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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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Cell Separation:
METHODS IN HEMATOLOGY

By **J. Harry Cutts**
Department of Anatomy
School of Medicine
University of Missouri, Columbia, Missouri

This book describes the various methods of cell separation that have been applied to the isolation of cell types from blood and hemopoietic organs, and discusses their principle and relative usefulness. Following an introductory chapter in which are outlined some of the initial considerations of cell separation including the choice of anticoagulant, the effects of temperature, treatment of glassware and equipment, each succeeding chapter is devoted to a specific method of cell separation. The principles on which each method is based are discussed, following which the various modifications used by different workers are presented. Pertinent data taken from the literature are used to illustrate the relative efficiencies of the methods presented, and details of special equipment are illustrated.

This volume will be of interest to microbiologists, cell biologists, pathologists, and comparative pathologists as well as a valuable addition to the libraries of research institutions and transfusion and pathological services in hospitals.

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CONTENTS

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Microchemical Journal, Vol. 17, No. 4

Briefs

A Rapid Fluorometric Determination of Cyanide. GERALD LEE MCKINNEY, HERBERT K. Y. LAU, AND PETER F. LOTT, *Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110.*

A new method is described based on the formation of the fluorescent product consisting of the selenium complex with 2,3-diaminonaphthalene, DANSe according to the following reaction:

$$Pd_2(DANSe)_2Cl_4 + 8CN^- \rightarrow 2Pd(CN)_4^{2-} + 4Cl^- + 2DANSe.$$

Of the cations tested, only mercury interfered. Of the anions tested, only sulfide interfered.

Microchem. J. **17**, 375 (1972).

The Spectrophotometric Determination of Molybdenum and Phosphorus by Carminic Acid Method. ALBERT LEE AND D. F. BOLTZ, *Department of Chemistry, Wayne State University, Detroit, Michigan 48202.*

A spectrophotometric method for the determination of molybdenum based on the use of carminic acid is described. Conformity to Beer's law was observed with an optimum concentration range of 1.5 to 8 ppm of molybdenum when absorbance measurements are made at 565 nm. The molar absorptivities for the determination of molybdenum are 1.4×10^4 mol l^{-1} cm^{-1} at 565 nm and 5.1×10^3 mol l^{-1} cm^{-1} at 336 nm. An indirect spectrophotometric procedure for the determination of orthophosphate based on the determination of the equivalent molybdenum in 12-molybdophosphoric acid is also described for the determination of 0.03 to 0.18 ppm of phosphorus.

Microchem. J. **17**, 380 (1972).

Spectrophotometric Determination of Cobalt with o-Mercaptobenzoic Acid. M. M. L. KHOSLA AND S. P. RAO, *Defense Laboratory, Jodhpur; and Chemistry Department, University of Jodhpur, Jodhpur, India.*

o-Mercaptobenzoic acid forms a bluish violet complex with cobalt having a maximum absorption at 570 mμ. Interferences due to a number of other metals have been overcome.

Microchem. J. **17**, 388 (1972).

Field Test for the Detection and Semiquantitative Estimation of Captan. (N-Trichloromethylthio-tetrahydrophthalimide) K. VISWESWARIAH, S. K. MAJUMDER, AND M. JAYARAM, *Central Food Technological Research Institute, Mysore-2A, India.*

A simple and rapid method of detecting and estimating captan, semiquantitatively, has been standardized. The method is based on the color development of

captan with monoethanolamine hydrolysis, both in the pure state as well as in the presence of aldrin, new BHC isomer, DDT, BHC, malathion, and thiram. These observations are further correlated with the color formations occurring with ferric alum and silver nitrate solutions.

Microchem. J. **17**, 396 (1972).

Colorimetric Determination of Iron(II) by Tetrazolium Salt. M. H. HASHMI, I. AHMAD, A. SUBHAN, AND A. A. AYAZ, *Pakistan Council of Scientific and Industrial Research Laboratories, Lahore, West Pakistan.*

A method for the determination of iron(II) with 2,3,5-triphenyltetrazolium chloride in basic medium is described. The method has advantages over other methods because of its sensitivity, selectivity, and wide range of pH over which Beer's law is obeyed, as well as the amount of interferences.

Microchem. J. **17**, 403 (1972).

Simultaneous Microdetermination of Solubility and Diffusivity of Dissolved Oxygen in Aqueous Electrolyte Solutions with Galvanic-Cell–Diaphram-Cell Technique. G. W. C. HUNG AND R. H. DINIUS, *Department of Chemistry, Auburn University, Auburn, Alabama 36830.*

Galvanic-cell–diaphram-cell technique and procedure are described for the simultaneous microdetermination of solubility and diffusivity of dissolved oxygen in aqueous electrolyte solutions. Electrolytes with surfactant properties, such as sodium 1-decanesulfonate, sodium 2,4-dimethylbenzenesulfonate, and sodium 4-dodecylbenzenesulfonate, were used. The effects of dissolved electrolytes on the solubility and diffusivity of dissolved oxygen, in aqueous systems are discussed in terms of the intramicroscopic structure of liquid medium.

Microchem. J. **17**, 410 (1972).

Extraction of Iron, Cobalt, and Manganese from Hydrochloric Acid with the Quaternary Amine, Aliquat-336. H. F. ALY, M. EL-GARHY, AND S. EL-REEFY, *Nuclear Chemistry Department, U.A.R. Atomic Energy Establishment, Egypt, U.A.R.*

The extraction of iron, cobalt, and manganese by Aliquat-336 from hydrochloric acid medium was investigated. Extraction depending on both HCl and amine concentration was studied. The separation factor between iron and both cobalt and manganese is determined and optimum conditions for separation is given. Two procedures for separation carrier free Fe-59 from irradiated cobalt target and Mn-54 from irradiated iron target are given.

Microchem. J. **17**, 431 (1972).

Microdetermination of Metals in Organometallic Compounds by the Oxine Method After Oxygen Flask Combustion. I. Cadmium, Magnesium, Uranium, and Zinc. A. B. SAKLA, S. W. BISHARA, AND S. A. ABO-TALEB, *Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt U.A.R.*

A simple, accurate, and relatively rapid procedure for the estimation of cadmium, magnesium, uranium, and zinc in organometallic compounds is presented. The oxygen-filled flask is recommended for decomposition of the organic sample. Determination of the metal concerned is carried out by three methods using a single sample weight. Both the gravimetric and titrimetric finishes are applied. The method has the advantage of a favorable conversion factor for the gravimetric determination besides and 8- or 12-fold amplification reaction, depending on the valency of the metal, in case of the titrimetric finish.

Microchem. J. **17**, 436 (1972).

Squaric Acid: Reactions with Certain Metals. HAROLD F. SCHAEFFER, *Department of Chemistry, Westminster College, Fulton, Missouri 65251.*

Various metals, in the form of sheet, foil, wire, or shot, were subjected to the action of a saturated solution of squaric acid [3,4-dihydroxy-3-cyclobutene-1,2-dione] for prolonged periods of time. The squarates were then subjected to microscopic study and photomicrographs were made of each. In general, the salt of a given metal appeared in several different forms. A few metals formed colored squarates.

Microchem. J. **17**, 443 (1972).

Hexosamine Content of Marine Biological Adhesives. ALAN F. KRIVIS AND MICHAEL D. MARTZ, *Department of Chemistry, University of Akron, Akron, Ohio 44325.*

Adhesives produced by some animals have distinct advantages over synthetic adhesives. The one secreted by the common mussel was studied and found to contain glucosamine and galactosamine.

Microchem. J. **17**, 456 (1972).

Direct Titrimetric Microdetermination of L-Aspartic and L-Glutamic Acids Separately. II. Determination of These Amino Acids in the Form of a Mixture Without Separating. O. C. SAXENA, *Chemical Laboratories, University of Allahabad, Allahabad-2, India.*

L-Glutamic and L-aspartic acids are quantitatively determined in micro amounts separately and in the form of a mixture against sodium tungstate and neodymium trichloride, respectively. In the form of a mixture L-glutamic acid is first titrated and in the same solution, without separating, L-aspartic acid is estimated.

Microchem. J. **17**, 462 (1972).

Use of Sodium Citrate as a New Titrant for the Microdetermination of Sulfanilic Acid. A. K. SAXENA, *Chemistry Department, University of Allahabad, Allahabad, India.*

Sulfanilic acid was determined in microquantities with a new titrant, i.e., sodium citrate solution using bromocresol purple as indicator. Estimations were carried out in the range of 0.574–0.096 mg with a maximum error of ±0.004 mg.

Microchem. J. **17**, 468 (1972).

A Technique for the Qualitative Spectrographic Analysis of Very Small Samples. A. J. CHRISTOPHER, *Royal Aircraft Establishment, Farnborough, Hants, England.*

Samples are attached to a graphite electrode together with carbon fibers which act as a bridge between the upper and lower electrodes. Background emission is greatly reduced by enclosing the arc in an argon atmosphere. Simple condensing optics are employed with a Hilger medium quartz spectrograph. Detection is of the order of 1 μg of element.

Microchem. J. **17**, 470 (1972).

Spectrophotometric Determination of Some Aromatic Nitro Compounds in Microgram Quantities. R. D. TIWARI AND U. C. PANDE, *Department of Chemistry, University of Allahabad, Allahabad, India.*

m-Dinitrobenzene, s-trinitrobenzene, *m*-nitrophenol, and 1-chloro-2,4-dinitrobenzene were determined in microgram quantities.

Microchem. J. **17**, 476 (1972).

Macro- and Microdetermination of Arsenic(III) Using Potassium Permanganate as an Oxidant in Acid Medium in Presence of Fluoride Ions. I. M. ISSA, M. H. HAMDY, AND A. S. MISBAH, *Chemistry Department, Assiut University, Assiut, U.A.R.*

A method is described for the determination of trivalent arsenic. It depends on the oxidation of As^{3+} to As^{5+} in H_2SO_4 solutions with $KMnO_4$ in presence of fluoride ions under the following conditions: acidity: 0.0–3.6 *N* H_2SO_4 in presence of 50 ml of 2% NaF; sodium fluoride: 25–75 ml of 2% solution/100 ml; temperature: should not exceed 60°C; amounts of As^{3+}, 0.0165–92.42 mg.

Microchem. J. **17**, 480 (1972).

MICROCHEMICAL JOURNAL 17, 375–379 (1972)

A Rapid Fluorometric Determination of Cyanide

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Received April 10, 1972

INTRODUCTION

The toxicity of the cyanide ion is well documented and although there is some uncertainty as to the toxicity of metal-complexed cyanides, they too are potential sources of free cyanide. "Standard Methods for the Examination of Water and Wastewater" (*1*) estimates the threshold limit of toxicity at infinite time for fish to be 0.1 mg CN^-/liter of water. The Public Health Service Drinking Water Standards call for less than 0.01 mg CN^-/liter in the water supply (*2*). Yet there appear to be no convenient, direct methods capable of measuring CN^- concentration accurately at or below 0.01 ppm in the presence of foreign ions. The standard methods most widely used employ titration with $AgNO_3$ if the [CN^-] is greater than 1.0 ppm and a colorometric procedure using the pyridine pyrazolone method if it is less than 1 ppm (*3*). The titration for higher concentration is "fairly" accurate, but the precision is poor. The colorometric method at lower concentrations is neither accurate nor precise. Both of these methods are subject to a large number of interferences which are circumvented by the Serfass distillation procedure in which the samples are distilled in the presence of acid and the HCN is collected in NaOH prior to its determination. The recovery, especially at low concentrations, by this distillation is poor. The need for an improved method for the determination of CN^- is exemplified in a study conducted by the Analytical Reference Service of the U. S. Department of Health, Education and Welfare (*4*). In this study, 59 laboratories analyzed a sample containing 1.10 ppm CN^- by titration with a standard deviation of 34%. Forty-seven laboratories analyzed a sample containing 0.02 ppm CN^- colorimetrically with a standard deviation of 99%.

Reported herein is a new method for the determination of CN^- which should prove to be superior to the existing methods. The procedure is based upon the following reaction in which the fluorescent product (DANSe) is formed.

$$Pd_2(DANSe)_2Cl_4 + 8CN^- \rightarrow 2Pd(CN)_4^{2-} + 4Cl^- + 2DANSe.$$

A somewhat similar two-step demasking reaction has been reported earlier in which cyanide reacts with potassium bis(5-sulfoxine)palladium II and releases 8-hydroxy-5-quinoline sulfonic acid, which is then allowed to react with magnesium to form a fluorescent chelate (*5*).

EXPERIMENTAL METHODS

Reagents

A standard palladium II chloride solution was prepared by dissolving 1.1276 g of reagent grade $PdCl_2$ in 1 liter of 0.01 *M* HCl and standardized by precipitation with noxime (6.30×10^{-3} *M*).

A standard selenium solution was prepared by dissolving 8.1680 g of H_2SeO_3 in 1 liter of distilled water (6.73×10^{-2} *M*).

A solution of 2,3-diaminonaphthalene (DAN) was prepared by dissolving 0.10 g (J. T. Baker) in 0.1 *M* HCl (6.4×10^{-3} *M*).

A standard cyanide solution was prepared by dissolving 2.5038 g reagent grade KCN in distilled water (1 ml = 1 mg CN^-). Working solutions were prepared by diluting the stock solution.

ACS reagent grade hexane was used for extraction.

Apparatus: Farrand filter fluorometer.

Preparation of Complexing Agent

To 100 ml DAN add 15 ml of H_2SeO_3; stir and allow to stand for 20 min. Filter the orange precipitate (DANSe) onto a sintered glass crucible; wash with 0.1 *M* HCl, followed by distilled water. Redissolve the precipitate by washing into a clean filtering flask with 95% ethanol; transfer to a 100 ml volumetric flask and dilute to volume with ethanol. Add this ethanolic DANSe solution to 200 ml $PdCl_2$ in a 400-ml beaker; stir and allow to stand 1 hr. Centrifuge and decant the liquid. Dry the Pd(DANSe) precipitate at 110°C in the centrifuge tube to avoid loss. Mix and powder with NaCl to give ~1% complexing agent mixture.

Analysis of Samples

Add a series of standards containing 10–100 μg CN^- to 30-ml separatory funnels. Add 25 mg complexing reagent to each. Shake and allow to stand 10 min. Add 3 ml hexane and extract 5 min. Allow layers to separate; decant the organic layer into quartz test tubes. Prepare a calibration curve by measuring the fluorescence intensity using 377 nm excitation (Corning 7-60 filter) and 520 nm emission light (Corning 3-71 filter). The calibration curve is linear with 12% relative standard deviation. Samples known to contain cyanide were obtained downstream

TABLE 1

Sample	CN^- (ppm) via	
	Titration	Fluorescence
1	0.54	0.60
2	1.05	0.66
3	1.25	1.49
4	2.06	2.00
5	3.05	3.52
6	3.65	7.37

from a gold mine in Whitewood, SD, by the Environmental Protection Agency. They were analyzed by the EPA using the titration procedure, and also by the fluorometric method. The results are shown in Table 1.

pH Effect

In the preparation of the complexing agent, the reaction is quantitative from pH 0 to 6, but the efficiency of the reaction drops rapidly above pH 6 (Fig. 1).

The reaction with CN^- is quantitative above pH 8 (Fig. 2). High results are obtained if the solution is too alkaline, due to competition between OH^- and CN^- to form DANSe. In acid solution the cyanide would be present in the form of HCN and unreactive to the complex;

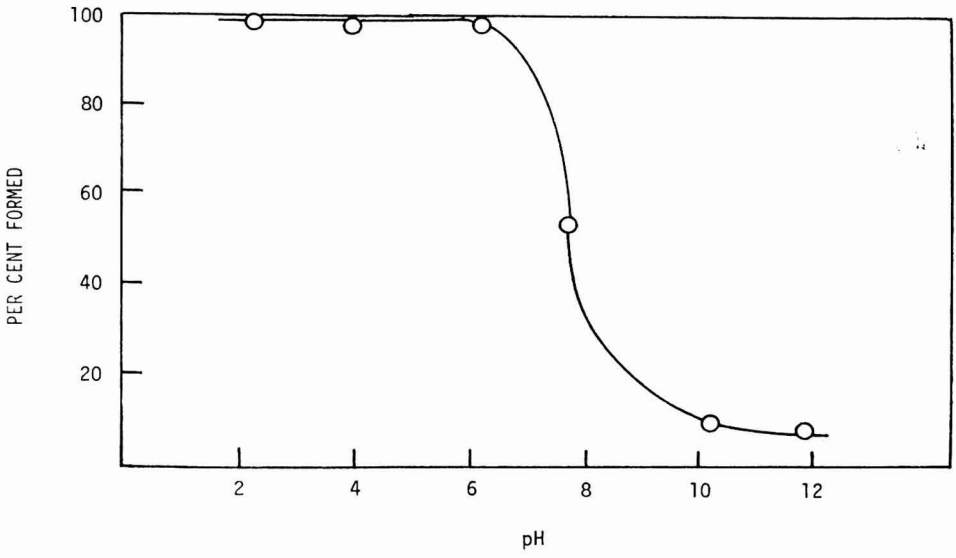

FIG. 1. Effect of pH on the formation of palladium DANSe.

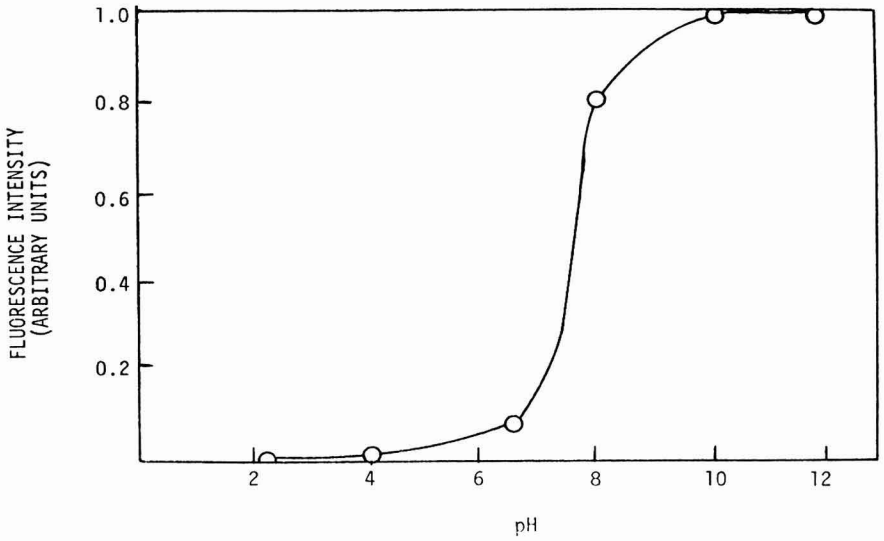

FIG. 2. Effect of pH on the reaction of palladium DANSe with cyanide.

in addition, the DANSe would be in the protonated form, which is more soluble in the aqueous layer and not fluorescent (pH $<$ 3). The extraction of DANSe with hexane is greatest near pH 8 (Fig. 3). Thus, the optimum condition for the reaction and extraction is at pH 8.

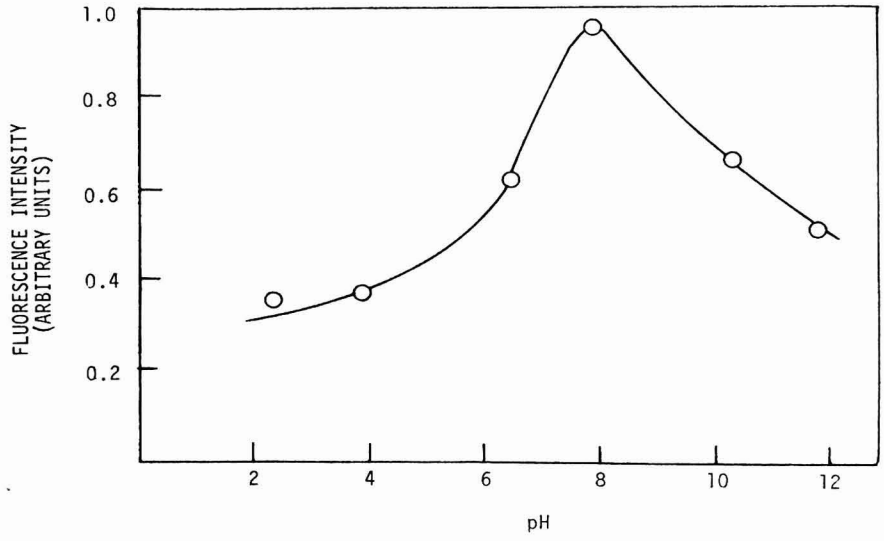

FIG. 3. Effect of pH on the extraction of DANSe.

Interferences

Few ions appeared to interfere with the reaction. Of the cations tested (Li^+, Na^+, K^+, Pb^{2+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Fe^{3+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}), only mercury interfered.

Of the anions tested (F^-, Cl^-, Br^-, I^-, PO_4^{3-}, NO_3^-, SO_4^{2-}, Ac^-, NO_2^-, $S_2O_3^{2-}$, $tart^{2-}$, CNO^-, S^{2-}), only sulfide interfered. Sulfide is a common interference in cyanide procedures due to similarity of sulfide and cyanide. This interference was removed by adding $KMnO_4$ to oxidize the S^{2-}, followed by addition of $SnCl_2$ to reduce the excess MnO_4^-, or by precipitation with $PbCO_3$ and filtering the PbS.

DISCUSSION

The first step in the preparation of the complex involves the quantitative reaction between 2,3-diaminonaphthalene and selenium (*6*). The reaction between this DANSe (naphtho-[2,3-*d*]-2-selena-1,3-diazole) and palladium has been studied as a method for Pd(II), and if an excess of Pd is used, a 1:1 complex is formed (*7*). This compound is relatively insoluble; it could be added conveniently as a solid reagent powdered with sodium chloride. As some blank fluorescence was observed, probably due to a dissociation of the complex at low concentrations, the amount added must be constant in all samples.

Because most of the interfering ions are not expected to be present in water and wastewater samples, this method should be applicable to analysis for CN^- in water from plating and mining wastes. The method will measure free cyanide and, as noted in the interference study, Fe(III) did not interfere.

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MICROCHEMICAL JOURNAL 17, 380–387 (1972)

The Spectrophotometric Determination of Molybdenum and Phosphorus by Carminic Acid Method

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INTRODUCTION

Carminic acid has been used as an analytical reagent in the determination or detection of lead (*9*), zirconium (*10*), germanium (*3*), and cerium (*6*). Kirkbright, West, and Woodward (*7*) developed spectrofluorometric methods for the determination of molybdenum and tungsten using carminic acid. This reagent has been investigated as chromogenic agent for boron in our laboratory and new ultraviolet and modified visible spectrophotometric methods have been developed (*4*). A study of the molybdenum(VI)–carminic acid reaction has resulted in the development of a new sensitive spectrophotometric method for the determination of molybdenum by either ultraviolet or light absorption spectrometry and a new sensitive indirect spectrophotometric method for the determination of phosphorus. Indirect methods based on the quantitation of the molybdenum associated with phosphorus in 12-molybdophosphoric acid have been reported based on the ultraviolet absorptivity of the molybdate (*8*), on the color developed with phenylfluorone (*5*) and on the color developed with either the chrome violet or acid chrome black special (*1*). The proposed procedure for the indirect spectrophotometric determination of phosphorus is based on the formation of 12-molybdophosphoric acid, the extraction of 12-molybdophosphoric acid into an immiscible organic solvent mixture, a retrograde extraction of the molybdate into a basic aqueous solution and the determination of the equivalent molybdenum by the carminic acid method.

MATERIALS AND METHODS

Reagents

Standard molybdenum(VI) solution ($10^{-3}M$): Dissolve 242.0 mg of reagent grade sodium molybdate dihydrate, $Na_2MoO_4 \cdot 2H_2O$, in double distilled water and dilute to volume in a 1-l volumetric flask. The con-

centration of this solution is $1.00 \times 10^{-3} M$, or 1 ml = 95.9 μg of molybdenum.

Carminic acid solution I ($10^{-3} M$): Dissolve 246.2 mg of reagent grade carminic acid in 10.0 ml of 0.100*N* sodium hydroxide. After complete dissolution of the carminic acid, add 10.0 ml of 0.200 *M* acetic acid and dilute to volume with double distilled water in a 500-ml volumetric flask. The concentration of this reagent is 1.00×10^{-3} *M*.

Acidic acetate buffer solution: Mix equal volumes of 0.486*M* solutions of sodium acetate and acetic acid. The pH of this buffer solution is 4.7.

Diverse ion solutions: Prepare all diverse ion solutions from reagent grade chemicals dissolved in double distilled water. Store diverse ion solutions in polyethylene bottles.

Standard phosphate solution (10 μg P/ml): Dissolve 2.198 g of potassium dihydrogen phosphate (KH_2PO_4) in double distilled water and dilute to 1 l. Transfer 20.00 ml aliquot of this stock solution to a 1-l volumetric flask and dilute to volume with double distilled water. This solution contains 10 μg P, as orthophosphate/ml.

Molybdate reagent solution (10% w/v): Dissolve 25.0 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, in double distilled water and dilute to 250 ml in a volumetric flask.

Carminic acid solution II ($5 \times 10^{-3} M$): Dissolve 246.2 mg of carminic acid in 10.0 ml of 0.10 *N* sodium hydroxide. Add 10 ml of 0.20 *N* acetic acid, mix well, and dilute to 100 ml with double distilled water.

Acidic washing solution: Add 50.0 ml of concentrated hydrochloric acid to double distilled water in a 500-ml volumetric flask and dilute to volume.

Buffer solution (1 *M* in ammonium chloride and 1 *M* in ammonium hydroxide): Dissolve 53.5 g of ammonium chloride in distilled water containing 70 ml of concentrated ammonium hydroxide and dilute to 1 l with distilled water.

Extractant: Spectrograde diethyl ether was used for the extraction of molybdophosphoric acid.

Apparatus

The absorbance measurements were made in 1.000-cm matched cells with a Cary 14 spectrophotometer. The pH measurements were made with a Corning Model 110 pH meter equipped with glass and calomel electrodes.

Procedure for Determination of Molybdenum

Transfer 50 ml of the $10^{-3} M$ carminic acid solution (if absorbance

is to be measured at 336 nm use only 20 ml) to a 100-ml volumetric flask. Add 20 ml of the buffer solution. Add 10–25 ml of sample solution containing no more than 1.00 mg of molybdenum(VI). Dilute to the mark with redistilled water and mix thoroughly. Prepare a blank solution by this same procedure but omitting addition of sample solution. Measure the absorbance at 565 nm or 336 nm using 1.000-cm matched cells and the blank solution in the reference cell.

General Procedure for Determination of Phosphorus

Transfer by microburet 0.50 to 2.00 ml of the standard phosphate solution to a 125-ml separatory funnel. (In the case of an unknown phosphate solution transfer an aliquot containing no more than 20 μg of phosphorus, as orthophosphate, in a 25-ml aliquot.) Add 1.0 ml of a 1:2 hydrochloric acid solution and dilute to about 40 ml with double distilled water. Add 4.0 ml of the 10% ammonium molybdate reagent, mix well and allow to stand for 10 min. The pH at this stage should be approximately 1.3. Add 5.0 ml concentrated hydrochloric acid, mix thoroughly, and allow solution to stand for another 5 min.

Add 45 ml of diethyl ether to the solution in the separatory funnel and shake the mixture for 3 min. Rinse the separatory funnel stopper with 2 ml of diethyl ether using a capillary pipet collecting rinsings in separatory funnel. After separation of the layers, remove carefully the lower aqueous layer and rinse the stem of the funnel with a jet stream of distilled water and wipe dry.

Add 10 ml of an acidic hydrochloric acid washing solution to the ether extract of molybdophosphoric acid and shake for 20 sec to remove excess molybdate. Discard aqueous wash layer. Again rinse the tip of funnel stem with distilled water and wipe dry. Repeat this washing procedure 3 times in order to ensure complete removal of excess molybdate.

Add 30 ml of the ammonium chloride–ammonia buffer to the ether extract and shake for 60 sec. Transfer the lower aqueous layer containing the equivalent molybdate and phosphate to a 80-ml beaker. Add 15 ml of the basic buffer to the ether layer and shake thoroughly for 30 sec. Withdraw this aqueous layer and combine wtih the molybdate solution in the 80-ml beaker.

Add glacial acetic acid dropwise with stirring as the pH is monitored by a pH meter until a pH of about 4.75 is obtained. Transfer this solution carefully to a 100-ml volumetric flask. Add 10.0 ml of the 5×10^{-3} *M* carminic acid reagent, mix well, and dilute to 100 ml with distilled water.

Measure the absorbance in 1.000-cm silica cells using a reagent blank solution, prepared in a similar manner except the extraction stages were

omitted, in the reference cell. The absorbance of 565 and 336 nm is used to prepare calibration plots for phosphorus and to compare with calibration plots for molybdenum.

RESULTS AND DISCUSSION

Determination of Molybdenum

Absorption spectra. Figure 1 shows the characteristic absorbance maxima obtained for the molybdenum–carminic acid complex. The prinicpal absorbance maxima are at 565 and 336 nm.

Molybdenum concentration. Conformity to Beer's law was observed when absorbance measurements were made at 336 nm or at 565 nm. Based on Ringbom plots (*2, 11*), the approximate optimum concentration range for the visible spectrophotometric method is 1.5 to 8 μg of molybdenum/ml while the corresponding range for the ultraviolet spectrophotometric method is 4 to 11 $\mu g\ ml^{-1}$. The molar absorptivities are 1.4×10^4 mol l^{-1} cm^{-1} when the absorbance is measured at 565 nm and 5.1×10^3 mol l^{-1} cm^{-1} when the absorbance is measured at 336 nm. Measurement of the absorbance at 565 nm is recommended because of the higher sensitivity obtained at this wavelength.

Reagent concentration. The effect of reagent was determined using the general procedure for the determination of molybdenum. For the

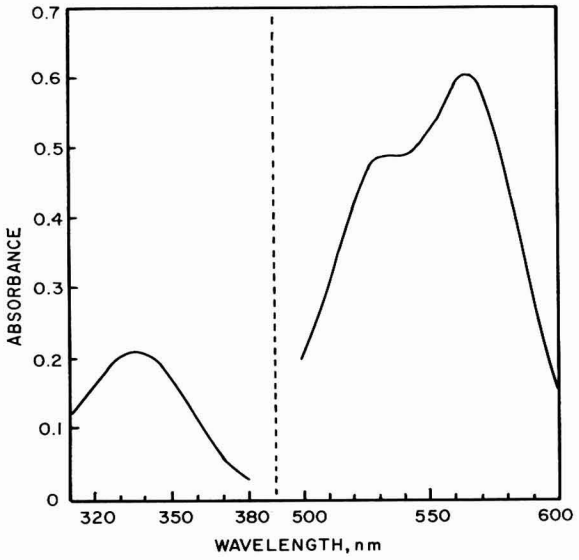

FIG. 1. Comparison of ultraviolet and visible absorbance maxima of molybdenum–carminic acid complex, 4.0 ppm molybdenum.

visible method 50 ml of carminic acid solution I is sufficient while for the ultraviolet method 20 ml of reagent is recommended.

pH. The influence of pH on the absorption spectrum of the carminic acid–molybdenum complex was investigated at pH 3.00, 4.00, 4.67, 5.00, 5.50, and 6.12. The buffered solutions were adjusted to these values by adding the required amount of either 0.200 *N* perchloric acid or 0.400 *N* sodium hydroxide. The absorbance at both 336 and 565 nm is pH dependent decreasing as pH 3 and 6 were approached. At pH 4 to 5 virtually constant absorbance readings were obtained.

Diverse ions. The effect of various diverse ions was studied using the general procedure for the determination of molybdenum and adding diverse ion solution prior to the addition of the molybdate solution. When the final solution was 10^{-3} *M* in respect to the diverse ion, the concentration of molybdenum was 5.0×10^{-5} *M* and the absorbance was measured at 565 nm. The following ions did not interfere: ammonia, cadmium, lithium, magnesium, manganese(II), potassium, sodium, thallium(I), acetate, arsenate, arsenite, bromide, carbonate, chloride, cyanate, fluoride, iodide, nitrate, perchlorate, sulfate, sulfite, tetraborate, thiocyanate, and phosphate. Those ions which cause interferences are listed in Table 1.

When absorbance measurements were made at 336 nm the following ions did not cause interference: ammonium, magnesium, manganese(II),

TABLE 1

INTERFERING IONS; ABSORBANCE MEASURED AT 565 nm

Ion	Added as	Amount added (ppm)	Relative error (%)	Permissible amount (ppm) [a]
Al^{3+}	$Al(NO_3)_3 \cdot 9H_2O$	25	+97	0
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	60	+7	5.9
Cr^{3+}	$Cr(NO_3)_3 \cdot 9H_2O$	50	+78	0.3
$Cr_2O_7^{2-}$	$K_2Cr_2O_7$	215	−92	0.2
Cu^{2+}	$CuCl_2 \cdot 2H_2O$	65	+33	0.3
$C_2O_4^{2-}$	$(NH_4)_2C_2O_4 \cdot H_2O$	90	−85	0.9
Citrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	100	−96	0.2
Ni^{2+}	$NiCl_2 \cdot 6H_2O$	60	+25	0.6
NO_2^-	$NaNO_2$	45	−88	2.3
Ge^{4+}	Na_2GeO_3	70	−6	3.7
Tartrate	$K_2C_4H_4O_6 \cdot 1/2H_2O$	170	−33	9
Th^{4+}	$Th(NO_3)_4 \cdot 4H_2O$	230	+96	0.2
U^{6+}	$UO_2(NO_3)_2 \cdot 6H_2O$	240	+17	2.4
WO_4^{2-}	$Na_2WO_4 \cdot 2H_2O$	250	+21	2.5

[a] Noninterference based on error less than 3 times the standard deviation.

potassium, sodium, thallium(I), acetate, arsenate, arsenite, bromide, carbonate, chloride, cyanate, fluoride, iodide, nitrate, perchlorate, sulfate, sulfite, tetraborate, thiocyanate, and phosphate. Those ions causing interference are listed in Table 2. Both iron(II) and tin(II) form precipitates with the carminic acid molybdenum complex.

Although a study of the effect of diverse ions on the determination of phosphorus was not made it would be expected that silicate, arsenate, and germanate would interfere. However, the possibility of using a strong cation exchange resin for removal of cations should eliminate many of the cation interferences cited in Table 1.

Precision. In a series of 4 samples containing 4.0 ppm of molybdenum an average absorbance of 0.606 at 565 nm was obtained with a relative standard deviation of 0.50%. In a series of 4 samples containing 8.0 ppm of molybdenum an average absorbance of 0.423 at 336 nm with a relative standard deviation of 0.47%.

Determination of Phosphorus

Phosphorus concentration. Figure 2 illustrates the characteristic absorbance maxima obtained for different concentrations of phosphorus using the proposed carminic acid method for the determination of the equivalent molybdenum present in the 12-molybdophosphoric acid. Conformity to Beer's law was observed for 0 to 0.2 ppm of phosphorus with the optimum concentration range being about 0.03 to 0.18 ppm of phosphorus.

TABLE 2

INTERFERING IONS; ABSORBANCE MEASURED AT 336 nm

Ion	Added as	Amount added (ppm)	Relative error (%)	Permissible amount (ppm) [a]
Cd^{2+}	$Cd(NO_3)_2 \cdot 4H_2O$	100	+8	11
Co^{2+}	$Co(NO_3)_2 \cdot 6H_2O$	60	+12	6
Cr^{3+}	$Cr(NO_3)_3 \cdot 9H_2O$	50	+180	0.5
$Cr_2O_7^{2-}$	$K_2Cr_2O_7$	210	+200	0.2
Cr^{2+}	$CrCl_2 \cdot 2H_2O$	60	+42	6
$C_2O_4^{2-}$	$(NH_4)_2C_2O_4 \cdot H_2O$	90	−83	0.9
Citrate	$Na_3C_6H_5O_7 \cdot 2H_2O$	190	−90	0.2
Ni^{2+}	$NiCl_2 \cdot 6H_2O$	60	+22	0.6
NO_2^{2-}	$NaNO_2$	45	+10	2
Tartrate	$K_2C_4H_4O_6 \cdot 1/2H_2O$	170	+31	9
U^{6+}	$UO_2(NO_3)_2 \cdot 6H_2O$	240	+160	0.2
WO_4^{2-}	$Na_2WO_4 \cdot 2H_2O$	254	+45	2.5

[a] Noninterference based on error less than 3 times the standard deviation.

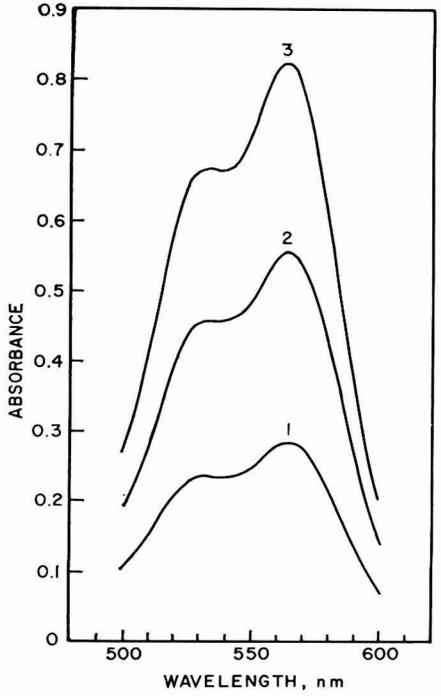

FIG. 2. Typical absorption spectra corresponding to 0.05, 0.10, and 0.15 ppm phosphorus.

Precision. An estimate of the precision obtainable by this indirect method for phosphorus was ascertained from the results of 4 samples each containing 0.15 ppm of phosphorus. These samples gave a mean absorbance or 0.811 with a relative standard deviation of 0.75%, the absorbance measurements being made at 565 nm.

SUMMARY

A spectrophotometric method for the determination of molybdenum based on the use of carminic acid is proposed. Conformity to Beer's law was observed with an optimum concentration range of 1.5 to 8 ppm of molybdenum when absorbance measurements are made at 565 nm. The molar absorptivities for the determination of molybdenum are 1.4×10^4 mol l^{-1} cm^{-1} at 565 nm and 5.1×10^3 mol l^{-1} cm^{-1} at 336 nm. An indirect spectrophotometric procedure for the determination of orthophosphate based on the determination of the equivalent molybdenum in 12-molybdophosphoric acid was developed suitable for the determination of 0.03 to 0.18 ppm of phosphorus.

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MICROCHEMICAL JOURNAL 17, 388–395 (1972)

Spectrophotometric Determination of Cobalt with o-Mercaptobenzoic Acid

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INTRODUCTION

o-Mercaptobenzoic acid has been reported as a reagent for the absorptiometric determination of nickel (*3*), fluroacetates (*13*), selenium (*5*), palladium (*7*), rhenium (*15*), as a metallochromo indicator (*2*), and as a precipitant for thorium (*14*). This reagent is also found to be very suitable for the determination of cobalt. The sensitivity of the proposed method is comparable to the most of the methods (*1, 4, 6, 9, 10, 16, 18*) for cobalt determination. *o*-Mercaptobenzoic acid forms a deep bluish violet complex at pH 5.2–7.2, having maximum absorption at 570 mμ. The complex is very stable at room temperature. It also forms a yellowish green complex with cobalt(II) having maximum absorption at 350 mμ in ammonia and pyridine. The formation and the extraction of the latter complex with chloroform is rapid and this makes for easy removal of the interferences of common base metals with EDTA. This paper reports the detailed investigation of the bluish violet complex formed between this reagent and cobalt(II) and the development of a general procedure for determining cobalt.

EXPERIMENTAL METHODS

Reagents

All the materials used were of analytical reagent grade. Twice distilled water was used throughout.

o-Mercaptobenzoic acid (Fluka). A 1% (w/v) solution was prepared by dissolving it in redistilled ethanol and it was stored in the refrigerator.

Stock aqueous solution of cobalt(II) was prepared by dissolving cobalt(II) sulfate heptahydrate, $CoSO_4 \cdot 7H_2O$ and standardized with EDTA using xylenol orange as indicator (*17*). More dilute solutions were prepared from this standard.

Buffer Solution

A saturated aqueous solution of hexamine (3.6 *M*) and dilute sulfuric acid were used for pH adjustment.

Ionic Strength

For spectrophotometric studies an ionic strength of about 0.1 was maintained with 1.0 *M* sodium perchlorate.

Apparatus

Absorbance measurements were made with Bausch and Lomb spectroconic 20. A Beckmann pocket pH meter, model 180 was used for pH measurements.

Procedure

Transfer, by pipette, 0.25 to 2.5 ml of 2×10^{-4} *M* solution of cobalt(II) (2.9 to 29 μg of cobalt) into a 50-ml beaker. Add 20 mg of ascorbic acid, 1 ml of 1% reagent and 0.5 ml of hexamine buffer. Allow the color to develop for 5 minutes with occasional shaking. Adjust the pH to 5.2–7.1 and transfer the solution to a 10-ml flask, and dilute to the mark, and mix. Measure the absorbance at 570 mμ using the distilled water as blank. Determine the cobalt concentration from a previously prepared calibration graph.

Removal of the Interferences

An aliquot of solution containing 2.9 to 29 μg of cobalt is transferred into a 50-ml separatory funnel. Add 1 ml of 2% reagent in pyridine, and shake. Then add 10 ml of 0.01 *M* EDTA. This is followed by the addition of 5 ml of chloroform. Shake for 1 minute. The phases were allowed to separate for another minute. The extraction is repeated with another 5-ml portion of chloroform. The combined extracts are evaporated over a water bath. The evaporated mass is treated with 1 ml of 10% ammonia solution and evaporated. This is again repeated. To the evaporated mass is added 1 ml of water and the color is developed as outlined under Procedure.

RESULTS AND DISCUSSION

Spectral Characteristics

The absorbance curve of Cobalt(II)–*o*-mercaptobenzoic acid complex is shown in Fig. 1. The complex shows maximum absorption at 570 mμ. The reagent has no absorption at this wavelength.

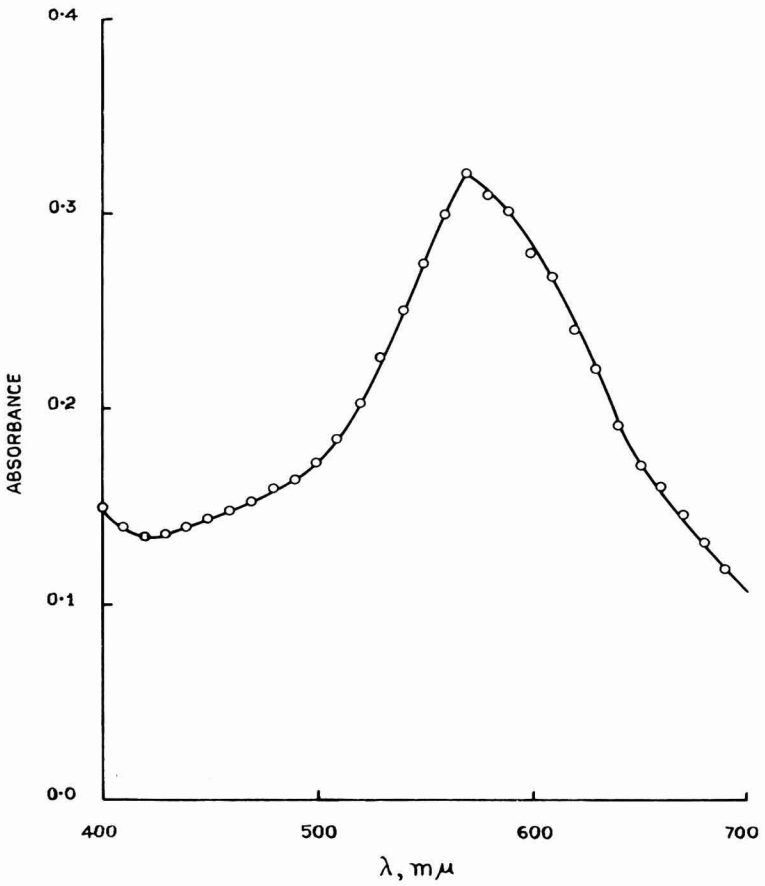


FIG. 1. Absorbance spectrum of the cobalt complex of *o*-mercaptobenzoic acid.

Calibration, Range, and Sensitivity

The Cobalt(II)–*o*-Mercaptobenzoic acid system conforms to Beer's law over the range 0.29–2.9 ppm of cobalt. The molar absorptivity at 570 mμ is 16.5 $\times$ 10^3 and the Sandell sensitivity is 0.0028 μg/cm^2. The molar absorptivity of cobalt–thioglycollic system (*1*) at 358 mμ is 8.4 $\times$ 10^3 and the Sandell sensitivity is 0.0066 μg/cm^2. The sensitivity of this method is comparable to those of many other methods for cobalt.

Stability and Effect of Reagent Concentration

The reagent solution in ethanol and pyridine was found to be stable if it was stored in a refrigerator. Only a 10-fold reagent excess was needed for full development of the color but a 20-fold reagent excess

was preferred. A 40-fold reagent excess was preferred in the removal of the interferences. A large excess of reagent had no deleterious effect on the system.

Color Development, Stability, and Effect of Ascorbic Acid

The maximum color of the complex was found to develop within 5 minutes with occasional shaking and to be very stable. The addition of ascorbic acid accelerated the development of the color and increased the sensitivity of the complex. The excess of ascorbic acid had no adverse effect on the system, and color developed at room temperature.

Effect of pH

Standard amounts of Cobalt(II) and *o*-mercaptobenzoic acid were buffered at varying pH values. The final pH of each solution was measured with a pH meter and the absorbance was measured at 570 mμ. A plot of absorbance against pH showed that the maximum absorbance was obtained at pH 5.2–7.2. A large pH range is a special significance of this method. The maximum color intensity in the case of cobalt–thioglycollic system was between pH 4.8 and 5.25 (*1*).

Effect of Diverse Ions

Several ions were added to the system 1.178 ppm of cobalt(II) to study interferences. Al^{3+}, Ca^{2+}, Mg^{2+}, Sr^{2+}, Na^{+}, K^{+}, Zn^{2+} did not interfere. F^{-}, CO_3^{2-}, NO_3^{-}, ClO_4^{-}, ClO_3^{-}, SO_4^{2-}, $S_2O_3^{2-}$, SCN^{-}, Cl^{-}, Br^{-}, PO_4^{3-} did not interfere. Cu^{2+}, Cd^{2+}, Ag^{+} which interfere can be effectively masked with 5% solution of thiourea. After the development of the color, EDTA, CN^{-}, tartarates, citrates, oxalates, did not interfere. Bi^{3+}, Fe^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, V^{5+}, and W^{6+} which interfere can be easily separated with EDTA as outlined under Removal of the Interferences and there was no interference from 500-fold excess of these ions. Tartrate (800 ppm), citrate (100 ppm), and oxalate (260 ppm) can be tolerated.

Composition of the Complex

The composition of the Cobalt(II)–*o*-mercaptobenzoic acid was determined by Job's method (*11, 12*) of continuous variation and molar ratio method (*19*). The composition of the cobalt was also determined by gravimetric method.

Continuous Variation Method

A series of equimolar solutions of metal ion and reagent were prepared using 0.0025 *M* solution of the metal and the indicator, in which

the ratio of the metal varied from 1 : 9 to 9 : 1, keeping the pH at 6.7. The final volume of the solution was 100 ml. The absorbance was measured at 570 mμ. The peak of curve (Fig. 2) obtained by plotting the optical densities against increasing amounts of metal, show that the cobalt(II) combines with the reagent in the ratio of 1 : 3.

Molar Ratio Method

In the method of Yoe and Jones (*19*) a series of solutions were prepared from 2 ml of 0.0025 *M* cobalt(II) and varying amounts of 0.0025 *M* reagent. The pH was maintained in each case at 6.7 and the final volume of the solution was 100 ml. In a plot of absorbance against mole-ratio of reagent to cobalt(II), the initial steep portion of the curve and final flat portion of the curve extrapolated to an intersection at a mole-ratio of 3 : 1 (Fig. 3).

Determination of Cobalt in the Complex by Ignition Method

The bluish violet precipitate was obtained by treatment of concentrated solution of cobalt(II) with the reagent. The precipitate was filtered and washed with absolute ethanol and dried at 105°C. Cobalt was determined as Co_3O_4 by ignition of the solid at 720°C in silica crucible. The percentage of cobalt was found to be 11.77 as against theoretical value of 11.51. The bluish violet precipitate was highly soluble in triethanolamine and propane-1,2-diol.

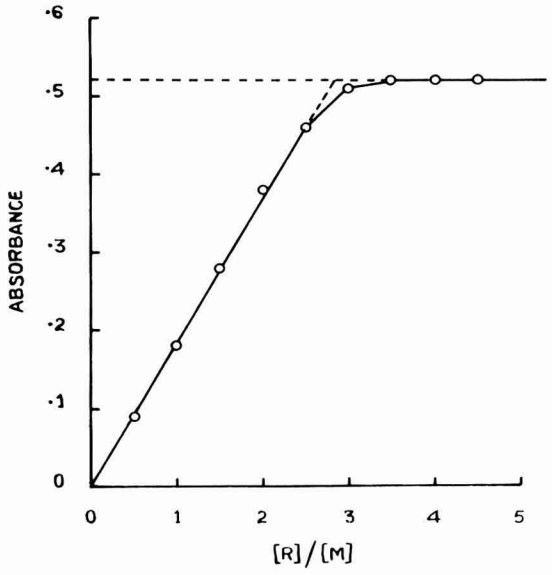


FIG. 2. Molar ratio method; final concentration of cobalt, 5×10^{-5} *M*.

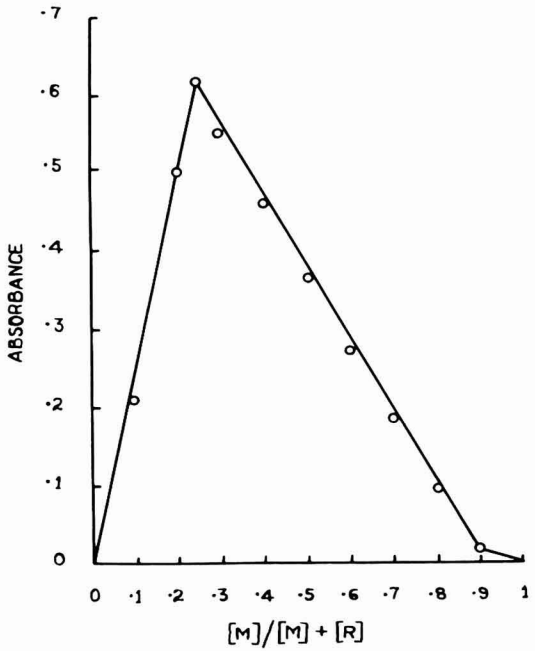
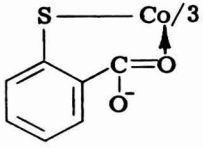


FIG. 3. Continuous variation study with equimolar solutions of cobalt and reagent.

Degree of Dissociation and Instability Constant

The value of α, the degree of dissociation was calculated from Harvey and Manning's equation (*8*), $\alpha = (E_m - Es)/Em$ where *Em* and *Es* have their usual significances. The instability constant calculated from the equation, $K = C(1 - \alpha)/(m\ \alpha\ c)^m\ (n\ \alpha\ c)^n$ with $m = 1$ and $n = 3$ is log $K = 18.21$ at room temperature (25°C) and at ionic strength = 0.1.

The complex may be represented by



Further work is in progress on the determination of bismuth, silver, osmium, molybdenum, copper, iridium, tungsten, uranium, chromium, etc., by this reagent.

SUMMARY

o-Mercaptobenzoic acid is shown to be a sensitive reagent for the determination of cobalt. The reagent forms a bluish voilet complex having maximum absorption

at 570 mμ. The color develops at room temperature, with a broad range of pH 5.2–7.2 and is very stable. The method is not sensitive to the concentration of the reagent. Beer's law conforms over the range of 0.29–2.9 ppm of cobalt. Copper, silver, and cadmium are effectively masked with thiourea. Interferences due to bismuth, iron, manganese, molybdenum, nickel, tungsten, and vanadium have been overcome by a simple and rapid separation. The molar absorptivity and Sandell sensitivity are 16.5×10^3 and 0.0028 $\mu g/cm^2$, respectively. The reagent forms 3:1 complex with cobalt with instability constant of log $K = 18.21$ at room temperature (25°C) and ionic strength = 0.1. A procedure for the determination of cobalt is described.

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Field Test for the Detection and Semiquantitative Estimation of Captan (N-Trichloromethylthio-tetrahydrophthalimide)

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INTRODUCTION

Captan has found a variety of applications both in industry and agriculture. It is most commonly used both as disinfestant and protectant of grains. It is active against a variety of soil borne pathogens only between 100 and 1000 ppm and very effective as a seed protectant about 1000 ppm (*1*). Recently the practice of including insecticides like aldrin, dieldrin, or lindane with captan as seed treatments for the dual protection against soil insects and soil borne pathogens has gained wide spread acceptance (*2*).

Many methods are put forward from time to time to detect and estimate captan based on colorimetry, infrared spectroscopy, gas chromatography, bioassay, and thin layer chromatography. Kittleson (*2*) has described a method that consists of heating resorcinol with captan at 135–140°C to form an intense red colored compound. After treating this colored complex with acetic acid, the yellow color is measured at 425 mμ and as little as 50 μg could be determined. Engst and Schnaak (*3*) have used silver nitrate solution spray followed by ultraviolet irradiation for thin layer chromatography analysis of captan and folpet in foods and the authors claim that 1% methanol in chloroform was the best solvent system. Irwin *et al.* (*4*) have studied the response of captan and its structurally related fungicides (folpet, captan, difolatan) to several chromogenic reagents such as resorcinol, potassium permanganate, dimethyl-*p*-phenylenediamine (DMPD) and chromic acid. The test is based on thin layer chromatography where the fungicides can be detected up to 2 μg. However, the reactions involved in the color development could not be explained. Burchfield and Stores (*5*) have suggested a volumetric method of determining captan which involves hydrolysis, oxidation, decomposition of hydrogen peroxide, etc., Though the method is quantitative, the procedure is tedious and time consuming. Majumder *et al.* (*6*) have developed a micro-thin layer chromatog-

raphy method of estimating and detecting captan at manogram levels. Visweswariah *et al.* (*7*) have indicated a method of estimating captan and certain pesticides by micro-thin layer chromatography at nanogram levels using visual extinction technique and spot diameter method. In field work and routine analysis, where simplicity and rapidity of the procedure are more important to gain preliminary knowledge of the nature and magnitude of the pesticide involved in the food material, visual semiquantitative estimations are of paramount importance. Therefore investigations were undertaken to develop a reaction where captan can be detected and estimated semiquantitatively by the visual color examination both in the pure state as well as in the presence of certain commonly used pesticides. This method will be helpful in captan formulations where it is used in the form of dust, spray, emulsion either alone or in combination with certain commonly used insecticides screened here.

REAGENTS

Monoethanolamine, DDT, New-BHC-isomer (*8*), BHC, aldrin, obtained from E. Merck-Darmstadt (made in Germany), malathion (supplied by Cyanamid India Limited). Captan (obtained from Chevron Chemical Company, California) were used.

0.1 *N* Silver nitrate solution was employed as chromogenic agent.

Ferric alum solution: 40 g of ferric ammonium sulfate crystals were dissolved in 100 ml of hot distilled water; and several milliliters of conc nitric acid were added to discharge the red color. The solution was filtered and used as chromogenic agent to distinguish various categories of pesticides.

PROCEDURE

Quantities of captan ranging from 0.1 to 5.0 mg were placed in separate test tubes and 1 ml of monoethanolamine was added to each. The tubes were kept in a boiling water bath for 10 minutes. They were cooled to room temperature and the volume was made up to 10 ml with distilled water. The colors were observed as noted in Table 1.

A constant quantity of each pesticide (0.5 mg) was mixed with varying quantities of captan ranging from 5.0 to 40 mg; and placed in separate test tubes; and 1 ml of monoethanolamine was added. They were kept in a boiling water bath for 10 minutes exactly. The tubes were removed from the water bath and cooled to room temperature. Volume was made up to 10 ml in each case with distilled water. The color changes were observed as detailed in Table 2.

Five mg of each insecticide was mixed with 5.0 mg of captan and 1

TABLE 1

VISUAL SEMIQUANTITATIVE ESTIMATION OF CAPTAN

Quantity of captan (mg)	Color observed
Control (monoethanolamine)	Colorless
0.1–0.5	Very faint yellow
0.5–1.0	Straw-yellow
1.0–2.0	Deep yellow
2.0–3.0	Pale orange
3.0–4.0	Deep orange
4.0–5.0	Red
5.0 and above	Blood red

ml of monoethanolamine was added. In each case, the experiment was conducted in duplicate. The reaction tubes were placed in a water bath for exactly 10 minutes; cooled; and volume was made up to 10 ml with distilled water. To one set, 1.0 ml of 0.1 *N* silver nitrate solution and to the other set, 0.2 ml of ferric alum solution were added and the color changes were observed as in Table 3.

DISCUSSION

Monoethanolamine hydrolysis in the estimation of hydrolyzable chlorine content of chlorinated pesticides like DDT, New-BHC-isomer, BHC, aldrin, dieldrin, endrin using Volhard's titration method has been reported to be satisfactory and ideal (*9–12*). Recent work (*13*) has shown that the above estimation can be made simple and sensitive by estimating the color developed by the unused monoethanolamine. However fungicides like captan which contains both sulfur and chlorine in the molecule behaved in a different way with monoethanolamine as compared to chlorinated pesticides like DDT, aldrin, New-BHC-isomer and endrin. Captan when hydrolyzed with monoethanalamine for 10 minutes in water bath produced different colors from yellow to blood red depending on the quantity present (Table 1). Clear yellow color was noticed between 0.1 to 2.0 mg of captan; and orange color between 2 to 4.0 mg, and above 5.0 mg, the color was blood red. Further the intermediate quantities of captan can also be assessed approximately by the different shades of yellow, orange, and deep red color. In fatty materials and highly colored fruits and vegetables, a detailed cleanup procedure is necessary before this method could be applied.

Captan can also quickly be detected and estimated approximately in the presence of DDT, lindane, New-BHC-isomer, BHC, aldrin, malathion, and thiram by the above procedure. The detection and estimation

TABLE 2

VISUAL SEMIQUANTITATIVE ESTIMATION OF CAPTAN IN THE PRESENCE OF CERTAIN INSECTICIDES

Insecticide (5.0 mg)	Captan (mg)				
	5.0	10.0	20.0	30.0	40.0
DDT	Milky white soln	Milky white soln	Faint orange soln: turbid.	Clear orange soln: turbid.	Deep orange soln: turbid.
New-BHC-isomer	Milky white soln	Very faint orange soln: turbid.	Pale orange soln: turbid.	Clear orange soln: turbid.	Deep orange soln: turbid.
BHC	Milky white soln	White turbid soln	Faint orange soln: turbid.	Clear orange soln: turbid.	Deep orange soln: turbid.
Aldrin	Milky white soln	White turbid soln	Pale orange soln: turbid.	Orange soln: *no turbidity.*	Orange soln: *no turbidity.*
Malathion	Faint orange soln: *no turbidity.*	Faint orange soln: *no turbidity.*	Pale orange soln: *no turbidity.*	Deep orange soln: *no turbidity.*	Intense orange soln: *no turbidity.*
Thiram	Faint greenish-yellow soln: *no turbidity.*	Faint green soln: *no trubidity.*	Yellow soln: *no turbidity.*	Deep yellow soln: *no turbidity.*	Intense yellow soln: *no turbidity.*

TABLE 3

COLOR CHANGES OF CAPTAN IN THE PRESENCE OF CERTAIN PESTICIDES USING CHROMOGENIC AGENTS

5.0 mg of insecticide + 5.0 mg of captan + 1 ml of monoethanolamine	Change of color after adding 0.5 ml of 0.1 *N* silver nitrate solution	Change of color after adding 0.2 ml of ferric alum solution
DDT	Dark brown turbid solution.	Yellowish green solution with brownish green precipitate.
New-BHC-isomer	Light brown turbid solution.	Faint yellow solution with brownish green precipitate.
BHC	Dark brown turbid solution.	Faint yellowish green solution with brownish green precipitate.
Aldrin	Dark brown turbid solution.	Clear green solution with brownish green precipitate.
Malathion	Chocolate color *clear solution.*	Faint leafy green solution; *no precipitate.*
Thiram	Intense chocolate colored turbid solution.	Intense bottle green solution; *no precipitate.*

were made only after the addition of 10 ml of distilled water in each case. The changes in the color of the solution and the presence or absence of turbidity were employed to distinguish captan among chlorinated, organophosphorous and carbomate pesticides. DDT, New-BHC-isomer and BHC, when treated with captan up to 10 mg, did not give any color when diluted with water (Table 3) but with 20 mg and above the color deepened from faint orange to deep orange. However, aldrin behaved in the same pattern up to 20 mgs but there was no turbidity in the orange colored solution with 30 mg and above. Therefore, the absence of turbidity with 30 mg and above in case of aldrin can be made use of to distinguish among BHC, New-BHC-isomer and DDT. Malathion with captan gave a clear orange colored solution with no precipitate as observed in case of chlorinated pesticides right from 5.0-mg level up to higher quantities. But the orange color deepened gradually, depending on the quantity present. It was observed that malathon gave only faint yellow color with monoethanolamine in contrast to orange color obtained with captan and malathion. In the same way thiram produced clear greenish-yellow colored solution up to 10 mg. Thus thiram, malathion, chlorinated insecticides can be distinguished by the different colors, as well as by the presence or absence of precipitate in the solution. These observations can also be further supported or confirmed by the use of chromogenic agents like 0.1 *N* silver nitrate solution or saturated ferric alum solution (Table 3).

DDT, New-BHC-isomer, BHC, and aldrin gave dark brown turbid solution in the presence of captan when treated with 0.5 ml of 0.1 N silver nitrate solution but malathion gave chocolate colored solution with no turbidity. Thiram produced intense chocolate colored solution with heavy precipitate. But malathion, thiram in the absence of captan gave faint yellow and turbid yellow solution, while the chlorinated insecticides gave milky white turbid solution under identical conditions. When ferric alum solution was treated in the place of silver nitrate solution; DDT and BHC gave the same colored solution with brownish green precipitate while New-BHC-isomer and aldrin gave yellow and green solution with brownish green precipitate (Table 3). These chlorinated pesticides can further be distinguished from malathion and thiram since they gave faint leafy green and intense bottle green solution without any precipitate. In the absence of captan, malathion and thiram gave faint yellow and faint greenish-yellow solution while the chlorinated pesticides gave pale yellow solution with turbidity.

These tests, being simple and rapid will help in detecting and estimating captan semiquantitatively both in the pure state as well as in combination with the other categories of pesticides screened here.

SUMMARY

Monoethanolamine hydrolysis of captan for 10 minutes in a water bath offered a quick and easy method to detect and estimate captan semiquantitatively, as the color of the hydrolyzed mixture changed from pale yellow to deep blood red color as the quantity increased. Captan can also be quickly detected and estimated approximately in the presence of DDT, New-BHC-isomer, BHC, aldrin, malathion, and thiram by the color changes, as well as the presence or absence of precipitate in the solution. This method of detection can also be supported or confirmed by the use of chromogenic agents like silver nitrate and ferric alum solutions. Since the method is quick and convenient it can be employed in field tests and routine analysis, especially in pesticide formulation laboratories.

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Colorimetric Determination of Iron(II) by Tetrazolium Salt

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2,3,5-Triphenyltetrazolium chloride has been used for the determination of iron(III), chromium(VI), thallium(III), gold(III), platinum, cerium, iodate, bromate, unsaturated aldehydes and isonicotinic acid hydrazide (*4–7*) in acidic medium. Determination of ascorbic acid with the same reagent has been reported (*8*). The color reaction of tetrazolium salt with iron is very peculiar. Iron(III) gives pink-red color at pH 2.03 (*4*) where iron(II) does not interfere; while iron(II) gives pink color at pH 8 or above where iron(III) becomes inactive.

In the present work, a new method for the determination of iron(II) with 2,3,5-triphenyltetrazolium chloride in basic medium is presented. The present method has advantages over many previously reported methods (*1, 2, 8, 11–16*) because of its high sensitivity, selectivity, wide pH range over which Beer's law is obeyed and minimum interferences by diverse ions. The method can be used for the determination of iron(II) in the presence of iron(III).

EXPERIMENTAL METHODS

REAGENTS

All reagents were of analytical grade or comparable purity.

Standard iron(II) solution. The stock solution of iron(II) was prepared by dissolving 49.643 mg of ferrous sulfate in distilled water acidulated with dilute sulfuric acid and the volume was made up to 100 ml.

Tetrazolium salt solution. This solution was prepared by dissolving 1 g of 2,3,5-triphenyltetrazolium chloride in distilled water to make 100 ml.

Citric acid solution. The solution was prepared by dissolving 20 g of citric acid in distilled water to make 100 ml.

Sodium arsenite solution. A 10% aqueous solution was prepared by dissolving 10 g of the reagent in distilled water to make 100 ml.

Dimethylformamide. Redistilled dimethylformamide at 152°C was used in all the experiments.

APPARATUS

All the absorbance measurements were made on a Unicam SP 1300 colorimeter with filter No. 3 in position and using 1-cm cells.

Spot test. To 1.0 ml of a neutral test solution, 0.2 ml of 2,3,5-triphenyltetrazolium chloride and 0.2 ml of sodium arsenite are added. Spontaneous appearance of pink color indicates the presence of iron(II). Two hundredfold of iron(III) does not interfere.

METHOD

Take 10 ml of the neutral test solution containing 50–160 μg of iron(II) in a 20-ml test tube. Add 0.2 ml each of 2,3,5-triphenyltetrazolium chloride and sodium arsenite. After shaking well for 20–30 sec, at room temperature (30–35°C), add 0.6 ml of citric acid. Make up the volume to 10 ml by the addition of dimethylformamide (DMF). Shake the solution and let it cool to room temperature before measuring the absorbance with SP 1300 Unicam colorimeter using filter No. 3. The color reaction is not influenced by change in pH when carried between pH 8.0 and 10.5.

Calibration curve. A standard graph is prepared by taking different amounts of standard iron(II) solution and treating as described above to produce pink-red color. Corresponding absorbances are plotted against standard amounts of iron(II) which obey Beer's law between 50–160 μg. Amount of iron(II) should be varied by weight only in each case and not the volume of iron(II) which will mean the variation of aqueous contents and thus introducing error in the determination, i.e., the amount of water in one set of experiments should remain constant in each case. A typical curve is shown in Fig. 1.

RESULTS AND DISCUSSION

Effect of pH. The color reaction is fairly stable between pH 8.0 to 10.5. The color intensity falls with the fall in pH but higher pH does not produce any bad effect; however, the intensity begins to fall with the passage of time.

Effect of time. The maximum time for complete color development is 15 sec. when other conditions are not varied.

Effect of temperature. The color intensity remains constant between 20–40°C. Decrease in color intensity is observed when the temperature is either way altered (below 20 or above 40°C).

Reagent concentration. The amount of 2,3,5-triphenyltetrazolium

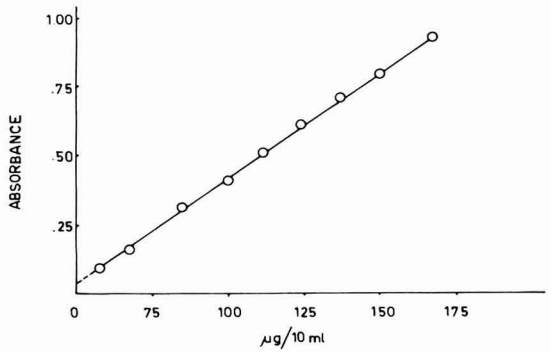

FIG. 1. Typical calibration curve for iron(II).

Order of addition of reagents. The order of mixing of 2,3,5-triphenyltetrazolium chloride and sodium arsenite with iron(II) solution has no effect on color intensity if added immediately one after the other. The best results are achieved when color producing agent is followed by sodium arsenite, otherwise, iron may get precipitated.

Effect of water contents. Sensitivity of the color reaction decreases with the increase in water contents, i.e., the quantity of water should remain constant in one set of experiments while plotting the standard calibration curve and the determination of iron(II) from an unknown solution.

Extinction coefficient. The extinction coefficients of the iron(II) complex with tetrazolium chloride have been calculated and the average value of ε has been determined to be 2.353×10^4.

chloride to react completely with 200 μg of iron(II) is 1.5 mg and higher amount of reagent does not produce any effect on the color intensity.

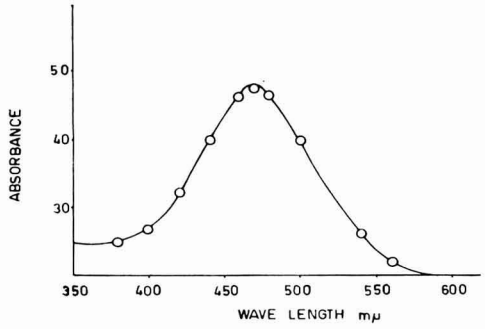

FIG. 2. Absorption characteristics of iron(II) complex with tetrazolium salt.

Direct determination formula. A formula for the direct determination of the amount of iron(II) without the use of a standard calibration curve has been developed.

$$A = \frac{Vd\ 10^3}{42},$$

where A = amount of iron(II) (μg) present in the sample;

V = final volume of the solution after developing color;

d = absorbance of the iron(II) complex formed from the above volume V.

Nature of the complex. Ferrous ions give red color with 2,3,5-triphenyltetrazolium chloride in the presence of sodium or potassium hydroxide. The red colored pigment begins to settle within a few seconds indicating the formation of diformazan (*3, 9, 10*). The absorbance of the red color thus produced cannot be measured due to the suspension formation. The suspension finally yields red colored thick precipitate which is soluble in DMF but the color becomes unstable. Red color is also developed by using certain alkaline salts instead of using a strong alkali. During these investigations it was found that sodium arsenite is the most suitable salt for this reaction and can replace all other alkalies successfully. It appears that sodium arsenite takes part in the actual chemical reaction, because the product becomes fairly water soluble; in addition to this, color gets stabilized. The red colored products obtained when using sodium hydroxide and sodium arsenite melt at 230°C. The absorption characteristics are similar to one another both showing maximas at 480 mμ. The diformazan produced when 2,3,5-triphenyltetrazolium chloride is reacted with reducing sugars and vitamin C in presence of sodium hydroxide shows maximum absorption at 490 mμ (*9*) and melts at 192°C.

From the above findings it is gathered that in the present case diformazan does not form but the role of sodium arsenite and the mechanism of the reaction is still unknown.

Citric acid helps in the formation of a soluble complex (*17*). Dimethylformamide has been found to be the best diluent in the present work.

Interferences. Gold when present in large amounts masks the color development. The maximum tolerable amounts of inorganic ions are given in Table 1. Twofold amounts of iron(II) do not interfere.

Sensitivity of the reaction. The reaction obeys Beer's law between 50 to 160 μg/ml of iron(II). The visual limit of detection is 5 μg/ml.

TABLE I

QUANTITATIVE ASSESSMENT OF TOLERABLE AMOUNTS OF DIFFERENT METAL IONS

Metal ion	Maximum amount not interfering $\mu g/10$ ml	Remarks
Ag^{+}	200	
Al^{3+}	500	
Au^{3+}	100	Compound itself is yellow colored. Increased amounts mask the color development of iron II
Ba^{2+}	500	
Be^{2+}	100	
Bi^{3+}	900	
Cu^{+}	5	Seriously interferes
Ca^{2+}	500	
Cd^{2+}	700	
Ce^{3+}	300	
Cu^{2+}	2	Seriously interferes
Ce^{4+}	50	
Co^{2+}	300	Pink colored compound
Cs^{+}	300	
Cr^{3+}	40	Increased amount gives blue color
Fe^{3+}	200	Yellow colored compound, yellow color interferes when amount is increased
Hg^{+}	1000	
Hg^{2+}	1500	
In^{3+}	700	
K^{+}	2000	
La^{3+}	900	
Li^{+}	500	
Mg^{2+}	300	
Mn^{2+}	600	
Ni^{2+}	200	Increased amount will produce thick white turbidity which interferes
Na^{+}	2500	
Pb^{2+}	900	
Pb^{4+}	900	
Pd^{2+}	200	Yellow colored compound
Pt^{4+}	260	Yellow colored compound
Rb^{+}	1000	
Ru^{3+}	70	Black colored compound
Sr^{2+}	1000	
Sb^{5+}	300	
Sn^{2+}	250	With the increase in amount, turbidity interferes
Sn^{4+}	1000	
Th^{4+}	1000	
Tl^{+}	500	
Te^{4+}	1000	
UO_2^{2+}	120	
VO^{2+}	2000	
Zn^{2+}	2000	
Zr^{4+}	2500	

TABLE 2

DETERMINATION OF Fe^{2+} FROM SYNTHETIC MIXTURES AND VARIOUS PHARMACEUTICAL PREPARATIONS

Iron preparation	Amount reported on labels (mg)	Recovery (%) of the added iron(II)	Composition of the preparation
Synthetic mixtures	—	97.5	About 50 mg of ferric chloride
	—	100.0	About 50 mg of ferric chloride
	—	98.3	About 50 mg of ferric chloride
Easton's syrup [a]	—	100.6	Ferr. Phons., Quinine and strychinine
		99.0	(Edruc. Ltd., Pabna Pakistan)
Syrup Minadex [a]	—	99.6	Vitamin A, 60,000; D 10,000; Fe-et-am. citrate 3 g/100 ml
		99.3	(Glaxo Laboratories; Karachi)
Ferrodin [a]	7.00	100.5	Fe-gluconate 300 mg; Ca-glycerophos, 41.0 mg; B HCl, 1.5 mg;
		100.2	B_2-5-phosphate Spd., 1.5 mg; B_6, 1 mg; B_{12}, 6.5 μg; Pantathenol, 3 mg;
Fersolate [b]	6.00	100.4	nicotinamide, 8 mg; Lysine monohydrochloride 15 mg; choline
		99.1	chloride, 8 mg; $MnCl_2$, 0.2 mg; $CuCl_2$, 0.11 mg; Alc. 2% (v/v), 5 ml
			(P.H.D. Laboratories, Lahore)
Calglufer [b]	8.00	100.0	Ferr. sulfate, 3 g; cupr. sulfate, 25 mg, Mang. sulfate 2.5 mg
		98.8	(Glaxo Laboratories, Karachi)
Fersamal [b]	65.00	99.6	Ca-gluconate BP, 250 mg; Fe-gluconate HP, 75 mg; Calceferol
		99.2	BP, 100 I.U.; B_{12}, 3 g (Sandoz Ltd., Basel, Switzerland)
Pregamal [b]	65.00	99.3	Fe-fumarate, 200 mg (Fe = 65 mg); B_{12}, 10 μg (Glaxo Laboratories,
		99.7	Karachi)

[a] Per milliliter.

[b] Per tablet.

SUMMARY

The present reaction is sensitive to iron(II) and the usually interfering cations in other procedures have greater tolerance limit. (cf. Table 1). The importance of the procedure has been ascertained by using it in the analysis of synthetic mixtures and various forms of pharmaceutical preparations. (cf. Table 2). The method is quick and suitable for routine analysis.

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Simultaneous Microdetermination of Solubility and Diffusivity of Dissolved Oxygen in Aqueous Electrolyte Solutions with Galvanic-Cell–Diaphragm-Cell Technique

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INTRODUCTION

The study of the transport process of dissolved gases in liquids, particularly the determination of the solubility and diffusivity of dissolved oxygen in aqueous systems, has been the subject of considerable theoretical and practical interest. Many useful methods for the determination of dissolved oxygen in water have been reported (*26, 29, 30, 34, 37*). The diffusivity of oxygen in water has been measured by various investigators (*10, 12*). However, solubility and diffusivity measurements of oxygen in aqueous solutions containing other dissolved solutes has received markedly less attention. We wish to report solubilities and diffusivities for oxygen in aqueous solutions of colloidal electrolytes with surfactant properties such as sodium 1-decanesulfonate, sodium 2,4-dimethylbenzenesulfonate, and sodium 4-dodecylbenzenesulfonate over extended concentration ranges of these electrolytes. A diaphragm cell technique with oxygen sensitive galvanic cells adapted to follow changes in dissolved oxygen concentrations was utilized. This technique, which provides a rapid, simple and direct way for simultaneous microdetermination of solubility and diffusivity of dissolved oxygen in aqueous systems, has several advantages over other tedious experimental methods.

Recently, Woolfolk and Dinius (*38*) described an acid-chromous titrametric procedure for the determination of dissolved oxygen in water. The procedure is based on the reaction of chromous ions with dissolved molecular oxygen in the presence of iodide ions in acid solution (pH ca. 1). Okuda, Inoue and Miwa (*23*) also reported a polarographic method for rapid microdetermination of the absolute amount of dis-

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solved oxygen in water. The method measured the ratio of oxygen consumed by the flavin enzyme in the presence of a limited amount of substrate as an internal standard to the total dissolved oxygen, which can be determined by the addition of an excess amount of substrate or sodium hydrosulfite. The two methods are both accurate and rapid, but they have been complicated because of the presence of other solutes. They measure the amount of dissolved oxygen indirectly from the equivalent amount of dissolved solutes which have reacted with the dissolved oxygen.

Over the past few decades, diffusion theory and diffusivities in various systems have contributed to a significant number of publications. [For a very excellent guide to the literature on diffusivities of gases in liquids, see Himmelblau's review (*10*)]. This is in part due to the desire to develop a satisfactory liquid-state theory. Since there is convincing evidence that aqueous solutions are structured (*15*), it might be expected that the transport of dissolved gases through such a medium would be influenced by the intramolecular structure. On this basis, dissolution (solubility) and diffusivity of oxygen in aqueous electrolyte solutions at different concentrations would reflect changes in the microstructure of the electrolyte solutions.

The amount of experimental work reported in the literature as to the effect of dissolved solutes on the diffusivity of another solute is meager. Ratcliff and Holdcraft (*31*) have measured the diffusivity of carbon dioxide in aqueous electrolyte solutions. Also, Jordan, Ackerman and Berger (*13*) have reported diffusion coefficients for oxygen in aqueous sucrose and glycerol solutions that were 0.1 *M* in KCl. The diffusivities reported by these workers varied inversely with the concentration of dissolved solutes. A correlation of the measured diffusivity with the solution intramolecular structure was not readily apparent in the work of Ratcliff and Holdcraft. Jordan, Ackerman and Berger interpreted their measurements qualitatively in terms of solution intramolecular structure. Their interpretation was handicapped by the presence of a constant 0.1 *M* KCl concentration.

In the present work solubilities and diffusivities for oxygen dissolved in aqueous electrolyte solutions have been measured utilizing a diaphragm cell technique. Oxygen concentrations in the compartments of the cell were measured with an oxygen sensitive galvanic cell. The electrolytes with surfactant properties such as sodium salts of 1-decanesulfonic acid, 2,4-dimethylbenzenesulfonic acid, and 4-dodecylbenzenesulfonic acid, respectively, were used. The concentration range was varied from 0 to 2000 ppm in microscale. All measurements were made at 25 ± 0.01°C.

EXPERIMENTAL METHODS

Reagents and Materials

All the reagents are of analytical grade. The surfactants were obtained commercially and were used as such without further purification. They include: the sodium salt of 1-decanesulfonic acid (Aldrich Chemical Co., Inc., Milwaukee, WI.); the sodium salt of 2,4-dimethylbenzenesulfonic acid (Eastman Organic Chemicals, Rochester, NY); and the sodium salt of 4-dodecylbenzenesulfonic acid (K & K Laboratories, Inc., 331 Plainview, NY). Doubly distilled water was used for all measurements.

Apparatus and Equipment

The diaphragm diffusion cell used in this work was constructed from two 50 ml round-bottom flasks with 20/40 ground glass joints. The two flasks were connected with a piece of 20 mm glass tubing into which was sealed a 2 mm fritted glass disc of approximately 40 nm pore size. The connecting tube was of minimum length, and the connection between the flasks and tubes were flared as much as possible to allow free circulation of the solution. Ports for filling, fitted with stopcocks, were sealed into both flasks. This diffusion cell was calibrated conductimetrically with 0.10 *N* KCl standard solution by the dipping conductivity cell and YSI Model 31 Conductivity Bridge (Yellow Spings Instrument Co., Inc., Yellow Springs, OH). The solubilities and the diffusion curves of oxygen in surfactant solutions were determined and monitored with an oxygen sensitive galvanic cell, Pb/KOH/Ag (supplied by Precision Scientific Co., Chicago, IL), incorporating with a bucking potential circuit in conjunction with an E. H. Sargent recorder. This recording system was calibrated with a Leeds and Northrup potentiometer. A deoxygenation apparatus was constructed from a graduated 250 ml Erlenmeyer flask. Nitrogen was used to purge oxygen from the diffusion cell and the deoxygenated solutions. A schematic diagram of these arrangements is shown in Fig. 1. The oxygen-saturated solutions were prepared by dissolving the accurate amount of sodium sulfonate samples into the preoxygenated water. The preoxygenated water sample was prepared by passing the clean air through a train of gas washing bottles for about 1–2 hr. Viscosities of various concentrations of surfactant solutions were measured with a Sargent Ostwald viscosimeter and a Sargent viscosimeter oil bath. Conductivities for a variety of surfactant solutions were determined with a Jones' type conductance cell and a Beckman Laboratory conductivity bridge. The temperature of the thermostat was controlled within $\pm$ 0.01°C by a Sargent thermonitor and a Sargent water cooler.

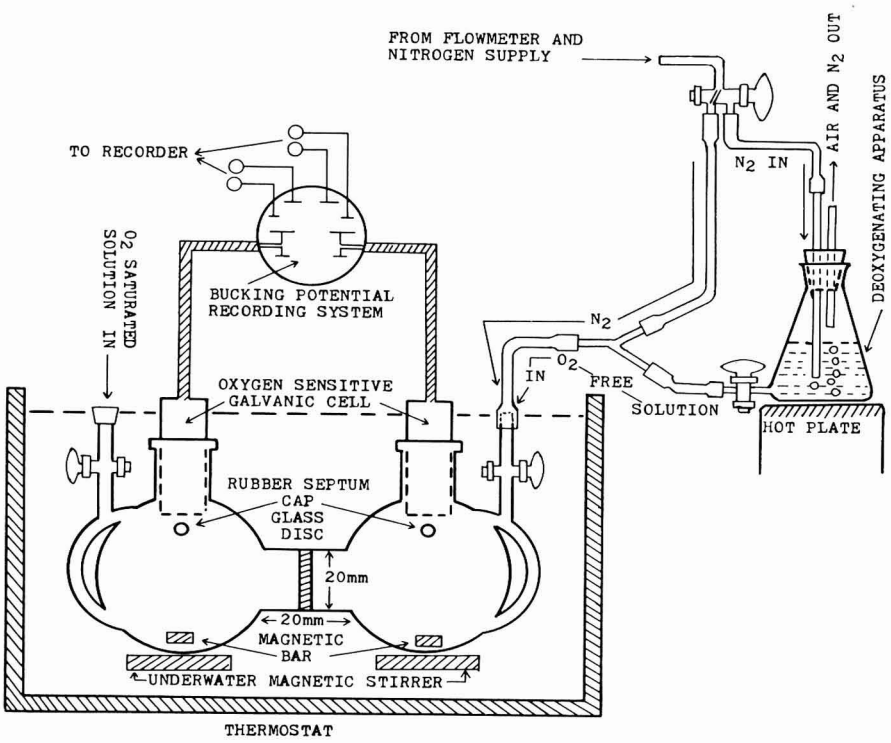

FIG. 1. Schematic diagram of galvanic-cell–diffusion-cell systems.

Procedure

A. Preparation and calibration of the galvanic cell. The detector, Pb/KOH/Ag galvanic cell, whose construction and characteristic function have been previously described by Mancy, Okun and Reilley (*20*), is very sensitive and selective to molecular oxygen. It consists of a silver cathode, a lead anode, a disc of lens paper saturated with 1 *N* supporting electrolyte solution, and a thin polyethylene membrane which covered on the top of the cell and was secured tightly by a fitting plastic sleeve. The electrode reactions of the cell involve:

Ag cathode: $O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$ (reduction) (1)

Pb anode: $Pb + 4OH^- \rightarrow PbO_2^{2-} + 2H_2O + 2e^-$ (oxidation) (2)

Cell: $2Pb + O_2 + 4OH^- \rightarrow 2PbO_2^{2-} + 2H_2O$ (3)

Thus, molecular oxygen is reduced at the silver cathode, and lead is oxidized to the lead oxide complex at the anode. The resultant electron flow is from lead to silver.

However, the probe performance is highly dependent on cleanliness and care must be taken to accomplish this properly. Therefore, each galvanic cell used in the present study was carefully prepared before each run as described below. First, the plastic sleeve, polyethylene membrane, and electrolyte pad were removed. Then, the surface of the lead and silver electrodes were wiped from one edge to another with an extra fine sandpaper. (Care was taken to ensure that all the cell surface was evenly rounded to maintain a uniform surface). The cell was dipped in water and with a twisting motion was wiped with filter paper cupped in the palm of the hand. Rinsing and drying were continued for several times until little residue remained on the paper. Using soft facial tissue and the same twisting motion, the cleaning was continued until no black residue was left on the tissue. The KOH supporting electrolyte was then substituted for water and the processes repeated a few more times. One electrolyte pad (1.5 cm in diam) saturated with 1–2 drops of 1 *N* KOH supporting electrolyte was then placed on the cell tip. The electrolyte pad was blotted with a tissue paper to remove any excess supporting electrolyte. A preformed polyethylene membrane (2.42 × 2.42 cm) was placed over the electrolyte pad and pulled down to remove any wrinkles or trapped bubbles. The plastic sleeve was then slipped over the membrane and the excess membrane was trimmed to match with the rubber collar. Finally, the cell was allowed to cure in water for 16–24 hr and was ready for use. (Care was taken throughout the process to avoid touching the membrane with fingers because a thin coat of oil or grease would be deposited which would impair the performance of the cell).

Acid-Chromous titration method (*38*) was employed to the calibration of the galvanic cell. Aliquots of standard oxygen solutions were analyzed for oxygen concentration and the equivalence of voltage generated to oxygen concentration was determined (Table 1). An oxygen calibration curve (Fig. 2) was prepared for conversion of

TABLE 1

CALIBRATION OF Pb/KOH/Ag GALVANIC CELL AT 25 ± 0.01°C

Oxygen concn (ppm)	Potential (mV)
4.44	125.2
5.87	159.4
6.78	172.4
6.94	185.9
7.64	199.2
8.42	212.6

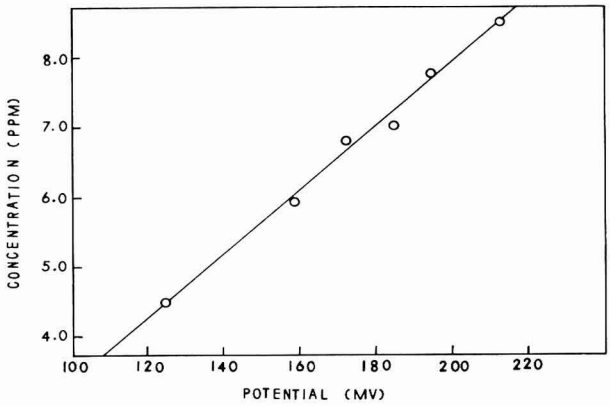

FIG. 2. Oxygen calibration curve [C (ppm) of dissolved oxygen vs potential (mV) measured by galvanic cell] at 25°C. (Slope = 0.045 ppm/mV; Y intercept = −1.134 ppm).

potential in millivolts into oxygen concentration in parts per million (ppm).

B. Calibration of diffusion cell to determine the cell constant. The calibration of the diffusion cell was accomplished by diffusion of 0.10 N KCl standard solution into distilled water at 25 ± 0.01°C. The left side of the diffusion cell was filled with 0.10 N KCl solution and the right side with distilled water (Fig. 1). The concentration changes of KCl in each compartment as the diffusion proceeded were measured conductimetrically (*11*). The cell constant of the diffusion cell was then evaluated from the slope of the plot of $\log(C_H - C_L)$ versus time by employing an integrated form of Fick's first law of diffusion in one dimension (*14*). That is,

$$\log(C_H - C_L) = \beta D t + K \tag{4}$$

where C_H and C_L are the concentration of diffusant on the high and low side of the cell, respectively. On the right side of the equation, β, D, and t are cell constant, diffusion coefficient (or diffusivity), and time in that order; K is an integration constant. The cell constant, β, represents the diffusion cross section (i.e., area and length of diffusion path). Since this constant cannot be measured directly, it is determined with KCl reference standard with a well-known diffusion coefficient of 1.838×10^{-5} cm²/sec at 25°C (*7*). Thus, a cell constant of -4.567×10^{-2}/cm² was obtained. Reproducibility of this value was 2.80%.

C. Determination of solubility and diffusivity. Before starting diffusion measurements, the oxygen-saturated solutions, the deoxygenated solutions, and the galvanic-cell–diffusion-cell systems (the cell systems)

were thermostated at 25 ± 0.01°C in water bath. The cell systems were flushed with nitrogen to remove the oxygen present. The right side of the cell systems was then filled with oxygen-free solution and the left side with oxygen-saturated solution. The filling was accomplished by connecting a short piece of tubing to the stopcock of the cell systems and to the deoxygenating apparatus for oxygen-free solution. A 50 ml syringe with a hypodermic needle through a rubber septum mounted on the stopcock was used to insert oxygen-saturated solution. Another hypodermic needle was inserted through the rubber septum during the filing operation to release the nitrogen from the closed system. This method of filling allowed the cell systems to be filled without exposing the samples to the atmosphere. Finally, the filled systems were mounted over the magnetic stirrers and thermostated in water bath, each galvanic cell connected to the recording circuit, and stirring of the cell systems was started. The cell systems was allowed to run for approximately 15–20 min to establish a steady state before the collection of data was begun. The diffusion data was taken from the recorder which was connected to the galvanic cell on the oxygen-saturated side of the cell systems. The other galvanic cell was used primarily to determine if the deoxygenated solution was initially oxygen-free. Each diffusion run was allowed to proceed for approximately 8.0×10^4 sec. The solubility of oxygen was taken from the value at the zero time of recorder before the diffusion run was started. All diffusivities were evaluated from the slopes of the plots of $\log(C_{II} - C_L)$ versus time by employing Eq. (*4*). The computation of the slopes were carried out by the method of least-squares utilizing IBM 360 Computer and Fortran IV.

RESULTS AND DISCUSSION

The Oxygen Sensitive Galvanic Cell and Oxygen Calibration Curve

As has been mentioned, the Pb/KOH/Ag galvanic cell is sensitive and selective to molecular oxygen. The cell response to dissolved oxygen was measured in terms of potential drop across a 30 kΩ load resistor with a recording potentiometer. The measuring circuit also contained a standard potentiometer circuit for bucking out definite increments of the potential developed across the 30 kΩ resistor (Fig. 1). Such a circuit has been described by Lipner, Witherspoon and Champeaux (*19*).

The cell was calibrated by determining the response in solution of known oxygen concentration. The oxygen concentration (ppm) in water equivalent to the potential generated (mV) by the galvanic cell at 25°C is given in Table 1 and plotted in Fig. 2. Figure 2 shows that the cell response is essentially linear with concentration because it is sensitive

to the activity of the dissolved oxygen, and Raoult's law is obeyed. The slope of such a plot is 0.045 ppm/mV or 1 mV = 0.045 ppm; and the Y intercept is -1.134 ppm.

This galvanic cell provides some distinctive advantages over the other techniques. It successfully overcomes the difficulty of measuring oxygen content or small changes in oxygen concentration in solutions at the microscale. It permits continuous monitoring of oxygen concentration during the course of diffusion. The polyethylene membrane eliminates any interference from ions and other substances to which it is impermeable. The dissolved oxygen in aqueous solution is analyzed directly without the necessity of adding supporting electrolytes or surface active agents in solutions which is impossible to determine by polarographic method. Thus, this detector offers a simpler, easier method for determining the dissolved oxygen in water, natural water, aqueous and nonaqueous solutions, water pollutants, waste effluents, and biological systems, etc.

However, one disadvantage of the cell is that its sensitivity decreases with time. This decrease is linear with time for about 2 wk so that sensitivity changes could be corrected in any given measurement. In this study, a freshly prepared and calibrated cell was used for each solubility and diffusion determination.

The Diaphragm Diffusion Cell Technique

The diaphragm cell technique for measuring diffusion coefficient has been described and analyzed by a number of investigators (*1, 7, 10, 24*). All of the diaphragm cells used previously utilized a horizontally mounted diaphragm. The stirrers for these cells were mounted just above and below the diaphragm; in some cases the stirrers in upper chamber actually rested on the diaphragm. This type of design usually requires a magnetic stirrer that surrounds the cell. The cell used here differs in design from that used by others. The primary difference is that the diaphragm is mounted between the two chambers vertically rather than horizontally. Also, the stirrers, which are usually directly above and below the diaphragm, are placed further away in the bottom of the chambers. This cell is operationally much more convenient with respect to mounting in a thermostat, and also conventional magnetic stirrers may be used by placing them beneath the cell compartments outside the water thermostat. The presence of galvanic-cell-type oxygen detectors in both chambers allows the simultaneous determination of solubility and diffusivity of dissolved oxygen in aqueous systems at isothermal condition. If two recording channels are used then both chambers may be monitored simultaneously (Fig. 1). The suitability

of the cell design and technique is demonstrated by the favorable comparison between the values of solubility and diffusivity of oxygen in aqueous systems obtained here (Table 2) and those published by other workers using conventional cell designs or other techniques (Tables 3 and 4).

The diaphragm diffusion cell, although widely accepted and used because of experimental simplicity, provides data that may be subject to some uncertainties. Among these uncertainties are the fact that it is a comparison technique and relies upon a preliminary standardization. The reliability of the standard diffusivity used for calibration then limits the measured diffusivities. Another point of uncertainty in diffusivities derived from the diaphragm cell measurements is the possibility of surface interaction occurring at the pore walls of the diaphragm. Such an interaction cannot be ascertained from the experimental data. Despite the various sources of uncertainty, diffusivities determined with a diaphragm cell have been widely accepted.

Solubility and Diffusivity of Oxygen in Aqueous Surfactant Solutions

Solubilities and diffusivities of oxygen in water and in various concentrations of surfactant solutions determined in this work are presented in Table 2. For comparison and judgement, several selected literature

TABLE 2

SOLUBILITY AND DIFFUSIVITY OF OXYGEN IN AQUEOUS SURFACTANT SOLUTIONS AT 25 ± 0.01°C

Concn of surfactant	(ppm)	Solubility of oxygen in surfactant solution (ppm)	Diffusivity of oxygen in surfactant solution ($D \times 10^5$ cm²/sec)
Pure water	0	8.38	3.40
Sodium 1-decanesulfonate	500	8.18	3.11
	1000	8.11	2.67
	2000	8.04	2.45
Sodium 2,4-dimethylbenzenesulfonate	500	8.30	2.93
	1000	8.20	2.60
	2000	7.96	2.05
Sodium 4-dodecylbenzenesulfonate	500	8.29	2.74
	1000	8.16	2.30
	2000	8.02	1.51

TABLE 3

SOME SELECTED LITERATURE VALUES OF SOLUBILITY OF OXYGEN IN WATER AT 25°C

Solubility (ppm)	Investigator	Ref.
8.26	Stone and Eichelberger	(*34*)
8.31 (26°C)	Woolfolk and Dinius	(*38*)
8.40	Amer. Public Health Ass.	(*33*)
8.40	Okuda, Inoue and Miwa	(*23*)
8.38	This work	—

values of solubility in water have been collected in Table 3, and those values of diffusivity in water and in aqueous systems are summarized in Table 4.

The values of solubility reported here were obtained simultaneously from the initial readings of the recorder just before the starting of each diffusion run. It was based on the assumption that saturation values were maintained in each of the corresponding concentrations of surfactant solutions at the zero time of diffusion. Table 3 shows that the solubility of oxygen in water at 25°C determined in this work is almost

TABLE 4

DIFFUSIVITY OF OXYGEN IN AQUEOUS SYSTEMS AT 25°C

Diffusivity $D \times 10^5$ (cm^2/sec)	Investigator	Method	Ref.
2.60	Kolthoff and Miller	Polarographic in 0.1 *N* KNO_3, 25°C.	(*16*)
2.12	Jordan, Ackerman and Berger	Polarographic in 0.1 *N* KCl and 0.01 *M* phosphate buffer, 25°C.	(*13*)
3.49	Krieger, Mulholland and Dickey	Optical bubble solution at 29.6°C.	(*18*)
3.82	Nakanishi, Voight and Hidelbrand	Diaphragm cell in CCl_4 at 25°C.	(*21*)
3.54	Tammen and Jessen	In agar, 25°C.	(*36*)
2.65	Ng and Walkley	Bubble solution, 25°C.	(*22*)
2.25	Pircher	Polarographic at 25°C.	(*27*)
2.42	Davidson and Cullen	Wetted Wall Column, 25°C.	(*3*)
2.42	Carlson	Capillary, in 1% KCl, 25°C.	(*2*)
1.90	Kreuzer	Surface active agent added, 25°C	(*17*)
3.40	This work	Pb/KOH/Ag galvanic cell, 25°C.	—

identical with those generally accepted. It is about 0.24% lower than that listed in the American Public Health Association Tables (*33*).

All diffusivities listed in Table 2 were evaluated from the slopes of the plots of $\log(C_H - C_L)$ vs time t by employing Eq. (*4*). Four typical examples of such plots are shown in Fig. 3. They are the diffusivity of oxygen in pure water, in 1000 ppm of sodium 1-decanesulfonate, in 1000 ppm of sodium 2,4-dimethylbenzenesulfonate, and in 1000 ppm of sodium 4-dodecylbenzenesulfonate solutions, respectively. The excellent linearity of all plots implies that the experimental results obey Fick's first law of steady-state diffusion equation in the integrated form. The computation of the slopes of all such plots were carried out by means of the method of least-squares. The correlation coefficients vary from 0.997 to 0.999. The data (points) on each plot for each measurement were taken over an extended period of time and treated with a resultant equation derived from integration without limits. Therefore, each value reported actually represents an average of several shorter diffusion measurements. The relative precision of duplicate measurements ranges from 2.41 to 5.02%.

The diffusivities obtained in this work fall within the range of values previously reported (Table 4). It is quite obvious that there is considerable uncertainty as to the true value for the diffusivity of oxygen in water. If the value of the present work (3.40×10^{-5} cm^2/sec) is

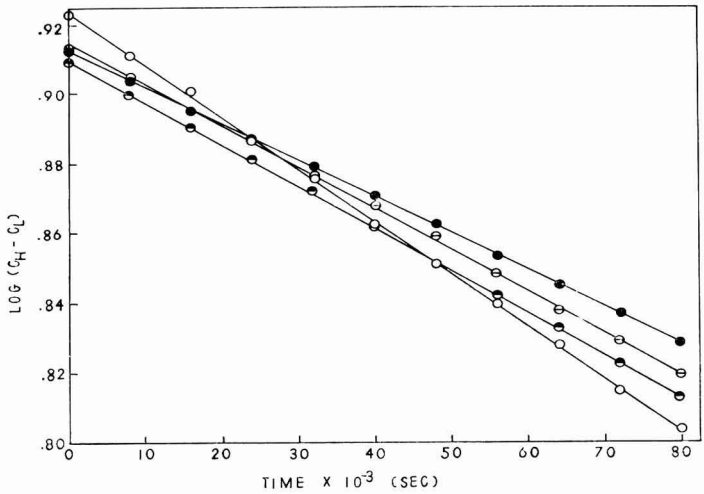

FIG. 3. Typical plots of $\log(C_H - C_L)$ vs time for evaluation of diffusivity of oxygen in aqueous systems at 25°C. (○) pure water; (◓) 1000 ppm of Na 1-decanesulfonate solution; (⊖) 1000 ppm of Na 2,4-dimethylbenzenesulfonate solution; (●) 1000 ppm of Na 4-dodecylbenzenesulfonate solution.

compared with those in Table 4 for the pure water case, it is almost 27% higher than the average. However, it should be pointed out that the several techniques from which the tabulated values were derived offer as much uncertainty as the diaphragm cell technique.

The presence of added electrolyte or surfactant increases the possibility that the measured diffusivities may include other effects. Kreuzer (*17*) value of 1.90×10^{-5} cm^2/sec determined for the water with surface active agent is comparable to the value of 2.05×10^{-5} cm^2/sec obtained here from the diffusion of oxygen in 2000 ppm of sodium 2,4-dimethylbenzenesulfonate solution (7.32% lower). This discrepancy is further discussed below.

Effect of Surfactant Concentrations on the Solubility of Oxygen

Table 2 and Figure 4 show that all three sodium sulfonates tend to decrease the solubility of dissolved oxygen in the corresponding solutions. This phenomenon is the well-known salting-out effect. However, instead of following the empirical Stetschenow relation as shown by Green and Carritt (*8*) that the solubility of oxygen in sea water declines exponentially with increase in salt concentration, in the present case the solubility of oxygen decreases linearly with increasing concentrations of sodium 2,4-dimethylbenzenesulfonate and sodium 4-dodecylbenzenesulfonate solutions, while a nonlinear effect was observed for sodium 1-decanesulfonate solutions (Fig. 4).

From a thermodynamic point of view, all these phenomena are expressions of the effect that the addition of salt increases the activity

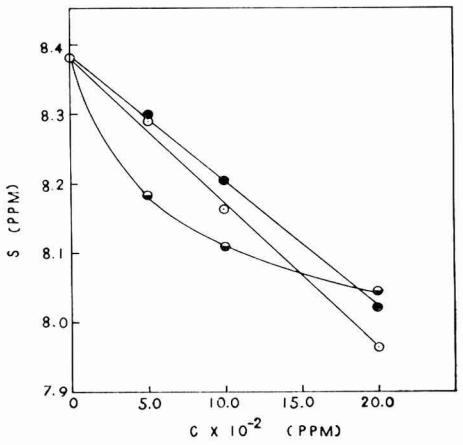

FIG. 4. Effect of surfactant concentration on the solubility of oxygen at 25°C. (○) pure water; (◒) Na 1-decanesulfonate solution; (●) Na 2,4-dimethylbenzenesulfonate solution; (⊙) Na 4-dodecylbenzenesulfonate solution.

coefficient of dissolved gas, while lowering that of water. Frank and Evans (*5*) and Frank and Wen (*5a*) have suggested that the salting-out effect is, to a great extent, a structural phenomena. That is, the observed entropies of solution for the inert gases are consistent with a model picturing the solute gas as producing a more highly structural order of the water molecules in its vicinity. However, the addition of salt results in disorganization of this induced structure. In other words, because of the salting-out effect, the nonpolar gas molecules are squeezed out of the neighborhood of the ions by the preferential attraction of the latter for the polar water molecules. This is consistent with the experimental results found here.

Effect of Surfactant Concentration on the Diffusivity of Oxygen

Figure 5 shows the effect of surfactant concentrations on the diffusivity of oxygen. In general, the diffusivity of oxygen decreases sharply and linearly with increasing concentrations of surfactant solutions from infinite dilution (pure water) up to 1000 ppm and then changes slowly but still linearly to 2000 ppm in all three cases. This effect decreases in the order: a long chain with an aromatic six-member ring>alkyl groups side chain on an aromatic six-member ring>a long straight hydrocarbon chain only. Since the influence of the Na^+ ions and the hydrophilic parts of sulfonate group, $-SO_3^-$, on the liquid medium properties may be considered to be the same for the three electrolytes, the differences in oxygen diffusivities (and also for solubility) may be attributed to the effect of the different (size and shape) hydrophobic

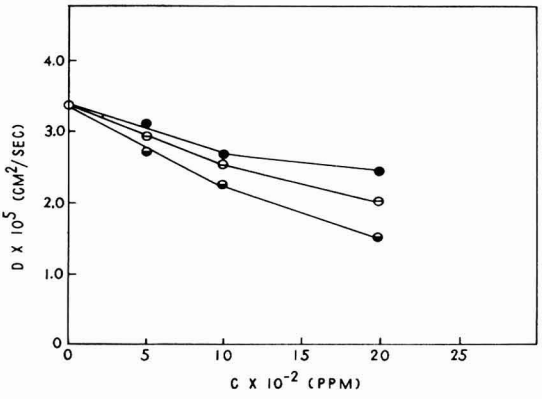

FIG. 5. Effect of surfactant concentration on the diffusivity of oxygen at 25°C. (○) pure water; (●) Na 1-decanesulfonate solution; (⊖) Na 2,4-dimethylbenzenesulfonate solution; (◒) Na 4-dodecylbenzenesulfonate solution.

hydrocarbon parts of the sulfonate anions upon the structure of liquid water.

In Figure 6, a perfect linear relationship between the specific conductance (Table 5) and the concentration of sodium sulfonate below 2000 ppm indicating no micelle formation is presented. In other words, it implies that the concentration of sodium sulfonates used in this study are far below the CMC (*32*). Consequently the charge produced in the solutions by this type of colloidal electrolyte with surfactant properties may be interpreted as the more simple ionic–solvent interactions of the ordinary strong electrolytes in dilute aqueous solutions (*11*). It might be anticipated with the bulky organic substituent attached to the sulfonate groups would induce other changes in the liquid water structure not found with simple strong electrolytes.

Using the predominantly cooperative phenomenon of the "flickering cluster" (5a) of the three dimensional hydrogen-bonding structure of

TABLE 5

SPECIFIC CONDUCTANCE OF SODIUM SULFONATE SOLUTIONS AT 25 ± 0.005°C

Sulfonate concn (ppm)		Specific conductance × 10^6 (mhos/cm)
Sodium 1-decanesulfonate	250	73.73
	500	140.55
	1000	274.17
	2000	541.43
	2500	675.05
	3000	808.68
Sodium 2,4-dimethylbenzenesulfonate	100	47.49
	250	101.03
	500	190.26
	1000	368.72
	1500	547.18
	2000	725.63
	2500	904.09
	3000	1082.54
Sodium 4-dodecylbenzenesulfonate	100	159.10
	250	282.72
	500	488.77
	1000	900.85
	2000	1751.02
	2100	1807.44
	2200	1889.86
	2400	2054.69
	2500	2137.11
	3000	2485.49

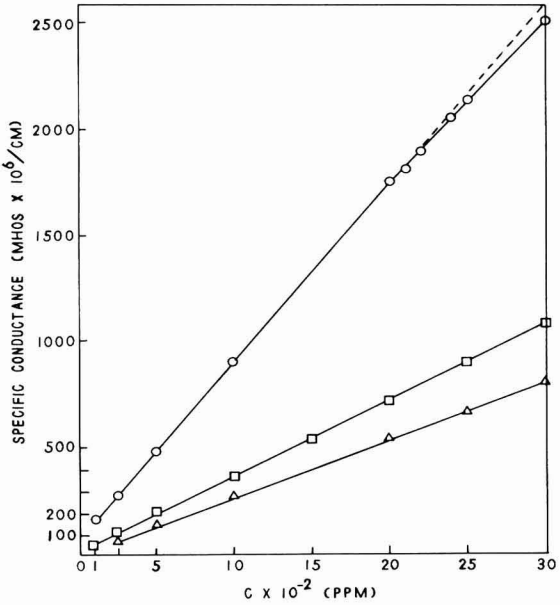

FIG. 6. Specific conductance as a function of surfactant concentration at 25°C. (△) Na 1-decanesulfonate solution; (□) Na 2,4-dimethylbenzenesulfonate solution; (○) Na 4-dodecylbenzenesulfonate solution.

liquid water model, both sodium and sulfonate ions interfere with the initiation of clusters (that is, hydrogen-bonding) and hasten cluster disruption through the torque which their electric fields exert on water dipoles. Also, a competition for occupying the more quasi-lattice ice-like structure space between water molecules occurs between nonpolar molecular oxygen and the hydrophobic part of the sulfonate anions when both are dissolved in water. The hydrocarbon chains with a larger ionic size and a greater number of molecules in solution than molecular oxygen (about 8.38 ppm of oxygen in saturated solution at 25°C compared to 500 ppm to 2000 ppm of sulfonate anions) tend to occupy the the greater parts of free space of liquid water. Furthermore, because of the net structure-breaking (*15*) and hydration [about 3.5 molecules of water are hydrated in straight chain of alkylsulfonate group for C_8 to C_{12} (*32*)] effects of the hydrophilic part, $-SO_3^-$, of the sulfonate anions, the ice-like structure of water is broken, and the water molecules are more closely packed. Thus, the sodium sulfonate solutions have a more closely packed structure with a decreased free quasi-lattice space available for molecular diffusion than in pure water. As a result, the rate of diffusion is reduced and a decrease in diffusivity of oxygen

with increasing concentration of sodium sulfonate solutions was observed.

Alternately, there is a strong interaction between biradical molecular oxygen and hydrogen atoms of the water molecules forming a possible five-membered ring (*9*); and, it forms a stronger even more plausible six-membered ring between the oxygen molecules and the hydrogen atoms on the adjacent carbon atoms of straight-chain hydrocarbons (*4*); while, a strongest interaction with the formation of an aromatic oxygen molecular complex was suggested (*4*). Following Eyring's "hole" theory of activated diffusion process (*6*), these gas–solvent and solute–gas interactions phenomena would indicate that a higher activation free energy is required for diffusion for the system with the stronger interaction and resulting in a lower diffusion coefficient. This is in excellent agreement with the previous interpretation.

Effect of Solubility of Oxygen on the Diffusivity of Oxygen

The dependence of the diffusivity of oxygen on the solubility of oxygen is shown in Fig. 7. It is evident that the D values of oxygen in various concentrations of sodium 1-decanesulfonate and sodium 4-dodecylbenzenesulfonate solution are a linear function of the corresponding S values of oxygen and passing through the D values in pure water. While, in sodium 2,4-dimethylbenzenesulfonate solution a good linear relationship exists between D and S values, the extrapolation to

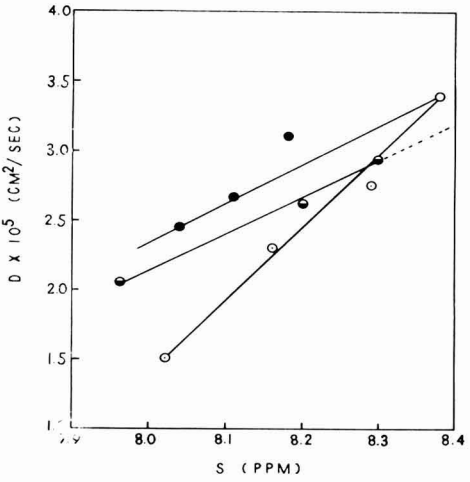

FIG. 7. Effect of solubility of oxygen on the diffusivity of oxygen in aqueous surfactant solutions at 25°C. (○) pure water; (●) Na 1-decanesulfonate solution; (◒) Na 2,4-dimethylbenzenesulfonate solution; (⊙) Na 4-dodecylbenzenesulfonate solution.

the infinite dilution does not pass through that of pure water. However, it should be pointed out that D and S values are varied by the changes in the surfactant concentrations and structural properties of liquid water medium as has been mentioned previously. Therefore, it is not clear to what extent D values are influenced primarily by the solubility of oxygen. At the present time, it can only be concluded that both factors, concentration of surfactant and solubility of oxygen, play equally important role in the diffusion behaviors of dissolved oxygen in aqueous systems.

Effect of the Long-Range and Short-Range Interactions in Electrolyte Solutions on the Diffusion Behaviors of Dissolved Oxygen

Figure 8 represents the characteristic plots showing the effects of long-range and short-range interactions in electrolyte solutions on the diffusion behaviors of dissolved oxygen which follows the specified equation in form (*25, 28*)

$$D/D_0 = 1 + A\,(c)_{1\,2} + Bc \tag{5}$$

The term A $(c)^{1/2}$ is the long-range interionic electrostatic interaction, and the linear term Bc is interpreted as the short-range ion–solvent interaction. The coefficients A and B depend on the solute species. The ratio of diffusivity, D/D_0, which actually represents the relative diffusivity of oxygen in electrolyte solutions compared to that in pure water (D_0), might be taken as the fraction of the changes in structure

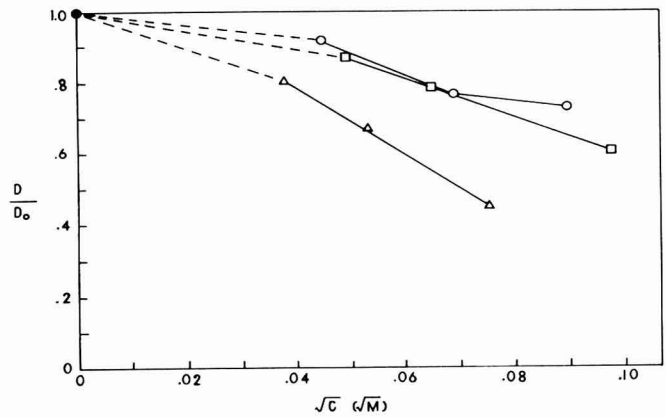

FIG. 8. Plot of ratio, D/D_0, as a function of $C^{1/2}$ of surfactant solutions at 25°C. (Where D is the diffusivity of oxygen in surfactant solutions, D_0 the diffusivity of oxygen in pure water). (●) pure water; (○) Na 1-decanesulfonate solution; (□) Na 2,4-dimethylbenzenesulfonate solution; (△) Na 4-dodecylbenzenesulfonate solution.

properties of diffusion medium caused by those two interaction effects. In solutions of sodium 2,4-dimethylbenzenesulfonate and sodium 4-dodecylbenzenesulfonate, the ratio, D/D_0, shows a good linear relationship with respect to $c^{1/2}$ indicating that the long-range interionic interaction effects predominate. Sodium 1-decanesulfonate solutions do not demonstrate a linear behavior. In the range of the extremely dilute to the infinite dilute solutions (in Fig. 8, broken lines) of three sulfonates the short-range ion–solvent effect is significant.

Testing of the Diffusion–Viscosity Behavior for the Stokes-Einstein Type Equation

The plots of D values versus fluidity, $1/\eta$, (reciprocal viscosity, numerical values are listed in Table 6), by applying Eq. (6) as shown in Fig. 9 represent the relationship of the Stokes-Einstein (*35*) type diffusion–viscosity behaviors of dissolved oxygen in the three sulfonate solutions. That is,

$$D = \frac{1}{\eta}\left(\frac{k_0 T}{4\pi r_1}\right), \tag{6}$$

where η is the viscosity of the diffusion medium, r_1 the radius of the spherical diffusing particle, k_0 Boltzman's constant, T the absolute temperature (°K), respectively.

Equation (6) has been used with moderate success in predicting diffusion of dissolved gases in water (*10*). In Fig. 9, the nonlinearity

TABLE 6

VISCOSITY AND FLUIDITY OF AQUEOUS SURFACTANT SOLUTIONS AT 25 ± 0.01°C

Concn of surfactant (ppm)		Viscosity $\eta \times 10^2$ (P)	Fluidity ($1/\eta$, P^{-1})
Pure water	0	0.8937 [a]	111.9
Sodium 1-decanesulfonate	500	0.8963 [b]	111.6
	1000	0.8980 [b]	111.4
	2000	0.9017 [b]	110.9
Sodium 2,4-dimethylbenzenesulfonate	500	0.8949 [b]	111.7
	1000	0.8965 [b]	111.5
	2000	0.9010 [b]	111.0
Sodium 4-dodecylbenzenesulfonate	500	0.9023 [b]	110.8
	1000	0.9089 [b]	110.0
	2000	0.9280 [b]	107.8

[a] Viscosity values obtained from Lange's Handbook of Chemistry, 8th ed., McGraw-Hill, New York, 1956.

[b] Experimentally determined viscosity values in this work.

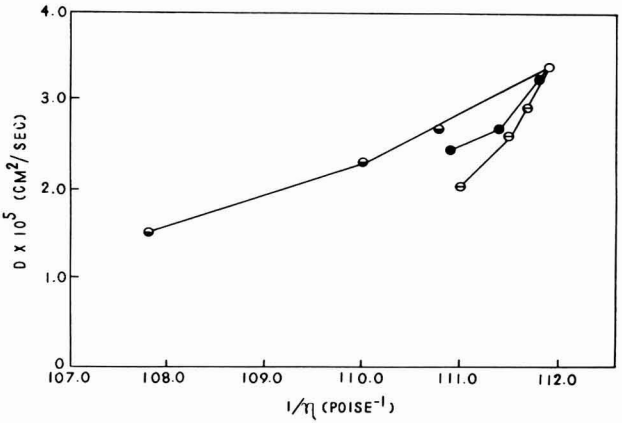

FIG. 9. Diffusivity of oxygen as a function of fluidity ($1/\eta$) of surfactant solutions at 25°C. (○) pure water; (●) Na 1-decanesulfonate solution; (⊖) Na 2,4-dimethylbenzenesulfonate solution; (◒) Na 4-dodecylbenzenesulfonate solution.

of the plots in all cases is interpreted as indicating that the Stokes-Einstein type equation is not completely applicable to the diffusion of ordinary dissolved molecules of the size of the oxygen molecules. This means that the process of molecular diffusion in the ionic media of aqueous electrolyte solutions is far more complicated than the Stokes-Einstein model. This is partly due to the nonfulfillment of Stokes' law by the diffusing molecules because the original assumption of a large spherical particle moving in a continuum medium and partly due to the "structural effect" of the ionic diffusion media.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 17, 431–435 (1972)

Extraction of Iron, Cobalt, and Manganese from Hydrochloric Acid with Quaternary Amine, Aliquat–336

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Separation of iron from cobalt and mangenese is of main importance in the production of carrier free iron and manganese from neutron-irradiated cobalt and iron. The most widely used methods for such separation are based mainly on ion exchange and solvent extraction (*1–5*). Related to solvent extraction procedures, some organic solvents such as tributyl phosphate, methyl isobutyl ketone and tridedocylamine were prepared to achieve such separation (*1*). In the present work the extraction of iron, cobalt, and manganese with the quaternary amine, Aliquat-336 was investigated and a proposed procedure for separation of carrier free iron-59 from cobalt, and manganese-54 from iron was adopted and tested.

EXPERIMENTAL METHODS

Reagents

The quarternary amine, Aliquat-336 was obtained from General Mills, Inc., Kankakee, Illinois. Aliquat-336 is [methyl tricapenyl] ammonium chloride. Xylene is obtained as A.R. grade from Fluka, Switzerland. All other reagents are of A.R. grade and used without any purification.

Radioactive Tracers

High specific activity ^{54}Mn, ^{59}Fe, and ^{60}Co were kindly supplied from the production of radioisotopes laboratory in U.A.R. Their radiochemical purity were checked by their ideal γ-ray spectrum. Radiometric assay was carried out with an EKCO scintillation counter connected to a well-type 2.5 × 2-cm NaI(Tl) crystal.

Solvent Extraction Measurements

The liquid–liquid extraction of different ions was performed by equilibrating a suitable volume of the preequilibrated organic phase and the aqueous phase containing the radioactive species by mechanical shaking

for 10 minutes. After equilibration, the two phases were separated by centrifugation and aliquot portions from both phases were taken for radiometric assay. The batch distribution coefficient was then measured using the expression:

$$D = \frac{A_{org}}{A_{aq}} \bullet \frac{V_{aq}}{V_{org}},$$

where A_{org}, A_{aq} are the total radioactivity in the organic and aqueous phases, respectively, and V_{org} and V_{aq} are the total volume of the organic and aqueous phases, respectively.

RESULTS AND DISCUSSIONS

The curves in Fig. 1 represent the distribution coefficient for the different ions investigated as a function of hydrochloric acid concentration using 0.3 F Aliquat-336 in xylene. It is clear that for trivalent iron, a rapid increase in the extraction is achieved at HCl concentration less than 5 *M* where a sort of constancy in the extraction coefficient is obtained. On the other hand, for Co^{2+}, Fe^{2+}, and Mn^{2+}, the distribution coefficient increased rather linearly in the HCl concentration investigated.

To find the extraction dependency on amine concentration, the distribution coefficient was investigated with different amine concentrations.

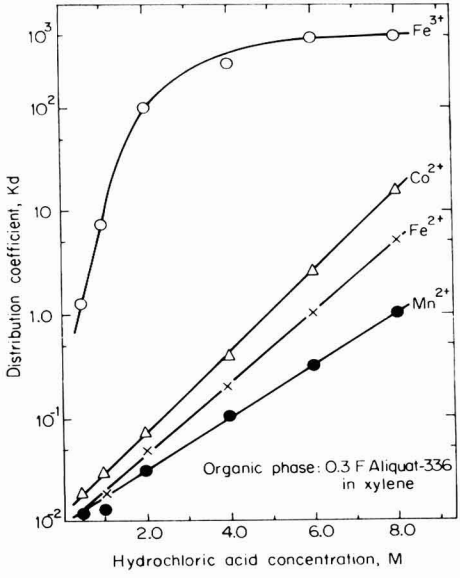

Figure 1

In Fig. 2, a log–log relation between the distribution coefficient of different cations and the amine concentration is presented. A linear relation was obtained for all the cations investigated with a slope of ~0.8. Since this slope may be used for determination of the number of extractant molecules reacting, one molecule of Aliquat-336 may be proposed as a solvation number for different extracted species.

When cobalt is irradiated with neutron flux, (n,p) reaction is expected to be of main importance to produce ^{59}Fe. On the other hand, when iron target is activated with neutrons in nuclear reactor, ^{54}Mn will result as a main product of the (*n, p*) reaction. In this respect, to isolate carrier free iron and manganese from cobalt and iron, knowledge of separation factor between iron and both elements is of main importance. In Table 1 the separation factor between iron and both cobalt and manganese is presented at different hydrochloric acid concentrations. As shown, maximum separation between iron and cobalt could be achieved by extraction with ca. 2 *M* HCl; whereas for separation of iron from manganese, ca. 4 *M* HCl is the required concentration. From these findings, a separation procedure for production of carrier free iron and manganese was developed.

Adopted Procedures

i. Separation of carrier free Fe-59 from cobalt target. 0.5 g of $2CoCo_3 \cdot 3Co(OH)_2 \cdot H_2O$, wrapped in cadmium sheet was irradiated for 48 hours with a neutron flux 1.3×10^{13} n/cm^2 second in the pile (UA-RR-1). The cobalt target was dissolved in HCl, 1 ml of conc

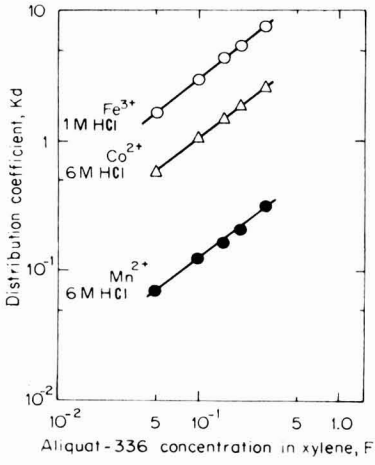

Figure 2

TABLE 1

HCl conc (M)	$S_1 = \frac{K_d Fe^{3+}}{K_d Co^{2+}}$	$S_2 = \frac{K_d Fe^{3+}}{K_d Mn^{2+}}$
8	30	480
6	175	1294
4	790	3400
2	2500	3330
1	577	410
0.5	325	100

HNO_3 acid was added (to oxidize any Fe^{2+} to Fe^{3+}); and boiled until dryness. The target is then dissolved in 10 ml of 4 *M* HCl and transferred to a separating funnel. To this aqueous phase, 10 ml of preequilibrated 0.3 *F* Aliquat-336 in xylene was added. The two phases were brought to equilibrium by vigorous stirring (using spiral type mechanical stirrer) for 10 minutes. After a resident time of 10 minutes, the aqueous phase containing cobalt was drained to the radioactive waste. Two more washings for the organic phase with 10 ml of 5 *M* HCl was found enough to achieve more than 99% radiochemical pure Fe-59. The carrier free iron-59 was finally stripped with 10 ml of 0.1 *M* HCl solution.

All the experiment procedures were undertaken behind a lead shield and using hand manipulators.

ii. Separation of carrier free Mn-54 from iron target. 1 g of Spec. pure iron sponge was irradiated for 48 hours with a neutron flux of 1.3×10^{13} n/cm^2 second in (UA-RR-1) reactor. The target was dissolved in HCl; 1 ml of conc HNO_3 was added; and the solution was boiled until dryness. The residue was then dissolved in 4 *M* HCl and transferred to a separating funnel containing 10 ml of the preequilibrated 0.3 *F* Aliquat-336 in xylene and the two phases were vigorously stirred. After equilibration (10 minutes) the solution was allowed to stand for 10 minutes for phase separation. The aqueous phase containing the carrier free Mn-54 was washed two times with a newly preequilibrated organic phase. A final product of Mn-54 of more than 99% purity was obtained in the aqueous phase.

SUMMARY

The extraction of iron, cobalt, and manganese by Aliquat-336 from hydrochloric acid medium was investigated. Extraction depending on both HCl and amine concentration was studied. The separation factor between iron and both cobalt and manganese is determined and optimum conditions for separation is given. Two procedures for separation of carrier free Fe-59 from irradiated cobalt

target and Mn-54 from irradiated iron target are given. The obtained products proved to be more than 99% radiochemically pure.

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MICROCHEMICAL JOURNAL 17, 436–442 (1972)

Microdetermination of Metals in Organometallic Compounds by the Oxine Method After Oxygen Flask Combustion

I. Cadmium, Magnesium, Uranium, and Zinc

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INTRODUCTION

In spite of the importance of organometallic compounds in the pharmaceutical and industrial fields, this branch of organic analysis has received scant attention (*1*). Two problems are involved in the analysis of such class of compounds: decomposition of the organic material and estimation of the metal after its dissolution.

Decomposition by incineration to sulfate, oxide, or the metal itself is not always so reliable (*7*). Wet decomposition in sealed Carius tubes using different digestion mixtures has been reported, but obviously proper selection of one of the various mixtures is necessary depending on the metal and on the method of determination to follow. Fusion in the nickel bomb has gained favor as a rapid means for decomposing organometallic compounds but the method suffers from the danger of co-precipitation due to the high salt concentration present in the final solution. Most recently, the striking simplicity and rapidity of the oxygen flask method of combustion suggested its use for the analysis of some organometallic compounds (*2*).

Methods of determination, though very extensive, are not usually recognized in standard textbooks (*7*). Some reviews have been published (*1, 6, 8, 10*). Organometallic compounds containing cadmium (0.5–3 mg) (*12*), magnesium (0.1–0.5 mg) (*9*), and zinc (less than 1 mg) (*4*) could be analyzed gravimetrically after precipitation of the metal hydroxyquinolate. Alternatively, the determination is carried out titrimetrically after dissolving the hydroxyquinolate compound in acid then

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titrating with standard potassium bromate solution. No such method has been reported for uranium. In fact, the method available for determination of organically-bound uranium is that depending on ignition to U_3O_8 (*1*). More recently (1958), Belcher *et al.* (*2*) investigated the possibility of analyzing some organometallic compounds using the oxygen flask combustion technique followed by an EDTA titrimetric finish. Only compounds of cadmium, magnesium, and zinc gave satisfactory results. Since that time, a very limited number of publications have appeared on the subject.

The favorable conversion factor in calculating the percentage of element, and consequently the high accuracy, attainable on using organic precipitant encouraged revival of the oxine method for the estimation of metals in organometallic compounds. Whereas previous methods (*4, 9, 12*) advocated digestion with sulfuric acid to decompose the organic sample, the present one makes use of the oxygen-filled flask. After combustion: (i) a measured excess of the oxine solution is added and the hydroxyquinolate compound is precipitated, filtered, and weighed; ii) to the filtrate, a known excess of potassium bromate–bromide solution is added followed by iodide. The liberated iodine is determined titrimetrically against thiosulfate; and (iii) the metal hydroxyquinolate is dissolved in hydrochloric acid and the liberated oxine treated, as under (ii).

EXPERIMENTAL METHODS

Reagents

All reagents used are of A.R. quality except where otherwise mentioned.

Ammonium or sodium acetate: a 10% solution.

Ammonium hydroxide: concentrated solution.

Hydrochloric acid: 1 and 5 *N* solutions.

8-Hydroxyquinoline: a 1% solution, either in ethanol or in *N* acetic acid.

Nitric acid: a 2 *N* solution.

Potassium bromate: a 0.05 *N* solution prepared from the M.A.R. grade.

Potassium bromide: a 10% solution.

Potassium iodide: a 10% solution.

Sodium thiosulfate: a 0.033 *N* solution prepared from the M.A.R. quality and standardized against the primary standard potassium bromate solution.

Starch: a 1% solution.

Procedure

Introduce an exactly weighed amount (3–6 mg) of the organometallic compound in 300-ml Erlenmeyer flask charged with 5 ml of N hydrochloric acid (or 5 ml of 2 *N* nitric acid in case of uranium). Fill the flask with oxygen, stopper, and burn in the usual way. Shake thoroughly for 1 or 2 minutes then let stand 10 minutes with intermittent shaking. Rinse stopper with ca. 15 ml of distilled water. In case of uranium, evaporate till nearly dryness then dissolve in 10 ml of distilled water. Warm to 70°C (if the organic sample contains nitrogen, the nitrogen oxides are removed by heating to 90°C then adding 1–1.5 g of urea with shaking; the flask then allowed to cool to 70°C).

Add, with constant stirring, 1 ml of 1% 8-hydroxyquinoline solution. Slowly add ammonium hydroxide till heavy precipitate appears then 1 ml of 10% ammonium or sodium acetate to bring the solution to pH 6–7 (in case of magnesium, the ammonium acetate solution is dispensed with and precipitation carried out at pH > 9.5). Heat the solution to ca. 80°C and allow to cool down over a period of 10 minutes.

Method I. Filter the crystalline precipitate, wash thoroughly with cold water (ca. 25 ml) until washings are colorless. For magnesium use hot 1% ammonium hydroxide solution for washing. Dry the precipitate to constant weight by heating for 30 minutes at 130, 110, 120, and 160°C for Cd, Mg, U, and Zn oxinates, respectively. The magnesium complex is weighed as the dihydrate, $MgQ_2 \cdot 2H_2O$, that of uranium as UO_2Q_2. HQ and those of cadmium and zinc as the anhydrous form, CdQ_2 and ZnQ_2; where Q stands for the 8-hydroxyquinoline radical.

Method II. To the filtrate and washings from Method I add 5 ml of 5 *N* hydrochloric acid followed by 1 ml of 10% potassium bromide. An accurately measured excess of potassium bromate solution is introduced, the flask is stoppered, shaken, and left in the dark for some time. Add 4 ml of 10% potassium iodide and titrate the liberated iodine with 0.033 *N* sodium thiosulfate solution to the starch end point.

Method III. The dry precipitate from Method I is dissolved in 5 ml of 5 *N* hydrochloric acid. The solution then is treated in the same way as in Method II.

For Methods II and III, a blank determination is carried out.

RESULTS AND DISCUSSION

The proposed general procedure provides an easy and reliable way to estimate cadmium, magnesium, uranium, and zinc in organometallic compounds. Regardless of some minor modifications, the procedure adopted is exactly the same for the four elements. For every sample

TABLE 1
MICRODETERMINATION OF CADMIUM, MAGNESIUM, URANIUM, AND ZINC IN SOME OF THEIR ORGANOMETALLIC COMPOUNDS USING THE OXINE METHOD AFTER OXYGEN FLASK COMBUSTION

Compound	Wt (mg)	Metal (%) Calc.	Found Method: I	II	III	Error (%) I	II	III
Cadmium anthranilate	4.095	29.22	29.45	29.29	29.60	+0.24	+0.07	+0.38
	5.745		29.53	29.70	29.18	+0.31	+0.48	−0.04
dipyridine thiocyanate	4.738	29.06	29.12	29.60	28.99	+0.06	+0.54	−0.07
	4.705		29.24	29.43	29.13	+0.18	+0.37	+0.07
Magnesium oxalate	3.238	16.40	16.59	16.19	16.77	+0.19	−0.21	+0.37
	4.640		16.06	16.17	16.58	−0.34	−0.23	+0.18
Uranyl acetate	3.607	56.13	56.46	56.90	56.03	+0.33	+0.77	−0.10
	4.068		55.50	55.90	56.27	−0.63	−0.23	+0.14
Zinc acetate	4.535	29.93	29.70	29.58	29.76	−0.23	−0.35	−0.17
	5.200		29.40	30.40	30.04	−0.53	+0.47	+0.11
anthranilate	4.674	19.36	19.30	19.37	19.30	−0.06	+0.01	−0.06
	5.061		19.21	19.48	19.38	−0.15	+0.12	+0.02
dipyridine thiocyanate	5.710	19.23	19.65	19.84	19.43	+0.42	+0.61	+0.20
	4.540		19.27	19.58	19.11	+0.04	+0.35	−0.12

weight, Methods I–III have been worked out. This constitutes a remarkable gain not only in the sample weight but also in time. The results obtained are given in Table 1 and are shown to be quite satisfactory; the mean error amounts to ±0.26, ±0.34, and ±0.15% for Methods I, II, and III, respectively.

The oxygen flask combustion technique proved suitable for the quantitative conversion of organically-bound Cd, Mg, U, and Zn to the corresponding ionic state; hydrochloric acid being used as the absorbent except for uranium, where nitric acid is employed instead. The nitric acid has a dual function; it acts as an efficient absorbent and also oxidizes any U(IV) to U(VI). Alternatively, hydrochloric acid can be used as absorbent for uranium, hydrogen peroxide then is added, and the

mixture is boiled to remove excess oxidant. It is necessary only in case of uranium to evaporate the absorption solution to dryness, then dissolve it in water; the other metals are easily convertible to the chloride form.

In order to maintain complete absorption of the combustion products, the flask is left for 10 minutes with frequent shaking. This seems less time-consuming compared with the method of Belcher *et al.* (*2*), who recommended standing for at least 20–30 minutes.

Precipitation of cadmium (*12*), magnesium (*9*), and zinc (*4*) metals as the corresponding hydroxyquinolates is already known in organic microanalysis. However, these methods advocated digestion of the organic sample in a Kjeldahl flask, adjusting the pH to the required value, addition of excess oxine, heating till near boiling, then setting aside for 15, 20, and 30 minutes, respectively, to allow complete precipitation. More recently, Gordon *et al.* (*3*) estimated some metals, e.g., U, Mg, and Zn, present in inorganic compounds, by precipitation of their hydroxyquinolinates from homogenous solution. The authors recommended heating the final reaction mixture at 75°C for 2 hours. Under the present experimental conditions, however, heating the mixture to ca. 80°C, then letting it cool down over a period of 10 minutes still give accurate results; cf. Table 1.

It is known that the particle size and ease of filtration of crystalline precipitates are important factors in any gravimetric procedure. 8-Hydroxyquinoline precipitates a considerable number of elements including Al, Bi, Cd, Co, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Th, U, and Zn (*11*). The precipitates usually filter easily, dry rapidly, and are usually weighed as the anhydrous form except the magnesium complex. This is weighed as the dihydrate and it is hard to remove the water molecules without decomposing or vaporizing some of the compound. The two water molecules are coordinated to the magnesium atom and raise its coordination number up to 6. Although the most accurate way to determine magnesium is to precipitate it as the magnesium ammonium phosphate complex, yet this co-precipitates K^+ badly due to the fact that the latter replaces NH_4^+ isomorphously (*11*).

The oxine method offers a very accurate determination of uranium in inorganic compounds (*5*). However, no such method has been reported for organically-bound uranium. Apart from the method of ignition to U_3O_8 (*1*), it seems there is no other way to analyze organometallic uranium compounds.

A remarkable advantage of the oxine method for the gravimetric determination of elements is the favorable conversion factor, e.g., 1 mg of $CdQ_2 \equiv 0.2807$ mg of Cd; 1 mg of $MgQ_2 \cdot 2H_2O \equiv 0.06979$ mg of

Mg; 1 mg of $UO_2Q_2 \cdot HQ \equiv 0.3384$ mg of U; and 1 mg of $ZnQ_2 \equiv 0.1902$ mg of Zn (4).

To check the accuracy of the results obtained gravimetrically or at least to have a confirmatory value, the metal–hydroxyquinolate precipitate is dissolved in acid and the oxine liberated is brominated through addition of potassium bromide followed by potassium bromate. Bromine is liberated and attacks the free oxine to give 5,7-dibromo-8-hydroxyquinoline. But, since bromination is somewhat slow, it is advantageous to add measured excess of bromate followed by potassium iodide; the iodine liberated then is titrated with standard thiosulfate to the starch end point.

If the organic compound contains nitrogen, the nitrogen oxides produced after combustion may liberate some iodine leading to high thiosulfate titers, e.g.,

$$2HNO_2 + 2I^- + 2H^+ = 2NO + I_2 + 2H_2O$$

Removal of nitrogen oxides is therefore necessary and could be achieved by addition of urea to the hot reaction mixture.

From the following equation:

$$C_9H_7ON + 2Br_2 = C_9H_5ONBr_2 + 2HBr,$$

it is evident that every mole of 8-hydroxyquinoline requires four equivalents of bromine for bromination. Therefore, the hydroxyquinolate salts of cadmium, magnesium, and zinc require 8 equivalents of bromine while that of uranium requires 12 equivalents. Such amplification reactions allow better accuracy and are quite favorable in analytical chemistry.

The present method is simple and accurate. The relatively time-consuming nature of the method is compensated by the fact that three results can be obtained for every sample weight; cf. Table 1.

8-Hydroxyquinoline precipitates almost every metal in the periodic table except the alkaline metals and, in low concentrations, the alkaline earth metals (*11*). Therefore, a large number of metals interfere and, if present, have to be removed beforehand.

Other metals in organometallic compounds can be determined following the present method and details will be published in due course.

SUMMARY

A simple, accurate, and relatively rapid procedure for the estimation of cadmium, magnesium, uranium, and zinc in organometallic compounds is presented. The oxygen-filled flask is recommended for decomposition of the organic sample. Determination of the metal concerned is carried out by three methods using a

single sample weight. Both the gravimetric and titrimetric finishes are applied. The method has the advantage of a favorable conversion factor for the gravimetric determination besides an 8- or 12-fold amplification reaction, depending on the valency of the metal, in case of the titrimetric finish.

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MICROCHEMICAL JOURNAL 17, 443–455 (1972)

Squaric Acid: Reactions with Certain Metals [1]

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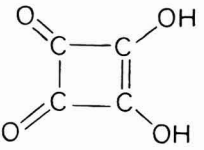
INTRODUCTION

The organic reagent to be considered in this paper is 3,4-dihydroxy-3-cyclobutene-1,2-dione,



however, because of its structure and its pronounced acidic properties, this aromatic compound has been assigned the more convenient trivial name "squaric acid" (*2*). While the behavior of squaric acid with various cations has been discussed elsewhere (*3*), the purpose of the present paper is a more detailed examination of the action of aqueous squaric acid on various metals which are more active than hydrogen, and which ordinarily are not appreciably attacked by cold water.

EXPERIMENTAL METHODS

In general, the squaric acid, or 3,4-dihydroxy-3-cyclobutene-1,2-dione, was applied as a saturated aqueous solution, prepared directly from the product as obtained from the manufacturer (Aldrich Chemical Co., Inc.). Most metals used were in the form of reagent grade sheet, foil, wire, or shot. To guard against any surface contamination which might have resulted from prolonged exposure to the laboratory atmosphere the specimens were cleaned with an abrasive, or by dilute acid, followed by thorough rinsing in distilled water.

Reactions were carried out in 75-mm test tubes. Whenever the dissolved squaric acid in a given assembly appeared to have been consumed, more of the solid was added to the mixture. In certain instances

[1] Adapted from a paper read at the Midwest Regional Meet. Amer. Chem. Soc., St. Louis, MO, Oct., 28–29, 1971.

specimens of a given metal from different batches appeared more reactive than others. As this might be attributed to the catalytic effect of impurities, certain metals which seemed nonreactive were used in contact with metallic copper.

RESULTS

Colored Salts

Of the metals subjected to the direct action of aqueous squaric acid, the only individuals which formed colored squarates were the members of the transition group, iron, cobalt, and nickel. All three were divalent. The specimens of iron were in the form of thin strips, and also in the form of wire spirals. In the first trials, very soon after immersing the samples in the aqueous squaric acid, the solution briefly acquired a color which ranged from lilac to deep purple. After 5–10 min, the color vanished, leaving a colorless liquid.[1] Meanwhile an accumulation of gas bubbles (hydrogen) began to form on the metal surface, but the rate soon decreased considerably. Even before bubbles were observed a sample of the colorless liquid gave a positive test for ferrous ion when treated with potassium ferricyanide. Depending upon conditions, the separation of small crystals of the ferrous squarate dihydrate became apparent within 12–48 hr. In order to consume the metallic iron more completely a quantity of solid squaric acid was eventually added to the mixture. Although the final solution remained colorless the macro appearance of the crystalline product was a pale yellow-green. As seen under the microscope (Fig. 1) the various forms which separated included long hair-like crystals, greenish yellow prisms, and long thin plates. Some of the latter presented a rectangular profile, while others exhibited tapered ends. Many of the individuals appeared to have a long modified sphenoid structure. The large hair-like forms measured as much as 0.75 mm in length. In polarized light the crystals exhibited pleochroism, changing from colorless to a pale greenish yellow when rotated. Extinction was frequently parallel, depending upon the orientation on the slide.

According to West and Niu (*4*) who prepared ferrous squarate by mixing solutions of ferrous salts and dipotassium squarate, the squarates of divalent cations are generally dihydrates, appearing in the form of chain polymers in which each squarate radical is chelated with two different metal ions. The composition of the ferrous dihydrate is represented by the formula $FeC_4O_4 \cdot 2H_2O$.

Metallic cobalt reacted more slowly than iron, but eventually yielded a pale pink solution and pink crystals (Fig. 2). Contact with metallic copper appeared to effect a slight increase in the rate of reaction. While

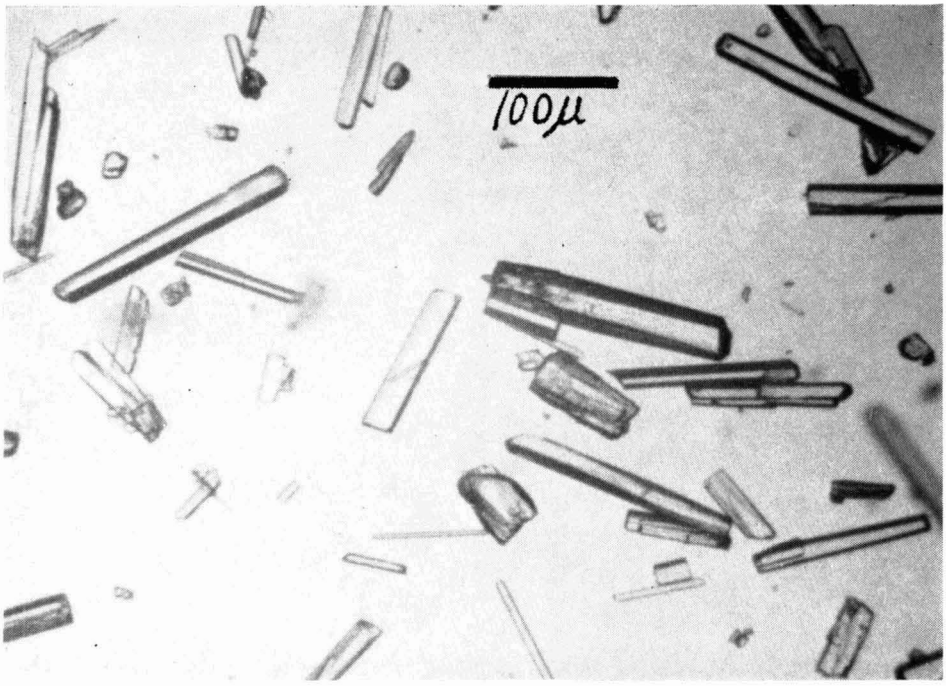


FIG. 1. Ferrous squarate dihydrate crystals.

crystals of the cobalt salt were generally acicular or prismatic, occasionally hexagonal plates or leaves were formed. Conditions favoring the separation of hexagonal crystals were not determined; all the various forms mentioned could appear in the same reaction mixture. In general, the shapes of the crystals, including the hexagonal forms, were similar to those obtained elsewhere by the interaction of solutions of dipotassium squarate and cobalt chloride. Individuals of appropriate dimensions were dichroic, passing from practically colorless (or a faint orange color) to pink when rotated in plane polarized light. As a rule the needles exhibited parallel extinction. Prisms and needles of cobalt squarate were comparatively small; i.e., even after 5–6 wk they did not exceed 0.7 mm in length. On the other hand hexagonal forms occasionally attained an overall length of 5–7 mm. While the latter could grow to as much as 3 mm in thickness, such structures appeared to consist of layers of very thin leaves.

With nickel the aqueous squaric acid reacted very slowly, a few gas bubbles being observed only occasionally. However, a sample drop of the colorless reaction liquid withdrawn at the end of 0.5 hr gave a definite test for nickel ion. Eventually a pale blue-green product began

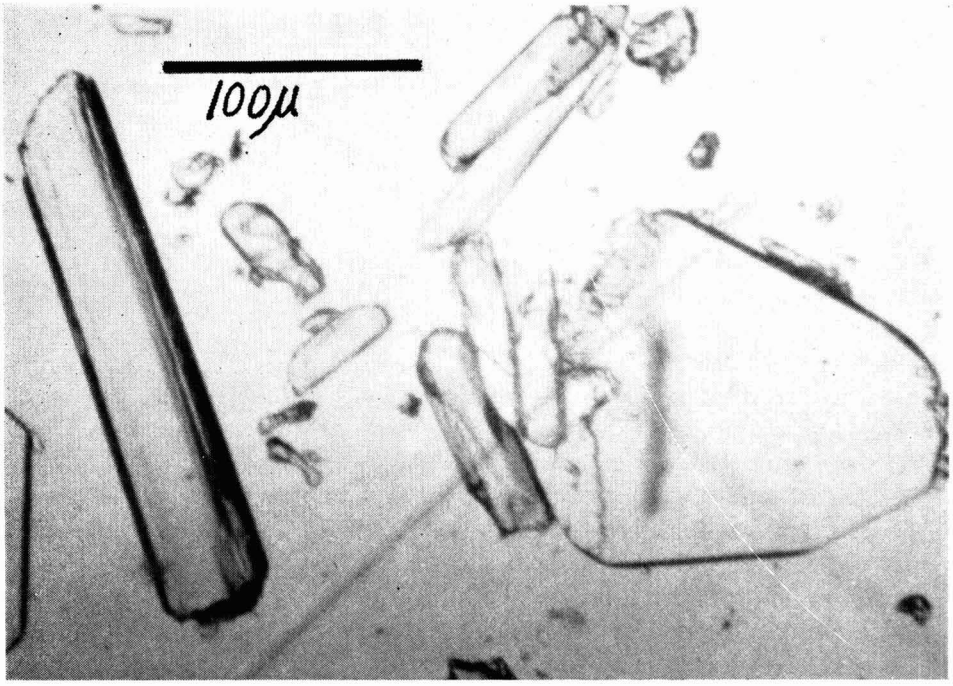


FIG. 2. Cobalt squarate dihydrate. The large thin leaves appear to be deformed hexagonal plates.

to accumulate, and by the end of 2 wk the crystalline product contained some individuals measuring as much as 0.06 × 0.02 mm. At this stage the reaction mixture still remained colorless, indicating very low solubility of the nickel salt. In fact, the residue left after the spontaneous evaporation of a small sample revealed only crystals of the solid squaric acid. As viewed through the microscope the nickel squarate which had collected on the metal appeared to consist of comparatively small colorless prisms, along with occasional parallelepipeds and laths (Figure 3). Some of the prisms presented a diamond-shaped cross-section. No pleochroism was detected, but for many of the crystals extinction was practically parallel.

Since the alloy, Chromel-A, consists of 80% nickel with 20% chromium, it may be of interest to mention that a spiral of Chromel wire immersed in squaric acid appeared immune to attack even after a period of 6 mo. However, a similar Chromel spiral in contact with copper was immersed in aqueous squaric acid only 5 days when faint signs of a deposit appeared on the copper; the Chromel still retained its bright surface. The separation of cupric squarate gradually increased,

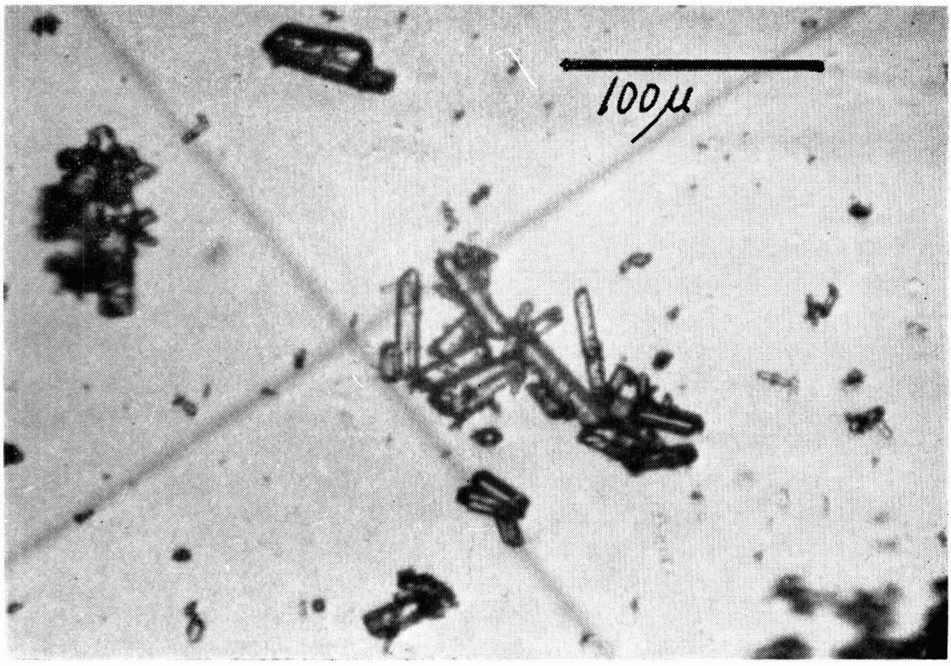


FIG. 3. Nickel squarate dihydrate.

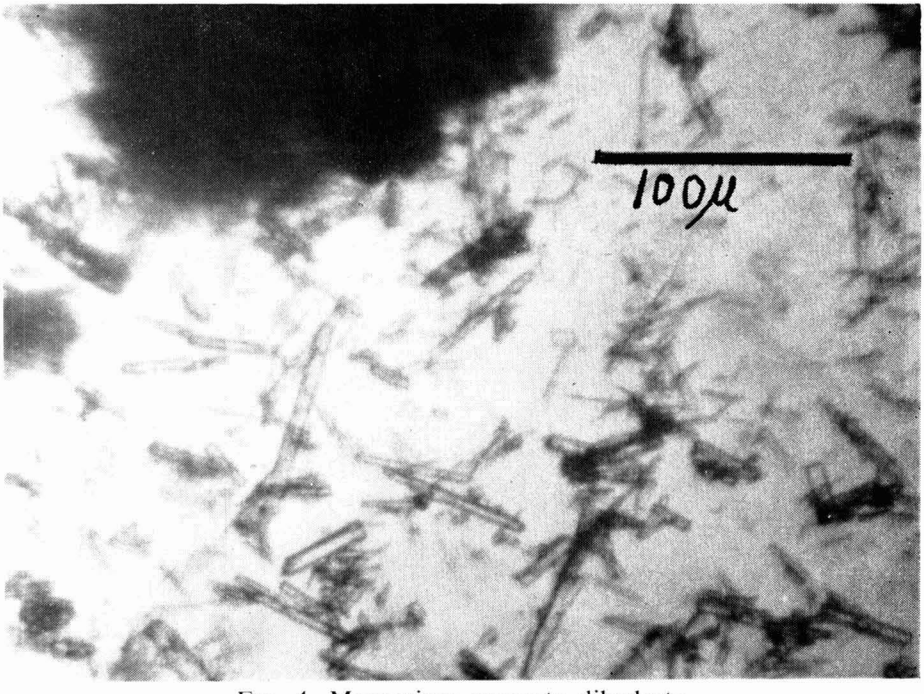


FIG. 4. Magnesium squarate dihydrate.

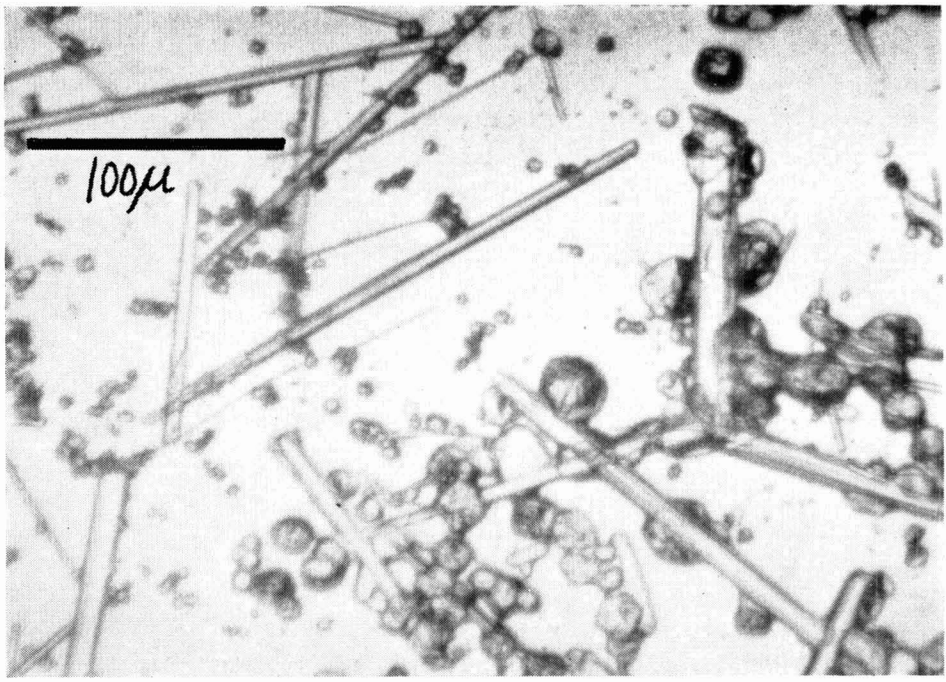


FIG. 5. A common form of zinc squarate dihydrate.

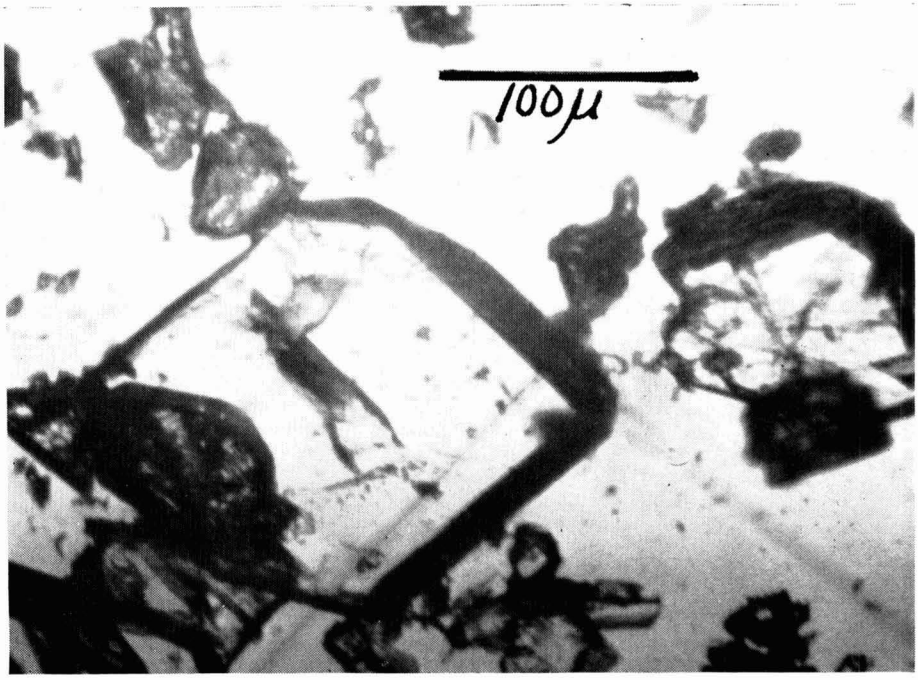


FIG. 6. Another common form of zinc squarate crystals.

with some actually forming on the bottom of the container 2 wk after immersion.

Colorless Salts

Of all the metals tested with aqueous squaric acid the most vigorous reaction was exhibited by magnesium, which promptly caused a rapid evolution of hydrogen accompanied by the accumulation of a fine, white, chalk-like precipitate (Fig. 4). The forms of magnesium squarate crystals appeared identical with those obtained when the acid reacted with a magnesium salt, but much smaller; even after remaining in contact with the mother liquor for 6 mo the dimensions of the larger lath-like forms rarely exceeded an area of 10 × 20 μ. The profiles of some crystals were elongated hexagons, although equilateral hexagons were found occasionally. Extinction was parallel. Solubility at room temperatures was in the 0.5% range.

Predictably, zinc reacted less rapidly than magnesium; even zinc dust reacted rather slowly. The resulting zinc squarate crystals eventually attained larger dimensions than those of the magnesium salt (Fig. 5, 6). Many acicular crystals measured 1 mm or more in length, while some other forms could eventually grow to an overall length of 5–7 mm.

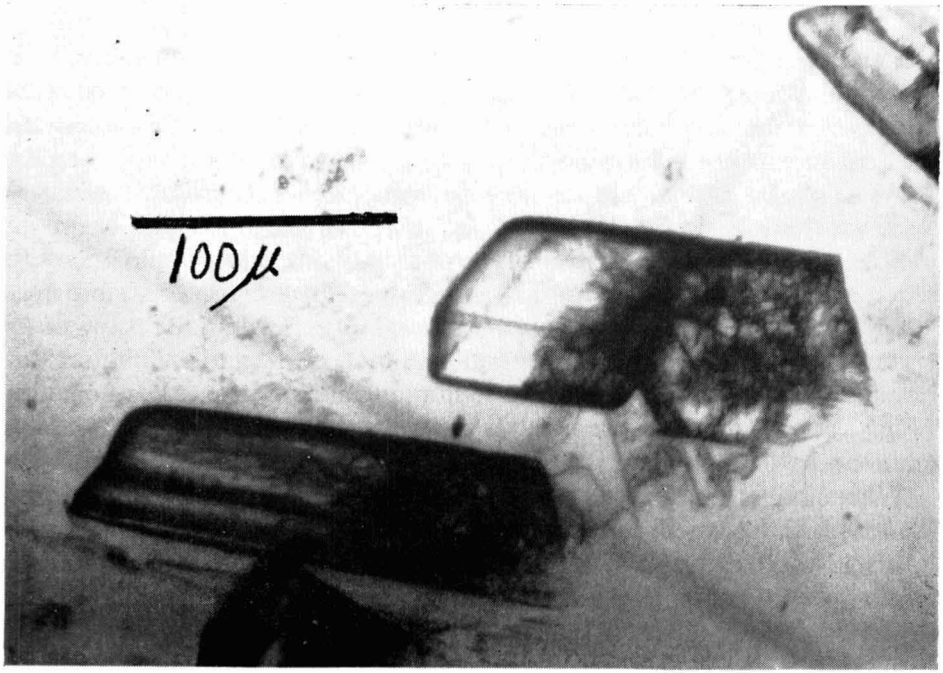


FIG. 7. Cadmium squarate dihydrate.

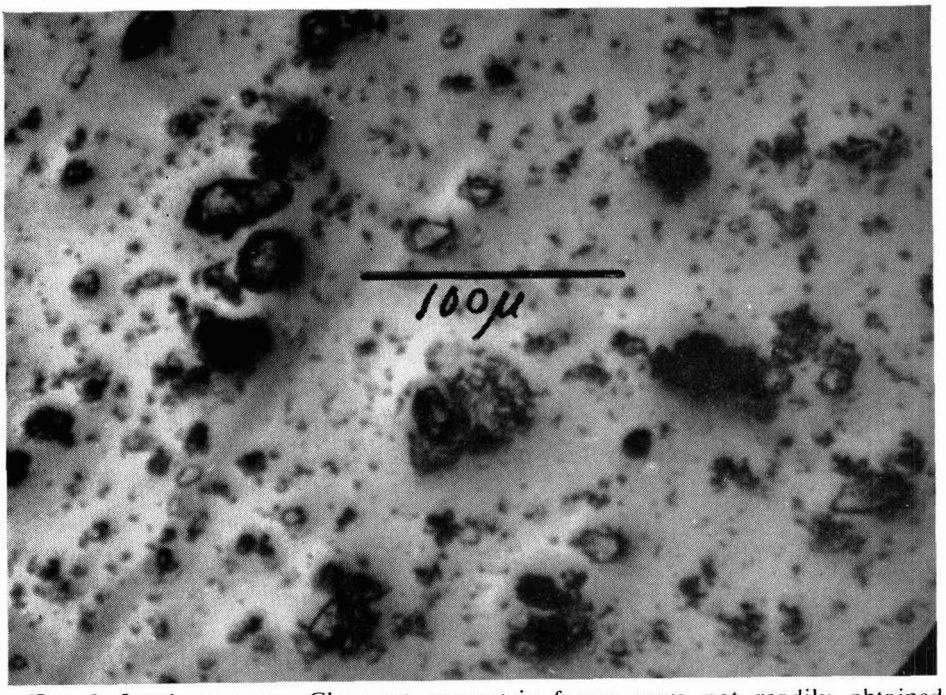


FIG. 8. Lead squarate. Clear-cut geometric forms were not readily obtained by the action of squaric acid on metallic lead.

Depending upon the orientation on the slide many crystals presented rectangular or hexagonal profiles. In a given batch of zinc squarate there was a tendency for part of the material to be slightly discolored; the reason was not determined.

As might be anticipated for cadmium, its rate of reaction with squaric acid was much slower than that for zinc, although a number of gas bubbles began to form within approximately 20 min. While the solubility of cadmium squarate is low, the appearance of small visible crystals in the reaction mixture required several days. Eventually some of the crystals attained a length of several millimeters. In general their forms resembled those of the zinc salt, including numerous needles and laths (Fig. 7). A number of the crystals were parallelepipeds which exhibited extinction when the shorter edges were parallel with one of the polars.

When bright metallic lead was immersed in aqueous squaric acid the original luster vanished within 3–5 min. Over a period of many hours a white deposit was formed on the surface of the metal and a small quantity of product accumulated on the bottom of the reaction tube. Eventually the reaction came to a halt because of the coating formed on the lead surface. In some of the experiments the lead

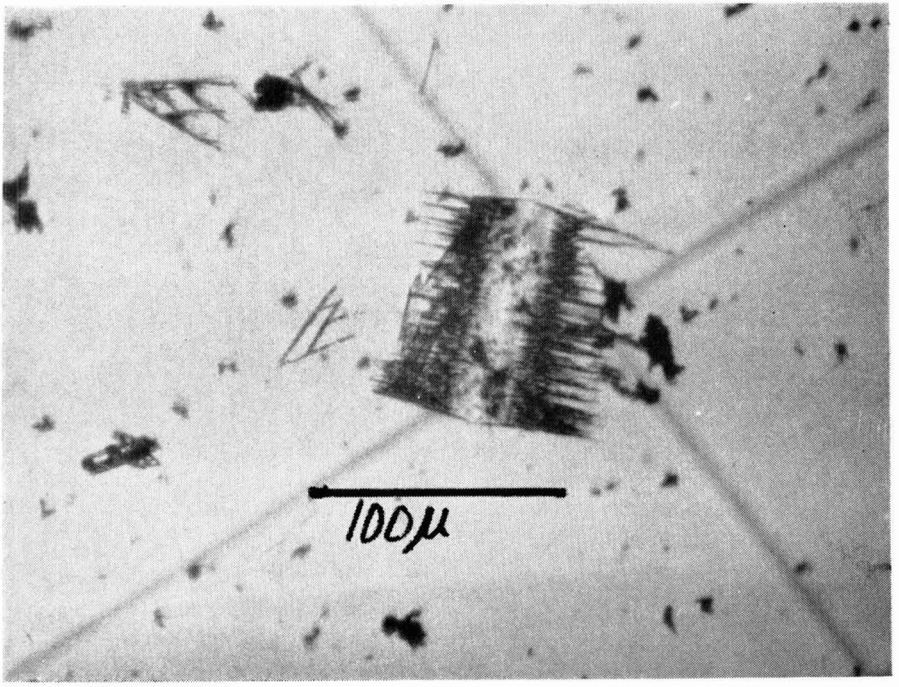


FIG. 9. Common form of crystals resulting from slow action of aqueous squaric acid on metallic tin. Other crystalline forms are frequently encountered.

squarate seemed to consist of amorphous particles, whereas some of the batches also contained fine crystalline material (Fig. 8).

Cleaned strips of metallic tin exposed to the aqueous squaric acid developed some minute glistening particles on the metal surface after approximately 12 hr, although there had been no visible evidence of gas evolution. After several days some small colorless crystals were observed growing out from the tin. Some of these appeared to be prisms capped by pyramids; there were occasional fine needles and other forms (Fig. 9). After several weeks some of the crystals attained a length of approximately 0.5 mm.

In some of the final reaction mixtures containing the tin salt an unexpected property was observed; after remaining undisturbed for several weeks the suspensions gradually assumed an off-white or buff color, and took on the consistency of a gel. Examination under the microscope no longer revealed the original crystalline forms. This tendency toward gel formation was not observed in the mixtures obtained with other metals.

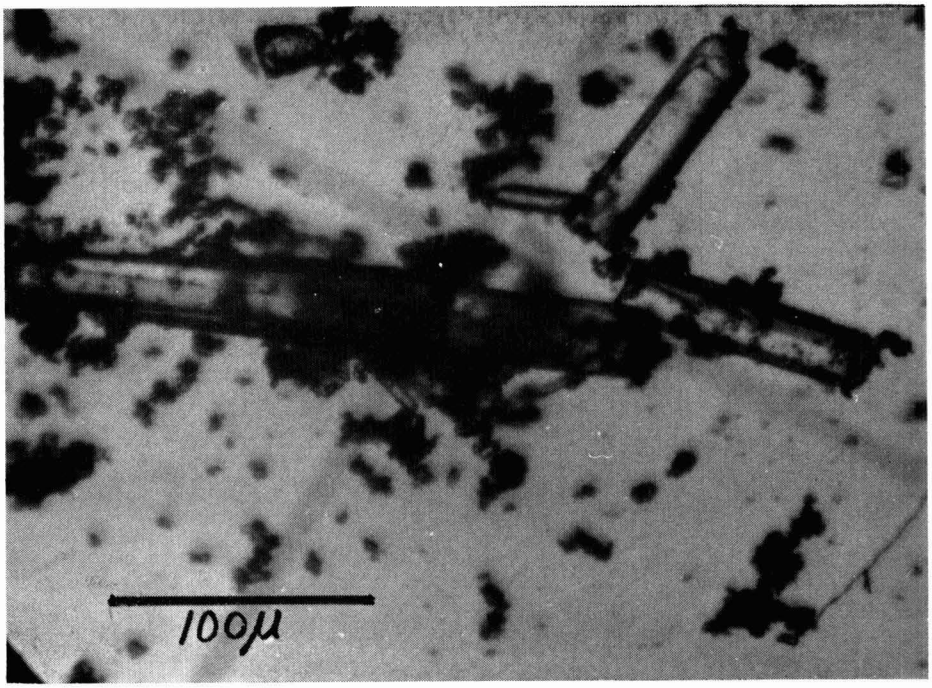


FIG. 10. Common forms of aluminum hydroxosquarate trihydrate.

Tervalent Metals

In the aluminum family the effects of squaric acid were observed on aluminum, gallium, and indium. With the first member of this group the reaction was very slow; although some samples gave rise to a few minute gas bubbles after approximately 20 min, this phenomenon was of short duration. The metal gradually lost its original luster, and after 2 days there were indications of a white coating beginning to form. After a considerable build-up of the coating, a white residue began to form on the bottom of the reaction vessel. The macro appearance of the product resembled that of chalk. According to West and Niu (*4*) the product is hydroxoaluminum squarate trihydrate, corresponding to the composition $M(C_4O_4)OH \cdot 3H_2O$, where M represents any tervalent cation such, as aluminum. The latter composition has also been confirmed by Ireland and Walton (*1*). Under the microscope the chalk-like deposit was seen to contain thin, colorless parallelepipeds, oblong rectangles, and laths, along with other forms, possibly prisms having a square cross-section (Figs. 10, 11). The larger crystals of the aluminum salt measured up to 0.4 mm in length.

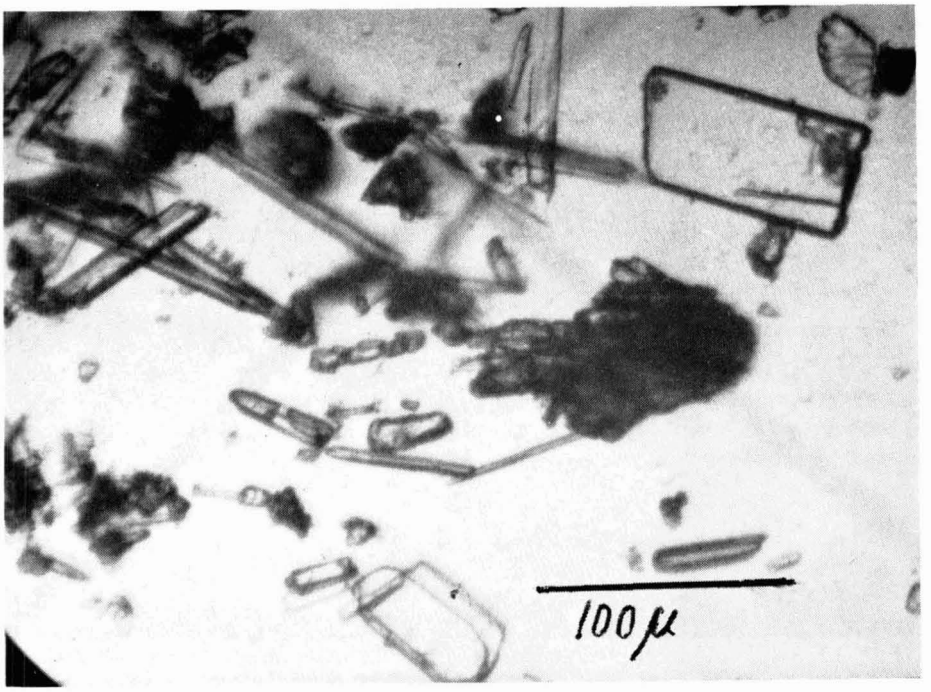


FIG. 11. Less common thin plates obtained by the action of aqueous squaric acid on aluminum.

For a long time after immersion gallium did not appear to exhibit any sign of reaction with the squaric acid; however, by introducing a short length of clean copper wire into the liquid gallium a very small quantity of white product could be observed within 12 hr. There was a considerable increase in amount during the following 24 hr. Although much of the product (probably hydroxogallium squarate trihydrate) seemed amorphous, there were also numerous crystals in the form of small laths and needles (Fig. 12). Profiles of some other forms included parallelegrams and hexagons. Generally the greatest overall dimension did not exceed 10 μ.

In some instances a strip of indium foil immersed in aqueous squaric acid acquired an accumulation of minute gas bubbles within approximately 15 min; if these bubbles were dislodged by jarring the container no sign of change could be observed on the metal surface; even after 48 hr of contact with the acid solution the metal exhibited no change in appearance. However, if immersed in contact with copper the indium surface became slightly etched within 12–16 hr. Under these conditions a small quantity of the hydroxoindium squarate could be collected

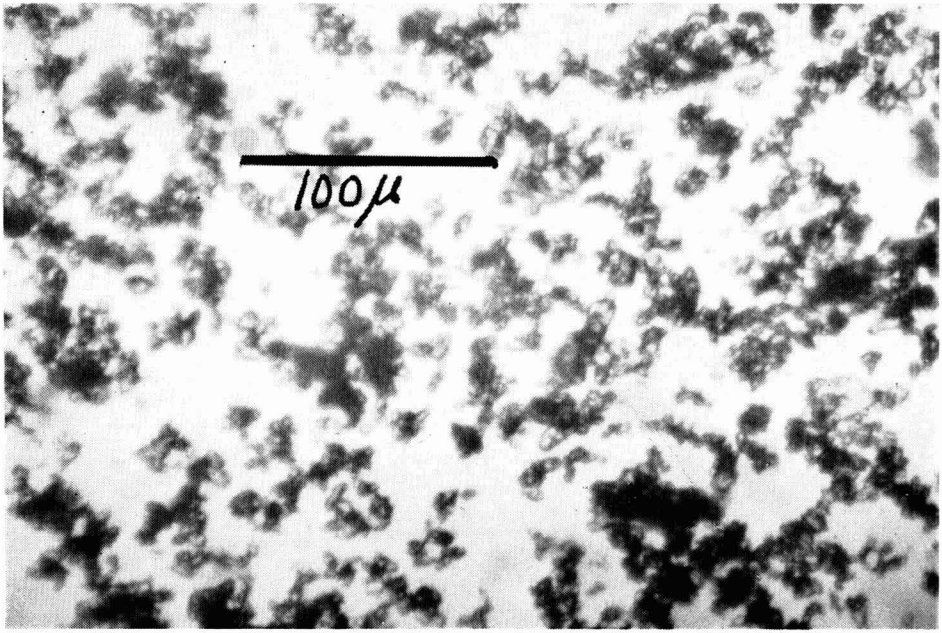


FIG. 12. Gallium hydroxosquarate trihydrate. No large forms of this salt have been observed in any of the author's preparations.

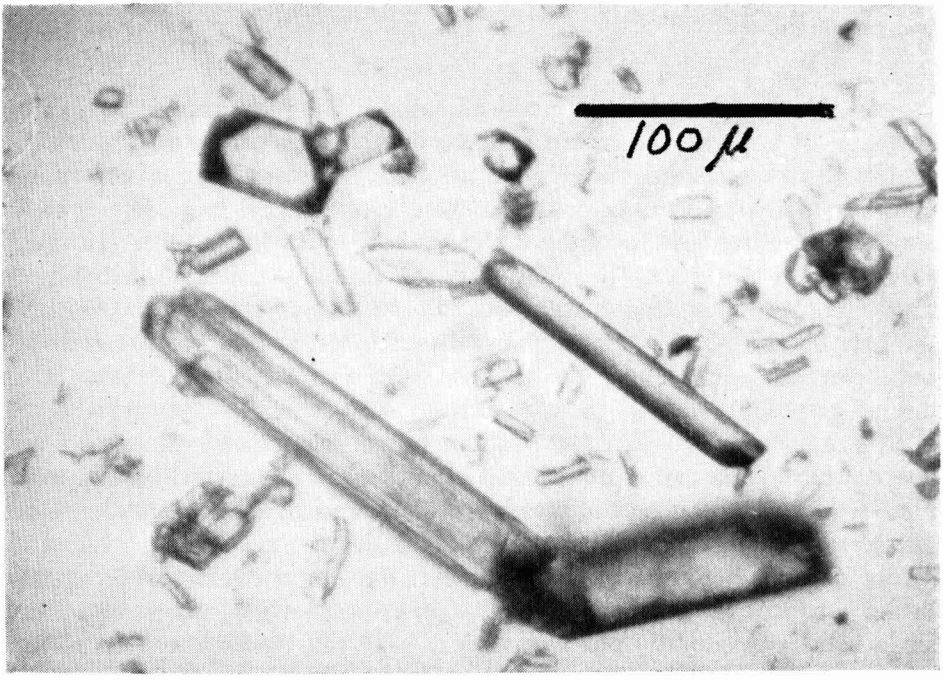


FIG. 13. Indium hydroxosquarate trihydrate.

after about 10 days. In form, the particles resembled those of the aluminum salt (Fig. 13).

SUMMARY

The metals which were exposed to the action of aqueous squaric acid formed salts of comparatively low solubility. At room temperatures the solubility of magnesium squarate dihydrate, for example, was in the 0.5% range. Of the metals investigated only magnesium and zinc could be said to react rapidly. Under the conditions imposed some of the metals, such as nickel, aluminum, and indium, required many hours, or even days, to react sufficiently to yield precipitates. The activity of certain metals could be increased by placing in intimate contact with metallic copper. Predictably, only iron, cobalt, and nickel formed colored squarates. In general the salt of a given metal appeared in several different forms. Although there was some similarity in the crystalline forms of the various squarates, there was a tendency for those of several metals, such as aluminum and lead, to be restricted to smaller dimensions than others, such as zinc and cadmium. An interesting property of the product formed by tin was that the spontaneous evaporation of part of the moisture from the reaction mixture yielded a gel, in which the original crystalline forms could no longer be detected. This property was not observed in connection with any of the other metals studied.

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MICROCHEMICAL JOURNAL 17, 456–461 (1972)

Hexosamine Content of Marine Biological Adhesives

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INTRODUCTION

Several marine organisms secrete an adhesive which permits the attachment of the creature to an undersea surface. The tenacity with which this kind of adhesive cements the organism to a substrate is well known by those who have had to remove marine fouling from a ship bottom, from pilings, or from buoys. In addition, contrary to the application requirements of most other adhesives, these marine adhesives are applied under water, to a wet surface. For these and a number of other reasons, studies to elucidate the composition and structure of several of these adhesives have been undertaken. In particular, the adhesive secreted by animals such as the mussel and the barnacle have been of interest (*2–4, 7*).

Preliminary reports on the composition of cured samples of this type of adhesive indicated the presence of a proteinaceous material, the amino acid content of which differed somewhat according to species and/or report (*3, 4, 7*). However, a marked difference appeared in these reports concerning other possible components of this kind of adhesive. Specifically, the presence or absence of one or more hexoses or hexosamines is a major point of difference. For example, it was proposed that the adhesive was a polysaccharide which did not contain any nitrogen or nitrogenous components (*2*), or at another extreme, the presence of carbohydrates could not be demonstrated with certainty (*7*). In between these extremes, the presence of one hexosamine was reported for the barnacle adhesive but none for the mussel adhesive (*3*), or the presence of more than one hexosamine was proposed (*4*).

The present report describes the experimental data confirming the presence of two hexosamines, glucosamine and galactosamine, as components of the mussel byssal thread and to offer a possible explanation for the disparities between the various reports cited.

EXPERIMENTAL METHODS

Live specimens of *Mytilus edulis* were obtained from Marine Biological Laboratory, Woods Hole, MA, and transferred to a salt water

aquarium in our laboratories. After allowing the animals to equilibrate in our laboratories, samples of byssal threads were cut from these specimens. The threads were cleaned thoroughly by abrasion with a brush, dried, and cut into suitably sized pieces.

Milligram amounts of dried byssal thread were hydrolyzed in a sealed tube in 4 *M* hydrochloric acid at 87°C. for 4 hrs. After removal of most of the remaining hydrochloric acid, a portion of the brownish residue was taken up in a minimum of distilled water and analyzed by means of a ligand exchange thin layer chromatographic procedure which was devised for the purpose of separating hexosamines (*6*). In essence, samples of hydrolysate were spotted on sheets of Mallinkrodt ChromAR 500 which had been impregnated with copper ion; the sheets were developed in an alcoholic ammonia solvent system. Spots were visualized with a Ninhydrin spray.

Other samples of hydrolysate were transferred to a column of Dowex-50 ion exchange resin and eluted with 2*M* hydrochloric acid (*1*). Aliquots of eluate fractions which were expected to contain hexosamines were chromatographed using the previously mentioned TLC procedure.

Further hydrolysate samples were analyzed by means of a TLC method which can separate amino acids from hexosamines (*5*); the latter method is not capable of separating the various hexosamines, however.

RESULTS AND DISCUSSION

Figure 1 is a photograph of a thin layer chromatogram of an aliquot of hydrolyzed mussel byssal thread. In addition to several other materials, galactosamine (spot No. 1), and glucosamine (spot No. 2), can be detected. In order to minimize possible interferences and establish the presence of these hexosamines even more firmly another aliquot of hydrolysate was subjected to a prior separtion via an ion exchange column. Eluate fractions which were expected to contain the hexosamines subsequently were chromatographed by means of the ligand exchange TLC procedure. Figure 2 shows a photograph of one of these chromatograms. The presence of galactosamine and glucosamine again can be detected (spot nos. 1 and 2, respectively) in several fractions.

Another chromatographic procedure was applied to add further confirmatory evidence for the presence of the hexosamines. A two-dimensional TLC procedure which had been developed for amino acid separations was utilized on aliquots of crude hydrolysate. This particular procedure separates the hexosamines, as a group, from the amino acids.

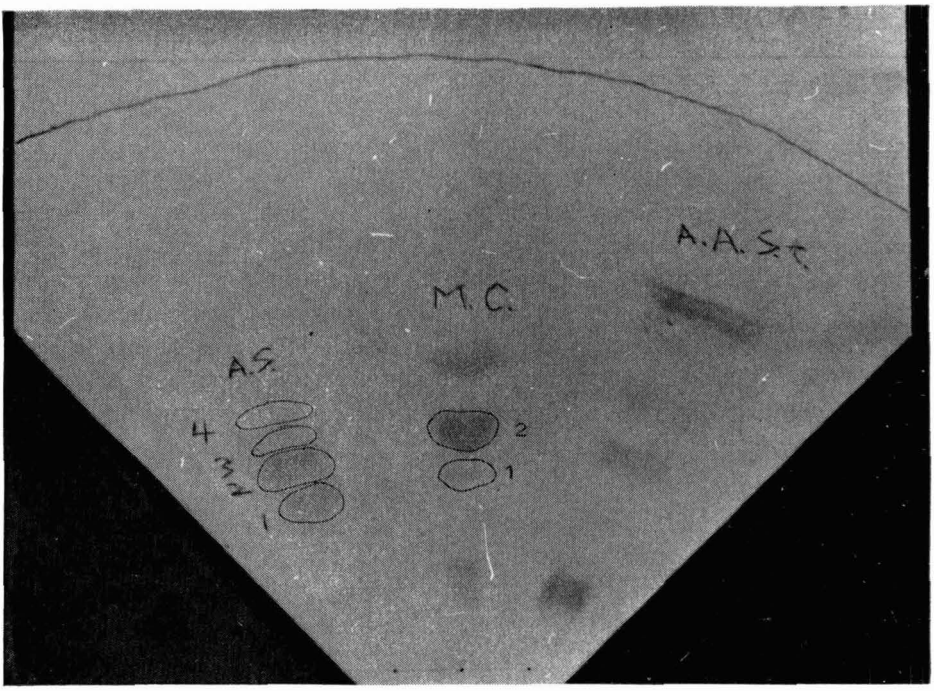

FIG. 1. Thin layer chromatogram of byssal thread hydrolysate. Spot 1 = galactosamine; Spot 2 = glucosamine.

The method, however, does not separate the different hexosamines from each other; several amino sugars appear as one spot on the chromatogram. When this technique was used to chromatograph an aliquot of byssal thread hydrolysate, a spot corresponding to the hexosamines appeared. Another chromatogram of an aliquot of hydrolysate with added known glucosamine showed the same spot slightly enlarged in diameter.

From these various and several results, we can conclude that both glucosamine and galactosamine are present as components of *Mytilus* byssal threads. We can now address ourselves to the differences between the reports cited previously.

The most logical reason for the disparities in the literature was gleaned from several unsuccessful experiments carried out in our laboratories. It was noted that when other samples of adhesive were hydrolyzed in 4 *M* hydrochloric acid for 4 hr at 92°C, only glucosamine could be detected in the hydrolysate (Fig. 3). Furthermore, when samples of byssal thread were hydrolyzed in 4 *M* hydrochloric acid for ca. 8 hr at 95–100°C, the chromatograms showed *no* hexosamine to be

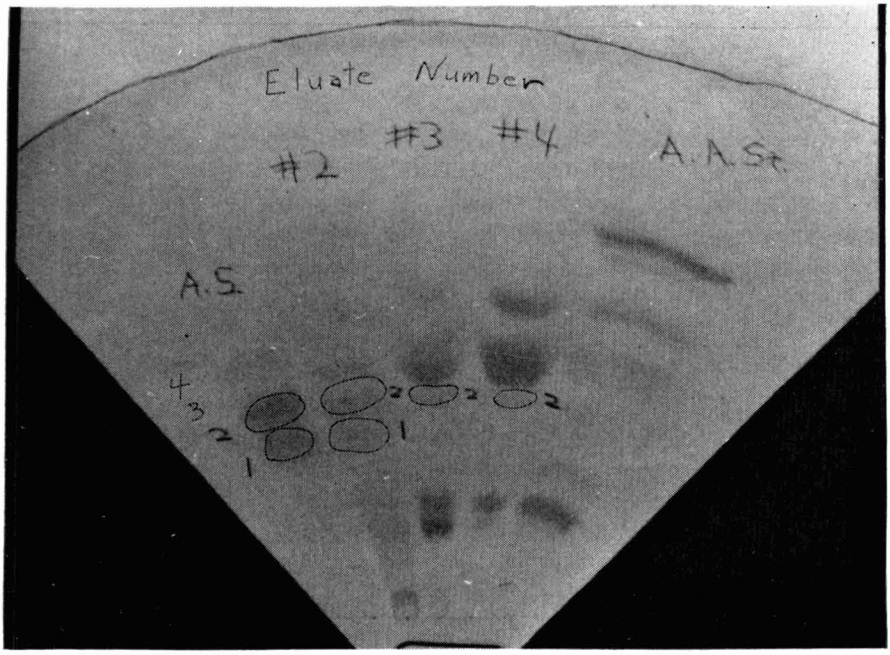

FIG. 2. Thin layer chromatogram of ionexchange eluates of byssal Thread hydrolysate. Spot 1 = galactosamine; Spot 2 = glucosamine.

present. In other words, a 5° elevation in the hydrolysis temperature could result in loss of one hexosamine while a 10° rise could destroy both hexosamines.

Behavior of the sort outlined is not totally unexpected in dealing with this class of biological material (*8*). However, in the reports cited (*2, 3, 7*), the hydrolytic conditions utilized were aimed at protein hydrolysis and ultimately amino acid content. This purpose requires rather vigorous conditions such as high temperatures and long times of reaction. From our experiments, these conditions would be most likely to degrade one or more of the hexosamines which are present. Therefore, it appears probable that the reported absence of hexosamines in these marine adhesives was caused by inadvertent degradation of the materials during hydrolysis.

SUMMARY

Mytilus edulis byssal threads have been shown to contain both glucosamine and galactosamine. Inadvertent degradation of the material probably is the reason for the reported absence of these hexosamines from the *Mytilus* adhesive; evidence for this explanation has been found.

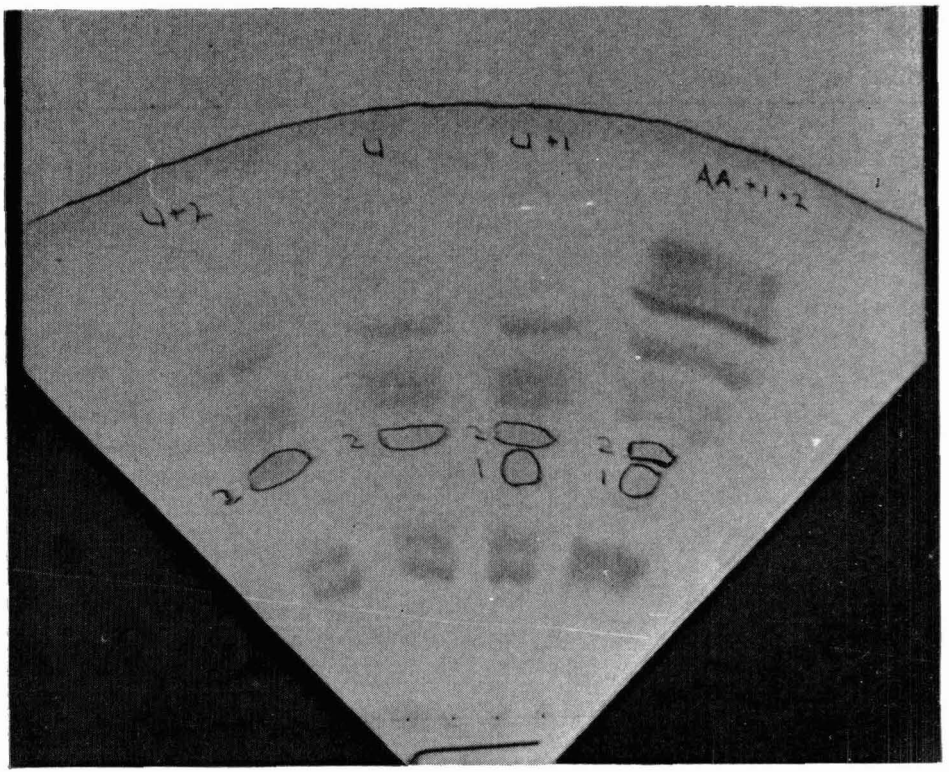

FIG. 3. Thin layer chromatogram of byssal thread hydrolyzed at elevated temperature. Spot 1 = galactosamine; Spot 2 = glucosamine.

ACKNOWLEDGMENT

The authors thank the National Institute of Dental Research for support through contract PH-43-67-1172.

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MICROCHEMICAL JOURNAL 17, 462–467 (1972)

Direct Titrimetric Microdetermination of L-Aspartic and L-Glutamic Acids Separately

II. Determination of These Amino Acids in The Form of a Mixture Without Separating

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INTRODUCTION

Available literature concerning the determination of L-aspartic and L-glutamic acids fails to show any direct and simple method, which could be used in determining these amino acids separately or in a mixture of these two. However, L-glutamic and L-aspartic acids are determined manometrically (*4*); by iodometric titrations of their copper salts (*12*); by oxidative bromination (*13*); enzymically (*7, 8*); chromatographically (*1, 3*); potentiometrically (*14*); colorimetrically (*6, 11*); by the action of periodate (*5*); by using Conway's method (*2*); separating and estimating 24-dinitrophenyl derivative acid (*9*); microbiologically (*15*); and by using densitometer for deciphering electrophoregram (*16*).

Most of the methods mentioned above are lengthy, difficult, time consuming, and in certain cases are either difficult to separate traces or quite impossible. Keeping in mind the difficulties encountered and elaborate procedures it was thought necessary to develop a simple and easy method for the determination of these two amino acids separately, as well as in the presence of each other without separating.

The present work deals with the determination of L-glutamic and L-aspartic acids in micro amounts by direct titration separately and in a mixture. L-glutamic acid has been determined, along, by directly titration against sodium tungstate solution using catechol violet, bromocresol green or chromazural red S as indicator. L-Aspartic acid is also determined by direct titration against neodymium trichloride solution using xylenol orange or alizarin red S as indicator. In a mixture of these two amino acids, L-glutamic acid is estimated first by titration against sodium tungstate solution using catechol violet as indicator; and then, in the same solution mixture, L-aspartic acid is titrated against

neodymium trichloride solution using xylenol orange as indicator. Xylenol orange is added after L-glutamic acid has been titrated.

EXPERIMENTAL METHODS

Reagents used

L-Glutamic acid and sodium tungstate (E. Merck grade); L-aspartic acid, neodymium trichloride, catechol violet, bromocresol green, chromazural red S, xylenol orange, and alizarin red S (B.D.H. grade).

Standard solutions of L-glutamic acid, L-aspartic acid, and sodium tungstate were prepared by dissolving the exactly weighed amounts in distilled water. Neodymium trichloride solution was standardized by complexing with disodium salt of rhodizonic acid (*10*). Indicator solutions were prepared by dissolving in distilled water.

Procedure

I. Determinations of L-*glutamic and* L-*aspartic acids separately.* By means of a micropipette (0.01 ml least count) a known volume of L-glutamic acid solution is placed in a beaker and diluted to 30 ml by adding distilled water. A few drops (2–3) of indicator—catechol violet solution, is added to the test solution and then a standard solution of sodium tungstate is run in from a microburette (with least count = 0.01 ml), at the end point the light yellow color changes sharply to ash color. In the case of chromazural red S the end point is marked by a sharp change in color from pink to orange rose. While with bromocresol green the change at the end point is from the color of oil of winter green to sky blue.

In the case of L-aspartic acid, a known volume of its standard solution is placed in a beaker through a micropipette, diluted to about 30 ml, and then a few drops (2 to 3) of xylenol orange are added, and the whole solution in the beaker becomes a light yellow color. From a microburette a standard neodymium trichloride solution is run into the beaker containing yellow colored solution, at the end point a sharp change in color takes place from light yellow to pink rose.

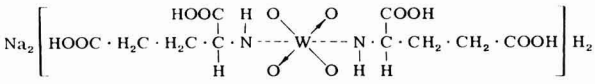
II. Determination in presence of each other. Known volumes of standard L-glutamic and L-aspartic acids are placed in a beaker and diluted with distilled water to 30 ml, which forms a solution mixture of two amino acids. First, a few drops of catechol violet are added to the mixture of the two amino acids, and the L-glutamic acid is first titrated against a standard sodium tungstate solution, where at the end point an ash color is observed. Then, in the same solution, a few drops of xylenol orange are added and L-aspartic acid is directly titrated against

a standard neodymium trichloride solution. At the end point the light yellow color sharply changed to pink rose.

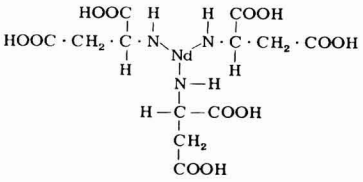
RESULTS AND DISCUSSION

Results are shown in Tables 1, 2, and 3. Ranges in which L-aspartic and L-glutamic acids are estimated vary from 6.7087×10^{-4} to 53.6696×10^{-4} mg/liter; and from 2.97×10^{-4} to 23.776×10^{-4} mg/liter, respectively. Tables 1 and 2 show the maximum errors in the estimations of L-aspartic and L-glutamic acids 0.8 and 0.9%, respectively. In Table 3 the two amino acids have been shown to be titrated together in one solution without separating. In a mixture, L-glutamic acid has been first titrated in the descending order from the top using catechol violet as indicator. Now, in the same solution xylenol orange indicator was added and L-aspartic acid was titrated against neodymium trichloride solution.

Present results, potentiometric titrations, and results of analysis show that in the reaction between sodium tungstate and L-glutamic acid a complex



is formed in the ratio of 1 : 2 at pH 5.2. In the reaction between neodymium chloride and L-aspartic acid a complex



is formed in the ratio of 1 : 3 at pH 3.7. Probably the following reactions take place:

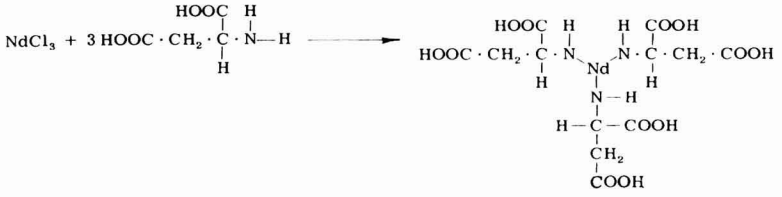


TABLE 1

MICRODETERMINATION OF L-ASPARTIC ACID SEPARATELY

L-Aspartic acid 0.05 *M* (ml)	$NdCl_3$ 0.024 *M* (ml)	Amount of L-aspartic acid ($\times 10^4$ mg/liter) Taken	Found	Error (%)
0.1	0.07	6.6556	6.7087	
0.3	0.21	19.9655	20.2161	
0.4	0.28	26.6220	26.8348	0.8
0.7	0.49	46.5885	46.9606	
0.8	0.56	53.4440	53.6696	

Calculations were made by multiplying the observed values by 2 and 3 in the cases of L-glutamic and L-aspartic acids, respectively. It was observed that 0.5 ml of 0.02 *M* L-asparagine, L-arginine, DL-valine and -alanine, glycine, L-leucine and isoleucine solutions do not interfere,

TABLE 2

MICRODETERMINATION OF L-GLUTAMIC ACID SEPARATELY

L-glutamic acid 0.02 *M* (ml)	Na_2WO_4 0.0101 *M* (ml)	Amount of L-glutamic acid ($\times 10^4$ mg/liter) Taken	Found	Error (%)
0.1	0.1	2.9426	2.970	
0.2	0.2	5.8852	5.944	
0.3	0.3	8.8278	8.916	0.9
0.4	0.4	11.7704	11.888	
0.6	0.6	17.6556	17.832	
0.8	0.8	23.5408	23.776	

neither in separate determinations of L-glutamic acid and L-aspartic acid, nor when these two amino acids are titrated together in a mixture. This method is direct, simple, less time consuming, and better than other methods described in the literature.

ACKNOWLEDGMENT

The author is grateful to the Council of Scientific and Industrial Research (Government of India) New Delhi for providing financial assistance.

SUMMARY

L-Glutamic and L-aspartic acids are quantitatively determined in micro amounts separately and in a mixture against sodium tungstate and neodymium trichloride, respectively. In a mixture, L-glutamic acid is first titrated and in the same solution,

TABLE 3

MICRODETERMINATION OF L-GLUTAMIC AND L-ASPARTIC ACIDS IN THE PRESENCE OF EACH OTHER WITHOUT SEPARATING

L-Glutamic acid 0.02 *M* (ml)	Na_2WO_4 0.0101 *M* (ml)	Amount of L-Glutamic acid ($\times 10^{-1}$ mg/liter) Taken	Found	L-Aspartic acid 0.05 *M* (ml)	$NdCl_3$ 0.024 *M* (ml)	Amount of L-Aspartic acid ($\times 10^{-1}$ mg/liter) Taken	Found
0.6	0.6	17.6556	17.832	0.1	0.07	6.6555	6.7087
0.4	0.4	11.7704	11.888	0.2	0.14	13.3110	13.6140
0.3	0.3	8.8278	8.916	0.3	0.21	19.9665	20.1261
0.2	0.2	5.8852	5.944	0.4	0.28	26.6220	26.8348
0.1	0.1	2.9426	2.972	0.5	0.35	33.2775	33.5435

without separating, L-aspartic acid is estimated. Complexes between L-glutamic acid and sodium tungstate, and between neodymium trichloride and L-aspartic acid are formed in the ratios of 1 : 2 and 1 : 3 at pH 5.2 and 3.7, respectively. Certain amino acids do not interfere, neither separately nor in a mixture of these two amino acids.

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MICROCHEMICAL JOURNAL 17, 468–469 (1972)

Use of Sodium Citrate as a New Titrant for the Microdetermination of Sulfanilic Acid

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Received February 1, 1970

Carboxylic acids are generally determined by titration with standard alkali in aqueous medium. In the present paper, a new method is described for the determination of sulfanilic acid in microchemical quantities, in which it is titrated with a standard sodium citrate (*1*) solution, using bromocresol purple as indicator. The end point is indicated, when the color of the solution changes from yellow to faint purple. In the earlier publications (*2, 3*) from this laboratory two new methods were suggested for the determination of this acid by titration with microcosmic salt and guanidine carbonate solutions.

EXPERIMENTAL METHODS

Reagents used. Sulfanilic acid (A.R. Italy), sodium citrate (E. Merck), and bromocresol purple (B.D.H.N.).

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 a 0.1% solution of bromocresol purple indicator. The solution is yellow at this point. Then titrate it with a standard sodium citrate solution till the yellow color is completely discharged and the solution becomes a faint purple.

RESULT

The results are given in Table 1; sulfanilic acid was estimated over a range of 0.096–0.574 mg. The results are concordant and precise and agree well with methods suggested earlier.

SUMMARY

Sulfanilic acid was determined in microquantities with a new titrant, i.e., sodium citrate solution using bromocresol purple as indicator. Estimation were carried out in the range of 0.096–0.574 mg with a maximum error of ±0.004 mg.

TABLE 1

MICRODETERMINATION OF SULFANILIC ACID

Sample no.	Vol of soln (ml)		Sulfanilic acid		
	0.001 *M* sulfanilic acid taken	0.001 *M* sodium citrate used	Found	Theoretical value	Error (mg)
1	3.00	3.00	0.574	0.574	0.000
2	2.00	1.98	0.379	0.382	0.003
3	1.00	1.02	0.195	0.191	0.004
4	0.50	0.50	0.096	0.096	0.000

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 17, 470–475 (1972)

A Technique for the Qualitative Spectrographic Analysis of Very Small Samples [1]

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INTRODUCTION

Atomic emission spectrography is frequently used in this laboratory as a convenient method for the qualitative analysis of a large number of elements in a wide range of materials. The sample size required is fairly small: trace elements can usually be detected in 10–20 mg of sample and major elements in less than 1 mg. If sufficient sample is available the normal practice is to burn separate portions in a dc arc between both carbon and copper electrodes. In this way, lines appearing in the region of the spectrum obscured by the cyanogen and Swan bands, formed by the carbon arc in air, can be sought in the copper electrode spectrum.

Problems have arisen in which the sample has been too small to give satisfactory spectra by the above technique. In other cases, analyses of discrete portions of samples would have been desirable: a knowledge of the compositions of individual particles in a sample can often provide considerably more information than an "average" analysis of the whole. To meet such needs a technique was sought for the qualitative spectrographic analysis of very small samples.

Evans and Waller (*2*) fixed small forensic samples to pointed graphite electrodes with "Durofix" (a nitrocellulose based solvent-loss adhesive). Harvey (*3*) used cupped electrodes and diluted the sample with powdered graphite or ammonium chloride. To increase the light-gathering power of the spectrograph he used a condensing system of a spherical mirror and a compound lens. Our technique combined the pointed electrode configuration with simple condensing optics. An argon sheath was used to reduce background emission and the arc was struck by bridging the gap with carbon fibers. In this way a simple

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method for the qualitative spectrographic analysis of very small samples was made available without resorting to expensive apparatus or difficult manipulation. A Hilger medium quartz spectrograph was used for all the work described. A current of 7–8 A for graphite and 2–3 A for iron, at 210 V dc, was passed in the arc.

USE OF POINTED GRAPHITE ELECTRODES

When using pointed graphite electrodes, Evans and Waller fixed the sample in a small hole at the point with Durofix. As an alternative they fixed cotton wool in a small step at the point and used the electrode as a "brush," with or without a solvent, to collect samples. They applied these methods to a wide range of forensic samples with a minimal mass of 25–30 μg.

Brush electrodes, made from 6.5 mm diameter graphite rods with a 20° point, have been used in this laboratory to obtain spectra of small samples of paint, soot, and inorganic residue from the combustion of carbon fibers. The brush electrode was placed in the lower position as cathode and a similar pointed electrode, with no cotton wool, in the upper position as anode. With a gap of 2 mm between the electrodes, a 7–10 sec exposure was made. Blank brush electrode and iron spectra were recorded contiguously above and below the sample spectrum, using the Hartmann diaphragm.

No detectable impurities were introduced into the spectra by either the Durofix or the cotton wool. Care must however be taken to avoid introducing wear particles produced by the steel pin in the nozzle of the Durofix tube.

ATTENUATION OF CYANOGEN BANDS

Cyanogen band emission can be reduced considerably by enclosing the arc in an atmosphere of argon or argon + oxygen. For quantitative work on larger samples, Shaw, Wickremasinghe and Yip (*4*) used a Stallwood air jet (*6*) to pass an argon–oxygen mixture around the electrodes. Dennen and Blackburn (*1*) designed a very simple substitute for the expensive Stallwood jet which they used to pass various gases.

A jet device of the Dennen and Blackburn type was made in stainless steel and a 2.5 cm diameter silica cylinder used to enclose the arc. To prevent entrainment of air, the lower edge of the cylinder was ground to a fine finish and greased. The absorption spectrum of the cylinder showed that about 35% of the arc emission would be absorbed at 210 nm, falling through about 10% at 300 nm to about 5% at 500 nm.

Argon was found to attenuate the background emission more efficiently than an argon–oxygen mixture and, as addition of oxygen should not be necessary to promote burning of samples of the size envisaged, the use of pure argon was adopted. Air was flushed out of the cylinder by passing argon at 5 l/min for at least 30 sec, and the flow rate was then reduced to 2 l/min during the arcing period to minimize dislodgement of the sample before burning was complete. The argon atmosphere used in this way was found to be satisfactory both for the usual scale of working and for the smaller scale.

IMPROVEMENT OF SENSITIVITY

No form of external condensing optics is usually employed for our qualitative work with the Hilger medium quartz spectrograph. Harvey (*3*) used a spectrograph with a condensing lens as standard, but improved the sensitivity by using a compound lens of shorter focal length and a spherical mirror at a distance equal to its radius of curvature behind the electrodes.

To increase the light-gathering power of the spectrograph for the present work, a single lens of 5 cm focal length was placed at that distance in front of the electrodes, ie between the electrodes and the slit, and the back half of the silica sheath was silvered on the outside, forming a cylindrical mirror at a distance equal to its radius of curvature from the electrodes. With this arrangement the sensitivity was improved by a factor of about 20 without introducing any noticeable aberrations.

IGNITION OF ARC

With the greatly increased sensitivity, it was found that very short exposure times could be used to reduce background. This meant that conventional techniques for striking the arc were unsuitable. Various circuits for igniting the arc with a high-frequency discharge have been described, e.g., by Sinclair (*5*), but these are complicated and expensive. The same effect was achieved by bridging the arc gap with a small yarn of carbon fibers (around 20–50 fibers, 1 cm long, lying closely side by side). The procedure is simple and rapid: a small blob of Durofix or other suitable adhesive is picked up on one side of the point of the lower electrode and the sample is picked up on it. While the adhesive is still tacky, one end of the carbon fiber yarn is also picked up and the adhesive is allowed to harden. The electrode is then placed in the recess in the jet device and the silica enclosure and stainless steel lid are fitted. The longer upper electrode is clamped into position so that the carbon fibers lie against the side of its pointed surface (see Fig. 1).

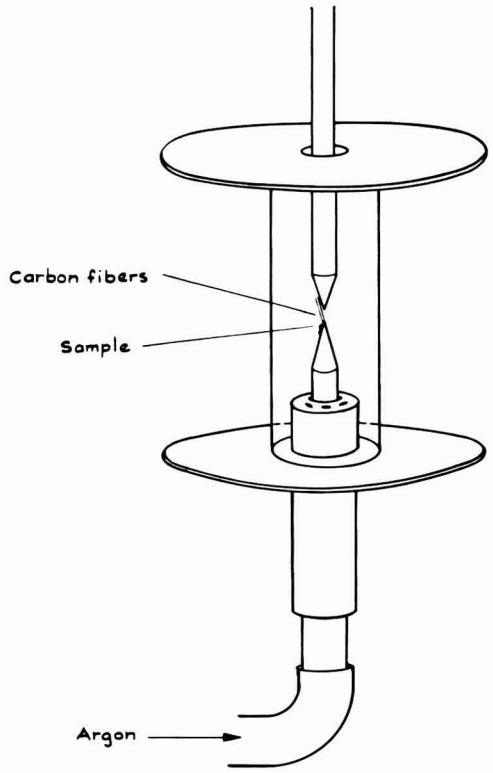

FIG. 1. Electrodes with carbon fiber yarn in enclosed jet device.

The arc gap is set to 2 mm. When the current is switched on, the arc strikes immediately. Experiments using a camera shutter in front of the slit showed that, if proper synchronization could be achieved, exposure times as short as 0.01 sec would probably be sufficient to record volatilization and excitation of the complete sample. It was found adequate, however, to open the shutter before striking the arc and close it an estimated 0.5 sec after striking. Even under these conditions the Durofix and carbon fibers added no extra lines to the spectra, nor did they cause extra cyanogen emission, despite the fact that Durofix contains nitrogen.

RESULTS

A number of spectrographically pure metal compounds were arced using the above small-scale technique and in most cases 1 μg of the metal was detected with ease. Weighing smaller samples than this was impracticable. In any case, the limit of detection varies considerably

with the matrix, so no quantitative assessment of the method has been attempted. An experiment carried out with RU powder (Johnson, Matthey and Co. Ltd, London, England) does, however, give some indication of the capabilities of the technique. Portions of the powder, weighing 90, 20, 10 and 1 μg, were arced by the method described. The elements detected in each portion are shown in Table 1.

TABLE 1

ELEMENTS DETECTED IN RU POWDER

	Sample wt (μg)			
Element	90	20	10	1
Zinc	+	+	+	+
Magnesium	+	+	+	+
Calcium	+	+	+	+
Iron	+	+	+	+
Boron	+	+	+	+
Silicon	+	+	+	
Arsenic	+	+		
Beryllium	+	+		
Lead	+	+		
Tin	+	+		
Copper	+	+		
Silver	+	+		
Phosphorus	+			
Antimony	+			
Bismuth	+			
Molybdenum	+			

In the table, detected (+) means that at least two persistent lines were observed. The zinc, magensium and calcium, as oxides, form the powder base. Another 50 elements are present in trace amounts: about seven persistent lines are said to appear for each element when 10–20 mg of RU powder is burned in the dc arc and the spectrum recorded on a Hilger large quartz spectrograph.

DISCUSSION

The technique described provides simple and rapid qualitative spectrographic analyses of very small samples. The Hilger medium quartz spectrograph is suitable without expensive accessories.

No quantitative estimate of the proportions or absolute amounts of elements in a sample should be attempted using the technique described: when spectra of samples containing 1 and 10 μg of various metals were recorded it was sometimes found that the 1 μg sample gave the more

intense lines. This may have been caused by detachment of the sample from the electrode before volatilization was complete. In all cases, however, sufficient spectral lines were recorded to identify the metals. When semiquantitative information or exact comparison between samples are required, the graphite powder pellet method described by Harvey (*3*) would probably be more suitable.

The present technique should be particularly useful in conjunction with microscopical examination. Complete identification of many small inorganic samples should be possible by relating optical and other physical properties to elemental composition. A spectrographic technique would be very much simpler than microchemical tests for this purpose.

SUMMARY

The sample and a small yarn of carbon fibers are stuck on to a pointed graphite electrode; the gap between this and the upper electrode is bridged by the carbon fibers which strike the arc when the dc source is switched on. Background emission is greatly reduced by enclosing the arc in an argon atmosphere. Simple condensing optics are employed with a Hilger medium quartz spectrograph. Many elements can be detected with ease in 1 μg amounts and five elements were detected in 1 μg samples of RU powder.

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Spectrophotometric Determination of Some Aromatic Nitro Compounds in Microgram Quantities

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Specific methods (*1–5*) for the determination of some individual nitro compounds have been reported in the literature. In the present work a general method, based on the observations of Nystrom and Brown (*6*), for the determination of aromatic nitro compounds has been described. Nystrom and Brown observed that when lithium aluminum hydride is added to aromatic nitro compound at room temperature, a colored azo compound is immediately formed according to the following:

$$2ArNO_2 + 2LiAlH_4 \rightarrow Ar{-}N{=}N{-}Ar + 2LiAlO_2 + 4H_2.$$

Gilman and Goreau (*7*) described a general qualitative test based on the above reaction for aromatic nitro compounds. The aromatic nitro compound was reduced with an excess of lithium aluminum hydride in the medium of tetrahydrofuran. The excess of lithium aluminum hydride was decomposed with a small amount of water and the hydroxides thus formed were dissolved in sulfuric acid. The clear solution which contains the reduction product was found to be colored. The spectrum of the colored solution of reduction products of *m*-dinitrobenzene, *sym*-trinitrobeneze, *m*-nitrophenol and 1-chloro-2,4-dinitrobenzene were examined in the visible region against a reagent blank and 355, 375, 360, and 350 mμ, respectively, were found to be the wavelength of maximum absorbance. It was also observed that solutions containing the reduction product obeyed Beer's law for the dilute solutions in the range of 100–600 μg. The proposed method has been used for the determination of aromatic nitro compounds in microgram quantities with precision and accuracy of $\pm 2.5\%$.

MATERIALS AND METHODS

Reagents

Sample solutions. Stock solutions of samples were prepared by dissolving exactly weighed 10 mg of compound (M.A.R. Hopkins and

Williams Ltd., England) in anhydrous tetrahydrofuran and solutions were made up to 100 ml. Different aliquots of these solutions were used.

Sulfuric acid. Fifty per cent sulfuric acid (A.R. B.D.H.)

Lithium aluminum hydride. About 50 mg of lithium aluminum hydride (E. Merck) were employed for each reduction.

Anhydrous tetrahydrofuran. Tetrahydrofuran (E. Merck) was treated with sodium wire and was kept overnight in a flask fitted with calcium chloride tube. Tetrahydrofuran was filtered and distilled. The sodium treatment was repeated again and distilled. This tetrahydrofuran was kept over lithium aluminum hydride to remove the last traces of moisture; distilled; and collected in a dry container.

Apparatus

Absorbance measurements were done with a Beckman model DU spectrophotometer; and Beckman 1.005-cm silica cells were used throughout these studies.

Procedure

A 4-ml aliquot from the stock solution and 5 ml of anhydrous tetrahydrofuran were placed in a 100-ml stoppered conical flask and about 50 mg of solid lithium aluminum hydride were added to it. Immediately a colored slurry was formed which was swirled and allowed to stand for 15 minutes. The excess lithium aluminum hydride was decomposed with 5 ml of distilled water. The hydroxides thus formed were dissolved in 3 ml of 50% sulfuric acid; and contents of the conical flask were quantitatively transferred to a 25-ml volumetric flask and volume was made up to the mark with distilled water. The absorbance of the solution was measured against a reagent blank in the region of 320–400 mμ.

Preparation of calibration curve

1-, 2-, 3-, 4-, 5-, 6-, and 7-ml aliquots from the stock solution were delivered separately in 100-ml stoppered flasks. Five ml of anhydrous tetrahydrofuran and about 50 mg of lithium aluminum hydride were added to each flask and the slurry was allowed to stand for 15 minutes. The excess of lithium aluminum hydride was decomposed with 5 ml of distilled water. The hydroxides were dissolved in 3 ml of 50% sulfuric acid. The contents of the flasks were transferred to 25-ml volumetric flasks and volume was made up to the mark. The absorbance of each solution was measured at a specified wavelength. The absorbance values were plotted against concentration of the sample.

RESULTS AND DISCUSSION

The proposed procedure has been applied for the determination of *m*-dinitrobenzene, *sym*-trinitrobenzene, *m*-nitrophenol, and 1-chloro-2,4-dinitrobenzene. The results are summarized in Tables 1, 2, 3, and 4.

TABLE 1

DETERMINATION OF *m*-DINITROBENZENE BY MEASURING THE EXTINCTION OF REDUCTION PRODUCT AT 355 mμ

	Sample (μg)		
Sample	Added	Found	Error (%)
1	120	123	+2.50
2	200	195	−2.50
3	280	276	−1.42
4	330	322	−2.42
5	440	446	+1.36

The reaction of lithium aluminum hydride is specific with aromatic nitro compounds, therefore, other reducible groups present in the compound do not interfere in determination.

ACKNOWLEDGMENT

One of us (U.C.P.) thanks the Council of Scientific and Industrial Research of India for the award of research fellowship.

TABLE 2

DETERMINATION OF *sym*-TRINITROBENZENE BY MEASURING THE EXTINCTION OF REDUCTION PRODUCT AT 375 mμ

	Sample (μg)		
Sample	Added	Found	Error (%)
1	123	125	+1.62
2	197	195	−1.01
3	276	270	−2.17
4	324	320	−1.23
5	448	450	+0.44

TABLE 3

DETERMINATION OF *m*-NITROPHENOL BY MEASURING THE EXTINCTION OF REDUCTION PRODUCT AT 360 mμ

	Sample (μg)		
Sample	Added	Found	Error (%)
1	135	133	−1.48
2	225	222	−1.33
3	315	319	+1.27
4	405	408	+0.74
5	585	589	+0.68

TABLE 4

DETERMINATION OF 1-CHLORO-2,4-DINITROBENZENE BY MEASURING THE EXTINCTION OF REDUCTION PRODUCT AT 350 mμ

	Sample (μg)		
Sample	Added	Found	Error (%)
1	122	124	+1.64
2	198	194	−2.02
3	360	360	—
4	380	386	+1.57
5	520	516	−0.58

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Macro- and Microdetermination of Arsenic(III) Using Potassium Permanganate as an Oxidant in Acid Medium in Presence of Fluoride Ions

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INTRODUCTION

The reaction between potassium permaganate and As_2O_3 in acid medium is usually beset with difficulties. Although Trautman (*1*) inferred that in H_2SO_4, Mn(II) is produced as the reduction product of $KMnO_4$, Willard *et al.* (*2*) observed that this was only the case during the early stages of the reaction, but later on there is the possiblity of formation of highly colored complex manganic arsenate which interferes with the accurate determination of the end point. According to Crimins *et al.* (*3*), the reaction gives various products, but proceeds normally and quantitatively in presence of osmic acid (*4*), KIO_3, HCl, or chlorides and less accurately in presence of KI (*3*). Accurate results are also obtained by adding Fe(II) after the first complex reaction with $KMnO_4$ and then completing the titration. Lang's method using iodate appears to be one of the most effective procedures (*5*). Several procedures using MnO_4^- or MnO_4^{2-} in alkaline solutions have also been developed (*6,7*).

In the present investigation, oxidation of As(III) with $KMnO_4$ in presence of fluoride ions is studied in order to overcome the difficulties which arise during the later stages of the titration (*8*). The reaction is liable to proceed quantitatively in accordance with the following equation:

$$Mn^{7+} + 2As^{3+} \rightleftharpoons Mn^{3+} + 2As^{5+},$$
$$MnO_4^- + 2AsO_3^{3-} + 2H^+ + 2HF_2^- \rightleftharpoons MnF_4^- + 2AsO_4^{3-} + 2H_2O,$$

since the equilibrium constant K and the degree of completion α amount to 2.63×10^{-65} and 6.35×10^{-10}, respectively. These were calculated using redox potential values amounting to 0.61 and 1.575 V for the As^{5+}/As^{3+} and Mn^{7+}/Mn^{3+} systems, respectively.

EXPERIMENTAL METHODS

Solutions

The potassium permanganate solutions were prepared by a method similar to that of Stamm (*9*) and standardized with sodium oxalate (*10*). Sodium arsenite solutions were prepared by dissolving the approximate amount of A.R. sodium arsenite in twice distilled water and standardized by the bromate method (*11*). Dilute solutions of sodium arsenite and potassium permangante were prepared by quantitative successive dilution with twice distilled water. Other solutions including 2% NaF, ~9 *N* H_2SO_4 were prepared from the C.P. products in twice-distilled water.

Electrical Equipment

The emf of the titration cell was measured by a millivoltmeter of the Radiometer type (Model PHM 28 b). The cell used consisted of the titration half cell and the calomel as the reference electrode. A platinum rod was used as indicator electrode which was always abraded with emery paper, etched with aqua regia and rinsed with water before using it in each titration.

RESULTS AND DISCUSSION

I. Macrodetermination of Arsenic(III)

i. The effect of acidity. Table 1, shows that good end points are obtained at acidities ranging from 0.9–3.6 *N* H_2SO_4 in presence of 50 ml of 2% NaF both in the visual and the potentiometric titrations. At lower acidities, e.g., 0.45 *N* or less, the end point is attained later due to partial production of the Mn(IV). The titration curves as shown in Fig. 1, are more or less smooth and characterized by sharp inflections at the end points.

The values of the formal redox potential depicted from the titration curves indicate that these values increase with rise of acidity and hence hydrogen ions must be involved in the reaction of trivalent arsenic with permanganate.

Acidity, *N* H_2SO_4	0.45	0.90	1.35	2.70	3.60
Redox potential of As^{5+}/As^{3+} system (V)	0.88	0.92	0.925	0.93	0.96

ii. The effect of fluoride ion concentration. Good end points are obtained in presence of 25 ml of 2% NaF. At higher concentrations of NaF, the end points coincide with the theoretical values within the limits of experimental errors as shown by the data in Table 2.

TABLE 1

EFFECT OF ACIDITY

Acidity N H_2SO_4	Vol of $KMnO_4$ consumed (ml)	Theoretical end point (ml)	Error (%; ±)	Inflection at end point (mV/0.1 ml of titrant)
Potentiometric titration [a]				
0.45	8.40	8.30	1.21	159
0.90	8.27	8.30	0.36	251
1.35	8.37	8.30	0.84	267
1.80	8.38	8.30	0.96	224
2.70	8.37	8.30	0.84	209
3.60	8.30	8.30	Nil	219
Visual titration [b]				
0.68	11.56	11.37	1.63	
0.90	11.43	11.37	0.53	
1.80	11.36	11.37	0.09	
2.70	11.34	11.37	0.26	

[a] 8 ml 0.08225 N As^{3+} titrated with 0.0991 N $KMnO_4$ in presence of 50 ml of 2% NaF. The solution was completed to 100 ml with twice-distilled water.

[b] 10 ml 0.1026 N As^{3+} titrated with 0.1130 N $KMnO_4$ in presence of 50 ml of 2% NaF, 5 ml of 0.25 M $CuSO_4$, and the solution was completed to 100 ml with twice-distilled water.

The redox potential values shown below as depicted from the titration curves also confirm the change of the values with the acidity of the solution since the latter is affected by the change in the NaF concentration.

Volume of 2% NaF	10	25	50	75
Redox potential of As^{5+}/As^{3+} system (V)	1.04	1.00	0.97	0.95

iii. The effect of $CuSO_4$. As shown in Table 3, and the titration curves in Fig. 1A, D, the inflection at the end point is not affected by the addition of $CuSO_4$ to the reaction medium. The titration curves possess the same character as in the absence of $CuSO_4$. In the visual titration the role played by $CuSO_4$ is to render possible the observation of the end point especially in the titration of relatively concentrated solutions. This is because the blue color of Cu^{2+} ions conceals the pink color of MnF_4^- complex which vitiates the end point. Although the potentiometric method overcomes this difficulty in the titration of higher concentrations of As(III), yet the reaction becomes sluggish when dilute solutions are titrated. In this case Cu^{2+} ions appear to catalyze the reac-

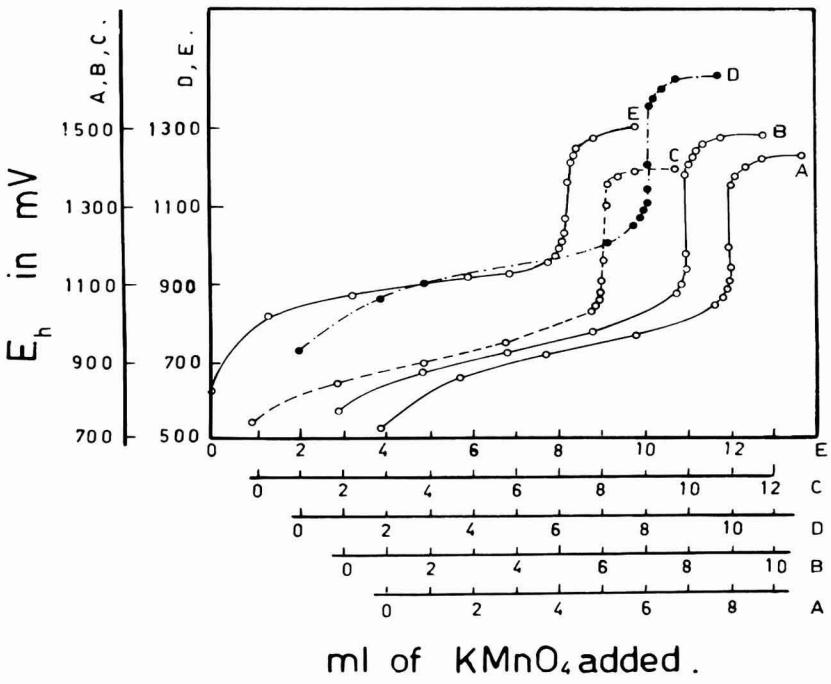

FIG. 1. Titration of 8 ml of 0.0823 *N* As^{+3} with 0.0991 *N* $KMnO_4$ using 50 ml of 2% NaF. The volume was completed to 100 ml with twice-distilled water. (A) 20 ml of 9 *N* H_2SO_4; (B) 10 ml of 9 *N* H_2SO_4; (C) 5 ml of 9 *N* H_2SO_4; (D) 20 ml of 9 *N* H_2SO_4 + 20 ml of 0.25 *M* $CuSO_4$; (E) 20 ml of 9 *N* H_2SO_4 at 60°C (0.0991 *N* $KMnO_4$ used).

tion which proceeds smoothly under these conditions.

iv. The effect of temperature. As far as the errors of the titration are concerned, it is apparent that one can titrate As(III) with $KMnO_4$ safely at temperatures up to 60°C, provided the optimum conditions of acidity and F^- ion concentration are applied (Table 4).

v. The effect of concentration of trivalent arsenic. On using the optimum conditions, the titration curves possess almost the same character irrespective of the concentration of As(III) (Table 5). Good results are obtained for amounts of arsenic up to 92.42 mg.

II. Application of the Method for the Microdetermination of Trivalent Arsenic

Table 6, lists the results of determining small amounts of trivalent arsenic in presence of sulfuric acid and sodium fluoride under the optimum conditions.

It was found essential that the total volume of the titration mixture should be kept at ~20 ml in order to obtain considerable inflections at

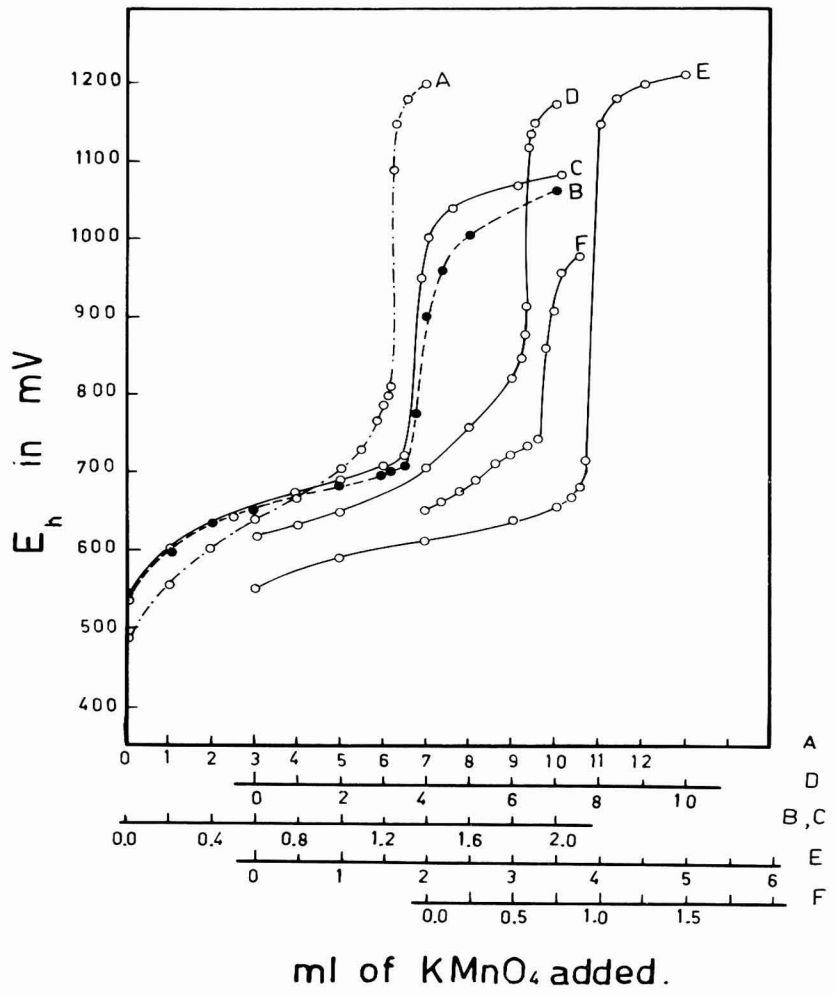

FIG. 2. (A) 5 ml of 8.81×10^{-3} *N* As^{3+}; 8.91×10^{-3} *N* $KMnO_4$; total volume, 100 ml. (B) 5 ml of 8.81×10^{-4} *N* As^{3+}; 8.91×10^{-4} *N* $KMnO_4$; total volume, 100 ml. (C) 5 ml of 8.81×10^{-4} *N* As^{3+}; 8.91×10^{-4} *N* $KMnO_4$; total volume, 50 ml. (D) 5 ml of 8.81×10^{-4} *N* As^{3+}; 8.54×10^{-4} *N* $KMnO_4$; total volume, 20 ml. (E) 3 ml of 8.81×10^{-4} *N* As^{3+}; 8.54×10^{-4} *N* $KMnO_4$; total volume, 20 ml. (F) 0.5 ml of 8.81×10^{-4} *N* As^{3+}; 8.54×10^{-4} *N* $KMnO_4$; total volume, 20 ml.

the end points. The total volume of the titration mixture has a considerable effect on the inflection at the end point, increasing with decreasing total volume. Thus on titrating 0.0330 mg of As(III) with 8.54×10^{-4} *N* $KMnO_4$ and using as total volumes 100, 50, and 20 ml, the inflec-

TABLE 2

EFFECT OF NaF CONCENTRATION

2% NaF solution added (ml)	Vol of $KMnO_4$ consumed (ml)	Theoretical end point (ml)	Error (%; ±)	Inflection at end point (mV/0.1 ml of titrant)
Potentiometric titration [a]				
10	8.16	8.3	1.68	178
25	8.32	8.3	0.24	121
50	8.38	8.3	0.96	224
75	8.33	8.3	0.36	215
Visual titration [b]				
5	11.14	11.37	1.96	
10	11.35	11.37	0.17	
50	11.38	11.37	0.09	
75	11.36	11.37	0.23	

[a] 8 ml of 0.08225 *N* As^{3+} titrated with 0.0991 *N* $KMnO_4$ in 1.8 *N* H_2SO_4, the solution was completed to 100 ml with twice-distilled water.

[b] 10 ml of 0.1026 *N* As^{3+} titrated with 0.1130 *N* $KMnO_4$ in 1.8 *N* H_2SO_4 and 5 ml of 0.25 *M* $CuSO_4$ (total volume 100 ml).

tions at end points amount to 50, 60, and 185 mV/0.05 ml of titrant, respectively.

DISCUSSION

The difficulties observed in the reaction between trivalent arsenic and permanganate (*3*) have been overcome in this investigation by carrying out the titration in presence of fluoride ions. When the optimum conditions are applied, the reaction proceeds smoothly up to the end point. The highly colored manganic arsenate observed by Willard *et al.* (*2*) in absence of F^- ions, no longer appears, since F^- ions act as complexing agent towards Mn(III). Under these conditions the titration can be carried out visually to a sharp end point when the amount of As(III) lies in the vicinity of ~19.21 mg. At higher concentrations, however, the pink manganic fluoride complex renders it difficult to perceive the end point easily. In presence of Cu(II) ions the pink color disappears and the end point is detected by a sky blue or faint violet color. Because a small amount of the oxidant is consumed before the appearance of this color, a blank titration has to be performed. The potentiometric method overcomes this difficulty, whereby, amounts of As(III) up to 92.42 mg can be determined without the addition of Cu(II) ions. In very dilute solutions, however, Cu(II) must be added in order to catalyze the reaction.

TABLE 3

EFFECT OF $CuSO_4$

Vol of 0.25 *M* $CuSO_4$ added (ml)	Vol of $KMnO_4$ consumed (ml)	Theoretical end point (ml)	Error (%; ±)	Inflection at end point (mV/0.1 ml of titrant)
Potentiometric titration [a]				
—	8.38	8.30	0.96	224
20	8.32	8.30	0.24	217
Visual titration [b]				
—	11.38	11.37	0.09	
5	11.38	11.37	0.09	
10	11.45	11.37	0.68	

[a] 8 ml of 0.08225 *N* As^{3+} titrated with 0.0991 *N* $KMnO_4$ using 50 ml of 2% NaF in 1.8 *N* H_2SO_4 (total vol, 100 ml).

[b] 10 ml of 0.1026 As^{3+} titrated with 0.1130 *N* $KMnO_4$ using 50 ml of 2% NaF in 1.8 *N* H_2SO_4 (total vol, 100 ml).

The reaction appears to take place according to the equation:

$$MnO_4^- + 2AsO_3^{3-} + 2H^+ + 2HF_2^- \rightleftharpoons MnF_4^- + 2AsO_4^{3-} + 2H_2O.$$

The reaction involves the uptake of H^+ ions, since the redox potentials of the MnO_4^-/Mn^{3+} and AsO_4^{3-}/AsO_3^{3-} systems are pH dependent.

TABLE 4

EFFECT OF TEMPERATURE

Temp (°C)	Vol of $KMnO_4$ consumed (ml)	Theoretical end point (ml)	Error (%; ±)	Inflection at end point (mV/0.1 ml of titrant)
Potentiometric titration [a]				
23	8.38	8.30	0.96	224
45	8.32	8.30	0.24	168
60	8.34	8.30	0.48	162
Visual titration [b]				
21	11.36	11.37	0.23	
40	11.31	11.37	0.51	
60	11.28	11.37	0.77	
70	11.25	11.37	1.12	

[a] 8 ml of 0.08225 *N* As^{3+} titrated with 0.0991 *N* $KMnO_4$ in 1.8 *N* H_2SO_4 using 50 ml of 2% NaF (total vol, 100 ml).

[b] 10 ml of 0.1026 *N* As^{3+} titrated with 0.1130 *N* $KMnO_4$ in 1.8 *N* H_2SO_4 using 50 ml of 2% NaF and 5 ml of 0.25 *M* $CuSO_4$ (total vol, 100 ml).

TABLE 5

EFFECT OF CONCENTRATION OF As^{3+}

Amounts of As^{3+} (ml)	(mg)	Vol of $KMnO_4$ consumed (ml)	Theoretical end point (ml)	Error (%; ±)	Inflection at end point (mV/0.1 ml of titrant)
Potentiometric titration [a]					
4	12.32	4.13	4.15	0.48	225
8	24.64	8.38	8.30	0.96	224
15	46.21	15.56	15.56	Nil	181
30	92.42	31.11	31.12	0.04	99

$NaAsO_2$ (ml)	(N)	0.25 M $CuSO_4$ added (ml)	Vol of $KMnO_4$ consumed (ml)	(N)	Theoretical end point (ml)	Error (%; ±)
Visual titration						
2.5 [b]	0.0205		2.28	0.02828	2.27	0.44
20 [c]	0.1026	10	22.70	0.1130	22.74	0.18

[a] 0.08225 N As^{3+} titrated with 0.0991 N $KMnO_4$ using 50 ml of 2% NaF in 1.8 N H_2SO_4.

[b] 1.921 mg of As.

[c] 76.847 mg of As.

TABLE 6

DETERMINATION OF MILLIGRAM AMOUNTS OF ARSENIC(III) [a]

No.	As(III) (mg) Taken	Found	Error (%; ±)	Inflection at end point (mV/0.05 ml of titrant)	Total vol of solution (ml)
1	16.497	16.497	Nil	235	100
2	1.650	1.653	0.20	280	100
3	0.165	0.166	0.60	125	100
4	0.165	0.166	0.60	210	50
5	0.165	0.165	Nil	230	20
6	0.132	0.132	Nil	220	20
7	0.0989	0.0987	0.10	220	20
8	0.0825	0.0826	0.12	120	20
9	0.0495	0.0497	0.40	190	20
10	0.0330	0.0330	0.00	185	20
11	0.0165	0.0166	0.60	115	20
12	0.0330	0.0331	0.30	50	100
13	0.0330	0.0331	0.30	50	50

[a] Nos. (1) 0.0881 N As(III), 0.08910 N $KMnO_4$; (2) 0.00881 N As(III), 8.91×10^{-3} N $KMnO_4$; (3–13) 8.81×10^{-4} N As(III), 8.54×10^{-4} N $KMnO_4$, determined in presence of optimum conditions of acidity and sodium fluoride concentration; (3–13) $CuSO_4$ was added except in No. 8.

$$AsO_4^{3-} + 2e + 2H^+ \rightleftharpoons AsO_3^{3-} + H_2O,$$
$$MnO_4^- + 4e + 8H^+ \rightleftharpoons Mn^{3+} + 4H_2O.$$

The redox potentials of both systems increase with rise of acidity, although the increase is higher in the MnO_4^-/Mn^{3+} than in the As^{5+}/As^{3+} system so that the reaction should be favored at higher than at lower acidities.

SUMMARY

A method is described for the determination of trivalent arsenic. It depends on the oxidation of As^{3+} to As^{5+} in H_2SO_4 solutions with $KMnO_4$ in presence of fluoride ions under the following conditions: Acidity: 0.9–3.6 *N* H_2SO_4 in presence of 50 ml of 2% NaF; sodium fluoride: 25–75 ml of 2% solution/100 ml; temperature: should not exceed 60°C; amounts of As^{3+}, 0.0165–92.42 mg.

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Book Reviews

The Origin of the Universe. By JAN ALEKSANDER PIASECKI. Philosophical Library, New York, 1972. VI + 56 pp. $3.75.

In spite of the intriguing title, this book does not present a scientific theory. According to the preface, the late Jan Piasecki "approached the enigma of the universe through observation, meditation, and logical analysis of the data . . . observed." What he has done is to put together 56 pages of words in a fantastic jumble of theology and pseudoscience. To the pragmatist, at least, the book does not make much sense.

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Techniques of Clinical Chemistry. By SAMUEL NATELSON. Thomas, Springfield, IL, 1971, XI + 965 pp. $37.00. 3rd edition.

This third edition of the book under review describes in detail tested and confirmed methods for clinical laboratory determinations. The methods presented are appropriate for general use in the average clinical laboratory and each has been standardized to maximize its usefulness and highlight any pitfalls to the user. The procedures are those which have shown themselves in thousands of analyses checked by standards and recoveries to be simple, reliable and easily performed.

The book is divided into three sections. The first section—Introduction to Microprocedures and Instrumentation—is a discussion of analytical chemical background, interpretation of the data, and description of the operation of newer instrumentation is also included. The second section comprising about 700 pages deals with chemical procedures. Techniques for the analysis of more than 140 components of blood, urine, and other biological fluids are included. Each procedure has been carefully checked—many are modifications of procedures not reported elsewhere. The methodology is presented first in a concise but detailed form. A literature review of the alternative methods applicable is documented for each procedure. The third and final section deals with instrumentation and analytical principles. Thoroughly covered are such topics as colorimetry, absorption spectrophotometry, emission spectrometry, gas chromatography, and automation. To sum up, the book is written in a clear and lively fashion, and anyone associated with clinical laboratory practice will find it useful and enjoyable reading. The main weakness of the book is the short discussion of enzyme assays. Failure to mention the most recent outstanding development, namely genetic disease diagnosis by enzyme analysis in cultured cells, particularly from skin fibroblasts is to be regretted. In spite of these deficiencies, the book is probably the best readily available comprehensive source of information for the laboratory desiring to add microtechniques to its routine. As such it should appeal to biochemists, clinical chemists, and all those associated with academic, hospital, or private laboratories.

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Organic Scintillators and Liquid Scintillation Counting. Edited by DONALD L. HORROCKS AND CHIN-TZU PENG. Academic Press, New York 1971. XVI + 1078 pp. $24.00.

This book contains 79 of the 83 papers presented at the International Conference on Organic Scintillation and Liquid Scintillation Counting held Jul. 7–10, 1970 at the University of California, San Francisco. The proceedings produced papers from two different but very interrelated fields; that of the theory and physics of organic scintillators, and radioactivity measurements using liquid scintillation methods. Subject material covered ranges from the academic and theoretical, to the practical and useful.

The book is divided into three sections. The first section containing "the theory of solvent excitation and energy migration, the correlations between molecular conformation and fluorescence properties, the use of lasers to study fluorescent properties in the picosecond time period, excimers, and the role of oxygen in the quenching process and discusses the techniques of evaluation of different compositions of liquid scintillator solutions, and the use of these for measuring radioactivity in animals and humans and bioluminescence."

The second section deals primarily with the physics of organic scintillators such as quenching effects, fluorescence yields and radiative lifetimes.

The last section deals with various liquid scintillation counting techniques, solubilizers, spectrometers and data analysis and reduction methods. This section touches on almost every type of application that the liquid scintillation process might be used for.

In general, there is more attention devoted to the academic aspects of organic scintillators, than the practical applied aspects of liquid scintillation counting. It would appear that some copying process was used to produce this book. The various papers appear to be in their original type set, providing the reader with several varieties of print, some of which were difficult to read due to the size reduction process used to fit the paper to the size of the book. Acknowledgements and references are provided with each paper providing the reader with an excellent collection of resource material.

These proceedings would be of use to the biologist, chemist, organic chemist, physicist, health physicist and solid state physicist.

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Handbook of Naturally Occurring Compounds. Vol. 2. By T. K. DEVON AND A. I. SCOTT. Academic Press, New York, 1972. XI + 576 pp. $21.00.

The Handbook of Naturally Occurring Compounds is scheduled to be produced in three volumes: Vol. 1, Acetogenins, Shikimates, and Carbohydrates; Vol. 2 Terpenes; and Vol. 3 Alkaloids and Related Nitrogenous Compounds. Annual supplements are projected for each volume.

In all, this is a monumental undertaking that will surely earn the gratitude of all natural products chemists. The authors' files contain some 11,000 compounds Of these, approximately 4000 are terpenoid and have been assembled in volume 2.

Each structure is recorded in the Handbook with its name, molecular formula, molecular weight, optical rotation, melting point, literature reference, and classification number. Compounds can be retrieved through the alphabetical, molecular weight, and molecular formula indices at the end of the book, and through the structural classification guides provided at the beginning of each section. The sections are based on the following classes: monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, steroids, carotenoids, polyprenoids, and miscellaneous compounds of terpenoid origin. Each section is subdivided into skeletal types.

Obviously a good deal of effort has gone into the arrangement of the sections and into the construction of the structural classification guides. Probably, the natural products chemist will turn first to these guides to find compounds of types of interest to him.

The authors enter a disclaimer that this handbook is free of errors of omission or commission, and they have undertaken some editing for structural and stereochemical correlations. They request that users who spot errors or omission forward them to the authors so that subsequent yearbooks can set the records straight.

I submit the following comments based on browsing through the sections of interest to me:

1. Geranial is not listed in the index. It is entered as citral, *trans-*, with a 1923 reference.
2. The literature reference to *cis*-verbenol gives the optical rotation as +4° (rather than +65°) and the melting point as 69° (rather than an oil).
3. The formula for ocimenone lacks a double bond.
4. The name tagitol is given by the authors to compound 4001-016. This designation does not seem appropriate for a compound that has a methylene substituent rather than the methyl substituent of tagetone.

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Inorganic Syntheses, Vol. 13. Edited by F. A. COTTON. McGraw-Hill, New York, 1972. XVI + 267 pp. $13.50.

This 13th volume, an excellent continuation of the well-known "Inorganic Synthesis Series," presents detailed and checked procedures for the preparation of 120 compounds. It retains, as all of the previous volumes, its traditionally well-established discipline to "present the best, independently tested methods for the synthesis of inorganic compounds—made available by contributors from all over the world." Thus, synthetic directions for the most current research interests of some 120 different substances listed under 46 different entries which were contributed from a total of 167 investigators are successfully compiled in this new volume.

In format, it follows the organizational patterns initiated in the previous volumes (such as Volumes 10, 11, and 12), syntheses being classified according to area of interest. Therefore, the synthetic procedures for the 120 compounds, which are of three main categories, are described and discussed comprehensively in seven chapters. The headings of these chapters are: compounds of the non-transition elements; organometallic compounds; compounds containing metal-to-

metal bonds; phosphine and phosphite complexes of low-valent metals; binary compounds of the transition metals; halo complexes of some metals; and various transition-metal compounds, etc. Furthermore, under each entry the following information is always provided for the preparation of each specific compound. A simple *Chemical Equation* represents the stoichiometric relations for the chemical reactions during the course of synthesis. An *Introduction* section includes a concise and critical summary of the available procedures for synthesis of the product; an estimate of the time required for the synthesis; an indication of the importance and utility of the product; and an admonition of any potential hazards (such as the precautions for the toxic, corrosive, flammable, explosive, pyrophoric, hygroscopic, or sensitive to air or moisture materials) which might be associated with the procedure. A *Procedure* section presents detailed and unambiguous laboratory directions; a clear description of any unusual equipment or procedure; a clear statement of all safety measures; sources of unusual starting materials and standards of purity of reagents and solvents; the discussion of the scale of procedure; the criteria for judging the purity of the final product; the yield and analytical results. The section on *Properties* lists and discusses those physical and chemical characteristics that are relevant to judging the purity of the product and permitting its handling and an intelligent use. Under *References*, all pertinent literature citations are listed in order. In addition, an alphabetical listing of the index of contributors; a cumulative subject index for Volumes 11, 12, and 13; and a cumulative formula index for Volumes 11, 12, and 13 are also available for helping the user to utilize the text conveniently and effectively.

Technically, several special methods that have been employed in the syntheses include the use of vacuum systems, gas phase reactions, low or high temperatures, nonaqueous solvents, dry box and controlled atmospheres, remote control manipulation, resolution of optical isomers by diastereoisomer formation, and fluorination by means of antimony and arsenic fluorides, and phosphorus pentafluoride.

The first chapter deals with the synthesis of compounds of the nontransition elements including the central atoms of B, Si, N, P, As, S, F, Cl, and Br (46 pp., 17 preparations). It is a rather diverse array, not strongly organized around any principal theme. However, it is devoted particularly to some organosilicon, organophosphorous, and organoboron compounds which have a considerable industrial and academic interest. For examples, $(CH_3)_3Si–P(C_6H_5)_2$, $(CH_3)_3Si–P(CH_3)_2$, $C_6H_5BCl_2$, $(C_6H_5)_2BCl$, CH_3PF_4, etc., have shown promise as catalysts, reagents and intermediates in the synthesis of other kinds of compounds. It thus challenges many chemists to work in this new and vigorous research area.

Chapter 2, Organometallic Compounds (34 pp., 24 preparations), describes the procedures for synthesis of some recently developed cyclic-chelating diolefins (that is, 1,5-cyclooctadiene) and cationic diene complexes of Pd(II) and Pt(II). These compounds, because of their unique bonding, have received considerable attention and have potential applications as intermediates or catalysts in a variety of reactions. Included in the chapter are also the preparation of polyhalo- and polyalkyl-silylcobalt tetracarbonyl complexes having the formulas $R_3SiCo(CO)_4$ (R = F, Cl, CH_3, C_2H_5, C_2H_5O) and of $H_3SiCo(CO)_4$ by interactions of a silicon hydride (R_3SiH) with a transition-metal carbonyl compound ($Co_2(CO)_8$). Another attractive feature of the chapter is the synthesis of many metal isoleptic (it indicates that all the ligands attached to the central metal atom are identical

in constitution) allyls and their derivatives from an allyl Grignard reagent. These compounds are $Sn(C_3H_5)_4$, $Si(C_3H_5)_4$, $Ge(C_3H_5)_4$, tri-h^3-allylchromium and di-h^3-allylnickel.

Chapter 3 presents a few procedures dealing with the nonclassical compounds involving metal atom clusters or very strong multiple metal–metal bonds (24 pp., 12 preparations). Many of these compounds are of considerable structural interest as well as of importance industrially. The representative species $(Re_2Cl_8)^{2-}$ was first recognized containing a quadruple bond, which (with the quadruply bonded Re_2 entity) has been shown a persistence through a variety of ligand substitution reactions. Besides, it is suggested that the easiest access to this class of compounds is via an $(Re_2Cl_8)^{2-}$ salt. Thus, general procedures for obtaining compounds with multiple metal–metal bonds of Re, Mo, Rh, Ru, Os, and Ir which interact with the ligands such as RCO_2, CO, RO are presented here (for examples, $Re_2(O_2CAr)_4Cl_2$, $Mo_2(O_2CR)_4$, $Os_3(CO)_{12}$, $Ir_4(CO)_{12}$).

A group of complexes involving low-valent metals coordinated by various phosphorous ligands, though they are transition-metal compounds, because these compounds have an exceptional degree of practical and fundamental interest, are discussed separately in Chapter 4 (30 pp., 16 preparations). Aryl phosphines and phosphites (X_3–P type ligands), in general, as carbon monoxide are pi-acceptor ligands, have the ability to stabilize metals in zero or other low formal oxidation states. Syntheses of this type of compounds included: triaryl phosphite complexes of Co(I), Ni(0), Pt(0), and Rh(I); tetrakis(triethyl phosphite) complexes of Ni(0), Pd(0), and Pt(0); tetrakis(diethyl phenylphosphonite) complexes of Ni(0), HCo(I), and FeH_2(II); tetrakis(triphenylphosphine) of Pd(0) and Ni(0); and $[Ir\{P(C_6H_5)_3\}_3(CO)H]$ and $[\{(C_6H_5)_3P\}_3RuHCl]$.

In Chapter 5 (30 pp., 16 preparations), procedures are given for the single-crystal growth of some transition-metal dioxides which possess electrical transport and magnetic properties such as RuO_2, IrO_2, OsO_2, β-ReO_2 and WO_2, using high temperature chemical transport techniques. Methods for preparing MoF_5, WCl_5, and several anhydrous Ni(II) halides and their tetrakis(ethanol) and 1,2-dimethoxyethane complexes [as useful sources of Ni(II) ion for the synthesis of nickel organic compounds in nonaqueous solvents] are also discussed in detail.

The shortest chapter, Chapter 6 (12 pp., 9 preparations), provides the general method for synthesis of some recent interest of halo complexes such as $[(C_6H_5)_4As][VCl_4]$ (first tetrahedral complex prepared), $[(C_2H_5)_4N]_3[V_2Cl_9]$, $[MoCl_5(H_2O)]^{2-}$, and $Na_2[PtCl_6]$.

The last and longest chapter of the volume, Various Transition-Metal Compounds (50 pp., 26 preparations), is devoted exclusively to the classical or Werner complexes. However, instead of following the old fashions, it places an emphasis on the modification or improvement of the synthetic procedures for new compounds. For examples, the preparation of $[V(i\text{-}C_3H_7OH)_4Cl_2]$ Cl from alcoholic solutions; the synthesis of five coordination complexes of $[VCl_3{\cdot}2N(CH_3)_3]$ by sealed-tube–dry-box techniques; the preparation of $[VO(CH_3CO_2)_2]$ from V_2O_5 in nonaqueous media of acetic anhydride; the synthesis of synthetically important complex of $[Cr(diamine)_3]$ $Cl_3{\cdot}3H_2O$ from methanol solution with zinc catalyst. Other special techniques such as the synthesis of cobalt and iron complexes of *cis*-1,2-disubstituted ethene-1,2-dithiol and their Lewis base adducts (that is, NO, pyridine, phosphine, etc.) which are capable of undergoing reversible one-electron transfer reactions; the resolution of the optically active forms of the (ethylenediamine)bis(oxalato)cobaltate(III) ion; and the transition-

metal hydride complexes of $[ReH_9]^{2-}$ and $[TcH_9]^{2-}$ (so far the only examples of transition-metal hydride complexes in which there is no ligand other than hydrogen) are also added to the text very attractive and noteworthy.

In summary, the editor has done a successful work collecting and compiling so many detailed and carefully checked synthetic procedures for preparation of a great number of important compounds. The book is well organized; the chapters are well written; each individual method or specific technique is clearly described and completely applicable; and, throughout the text it is free of significant typographical errors. It is thus highly recommended not only for synthetic chemists or research workers as an invaluable reference book but also for both undergraduate and graduate students as a useful preparative guidebook so long as synthesis problems are concerned. At a cost of only $13.50, libraries should keep one copy and the individual user, whether he has previous volumes or not, would be smart to have one on his desk.

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Analytical Profiles of Drug Substances. Edited by KLAUS FLOREY. Vol. 1. Academic Press, New York, 1972. XL + 480 pp. $14.00.

This series of books is concerned with the in-depth analytical chemical study of drugs appearing in the official compendia (United States Pharmacopeia and National Formulary) and is sponsored by the Pharmaceutical Analysis and Quality Control Section of the Academy of Pharmaceutical Sciences.

Volume 1 deals with the following drugs: acetohexamide, chlordiazepoxide, chlordiazepoxide hydrochloride, cycloserine, cyclothiazide, diazepam, erythromycin estolate, halothane, levarterenol bitartrate, meperidine hydrochloride, meprobamate, nortriptyline hydrochloride, potassium phenoxymethyl penicillin, propoxyphene hydrochloride, sodium cephalothin, sodium secobarbital, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, vinblastine sulfate, and vincrystine sulfate. The monographs are more or less standardized according to the following sections: description, physical properties, synthesis, stability-degradation, drug metabolic products, methods of analysis, pharmacokinetics, classification, and references.

Since our national compendia are quality control oriented, a book of this scope is very useful for everyone engaged in pharmaceutical or biochemical research. One would be hard pressed to find all these physical chemical, analytical, and other data compiled in one book.

The book, however, has too many shortcomings which is distracting. The pages are printed in typewritten letters of different size. It would be better if in the next volume only one single brand of typewriter is used. There are several errors such as on page 103 where the chemical representation of erythromycin estolate as an ammonium alkyl sulfate salt is incorrect. Also erythromycin is misspelled as esthromycin. On page 240 nortriptyline is misspelled and the acetylene bond is designated twice as an ethylene bond. A more serious error is found on page 243 where the development system of a paper chromatographic procedure is misrepresented as follows:

Developer
15%
$BuOH–HC_{02}H–H_2O$ (12:1:7)
This should be: $BuOH–15\%HCO_2H–H_2O$(12:1:7)

Another weakness is the nonuniformity in the journal abbreviations. On page 247 Chemical Abstracts is abbreviated two ways namely: Chem. Abstr. and Chem. Abstracts. The Journal of the American Chemical Society is abbreviated as J. Amer. Chem. Soc. on page 291 and as J. Am. Chem. Soc. on page 339. Using the Chemical Abstracts recommendations for the journal abbreviations will correct these inconsistencies.

Nevertheless, with all these errors and nonuniformity, this book will be of great value to the research pharmacist as well as the research pharmaceutical chemist. A tighter editing is all what is needed to improve the quality of this book.

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Orbital Symmetry, A Problem Solving Approach. By ROLAND E. LEHR AND ALAN P. MARCHAND, Academic Press, New York, 1972. X + 190 pp. $4.95 (paper).

This book is a useful extension of the now classic monograph "The Conservation of Orbital Symmetry" by R. B. Woodward and R. Hoffmann. An extension, because on its own this book provides only an inadequate theoretical background which is needed in the construction of energy correlation diagrams, such an essential part of the application of orbital symmetry rules.

As an extension, however, the book serves a useful purpose on several counts.

First of all, introduction of the selection rules in sigmatropic, electrocyclic and cycloaddition reactions, all illustrated with numerous examples should serve to encourage use of these rules even by chemists not possessing a substantial theoretical foundation. (Parenthetically, many organic chemistry students appear to find Woodward and Hoffmann's book difficult to follow.)

Secondly, besides discussing numerous examples adopted from Woodward and Hoffmann's book, the authors also did an excellent job of compiling an impressive updated list of further examples proving the general applicability of the symmetry rules (literature survey to mid-1971 is apparent). As the title suggests, the principal merit of this work is the clear statement of many synthetic results and a rationalization of these results based on orbital symmetry rules. As such, it is very useful pedagogically since a student trying to familiarize himself with the application of the orbital symmetry rules will find worked out answers and explanations as well as literature references in the book.

There is an effort to introduce other theoretical methods available for the treatment of the problem of reactivity of unsaturated organic compounds (such as Dewar's Perturbation Molecular Orbital method, Fukui's Frontier Electron Theory and Zimmerman's Mobius vs Huckel Aromaticity concept). While for sake of completeness this is of value, the brief treatments are quite insufficient.

The book is highly recommended to those unfamiliar with orbital symmetry rules and possessing a weak background in molecular orbital theory. It is

also recommended as a useful problem source to those teaching the Woodward–Hoffmann treatment.

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Announcements

California Association of Criminalists

FALL 1972

California Association of Criminalists, 40th Semiannual Seminar, October 19–21, 1972, Mansion Inn, Sacramento, California. For further information contact Seminar Chairman, Alan Gilmore, Sacramento County District Attorney, Criminalistics Lab, 4400 "V" Street, Sacramento, California, 95817 (916) 454-5704.

SPRING 1973

California Association of Criminalists, 41st Semiannual Seminar, May 17–19, 1973, Sheraton Inn, Harbor Island, California. For further information contact Richard Shaw, San Diego Coroner's Office, 5555 Overland Avenue, Building 14, San Diego, California, 92123 (714) 278-9600.

International Symposium on Microchemical Techniques—1973*

"Progress and Projections for Microchemistry," will be the general theme for the International Symposium on Microchemical Techniques—1973. The symposium will be held at The Pennsylvania State University, University Park, Pennsylvania, on 19 August to 24 August 1973 and will be conducted by the American Microchemical Society, with the sponsorship of the International Union of Pure and Applied Chemistry.

The scientific program will consist of sessions dedicated to topics of current interest, general papers, discussion groups, practical demonstrations, an equipment exhibit, and will also include a number of instructional workshop sessions. Special sessions will be included on such topics as:

Automated Elemental Analyzers—Ten Years Later
Computers in Elemental Analysis
Organic Elemental Analysis: New Methods and Equipment

* Due to a change in the scheduling of educational activities at The Pennsylvania State University, it has become necessary to change the dates of the International Symposium on Microchemical Techniques to 19 August to 24 August, 1973.

Environmental Microanalysis: New Sensors and Techniques
Microelectrodes
Forensic Analysis: Narcotics and Drugs of Abuse
Organic Functional Group Analysis: New Directions
Electroanalytical Advances, including Ion Selective Electrodes
Microscale Separations: Advances in Techniques and Methods
Standards and Standardization for Microchemistry and Microanalysis
Trace Analysis: Advances in Organic and Inorganic Analysis
New Techniques in Microchemistry

Persons interested in presenting a paper under any of the above topics, or a paper on the general topic of microchemistry, should submit their paper to:

Mr. Howard J. Francis, Jr.
Pennwalt Corporation
900 First Avenue
King of Prussia, Pennsylvania 19406, U.S.A.

Included in the program of scientific presentations will be classroom workshops on the topics of:

Applications of Ion Selective Electrodes
Theory and Applications of Thermal Methods of Analysis

A number of semitechnical and social events are planned, an introductory evening mixer, a banquet, a social evening, an evening demonstration of gadgets, and an evening session on "The Art of Presenting a Paper."

Ladies and families are welcome to attend.

Utilizing the excellent facilities of The Pennsylvania State University for both the technical program and housing, expenses will be minimal.

A later announcement will be made concerning the program details.