are cooled under tap water and the green color is measured at 620–630 nm using SP 600 Unicam spectrophotometer and 1-cm cells with water as blank. The

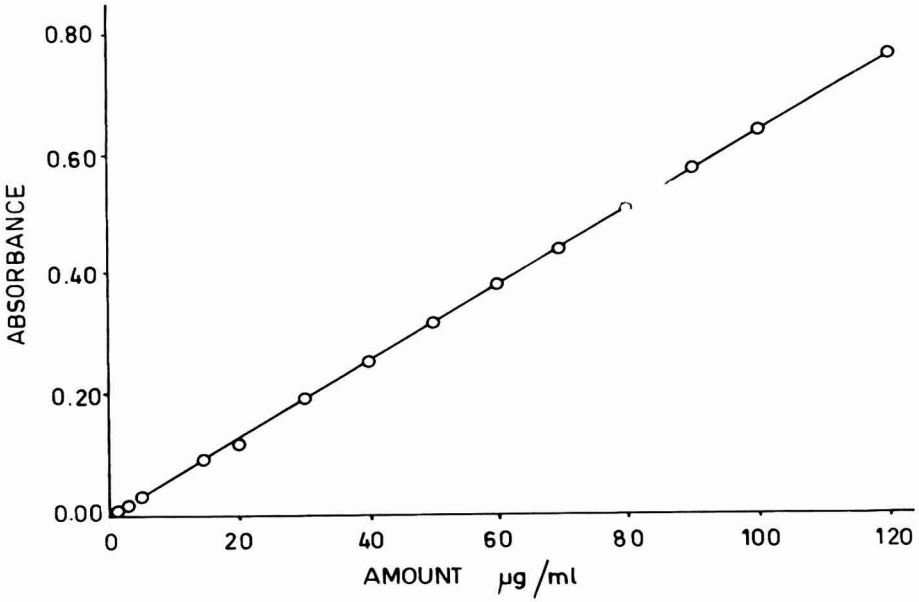

FIG. 1. Typical calibration curve for palladium.

experiment was repeated with different volumes of standard palladium solution; and a calibration curve was prepared (Fig. 1). The color reaction obeys Beer's law.

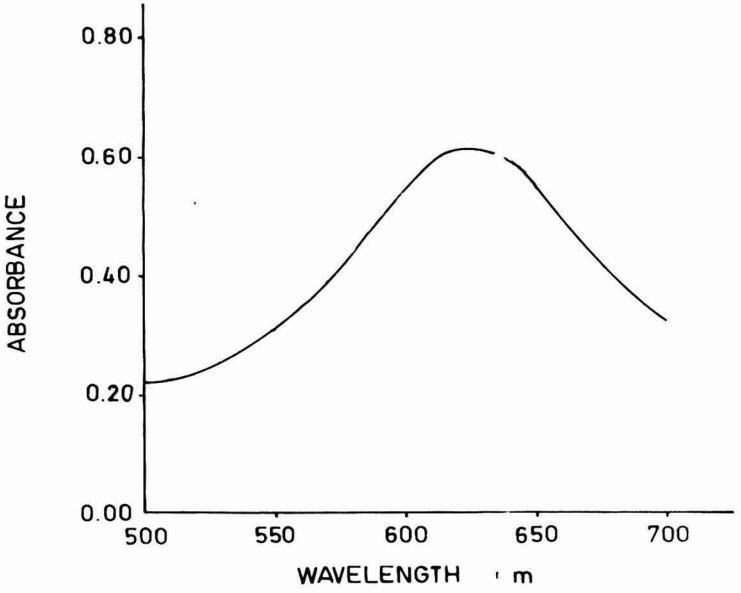

FIG. 2. Absorption spectra of palladium with melamine.

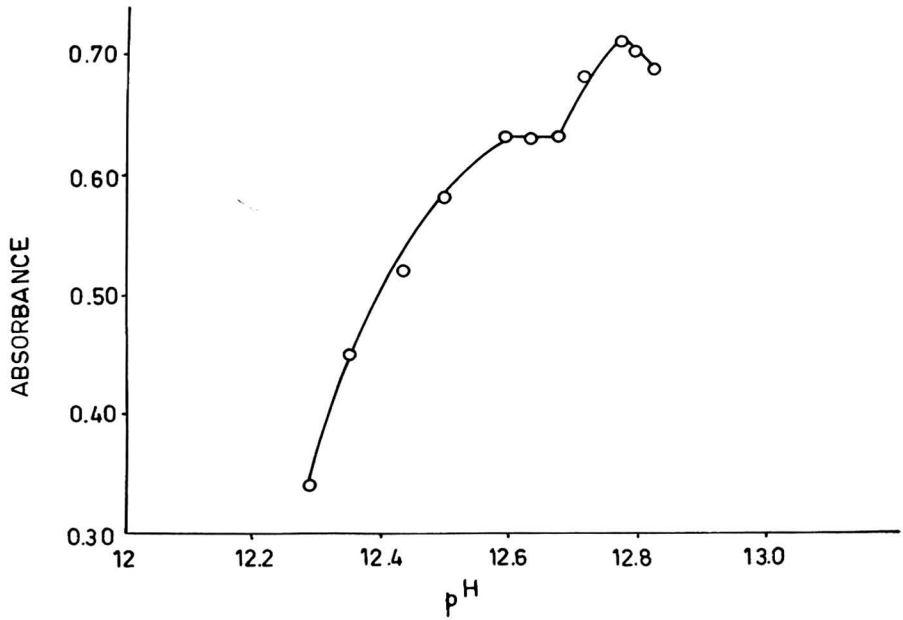

FIG. 3. Effect of pH on color intensity.

RESULTS AND DISCUSSION

The color reaction has maxima at 620–630 nm (Fig. 2); hence, all absorbance measurements were carried out at this wavelength.

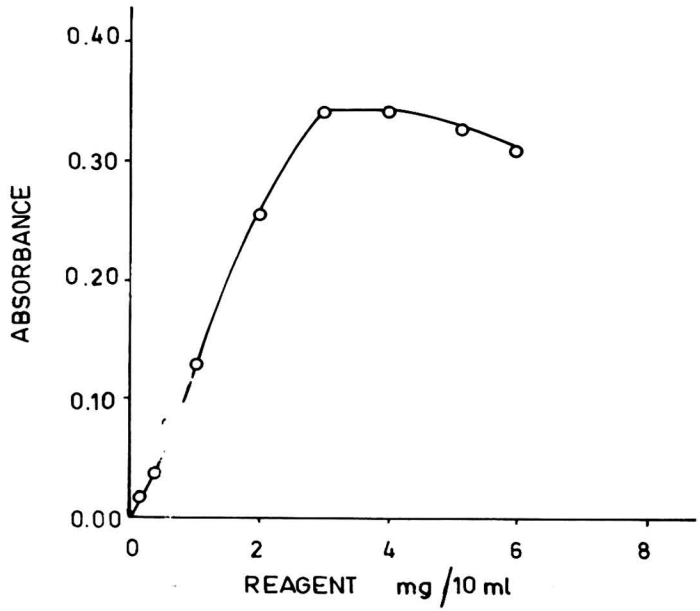

FIG. 4. Effect of reagent concentration on color intensity.

TABLE 1

DETERMINATION OF PALLADIUM FROM PURE SOLUTION AND IN PRESENCE OF OTHER IONS

Compound present in addition to Pd^{2+} to be determined	Other metal ions present with respect to Pd^{2+} to be determined (%)	Pd^{2+} determined (μg/10 ml)	
		Present	Found
—	—	50	50
—	—	80	81
—	—	100	100
—	—	110	110
—	—	120	119
Cobalt nitrate	80		
Copper nitrate	4		
Nickel nitrate	80		
Zinc nitrate	400		
Bismuth nitrate	400	60.0	60.0
Gold chloride	400		
Platinum chloride	400		
Calcium nitrate	80		
Zirconium nitrate	400	35.0	34.0
Manganese nitrate	40		
Mercurous nitrate	80		
Lanthanum nitrate	80		
Strontium nitrate	100	45.0	45.0

The effect of pH is shown in Fig. 3. The color intensity between pH 12.6 and 12.68 remains constant; therefore, all determinations were carried out between these pH values.

Since the color intensity remains constant at 95–100°, all absorbance measurements were carried out at this temperature. Below this temperature, the color intensity is not maximum. Ten minutes heating time was found optimum.

The effect of reagent concentration is shown in Fig. 4.

The results for the determination of palladium are shown in Table 1 which shows the reliability of the method.

The color reaction is specific for palladium and no other cation or anion gives any color with the color producing reagent. The quantitative assessment of the tolerable amounts of different ions under experimental conditions is given in Table 2.

The color producing reagent is available easily at low cost. The method does not involve the use of any organic solvent for rigid conditions and the color is stable for a sufficiently long time. This procedure

TABLE 2

QUANTITATIVE ASSESSMENT OF TOLERABLE AMOUNTS OF DIFFERENT IONS

Ion	Maximum amount not interfering [a] (%)	Ion	Maximum amount not interfering [a] (%)
Mg^{2+}	80	In^{3+}	40
Ga^{3+}	400	Au^{3+}	400
Ca^{2+}	80	W^{6+}	400
Te^{4+}	400	As^{3+}	200
La^{3+}	80	Ni^{2+}	80
U^{6+}	200	Co^{2+}	80
Th^{4+}	80	Pt^{4+}	400
Zr^{4+}	400	Ru^{3+}	40
Cu^{2+}	4	Sn^{4+}	400
Cr^{3+}	10	Rh^{3+}	10
Pb^{2+}	80	Fe^{3+}	10
Bi^{3+}	400	V^{5+}	60
Cd^{2+}	80	Zn^{2+}	400
Mn^{2+}	40	Sb^{5+}	2
Ag^{+}	80	Ge^{4+}	20
Mo^{6+}	80	Se^{4+}	300
Tl^{3+}	80	Ti^{4+}	400
Ce^{3+}	80	I^{-}	400
Hg^{2+}	80	Cl^{-}	400
Be^{2+}	200	Br^{-}	400
Sr^{2+}	100	NO_3^{-}	300
		SO_4^{2-}	400
		SO_3^{2-}	400
		SCN^{-}	400
		CH_3COO^{-}	200
		CrO_4^{2-}	400

[a] Solution containing 50 μg/ml of Pd^{2+} was taken and different amounts of various compounds were added under experimental conditions of temperature and pH. The percentage of various ions is with respect to the amount of palladium.

has an advantage over other methods because palladium can be determined in the presence of platinum, gold, copper, ruthenium, and selenium, etc.; and its sensitivity is comparable to most sensitive methods reported in the literature (*12, 20, 25*). The mechanism of the color reaction is not clear.

SUMMARY

Palladium reacts with melamine in an alkaline medium to give a green color having maximum absorbance at 620–630 nm. The color reaction is specific for palladium, has 1 μg/ml as visual limit of identification, and provides the basis of a new spectrophotometric method for the determination of palladium. The quantitative assessment of the tolerable amounts of different ions which do not interfere with the determination is reported. The mechanism of the color reaction is not clear.

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MICROCHEMICAL JOURNAL 17, 26–30 (1972)

A Colorimetric Method of Estimating Hydrolyzable Chlorine in Lindane (γ-Hexachlorocyclohexane)

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INTRODUCTION

Many methods, physical, chemical, and biological, are reported in the literature for estimation of lindane either in pure form or along with food materials. Among the chemical methods, estimation of DDT and BHC, the common chlorinated insecticides through hydrolyzable chlorine are reported by Gunther (*1*) and Howard (*2*). For dehydrohalogenation, Gunther suggested alcoholic potassium hydroxide and Howard recommended monoethanolamine. Butterfield *et al.* (*3*) considered that monoethanolamine is superior to alcoholic potassium hydroxide since it hydrolyzes only organochlorine insecticides but not the associated interfering fatty materials in foods. Majumder and Pingale (*5*) indicated the specificity and suitability of the method for the estimation of residues of mixtures of insecticides. The moles of chlorine labile to monoethanolamine at temperatures as observed for some of the insecticides are: BHC (25°C), 3; DDT (70°C), 1; chlordane (70–100°C), 2; Toxaphane (70–100°C), 1; and Dieldrin (140°C under vacuum), 2. Majumder and Pingale (5) reported the applicability of a labile chlorine method for the estimation of aldrin by using monoethanolamine as the dehydrohalogenating agent. The insecticide is hydrolyzed with the reagent in vacuum-sealed tubes at 140°C for 16 hours when six chlorine atoms are labilized. Labilization of chlorine from polychlorinated dimethanonaphthalene insecticides has further been simplified by Krishna *et al.* (*6*), using metallic sodium along with monoethanolamine. The improvement suggested requires only 2 hours' hydrolysis at 100–110°C (without vacuum) as compared to 16 hours' hydrolysis under vacuum at 140°C. Chlorine liberated in the above technique is measured by Volhard's method and in routine work the above procedure is found satisfactory (*4*). It has been observed by Peters *et al.* (*8*) that the estimation of chloride ion of the insecticide by Volhard's method gives rise to a large error in the microgram scale because of end point and solubility fac-

tors. For increased sensitivity, potentiometric method for chloride ion has been recommended (*7*). In residues involving potentiometric method for chloride ion titrations the method is insensitive (*8*) below about 50 μg of chloride ion but quite convenient above this level. Also large amounts of salts involved in this estimation, due to the interfering organic and inorganic chlorine containing compounds, will interfere with potentiometric titrations (*9*).

To estimate by Volhard's titration which requires a lot of time and manipulations and to increase the sensitivity of the estimation, the suitability of a colorimetric method based on the interaction of *p*-nitrobenzenediazonium fluoborate (*10*) with unused monoethanolamine in the hydrolyzed reaction mixture of the insecticide is investigated. The method has been verified with the estimation of known samples of lindane as well as with the determination of lindane by Volhard's method.

EXPERIMENTAL METHODS

Spectronic-20 (Bausch and Lomb) colorimeter is used to record the percentage transmission.

Monoethanolamine (supplied by 'Narden' company Holland) is used.

P-Nitrobenzenediazonium Fluoborate) (supplied by Eastman Organic Chemicals, Rochester, New York is employed. One of the chemicals is dissolved in about 50 ml of ethylalcohol and warmed in a waterbath for about 15–20 minutes. It is then made up to 100 ml with ethyl alcohol. This reagent is found to develop blood red color immediately with primary and secondary amines and the reaction has been made use of by Visweswariah *et al* (*10*) in the estimation of diphenylamine.

Dehydrochlorination of lindane. Specified quantity of lindane (Merck grade) is taken in a stoppered test tube and is used.

Procedure

Quantities of lindane varying between 10 and 100 mg are separately transferred to 10 glass stoppered test tubes, 1 ml of monoethanolamine is added to each of the test tubes. While adding monoethanolamine, care is taken that all the particles of lindane adhering against the walls of the test tube are washed to the bottom. The stoppers are closed and the test tubes are immersed for 45 minutes in a water bath kept continuously boiling. After the hydrolysis, the reaction mixture is cooled to room temperature and the test tubes are washed with distilled water to transfer the contents to a 50-ml volumetric flask and finally made up to the volume; 10 ml of this made up solution is taken in a 50-ml volumetric flask and 1 ml of 0.1% *p*-nitrobenzenediazonium fluoborate solu-

tion is added and shaken well. The colorless reaction product turned blood red color on the addition of p-nitrobenzenediazonium fluoborate solution. It is then made up to 50 ml and the color is read against a reagent black, at 520 mμ when it showed maximum absorption (Fig 1). The color faded gradually after 15–20 minutes' exposure. The color complex changed violet red on the addition of concentrated nitric acid, hydrochloric acid, and sulfuric acid, while weak acids like formic acetic and propionic did not alter the color. Ammonium hydroxide, potassium hydroxide, and sodium carbonate turned the blood red color complex into brick red color.

DISCUSSION

The method obeys Beer's law between 80 to 320 μg (Fig. 2). Above 320-μg concentration, the color faded quickly due to higher concentration of trichlorobenzene released during hydrolysis. Also, the comparative high turbidity in the reaction mixture interfered in the colorimetric measurement.

The colored complex obtained by the reaction of *p*-nitrobenzenediazonium fluoborate with monoethanolamine is stable for 15–20 minutes, and faded gradually. This is found to be due to the presence of 1,2,3- and 1,2,4-trichlorobenzene released by lindane during hydrolysis with monoethanolamine. In lower concentrations of lindane estimation, the color is found to be stable up to 20 minutes. But as the concentration of lindane is increased, a proportionately larger quantity of trichlorobenzene is released during hydrolysis, which in turn caused quick disappearance of color complex. In contrast to the diphenylamine estimation (*10*), and also ethylene dibromide and methyl bromide estimations, the unstability of the colored complex, in presence of trichlorobenzenes, indicates that it has to be separated from the hydrolyzed

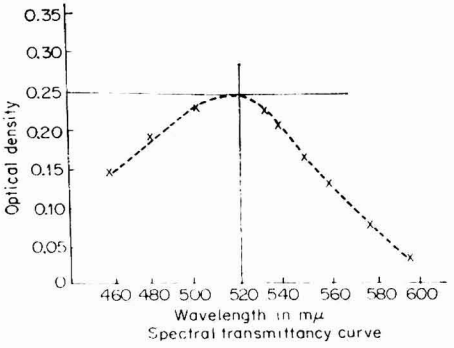

FIG. 1. Spectral transmittancy curve.

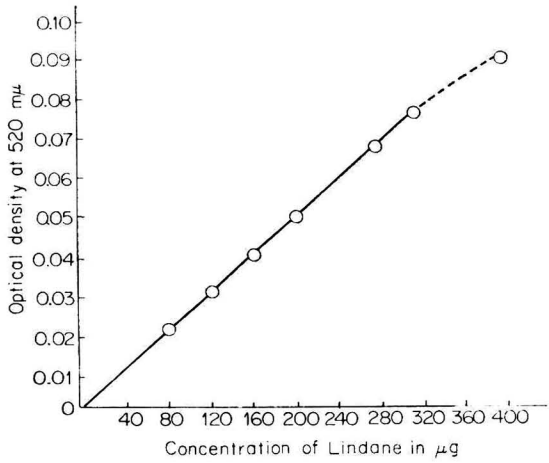

Figure 2.

reaction mixture before the addition of color-developing reagent. The change in the colored complex into brick red color on the addition of alkali is due to the alkali reacting with the unreacted *p*-nitrobenzenediazonium fluoborate. Since the red color is also developed with primary, secondary, and tertiary amines, their presence will interfere in the estimation of lindane.

Lindane is estimated in a few samples by the standardized method and comparing with Volhard's method (*1*) (Table 1). In case of colorimetric method, the percentage of error varied between (±) 0.8 to 1.9%; whereas Volhard's method gave an error ranging between (±) 2.4 and 5.2%. The comparative high percentage in Volhard's method is due to the visual end point difficulty and solubility factors as evidenced by Peters *et al.* (*8*). The recommended method saves much time, labor

TABLE 1

COMPARATIVE STUDY OF HYDROLYSABLE CHLORINE CONTENT OF LINDANE BY THE COLORIMETRIC AND VOLHARD'S METHOD

	Hydrolyzable chlorine content of lindane (mg)			
	I	II	III	IV
Calculated value	365	365	365	365
Colorimetric method	359	368	360	372
Volhard's method	353	356	350	384
Error (%)				
Colorimetric method	(−) 1.6	(+) 0.8	(−) 1.5	(+) 1.9
Volhard's method	(−) 3.3	(−) 2.5	(−) 4.3	(+) 5.2

in preparing standard solutions, titration and other manipulations, which are normally encountered in Volhard's method. Above all, the sensitivity in 100% more than Volhard's method.

This method of determining the hydrolyzable chlorine content in polychlorinated insecticides like DDT, BHC, and any of the isomers of BHC can be followed wherever monoethanolamine is used as dehydrohalogenating agent. The estimation of lindane residues in treated food materials can be estimated by this method after the usual cleanup technique involving solvent extraction and column chromatography separation of the pesticide.

SUMMARY

A rapid colorimetric method of determining hydrolyzable chlorine content of lindane has been developed. The region of maximum absorption, Beer's law is obeyed between 80 and 320 μg/ml of solution. The sensitivity of this method is compared with Volhard's titration method and is found to be superior. With the above method, the insecticide can be estimated in pure form with an accuracy of ±0.8 to 1.9%.

ACKOWLEDGMENT

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MICROCHEMICAL JOURNAL 17, 31–60 (1972)

Qualitative Analysis of Primers, Tracers, Igniters, Incendiaries, Boosters, and Delay Compositions on a Microscale by Use of Infrared Spectroscopy

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INTRODUCTION

The constituents of primers, tracers, igniters, incendiaries, boosters, and delay compositions are ordinarily identified by wet chemical tests for the organic compounds, anions, and metals (frequently emission spectrography is used for the metals). These wet tests have been found to be untrustworthy, especially if applied on a mircroscale.

This laboratory undertook an investigation on the application of infrared spectroscopy to the detection of the organic compounds and inorganic compounds present in primers, tracers, igniters, incendiaries, boosters, and delay compositions. It was believed that infrared spectroscopy, together with emission spectroscopy (for inorganic materials that do not give infrared spectra), would provide an excellent means for the qualitative analysis of the constituents in question.

Pristera and Fredericks (*1*) have compiled spectra of most constituents of propellants and explosives. However, their compendium does not include many of the very sensitive explosive compounds found in primers, tracers, igniters, incendiaries, boosters, and delay compositions and it does not cover the far infrared. Miller and Wilkins (*2*) and Miller *et al.* (*3*) have compiled spectra of inorganic compounds. Their collection does not include the organic compounds that would be found in primers, tracers, igniters, incendiaries, boosters, and delay compositions and does not cover the far infrared. Pristera and Fredericks (*1*) and Miller et al. (*2*, *3*) used a prism-type infrared spectrophotometer. The present authors used a grating type.

EXPERIMENTAL METHODS

Apparatus and Reagents

Perkin-Elmer Model 621 infrared spectrophotometer.

Evacuable potasssium bromide die (Model 186-0025, Perkin-Elmer Corp., Norwalk, CT).

Adapters for mounting KBr discs on spectrophotometer.
Twenty-ton hydraulic press.
Vacuum pump.
Mortar (o.d. 50 mm) with pestle.
Plastic microspatula.
Potassium bromide, infrared grade (Harshaw Chemical Co., Cleveland, OH).

Preparation of Potassium Bromide Pellets

Grind the KBr (in small portions) with the mortar and pestle and store in a desiccator until needed.

Add 1–2 mg of the sample to the mortar with the plastic microspatula, add 300 mg of the preground KBr, and mix thoroughly. Grind the mix for about 3 minutes and then scrape it loose from the mortar surface. Transfer to the die. insert the plunger, evacuate for 3 minutes without applying pressure, and then apply 18,000 lb. pressure with the hydraulic press for 5 minutes. Disassemble the die and push out the KBr pellet with a gentle application of the hydraulic press. Mount the pellet on an adapter and scan the spectrum from 2.5 to 50 μ with a blank KBr pellet in the reference beam. Use a dry air purge for the far infrared region (30 to 50 μ). The following settings were used on the Perkin-Elmer Model 621 infrared spectrophotometer: slit program, 1000; gain 5.1; attenuator speed, 1100; scan drive, 1; scan time, 16; supression, 8; scale, 1×; source current, 0.8.

RESULTS AND DISCUSSION

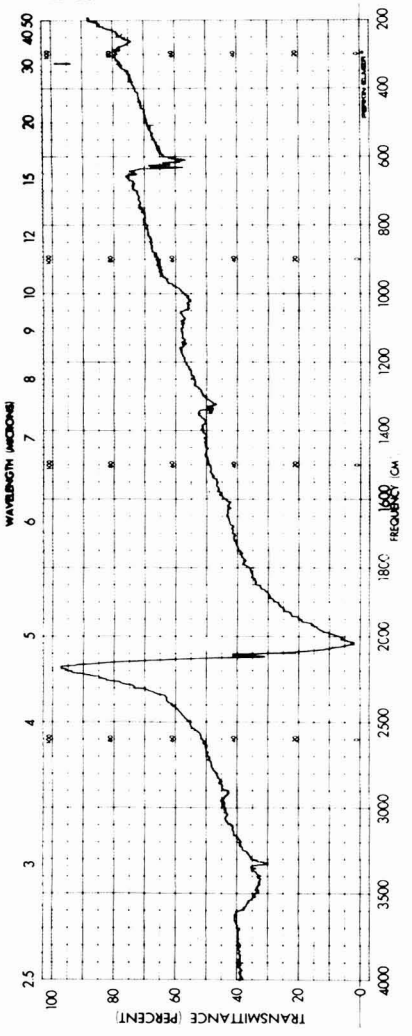
The spectra obtained for the 43 commonly used ingredients of primers, tracers, igniters, incendiaries, boosters, and delay compositions are shown in Figs. 1 to 43. These ingredients are arranged (Table 1) according to function as follows: primary explosives, high explosives, oxidizers, color intensifers, binders, and miscellaneous. Compounds having more than one usage are listed according to their major function; for example, polyvinyl chloride is used as a binder or color intensifier but is listed under binders. Lead thiocyanate, the only fuel that gives a spectrum, is listed under miscellaneous; other fuels (magnesium, aluminum, zirconium, titanium, boron, silicon, and metallic hydrides) do not give infrared spectra.

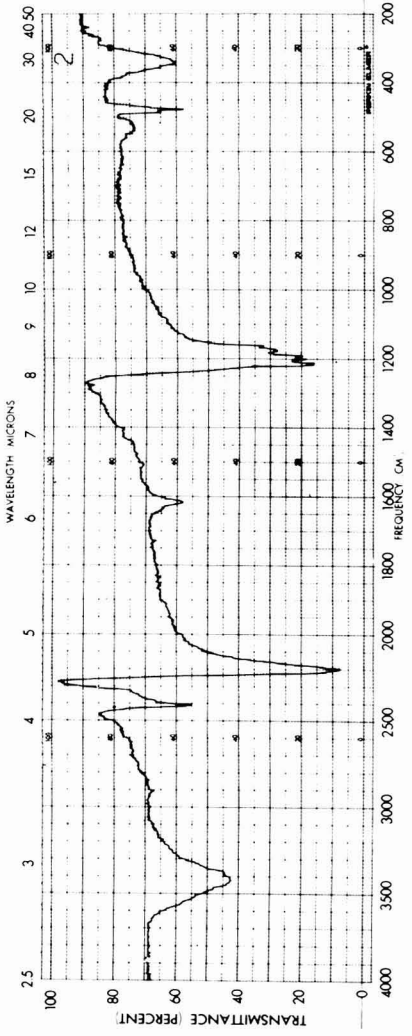
The spectra of 7 typical explosive compositions, together with the notation of the peaks identifying the principle constituents, are shown in Figs. 44 to 50. The conclusions concerning the analysis of these compositions drawn from these spectra and the emission spectrographic analysis are shown in Table 2.

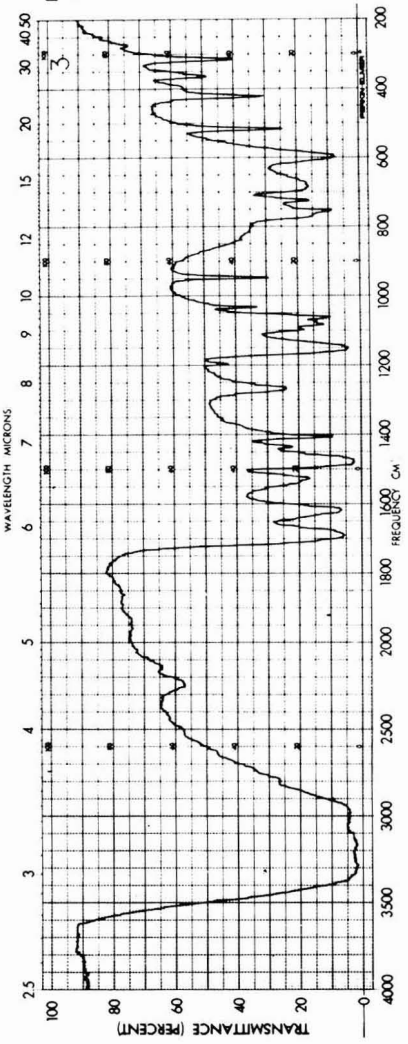
TABLE 1

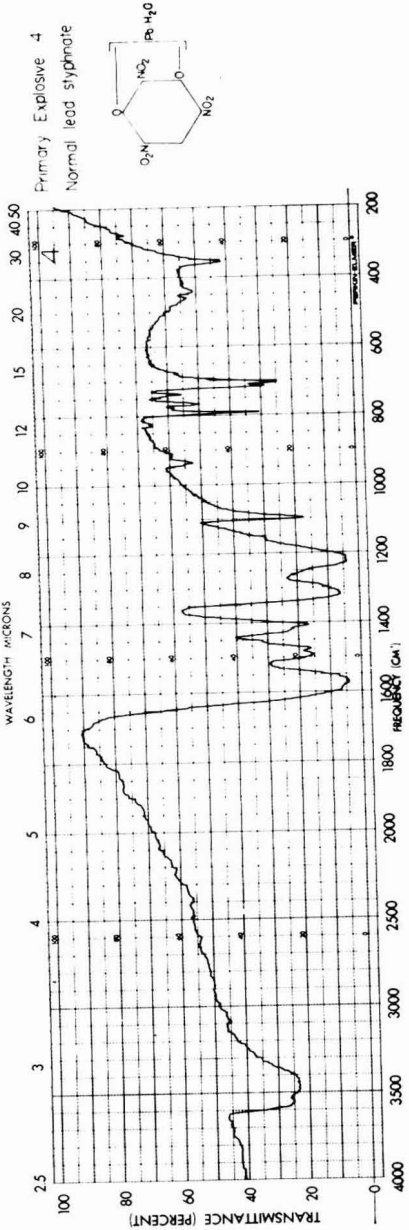
IDENTIFICATION OF SPECTRA

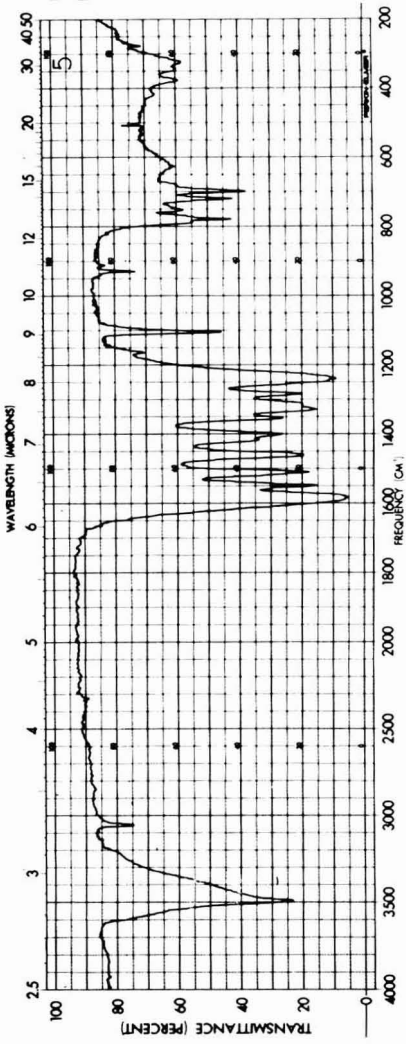
Spectrum	Code
1. Lead azide	Primary explosive 1
2. Mercury fulminate	Primary explosive 2
3. Tetracene	Primary explosive 3
4. Normal lead styphnate	Primary explosive 4
5. Basic lead styphnate	Primary explosive 5
6. RDX (cyclotrimethylenetrinitramine)	High explosive 1
7. HMX (cyclotetramethylenetetranitramine)	High explosive 2
8. Tetryl (2,4,6-trinitrophenylmethylnitramine)	High explosive 3
9. PETN (pentaerythritol tetranitrate)	High explosive 4
10. TNT (2,4,6-trinitrotoluene)	High explosive 5
11. Nitrocellulose	High explosive 6
12. Styphnic acid (2,4,6-trinitroresorcinol)	High explosive 7
13 Ammonium nitrate	Oxidizer 1
14. Sodium nitrate	Oxidizer 2
15. Potassium nitrate	Oxidizer 3
16. Barium nitrate	Oxidizer 4
17. Strontium nitrate	Oxidizer 5
18. Lead nitrate	Oxidizer 6
19. Potassium chlorate	Oxidizer 7
20. Ammonium perchlorate	Oxidizer 8
21. Potassium perchlorate	Oxidizer 9
22. Barium chromate	Oxidizer 10
23. Lead chromate	Oxidizer 11
24. Barium peroxide	Oxidizer 12
25. Strontium peroxide	Oxidizer 13
26. Lead monoxide	Oxidizer 14
27. Red lead (Pb_3O_4)	Oxidizer 15
28. Ferric oxide	Oxidizer 16
29. Molybdenum trioxide	Oxidizer 17
30. Dechlorane (perchloropentacyclodecane)	Color intensifier 1
31. Sodium oxalate	Color intensifier 2
32. Strontium oxalate	Color intensifier 3
33. Gum arabic	Binder 1
34. PVC (polyvinyl chloride)	Binder 2
35. Parlon (chlorinated rubber)	Binder 3
36. Stearic acid	Binder 4
37. Wax (hydrocarbon type)	Binder 5
38. Calcium resinate	Binder 6
39. Ethylcellulose	Binder 7
40. Oxamide	Misc 1
41. Barium monoxide	Misc 2
42. Lead thiocyanate	Misc 3
43. Calcium carbonate	Misc 4
44. Detonator	Mix 1
45. Igniter	Mix 2
46. Primer	Mix 3
47. Primer	Mix 4
48. Primer	Mix 5
49. Detonator	Mix 6
50. Detonator	Mix 7

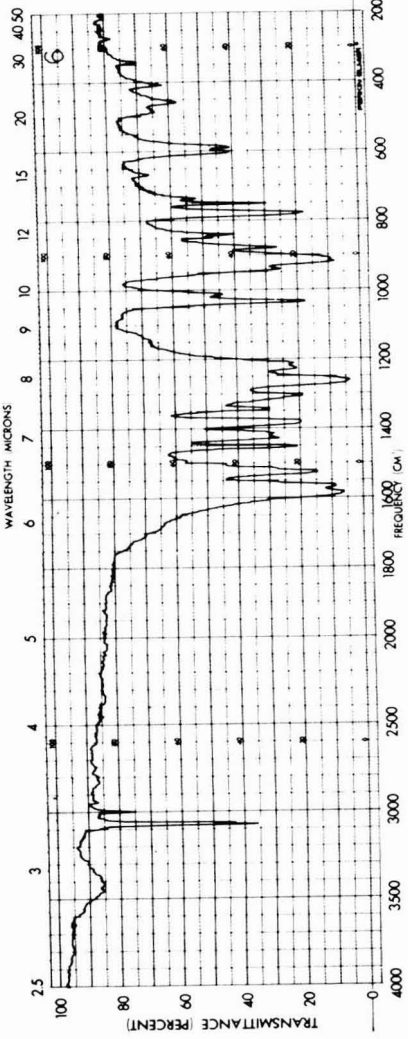

Primary Explosive 1
Lead azide
$Pb(N_3)_2$
WAVELENGTH (MICRONS)
FREQUENCY (CM)
TRANSMITTANCE (PERCENT)

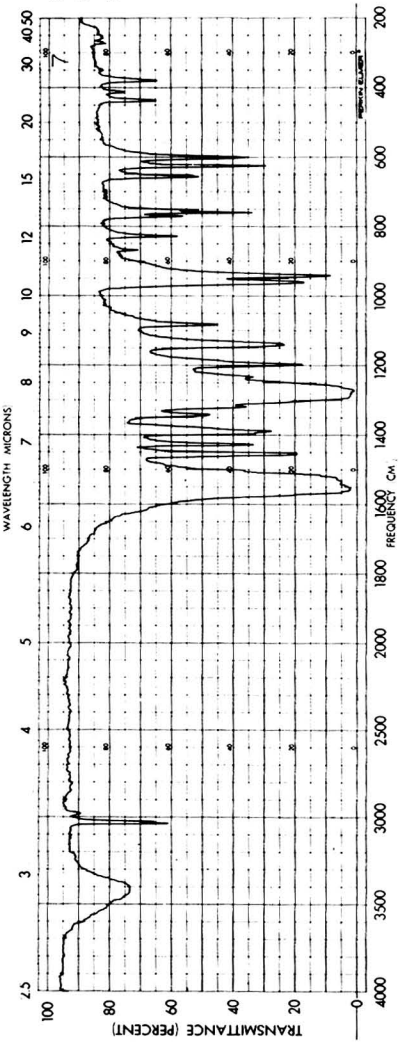

Primary Explosive 2
Mercury fulminate
$Hg(ONC)_2$
WAVELENGTH MICRONS
FREQUENCY (CM)
TRANSMITTANCE (PERCENT)

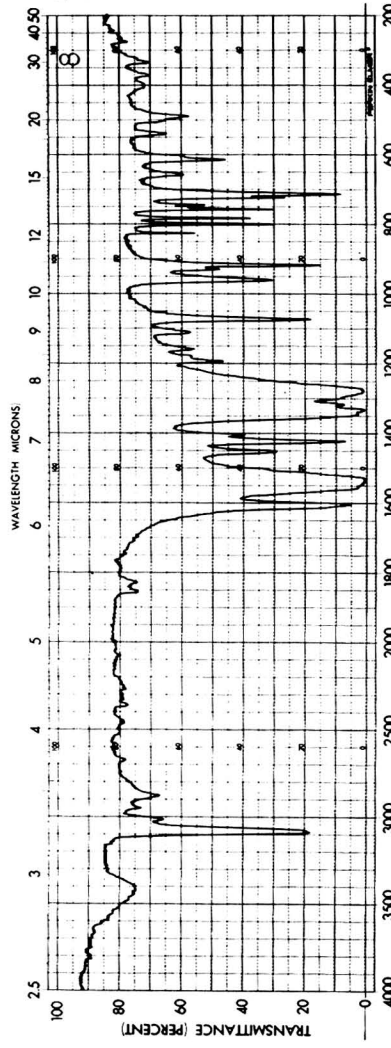

Primary Explosive 3
Tetracene
3
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)

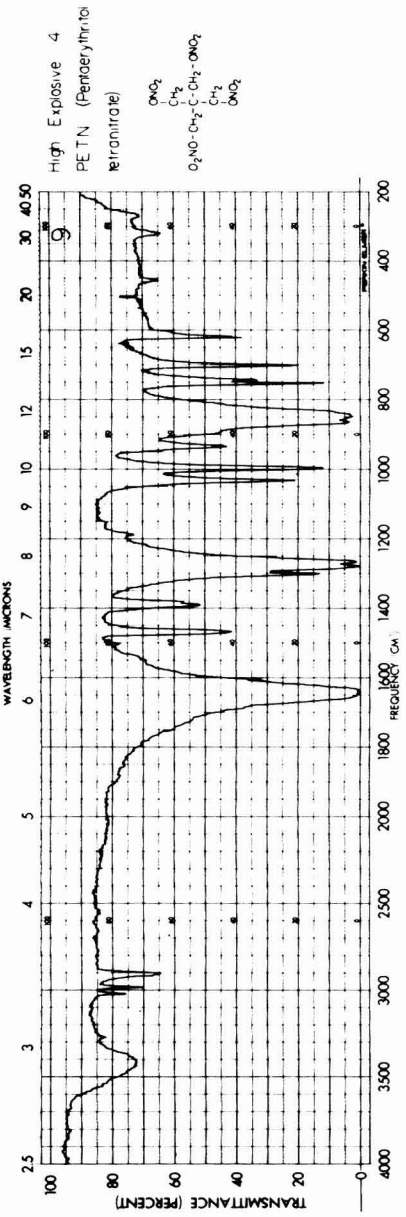

Primary Explosive 4
Normal lead styphnate
4
WAVELENGTH MICRONS
FREQUENCY (CM)
TRANSMITTANCE (PERCENT)

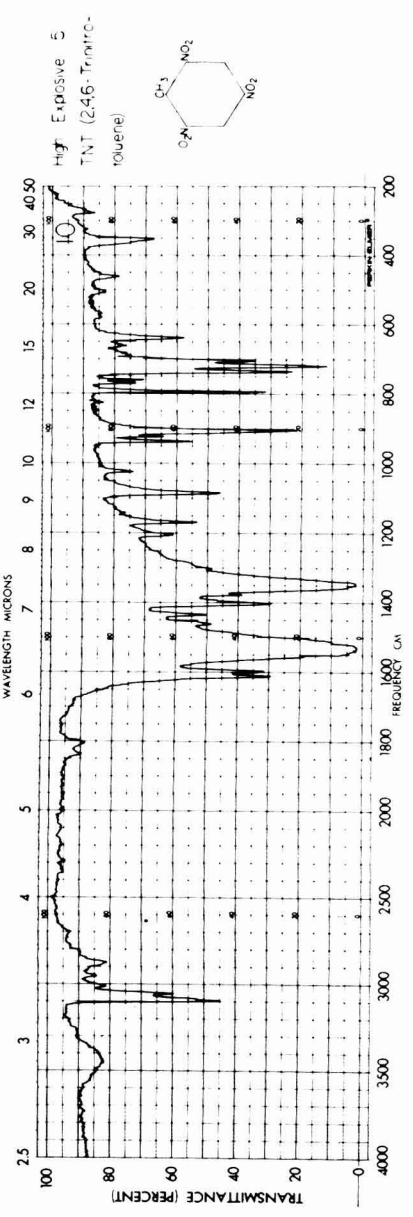

Primary Explosive 5
Basic lead styphnate
5
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

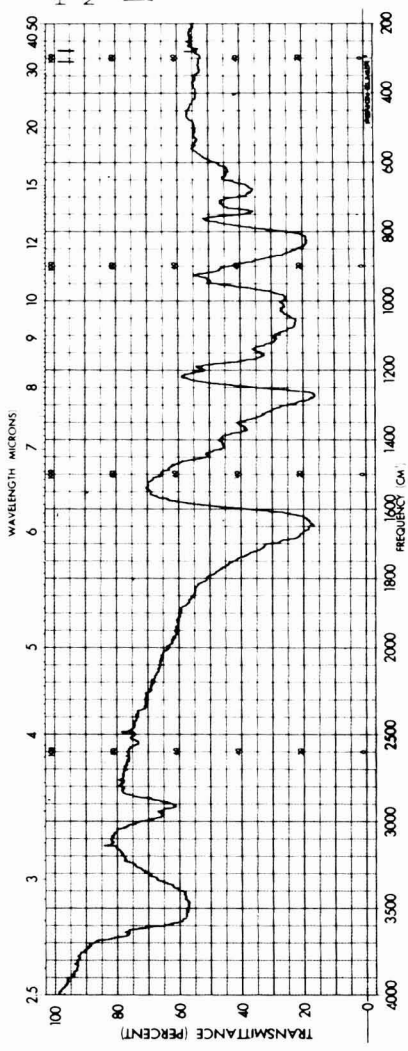

High Explosive I
RDX (Cyclonite,
Cyclotrimethylenetrinitramine)
6
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

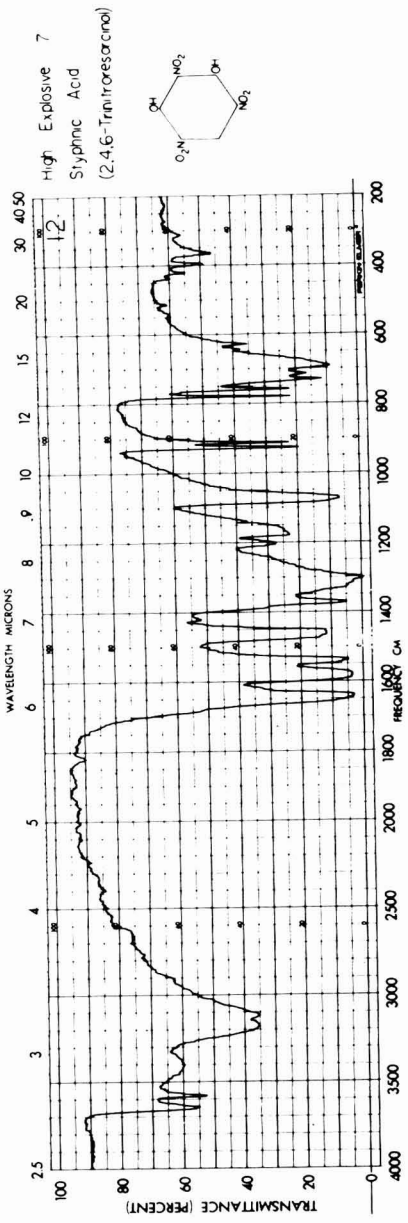

High Explosive 2
HMX (Cyclotetra-
methylenetetranitramine)
7
TRANSMITTANCE (PERCENT)
WAVELENGTH MICRONS
FREQUENCY CM.

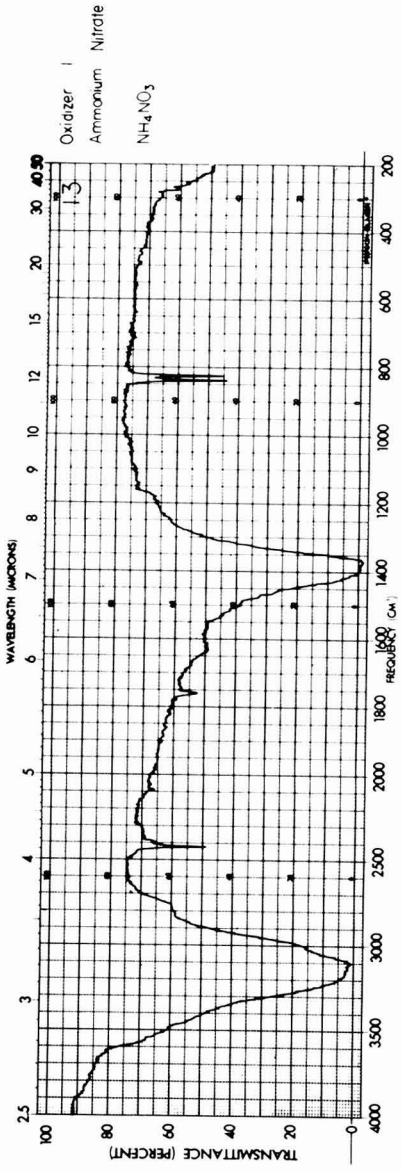

High Explosive 3
Tetryl (2,4,6-Trinitro-
phenylmethylnitramine)
8
TRANSMITTANCE (PERCENT)
WAVELENGTH MICRONS
FREQUENCY (CM.)

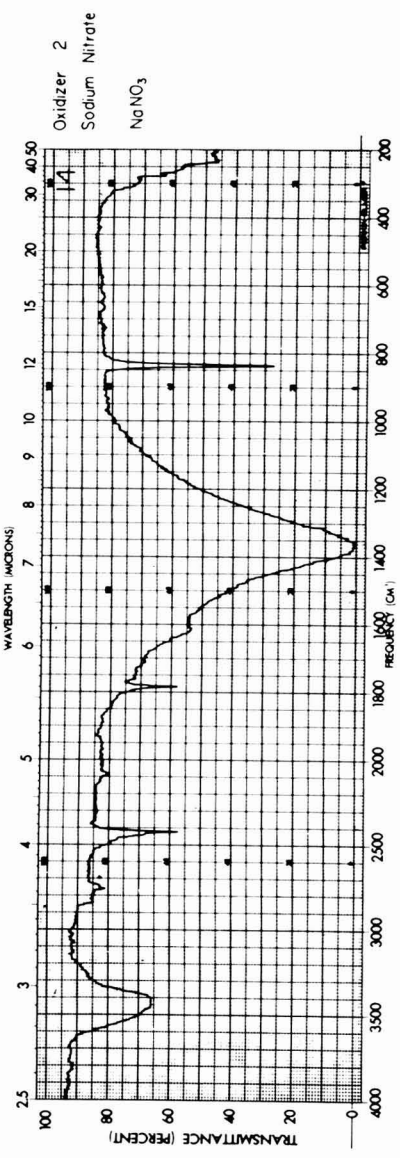

9
High Explosive 4
PETN (Pentaerythritol
Tetranitrate)
ONO2
CH2
O2NO-CH2-C-CH2-ONO2
CH2
ONO2
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)

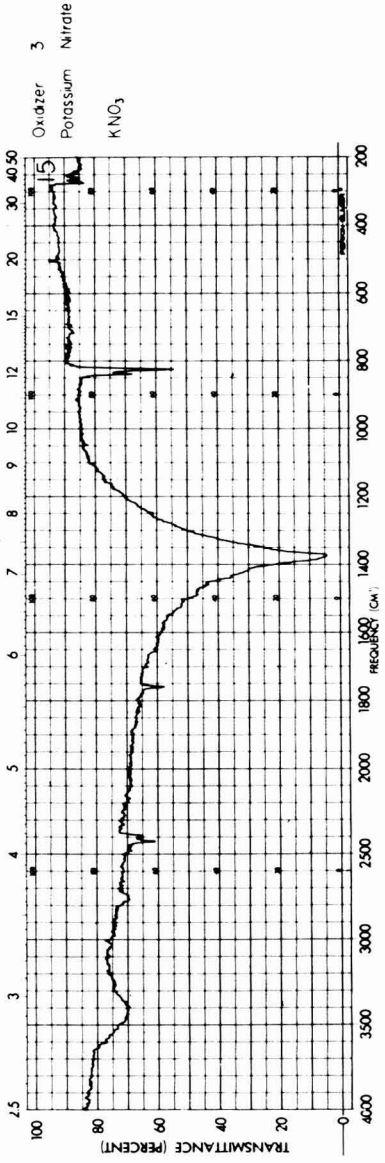

10
High Explosive 5
TNT (2,4,6-Trinitro-
toluene)
CH3
NO2
O2N
NO2
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)

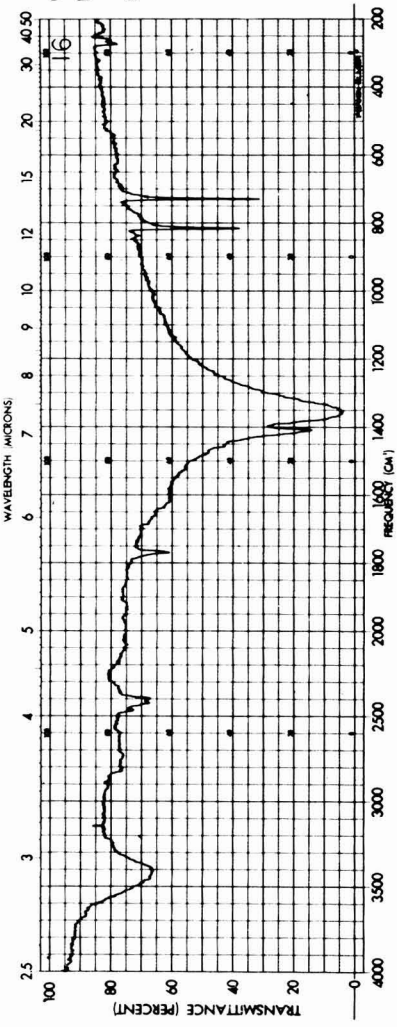

High Explosive 6
Nitrocellulose
$[C_6H_7O_5(NO_2)_3]_x$
11
WAVELENGTH MICRONS
FREQUENCY CM.
TRANSMITTANCE (PERCENT)

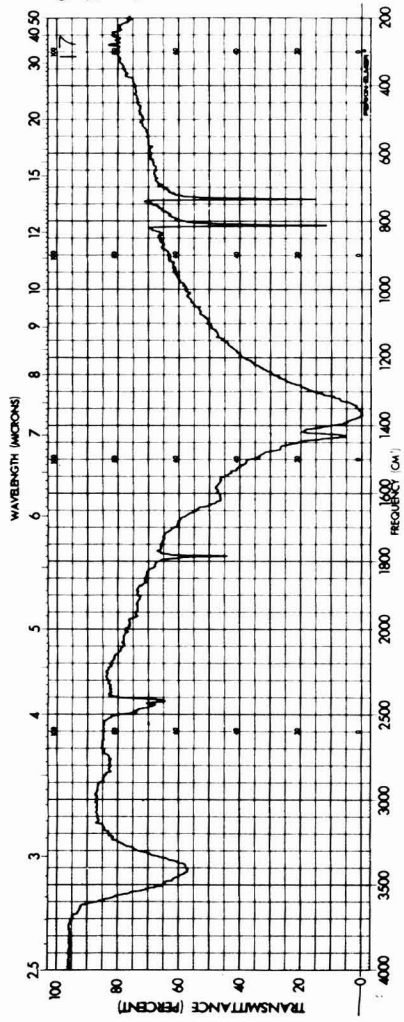

High Explosive 7
Styphnic Acid
(2.4.6-Trinitroresorcinol)
12
WAVELENGTH MICRONS
FREQUENCY CM.
TRANSMITTANCE (PERCENT)

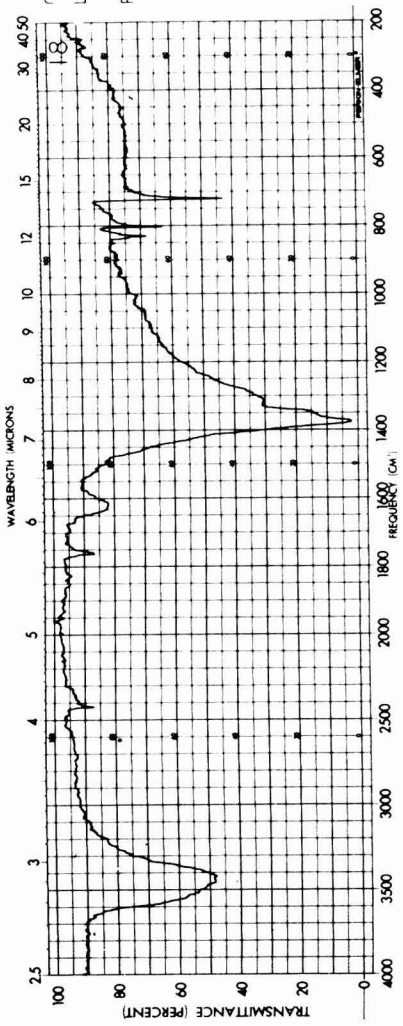

Oxidizer 1
Ammonium Nitrate
NH_4NO_3
13
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

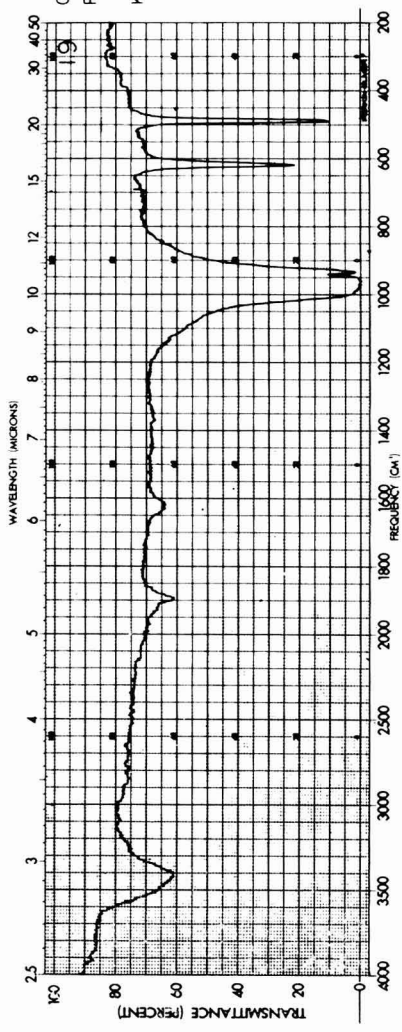

Oxidizer 2
Sodium Nitrate
$NaNO_3$
14
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

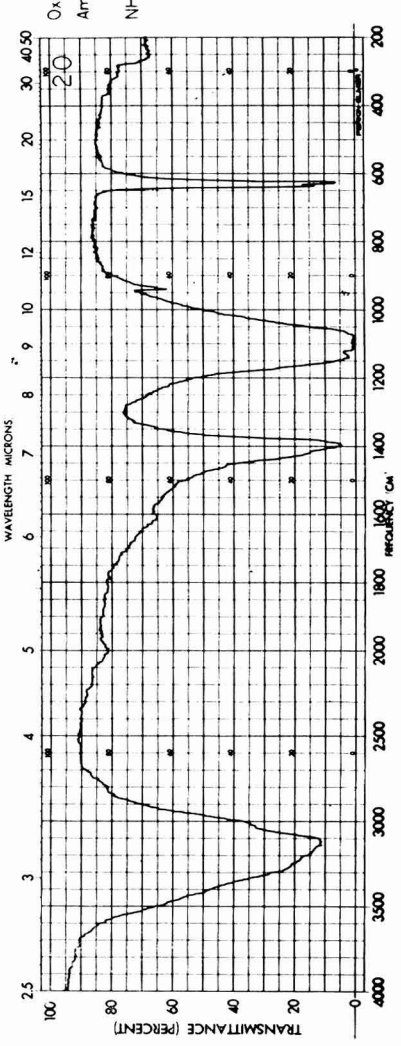

Oxidizer 3
Potassium Nitrate
KNO_3
15
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

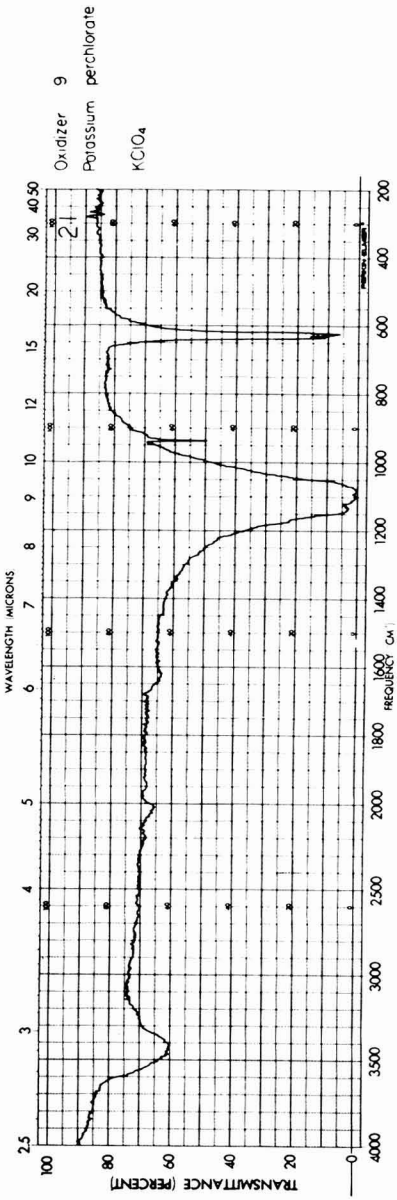

Oxidizer 4
Barium Nitrate
$Ba(NO_3)_2$
16
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

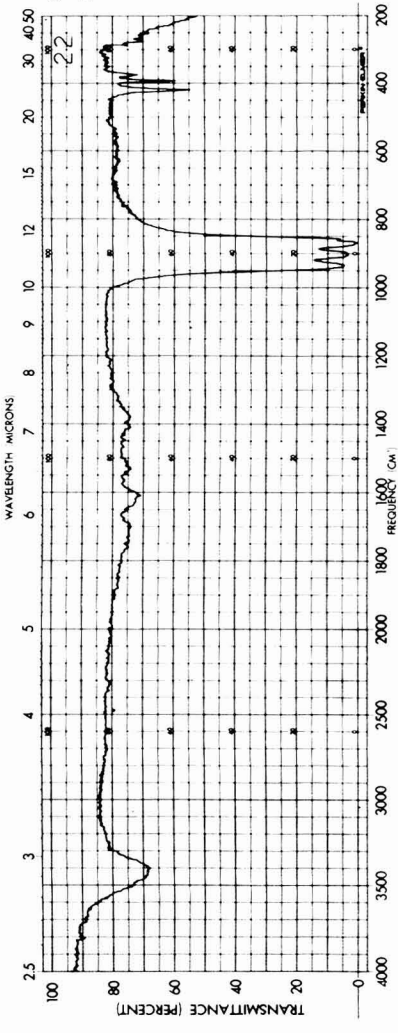

Oxidizer 5
Strontium Nitrate
$Sr(NO_3)_2$
17
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

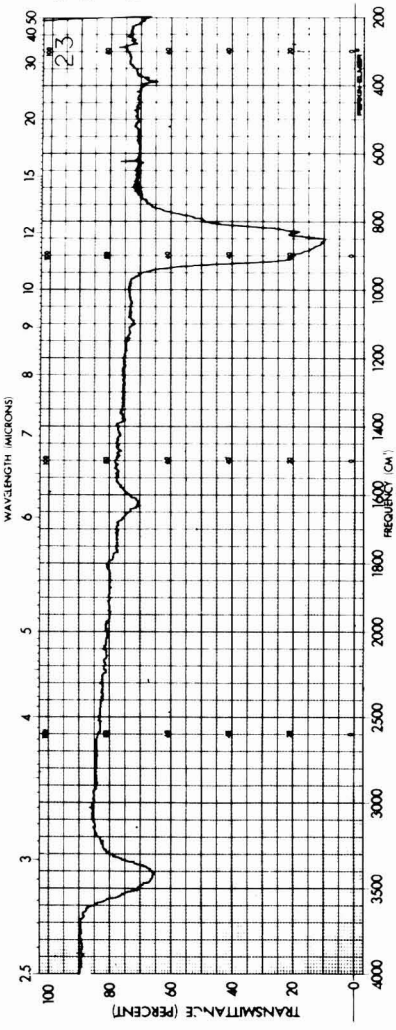

Oxidizer 6
Lead Nitrate
$Pb(NO_3)_2$
18
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

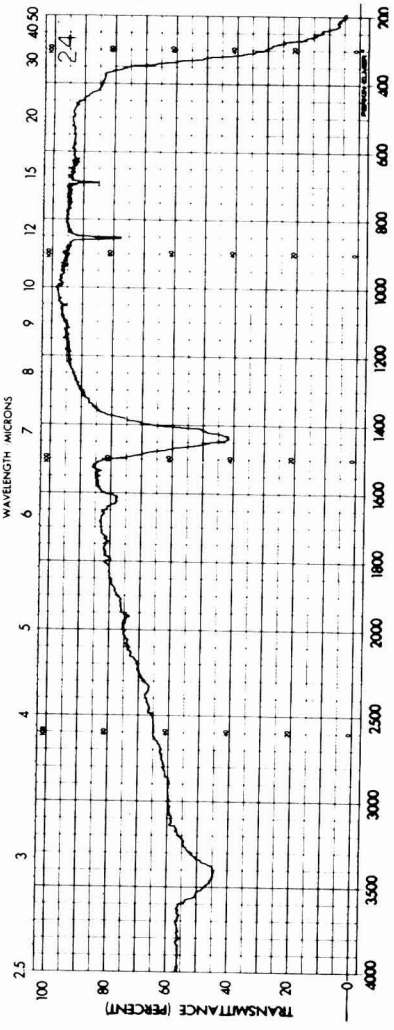

Oxidizer 7
Potassium chlorate
$KClO_3$
19
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

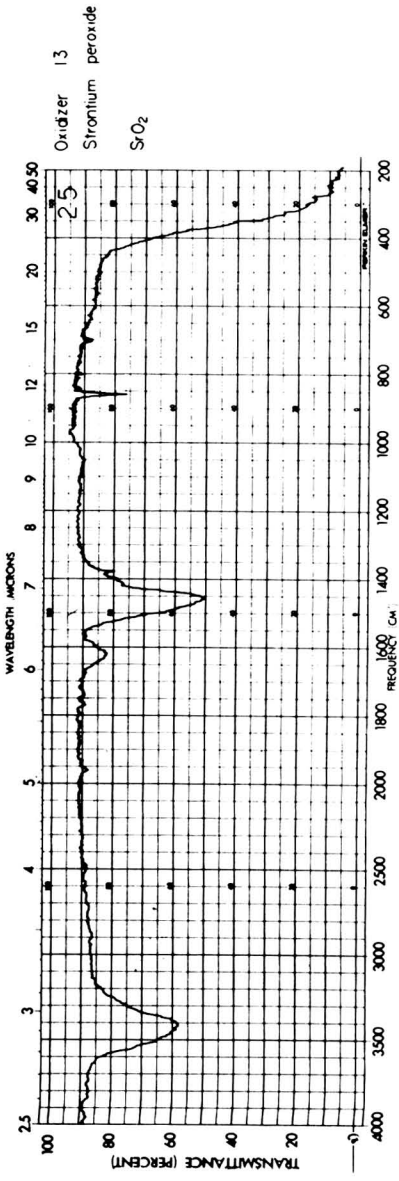

Oxidizer 8
Ammonium perchlorate
NH_4ClO_4
20
WAVELENGTH MICRONS
FREQUENCY CM⁻¹
TRANSMITTANCE (PERCENT)

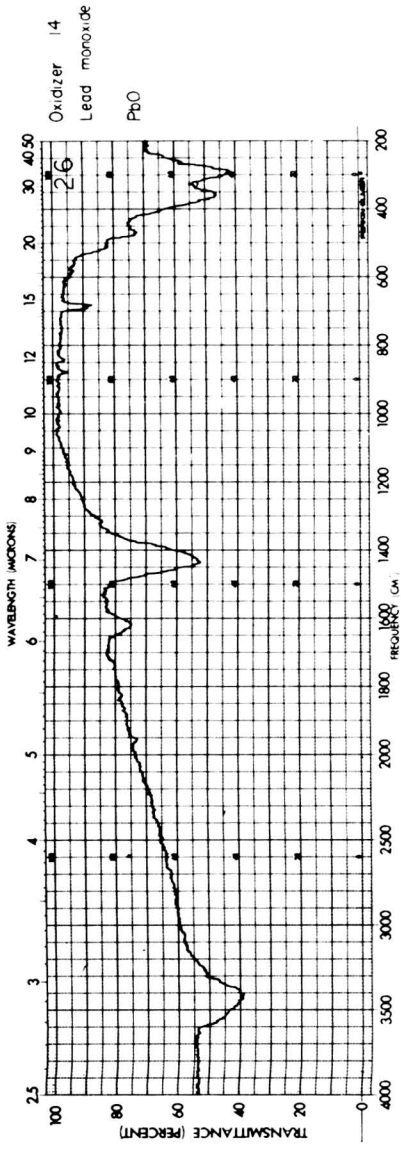

Oxidizer 9
Potassium perchlorate
$KClO_4$
21
WAVELENGTH MICRONS
FREQUENCY CM⁻¹
TRANSMITTANCE (PERCENT)

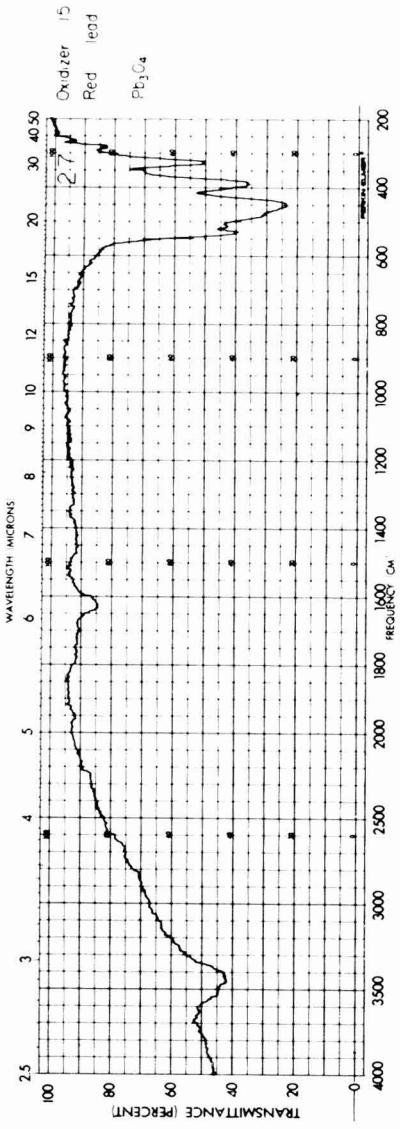

Oxidizer 10
Barium chromate
$BaCrO_4$
22
WAVELENGTH MICRONS
FREQUENCY CM⁻¹
TRANSMITTANCE (PERCENT)

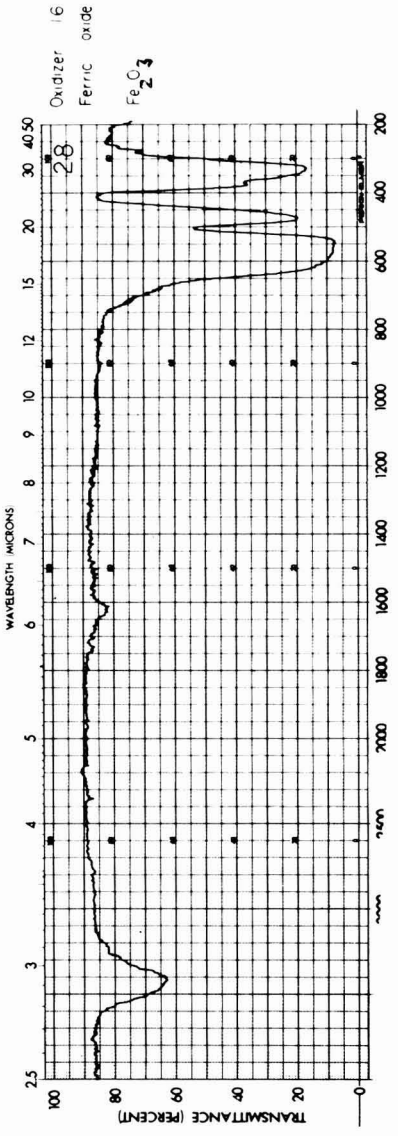

Oxidizer 11
Lead chromate
$PbCrO_4$
23
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

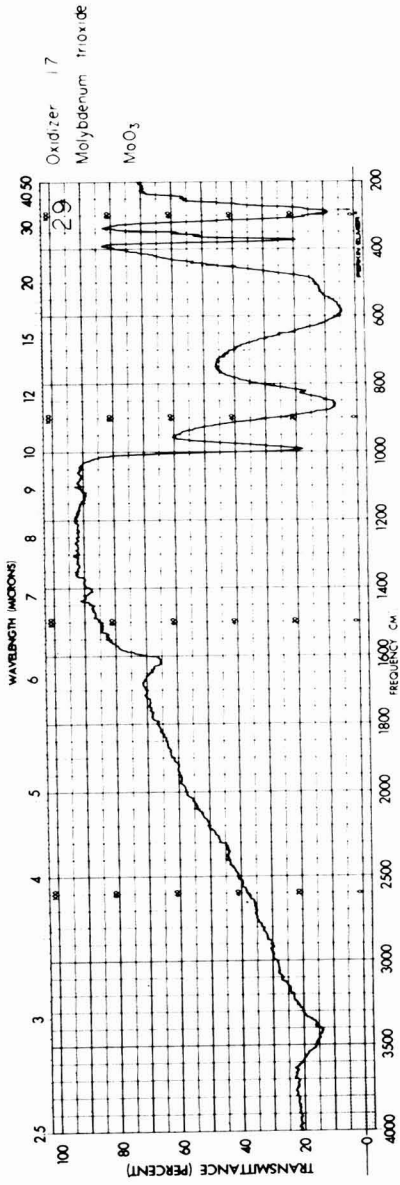

Oxidizer 12
Barium peroxide
BaO_2
24
WAVELENGTH MICRONS
TRANSMITTANCE (PERCENT)

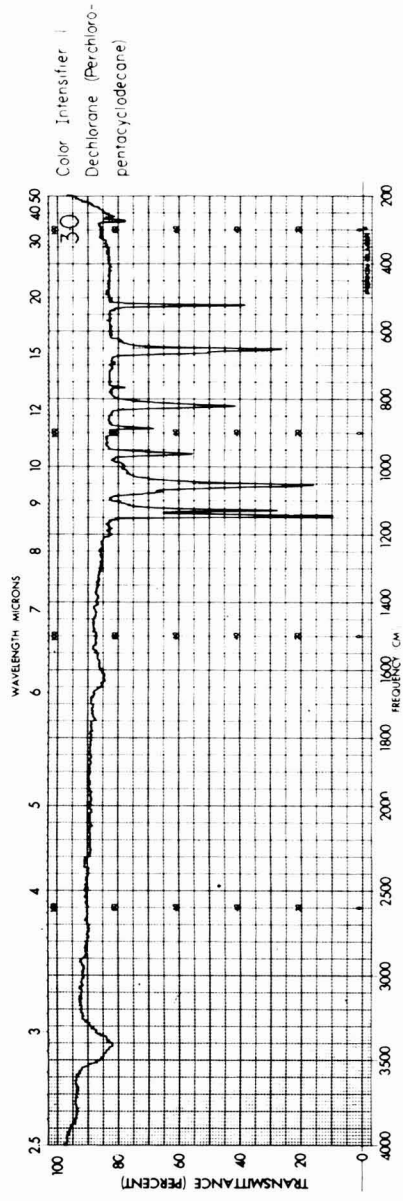

Oxidizer 13
Strontium peroxide
SrO_2
25
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

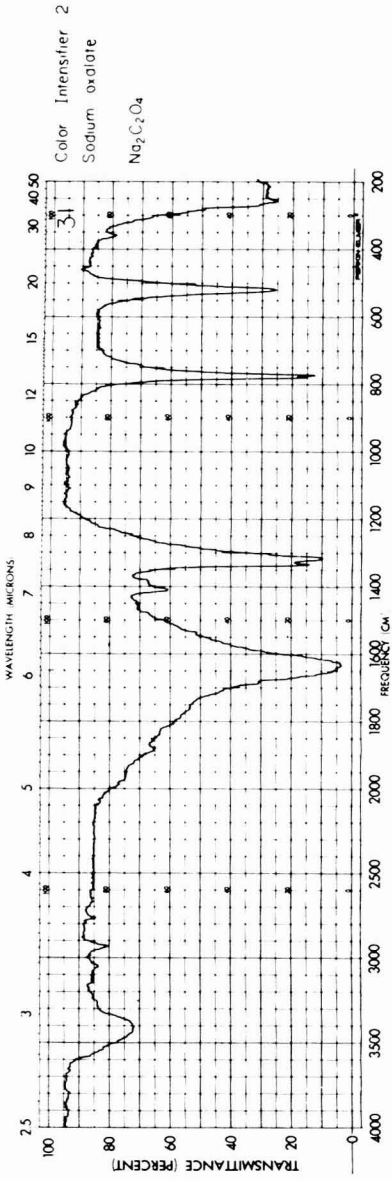

Oxidizer 14
Lead monoxide
PbO
26
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

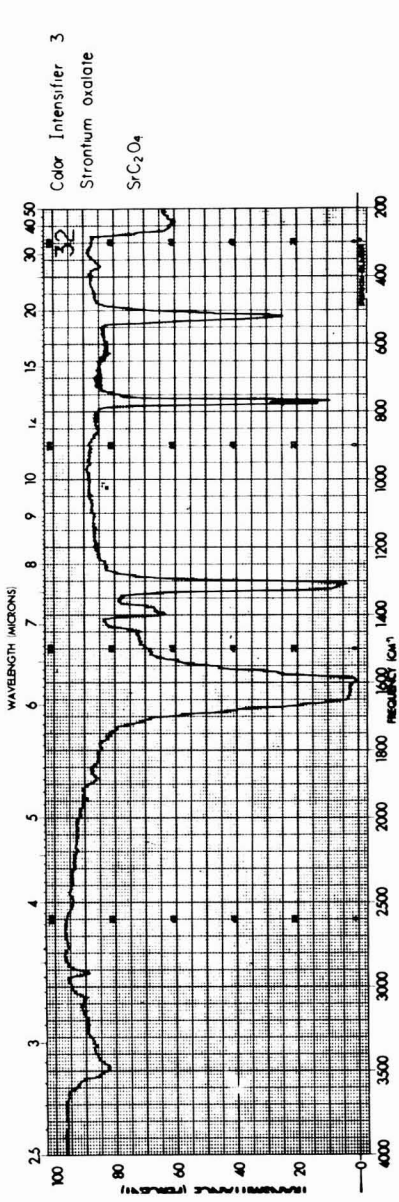

Oxidizer 15
Red lead
Pb_3O_4
27
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)

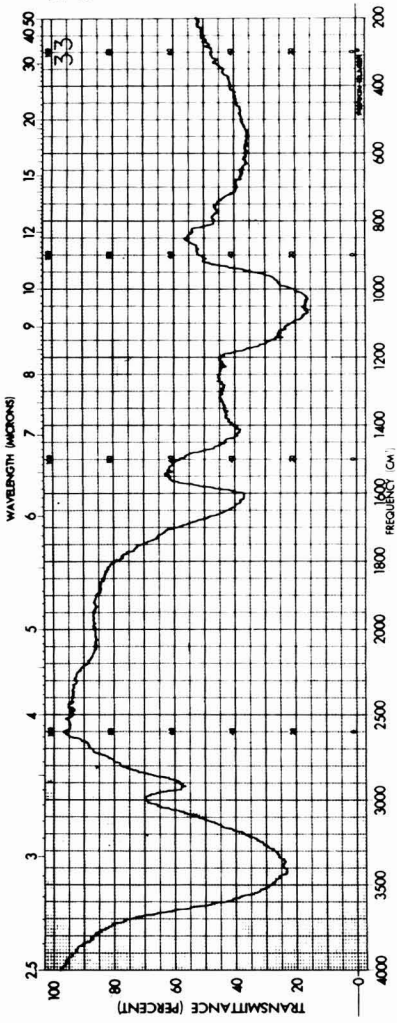

Oxidizer 16
Ferric oxide
Fe_2O_3
28
WAVELENGTH MICRONS
TRANSMITTANCE (PERCENT)

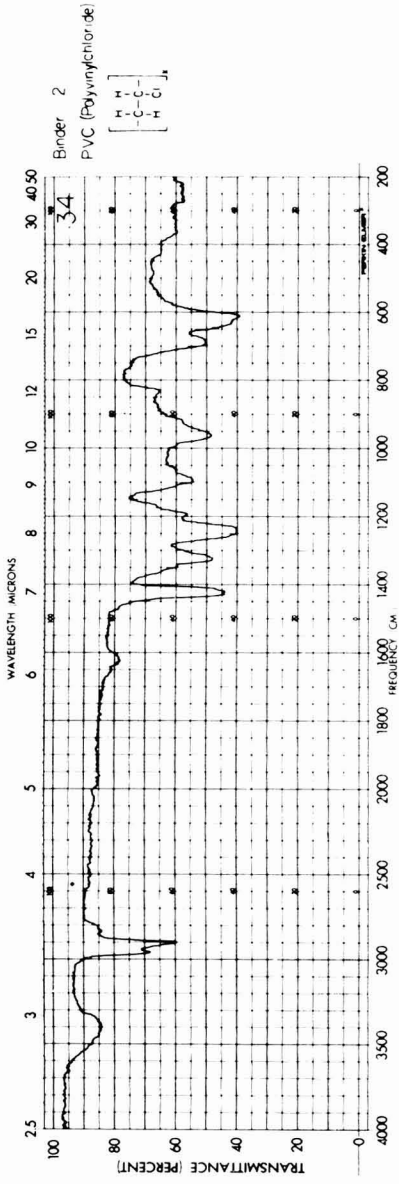

Oxidizer 17
Molybdenum trioxide
MoO_3
29
TRANSMITTANCE (PERCENT)
WAVELENGTH (MICRONS)
FREQUENCY CM

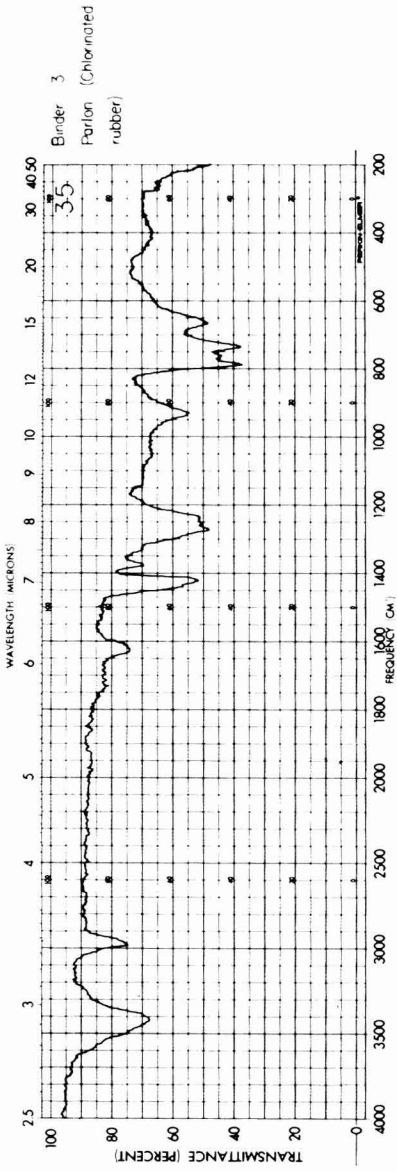

Color Intensifier 1
Dechlorane (Perchloro-pentacyclodecane)
30
TRANSMITTANCE (PERCENT)
WAVELENGTH MICRONS
FREQUENCY CM

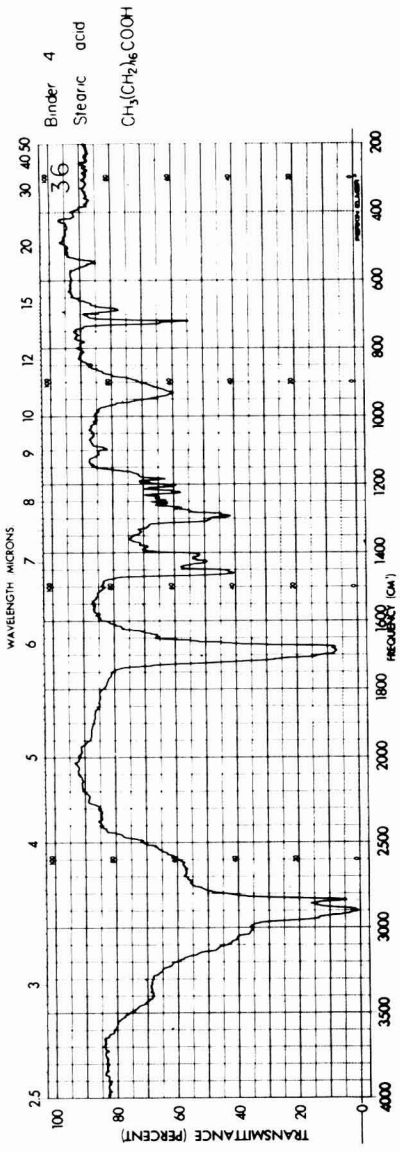

Color Intensifier 2
Sodium oxalate
$Na_2C_2O_4$
31
WAVELENGTH MICRONS
FREQUENCY /CM
TRANSMITTANCE (PERCENT)

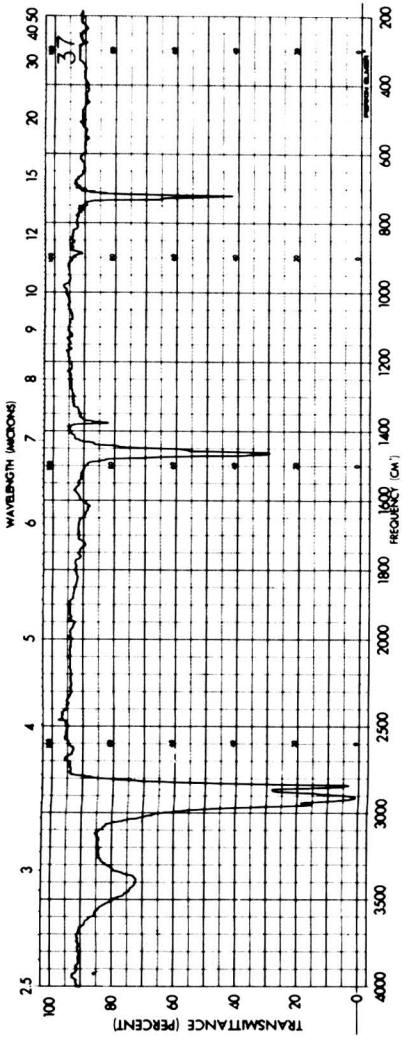

Color Intensifier 3
Strontium oxalate
SrC_2O_4
32
WAVELENGTH (MICRONS)

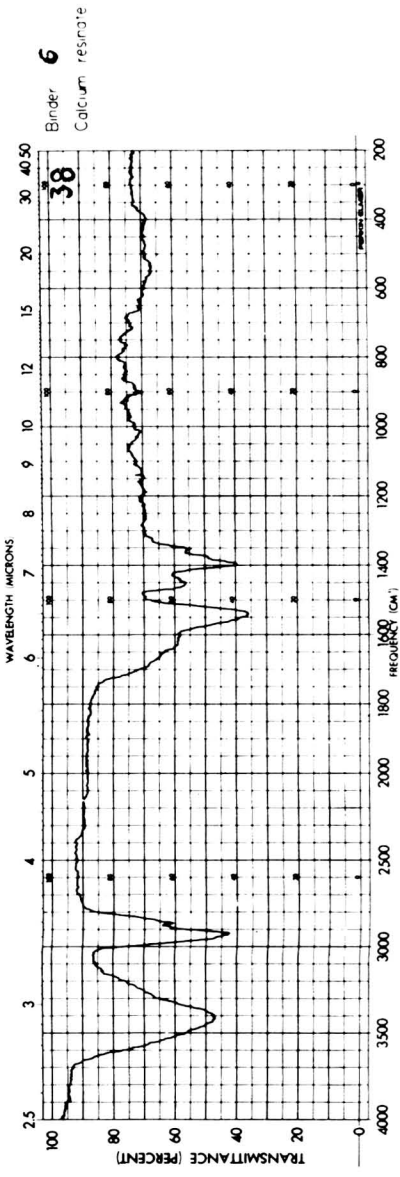

Binder 1
Gum arabic
33
WAVELENGTH (MICRONS)
FREQUENCY (CM.)
TRANSMITTANCE (PERCENT)

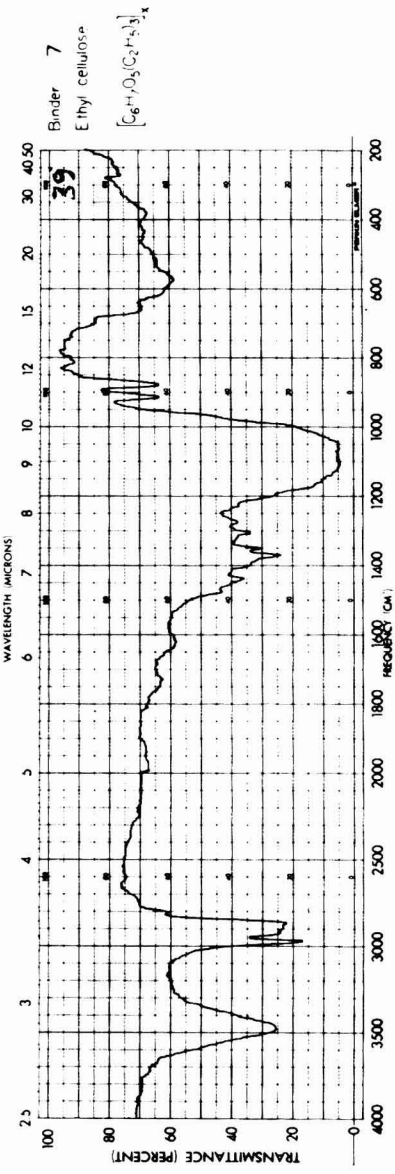

Binder 2
PVC (Polyvinylchloride)
34
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)

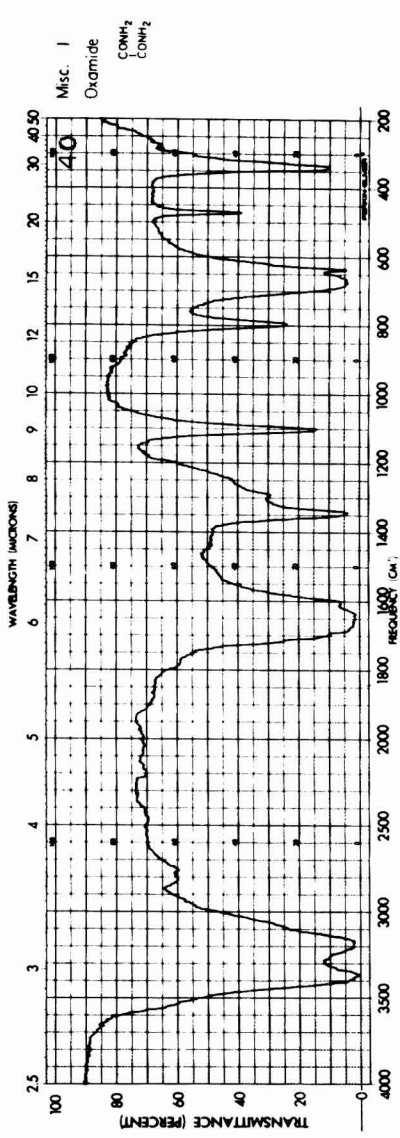

Binder 3
Parlon (Chlorinated rubber)
35
WAVELENGTH MICRONS
TRANSMITTANCE (PERCENT)
FREQUENCY (CM⁻¹)

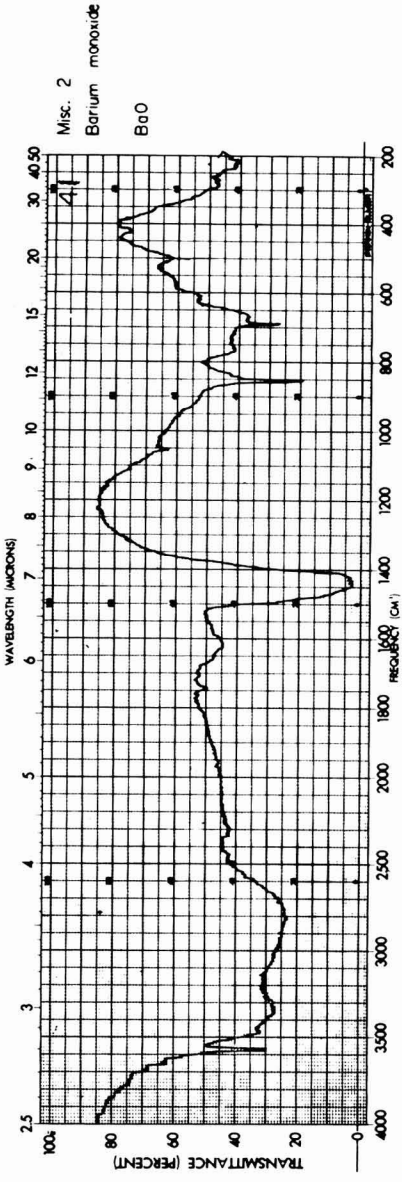

Binder 4
Stearic acid
$CH_3(CH_2)_{16}COOH$
36
WAVELENGTH MICRONS
TRANSMITTANCE (PERCENT)
FREQUENCY (CM⁻¹)

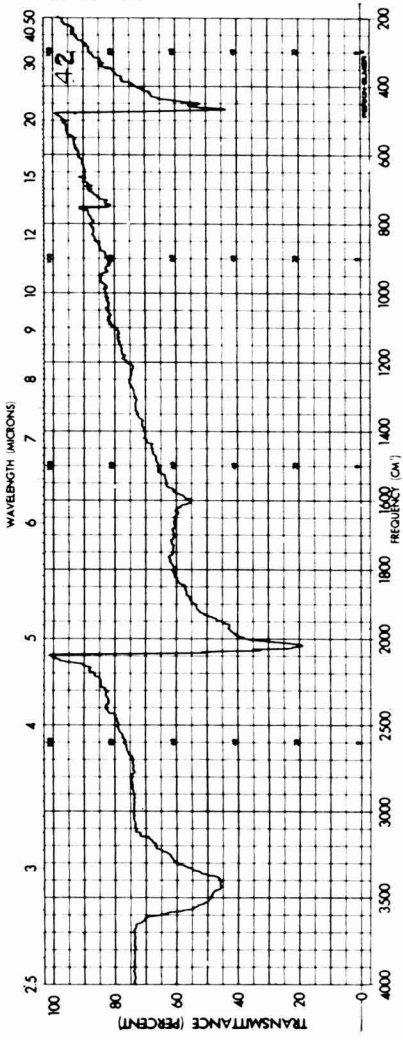

Binder 5
Wax
37
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

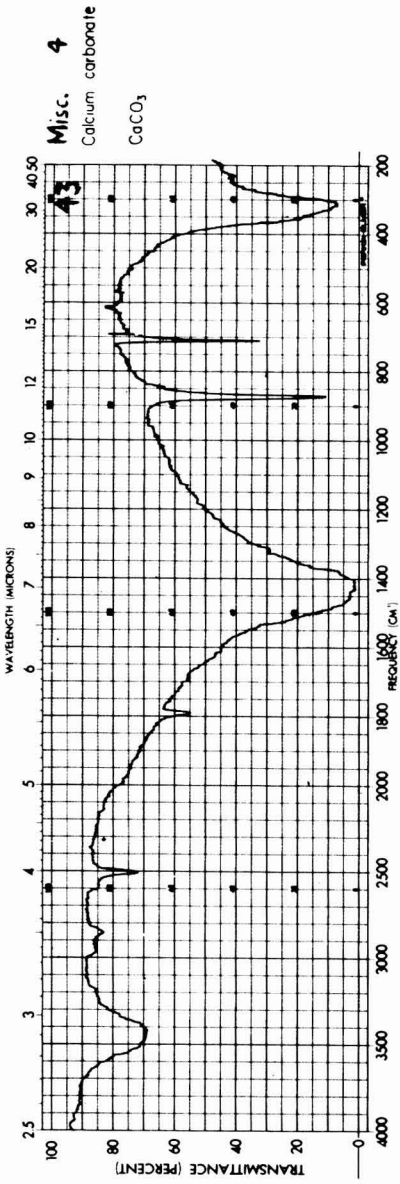

Binder 6
Calcium resinate
38
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

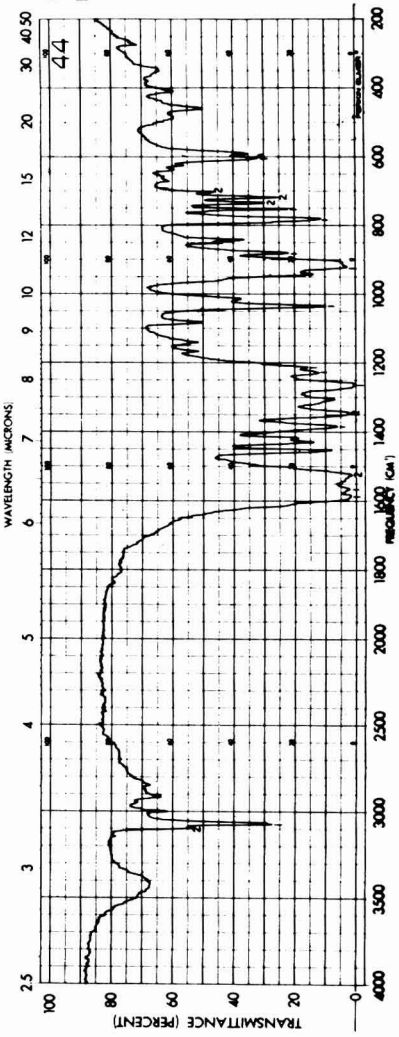

Binder 7
Ethyl cellulose
39
TRANSMITTANCE (PERCENT)
WAVELENGTH (MICRONS)
FREQUENCY (CM.)

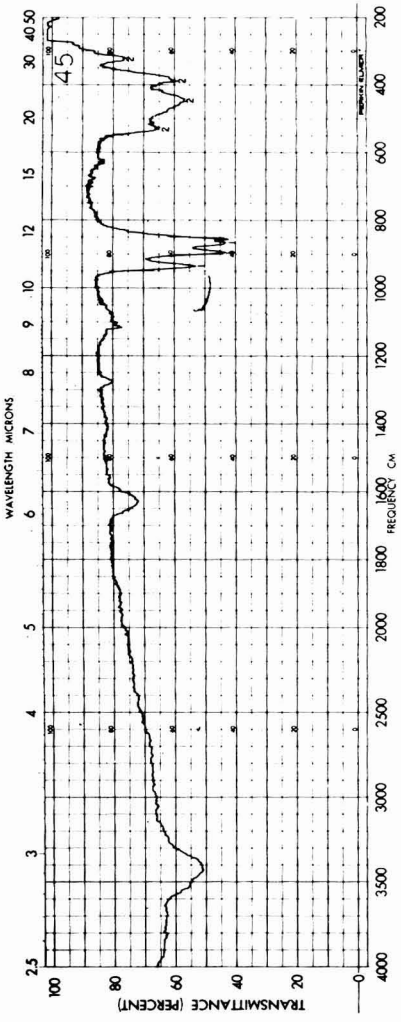

Misc. 1
Oxamide
CONH2
CONH2
40
TRANSMITTANCE (PERCENT)
WAVELENGTH (MICRONS)
FREQUENCY (CM.)

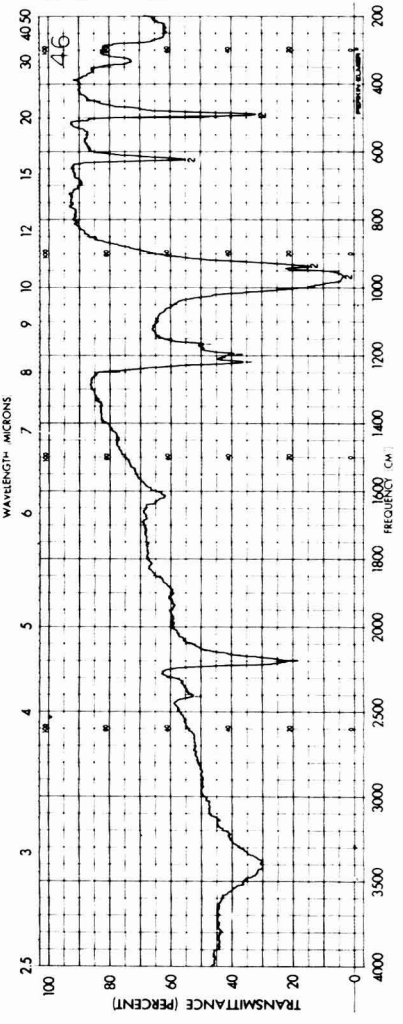

Misc. 2
Barium monoxide
BaO
41
TRANSMITTANCE (PERCENT)
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)

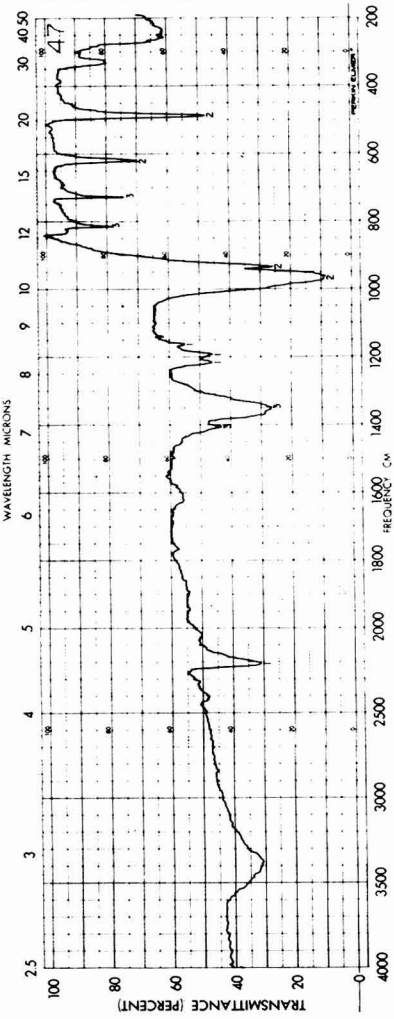

Misc. 3
Lead thiocyanate
Pb(SCN)2
42
TRANSMITTANCE (PERCENT)
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)

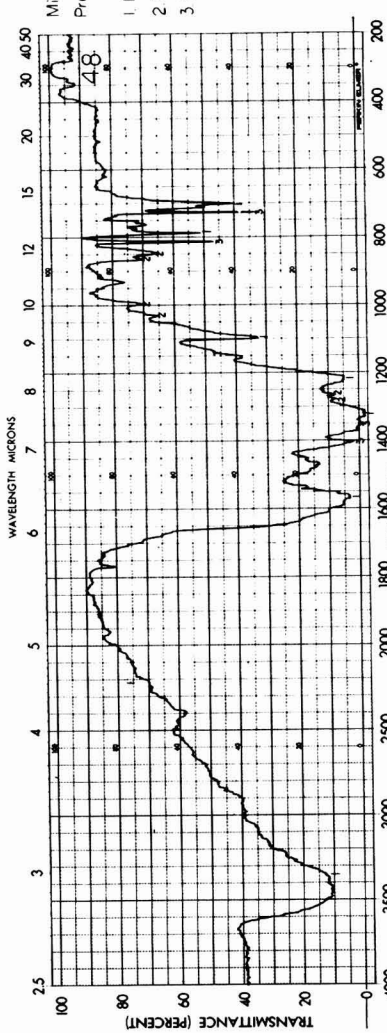

Misc. 4
Calcium carbonate
$CaCO_3$
43
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)

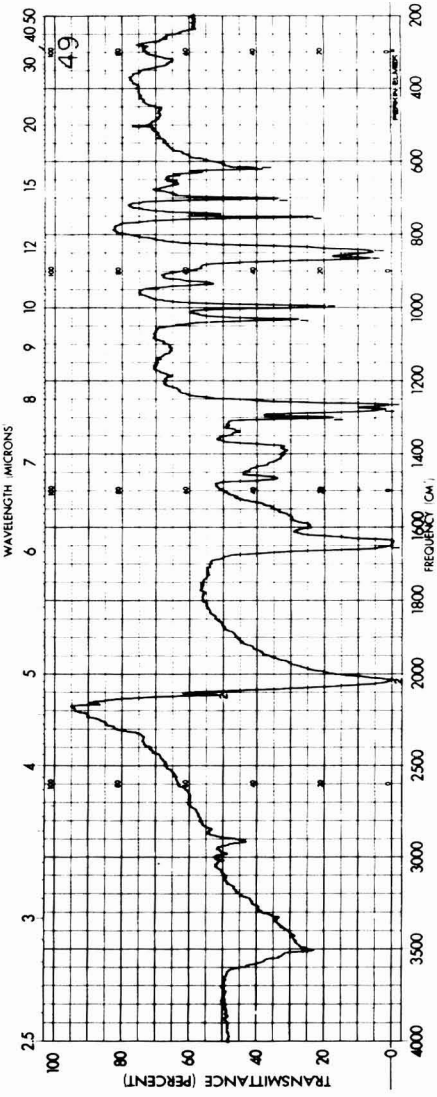

Mix 1
Detonator
1. RDX
2. TNT
44
WAVELENGTH (MICRONS)
FREQUENCY (CM⁻¹)
TRANSMITTANCE (PERCENT)


Mix 2
Igniter
1 $BaCrO_4$
2 Pb_3O_4
45
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)


Mix 3
Primer
1 Mercury fulminate
2 $KClO_3$
46
WAVELENGTH MICRONS
FREQUENCY CM
TRANSMITTANCE (PERCENT)


Mix 4
Primer
1. Mercury fulminate
2. KClO3
3. Ba(NO3)2
47
WAVELENGTH MICRONS
FREQUENCY CM-1
TRANSMITTANCE (PERCENT)


Mix 5
Primer
1. Normal lead Styphnate
2. PETN
3. Ba(NO3)2
48
WAVELENGTH MICRONS
FREQUENCY CM-1
TRANSMITTANCE (PERCENT)


Mix 6
Detonator
1. PETN
2. Lead azide
49
TRANSMITTANCE (PERCENT)
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)

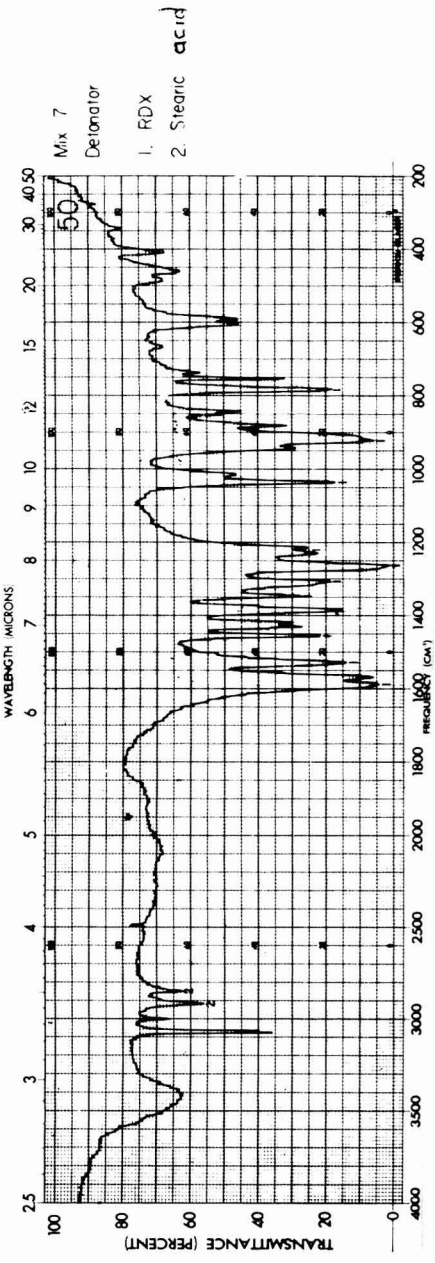

Mix 7
Detonator
1. RDX
2. Stearic acid
50
TRANSMITTANCE (PERCENT)
WAVELENGTH MICRONS
FREQUENCY (CM⁻¹)

TABLE 2

ANALYSIS OF TYPICAL COMPOSITIONS

Spectrum	Infrared	Emission spectroscopy	Combined analysis
44	RDX TNT		RDX TNT
45	$BaCrO_4$ Pb_3O_4	Pb, Cr, Ba, Zr	$BaCrO_4$ Pb_3O_4 Zr
46	Hg fulminate $KClO_3$	K, Sb, Hg	Hg fulminate $KClO_3$ Sb_2S_3
47	Hg fulminate $KClO_3$ $Ba(NO_3)_2$	K, Ba, Sb, Hg	Hg fulminate $KClO_3$ $Ba(NO_3)_2$ Sb_2S_3
48	Normal Pb styphnate PETN $Na(NO_3)_2$	Al, Sb, Pb, Ba	Normal Pb styphnate PETN $Ba(NO_3)_2$ Al Sb_2S_3
49	Pb azide PETN	Pb	Pb azide PETN
50	RDX Stearic acid		RDX Stearic acid

As shown, normal lead styphnate can be distinguished from basic lead styphnate by infrared. The different oxides of lead, iron, and barium may also be distinguished (PbO, Pb_3O_4, Fe_2O_3, BaO, and BaO_2 give unique spectra, while PbO_2 and Fe_3O_4 give no spectra).

In some spectra there is evidence of light scattering in the high frequency end of the spectrum. This is caused by inadequate grinding or the presence of such ingredients as antimony sulfide, magnesium, aluminum, or zirconium. However, in no instance was the problem severe enough to interfere with the qualitative use of the spectrum.

The majority of spectra indicated the presence of small amounts of moisture; hence, care must be taken to avoid misinterpreting peaks occurring in the regions of 3420 and 1630 cm^{-1}.

A special concentration procedure must be used to detect tetracene (2 to 4%) present in some lead styphnate primers. In this concentration procedure, about 50 mg of the primer contained in a sintered glass crucible is washed successively with ammonium acetate solution

(20%), water, and acetone to remove lead styphnate, barium nitrate, and PETN. About 3 or 4 mg of the residue, which contains the tetracene antimony sulfide, is then used for the pellet.

It might be thought that preparing potassium pellets from very sensitive explosive materials would be hazardous. This did not prove to be the case because a very small amount of the explosive is used and the potassium bromide acts as a diluent.

In the analysis of explosives by emission spectroscopy, it is customary in this laboratory to first destroy the explosive by treatment with nitric acid and evaporation to dryness.

SUMMARY

The application of infrared spectroscopy to the detection of the constituents of primers, tracers, igniters, incendiaries, boosters, and delay compositions on a microscale was investigated. It is shown that these constituents can be identified quickly and with certainty, using infrared pellet technique to detect organic and inorganic compounds and emission spjectroscopy to identify the metals. In making the pellet, 1 to 2 mg of the material is ground with 300 mg of potassium bromide; and the pellet is formed in the die press. The grinding and pressing operation has been found to be completely safe even with the most sensitive explosives. The infrared spectra of 43 of the most common ingredients of primers, tracers, igniters, incendiaries, boosters, and delay compositions are given over the range 2.5 to 5 μ. The qualitative analysis of 7 typical compositions is demonstrated.

REFERENCES

1. Pristera, F., and Fredericks, W., Compilation of infrared spectra of ingredients of propellants and explosives. In "Picatinny Arsenal Technical Memorandum 1887." (Sept.) (1969).
2. Miller, F. A., and Wilkins, C. H., Infrared spectra and characteristic frequencies of inorganic ions; their use in qualitative analysis. *Anal. Chem.* **24**, 1253–1294 (1952).
3. Miller, F. A., Carlson, G. L., Bentley, F. F., and Jones, W. H., Infrared spectra of inorganic ions in the cesium bromide region (700-300 cm^{-1}). *Spectrochim. Acta* **16**, 135–235 (1960).

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Microdetermination of Benzoic and Salicylic Acids with Guanidine Carbonate as a Titrant

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Received October 27, 1969

In an earlier communication, the use of guanidine carbonate as a titrant for the microdetermination of a carboxylic acid (*1*) was described. The present paper describes a similar procedure for the determination of benzoic and salicylic acids. It is observed that the results are concordant and precise, and compare well with another method (*2*) proposed earlier.

EXPERIMENTAL METHODS

Reagents used. Benzoic acid (M.A.R.H.W. England); salicylic acid (Analar R. B.D.H.), guanidine carbonate (Fluka) and bromcresol purple (B.D.H.).

Stock solution of salicylic acid was prepared in water and standardized by standard method. Benzoic acid was dissolved in a 1:1 water–alcohol mixture and standardized. The solutions were suitably diluted to required strength.

Procedure. To a given volume of a solution of any of these acids, add some distilled water to raise its volume to about 15 ml, followed by 1 or 2 drops of 0.1% solution of bromcresol purple indicator. The solution is yellow at this point. Now titrate it with a standard guanidine carbonate solution until the yellow color is completely discharged and the solution is a faint purple.

RESULTS

The results are given in Tables 1 and 2; these acids were estimated over a range of 0.061–0.691 mg. The results are concordant and precise.

SUMMARY

Benzoic and salicylic acids were determined in microquantities with a titrant, i.e., guanidine carbonate, using bromcresol purple as indicator. Estimations were made in the range of 0.061–0.610 mg of benzoic acid and 0.069–0.691 mg of salicylic acid with maximum error of ±.005 mg.

TABLE 1

MICRODETERMINATION OF BENZOIC ACID

Sample no.	Vol of soln (ml): 0.001 *M* Benzoic acid taken	Vol of soln (ml): Guanidine carbonate soln used 0.001 *M*	Benzoic acid (mg): Found	Benzoic acid (mg): Theoretical value	Error (mg)
1	5.00	5.04	0.615	0.610	0.005
2	3.00	3.00	0.366	0.366	0.000
3	1.00	0.96	0.117	0.122	0.005
4	0.50	0.50	0.061	0.061	0.000

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TABLE 2

MICRODETERMINATION OF SALICYLIC ACID

Sample no.	Vol of soln (ml): 0.001 *M* Salicylic acid taken	Vol of soln (ml): Guanidine carbonate soln used 0.001 *M*	Salicylic acid (mg): Found	Salicylic acid (mg): Theoretical value	Error (mg)
1	5.00	5.00	0.691	0.691	0.000
2	3.00	2.98	0.412	0.414	0.002
3	1.00	0.96	0.133	0.138	0.006
4	0.50	0.50	0.069	0.069	0.000

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MICROCHEMICAL JOURNAL 17, 63–67 (1972)

Methods for the Isolation and Characterization of Constituents of Natural Products

XIII. Regeneration of 2,4-Dinitrophenylhydrazones on a Periodic Acid Column

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Regeneration of 2,4-dinitrophenylhydrazones (DNPH) has been accomplished in a number of ways. An early method attributable to Conant and Bartlett (*2*) employed pyruvic acid as regenerator-acceptor; Demaecker and Martin (*4*) and Cullinane and Edwards (*3*) employed hydrochloric acid and acetone for cleavage and acceptor after reduction of the nitro groups with $SnCl_2$. Robinson (*10*) suggested the use of at least 80% formic acid solution and copper carbonate as a regenerating system for ketosteroids. Reduction of the nitro groups with chromous chloride and simultaneous cleavage of ketosteroid hydrazones with hydrochloric acid was described by Elks and Oughton (*6*). Keeney (*8*) outlined a method of cleavage which utilized levulinic and hydrochloric acids. DePuy and Pender (*5*) extended this method to other classes of DNPH and also to oximes. Ralls (*9*) was able to get limited regeneration of DNPH by heating with α-ketoglutaric acid. Bassette and Day (*1*) used concentrated H_2SO_4 and water to liberate the parent carbonyl from the derivative. The recent method of Wong and Schwartz (*13*) regenerates the parent carbonyl in the injection port of the gas chromatograph.

A statement by Feigl (*7*) that hydrazides, oximes, and hydrazones can be attacked by periodic acid prompted us to investigate the use of this reagent for cleaving 2,4-dinitrophenylhydrazones, especially since we had already developed a simple, two-phase periodic acid column technique for cleaving periodic acid-susceptible structures (*12*).

EXPERIMENTAL METHODS

Reagents and Apparatus [1]

Paraperiodic acid (G. Frederick Smith Co., Columbus, OH); magnesium sulfate, anhydrous (J. T. Baker Chemical Co., Phillipsburg, NJ, cat. no. 2506); CCl_4 (Baker, cat no. 1512); Silicone fluid 200, viscosity grade 2.0 (Dow Corning Corp., Midland, MI); medicine droppers, approximately 10 × 0.5 cm (i.d.).

Preparation of Periodic Acid–Sulfuric Acid Column

One milliliter of a saturated solution of periodic acid and 1 ml of 2 *N* H_2SO_4 were pipetted onto 8 g of $MgSO_4$ in a mortar. The mortar was shaken gently to cover the liquid with $MgSO_4$ and was then ground with a pestle until homogeneous. The impregnated $MgSO_4$ was sieved through an 80 mesh screen and the material which passed stored at 0°C. To prepare a column, approximately 300 mg of the powder was transferred to a medicine dropper and packed by tapping on a bench top. The column was wetted with CCl_4 and inspected for voids. These were removed by tapping or by stirring with a wire. Columns prepared in this manner will allow 0.5 ml of CCl_4 to completely enter in 15–20 minutes.

Regeneration Procedure

One micromole or less of the 2,4-dinitrophenylhydrazone was dissolved in CCl_4 and 0.5 ml was pipetted on the column. The course of the reaction was easily followed by observing the accumulation of an orange-yellow component at the top of the column. The colorless effluent was collected together with a column volume of CCl_4 used to wash out residual carbonyl.

Quantitative Aspects

The quantitative aspects of the reaction were followed by reforming the DNPH of the carbonyl present in the colorless effluent. The exit tip of the periodic acid column was positioned on top of a small (1 g) column of Celite impregnated with a 60% H_3PO_4 solution of 2,4-dinitrophenylhydrazine prepared as described by Schwartz and Parks (*11*). Following derivatization, the CCl_4 effluent was passed directly over a 400 mg column of Dowex 50 × 8 (H^+) (used directly from the bottle and merely washed with 2 column volumes of CCl_4) to remove 2,4-dinitrophenylhydrazine. The colored effluent was then adsorbed onto

[1] Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

alumina (*11*) and the DNPH eluted with benzene. Recovery was estimated spectrophotometrically in $CHCl_3$ by comparing the absorbance to that of a standard.

Experiments Using Silicone Oil as Solvent

A major disadvantage of regenerating the DNPH by the above method was apparent when one wished to evaluate the odor of the regenerated carbonyl. To circumvent this situation we have used silicone oil with the specified viscosity as solvent for the DNPH. This oil has no odor and this attribute, together with its low viscosity, makes it quite suitable for odor evaluation of the regenerated carbonyl. The procedure was exactly the same except that silicone oil was substituted throughout. For some DNPH it was necessary to heat the silicone oil to effect solution.

RESULTS AND DISCUSSION

Table 1 summarizes the regeneration information. The absence of data on the regeneration of unsaturated DNPH should be noted. This is due to the fact that oxidation of the double bond occurs to some extent. Although this is a serious limitation, it is felt that the procedure will still be of considerable utility due to its simplicity.

All of the compounds listed in Table 1 gave good yields of the parent carbonyl except formaldehyde which gave no yield. The aldehyde and keto esters originally gave yields in the vicinity of 60–80%, when run at a flow rate of 15–20 minutes/0.5 ml. This has been attributed to hydrolysis of the ester bond since decreasing the flow rate gave even lower yields whereas increasing the flow rate (through the use of a wider diameter column) gave much better yields.

The only DNPH investigated which did not regenerate were benzophenone and methyl-α-ketostearate. These came through the column unchanged. Formaldehyde DNPH was apparently regenerated but no free formaldehyde was detected in the effluent. It has been reported that formaldehyde is not detected in the effluent when terminal glycols are oxidized on a periodic acid column (*12*).

In order for a 2,4-dinitrophenylhydrazone to be cleaved, adsorption on the surface of the $MgSO_4$ must first take place. Thus, no regeneration takes place when Celite is substituted for $MgSO_4$ as the support. This is also probably a prerequisite for the oxidation of glycols and related compounds and explains the observation of Schwartz *et al.* (*12*) that Celite and glass beads impregnated with periodic acid fail to cleave periodate-susceptible structures.

Solvents with greater polarities than CCl_4 do not work well or not at all because of the adsorption requirement. However, a 20% (v:v)

solution of benzene in CCl_4 is satisfactory for most of the DNPH listed in Table 1. Short chain DNPH and DNPH with other functional groups in the parent compound can be regenerated using more polar solvents since they are more strongly adsorbed than are the longer chain DNPH.

Analysis of the regenerated carbonyl compounds as DNPH by thin-layer partition chromatography showed that no colored artifacts are produced in the procedure. With unsaturated DNPH, other spots, principally aldehydes produced from the oxidation of the double bond, were apparent.

Regeneration has also been accomplished on microgram quantities of DNPH by preparing microcolumns in melting point capillaries. In these instances, we have been able to inject the effluent from the column

TABLE 1

REGENERATION OF 2,4-DINITROPHENYLHYDRAZONES ON A PERIODIC ACID-SULFURIC ACID COLUMN

2,4-Dinitrophenylhydrazone	Flow rate (min/0.5 ml)	Amount over column (μmoles)	Recovery of free carbonyl (%)
Ketones			
2-Nonadecanone	20	0.07	96
2-Undecanone	20	0.13	101
2-Hexanone	15	0.07	103
2-Butanone	15	0.17	96
2-Propanone	15	0.15	90
Acetophenone	18	0.08	102
3-Cholestanone	20	0.21	99
Aldehydes			
Octadecanal	18	0.08	102
Tridecanal	18	0.05	101
Butanal	15	0.35	98
Acetaldehyde	20	0.34	96
Formaldehyde	15	0.29	0
Benzaldehyde	18	0.06	99
Phenylacetaldehyde	17	0.27	82
Carbonyl esters [a]			
Ethyl levulinate	4.5	0.11	105
Methyl-12-ketostearate	6.5	0.15	90
Methyl-7-ketostearate	6.5	0.07	92
Glycolaldehyde stearate	6.5	0.07	92

[a] Hydrolysis of esters takes place on the periodic acid column when slow flow rates are used. Data on these esters were obtained using a 400 mg column in a chromatography tube 0.8 cm, i.d.

directly into a gas chromatograph for purposes of confirming the identity of the parent carbonyl.

SUMMARY

A method is described for regenerating carbonyl compounds at room temperature from 2,4-dinitrophenylhydrazones. A column of $MgSO_4$ impregnated with periodic and sulfuric acids is used as the regenerant and CCl_4 is used as the solvent for the 2,4-dinitrophenylhydrazone. Examples are given for saturated aliphatic and aromatic ketones, saturated aliphatic and aromatic aldehydes, keto- and aldehydo-esters, and a keto-steroid. Some oxidation of the double bond in unsaturated 2,4-dinitrophenylhydrazones occurs. The use of a low-viscosity silicone oil as solvent allows for odor evaluation of the regenerated carbonyl compound.

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MICROCHEMICAL JOURNAL 17, 68–71 (1972)

Titrimetric Microdetermination of Yttrium and Scandium: Disodium Salt of Rhodizonic Acid as Complexing Agent

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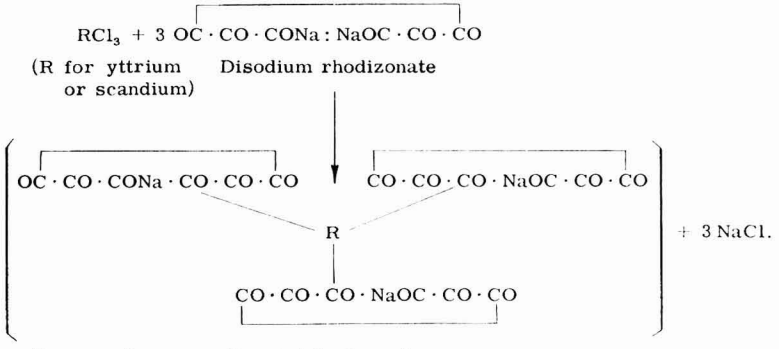
The literature concerning the determination of yttrium and scandium is quite plentiful, but titrimetric methods are less frequently cited. However, different techniques that have been employed for the determination of yttrium are spectrophotometric (*4, 10*); flame photometric (*11*); by EDTA titration (*2*); chromatographic (*8*); with potassium ferrocyanide (*12*); microanalysis by focusing ion-exchange (*16*); and iodometrically (*15*).

In general scandium is determined colorimetrically (*2, 17, 18*); with polyamino carboxylates (*1*); spectrophotometrically (*6, 9, 13, 14*); by EDTA titration (*5*); and gravimetrically (*3*).

Present work deals with the determination of yttrium and scandium separately in micro amounts: first, by forming a complex between yttrium or scandium and disodium salt of rhodizonic acid in the ratio of 1:3; and next, decomposing it with dilute mineral acid. After decomposition the attached rhodizonic acid salt with the metal ion is titrated against ceric sulfate solution using *N*-phenylanthranilic acid as indicator. In both cases the rare earth rhodizonate complexes are formed at pH 4.9. Potentiometric data and results of analysis confirm the titrimetric results that the ratios in which these complexes are formed is 1:3. Probably the following reaction takes place:



Rare earth monosodium trirhodizonate.

EXPERIMENTAL METHODS

Chemicals and apparatus used. Yttrium and scandium trichlorides (E.Merck grade); disodium salt of rhodizonic acid, sodium carbonate, and *N*-phenylanthranilic acid (B.D.H. grade); ferrous ammonium sulfate and sulfuric acid (AnalaR B.D.H. grade); and ceric sulfate (Technical B.D.H. grade).

Micropipettes and microburettes used had least count = 0.01 ml.

0.0022 *M* ceric sulfate (in 4 *N* H_2SO_4) solution was standardized by titrating against a standard solution of ferrous ammonium sulfate (in NH_2SO_4) using *N*-phenylanthranilic acid as indicator.

Yttrium and scandium trichloride solutions were standardized by EDTA titrations (*5, 7*), respectively.

PROCEDURE

Since the procedure for both yttrium and scandium is the same, hence R would represent them in the following description:

A known volume of a known standard R-trichloride solution, through a micropipette, is placed in a beaker and the volume is raised to 20 ml. To this solution, a known excess of a standard disodium rhodizonate solution is added, boiled for 2 to 3 minutes and then cooled at room temperature between 30 to 35°C. After cooling at room temperature, the dark violet colored rhodizonate complex is filtered. The filtrate is discarded and the precipitate is washed thoroughly, until the filter paper shows no brown or yellow color. The precipitated complex is now dissolved in 0.1 *N* H_2SO_4 by adding the acid onto the filter paper and then piercing it with pointed thin glass; wash the glass and the filter paper so that all the acid may pass down the funnel into the beaker. Now, the dissolved rhodizonate complex is titrated for the attached monosodium salt of rhodizonic acid to R by running in a standard ceric sulfate (in 4 *N* H_2SO_4) solution, through a microburette, using *N*-phenylanthranilic acid as indicator. At the end point a reddish brown color appears sharply.

RESULTS AND CONCLUSION

Results are given in Tables 1 and 2. Yttrium and scandium were estimated in ranges of 0.7238×10^{-4} to 1.7997×10^{-4} mg/liter; and from 0.9495×10^{-4} to 2.3739×10^{-4} mg/liter, respectively.

Since the R-rhodizonate complex between RCl_3 and disodium rhodizonate is formed in the ratio of 1:3, the observed values are divided by 3. In the case of yttrium it is not possible to estimate below 0.7238×10^{-4} mg/liter; and in the case of scandium not below 0.9495×10 mg/l. One of the most important precautions while precipitating the R-

TABLE 1

MICRODETERMINATION OF YTTRIUM

YCl_3 0.004 *M* (ml)	$Ce(SO_4)_2$ 0.0022 *M* (ml)	consumed	Amount of yttrium ($\times 10^4$ mg/liter) Taken	Found	Error (%)
0.2	1.12		0.7113	0.7238	1.7
0.3	1.66		1.0670	1.0818	1.3
0.4	2.20		1.4226	1.4280	0.3
0.5	2.76		1.7784	1.7997	1.1

rhodizonate complex by boiling, is that even after boiling the solution, the solution must be dark red yellow or ornage—which ensures complete precipitation. It has been observed that 0.0002 *N* H_2SO_4 is sufficient to affect the complexes mentioned above. In the cases of yttrium and scandium the maximum error is 1.7 and 1.1%, respectively. It has been observed that in both the cases Ag, Tl, Au, Co, Ni, Mn, Ca, Mg, Sr, Er, Gd, Ba, V, La, Mo, U, Ga, Sm, Nd, Zn, Zr, Th, In, Ge, Nb, and Be interfere.

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SUMMARY

Yttrium and scandium are determined separately in micro amounts by forming and decomposing the complex, rare earth monosodium trirhodizonate, with ceric sulfate. The complexes between rare earths and disodium salt or rhodizonic acid are formed in the ratio of 1:3 at pH 4.9. Maximum error for yttrium and scandium is 1.7 and 1.1%, respectively. 0.0002 *N* H_2SO_4 is sufficient to affect the complexes. Interference by metal ions is, also, observed.

TABLE 2

MICRODETERMINATION OF SCANDIUM

$ScCl_3$ 0.0105 *M* (ml)	$Ce(SO_4)_2$ 0.0022 *M* (ml)	consumed	Amount of scandium ($\times 10^4$ mg/liter) Taken	Found	Error (%)
0.2	2.90		0.9441	0.9495	0.5
0.3	4.32		1.4162	1.4243	0.5
0.4	5.80		1.8882	1.9090	1.1
0.5	7.20		2.3604	2.3739	0.5

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MICROCHEMICAL JOURNAL 17, 72–74 (1972)

Simple Modification of the Cary 14 Recording Spectrophotometer for Visible Photometric Titrations

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Several textbooks on instrumentation describe photometric titrations and their usefulness (*1, 6, 8*). Nevertheless, few commercially available instruments have the necessary accessories for performing these titrations. Examples of individualized titration assemblies for different spectrophotometers have been described (*2–5, 7*). Among these is one by Malmstadt and Gohrbandt (*4*) for automatic titrations. These authors modified a Cary spectrophotometer by making a new cover for the titration cell compartment, constructing a new cell, and inserting a brass stirrer through the cover.

A simpler modification for the Cary 14 spectrophotometer for visible work has been used successfully in our laboratory (Fig. 1). The cell holders from both the reference and sample cell compartments were removed; the center of each cell holder mounting plate was marked for future placement of the cells. The handle from the mounting plate in the sample compartment was removed along with the mounting plate itself. An air-driven magnetic stirrer (such as Scientific Glass Apparatus Co., Bloomfield, N.J., S-6962) was placed in the sample compartment and supported by a "Little Jack" or a wooden or Styrofoam block 5.9 cm high by 8.3 cm wide by 11.7 cm long. The input and exhaust lines for the air-driven stirrer were fed through the two knockout plugs of the sample cell compartment base. The stirrer was propelled by compressed air (12 psi minimum was needed to stir 50 ml of H_2O efficiently). In order to keep the stirrer from moving, it was wired in place with copper wire to the two slides which support the mounting plate. The mounting plate was then replaced along with its handle, and centered over the stirrer.

The reference and sample cells were placed in their respective compartments on the center marks on the mounting plates. The sample cell was secured with Apiezon Q sealing compound at its base to eliminate movement while stirring the sample. The cells used were sinter-

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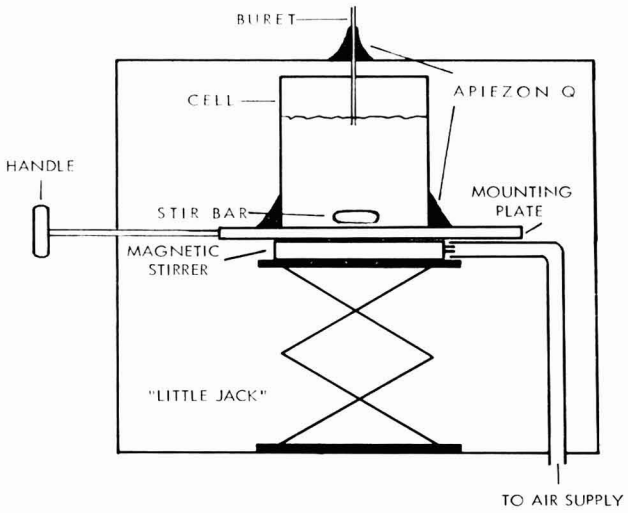

FIG. 1. Side view schematic of cell compartment.

fused glass 20 × 40 × 80 mm high, with plane parallel sides (such as Arthur H. Thomas Co., Philadelphia, Pa. 3790-F). These cells can be used from 360 to 800 nm, and in the near ir from 800 nm to 1.35 μ. This size cell is required to accommodate an octagonal 0.5-inch long, 0.31-inch o.d. magnetic stir bar. The minimum starting volumes are: 35 ml in the sample cell, and 50 ml in the reference cell.

The handle on the sample compartment cover was removed and the drawn-out tip of a 2, 5, or 10 ml buret was inserted into the compartment through the screw hole, and down into the sample itself. Apiezon Q sealing compound was used around the buret tip to eliminate as much stray light as possible. Since the data sought is the change in absorbance versus titrant volume added, the initial absorbance of the sample solution can be arbitrarily set by the balance control of the instrument.

This assembly has the following distinct advantages: it is fairly inexpensive, no vortex is formed while stirring, no permanent change is made to the Cary 14, and, the stirring assembly can be left in the instrument under the mounting plate while the instrument is used in its normal mode of operation.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL **17**, 75–76 (1972)

Microdetermination of Sulfanilic Acid with Guanidine Carbonate as a Titrant

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In an earlier communication, the use of guanidine carbonate as a titrant for the microdetermination of carboxylic acid (*1*) was described. The present paper describes a similar procedure for the determination of sulfanilic acid. It is observed that the results are concordant and precise, and compare well with another method (*2*) proposed earlier.

EXPERIMENTAL METHODS

Reagents used. Sulfanilic acid (A. R. Italy); guanidine carbonate (Fluka) and bromocresol purple (B.D.H.).

Procedure. To a given solution of this acid, add some distilled water to raise its volume to about 15 ml, followed by 1 or 2 drops of 0.1% solution of bromocresol purple indicator. The solution is yellow at this point. Now titrate it with a standard guanidine carbonate solution until the yellow color is completely discharged and the solution is a faint purple.

RESULTS

The results are given in Table 1; this acid was estimated over a range of 0.096–0.956 mg. The results are concordant and precise.

SUMMARY

Sulfanilic acid was determined in microquantity with a titrant, i.e., guanidine carbonate, using bromocresol purple as indicator. Estimations were made in the range of 0.096–0.956 mg with maximum error of ±0.004 mg.

ACKNOWLEDGMENT

The author is grateful to Drs. B. B. L. Saxena and M. N. Srivastava for their kind guidance and to U.G.C. (Govt. of India) for providing financial assistance.

TABLE I

MICRODETERMINATION OF SULFANILIC ACID

Sample no.	Vol of soln (ml)		Sulfanilic acid (mg)		
	Sulfanilic acid taken (0.001 *M*)	Guanidine carbonate soln used (0.001 *M*)	Found	Theoretical value	Error (mg)
1	5.00	5.02	0.960	0.956	0.004
2	3.00	2.98	0.570	0.574	0.004
3	2.00	2.00	0.382	0.382	0.000
4	1.00	1.02	0.195	0.191	0.004
5	0.50	0.50	0.096	0.09	60.000

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MICROCHEMICAL JOURNAL 17, 77–90 (1972)

The Introduction of Microtechniques in Elementary Science Courses

II. Serum Cholesterol and Cholesterol Ester Determination

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Since previous attempts by the authors to introduce quantitative microanalytical techniques into beginning curricula of biological and chemical studies proved successful (*1*), the program was continued using microtechniques for serum cholesterol and cholesterol ester analysis.

As in the previous study, a single method was chosen and three instruments were utilized to determine the milligrams of cholesterol and cholesterol esters per 100 ml of blood (mg %). The method chosen for the colorimetric determinations was the modified method of Bowman and Wolf [cited in Ref. (*2*)]. The instruments utilized to make the colorimetric analysis were the Spectronic 20 photoelectric colorimeter, the Spectronic 505 spectrophotometer, both manufactured by Bausch and Lomb (Bausch and Lomb, Inc., Rochester, NY), and the color-comparator block.

College students enrolled in both biology and chemistry curricula performed the colorimetric determinations. Reagent and standard solutions were prepared ahead of time so that the procedures could be done in the usual laboratory time periods where possible. Those procedures requiring lapses of time between the steps were carried on beyond the regular laboratory periods by student volunteers who returned to the laboratory area during free periods to process their own samples and those of their colleagues.

Small samples were used in the microchemical procedures, so accuracy in measurement was essential to the success of the technique. Microvolumetric pipettes were required; and the techniques of using such equipment was part of the instructional preparation given to the students. Blood drawing techniques and operation of the centrifuge and the colorimetric instruments were also reviewed.

MATERIALS AND METHODS

Preparation of Animal Subjects

Cockerels and pullets, raised from the time that they were 3 days old, were maintained at 85–90°F in rabbit cages and were fed chick-starter mash twice a day and water *ad libitum*. When the chicks were 4 days old, they were divided into three groups. Group 1 received 2.5 mg of cortisone acetate subcutaneously twice a week for 3 weeks. Group 2 received 3.0 mg of cortisone acetate by subcutaneous injection twice a week for 3 weeks. Group 3 served as untreated controls. Each group was housed in separate cages.

Physical Observations

During the experimental period, the chicks were observed with respect to their physical condition and growth. The experimental animals showed a decreased rate of body weight and height gain and a decrease in comb growth and feather maturation (Fig. 1). There was also a decrease in overall activity in the experimental chicks but they showed an increased excitability and a slightly increased BMR (*3*). The experimental animals continued immature high-pitched cheeping, while the controls had developed a deeper pitched crowing. During the last week of the 3 week injection period, the experimental chicks showed an increase in food consumption and the excretion of watery feces. Urine glucose tests proved negative.

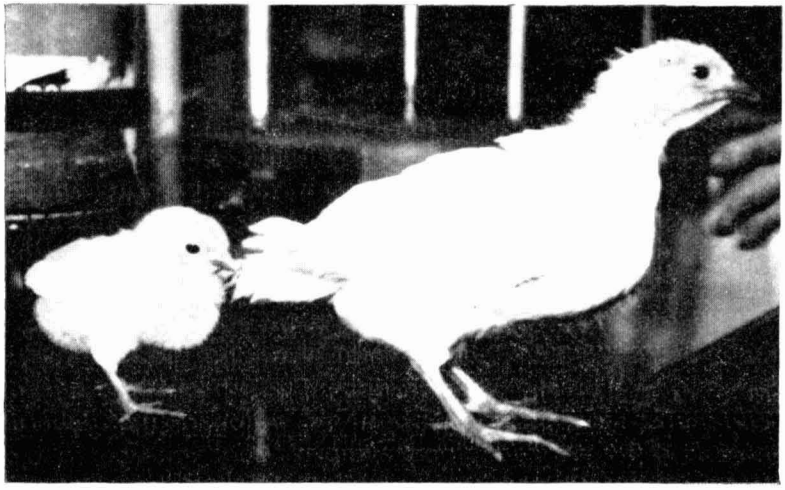

FIG. 1. Experimental and normal chicken.

Animal Blood Drawing Procedure

At the end of the 3 week injection period the experimental and control chicks were weighed and then were sacrificed by decapitation. Seven milliliters of blood were collected from the carotid arteries directly into centrifuge tubes. Since chicken blood has a tendency to contain a large cellular fraction, it was necessary to collect a large blood sample to obtain an adequate serum volume for the chemical tests. The animals could not be etherized since this would have interfered with their cholesterol levels. The blood samples were allowed to clot and were then centrifuged at 2500 rpm. At autopsy, the experimental animals showed a decrease in both adrenal and pancreatic weight; and the pancreas had a very pale color compared with those of the control animals.

Human Blood Drawing Procedure

Several students volunteered to come to class in a fasting condition. Their fingers were placed in moderately hot water for a few minutes to stimulate the circulation in the extremities. The fingers were wiped dry and the middle finger of each volunteer was then wiped with a gauze sponge saturated with a 70% aqueous solution of ethyl alcohol. Sterile, disposable lancets were used to prick the fingers to a depth that permitted free flow of blood. The first drop of blood was wiped away with a sterile gauze pad (the first drop is usually diluted with tissue fluid). Blood from each student was collected in capillary tubes approximately 8.5 cm in length. These capillary tubes are marketed by the glassware companies as Coagulation Determination Tubes.[1]

Each end of the blood-filled capillary tube was sealed with sealing wax. In order to prevent hemolysis of the blood sample inside the capillary tube, the blood was made to run into the tube by quickly leveling the tube after collection so that the center part of the tube was filled with blood, leaving each end of the tube clear of blood. The sealing wax was warmed in the flame of a microburner and, at the same time, one end of the capillary tube was carefully warmed in the same flame. Care must be used so that only the clear glass end becomes warm but not the area holding the blood sample. Heating the blood sample would cause hemolysis of the blood cells. The tube is held perfectly horizontal during the whole process so that no movement of the blood into the warmed end of the tube is possible. The sealing wax is then applied to the end of the tube and some of the wax runs into the warmed end of the tube making a firm seal. The process is repeated on the other end of

[1] Corning Glass Company.

the capillary tube. The capillary tubes kept in a horizontal position until the glass and the wax on each end is cool (Fig. 2).

Small pieces of paper were folded to make envelopes to contain the sealed blood sample tubes (Fig. 3). Each paper envelope can be used to label the blood samples with the name of the person from whom the blood was drawn. All envelopes and blood samples were refrigerated for about 1 hour to allow the blood to coagulate and the blood clots to shrink and free the serum.

The envelopes and blood samples were placed in the cups of the centrifuge and were centrifuged for 10 minutes at 2500 rpm. Care

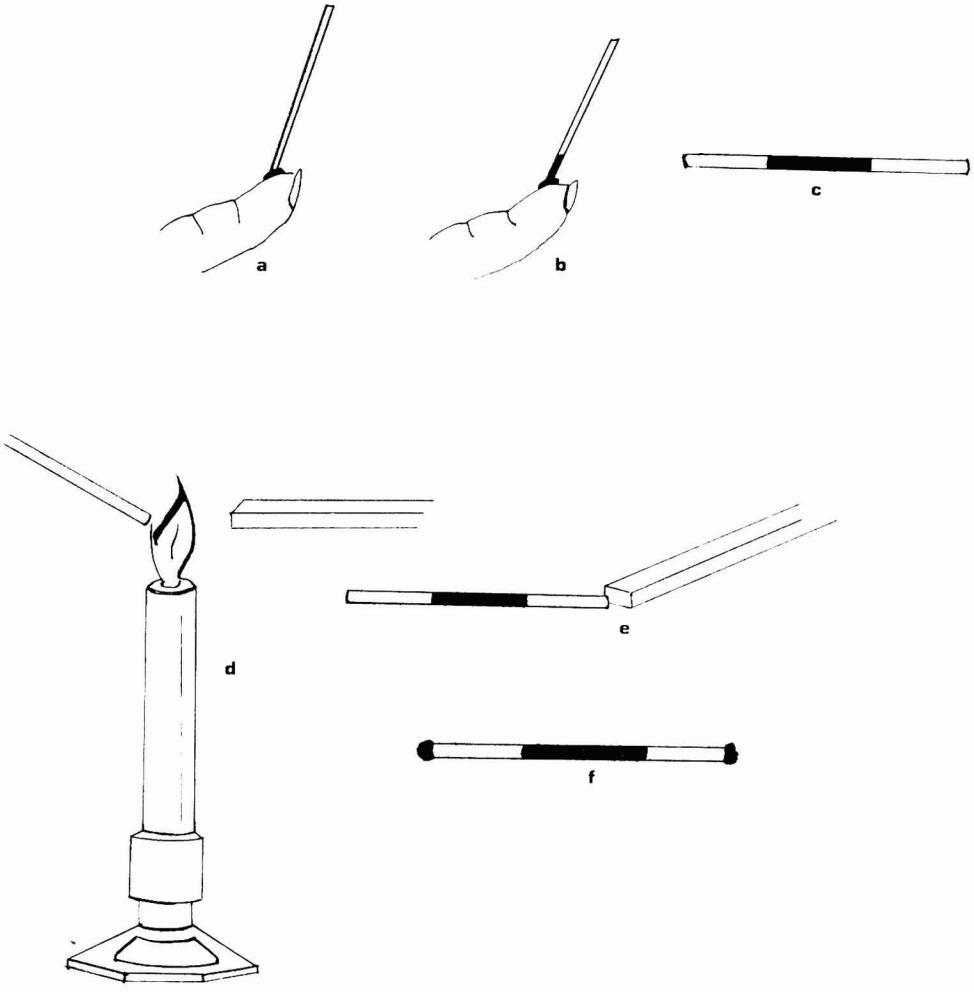

FIG. 2. Collecting fingertip blood samples.

must be taken so that the samples are not packed too tightly or the capillary tubes will break during centrifugation.

After centrifugation, the tubes are carefully removed from the envelopes one envelope at a time to avoid mixing the samples of one donor with that of another. Each capillary tube is scored with a diamond pointed pencil just below the top of the seal and just above the sedimented blood clot. The portion of the tube containing the clear serum is broken free and the aliquots of blood serum are drawn from these sections by means of micropipettes.

Test Procedure

Reagents. Ferric chloride stock solution, containing 15 g of ferric

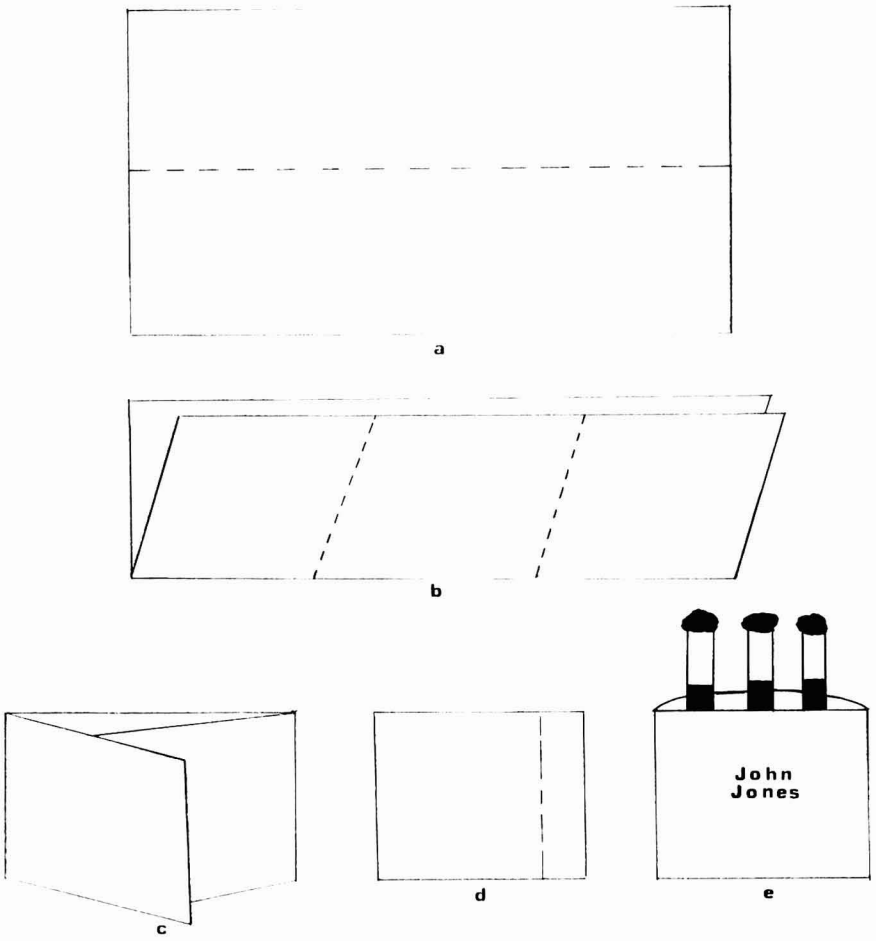

FIG. 3. Paper envelopes for capillary tubes.

chloride/liter of pure 87% orthophosphoric acid. Store in plastic bottle in refrigerator (keeps indefinitely).

Working color reagent, made by diluting 8.0 vol of the ferric chloride stock solution up to 100 vol with reagent-grade concentrated H_2SO_4. Store in plastic bottle in refrigerator (keeps approx 2 months).

Stock cholesterol standard solution, containing 1 g of dry reagent-grade pure cholesterol [2]/liter of absolute ethyl alcohol (keep refrigerated).

Working cholesterol standard solutions were prepared containing the following amounts of stock standard cholesterol solution diluted to 100 ml with absolute ethyl alcohol (ml): 1.0, 1.5, 2.0, 2.5, 3.0. These correspond to 100, 150, 200, 250, 300 mg % cholesterol in the test procedure. One milliliter of distilled water is added to each flask to correct for a small amount of serum water present in the blood serum aliquot.

Absolute ethyl alcohol, reagent grade, anhydrous.

Isopropyl alcohol, reagent grade, anhydrous.

Acetone, reagent grade.

Digitonin solution, containing 50.0 mg of pure digitonin in 10.0 ml of 50% (v/v) ethyl alcohol (make just before using).

Total Serum Cholesterol Method

1. Transfer 0.05 ml of serum into the bottom of a 12.0 ml glass-stoppered tube using a 50 μ Lang-Levy pipette.

1. Add 5.0 ml of absolute ethyl alcohol directly into the serum to insure a fine protein precipitate.

3. Stopper centrifuge tube and shake vigorously for 10 seconds.

4. Centrifuge for 10 minutes at 2500 rpm.

5. Stoppered 25 ml Erlenmeyer flasks are set up and marked for a blank, the tests to be run, and the five standards to plot the standard curve. (Once the standard curve has been established, only two standards need be run to check the validity of the standard curve from day to day.)

6. Transfer 3.0 ml of the supernatant from each centrifuge tube into the appropriate test flask.

7. To the flask marked "blank" add 3.0 ml of absolute ethyl alcohol.

8. Into each standard flask pipette 3.0 ml of the appropriate working standard which has been allowed to reach room temperature after removal from the refrigerator.

[2] Standard Reference Material 911 Cholesterol, Office of Standard Reference Materials, National Bureau of Standards, Washington, DC.

9. To all flasks, add 2.0 ml of working color reagent. Each flask is swirled in the same way for the same amount of time after this addition. It is suggested that the reagent be added to each flask, that the flask then be swirled and stoppered before the next flask receives the reagent.

10. Allow the flasks to cool for 30 minutes.

11. Read the contents of the flasks at 550 ml in the spectrophotometers, setting the instruments at zero absorbance with the reagent blank. The color-comparator block may be used as outlined in the previously published instructions for its use (*1*).

Free Serum Cholesterol Method

1. Pipette 0.2 ml of serum into the bottom of a 12.0 ml glass-stoppered centrifuge tube.

2. Add 5.0 ml of absolute ethyl alcohol directly into the serum to insure a fine protein precipitate.

3. Stopper centrifuge tube and shake vigorously for 10 seconds.

4. Centrifuge for 10 minutes at 3000 rpm.

5. Pipette 2.0 ml of clear supernatant into a 6.0 ml glass-stoppered centrifuge tube.

6. Add 1.0 ml of freshly prepared digitonin solution.

7. Stopper the centrifuge tubes, shake well, and let stand for at least 2 hours.

8. Centrifuge the tubes for 10 minutes at 3000 rpm.

9. Decant and discard the supernatant fluid; and drain the tube on filter paper for 2 minutes before returning the tube upright.

10. Wash the precipitant by adding 3.0 ml of acetone from a syringe with a fine needle to resuspend the precipitated particles.

11. Recentrifuge for 10 minutes at 3000 rpm.

12. Decant and drain as in step 9 above.

13. Add 3.6 ml of isopropyl alcohol, stopper, and shake well to dissolve the precipitate.

14. Stoppered 25 ml Erlenmeyer flasks are set up and marked for a blank, the tests to be run and the five standards to plot the standard curve. (Once the standard curve has been established, only two standards need be run to check the validity of the standard curve from day to day.)

15. Transfer 3.0 ml of the supernatant from each centrifuge tube into the appropriate test flask.

16. To the flask marked "blank" add 3.0 ml of isopropyl alcohol.

17. Into each standard flask, pipette 2 ml of the appropriate working standard containing 1.0, 1.5, 2.0, 2.5, or 3.0 mg % cholesterol.

18. To each flask containing a standard solution, add 1.0 ml of ethyl

alcohol. This dilution, together with the differences in aliquot size taken for the free serum cholesterol determination in contrast to the total cholesterol determination, now makes each of the standards equivalent to 30, 45, 60, 90, and 120 mg % free cholesterol.

19. Proceed as in steps 9–11 of the total cholesterol method.

Calculations:

total cholesterol − free cholesterol = ester cholesterol
ester cholesterol ÷ total cholesterol = ester (%)

Modifications for Free Serum Cholesterol Determination (human studies)

In steps:

1. Use 0.05 ml of serum in a 6 ml glass-stoppered centrifuge tube.
2. Add 1.5 ml of absolute ethyl alcohol.
5. Use 0.5 ml of the supernatant fluid.
6. Add 0.25 ml of digitonin solution.
10. Wash with 0.8 ml of acetone.
13. Add 0.9 ml of isopropyl alcohol.
15. Transfer 1.5 ml of fluid into test flask and use 1.0 ml of color reagent/test flask.

The colorimetric procedure for this determination was the same as that outlined previously (*1*). The absorption curve and the readings for the total cholesterol and the free serum cholesterol standards are shown in Figs. 4, 5, and 6. In this procedure, the color-comparator block proved less accurate than it had with the previous method. The reddish color that developed in the cholesterol solutions was found to be a difficult color to match by visual methods. As formerly stated, a greater accuracy might have been obtained from the block if larger numbers of standards with smaller intervals between their concentrations had been prepared.

MICROCHEMICAL RESULTS

Experimental Animal Results

To make the results more meaningful to interpret, all of the values were converted to values per 100 g of body weight. Although the chicks making up each group were randomly picked, it developed that all of the animals in groups 1 and 2 were cockerels and those in group 3 were both cockerels and pullets. The results of the tests are shown in Tables 1, 2, and 3. A summary of the findings is given in Table 4.

In man, it is known that 70–80% of the plasma cholesterol is in the form of esters and this percentage is true of other mammals as well (*4*). As can be seen, this percentage is somewhat lower in the month-old

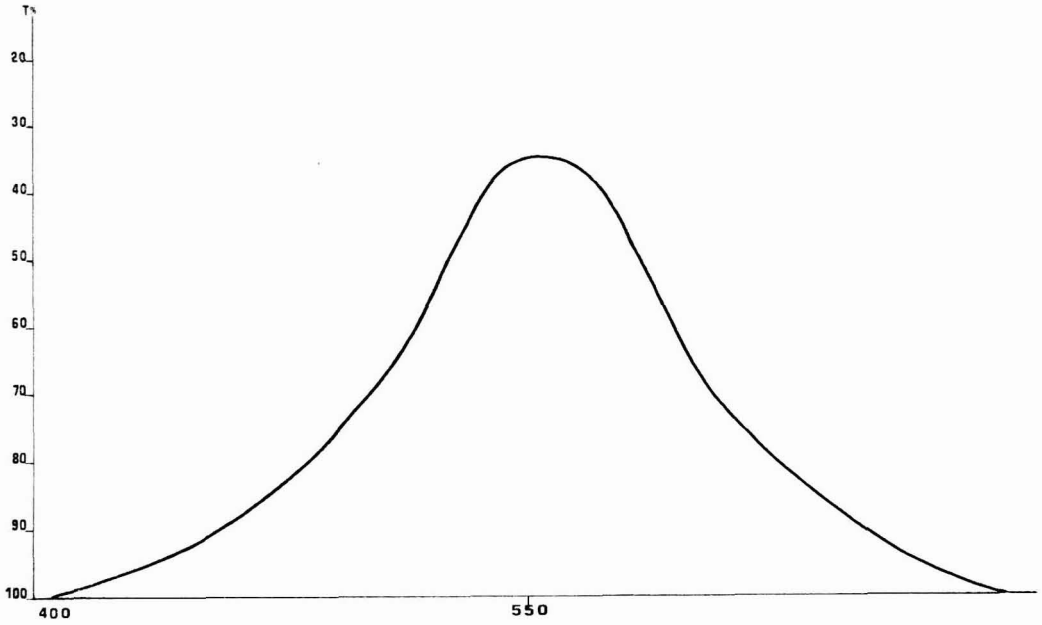

FIG. 4. Cholesterol standard absorption curve: (abscissa) mμ.

cockerels and pullets and there was no significant difference by sex in the control group (group 3). The total serum cholesterol levels in the group which received the 2.5 mg dosage of cortisone acetate did not differ from the values of the control cockerels, but those chicks which received the larger dosage (3.0 mg) had a markedly lower total cholesterol level and their ester percentage was also noticeably lower. From these results, it would seem that the exogenous cortisone had a greater effect on the esterification process or on the rate of ester breakdown by the liver.

Laboratory animals other than the chick may be used for these determinations. The following are the total blood cholesterol levels for some of them (*5*):

	mg % serum
Cat	95
Guinea pig	40
Hamster	57
Hog	110
Mouse	97
Rabbit	40
Rat	50
Sebus monkey	93

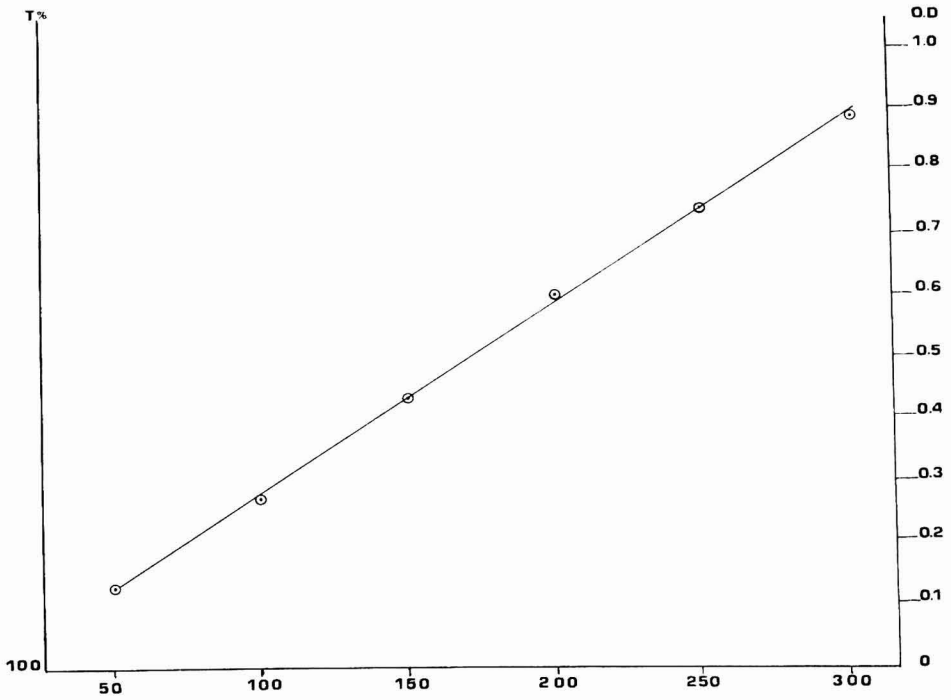

FIG. 5. Standard curve for total cholesterol: (abscissa) mg.

It is interesting to see the wide disparity in these results. They seem to have little significance as a criterion of comparative physiology. Human values for total blood cholesterol vary with age as follows:

	mg % serum
Infant	45
1 year to puberty	110–250
Adult (years)	
40 Males	135–315
Females	135–240
50 Males	150–340
Females	145–330
60 Males	140–321
Females	156–356

Further study remains to be done on any age-related variation in the chicken.

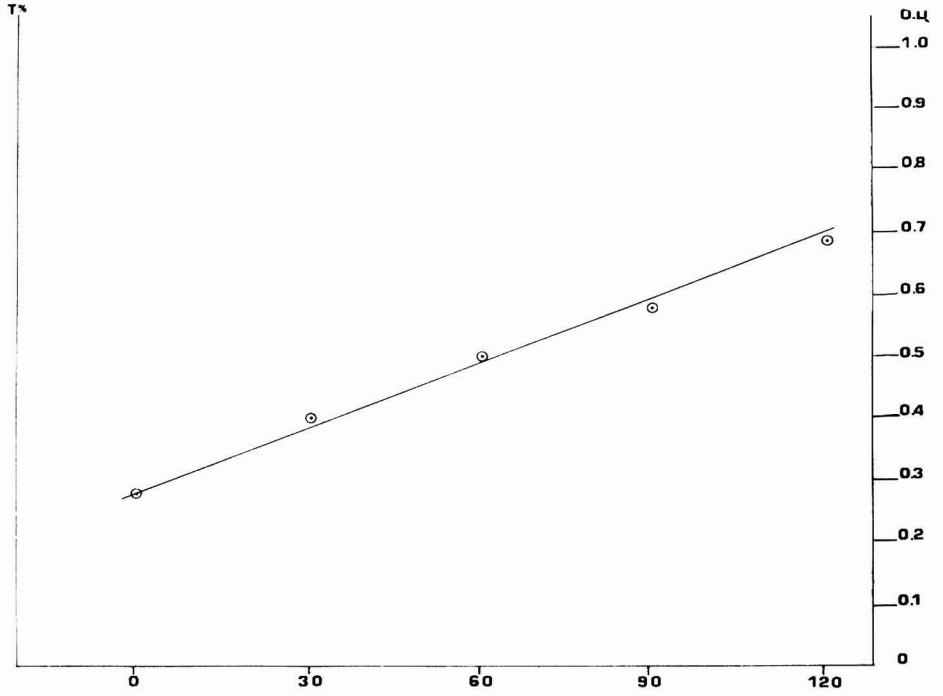

FIG. 6. Standard curve for free serum cholesterol: (abscissa) mg.

RESULTS OF HUMAN BLOOD ANALYSIS

The results on the human studies (Table 5) were all low normals as would be expected of samples from young, healthy adults. The human experiments were included in this study to demonstrate the possibility

TABLE 1

GROUP 1, CORTISONE ACETATE (2.5 mg)

Serum cholesterol (mg/100 ml of serum)		Cholesterol ester	
Total Cockerels, 28 days old	Free	(mg/100 ml of serum)	(%)
95.6	34.56	61.04	63.8
89	20.04	68.96	77.5
117.2	16.12	101.08	86.4
92	28.14	63.86	69.4
93	32.98	60.02	62.4
102.1	27.34	74.76	73.3
94.4	32.19	62.21	65.9

TABLE 2

GROUP 2, CORTISONE ACETATE (3.0 mg)

Serum cholesterol (mg/100 ml of serum)		Cholesterol ester	
Total	Free	(mg/100 ml of serum)	(%)
Cockerels, 28 days old			
65	25	40	61.5
90	35	55	61
63	31	32	50.8
68	25	43	63.2
72	35	37	51.4
86	40	46	53.5

of using fingertip blood for serum cholesterol and serum cholesterol ester determinations.

Educational Implications

As in the preceding study (*1*), the microanalytical techniques proved no barrier to proper performance on the part of the students executing the tests. This reemphasized the authors' belief that microanalytical techniques can be introduced profitably to the early science experience of the average student.

TABLE 3

GROUP 3, CONTROLS

Serum cholesterol (mg/100 ml of serum)		Cholesterol ester	
Total	Free	(mg/100 ml of serum)	(%)
Cockerols, 28 days old			
103	39	64	62.1
105	44	61	58.1
113.8	43.9	69.9	61.4
106	40.6	65.4	61.7
98.2	31.8	66.4	67.6
119.4	46.0	73.4	61.5
117.8	37.0	80.0	68.6
93	44.1	48.9	52.5
Pullets, 28 days old			
95.6	34.6	61.0	63.8
101	41	60	59
105	35	70	66.7

TABLE 4

AVERAGE RESULTS

	Group		Control	
	1	2	Cockerels	Pullets
Serum Cholesterol (mg %)				
Total	97.6	74.0	107.2	100.5
Free	27.3	31.8	41.0	36.8
Cholesterol esters				
(mg %)	70.3	42.0	66.2	63.7
(%)	71.2	56.9	61.7	63.2

Adaptations of Methods for High School Use

Comparison of any available equipment can be the basis for the introduction of microanalytical techniques into high school science courses. In those school systems where neither the students themselves nor an animal may be used for experiments, such as outlined in this article, it is suggested that the following fluid be used in place of serum: 8.5 g of sodium chloride, 70.0 g of albumen (egg or other), dissolved in distilled water and then made to 1 liter. "Serum" cholesterol solutions may be made up from this solution by placing weighed amounts of cholesterol (from 140 to 240 mg) in 100 ml volumetric flasks and diluting to the proper volume with the sodium chloride–albumen solution. Food dye can be used to color the solution yellow to give the appearance of blood serum.

ACKNOWLEDGMENTS

The authors express their thanks to Mr. Kenneth Iandoli, senior Biology major at Jersey City State College, for his help with the diagrams. Development of the black and white photograph from a color print was done by Action Photos, 405–37th Street, Union City, NJ.

TABLE 5

RESULTS OF HUMAN BLOOD ANALYSIS

	Serum cholesterol (mg/100 ml of serum)		Cholesterol ester	
	Total	Free	(mg/100 ml of serum)	(%)
E. K.	130	30	100	77
M. M.	120	29	91	76
M. J.	125	30	95	76

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MICROCHEMICAL JOURNAL 17, 91–92 (1972)

Microdetermination of Aspartic and Glutamic Acids with Guanidine Carbonate as a Titrant

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Received November 19, 1969

In earlier communications from this laboratory the use of guanidine carbonate as a titrant for the microdetermination of organic acids (1–3) was described. The present paper describes a similar procedure for the determination of dicarboxylic amino acids. In this titration the end point occurs at one equivalence, i.e., only one COOH group is titrated. It is observed that the results are concordant and precise and compare well with the other standard method (*4*) proposed earlier.

EXPERIMENTAL METHODS

Reagents used. Aspartic acid (E.Merck); glutamic acid (B.D.H.); guanidine carbonate (Fluka); and bromocresol purple (B.D.H.).

Procedure. To a given volume of a solution of any of these acids, add some distilled water to raise its volume to about 15 ml, followed by 1 or 2 drops of 0.1% solution of bromocresol purple indicator. The solution is yellow at this point. Now titrate it with a standard guanidine carbonate solution until the yellow color is completely discharged and the solution is a faint purple.

RESULTS

The results are given in Tables 1–2, these acids were estimated over a range of 0.067–0.735 mg. The results are concordant and precise.

SUMMARY

Aspartic and glutamic acids were determined in microquantities with a titrant, i.e., guanidine carbonate, using bromocresol purple as indicator. Estimations were made in the range 0.067–0.666 mg of asparic acid and 0.074–0.735 mg of glutamic acid with maximum error of ± 0.008 mg.

ACKNOWLEDGMENT

The author is grateful to Drs. B. B. L. Saxena and M. N. Srivastava for their kind guidance and to U.G.C. (Govt. of India) for providing financial assistance.

TABLE 1

MICRODETERMINATION OF ASPARTIC ACID

Sample no.	Vol of soln (ml) 0.001 *M* Aspartic acid taken	Guanidine carbonate soln used 0.001 *M*	Aspartic acid (mg) Found	Theoretical value	Error (mg)
1	5.00	5.04	0.671	0.666	0.005
2	2.00	2.02	0.269	0.266	0.003
3	1.00	0.94	0.125	0.133	0.008
4	0.50	0.48	0.064	0.067	0.003

TABLE 2

MICRODETERMINATION OF GLUTAMIC ACID

Sample no.	Vol of soln (ml) 0.001 *M* Glutamic acid taken	Guanidine carbonate soln used 0.001 *M*	Glutamic acid (mg) Found	Theoretical value	Error (mg)
1	5.00	5.02	0.738	0.735	0.003
2	3.00	2.98	0.438	0.441	0.003
3	1.00	0.96	0.141	0.147	0.006
4	0.50	0.50	0.074	0.074	0.000

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MICROCHEMICAL JOURNAL 17, 93–104 (1972)

Application of Differential Thermal Analysis to Organic Elemental Microanalysis

I. Combination with Carbon and Hydrogen Determination Apparatus

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INTRODUCTION

During the past several decades, numerous approaches have been attempted for the improvements of the methods of organic elemental microanalysis resulting in both of faster operations and higher quality of the analytical results (*1*). Automatic instrumentations incorporated with fine electronic finish up are therefore a recent trend of the quantitative microanalysis. However, it must be noticed that the quality of the analytical results is not essentially functioned by the mechanical grade of the given apparatus; but it is partly or, occasionally, seriously affected by the properties of the test samples. For example, anomalous analytical results are often obtained with samples containing impurities, unstable crystal water, or surface moisture. Explosives and refractory substances introduce other types of trouble. Therefore, the analytical results may be more reasonably interpreted by preliminary knowledges of the properties of the samples being tested.

The present authors have introduced a new technique in organic elemental microanalysis with a simultaneous operation of the differential thermal analysis (DTA). The DTA apparatuses (*2–4*) combined with gas chromatography, mass spectrometry, and infrared absorption spectroscopy have been extensively reported in recent years, but no attempt has been made for an application to the organic elemental microanalysis. It should be naturally considered that the sample heating technique with programmed temperature employed in elemental analysis is similar to that of DTA, unless the former requires a rapid combustion process.

The present paper describes an application of DTA to microdetermination of carbon and hydrogen in which a sample is slowly heated on a thermocouple detector, the thermal characteristics of the sample during the heating process such as dehydration, fusion, boiling, vapori-

zation, sublimation, explosion, decompositions, and oxidation being indicated as the exothermic peak or endothermic negative peak in the differential thermogram. These thermal changes within the substance often explain the anomalous carbon and hydrogen values.

The authors have also inserted the thermocouple detectors into the layer of oxidizing catalyst, a water absorption tube, and a carbon dioxide absorption tube. Heat evolution due to chemical reactions at these test points signified the distribution patterns of the gaseous components in the effluent gas, which presented much information concerning the type of thermal decomposition of the test sample, when the DTA curve of the same sample was referred to. Additionally, the new technique has enabled an estimation of the decomposition product of the sample at a certain temperature where the DTA indicated a thermal change in the thermogram.

MATERIALS AND METHOD

Apparatus and Procedure

Schematic diagram of the instrumentation is illustrated in Fig. 1. Two quartz combustion columns filled with oxidizing catalyst—cobaltic oxide (*5*) and silver granules (*6*)—are closely installed with upright position being fused upon a quartz buffer column. The catalyst is heated by a cylindrical furnace of 20 cm in length. The buffer column is provided for both of sample ignition and a DTA detector with a short furnace of 10 cm in length, which operates temperature programming by a remote control circuit. The bottom of the buffer tube is closed by a ground glass cap holding two thermocouples in the heating zone of the buffer tube.

A carrier gas of air with a flow rate of 10 ml/min is introduced into the buffer column and divided into two equal portions for both sensing and reference sides. A quartz vessel containing 2–5 mg of accurately weighed sample is placed upon the alumel–chromel thermocouple at the sensing side as illustrated in Fig. 2, while the other vessel, with the same dimension but without sample, is placed at the reference side. The quartz vessels are slowly heated by the sample heater normally at a rate of 15°C/min, but any other constant rate may be programmed by adjusting the series of variable resistances in the thermoregulator shown in Fig. 3.

Two thermocouples are located at the bottom of the oxidizing catalyst as illustrated in Fig. 1, where heat evolution by the combustion of sample mainly takes place. Two sets of the metal absorption train, namely, water absorption tubes, nitrogen oxide removal tubes and carbon dioxide absorption tubes are symmetrically connected at the top of the combustion columns. The absorption tubes at the reference side play an important role to cancel out the blank values if they are used

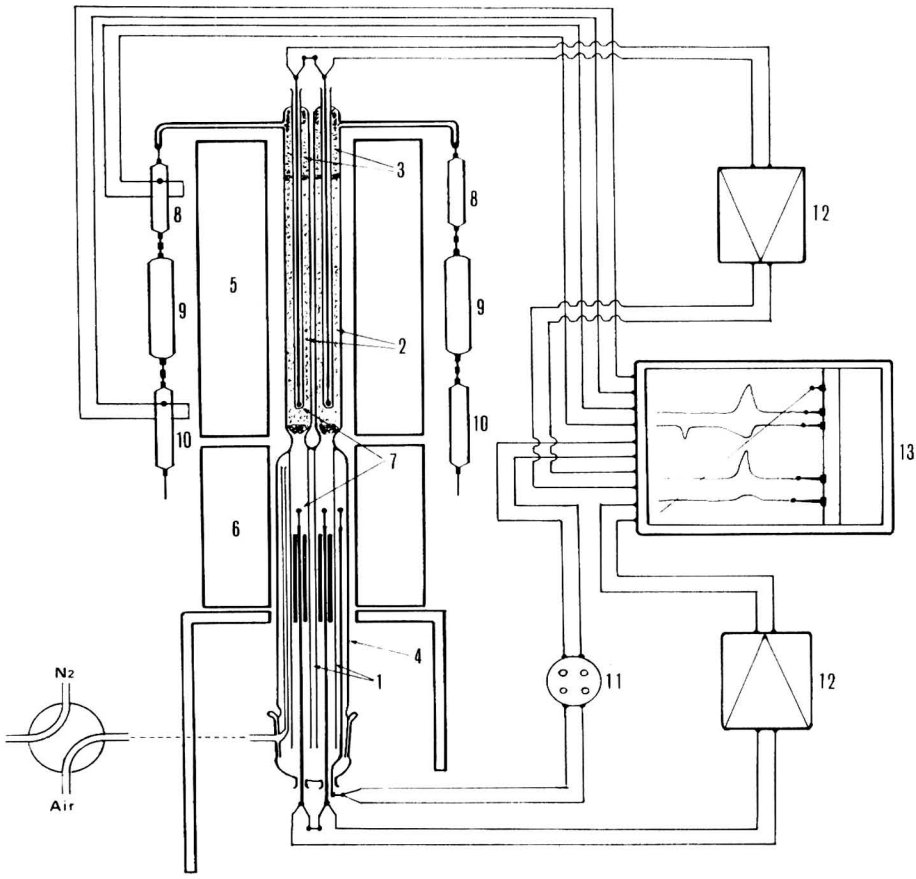

FIG. 1. Schematic diagram of the instrumentation: (1) combustion column; (2) cobaltic oxide; (3) silver granules; (4) buffer column; (5) cylindrical furnace; (6) sample furnace; (7) alumel–chromel thermocouple detector; (8) water absorption tube; (9) nitrogen oxides removal tube; (10) carbon dioxide absorption tube; (11) cold junction; (12) amplifier; (13) recorder.

as the tare weights in a microbalance. Heat of absorption of water and of carbon dioxide are also detected with the alumel–chromel thermocouples which are inserted into the layer of absorption agents—magnesium perchlorate and soda asbestos—as shown in Fig. 4.

The DTA curve accompanied with the scanning curve of the temperature programming of the sample heater, the curve indicating the differential temperature of the catalyst, and the curves of the water and carbon dioxide absorption tubes are traced on a strip chart of a 5-pen recorder. It is also possible to use nitrogen as a carrier gas instead of air to observe the true heat decomposition of the test sample in the absence of oxygen.

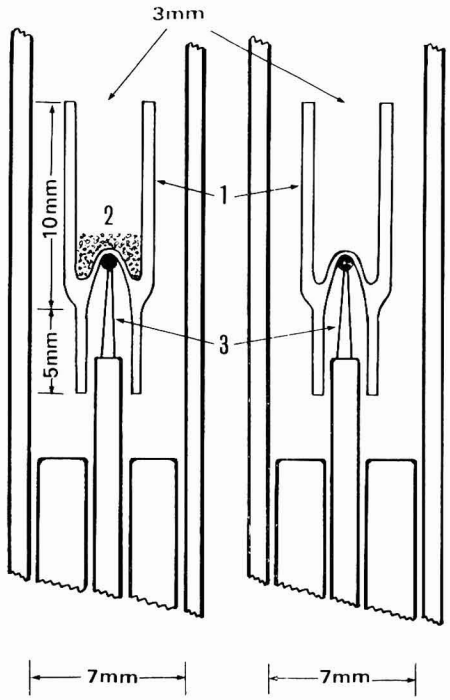

FIG. 2. Thermocouple detector: (1) quartz vessel; (2) sample; (3) alumel–chromel thermocouple.

RESULTS AND DISCUSSION

Representative compounds having different properties during heat decomposition were analyzed by the proposed method. The results are summarized below.

Benzoic Acid

Benzoic acid was analyzed under the normal heating rate of 15°C/min in an air stream of 10 ml/min. The thermograms and the results of carbon and hydrogen determination are shown in Fig. 5. In the DTA curve I, an endothermic negative peak at 120°C appeared at the melting point and a successive negative peak at 220° was observed at the boiling point, although any impurity in the sample would lower these temperatures and weaken the sharpness of the negative peaks depending upon the grade of impurity.

Immediately after the liquefaction of the sample, an exothermic peak commenced in the catalyst curve II due to the combustion of organic vapor from the sample. Water and carbon dioxide curves III and IV practically coincided with the catalyst curve II. When the temperature

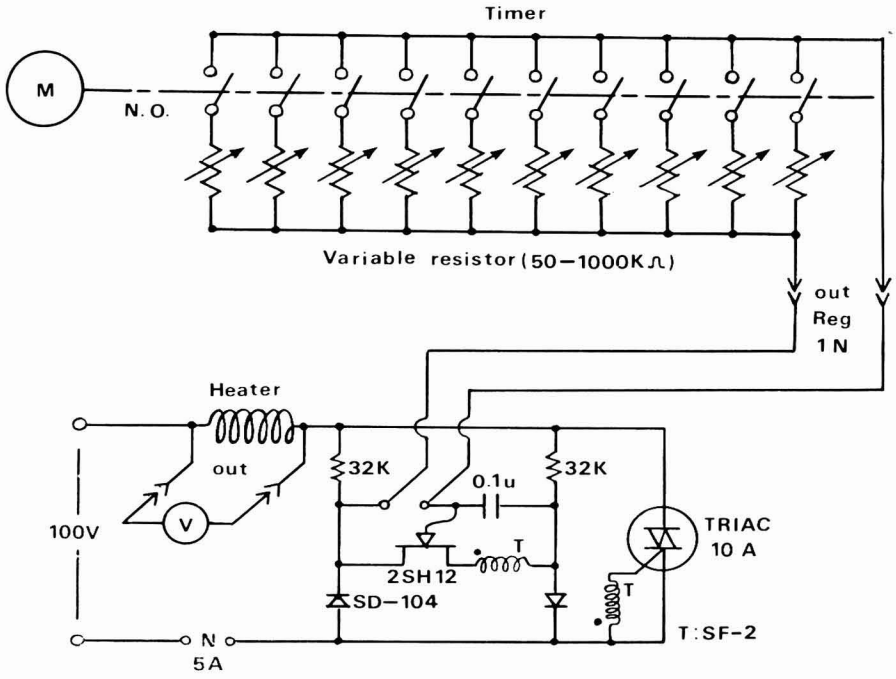

FIG. 3. Thermoregulator.

passed the boiling point, either curve II or IV exhibited their maxima, though an insignificant maximum point was observed in the curve III. Therefore, it was evidently accepted that benzoic acid could be almost evaporated during the temperature of 120–220°C.

In case of such volatile compounds as benzoic acid and acetanilide, sharp exothermic peaks are characteristically obtained in the catalyst curve II. This also suggests that the oxidizing catalyst must be as active as possible to avoid incomplete combustion against such rapid evaporation of the compounds. A similar view point must be considered for the carbon dioxide tube, which requires complete absorption of carbon dioxide in high concentration.

The observation of the thermogram data confirmed that the combustion–absorption process was completed before elevated temperature at 500°C. Satisfactory results of carbon and hydrogen determination with the maximum sample heating temperature of 500°C could be therefore obtained in Fig. 5.

Sucrose

The thermograms and the analytical result of sucrose are shown in Fig. 6. The sample was slowly heated up to 700°C under the same con-

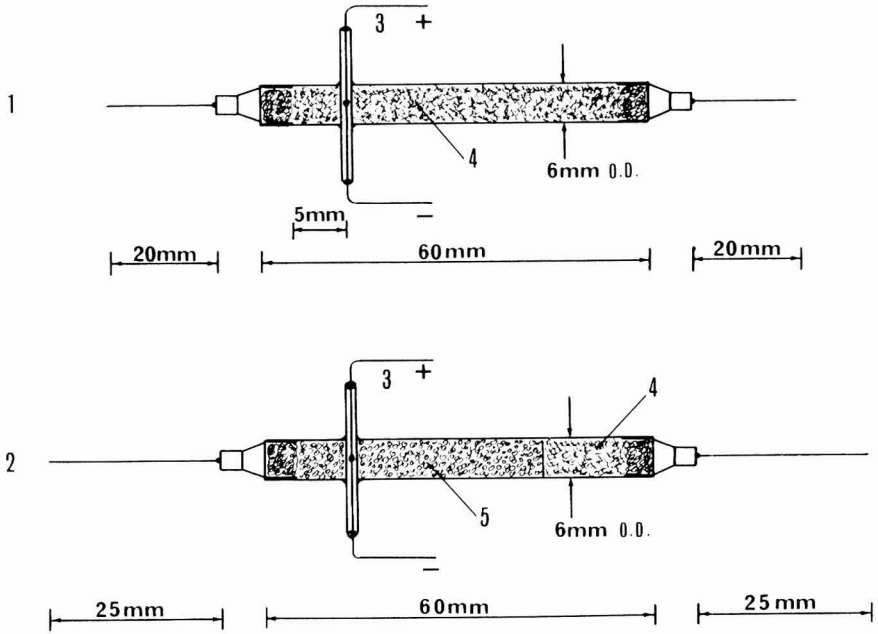

FIG. 4. Stainless steel absorption tube: (1) water absorption tube; (2) carbon dioxide absorption tube; (3) alumel–chromel thermocouple; (4) magnesium perchlorate; (5) soda asbestos.

dition described above. DTA curve I exhibited a endothermic negative peak at 170°C and a smaller negative peak followed at about 210°C probably because of some endothermic decomposition of the sample. A broad exothermic peak took place during the temperature of 210–550°C in the DTA curve I, while no significant exothermic peak appeared in the catalyst curve II. Therefore, it has been assumed that the sample was not vaporized from the quartz vessel but mostly formed carboneous residue which gently evolved heat by surface oxidation during the programmed temperature of 210–550°C. Broad signals of heat evolution identical with the DTA curve I were also observed in the water and carbon dioxide curves III and IV. Analytical results with a heating range of the sample up to 600°C was satisfactorily obtained as listed in Fig. 6.

Behavior of pure organic materials under the programmed temperature was roughly classified with two groups, namely, the vaporization type, such as benzoic acid and acetanilide, and the carboneous residue type, such as sucrose and high molecular compounds. The former type exhibits two endothermic negative peaks at the melting and boiling points in the DTA curve I, while narrow and high peaks appear in other curves II, III, and IV. On the other hand, broad exothermic peaks are

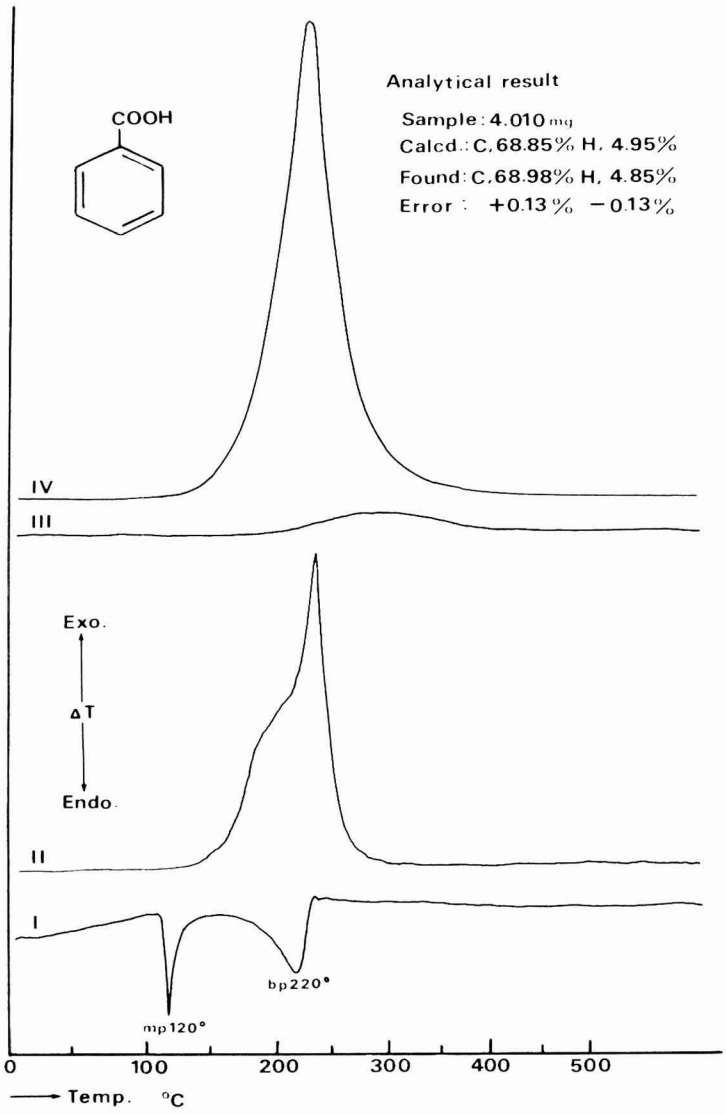

FIG. 5. Thermogram of benzoic acid.

normally obtained in the DTA curve I for the samples of latter type being characterized by insignificant heat evolution in the catalyst curve I.

Cupric Sulfate

Cupric sulfate containing 5 moles of crystal water at ordinary temperature was taken up as an example of dehydration, although it does not belong to organic compounds. The sample was heated at a rate of

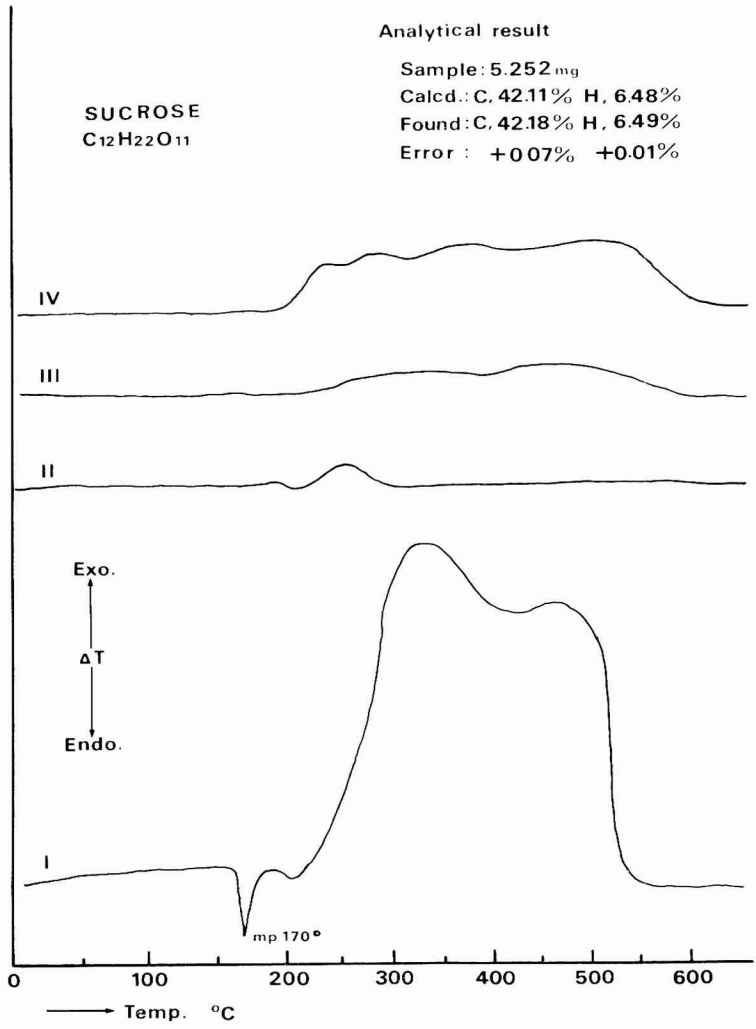

FIG. 6. Thermogram of sucrose.

10°C/min in a nitrogen stream of 10 ml/min. Three negative peaks with minima at 95, 110, and 230°C in the DTA curve I of Fig. 7 indicated the three steps of dehydration of the crystal water. The temperature of the oxidizing catalyst might have been somewhat lowered by the water vapor, so that a broad endothermic negative peak appeared in the catalyst curve II. Two exothermic peaks in the water curve III and the straight line of the carbon dioxide curve IV were naturally explained with the chemical structure of the compound.

The determination of water by weighing the absorption tube resulted in

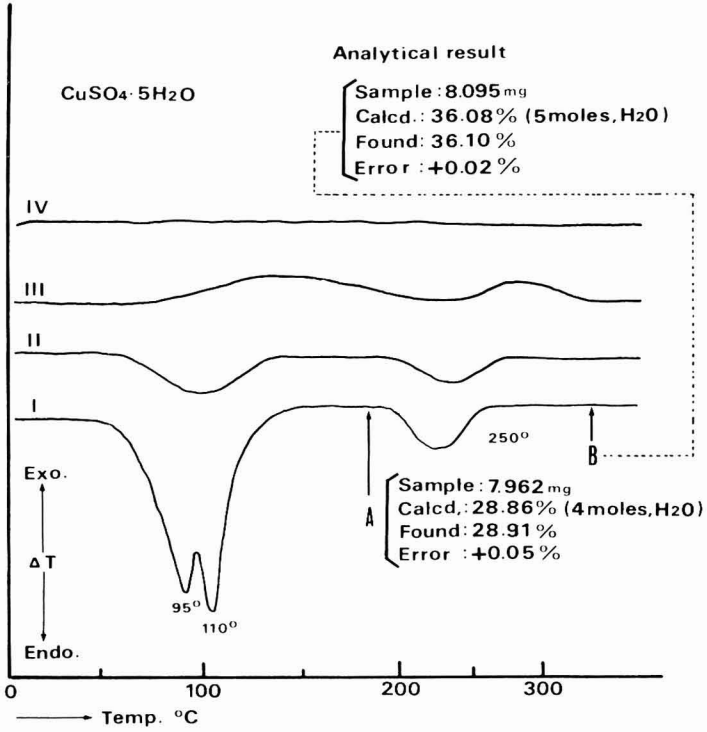

FIG. 7. Thermogram of cupric sulfate.

4 moles of water at point A (195°C) and 5 moles of water at point B (350°C) in Fig. 7. Even in case of a sample of unknown material, it is possible to find out the temperature of dehydration so that the simultaneous determinations of the crystal water and the carbon and hydrogen values will be feasible.

Zygosporin·A Derivative (7)

A determination of crystal solvent was attempted as another example of estimating the composition of effluent gas. Figure 8 is the thermogram of zygosporin·A derivative, an extract of certain bacterial product, containing 1 mole of isopropanol as the crystal solvent. The sample was heated in a nitrogen stream with the normal heating rate up to 350°C. An endothermic negative peak at 160°C which appeared in the DTA curve I might be misconceived as the melting point; but, according to the catalyst curve II, which exhibited also an endothermic negative peak at the same temperature, a rapid release of crystal solvent should rather be implied. It would be of some interest that the release of isopropanol gave an endothermic negative peak at the oxidizing catalyst,

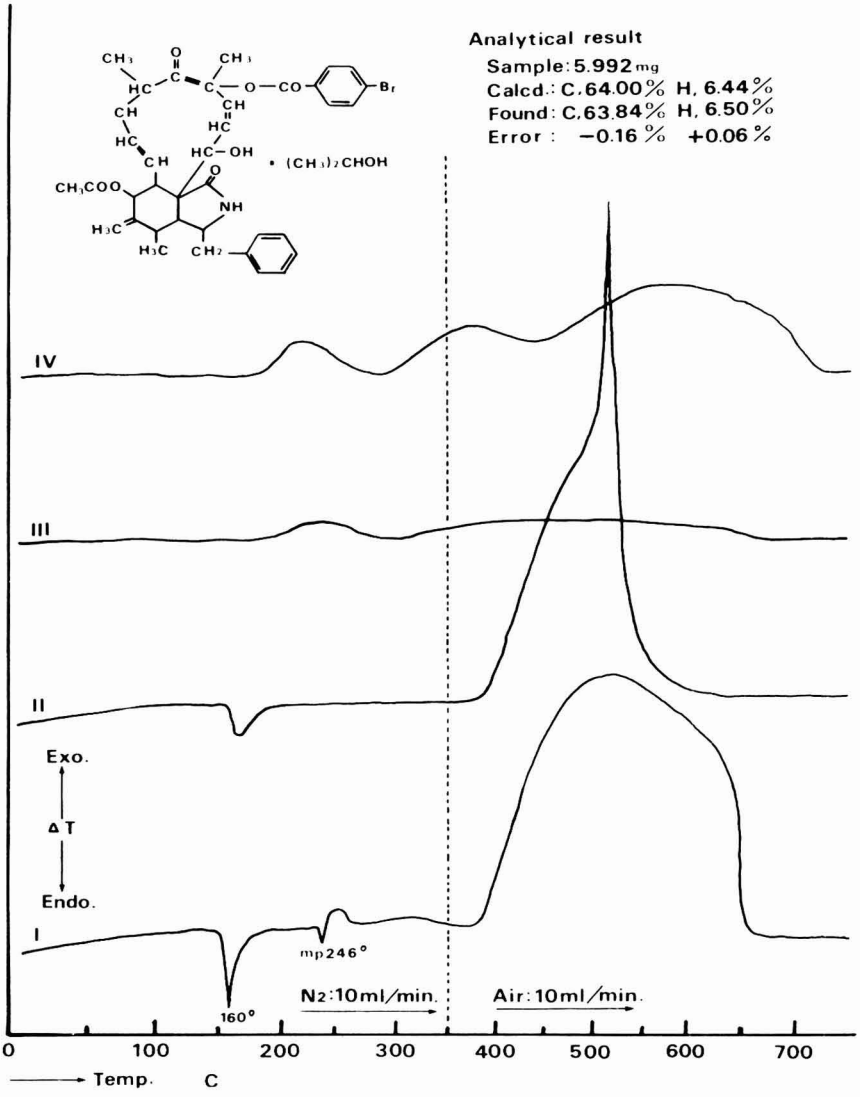

FIG. 8. Thermogram of zygosporin • A derivative.

in spite of supposed exothermic peak by combustion. The reason was assumed because of a very rapid release of isopropanol from the zygosporin • A lowering the temperature of effluent gas towards the oxidizing catalyst. This effect might have been more extensive than the heat evolution due to combustion using the carrier gas of nitrogen. The release of crystal solvent was further ascertained by the exothermic peak in the water and carbon dioxide curves III and IV.

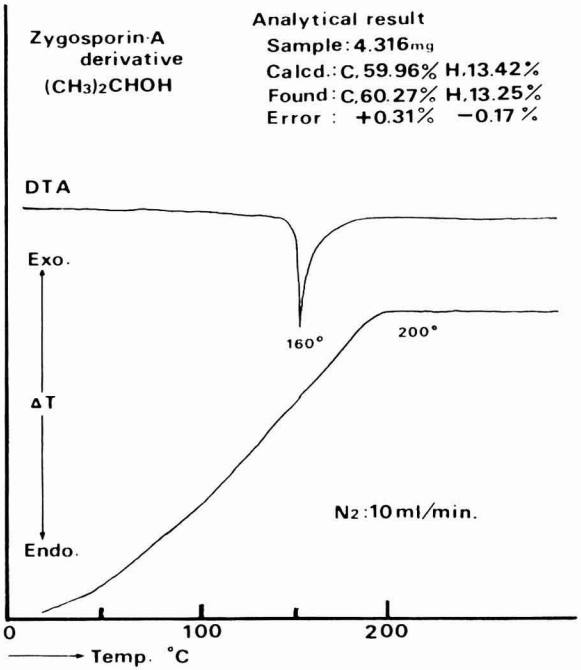

FIG. 9. Thermogram of zygosporin • A derivative.

Figure 9 shows a short-range thermogram with the maximum sample temperature of 200°C. Water and carbon dioxide formed by the combustion were determined by means of weighing the absorption tubes. The analytical data obtained was fairly identical with the calculated value of isopropanol as listed in Fig. 9.

CONCLUSION

The combination of DTA with the carbon and hydrogen determination presented much valuable information, i.e., the individual thermal behavior of the sample during the combustion, release temperature of crystal water or crystal solvent, interpretation of anomalous analytical results due to the property of the same being tested. Grade of impurity of the given sample may be sensitively estimated by referring to the pure standard material.

Although this method could never be a rapid process being based upon the principle, still it may function as a powerful tool for the samples which given anomalous results by other conventional carbon and hydrogen apparatus. Quality control of the industrial products with the method is also a profitable application.

SUMMARY

An application of differential thermal analysis to microdetermination of carbon and hydrogen is described. A sample in a quartz vessel was slowly combusted on a thermocouple detector in an air stream, and carbon–hydrogen values were determined gravimetrically. During the heating process the thermal characteristics of the sample such as dehydration, fusion, boiling, vaporization, sublimation, explosion, decomposition, and oxydation were indicated as the exothermic peak or endothermic negative peak in a differential thermogram. The differential thermocouples were installed in the layer of oxidizing catalyst and additional thermocouples were placed both in water- and carbon dioxide-absorption tubes to monitor the combustion process of organic vapor in the effluent gas. Anomalous analytical results may be reasonably interpreted by referring to numerous data concerning the type of thermal decomposition of the sample.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 17, 105–108 (1972)

Separation of $BiPO_4$ Carried Actinides by BiOCl Precipitation [1]

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1. INTRODUCTION

Health monitoring of persons engaged in plutonium processing plants for accumulated body burden is routinely carried out by radioanalysis of body excretions (*1*, *2*).

Alpha emitters in the process effluents of uranium which has undergone short-term irradiation are predominantly plutonium, uranium, and to a minor extent ionium and americium. Long irradiated fuel will contain higher amounts of transplutonium elements. The present study was carried out to evolve a rapid determination of uranium, thorium, and americium sequentially in a single urine sample. The excretion rates of these heavy elements in urine are of the order of 0.01% (except for uranium) and it is required to take the full volume of urine sample for determination of each element. Sequential analysis avoids repetitive urine collection and processing.

2. OUTLINE OF METHOD

Measure the volume of urine (16-hour collection). Use 1 ml for creatinine determination (*1*).

Add 15.6 ml of HNO_3 (for each 500 ml of urine) to the urine sample and 2 ml of calcium carrier (200 mg). Add 2 ml of H_3PO_4. Heat. Add NH_4OH to precipitate calcium phosphate. Stir well and keep for settling. Centrifuge.

Dissolve the precipitate in 10 ml of 0.5 *M* HNO_3. Add 5 ml of 5% NH_2OH HCl. Keep for 10 minutes. Add 1.5 ml of H_3PO_4. Heat to boiling. Precipitate $BiPO_4$ by adding 1 ml of Bi carrier (100 mg) drop by drop. Stir and keep for settling. Centrifuge (uranium is in the supernatant, thorium, plutonium, and americium remain in the precipitate).

[1] Presented: Atomic Energy Chemistry Symposium September 23–26, 1969. Chandigarh; Sect.: Nuclear and radiation chemistry.

2.1 Uranium (supernatant)

Evaporate supernatant: add 0.5 ml of HNO_3, 2 ml of water, and 2.5 ml of $Fe(NO_3)_3$ (122 mg of Fe/ml). Transfer the solution to a 100-ml separating funnel. Add 30 ml of ether–nitric acid mixture (100 : 5) and extract U. Separate, repeat ether extraction. Filter combined ether extracts through a cellulose powder column, soaked previously in ether–nitric acid mixtures, into a beaker and evaporate off ether. Transfer residue with 1 *N* HNO_3 to a platinum planchet for fluorimetric determination of U (*1*, *3*).

2.2 Thorium and Plutonium (precipitate)

2.2.1. Dissolve $BiPO_4$ in 4 ml of HNO_3; and add 3 ml of water and 1 ml of $NaNO_2$ (1 *M* solution). Pass through 2.5 g of Dowex 1 × 8 anion exchange column conditioned with 8 *N* HNO_3. Wash with 20 ml. of 8 *N* HNO_3. Collect together effluent and washings (containing americium).

2.2.2. Thorium. Elute Th from the column by passing 20 ml of 8 *N* HCl. Determine Th (Ionium) by alpha counting in the eluate (*4*).

2.2.3. Plutonium. Elute Pu with 1.5 *M* NH_2OH HCl in 1 *M*. HCl. (Eluate is taken up with iron for precipitation and alpha counting (*5*) or for electrodeposition (*6*).

2.2.4. Americium. Evaporate effluent and washings from process 2.2.1 to dryness. Dissolve residue in 10 ml of 2 *N* HCl and add 80 ml of 0.01 *N* HCl to hydrolyze. Centrifuge off BiOCl. Evaporate supernatant to dryness and take in 10 ml of 4 *N* HCl. Add 1 ml of La carrier and 2 ml of conc HF. Centrifuge and wash the precipitate (*1*). Transfer to a weighed stainless steel planchet; weigh and count for alpha activity.

Table 1 gives analytical results for urine spiked with different alpha emitters.

TABLE 1

RESULTS OF URINE SAMPLES SPIKED WITH DIFFERENT ALPHA EMITTERS

Tracer	Spiked activity (cpm)	Actual recovery [a] (cpm)	Recovery (%)
Pu	69	58.7	85
Th	16	14.3	89
Am	22	18.3	83
U	392.6 mμg	338.0 mμg	86

[a] Average of 3 sets of urine samples; $1\sigma = 10\%$.

TABLE 2

RESULTS OF HYDROLYSIS OF $BiPO_4$ USING DIFFERENT REAGENTS

Reagents used for hydrolysis	Supernatant: Phosphate (as $MgNH_4PO_4$) (mg)	Supernatant: Bi (as $BiPO_4$) (mg)	Analysis for Bi in precipitate; wt of BiOCl (ppt) (mg)
0.01 *N* HCl	42.2	0.68	117.5
0.1 *M* NH_4Cl	40.6	4.4	114.1
Water	38.6	Nil	120.2

3. DISCUSSION

References (*1–5*) describe methods for the determination of uranium, thorium, and plutonium adopted in the laboratory.

The dissolution of $BiPO_4$ and hydrolysis were studied as follows:

$BiPO_4$ precipitate is dissolved in 10 ml of 2 *N* HCl and warmed. 80 ml of 0.01 *N* HCl is added. The precipitate and supernatant are collected. The precipitate is weighed. Table 2 gives the recoveries of BiOCl. Supernatant is made up to 100 ml and divided into two parts for determination of phosphate and bismuth.

The above experiment is repeated with 0.1 *M* NH_4Cl and water for hydrolysis. The results obtained for PO_4^{3-} and Bi content are shown in Table 2. High $(PO_4)^{3}$ and low Bi in the supernatant, and high BiOCl recovery are considered most favorable for separation.

Of the three techniques 0.01 *N* HCl dilution was the most effective and was adopted for final separation. Low $(PO_4)^{3-}$ in the aqueous treatment are likely to result in the holdback of Am recoveries.

Tracer separation by the hydrolysis method was carried out for $BiPO_4$ carried thorium, plutonium, and americium. Results are given in Table 3.

TABLE 3

TRACER RECOVERY IN SINGLE PRECIPITATION OF BiOCl FROM $BiPO_4$

Tracer	Spiked activity (cpm) present in $BiPO_4$	Tracer in BiOCl precipitate (cpm) (a)	Tracer in supernatant after BiOCl precipitation (cpm) (b)	Total (a) + (b)
Pu	66.7 ± 10%	6.7	60.2	66.9
Am	21.9 ± 10%	0.9	21.3	22.2
Th	24.3 ± 10%	2.7	20.3	23.0

High recoveries indicated that the BiOCl separation technique could be recommended for separation of actinides from bulk carrier bismuth phosphate used in collection of the individual trace element.

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MICROCHEMICAL JOURNAL 17, 109–118 (1972)

Studies on the Distribution and Concentration of Thiamine in Blood and Urine [1]

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The determination of thiamine levels in blood is diagnostic of the nutritional state of the individual, and is important in screening populations for estimating their nutritional well being (*22*). For this reason, we decided to explore and attempt to solve the problems associated with this assay. An additional impetus was the fact that thiamine deficiency is commonly found associated with alcoholic cirrhosis of the liver. Blood thiamine levels are related to the extent of liver damage in these patients (*21*).

The estimation of thiamine in whole blood presents several problems which make this procedure difficult to perform. Most of the thiamine is in the erythrocyte in the form of the pyrophosphate (*2*). This, in turn, is apparently tightly linked to erythrocyte proteins from which it is not readily separated. Trichloroacetic acid (*3, 4*) and metaphosphoric acid (*6, 7*) used to precipitate the proteins do not liberate the thiamine pyrophosphate completely, some of it being carried down with the precipitate (*17*). If blood is allowed to stand with trichloroacetic acid for extended periods, other fluorescent substances, which interfere in the procedure, are generated (*17*). An additional problem is the low concentration at which thiamine occurs in the blood. For this reason only microbiologic (*1, 2*), enzymic (*19*) and fluorometric methods (*3, 5–10, 16, 17*) have served to detect thiamine at this level. Colorimetric methods are too insensitive for this purpose (*12–15*).

In order to isolate the thiamine, it is advantageous to adsorb the material to a column and elute it. Zeolite (*6, 9*), Permutit (*16*), and Decalso (*5*) have all been utilized for this purpose. It is noted in the literature that adsorption and elution on columns is inefficient at the low

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[2] Postdoctorate Research Fellow.

concentration required for assay of blood thiamine levels (*17*).

We first investigated the various procedures for hydrolyzing thiamine pyrophosphate. For this purpose, we used paper chromatography (*11*) to separate the mono- and diphosphate from thiamine itself so as to judge the efficacy of the various phosphatase preparations recommended in the literature. "Active" yeast powder (*14*), kidney powder (*9*), and diastase (*7, 17*) have been recommended for this purpose. We extended the study to the commercially available acid and alkaline phosphatases. Using the preparations previously reported, complete hydrolysis could not be achieved in less than 6 hours, usually requiring incubation overnight. Using a relatively inexpensive wheat germ acid phosphatase, we found that we could readily accomplish this hydrolysis in less than 1 hour.

Exploration of the various procedures for separation of thiamine pyrophosphate from the erythrocytes, including various protein precipitants, demonstrated that this could be quantitatively accomplished only by heating at 100°C for 1 hour, at pH 2. This pH was reached by adding 0.3 *N* HCl solution to blood. This served to hemolyze the blood, liberate the thiamine pyrophosphate, and precipitate the proteins when sodium acetate was added.

In order to remove interfering substances, especially to clarify the solutions, it was found that it was necessary to adsorb the thiamine to a column. For this purpose we found that use of the carboxy resin, CG-50, an acrylamide resin, resulted in excellent recovery of nanogram quantities of thiamine added.

The combination of the use of boiling temperatures at acid pH to liberate the thiamine pyrophosphate, and the use of wheat germ acid phosphatase to liberate the thiamine, and the application of the CG-50 resin column to isolate the thiamine, permitted the ready assay of the thiamine content of whole blood. Readout was by oxidation of the thiamine to thiochrome and estimation by fluorescence.

Study of the thiamine distribution in urine indicated that practically all of the thiamine was in the free state and not phosphorylated. Treatment with the phosphatase was therefore unnecessary. Preliminary extraction of the urine with butanol (*5, 22*) or treatment with benzenesulfonyl chloride (*8*), to remove interfering substances, was unnecessary if one resorted to column purification.

METHODS AND MATERIALS

1-Butanol. Analytical reagent grade.

Potassium ferricyanide (0.15%). 150 mg of $K_3Fe(CN)_6$ to 100 ml with water.

Sodium acetate, 2 N. 27.2 g of the trihydrate to 100 ml with water. Dilute 1:1 with water to make 1 *N* sodium acetate solution.

Acid phosphatase solution. Dissolve 150 mg of wheat germ acid phosphatase (Sigma, 0.3–0.5 units/mg) in 20 ml of 2 *N* sodium acetate.

Acetate buffer (0.2 N, pH 5): To 7 ml of 2 *N* sodium acetate, add 0.4 ml of glacial acetic acid, and make up to 100 ml.

Thiamine standards. Dissolve 56.1 mg of thiamine hydrochloride (Mann Res. Lab, New York, NY) and make to 100 ml with 0.5% oxalic acid. This stock solution contains 500 μg/ml of thiamine and is stable in the refrigerator indefinitely. Prepare a 2.5 μg/ml working standard by diluting 0.5 ml of the stock standard to 100 ml with water. To Erlenmeyer flasks, add 25 ml of 1 *N* H_2SO_4. To each, add the volume of working standard indicated in Table 1 to make the standard curve, treating subsequently as for the column eluates.

Ion exchange column. The column was 1 × 10 cm prepared from the resin C. G. 50, Amberlite (Mallinckrodt), 100–200 mesh. The resin was washed with 1 NH_2SO_4, water and 1 *N* sodium acetate. It was then equilibrated with a pH 5.0 1 *N* acetate buffer.

Method (whole blood). To 4 ml of 0.3 *N* HCl solution add 3 ml of heparinized blood, mixing vigorously. Heat at 100°C for 30 minutes and cool to below 37°. Add 4 ml of the acid phosphatase solution, mix, and incubate for 1 hour at 37°. The pH at this point is approximately 5.0. Centrifuge for 20 minutes at 2000 rpm in an International No. 1 centrifuge. Transfer a 6.5 ml aliquot of the clear supernatant to a 50 ml Erlenmeyer and add 10 ml of 0.2 *N* acetate buffer, pH 5.0, and mix. Pass the solution through the resin column and wash in with 40 ml of water. Set up a reagent blank using 3 ml of water instead of the blood and proceed in the same manner.

TABLE 1

STANDARDS FOR THIAMINE ESTIMATION

Amounts of the 2.5 μg/ml solution listed are added to 25 ml of 1 *N* H_2SO_4 and treated as the column eluate.

Whole blood			Urine		
Std added (ml)	Added (μg)	Blood equiv (μg/100 ml)	Std added (ml)	Added (μg)	Urine equiv (μg/100 ml)
0	0	0	0	0	0
0.02	0.05	2.8	0.04	0.1	2
0.03	0.075	4.2	0.20	0.5	10
0.04	0.100	5.6	0.40	1.0	20
0.05	0.125	7.1	0.80	2.0	40
0.06	0.150	8.5	1.00	2.5	50

Elute the thiamine from the column by passing 25 ml of 1 *N* H_2SO_4 through and collecting 25 ml in a 50 ml centrifuge tube fitted with a ground glass stopper. Add 0.5 ml of potassium ferricyanide solution to the eluate. Mix and let stand for 2 minutes. Now add 4 g of anhydrous Na_2HPO_4, mixing vigorously to dissolve. Adjust to pH 9.8–10.0 by adding 20% NaOH by drop (approx 4 ml). Add 7 ml of 1-butanol and shake vigorously to extract the thiochrome. Centrifuge to clarify the solution and read the butanol layer in the fluorometer. Excitation is at 371 nm and emission is at 450 nm (see Fig. 1).

Prepare a standard curve from the solutions made in Table 1. Set the instrument to read 100 with the highest standard and read the other standards to prepare a standard curve for evaluation of the unknowns. Subtract the concentration equivalent found for the reagent blank from that found for the unknown to obtain the concentration in he blood.

Method (urine). The 24 hour urine is collected over 15 ml of glacial acetic acid. To 10 ml of urine add 10 ml of deionized water. Adjust to pH 4.5–5.5 by adding 10% NaOH dropwise. Add this solution directly to the column; and wash the column with 50 ml of water, discarding the washings. Elute with 50 ml of 1 *N* H_2SO_4 and divide into two portions of 25 ml each. To one (unknown), add 0.5 ml of the ferricyanide solution. The other is the blank. To the blank and the unknown, add the disodium phosphate, proceeding as for whole blood but using the standard curve described in Table 1. Subtract the urine equivalent (μg/100 ml) for the blank from that of the unknown to obtain the thiamine concentration in the urine. The micrograms per 100 ml, divided by 100 and multiplied by the 24 hour volume (ml) yields the total amount of thiamine excreted.

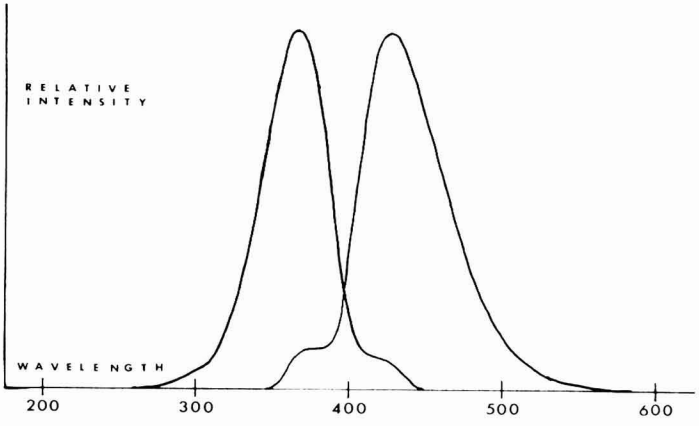

FIG. 1. Excitation and emission spectrum for thiochrome.

Comparison of phosphatase preparations in hydrolyzing thiamine pyrophosphate. The phosphatase preparations compared were diastase from hog pancreas (500–1000 units/mg, Sigma); diastase from malt (Sigma, Type V-A); acid phosphatase from wheat germ (0.3–0.5 units/mg); potato acid phosphatase (0.3–0.5 units/mg); alkaline phosphatase from hog intestinal mucosa (1 unit/mg, Sigma) and alkaline phosphatase from *E. coli* (Type III, Sigma). The acid phosphatases were assayed at pH 5 in an 0.1 *M* acetate buffer and the alkaline phosphatases at pH 9 in 0.1 *M* borate buffer.

In each case 60 μg of thiamine pyrophosphate (cocarboxylase, Mann) were dissolved in 6 ml of the buffer. In the case of the malt and hog diastase preparations, both of which crude preparations are prepared for their amylase activity, 40 mg were dissolved in 6 ml of the acetate buffer containing 60 μg of cocarboxylase, at pH 5. In the cases of the purified phosphatases, 30 mg of the preparation/6 ml of buffer containing 60 μg of thiamine pyrophosphate were used. In the case of the alkaline phosphatases from hog mucosa and *E. coli,* the 0.1 *M* borate buffer (pH 9) was used. For the wheat germ acid phosphatase, the acetate buffer was used at pH 5.0. In each case incubation was at 37°C, samples being taken at hourly intervals for the first 6 hours and at 24 hours.

In addition to the studies on the acid phosphatase preparations, a KCl–HCl buffer was prepared (0.1 *M*) at pH 2.0. The thiamine pyrophosphate was heated at 100°C with this solution to see whether hydrolysis could be achieved nonenzymatically.

Paper chromatography. After hydrolysis, the effectiveness of the procedure was determined by paper chromatography. Samples were taken at periodic intervals for this purpose; and the minimum period required for complete hydrolysis of both the pyrophosphate and the intermediate monophosphate was determined. For this purpose, a previously described method (*11*) was employed.

A sample of the incubated mixture was spotted on Whatman 3MM paper. Ascending chromatography was used; 2 hours was sufficient for a rise of approximately 8 cm. The developing solution was a mixture (by volume) of 1-propanol, water, and 1 *M* acetate buffer (pH 5) in the ratio of 7:2:1. The paper was air dried and then sprayed lightly with a mixture consisting of 1 ml of 2% $K_3Fe(CN)_6$, 15 ml of 15% NaOH, and 76 ml of water. The sheet were air dried and then examined under an ultraviolet ray lamp (long wave, UVL-22 lamp, Ultraviolet Products Inc., San Gabriel, CA). Hydrolysis was considered complete when no mono- or diphosphates of thiamine were visualized. The R_f values observed were 0.11, 0.27, and 0.55 for the thiamine pyrophosphate, thiamine phosphate, and thiamine, respectively.

RESULTS

Using thiamine pyrophosphate as substrate and the crude diastases as the source of the acid phosphatase, only malt diastase had any acid phosphatase activity. The hog pancreas diastase was inactive in this regard. The alkaline phosphatase preparations (hog intestinal mucosa and from *E. coli*) were of no value since the alkaline pH required, split the thiamine into its two components so that it could not be subsequently converted to thiochrome for visualization on the paper. After incubation at alkaline pH (9.0–9.2), no thiamine, thiamine phosphate, or thiamine diphosphate could be detected by paper chromatography. Nonenzymic acid hydrolysis was also ineffective in hydrolyzing the pyrophosphate.

Of the three acid phosphatase preparations (malt, potato, and wheat germ), the wheat germ preparation was the most active. With malt diastase, hydrolysis approached completion at 6 hours at the 6 mg/ml level of the crude enzyme. Potato acid phosphatase exhibited approximately $\frac{1}{5}$ the activity of the wheat germ preparation. With wheat germ acid phosphatase, approximately 1 hour was sufficient to complete the hydrolysis at the 3 mg/ml level. Since the wheat germ acid phosphatase is also the least expensive, its advantages dictate that it be the preparation of choice for thiamine assay.

The method was applied to establish the range for both urine and blood. These results are shown in Table 2.

Urine analysis, comparing urine treated with acid phosphatase before adding to the column and urine not so treated, yielded results that were not significantly different and within experimental error.

Once the conditions had been established for thiamine assay, recovery studies were made by adding 0.05 μg of thiamine as the pyrophosphate to 3 ml of blood. This is equivalent to 1.69 μg/100 ml of whole blood. The results of these studies are shown in Table 3. Note that the mean recovery was 94%.

TABLE 2

VALUES OBTAINED FOR THIAMINE ON WHOLE BLOOD AND URINE SPECIMENS FROM APPARENTLY HEALTHY ADULTS

Range of values is based on an observed log normal distribution. Mean values are arithmetic means.

Whole blood			Urine			
No. of specimens	Mean (μg/100 ml)	Range (μg/100 ml)	No. of specimens	Mean (μg/100 ml)	Range (μg/100 ml)	Mean (μg 24 hr)
18	2.8 ± 0.48	1.9–3.9	11	19.2 ± 16.2	5.6–78	346 ± 291

TABLE 3

RECOVERY OF THIAMINE ADDED AS THE PYROPHOSPHATE TO WHOLE BLOOD OF HOSPITAL PATIENTS

	Mean			
No. of specimens	In blood (μg/100 ml)	In blood + added thiamine (μg/100 ml)	Recovery (μg/100 ml)	Recovered (%)
12	4.35 $\pm$ 2.06	5.87 $\pm$ 2.28	1.59 $\pm$ 0.28	94.1 $\pm$ 16.5

The amount added corresponded to 1.69 μg of thiamine/100 ml of blood.

DISCUSSION

Thiamine pyrophosphate can be oxidized to thiochrome pyrophosphate. However, this cannot be extracted into organic solvents. It is for this reason that hydrolysis is required before assay for thiamine can be accomplished. The wheat germ acid phosphatase serves admirably for this purpose.

It has been only in relatively recent times that acid phosphatases have become commercially available. For this reason, other preparations such as the crude diastases, prepared for their amylase activity, were utilized for their acid phosphatase content, in order to hydrolyze thiamine pyrophosphate. As shown above, some diastases available today are free of acid phosphatase activity. This emphasizes the hazard in utilizing a diastase for its acid phosphatase activity.

Examinations of the thiamine recovered before, and after, the action of acid phosphatase revealed that approximately 90% of the thiamine in the blood is in the form of the pyrophosphate. This conforms to observations made by others (*2*). On the other hand, the thiamine in urine is unconjugated. This is to be expected since the pH of urine is usually at approximately 5 and in addition, acid phosphatase activity is usually present in normal urine.

The methods, which assay for thiamine without adsorption to the column, suffer from the fact that interfering substances are not removed. This is especially significant in urine analysis. In the case of whole blood, color which develops, extracts into the butanol, causing significant decrease in fluorescence due to the absorption of the exciting light by the colored impurity. These problems are readily bypassed by utilization of the column.

Table 3 indicates that, contrary to the experience of others, thiamine can be adsorbed and eluted from a column fairly efficiently. The pH at which the material is placed on the column is critical. For example,

in the procedure, the column is loaded at pH 5 to obtain a recovery of 94.1%. If the column is loaded at pH 6.5, recovery drops to only 40%, in our experience. Similarly a pH significantly less than 3.5 is also undesirable in loading the column. Difficulties experienced by other experimentors probably stem from attempts to fix the thiamine on the column outside the range of optimum pH (3.5–5.5) for this purpose.

Table 2 also shows that thiamine levels in blood (1.9–3.9 μg/100 ml at the 95% confidence limits) are generally in the vicinity of the mean values reported by others. However, our range (2σ) is narrower and suggests a more reproducible procedure. Note that hospital patients show higher levels (Table 2) than the apparently normal healthy adult. This is probably the result of supplementary vitamin administration to almost every patient in the hospital even those on intravenous fluids.

The levels in urine (Table 2) show a much broader range, the value apparently depending upon the thiamine intake in the diet (*18, 20*). The highest levels were found in those taking vitamin pills.

The procedure described above should be useful in other applications such as for tissues and foods and is recommended as a general specific procedure for thiamine estimation.

SUMMARY

Studies are reported resulting in a reliable procedure for estimating the thiamine content in human blood and urine. For the determination in blood, heparinized blood is hemolyzed with 0.3 *N* hydrochloric acid at 100°C. Cocarboxylase is then converted to free thiamine by means of wheat germ acid phosphatase at pH 5.0 in an acetate buffer. The liberated thiamine is adsorbed to a CG-50 (Rohm & Haas) carboxylic acid ion exchange acrylic resin column and then eluted with 1 *N* H_2SO_4. The thiamine is then oxidized to thiochrome and extracted with *n*-butyl alcohol, at pH 9.8–10.0, in the presence of disodium phosphate. Readout is by fluorometry at an excitation wavelength of 371 nm and an emission wavelength of 425 nm. The range found for thiamine in whole blood by this procedure on 18 normal adults was 1.9–3.9 μg/100 ml, with a mean value of 2.77 μg/100 ml of whole blood. The mean recovery of 12 recovery experiments was 94.1%. The same procedure is applicable to the determination of thiamine in urine. Conversion of cocarboxylase to free thiamine is not necessary since it was determined that practically all of the thiamine found in urine is not phosphorylated. Urine values were variable, the range for 11 healthy adults being 5.6–77.9 μg/100 ml with a mean value of 19.2 μg/100 ml. This corresponds to a value of 346 μg of thiamine/24 hours.

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MICROCHEMICAL JOURNAL 17, 119–124 (1972)

Simultaneous Determination of Copper and Manganese with Sodium Diethyl Dithiocarbamate

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Among the several reagents which have been reported for the spectrophotometric determination of copper in the presence of interfering cations, the use of the diethyl dithiocarbamate reagents has recently received some attention (*3, 4*). La Coste (*4*) reported on the determination of copper from acid solutions of pH 1–3.5 using sodium diethyl dithiocarbamate while Claassen and Bastings (*1*) reported on the determination of copper with lead diethyl dithiocarbamate from alkaline solutions at pH 8.5. In all these studies interfering cations were masked by EDTA, citrate, tartrate, or cyanide, either alone or in various combinations of each other. The use of sodium diethyl dithiocarbamate for the spectrophotometric determination of manganese has also been reported. Thus, Specked (*5*) used it to determine manganese in the presence of copper, iron, cobalt, and vanadium after selective solvent extraction of the thiocyanate complexes of the interfering cations from a solution which was 3 *N* in hydrochloric acid, followed by the determination of the unextracted manganese with sodium diethyl dithiocarbamate at pH 8–9. Dittel (*2*) determined manganese in the presence of iron by formation and extraction of the manganese and iron diethyl dithiocarbamates at pH 8–9, followed by selective reextraction of the manganese alone with 2.5 *N* hydrochloric acid, and reformation and reextraction of the manganese diethyl dithiocarbamate complex at a final acid concentration of 0.1 *N* hydrochloric acid containing trisodium citrate and potassium cyanide. However, no method has been reported hitherto, for the simultaneous determination of copper and manganese as their diethyl dithiocarbamate complexes. This paper describes such a method.

[1] Taken from a thesis submitted in partial fulfillment of the MS degree.

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MATERIALS AND METHODS

Reagents

All reagents were of Analar Grade. Distilled water was used throughout. All reactions were carried out at room temperature.

Copper Solutions

Prepare a stock solution of copper by dissolving 0.3929 g of copper(II) sulfate (pentahydrate) in water and diluting to 100 ml. Prepare further solutions from this by dilution.

Manganese Solutions

Prepare a stock solution of manganese by dissolving 0.3602 g of manganese(II) chloride (tetrahydrate) in water and diluting to 100 ml. Prepare further solutions from this by dilution.

Sodium diethyl dithiocarbamate solutions, 2% aqueous (w/v)
Perchloric acid, 70% (sp gr 1.6)
Ammonium hydroxide solution, 1:1 (v/v)
n-butyl acetate
Isobutyl acetate

Recommended Procedure

Pipette 2.0 ml of the test solution into a 50 ml separating funnel, add 1.0 ml of perchloric acid and mix. The measured pH of this solution is 0.7. Now, add 2.0 ml of sodium diethyl dithiocarbamate solution and mix. Add 10.0 ml of *n*-butyl acetate and extract the copper diethyl dithiocarbamate complex by shaking for 30 seconds. Carefully transfer the lower aqueous layer into another 50 ml separating funnel and retain for manganese determination. Read the brown *n*-butyl acetate layer after 5 minutes at 440 nm against a similarly treated blank. Copper is thereby determined.

To the separated aqueous layer *supra*, add ammonium hydroxide from a burette until the solution is just alkaline to a small portion of red litmus paper placed in the solution in the separating funnel. Cool the contents of the separating funnel to room temperature by allowing its contents to stand at room temperature, then add 2.0 ml of sodium diethyl dithiocarbamate solution, mix, and extract by shaking for 30 seconds with 10.0 ml of Isobutyl acetate. Read at 345 nm, 10 minutes after extraction against a similarly treated blank. Manganese is thereby determined.

RESULTS AND DISCUSSION

Tables 1 and 2 are composite tables showing the efficiency of extraction and the stability of the copper and manganese diethyl dithiocarba-

TABLE 1

A COMPOSITE TABLE SHOWING THE EFFICIENCY OF EXTRACTIONS AND THE STABILITY OF COPPER DIETHYL DITHIOCARBAMATE COMPLEX IN SELECTED ORGANIC SOLVENTS [a]

Solvent	Optical density of aqueous phase after solvent extraction	Stability of copper diethyl dithiocarbamate in solvent phase: optical density of solvent phase after extraction (minutes): 2	5	10	15
Carbon tetrachloride	0.004	0.767	0.810	0.824	0.796
Chloroform	0.002	0.770	0.810	0.824	0.796
Methyl isobutyl ketone	0.000	1.222	1.126	0.824	0.288
Isoamyl alcohol	0.000	0.783	0.796	0.854	0.824
n-Octanol	0.000	0.839	0.854	0.854	0.854
n-Butyl acetate	0.000	0.796	0.810	0.810	0.810
Isobutyl acetate	0.000	0.745	0.762	0.762	0.762
n-Amyl acetate	0.000	0.870	0.903	0.870	0.824
Diethyl ether	0.004	nd	nd	nd	nd
	0.131	nd	nd	nd	nd
Benzene	0.187	nd	nd	nd	nd
Toluene	0.086	nd	nd	nd	nd

[a] Test solution (2.0 ml) containing 40μg/2.0 ml of Cu were treated with 1.0 ml of perchloric acid and 2.0 ml of sodium diethyl dithiocarbamate solution, followed by extraction with 10.0 ml of solvent.

[b] nd = not done.

mate complexes, respectively, in selected organic solvents. The results in Table 1 show that although methyl isobutyl ketone gives the most sensitive procedure for the extraction of the copper diethyl dithiocarbamate complex at the measured pH 0.7, nevertheless, this solvent may not be so employed because of the instability of the color of the copper complex in it. Among the solvents tested, *n*-butyl acetate, isobutyl acetate, and *n*-octanol were the only solvents which combined both efficiency of extraction and stability of the color of the copper diethyl dithiocarbamate in the organic phase. Due to the slower rate of separation of the *n*-octanol phase after extraction, *n*-butyl acetate was selected as the preferred extracting solvent for the copper complex. Furthermore, the experimental data showed the maximum absorbance of the copper diethyl dithiocarbamate complex in *n*-butyl acetate to be 440 nm and the absorbance reading time to be at least 5 minutes after solvent extraction of the complex.

The results in Table 2 demonstrate that, except for the two aromatic hydrocarbons tested, all the recorded solvents efficiently extracted the manganese diethyl dithiocarbamate and that the color of the complex

TABLE 2

A COMPOSITE TABLE SHOWING THE EFFICIENCY OF EXTRACTION AND THE STABILITY OF MANGANESE DIETHYL DITHIOCARBAMATE COMPLEX IN SELECTED ORGANIC SOLVENTS, IN THE PRESENCE OF AMMONIUM PERCHLORATE [a]

Solvent	Optical density of aqueous phase after solvent extraction	Stability of manganese diethyl dithiocarbamate in solvent phase: optical density of solvent phase after extraction (minutes):			
		2	5	10	15
Carbon tetrachloride	0.000	0.615	0.615	0.615	0.615
Chloroform	0.000	0.699	0.699	0.699	0.699
n-Octanol	0.000	0.710	0.721	0.678	0.658
Isoamyl alcohol	0.000	0.783	0.854	0.699	0.569
2-Octanol	0.000	0.699	0.699	0.658	0.620
n-Butyl acetate	0.000	0.678	0.678	0.678	0.678
Isobutyl acetate	0.000	0.770	0.770	0.810	0.810
Diethyl ether	0.000	0.770	0.699	0.620	0.569
Benzene	0.783	nd [b]	nd	nd	nd
Toluene	0.783	nd	nd	nd	nd

[a] Test solution (2.0 ml) containing 40μg/2.0 ml of Mn were treated with 2.0 ml of ammonium perchlorate solution (10%) and 2.0 ml of sodium diethyl dithiocarbamate solution (2%), followed by extraction with 10.0 ml of solvent.

[b] nd = not done.

was stable in carbon tetrachloride, *n*-butyl acetate and isobutyl acetate. These results were obtained by formation and extraction of the manganese diethyl dithiocarbamate complex in the presence of 2.0 ml of 10% ammonium perchlorate solution instead of forming the ammonium perchlorate *in situ* by adding 1.0 ml of perchloric acid followed by neutralization with ammonia solution as given in Recommended Procedure. Under this circumstance, it was observed that the blanks were unduly high, indicating that excess of the diethyl dithiocarbamate reagent was also being extracted, presumably, due to the presence of such a large excess of perchlorate ions. From these results, isobutyl acetate was selected as the preferred solvent for the extraction of the manganese complex, while further experimental data showed the maximum absorbance of the manganese diethyl dithiocarbamate complex in isobutyl acetate to be 345 nm and the absorbance reading time to be 10 minutes after solvent extraction of the complex.

The procedures were applied as outlined for the simultaneous determination of copper and manganese and straight-line calibration graphs were obtained for both copper and manganese as their diethyl dithio-

TABLE 3

ACCURACY OF CALIBRATION CURVE FOR COPPER DETERMINATION AS THE COPPER DIETHYL DITHIOCARBAMATE COMPLEX AT pH < 1.0

Cu content (μg)	
Present	Found
4	4
8	8
12	12
16	16
24	24
30	30

carbamates over the concentration range of 0–30 μg/ml as Cu and 0–30 μg/ml as Mn. These calibration graphs were tested at various concentrations of copper (Table 3) and manganese (Table 4) and were found to give accurate and reproducible results.

To complete these studies, the recoveries of copper and manganese from mixtures of the two were determined by the recommended procedure described. The results of these experiments (recorded in Table 5) show excellent recoveries of both copper and manganese by the procedure. They further demonstrate that this method of simultaneous determination of copper and manganese is simple, rapid, accurate, and reproducible.

SUMMARY

A simple, rapid, accurate, and reliable method for the simultaneous determination of copper and manganese in the presence of each other is reported. The method involves the selective formation of copper diethyl dithiocarbamate complex at pH < 1.0 in the presence of perchloric acid and solvent extraction of the

TABLE 4

ACCURACY OF CALIBRATION CURVE FOR MANGANESE DETERMINATION AS THE MANGANESE DIETHYL DITHIOCARBAMATE COMPLEX

Manganese content (μg)	
Present	Found
4	4
8	8
12	11
16	17
24	24
30	31

TABLE 5

SIMULTANEOUS DETERMINATION OF COPPER AND MANGANESE IN MIXTURES OF EACH OTHER

Mixture	Present in mixture (μg)		Found in mixture (μg)	
	Copper	Manganese	Copper	Manganese
A	8	8	8	9
B	4	12	4	12
C	12	8	12	8
D	20	40	21	40
E	40	8	41	8
F	20	20	20	21
G	40	20	42	20
H	8	40	8	39
I	40	40	40	40

copper complex with *n*-butyl acetate. The separated aqueous layer is neutralized with ammonia solution followed by formation of the manganese diethyl dithiocarbamate and subsequent solvent extraction of this complex by isobutyl acetate in the presence of perchlorate ions.

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MICROCHEMICAL JOURNAL 17, 125–134 (1972)

Book Reviews

Fluorescence Spectroscopy. By A. J. PESCE, C-G. ROSEN, AND T. L. PASBY. Dekker, New York, 1971. xiii + 247 pp. $16.50.

The first chapter of Fluorescence Spectroscopy provides a very concise, beautifully written review of the nature of light, quantum chemistry, molecular orbital theory, electronic transitions, and absorption and emission spectra.

The next four chapters develop in detail the principles of fluorescence, and the final chapter describes the use of fluorescence in protein binding studies, obviously the major research area of the editors.

The editors succeed in their major objective of providing a "thorough grounding in the theoretical approach to fluorescence spectroscopy." They have managed to edit the individual chapter contributions to provide a coherent treatment with a uniform style. However they may be somewhat overly optimistic in expecting most biologists to follow some of the mathematical treatment and derivations.

The applications of fluorescence are not discussed in detail except for protein binding, but the reader who masters the fundamentals presented in this treatment should be able to move into any of the applied areas.

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Modern Analytical Chemistry, Vol. 1. By W. F. PICKERING. Dekker, New York, 1971. xii + 622 pp. $13.75.

As the title of the book indicates, this publication represents a description of nearly all the modern instrumental and noninstrumental methods currently used in analytical chemistry. This the author does in over 600 pages. Although these reviewers possess great admiration for the author's tremendous effort, we feel that, in many instances, the material is superficially developed, making us doubtful of the benefit it can offer beginners in the field.

The volume contains 16 chapters with a total of over 80 subheadings. Each chapter contains valuable references and problems. Because there are no laboratory experiments described, we are of the opinion that many academic instructors will hesitate in using this book as a manual for undergraduate classes. Actually, the text offers an excellent review of the field and as such it would be more suitable for graduate courses in analytical chemistry, biology, and medicine. Furthermore, because of the extensive coverage of topics and references, it should find wide use as a reference text by individuals interested in analytical chemistry and should be on the shelf of every chemistry library.

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Lens Aberration Data. By J. M. PALMER. **Monographs on Applied Optics, No. 1.** Amer. Elsevier, New York, 1971. xi + 118 pp. $17.00

This is the first of a proposed series of monographs on optical design. A preface by Professor W. D. Wright explains that the series is to consist of specialized studies on selected areas of optics, written by young and critical authors; a potential source of manuscripts is the library of MSc reports prepared by optics students at Imperial College. We should therefore expect in-depth treatments for experts rather than elementary expositions directed toward the general reader.

Volume 1 of the series indeed fits this prescription. It plunges into the subject of aberrations (deviations from paraxial optics) with the implicit assumption that the reader is competent in the field. A professional interest is certainly a prerequisite for persisting through this little book.

The four pages of Chapter 1 provide all of the introduction deemed necessary and also describe the double Gauss objective to be used as an example. Chapter 2, entitled The Aberrations of Geometrical Optics, presents the principles of ray tracing in sufficient detail to refresh the memory of anyone who has already learned the subject. Image analysis is treated with respect to total ray aberrations, particular aberrations, and wave front aberrations.

Chapter 3 discusses tolerances for aberration, and several tolerance functions are considered: figure of merit functions, the Rayleigh limit, the Strehl intensity ratio, the optical transfer function, and encircled energy. Chapter 4 is devoted mainly to description of a Fortran computer program for generating interferograms. Chapter 5 appraises the various methods of analysis of aberration.

This book is suited for the professional designer of optical systems and may be recommended to this narrow circle of experts. It is not appropriate for casual reading or instructional use. There are numerous typographical errors, mostly of a trivial nature.

DONALD E. SANDS, *Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506*

Identification Techniques in Gas Chromatography. By D. A. LEATHARD and B. C. SHURLOCK. Wiley, New York, 1970. x + 282 pp. $13.95.

The book under review covers virtually all the techniques which can be used to identify gas chromatographic effluents. Many examples of their application are given and the methods range from those which enable an identity to be confirmed to those which indicate the general nature of a poorly characterized sample. Not only are the more sophisticated techniques, such as mass spectrometry, described; but many simple, yet effective, methods are considered which are suitable for the smallest laboratory. The aim of the book, it seems to this reviewer, is to bridge these extremes and to try to bring some coherence to a field which straddles many subjects and expertises.

The book is divided into 11 chapters dealing with the following topics: principles of retention and column selectivity, a large chapter devoted to the fundamentals of retention, selective abstraction as a means of identification involving chemical modification of the sample, pyrolysis gas chromatography and other degradative methods of identification, measurement of molecular weight in gas chromatography, identification from detector response, peak trapping, IR and

other spectroscopic techniques of identification, and combined GLC-MS spectrometry. The chapter on retention fundamentals is quite long since, in the view of the authors, retention data are not fully appreciated in analytical circles, and yet these data are crucial for the understanding of basic retention coincidence techniques. The chapter on selective abstraction is particularly useful. The precautions to be exercised when using columns of boric acid and firebrick, or similar support material, are noteworthy. Although ancillary techniques for identification are well covered, it is to be regretted that thin-layer chromatography, a very valuable separation tool, receives only scant mention in chapter 3. It is good to see considerable discussion devoted to little used, but potentially valuable, detectors such as the piezoelectric detector, the mass detector, and the often neglected gas-density balance. The gas-density balance diagrams are exceptionally clear and lucid. While it is correct to state that the mass detector has been used to weigh 10 μg samples, it is also true that the limit of detection is 0.5 μg. Development of the piezoelectric detector as a quantitative tool is feasible, but changes in frequency resulting from stationary phase bleed from the column on to the detector, and from the detector itself may make the long-term stability questionable. One of the book's shortcomings is that the main portion of material presented is devoted to the identification of gross amounts of materials. It would have been desirable to give more explicit and detailed information on the somewhat more difficult task of identifying and separating trace constituents.

To sum up, the book is well documented, adequately indexed, and as up-to-date as possible. The chapters are well written, the diagrams are clear, and free from significant errors. The volume is a forward looking one and is not merely a collection of works of other people, and, as such, it should be essential reading for anybody, expert and novice alike, engaged in separation work and who wants to master the intricacies of gas chromatography.

GEORGE WIENER, *Pfizer, Inc., Brooklyn, New York 11206*

Atomic Absorption Spectroscopy. By GARY D. CHRISTIAN AND FREDRIC J. FELDMAN. Wiley (Interscience), New York, 1970. xix + 490 pp. $16.50.

This book presents atomic absorption spectroscopy for all but the most experienced worker in the field. It is divided into two general sections: Part I, Principles and Theory; Part II, Applications.

In Part I, the authors have presented the theory of absorption of light by atoms in such a way that the beginner will understand. Instrumental parameters such as various sources, various types of atomization processes, monochromators, and detection systems, are then discussed in detail. The chapter on various types of interferences, spectral, excitation, ionization, chemical, and light scatter and molecular absorption, is useful. The chapter presenting an explanation of terms, and also the methods used in increasing detection limits, is well done. Chapter 6 compares about 25 commercial instruments for their specifications and prices; also given are the addresses of the company producing such instruments. This kind of information is not usually found in texts such as this. I think that the 20-odd pages used to present this material were well spent. Recent developments, such as the sampling boat and laser microanalysis, are covered; there is a chapter on atomic emission and atomic fluorescence.

The first chapter of Part II describes some methods of sample preparation. The remainder of the book, about half of the total pages, is devoted to atomic absorp-

tion of specific elements. These are grouped according to periodicity: the alkali metals, the alkaline-earth metals, etc. For each metal, its occurrence in biological environments is presented; this is followed by an analysis section giving specific absorption lines, sensitivities, interferences, special problems that may arise, and literature citations of recent work on that particular element.

Appendix 1 gives the detection limits of each element. There are about 1800 references to authors, giving a very comprehensive bibliography.

This book presents up-to-date techniques and instrumentation available in atomic absorption spectroscopy, and will be useful to both the beginner and the advanced spectroscopist.

ROGER A. LALANCETTE, *Department of Chemistry, Rutgers University, Newark, New Jersey 07102*

Qualitative Organic Analysis. By WILLIAM KEMP. McGraw-Hill, New York, 1970. ix + 2 unnumbered + 183 pp. $7.50.

This valuable book makes simultaneous use of chemical organic analysis and spectroscopic analysis to identify functional groups in a molecule. In fact, the book is predominantly spectroscopic analysis and according to accepted ideas about the subject, the reviewer believes that the title should have been expanded somewhat. However, Dr. Kemp is correct in his idea that both chemical and spectroscopic methods should be presented to the student.

The book is divided into eight chapters, namely, Chemical Examination of an Unknown Compound; Classification of an Unknown Compound—Chemical and Infrared Evidence; The Infrared Spectrum; Identification, Derivatives, Tables of Physical Constants; The Nuclear Magnetic Resonance Spectrum; The Electronic (Ultraviolet–Visible) Absorption Spectrum; The Mass Spectrum; The Chemistry of the Class Reactions. In addition, two pages are devoted to a section called Separation of Mixtures, which includes chemical and physical means.

The author assumes that the student has had introductory courses on the theory and practice of spectral methods of analysis and also that he has access to the various spectra of the unknown compounds (either recorded by the student or by a technician). This would tend to limit the use of the book as a text to courses for which other courses on spectral analysis are prerequisite, as well as to larger colleges and universities that have the necessary equipment.

Other places where the author assumes that the student knows more than he probably would, are in Chapters 1 and 4. In Chapter 1, a number of tests make no mention of the amount of unknown to be used, which the reviewer considers to be very important to the success of the tests. Likewise, in Chapter 4, no formulas are given for the calculation of the equivalent weights of the compounds containing 15 different functional groups.

A number of fine features are present in the book. Chapter 3 concludes with an 11-page correlation chart which contains a wealth of information regarding infrared spectra. This should be most useful to anyone in the field.

Chapter 4 contains 56 tables of the type usually found in books on qualitative organic analysis. These list compounds with physical constants and physical constants of likely derivatives.

Chapter 5, although rather short, provides considerable information on nuclear magnetic resonance spectra. Figure 5.1 in this chapter shows single spin splitting

systems, presented quite clearly. The chapter concludes with five tables. These show values for the protons of CH_3, CH_2, and CH attached to various groups and to saturated hydrocarbon residues; values for the protons of CH_2 and CH groups bearing more than one functional substituent; values for H attached to unsaturated and aromatic groups; proton–proton spin coupling constants; and shifts in the position of benzene protons caused by constituents.

Chapter 6 deals with ultraviolet and visible spectra and includes four tables dealing with data on conjugated dienes and trienes, unsaturated carbonyl compounds, aromatic hydrocarbons, and heterocyclic systems.

Chapter 7 on mass spectroscopy is one of the best chapters in the book. It discusses the molecular ion, the molecular formula from the molecular ion, fragmentation patterns, and the cleavage of a number of types of compounds.

The final chapter deals with the chemistry of the class reactions. This chapter is much too short, omitting much information which should be present in a book of this title. Here, too, indicates the importance of the spectral work over the chemical analysis portion. Following Chapter 8 is a section of two pages entitled "Separation of Mixtures." A few chemical and physical means are mentioned, but too briefly.

Throughout the book, the author lists reference texts for the reader's information. This, too, is most helpful to one involved in investigative work.

In general, this book will be very valuable to anyone involved in any branch of organic chemistry, analytical or preparative, and the reviewer recommends it highly.

AL STEYERMARK, *Department of Chemistry, Newark College of Arts and Sciences, Rutgers University, Newark, New Jersey 07102*

"Progress in Thin-Layer Chromatography and Related Methods." Vol. 2. Edited by A. NIEDERWIESER AND G. PATAKI. Ann Arbor Sci. Pub., Ann Arbor, MI, 1971. xii + 259 pp. $18.75.

The second volume in this series, international in scope, contains seven contributions. Three papers deal with general problems: reflectance spectroscopy, the combination of TLC with gas chromatography, and the use of azeotropic mixtures as solvents. The other four contributions treat more specialized subjects: the chemistry, physical properties, and chromatography of lipids containing ether bonds, the analysis of molecular species of polar lipids, the application of TLC in pharmacognosy, and TLC of amino acids in urine.

One of the more interesting and fascinating chapters in the volume under review is, in this writer's opinion, the paper dealing with TLC in pharmacognosy. Largely because of TLC the number of investigated plant species and of isolated chemical compounds has greatly increased during the past decade. Nevertheless, the number of investigated plant species is still negligibly small compared to the tremendous number which make up the plant kingdom. Consequently, the preparation of pure compounds also lags considerably behind the possibilities. Through further improvements in TLC technique, it is hoped that considerable advances may be made with respect both to investigated species and the isolated compounds—and consequently to one of the aims of medical plant and drug research: the preparation of plant substances capable of new therapeutical action.

To sum up, the editors have presented their particular expertise in a manner

to benefit the novice and expert alike. This is basically a very readable book in spite of changes in styles between chapters. It is well edited, clearly written and presented. While no author index is provided, a regrettable omission in an otherwise high quality book, the abundant references at the end of each chapter will bring the reader very up-to-date in the topics covered. In view of the volume's moderate price, as cost of books go these days, its acquisition by a wide variety of workers in various scientific disciplines is recommended, and it should go a long way in providing help in the practical performance of their work.

GEORGE WIENER, *Pfizer Inc., Brooklyn, New York 11206*

Fundamental Chemical Equilibria—Nonionic—Ionic. By KELSO B. HOWARD. Gordon and Breach, New York, 1971. x + 110 pp. $12.50.

This four chapter monograph includes sections on: (a) certain principles of thermodynamics related to equilibrium processes; (b) acid–base equilibria in aqueous solution (as interpreted by the Bronsted theory); (c) solubility, precipitation, and complexation; and (d) oxidation–reduction equilibria and voltaic cells

Despite the fact that authors of textbooks on general chemistry, analytical chemistry, and physical chemistry devote large amounts of space to several aspects of the topic, the publisher feels that the monograph will be of maximum value to students of general chemistry, introductory analytical and physical chemistry, introduction to chemical engineering and biochemistry for medical and dental students.

Exercises are given at the end of each chapter; answers are provided for some of these in a two page appendix. Another appendix includes brief tables of ionization constants, solubility product constants, formation constants for complex ions, and instability constants for complex ions. There is an alphabetical list of the chemical elements, with symbols, atomic numbers, and atomic weights; a table of four place common logarithms; and a subject index.

Unlike most monographs no references are provided and the text is free of citations to the primary literature. The dust jacket indicates that a paper bound edition is also available, presumably at lower cost.

ROBERT A. HARTE, *American Society of Biological Chemists, Bethesda, Maryland 20014*

Physicochemical Characteristics of Oligonucleotides and Polynucleotides. By BOREK JANIK. Plenum, London, 1971. viii + 213 pp. $14.50.

This book represents a successful survey and compilation of the literature, for the first time in a single volume, of the vast amount of selected physicochemical characteristics of oligonucleotides, synthetic polynucleotides, and their complexes into a useful format, tabulated in 26 tables. A total of 979 compounds from the sources of 289 references has been collected and compiled. The information thus cited in the tables covers the literature up to the end of 1970.

The tables consist of three main sections, presenting data on dissociation properties for 125 different compounds, spectral characteristics for 474 various compounds, and melting temperature for 380 different compounds, respectively. Included in the tables are: the values of pK_{mono}, pK_{oligo}, pK_{poly}, and pK_{polym} in the section of dissociation properties; pH values, λ_{max}, molar absorptivity (E),

and percentage hypochromicity or hyperchromicity (h) in the section of spectral data; pH values and T_m (that is, the melting temperature, which is defined as a temperature at which the temperature-induced change of an experimental quantity is 50% complete) in the section of melting temperature. In addition, the following common information is also included in all tables under each specific compound: methods of investigation, the appropriate experimental conditions, temperature (where available), original literature references, and pertinent supplemental information (under notes or footnotes in each section).

In order to have a convenient and effective use of the tables, the author has given a brief but clear illustration, "Explanation of Tables," in the beginning of the book. For examples, the selection of materials, that is, "Content of Tables," "Nomenclature and Symbols," "How to Use the Tables," and "Abbreviations," etc., are described so clearly that they are essentially serving as a general guideline for using the tables. Thus, the book becomes a highly readable and valuable working reference, facilitating the understanding of the role of structure, conformation, and other properties of oligonucleotides, polynucleotides, and their complexes in various chemical and biological events.

In summary, the author has done an excellent job summarizing and compiling such a great number of physicochemical data together with the necessary pertinent information such as the proper methods of investigation, the optimum experimental conditions, and the additional references for the information of those compounds not including in the tables. This book is intended for both graduate students and research workers in the biological and chemical sciences. At a cost of $14.50 this book will be found not only on library shelves, but also placed on the individual desk of biochemists, biophysicists, physical chemists, organic chemists, and molecular and quantum biologists.

GEORGE W. C. HUNG, *Materials Science Toxicology Laboratories, The University of Tennessee, Medical Units, Memphis, Tennessee 38103*

Microbial Aspects of Metallurgy. Edited by J. D. A. MILLER. Amer. Elsevier, New York, 1971. 202 pp. $11.25.

Metallic corrosion is a chemical reaction between a surface and its environment. Similarly, the life processes of microorganisms consist of chemical reactions between the living cells and their environment. Inevitably, interactions between these two types of chemical reactions develop in nature and frequently result in deterioration and degradation of metallic substrates. Not all microbes are involved in metallic corrosion, of course, and not all metals corrode. Only under very specific conditions of nutrients, temperature, moisture, acidity, oxygen access, etc., do certain microbes promote corrosion. This book undertakes to examine the pertinent factors involved in metallic corrosion by microbes with a view to explaining the mechanisms involved and, more importantly, to devise methods of combating the same. Additionally, other microbe-related phenomena are discussed that do not directly involve metallic corrosion, but do entail undesirable consequences, e.g., the development of slime by algae in closed-loop water cooling towers. Finally, there is a discussion of the beneficial aspects of certain microbes in connection with the extractive metallurgy of some metals.

This book is presented in eight chapters written by seven authors in addition to the editor. All are in English and the writing style is clear and concise. In view

of the somewhat unusual combination of disciplines involved in microbe-promoted corrosion, it was found desirable by the editor to provide chapters on Introductory Microbiology and Introductory Corrosion for the edification of those readers not conversant with these topics. Other chapters are devoted to microbial corrosion of buried or immersed metal, aircraft fuel systems and corrosion related to metal machining and deformation. Another chapter deals with corrosion in industrial situations by mixed microbial floras; and includes such topics as microbial infection effects on cooling towers, heat exchangers and pipework. This is an important chapter to those concerned with the design and operation of closed-system water cooling towers, as the number and size of such devices will proliferate in the present climate of ecological awareness. Each chapter is provided with a list of pertinent references and suggestions for further reading. The Appendix to Chap. 5, Microbial Infections in Relation to Corrosion During Metal Machining and Deformation, gives a detailed procedure for the microbiological examination of petroleum products.

WILLIAM H. GRAFT, *La Salle Steel Co.*
Hammond, Indiana

Encyclopedia of Industrial Chemical Analysis, Edited by F. D. SNELL AND L. S. ETTRE. Wiley (Interscience), New York, 1971, Vol. 12, Dyes to Flour, xiv + 618 pp.; Vol. 13, Fluorine to Glycols, xiv + 607 pp. Each volume $45.00 ($35.00 by subscription).

Earlier volumes of this significant compendium have been reviewed by this journal (see *Microchem. J.* **16**, 171 (1971)). With Volume 12, the chemical alphabet for products and groups of products extends from dyes and reaches flour. Intermediate topics include EDTA and related compounds; eggs and egg products; elastomers, synthetic; embalming chemicals; enamels; epoxy compounds; epoxy resins; essential oils; ethanolamines; ethers; ethylene oxide; ethylene and propylene polymers; europium; explosives; fats and fatty oils; felt; ferrites; and films, photographic.

In Volume 13, topics treated include, fluorine, inorganic fluorine compounds, inorganic fluoro acids, fluorocarbons, (and polymers), formaldehyde, formic acid, fumigants, fungicides, furan and derivatives, gallium (and its alloys and compounds), noble gases, natural gas, gasoline, gelatin, germanium, glass and glass-ceramics, glues of animal origin, glycerol, and glycols and polyhydric alcohols.

The article on dyes in Volume 12 effectively compliments the article on colors for foods, drugs, and cosmetics in Volume 10 and provides ready-to-use procedures for the preliminary study of colorants and their identification. The article on EDTA and related compounds is timely and brings together useful procedures for the identification and determination of these chelating agents, when present both as major components and in trace concentrations in such products as soaps, detergents, food products, water, and biological fluids.

The 30-page article on fungicides, in Volume 13, provides physical properties and general information on over 25 commercial fungicides, and also full analytical procedures for determination of their residues in fruit, plants, and seeds. It is noteworthy that quantification is largely based on photometry (15 procedures) and less frequently on gas chromatography (3 procedures). Isotope dilution, fluorimetry, polarography, and bioassay are each employed in a single procedure only.

Separation techniques involved, either in concentrating fungicides or their reaction products or in removing major interferences, include extraction or extractive leaching (15 procedures), column chromatography (3), steam distillation (3), thin-layer chromatography (2), liquid chromatography (1), and acid digestion (1).

In the organization of this encyclopedia, general techniques were treated in Volumes 1 through 3; however, methods that have only recently attained prominence are considered, as appropriate, in the alphabetical treatment of products. Volume 13 thereby includes a 38-page article on Fourier transform spectroscopy. This technique can come of age now that digital data reduction and computer-based data analysis are available. Fourier transform spectrography appears to offer certain advantages over dispersion spectrometry, including improved signal-to-noise ratio and ease of multiple scan. At least five companies are now offering "FTS" systems, some covering the spectrum from the ultraviolet to the far-infrared.

This encyclopedia continues to be recommended to any library having collections and clientele oriented to chemistry and chemical technology. With 13 volumes (released in the period 1965–1971) now available, the analyst should more and more think of this work as first place to look for a ready-to-use procedure.

A. J. BARNARD, JR., *J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865*

Biological Techniques in Electron Microscopy. By CLINTON J. DAWES. Barnes and Noble, New York, 1971. xiv + 193 pp. Paperback, $9.95.

This book provides a concise summary of the methods of preparing biological specimens for electron microscopy. The pace is swift, the topics are many, and the treatment is variable in this catalog of preparative techniques. The fifteen pages of Chapter 1 include a historical review of electron microscopy, the principles of construction of an instrument, a discussion of resolution, a listing of preparative methods and what each can accomplish, and instructions for designing and staffing an electron microscopy laboratory.

Subsequent chapters treat fixation (both chemical and mechanical), dehydration, embedding in plastics, plastics and epoxy resins, trimming of plastic blocks and knife making, ultrathin sectioning and the ultramicrotome, preparation of specimen grids, evaporation and replication, staining, and photography. Appendices are devoted to commercial sources of equipment, the chemistry of epoxy resins, the schedule of sample preparation, localization techniques, and general references. Most of the treatment tends to be specific and detailed; for example, the chapter on staining includes several recipes for preparing stains, and the section on photography goes into the properties of certain commercial films and gives lengthy instructions on how to develop and print photographs.

Some statements in the book seem superfluous: does an electron microscopist need to be told that the sine of an angle cannot exceed 1, or that 95% alcohol is cheaper than absolute alcohol, or that boxes of photographic printing paper should not be opened in the light? There are instances of careless construction (on p. 22 $KMnO_4$ seems to be classified as an aldehyde, on p. 34 HCHO is used in a heading as a generic formula for aldehydes). Some sections are so error laden as to completely undermine the reader's confidence in what is being said, and on a single page we can find the statements or implications that the ratio of 0.2 μm to 0.1 nm is almost 1000, that the number 0.612 varies from 1.22 to 0.61, that the index of refraction of free space is 0, and that diffraction is the bending of

light. There is frequent repetition of minor points; surely one reminder that 1 mm of Hg equals 1 torr is enough, and I believed the author the first time he said that a knife edge is weak if the bevel angle is too small. There are also several misprints (or does ice sublime at 105°C?) of varying degrees of distraction. While some of the illustrations are very helpful, others such as 1-3, 6-5, and 9-4, seem unnecessary and useless.

In spite of these criticisms, this book should prove extremely useful to the electron microscopist. It is derived from practical experience, and the expertise of the author is made available to other practitioners. An enumeration of some of its defects should not obscure the fact that this is a useful book that fulfills a definite need.

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Methods of Biochemical Analysis—Volume 20. Edited by DAVID GLICK. Wiley (Interscience), New York, 1971. viii + 393 pp. $17.50.

This continuing review covers in detail six additional areas of biochemical analysis written by invited international specialists. In keeping with the editor's desire to report the techniques and methods, as well as the results of developing fields, emphasis is placed on methodology and instrumentation.

Well established methods of biochemical analysis involving marked changes and new promising approaches are covered. The contents of this volume are: analysis of cyclic adenosine and guanosine; separation and determination of nucleic acids and measurement of their enzymes; determination of glutamic and aspartic acids and their amides; determination of phytate and inositol phosphates; hydrogen isotope exchange measurement in globular proteins; and a guide to temperature-jump methods for fast reactions.

As in past volumes, the taste and style of the individual authors is evident yet consistency in presenting the technical details, documentation, and reference citations is maintained. Also the excellent feature of cumulative author and subject indexes for all 20 volumes is included. Because of the explicit detailed procedures in each subject, this book is of special value to the novice. Yet it remains extremely valuable to the expert for keeping abreast of the very latest developments in biochemical analysis.

GRANT GUSTIN, *The Norwich Pharmacal Company, Norwich, New York 13815*

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(Signed) R. Coviello, Asst. Vice President

The Chemistry of Nonaqueous Solvents

Volume 3 INERT, APROTIC, AND ACIDIC SOLVENTS

Edited by J. J. LAGOWSKI
Department of Chemistry
University of Texas
Austin, Texas

CONTENTS:

Marion Maclean Davis, Bronsted Acid-Base Behavior in "Inert" Organic Solvents. **D. F. Burow,** Liquid Sulfur Dioxide. **Ram Chand Paul** and **Sarjit Singh Sandhu,** Acyl Halides as Nonaqueous Solvents. **F. Fehér,** Liquid Hydrogen Sulfide. **Alexander I. Popov,** Anhydrous Acetic Acid as Nonaqueous Solvent. **Alexander I. Popov,** Other Carboxylic Acids.
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