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247

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CONTENTS

M. GUZMÁN, A. M. CAMEÁN, AND J. BAUTISTA. 6-Methylpyridine-2-Aldehyde *p*-Nitrophenylhydrazone as an Indicator for Colorimetric pH Measurements. . . 1
DA. MALJKOVIĆ, DU. MALJKOVIĆ, AND J. HEDŽET. The Study of Iron(III) and Gold(III) Separation by Paper Chromatography Using Diisopropyl Ether. . . . 6
CZESŁAW WYGANOWSKI. Sensitive Spectrophotometric Determination of Gallium with Bromopyrogallol Red in the Presence of Surfactants. 13
A. A. ELSIRAFY. A Water Purification System for Microanalysis. 18
WADALA A. BASHIR, GHAZAR W. HAGOP, AND S. FALAMERZ. Indirect Spectrophotometric Determination of Cerium(IV). 21
F. SALINAS, F. GARCIA-SANCHEZ, AND C. GENESTAR. Fluorimetric Determination of Vanadium Based on Its Reaction with 1-Amino-4-hydroxyanthraquinone. . . 26
F. SALINAS, C. GENESTAR, AND F. GRASES. Kinetic–Fluorimetric Determination of Cerium with 1-Amino-4-hydroxyanthraquinone. 32
CZESŁAW WYGANOWSKI AND MIROSŁAWA KOLCZYNSKA. Spectrophotometric Determination of Aluminum with Bromopyrogallol Red in the Presence of Surfactants. 37
S. W. BISHARA AND F. M. EL-SAMMAN. Simultaneous Microdetermination of Sulfur and Phosphorus in Organic Compounds. 44
J. BAREK, A. BERKA, AND V. BOREK. The Use of Redox Reactions in the Analysis of Dyes and Dye Intermediates. XI. A Chromometric Determination of Some Azobenzene Derivatives in an Acetonitrile–Methanol–Water Mixed Medium. . 49
J. BAREK, A. BERKA, AND K. JAKUBEC. The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XVIII. A Contribution to the Coulometric Generation of Manganese(III) Acetate in an Acetic Acid Medium. 55
J. BAREK, A. BERKA, AND A. HLADÍKOVÁ. The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XXI. The Oxidation of Glycoaldehyde with Hexaquomanganese(III) Ions in a Noncomplexing Perchloric Acid Medium. 62
J. BAREK, A. BERKA, AND A. HLADÍKOVÁ. The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XXII. The Oxidation of Glyceraldehyde with Manganese(III) Sulfate. 66
KATSUYA UESUGI AND SHIGEROKU YAMAGUCHI. Extraction–Spectrophotometric Determination of Nickel with 2-Hydroxy-1-naphthaldoxime. 71
DANDINASIVARA S. MAHADEVAPPA, K. S. RANGAPPA, B. T. GOWDA, AND NETKAL M. MADE GOWDA. Some Analytical Applications of Aromatic Sulfonyl Haloamines: Determination of Thiocyanate and Cyanide Ions in Metal Complexes and Salts with Bromamine-B and Dichloramine-B. 77
NETKAL M. MADE GOWDA, NORMAN M. TRIEFF, DANDINASIVARA S. MAHADEVAPPA, V. M. SADAGOPA RAMANUJAM, AND ROBERT S. TRIEFF. Sodium *N*-Chloro-*p*-Nitrobenzenesulfonamide (Chloramine-N) as a New Redox Titrant. I. The Status of Chloramine-N as a Titrimetric Reagent. 87
ALENA ŠPAČKOVÁ AND MOHAMMED S. CHAUDHRI. Spectrochemical Determination of Tungsten and Boron in Mineral Matrix. 97
WALTER SELIG. Microdetermination of Aliphatic Diamines and Polyamines with Cupric Ion. 102
M. C. MEHRA AND J. RIOUX. Mercuric Chloranilate as Solid Colorimetric Reagent in Microanalysis of Complex Ions. 112

CAROLE L. WALTERS. Determination of Carbon Monoxide with Palladium Chloride. 116
THOMAS L. MCDONALD. A Microcell for Small-Volume Dialysis. 124
ADELA BERMEJO-BARRERA, F. BERMEJO-MARTINEZ, AND MERCEDES MOLINA-POCH. Spectrophotometric Determination of Cadmium with Uranyldiacetic Acid. . . . 127
G. A. MILOVANOVIĆ, M. A. SEKHETA, AND I. M. PETROVIĆ. Kinetic Determination of Microquantities of D(−)-Arabinose. 135
E. S. BAGINSKI, S. S. MARIE, R. E. KARCHER, AND B. ZAK. Microdetermination of Magnesium in Biological Fluids. 141

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Microchemical Journal, Volume 27, Number 1, March 1982

Briefs

6-Methylpyridine-2-aldehyde-p-nitrophenylhydrazone as an Indicator for Colorimetric pH Measurements. M. GUZMAN, A. M. CAMEAN, AND J. BAUTISTA, *Department of Applied Analytical Chemistry and Food Science, Faculty of Pharmacy, University of Sevilla, c/ Palos de la Frontera s/n, Sevilla-4, Spain.*

The indicator is suggested as an indicator for colorimetric pH measurements in the high-alkaline range.

Microchem. J. **27**, 1–5 (1982).

The Study of Iron(III) and Gold(III) Separation by Paper Chromatography Using Diisopropyl Ether. DA. MALJKOVIĆ, DU. MALJKOVIĆ, AND J. HEDŽET, *Faculty of Metallurgy, Aleja narodnih heroja 1, 44000 Sisak, Yugoslavia.*

In this paper the separation of iron(III) and gold(III) by paper chromatography has been described along with the study of factors influencing the chromatographic separation in systems containing diisopropyl ether.

Microchem. J. **27**, 6–12 (1982).

Sensitive Spectrophotometric Determination of Gallium with Bromopyrogallol Red in the Presence of Surfactants. CZESŁAW WYGANOWSKI, *Institute of General Chemistry, Technical University, 90-924 Lodz, Poland.*

Gallium in the presence of surfactants forms with bromopyrogallol Red a violet ternary complex which can be used for the determination of metal.

Microchem. J. **27**, 13–17 (1982).

A Water Purification System for Microanalysis. A. A. ELSIRAFY, *Faculty of Science, Cairo University, Giza, Egypt A.R.E.*

Apparatus is described for the preparation of high purity water suitable for microanalysis.

Microchem. J. **27**, 18–20 (1982).

Indirect Spectrophotometric Determination of Cerium(IV). WADALA A. BASHIR AND GHAZAR W. HAGOP, *Department of Chemistry, College of Science,* AND S. FALAMERZ, *Department of Chemistry, College of Education, University of Mosul, Mosul, Iraq.*

Use has been made of the reaction in which iron(II), on oxidation with cerium(IV), gives iron(III) which is complexed with tiron.

Microchem. J. **27**, 21–25 (1982).

Fluorimetric Determination of Vanadium Based on Its Reaction with 1-Amino-4-Hydroxyanthraquinone. F. SALINAS, F. GARCIA-SANCHEZ, AND C. GENESTAR, *Department of Analytical Chemistry, University of Palma de Mallorca, Palma de Mallorca, Spain.*

The anthraquinone derivative is oxidized by the ion which yields a product exhibiting an intense yellow fluorescence in acid solution.

Microchem. J. **27**, 26–31 (1982).

Kinetic–Fluorimetric Determination of Cerium with 1-Amino-4-hydroxyanthraquinone. F. SALINAS, C. GENESTAR, AND F. GRASES, *Department of Analytical Chemistry, Faculty of Sciences, University of Palma de Mallorca, Palma de Mallorca, Spain.*

The determination is based on the oxidative reaction between cerium(IV) and the reagent. About 20 cations and anions show neglible interference effects.

Microchem. J. **27**, 32–36 (1982).

Spectrophotometric Determination of Aluminum with Bromopyrogallol Red in the Presence of Surfactants. CZESŁAW WYGANOWSKI AND MIROSŁAWA KOLCZYNSKA, *Institute of General Chemistry, Technical University, 90-924 Łódź, Poland.*

Microgram amounts of aluminum are determined based on the formation of a ternary complex between the metal, bromopyrogallol red, and cetyltrimethylammonium bromide or nonylphenol tetradecaethylene glycol ether.

Microchem. J. **27**, 37–43 (1982).

Simultaneous Microdetermination of Sulfur and Phosphorus in Organic Compounds. S. W. BISHARA AND F. M. EL-SAMMAN, *Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq.*

Sulfate is precipitated with a known amount of Ba and the excess of the latter measured polarographically. After filtration, phosphorus is determined by the quinoline phosphomolybdate method and the unreacted Mo(VI) is determined polarographically.

Microchem. J. **27**, 44–48 (1982).

BRIEFS

The Use of Redox Reactions in the Analysis of Dyes and Dye Intermediates. XI. A Chromometric Determination of Some Azbenzene Derivatives in an Acetonitrile–Methanol–Water Medium. J. Barek, A. Berka, and V. Borek, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The work deals with a chromometric determination of some azobenzene derivatives in a medium in which these substances are sufficiently soluble.

Microchem. J. **27**, 49–54 (1982).

The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XVIII. A Contribution to the Coulometric Generation of Manganese(III) Acetate in an Acetic Acid Medium. J. Barek, A. Berka, and K. Jakubec, *Department of Analytical Chemistry, Faculty of Natural Sciences, Charles University, 128 40 Prague 2, Czechoslovakia.*

After finding the conditions under which the generation of manganese(III) acetate proceeds with the 100% current efficiency, the possibility of direct and indirect coulometric determinations of organic substances with the reagent was investigated.

Microchem. J. **27**, 55–61 (1982).

The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XXI. The Oxidation of Glycolaldehyde with Hexaquomanganese(III) Ions in a Noncomplexing Perchloric Acid Medium. J. Barek, A. Berka, and A. Hladíková, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The reaction is quantitative and stoichiometric after 2 hr, the oxidant consumption being 2 equivalents per mol.

Microchem. J. **27**, 62–65 (1982).

The Oxidation of Organic Substances with Compounds of Trivalent Manganese. XXII. The Oxidation of Glyceraldehyde with Manganese(III) Sulfate. J. Barek, A. Berka, and A. Hladíková, *Department of Analytical Chemistry, Charles University, 128 40 Prague 2, Czechoslovakia.*

The mechanism of the oxidation was studied. The oxidation was found to be complete after 4 min.

Microchem. J. **27**, 66–70 (1982).

Extraction–Spectrophotometric Determination of Nickel with 2-Hydroxy-1-naphthaldoxime. Katsuya Uesugi and Shigeroku Yamaguchi, *Laboratory of Chemistry, Himeji Institute of Technology, Shosha, Himeji, Hyogo, 671-22 Japan.*

The method is based upon the formation of the nickel complex which is extracted into chloroform from an aqueous solution.

Microchem. J. **27**, 71–76 (1982).

BRIEFS

Some Analytical Applications of Aromatic Sulfonyl Haloamines: Determination of Thiocyanate and Cyanide Ions in Metal Complexes and Salts with Bromamine-B and Dichloramine-B. DANDINASIVARA S. MAHADEVAPPA,[1] K. S. RANGAPPA,[1] B. T. GOWDA,[1] AND NETKAL M. MADE GOWDA.[2] *[1]Department of Post-Graduate Studies and Research in Chemistry, University of Mysore, Manasagangotri, Mysore-570006, India, and [2]Department of Preventive Medicine and Community Health, Division of Environmental Toxicology, The University of Texas Medical Branch, Galveston, Texas 77550.*

The oxidation involves eight and two electron changes, respectively, with thiocyanate and cyanide ions. The method could be employed for computing the number of ligands present in the metal complexes.

Microchem. J. **27**, 77–86 (1982).

Sodium *N*-Chloro-*p*-Nitrobenzenesulfonamide (Chloramine-N) as a New Redox Titrant. I. The Status of Chloramine-N as a Titrimetric Reagent. NETKAL M. MADE GOWDA,[1] NORMAN M. TRIEFF,[1] DANDINASIVARA S. MAHADEVAPPA,[2] V. M. SADAGOPA RAMANUJAM,[1] AND ROBERT S. TRIEFF,[1] *[1] Division of Environmental Toxicology, Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, Texas 77550, and [2] Department of Chemistry, University of Houston, Houston, Texas 77004.*

Analytical applications of the reagent as a potential titrimetric reagent in the direct determination of diverse reducing agents such as glutathione, thioglycolic acid, thiourea, ascorbic acid, methionine, sulfite, and arsenite are described.

Microchem. J. **27**, 87–96 (1982).

Spectrochemical Determination of Tungsten and Boron in Mineral Matrix. ALENA ŠPAČKOVÁ, *Geological Survey, Prague, Czechoslovakia,* AND MOHAMMED S. CHAUDHRI, *PCSIR Laboratories, Lahore, Pakistan.*

Copper (II) oxide was used to prevent the carbide formation and to increase the volatilization rate of the analysis elements.

Microchem. J. **27**, 97–101 (1982).

Microdetermination of Aliphatic Diamines and Polyamines with Cupric Ion. WALTER SELIG, *Lawrence Livermore National Laboratory, University of California, Livermore, California 94550*

The reaction was investigated potentiometrically, using a copper ion-selective indicator electrode and a single-junction reference electrode.

Microchem. J. **27**, 102–111 (1982).

BRIEFS

Mercuric Chloranilate as Solid Colorimetric Reagent in Microanalysis of Complex Ions. M. C. MEHRA AND J. RIOUX, *Chemistry Department, Université de Moncton, Moncton, New Brunswick E1A 3E9, Canada.*

The colorless complex anions triphenylcyanoborate, azide, and monothiophosphate were determined in an acidic aqueous medium through colorimetric exchange on solid mercuric chloranilate.

Microchem. J. **27**, 112–115 (1982).

Determination of Carbon Monoxide with Palladium Chloride. CAROLE L. WALTERS, *Emissions Measurement and Characterization Division, Environmental Sciences Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.*

A gas sample transfer apparatus was designed to facilitate work. Tests were conducted to assess the performance of the palladium chloride method.

Microchem. J. **27**, 116–123 (1982).

A Microcell for Small-Volume Dialysis. THOMAS L. MCDONALD, *Department of Medical Microbiology, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, Nebraska 68105.*

A microcell was designed for use in the dialysis of microliter sample volumes.

Microchem. J. **27**, 124–126 (1982).

Spectrophotometric Determination of Cadmium with Uramyldiacetic Acid. ADELA BERMEJO-BARRERA, F. BERMEJO-MARTINEZ, AND MERCEDES MOLINA-POCH, *Department of Analytical Chemistry, Faculty of Chemistry, University of Santiago de Compostela, Santiago de Compostela, Spain.*

The metal reacts at pH 6 with uramyldiacetic acid to give a complex with the maximal absorbance at 265 nm and follows Beer's law in the range of 0.12–3.6 μg Cd(II)/ml.

Microchem. J. **27**, 127–134 (1982).

Kinetic Determination of Microquantities of D(−)-Arabinose. G. A. MILOVANOVIĆ, M. A. SEKHETA, AND I. M. PETROVIĆ, *Chemical Institute, Faculty of Sciences, University of Belgrade, Studentski trg 16, POB 550, 11001 Belgrade, Yugoslavia.*

The method is based on the accelerating effect of D(−)-arabinose on the reaction between molybdenum (VI) and hydrogen peroxide in solution containing 50 vol% of acetonitrile.

Microchem. J. **27**, 135–140 (1982).

BRIEFS

Microdetermination of Magnesium in Biological Fluids. E. S. Baginski,* S. S. Marie,* R. E. Karcher,† and B. Zak,‡ **Department of Laboratories, Doctors' Hospital, Detroit, Michigan, †Department of Clinical Pathology, William Beaumont Hospital, Royal Oak, Michigan, and ‡Department of Pathology, Wayne State University School of Medicine, Detroit Receiving Hospital and University Health Center, Detroit, Michigan 48201.*

A modified procedure for the spectrophotometric determination of serum magnesium is described in which a direct addition of serum is made to a two-piece color reagent system.

Microchem. J. **27,** 141–150 (1982).

MICROCHEMICAL JOURNAL 27, 1–5 (1982)

6-Methylpyridine-2-Aldehyde *p*-Nitrophenylhydrazone as an Indicator for Colorimetric pH Measurements

M. GUZMÁN, A. M. CAMEÁN, AND J. BAUTISTA

Department of Applied Analytical Chemistry and Food Science,
Faculty of Pharmacy, University of Sevilla, Sevilla, Spain

Received January 13, 1981

INTRODUCTION

Colorimetric determination is an advantageous practice that can be used in the high and low pH ranges. With this aim some indicators were proposed (*5*, *6*, *7*). In a previous work (*1*), one of the authors proposed a new indicator, pyridine-2-aldehyde *p*-nitrophenylhydrazone (PANPH), which displays undoubted advantages over benzaldehyde *p*-nitrophenylhydrazone (*8*). In the present work, a preliminary study of 6-methylpyridine-2-aldehyde *p*-nitrophenylhydrazone is performed, showing consideration for the inductive (+*I*) and mesomeric effect of the methyl group on the indicator's characteristics.

MATERIALS AND METHODS

Reagents. 6-Methylpyridine-2-aldehyde *p*-nitrophenylhydrazone was synthesized by refluxing equimolar quantities of *p*-nitrophenylhydrazone with 6-methylpyridine-2-aldehyde in ethanolic solutions. The crude product, yellow-brown crystals, was separated and recrystallized from ethanol (mp, 222–225°C). Found: 60.69% C, 21.62% H, 4.90% N. Calculated for $C_{13}N_4H_{12}O_2$: 60.93% C, 21.87% H, 4.68% N. The infrared spectrum was obtained (KBr discs), and the bands were assigned (cm^{-1}) to the stretching vibrations of –NH– (3000 w), C=N (hydrazone) (1620 w), C=N (aromatic) (1300 m, 1270 m), pyridine ring (1170 m, 1110 s, 800 m), $-NO_2$ (1510 s, 1330 s, 760 m); skeletal vibrations of aromatic ring (1460–1610) and the bending vibrations of $-CH_3$ (1385 m, 1460 s). A 0.05% (w/v) ethanolic solution of 6-methylpyridine-2-aldehyde-*p*-nitrophenylhydrazone (6-Me-PANPH) was used.

Buffer solutions of constant ionic strength were prepared by using appropriate mixtures of sodium borate, hydrochloric acid, sodium hydroxide, and potassium chloride (*9*). Likewise, acetate buffer, ammonia–ammonium chloride buffer, and ammonium acetate were used.

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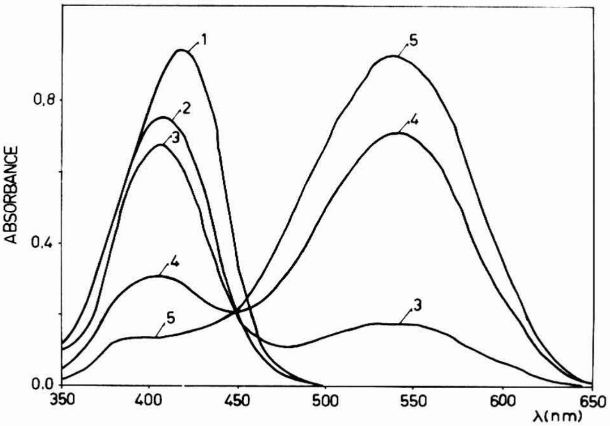


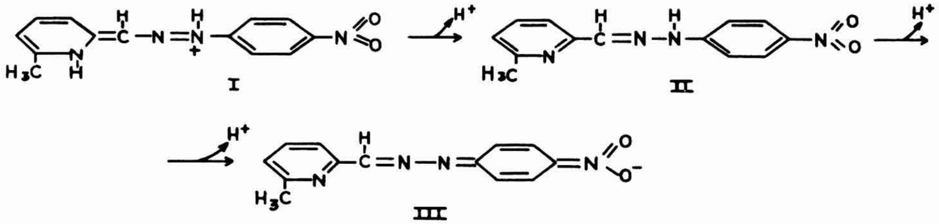
FIG. 1. Visible absorbance spectra of 6-Me-PANPH in aqueous-ethanolic (3 – 2) solutions at various pH values (4.10^{-5} M): (1) pH 2.5, (2) pH 5.8, (3) pH 11.4, (4) pH 11.8, (5) pH 12.8.

All solvents and reagents were of analytical grade.

Apparatus. Pye – Unicam SP 800 and Perkin – Elmer Coleman 55 spectrophotometers with 1.0-cm glass cells, a Metrohm E 1009 photometric titrator with a 4.0-cm glass cell, and a Phillips PW 9408 digital pH-meter with a combined glass – calomel electrode, were used.

RESULTS

Absorption spectra and indicator constant. Absorption spectra of 6-Me-PANPH, at various pH, are shown in Fig. 1. The isosbestic point was found at 450 nm. As the pH is raised, deprotonation of pyridin nitrogen occurs first, then hydracinic hydrogen is deprotonated and structural readjustment takes place (I, II, III), causing the quinoidal chromophore.



These structural modifications are related to a hypsochromic and hypochromic shift from 430 to 420 nm, continued in a bathochromic shift from 420 to 550 nm. In Fig. 2 absorbance versus pH, in the alkaline range, is recorded at 550 nm. From the resulting curve, the pH value corresponding to one-half color development, i.e., the apparent indicator constant, may

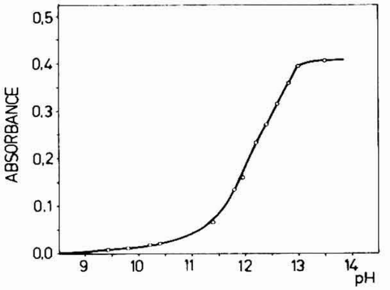


FIG. 2. Absorbance plotted against pH for 6-Me-PANPH solutions at 550 nm.

be obtained (pK_i^{ap} = 12.20). A rigorous estimate is carried out (*3*), showing the experimental data in Fig. 3. The obtained value $pK_i = 12.15 \pm 0.01$ (20°C, ethanol–water 2:3, ionic strength 0.01) was assigned to removal of the hydracinic hydrogen by base.

Color change interval. The determination of the transition interval was achieved by processing a buffer series, according to the transition interval to be expected, in 10 test tubes of equal color and diameter. Into every test tube 10 ml of buffer and 1.0 ml of indicator solution were pipetted. It was found that the transition interval (yellow to red-violet) lies between pH 10.4 and 12.4.

Intensity of color. The color intensity was measured by determining the specific extinction ($E_{1\%}^{1\,\mathrm{cm}}$) at the lower and upper pH limits. The results were

$$E_{1\%}^{1\,\mathrm{cm}}(420\ \mathrm{nm}) = 742, \qquad E_{1\%}^{1\,\mathrm{cm}}(550\ \mathrm{nm}) = 795.$$

Reversibility and stability of indicator solutions. Drop additions of 0.1 *M* hydrochloric acid into an alkaline solution of the indicator, and continue to add 0.1 *M* sodium hydroxide solution. This results in the solutions changing from red-violet to yellow and back to red-violet again.

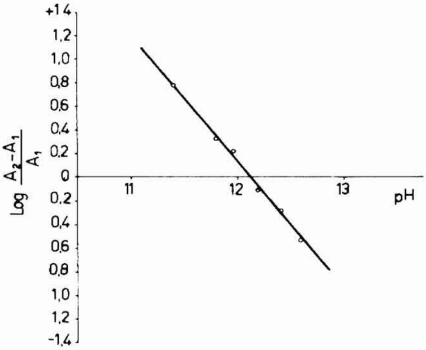


FIG. 3. pH-log($A_2 - A_1$)/A_1 plot for determining pK_i.

Indicator solutions were stored in the dark and diffuse daylight for some weeks. Absorption spectra recorded periodically showed the solutions to be stable. Red-violet dilute solutions ($4.0 \cdot 10^{-5}$ M) of 6-Me-PANPH, at pH 13, remain stable for 3½ hr.

DISCUSSION

In the alkaline range of pH, excluding some indicators like alizarine yellow, nitramine, and tropaeoline, no excellent indicator substances have been found. Those proposed in previous studies suffer from having a narrow transition interval, slight stability, and faint color intensity. Osazones and nitrophenylhydrazones (*2, 4*) can be regarded as suitable colorimetric indicators for the alkaline range. Modifications in the electronic density around the hydracinic hydrogen cause structural disturbance affecting transition interval, stability, and other properties. In this sense, the pyridine ring brings remarkable improvements in the indicator attributes. Thus, PANPH offers, in comparison with benzaldehyde *p*-nitrophenylhydrazone, a greater stability of red-violet color, a sharp change of transition interval of color, and a remarkable shift of maximum wavelengths.

6-Me-PANPH keeps the preceding characteristics and even enhances them, since the red-violet color remains stable for several hours. The inductive effect ($\pm I$) of the methyl group and the inductive and mesomeric effects of the aromatic and pyridine rings result in a decrease of hydracinic nitrogen basicity and, therefore, a decrease of pK_i.

SUMMARY

6-Methylpyridine-2-aldehyde *p*-nitrophenylhydrazone is suggested as an indicator for colorimetric pH measurements in the high-alkaline range. Absorption spectra and indicator constant have been reported. Color change interval, reversibility, and intensity of color have been established. A comparative study with regard to previously proposed pyridine-2-aldehyde and benzaldehyde *p*-nitrophenylhydrazones is made. A good stability of color is obtained.

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MICROCHEMICAL JOURNAL **27**, 6–12 (1982)

The Study of Iron(III) and Gold(III) Separation by Paper Chromatography Using Diisopropyl Ether

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INTRODUCTION

Diisopropyl ether (IPE) as a solvent or as a constituent of a solvent has not been adequately examined in chromatography, although metal as iron(III), indium(III), gallium(III), thallium(III), and gold(III) are readily extracted with IPE in the presence of a sufficiently high concentration of acid. Systems containing IPE are interesting due to their nonideal behavior expressed during extraction experiments. They form three liquid phases under certain conditions (*1*), show appreciable changes in the phase volumes depending on the composition of the system (*2*), and indicate strong sensitivity to temperature changes in a very narrow range (*3*).

In this paper the separation of iron(III) and gold(III) by paper chromatography has been described along with the study of factors influencing the chromatographic separation in systems containing diisopropyl ether.

MATERIALS AND METHODS

Reagents. Chemicals used in preparing the solvent were: benzene, Merck, p.a. (0.03% water maximum), hydrochloric acid, Merck, p.a. (d^{20}_{4} = 1.16), and diisopropyl ether (IPE), Merck, C.P. IPE was purified, distilled, and dried before use. Purification was achieved with successive treatments by iron(II) sulfate (saturated solution), potassium permanganate (0.5% solution), sodium hydroxide (0.5% solution), and distilled water. After drying by calcium chloride, IPE contained 0.85 mg H_2O/ml. Samples were prepared from iron(III) chloride hexahydrate, Hopkins and Williams, p.a., and gold(III) chloride tetrahydrate, Merck (48% Au minimum). Locating reagent solutions were prepared from p.a. chemicals. The iron(III) spot was located by a solution of ammonium tiocyanate and gold(III) spot by a solution of zinc sulfate and stannum(II) chloride mixture.

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Apparatus. Development by ascending technique was performed in cylindrical chambers. Two types of chambers were constructed and their dimensions can be seen in Fig. 1. The first type (Fig. 1a) is a combined chamber of changeable height which enables experiments with various paper lengths. The second type (Fig. 1b) is a jacketed and thermally insulated chamber which was connected to a thermostat which enables experiments at a predetermined constant temperature (t ± 0.05°C). The chambers were equipped with a simple device for the addition of the developing solvent. Thus the opening of the chamber after achieving equilibrium was avoided.

Procedure. Samples of iron(III) and gold(III) were prepared as 0.001 *M* solutions. Spotting was done by micropipet (2 μl) on Whatman No. 1 paper strips (2 × 27 cm). Samples were started 20 mm from the bottom end of a strip of paper. Spots were allowed to dry in air. The components of the heterogeneous solvent system were equilibrated in a separation funnel using equal initial volumes of aqueous phase and organic phase. Saturation of the atmosphere in the chamber was achieved by placing the equilibrate aqueous phase at the bottom of the chamber around the beaker

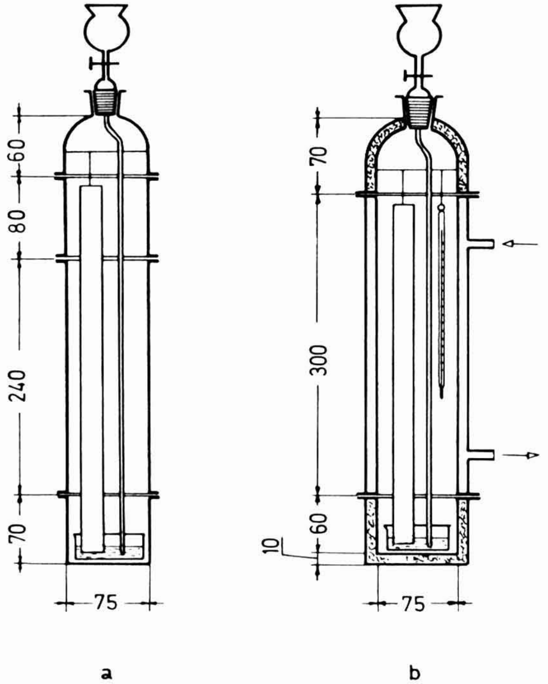

FIG. 1. Cylindrical chambers for ascending paper chromatography. (a) Combined chamber of changeable height. (b) Thermostatically controlled jacketed chamber.

holding the developing solvent (equilibrate organic phase). The paper strip was hung over the beaker. After 1 hr of equilibration the addition of the developing solvent through the long neck funnel raised the level of solvent 3 mm over the bottom end of paper strip and started a run. Development was always carried out over a distance of 20 cm. After removing the paper strip from the chamber it was allowed to dry in air and spots were developed by spraying with a locating reagent.

All experiments were carried out at 20 ± 0.5°C, except those where the temperature effect was investigated. Temperature effect was investigated in the range from 10 to 50°C by steps of 1 to 10°C.

Experiments with diluted IPE were carried out using benzene as an inert diluent. Content of IPE in the organic solvent was varied in the range from 0.71 to 6.40 *M* (pure IPE is 7.11 *M*).

Factors such as initial volume ratio of the organic phase to the aqueous phase volume, length of the paper strip, distance from the start line to the developing solvent level, and amount of substance were chosen on the basis of the experimental investigation of the system examined.

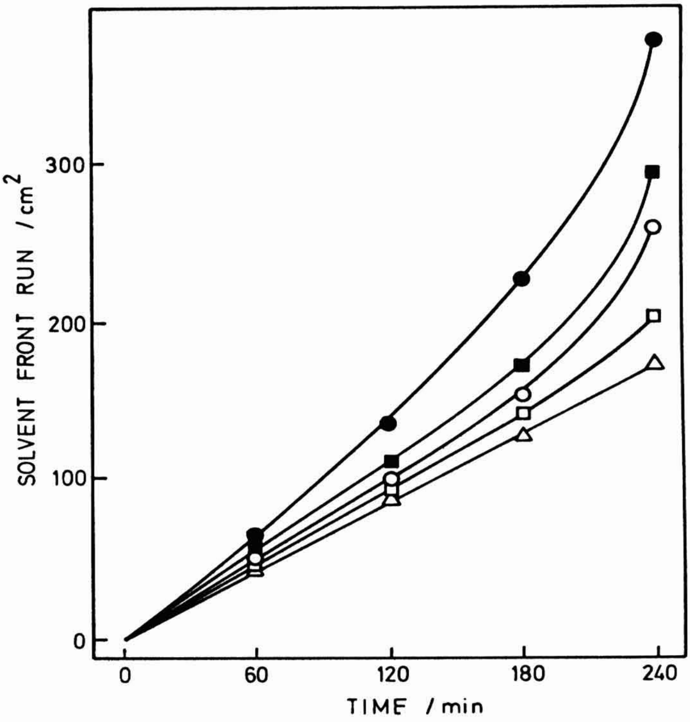

FIG. 2. Front run vs the time at different initial hydrochloric acid concentrations (△, 3 *M;* □, 5 *M;* ○, 7 *M;* ■, 9 *M;* ●, 11 *M*). Ascending technique. System: hydrochloric acid–IPE. Temperature, 20°C. Whatman No. 1 paper.

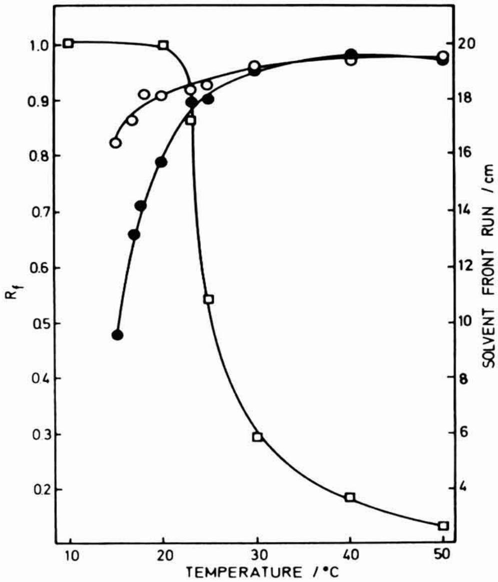

FIG. 3. R_f values of iron(III) (○) and gold(III) (●) and solvent front run (☐) vs the temperature. Ascending technique. System: hydrochloric acid (10 *M*)–IPE. Whatman No. 1 paper.

RESULTS AND DISCUSSION

Blank run investigations of initial acid concentration influence on the velocity of the solvent front movement indicate that an increase in the initial hydrochloric acid concentration increases the travelled distance of solvent front (Fig. 2). But in sample runs the mentioned influence was less regular and the results were poorly reproducible. This can be explained by the irregular change of the mobile phase viscosity in the sample run caused by the presence of extractable complex metallohalogenic acid $HFeCl_4$ and (or) $HAuCl_4$ and an enhanced acid transfer into the mobile (organic) phase; and by the strong change of the phase cross section, resulting in a change of phase number. Such an explanation was supported by the extraction data. For instance, at a 10 *M* initial hydrochloric acid concentration, in $H_2O-HCl-IPE$ and $FeCl_3-H_2O-HCl-IPE$ systems, the concentration of hydrochloric acid in the organic phase was 0.09 and 2.05 *M*, respectively. Another example illustrating the change of number of phases: the $AuCl_3-H_2O-HCl-IPE$ system was three phases at 10.0 *M* initial hydrochloric acid concentration, two phases at 10.6 *M*, and one phase at 11.2 *M*.

The temperature effect was investigated in the range of 10 to 50°C and the developing solvent was equilibrated by 10 *M* hydrochloric acid. From

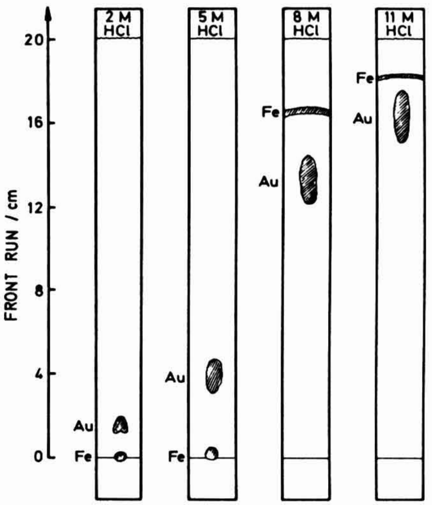

FIG. 4. Paper chromatograms of iron(III) and gold(III). Ascending elutions of Whatman No. 1 paper strips. Influence of the acid concentration on the size and shape of the spot. Temperature, 20°C.

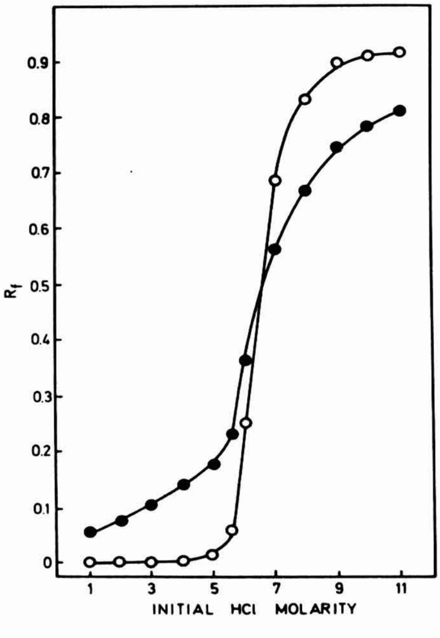

FIG. 5. R_f values of iron(III) (○) and gold(III) (●) vs the initial hydrochloric acid concentration. Ascending technique. System: hydrochloric acid–IPE. Temperature, 20°C.

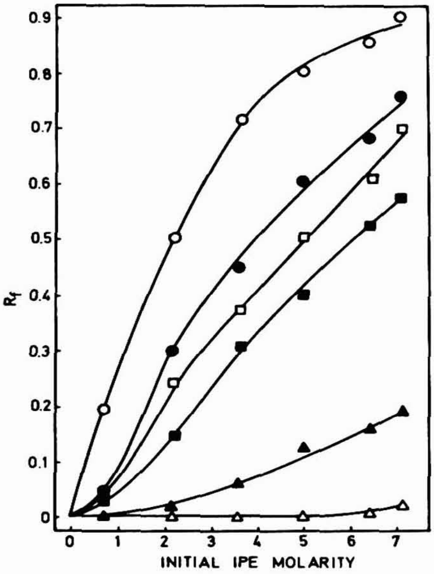

FIG. 6. R_f values of iron(III) and gold(III) vs the initial IPE concentration at different initial acid concentrations. Ascending technique. System: hydrochloric acid–IPE–benzene. Temperature, 20°C. Whatman No. 1 paper. Iron(III): △, 5 *M* HCl; □, 7 *M* HCl; ○, 9 *M* HCl. Gold(III): ▲, 5 *M* HCl; ■, 7 *M* HCl; ●, 9 *M* HCl.

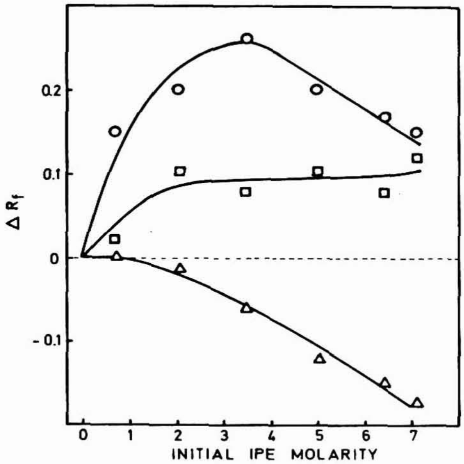

FIG. 7. Difference between R_f values of iron(III) and gold(III) vs the initial IPE concentration at different initial hydrochloric acid concentrations (△, 5 *M;* □, 7 *M;* ○, 9 *M*). Ascending technique. System: hydrochloric acid–IPE–benzene. Whatman No. 1 paper. Temperature, 20°C.

Fig. 3 it can be seen that the solvent front run strongly decreases with an increase in temperature from 21–50°C and at 50°C the travelled distance was only 2.5 cm. R_f values of iron(III) and gold(III) increase with an increase in temperature. This is strongly expressed in the range from 15 to 30°C especially in the case of gold(III). One can conclude that under the investigated conditions a decrease of temperature is favorable to the separation of iron(III) and gold(III).

Some of the typical chromatograms representing the size and shape of spots at various initial concentrations of acid are shown in Fig. 4. R_f values of iron(III) and gold(III) increase with an increase in the initial hydrochloric acid concentration (Fig. 5). A very strong change was found in the range from 6.0 to 8.0 *M*, and the best separation was at a 5.5 *M* initial acid concentration.

The experiment with diluted IPE shows that an increase in the IPE concentration increases the R_f values of both metals, and such an effect is stronger at higher acid concentrations (Fig. 6). The best separation was achieved with 9.0 *M* initial acid concentration and 3.55 *M* initial IPE concentration (50% volume) when the R_f difference (ΔR_f) was 0.26 (Fig. 7).

By proper choice of conditions it is possible to achieve good separation in a desired region of R_f values.

SUMMARY

The influence of experimental conditions on the chromatographic behavior and separation efficiency of iron(III) and gold(III) in partition paper chromatography was investigated, when solvents containing diisopropyl ether (IPE) were used. The influence of temperature (range investigated from 15 to 50°C), initial acid concentration (1–11 *M* HCl), and concentration of active solvent component (0.7–7.1 *M* IPE, benzene as diluent) on the R_f values were particularly studied. Ascending technique using Whatman No. 1 paper was employed. Considerable influence of temperature was found. The best separation ($\Delta R_f = 0.26$) was achieved with 9 *M* concentration of hydrochloric acid and 3.6 *M* (50% volume) concentration of IPE.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 27, 13–17 (1982)

Sensitive Spectrophotometric Determination of Gallium with Bromopyrogallol Red in the Presence of Surfactants

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INTRODUCTION

This paper is part of general research about the use of xanthene dyes as reagents in the presence of surfactants for very sensitive spectrophotometric determination of metal ions (*1–3*). The sensitizing effect of surfactants on color reaction is now well known. Previously, a sensitive method for the determination of gallium, based on the ternary gallium–pyrogallol red–cetyltrimethylammonium bromide system was described (4).

This paper presents a sensitive method for the determination of gallium on the basis of the ternary color systems formed with brompyrogallol red in the presence of surfactants. The following surfactants were used: cetyltrimethylammonium bromide and nonionic surfactant, e.g., nonylphenol tetradecaethylene glycol ether.

EXPERIMENTAL

Reagents and Apparatus

Gallium stock solution. 1 mg Ga/ml. Dissolve 0.1000 g of the metal in 10 ml of 7 *M* hydrochloric acid and dilute the solution with 1 *M* hydrochloric acid to 100 ml. Dilute further with 1 *M* hydrochloric acid as required.

Bromopyrogallol red (*BPR*). 3.4×10^{-4} *M*. Dissolve 50 mg of the reagent in 125 ml of ethanol and dilute with water to 250 ml.

Cetyltrimethylammonium bromide (*CTA*). 4×10^{-3} *M*. Dissolve 0.3750 g of the reagent in water and dilute with water to 250 ml.

Nonylphenol tetradecaethylene glycol ether (*NTGE*). 1.8×10^{-3} *M*. Dissolve 0.3750 g of the reagent in water and dilute with water to 250 ml.

Buffer solutions (*pH 6.9 and 5.0*). An aqueous 10% hexamine solution, adjust the pH to 6.9 or 5.0 with dilute hydrochloric acid.

Apparatus. Spectrophotometer Varian Techtron Model 635, 10-mm cells, N-517 pH meter (Polish).

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Procedure I

Dilute a sample of test solution, containing 5 to 25 μg of gallium to about 10 ml in a beaker. Add 5 ml of BPR and adjust the pH to 2.5 with ammonia. Transfer this solution to a 25-ml standard flask, fill with water to the mark, and mix. After 10 min, measure the absorbance of the solution at 525 nm against a blank as a reference.

Procedure II

Dilute a sample of test solution, containing 2.5 to 10 μg of gallium to about 10 ml in a beaker. Add 3 ml of BPR and 3 ml of CTA and adjust the pH to approx. 5.0 with ammonia. Transfer this solution to a 25-ml standard flask, add 3 ml of buffer solution, fill with water to the mark, and mix. After 15 min, measure the absorbance of the solution at 625 nm against a blank as a reference.

Procedure III

Add 3 ml of BPR solution and 4 ml of NTGE solution to acidic sample solution containing 5 to 15 μg of gallium. Adjust the pH to approx. 6.9 with ammonia, and transfer this solution to a 25-ml standard flask. Add 3 ml of buffer solution, fill with water to the mark, and mix. After 15 min, measure the absorbance of the solution at 615 nm against a reagent blank.

RESULTS AND DISCUSSION

Binary System

Gallium ions react with bromopyrogallol red in the acid range (pH 1.5–4.0) and forms an orange-red complex. The optimum pH is 2.5 ± 0.1, at which $\lambda_{\max}$ of the complex is 525 nm.

The maximum absorbance was observed in the range $6.8 \times 10^{-5}-9.1 \times 10^{-5}$ M of BPR. A Job plot shows the molar ratio of gallium to BPR is 1:1. Under the conditions used in Procedure I Beer's law is obeyed up to 1 μg of Ga/ml. The molar absorptivity at 525 nm is 3.5×10^4 liters mol^{-1} cm^{-1}.

Ternary System

An excess of surfactants causes a bathochromic shift of the absorption maximum from 525 to 615 nm in the presence of NTGE, and 525 to 625 nm in the presence of CTA. The absorption spectra of BPR and the binary and ternary complexes are shown in Fig. 1.

Effect of pH

The absorbance and optimum wavelength of the ternary complex changes with pH (3.5–8.0). This is illustrated by the absorption spectra of the Ga–BPR–CTA complex shown in Fig. 2. However, the

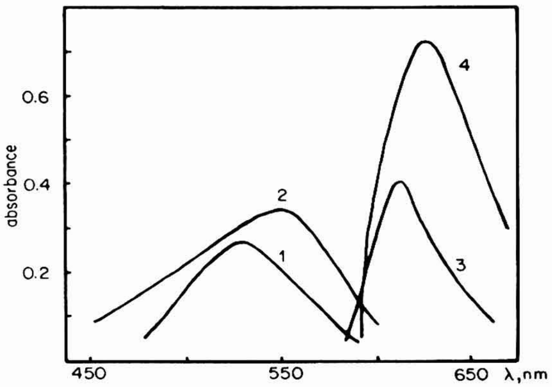

FIG. 1. Absorption spectra of pH 2.5: (1) Ga–BPR complex, $C_{Ga} = 8.3 \times 10^{-6}\ M$, $C_{BPR} = 6.8 \times 10^{-5}\ M$ measured against a blank. pH 5.0: (2) bromopyrogallol red, $C_{BPR} = 2.5 \times 10^{-5}\ M$ measured against water: (3) Ga–BPR–NTGE complex, $C_{Ga} = 5.7 \times 10^{-6}\ M$, $C_{BPR} = 4.0 \times 10^{-5}\ M$, $C_{NTGE} = 2.8 \times 10^{-4}\ M$ measured against a blank: (4) Ga–BPR–CTA complex, $C_{Ga} = 5.7 \times 10^{-6}\ M$, $C_{BPR} = 4.0 \times 10^{-5}\ M$, $C_{CTA} = 4.8 \times 10^{-4}\ M$. pH 6.9.

Ga–BPR–NTGE complex does not change optimum wavelength in this pH range. Further measurements were carried out at 615 nm in the presence of NTGE and at 625 nm in the presence of CTA. The optimum pH range for formation of the Ga–BPR–CTA complex is 4.8–5.2 and for the Ga–BPR–NTGE complex 6.8–7.2 (Fig. 3).

Hexamine buffer can be used.

Effect of BPR and Surfactants Concentration

The dependence of the absorbance of the ternary complexes on the BPR concentration was examined at large excess of surfactants (40-fold

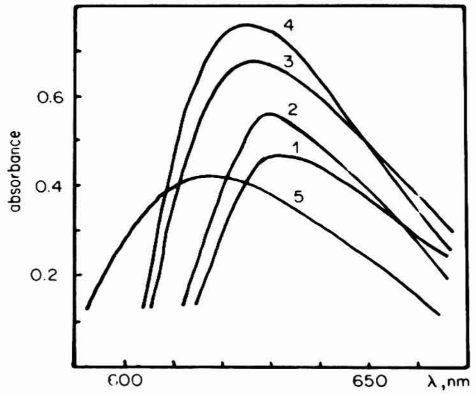

FIG. 2. Absorption spectra of gallium complex with BPR and CTA at different pH's: $C_{Ga} = 0.4\ \mu g/ml$, $C_{BPR} = 4.0 \times 10^{-5}\ M$, $C_{CTA} = 4.8 \times 10^{-4}\ M$; (1) pH 3.5, (2) pH 4.0, (3) pH 6.0, (4) pH 5.0, (5) pH 8.0.

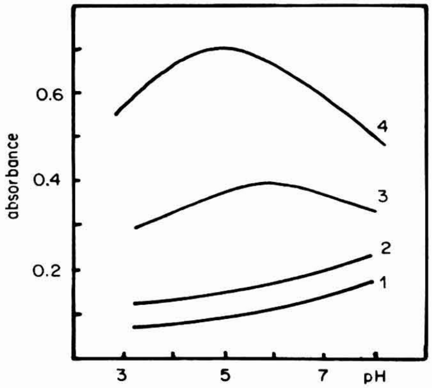

FIG. 3. Effect of pH at 615 and 625 nm, $C_{Ga} = 0.4\ \mu g/ml$, $C_{BPR} = 4.0 \times 10^{-5}\ M$, $C_{CTA} = 4.8 \times 10^{-4}\ M$, $C_{NTGE} = 2.8 \times 10^{-4}\ M$ of: (1) BPR + CTA at 625 nm, pH 5.0; (2) BPR + NTGE at 615 nm, pH 6.9; measured against water: (3) Ga–BPR–NTGE at 615 nm, pH 6.9, (4) Ga–BPR–CTA at 625 nm, pH 5.0 measured against blank.

ratio to Ga). The concentration of the BPR varied in the range 1.3 to $6.8 \times 10^{-5}\ M$. The maximum absorbance was observed when the molar excess of BPR over gallium was somewhat higher than fourfold.

The effect of changes in the concentration of surfactants on the absorbance of the ternary complex was determined by measuring the absorbance at 615 and 625 nm. Optimum wavelength of the ternary complexes does not change on the surfactants concentration. The maximum absorbance of the ternary complexes was observed when molar excess of CTA to Ga is 60-fold and NTGE 35-fold. At low CTA concentration (twofold ratio to Ga) the solution becomes turbid.

Also cetyldimethylbenzylamonium chloride has been used to the determination of gallium with BPR. At low and high CDBA concentrations the solutions are not clear, because the ternary complex precipitates.

Beer's Law

Under the conditions used in Procedure II Beer's law is obeyed over the range 0.1–0.4 μg/ml of gallium. The molar absorptivity is 1.3×10^5 liters $mol^{-1}\ cm^{-1}$. However according to Procedure III Beer's law is obeyed over the range 0.2–0.6 μg of Ga/ml and molar absorptivity is 7.0×10^4 liters $mol^{-1}\ cm^{-1}$.

The present method (Procedure II) is very sensitive. Its sensitivity is greater than that of the method using pyrogallol red with CTA (molar absorptivity = 1.0×10^5).

Effect of Foreign Ions

EDTA and oxolates interfere. Tartrates, citrates, and acetates do not interfere as well as fluoride and phosphate at a concentration of 1 mg/25 ml can be tolerated in the determination of gallium. Numerous metals interfere, e.g., Fe(III), Fe(II), W, Sn, Mo, Ti, Bi, V, but these interferences can be easily eliminated by preliminary extraction of gallium from 7 *M* hydrochloric acid with diisopropyl ether. This method was described previously (*4*).

Complex Formation

Job's method of continuous variations has been used to establish the molar ratio of the gallium complex in the presence of surfactants. A Job plot shows the molar ratio of gallium to BPR is 1:3.

SUMMARY

Gallium in the presence of surfactants (NTGE and CTA) forms with BPR a violet ternary complexes with λ_{max} at 615 and 625 nm, respectively, with molar absorptivities of 7.0×10^4 and 1.3×10^5 liters mol^{-1} cm^{-1}. These complexes can be advantageously used for the determination of gallium. The molar ratio of gallium to BPR, which is 1:1 in the binary complex, increases to 1:3 in the ternary complex. The presence of surfactants changes the number of BPR molecules bonded to gallium. This is the main factor in the increase in color intensity. Numerous metals interfere. Gallium can be separated by extraction of gallium from 7 *M* hydrochloric acid with diisopropyl ether.

ACKNOWLEDGMENT

The author would like to thank Prof. A. Cygański for discussions.

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A Water Purification System for Microanalysis

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INTRODUCTION

Water purification has been treated extensively for laboratory or manufacturing purposes. A conductivity water level is required (*1*). Essentially two principles are applied, distillation and/or deionation. Either of these alone does not achieve efficienctly the purification level required for microanalysis. Organic matter contaminated with the main water cannot be removed by one distillation process; much of the materials used for destruction are consumed (*3*). Distillation alone is not effective in demineralization. Deionation alone gives sufficiently salt-free water with fast exhaustion of the resins and incomplete removal of the nonionic organic matter; the resin may adsorb some of it leading to decrease in capacity (*6*).

The most efficient system involves distillation followed by deionation. One of the best purification systems for analytical purposes is that constructed by Hole *et al.* (*2*), in which a conductivity water level is achieved. The system is relatively expensive and not simple, but the amount of purified water is satisfactory.

A simpler system is described which could be assembled in a laboratory from known Quickfit units with some glass elaboration. Moreover it involves double distillation and deionation. The organic matter is destroyed in the second distillation step and the remainder is adsorbed by the resins. Thus chemically, economically, and in regard to simplicity in design and construction, it is better than the former system.

CHEMICAL PROCESSING

Tap water introduced to the system (Fig. 1) at a certain dropping rate is first distilled to remove as far as possible the salts contained. A second distillation follows from 1 g potassium permanganate/2 liters in 2 ml conc. sulfuric acid/2 liters. The double-distilled water is passed through a column of Amberlite IR-120 resin in the acid form, then through another of Amberlite IRA-400 resin in the hydroxyl form. Exhaustion is indicated by the color change of the anion resin from yellow to brownish-orange. The production rate of the apparatus ranges between 1 and 2 liters per hour depending on the efficiency of the heaters.

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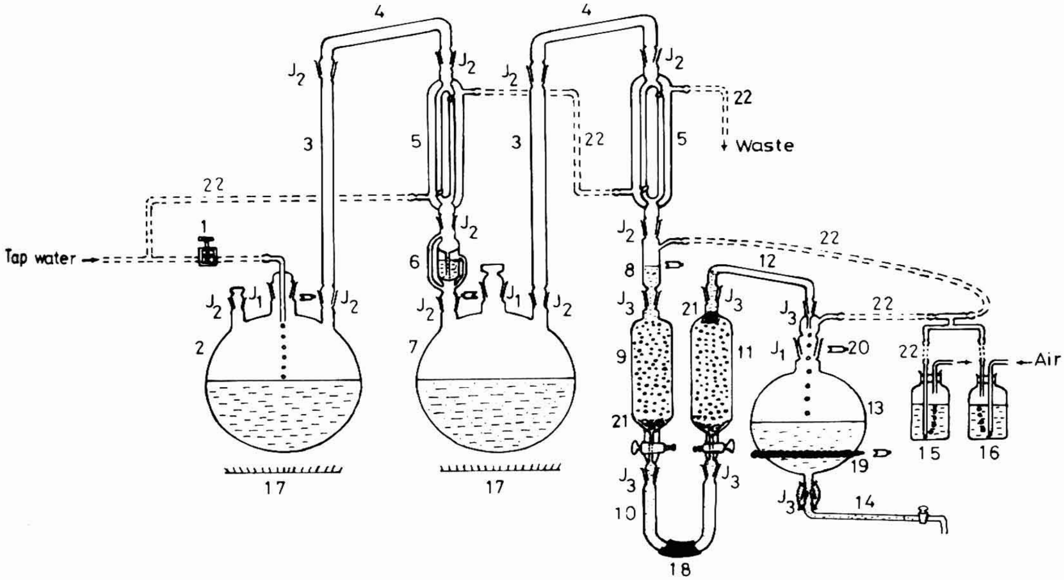

FIG. 1. The water purification apparatus. Detailed units are given in Table 1.

The purified water by this system proved the validity for micro and submicroanalysis by conductivity and spectrophotometry. The conductance amounted to 6 – 10 × 10^{-6} mho. Conductance does not indicate the content of the nonionic species (*1*, *5*). The copper spectrophotometric method (*4*) obeys Beer's law in the low range 0.1 – 1 ppm; using a second distilled water (alkaline medium) the points of the calibration curve are scattered around a straight line, while under the same conditions but using the water produced by the present system the construction points are strictly aligned. This indicates the complete removal of organic matter, a new test.

APPARATUS

The apparatus (Fig. 1 and Table 1) consists of a first distillation unit fed with tap water through a dropper. The stills of the first and second distillations are of similar units. To prevent back second distillation a regulating valve is placed in between the two stills, a distinguishing feature of the design. Air outlet during warm up is allowed through a water bubbler; a ganged inlet bubbler containing 50% sodium hydroxide protects the system from the atmospheric carbon dioxide which could be introduced with air during cooling. The dropping rate of the feed water is adjusted to nearly that of the deioner outlet in the reservoir. The first fill of the latter is rejected. Preferably the reservoir has to be coated internally with silicon. Cleaning of the boiler flasks is performed in place through a suction pump.

TABLE 1

No	Type of unit	No	Type of unit
1	Screw clips of tap.	11	Separating funnel[a] (D 12/200) for anion resin
2	Boiler flask[a] (5 liters) with dropper[b]	12	Link tube[a] (SH 2/22)
3	Vigreux air condenser (FC 2/28 or FC 7/43)[a]	13	Reservoir[a] (5 liters), with dropper and socket for the tap[b]
4	Link tube (SH 2/22)[a]	14	Flexible tap with spirals[b]
5	Water condenser, coil type (CG/31)[a] or Davis type	15	Water bubbler[b]
		16	50% NaOH bubbler[b]
6	Pressure regulating valve[b]	17	Heating mantle or ring flame
7	Boiler flask[a] (5 liters)	18	Rubber shield
8	Adapter with side tube[b]	19	Iron ring coated with asbestos
9	Separating funnel[a] (D 12/200) for cation resin	20	Clamp to stand
10	U tube[a]	21	Glass wool, or sintered glass disc at the bottom
		22	Polyethylene or rubber tubing

[a] Quickfit unit.

[b] Glass elaborated. For non-Quickfit glass, joints J_1, B34; J_2, B24; J_3, B19.

SUMMARY

A water purification system producing conductivity water is described. It involves first distillation, second distallation from acidified potassium permanganate, and deionation by separate bed strong cation and anion-exchange resins. The purified water proved the validity for microanalysis by conductivity and spectrophotometry. The apparatus can be assembled easily in a laboratory from all Pyrex glass Quickfit units with some glass elaboration.

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MICROCHEMICAL JOURNAL 27, 21–25 (1982)

Indirect Spectrophotometric Determination of Cerium(IV)

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INTRODUCTION

Although numerous procedures are available for the spectrophotometric determination of cerium(IV), they are all in some way unsatisfactory (*1*–*3*). Accordingly, it was thought advantageous to propose a method that eliminates many of the difficulties encountered in the literature methods used for the ion under investigation.

In the course of studies in our laboratories, it was found that after the reaction of the titled ion with excess ferrous ion in acidic buffer solution, an immediate, stable, water-soluble, blue-colored complex with tiron is formed, the intensity of which is proportional to the amount of cerium(IV) originally present in the sample solution. Moreover, the developed method resorts neither to a temperature control nor to a time-consuming and tedious extraction step.

MATERIALS AND METHODS

Reagents

All chemicals used were of analytical-reagent grade.

Standard cerium(IV) solution (100 μg/ml). A 0.28853-g sample of ceric sulfate tetrahydrate was dissolved in distilled water; 2 ml of concentrated sulfuric acid was added; the solution was heated until clear and then cooled; and the volume was completed to 1000 ml with distilled water.

Iron(II) solution (3×10^{-3} *M*). A 0.12-g sample of ammonium ferrous sulfate was dissolved in distilled water and the volume was diluted to 100 ml.

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Buffer(pH 3) solution. To 50 ml of 0.1 *M* potassium hydrogen phthalate solution, 22.3 ml of 0.1 *M* hydrochloric acid was added and the volume was diluted to 100 ml.

Tiron solution (7×10^{-3} M). A 0.224-g sample of the disodium salt of the compound was dissolved in distilled water and the volume was made up to 100 ml.

Interfering ion solutions. A 1000 μg/ml solution of each tested ion was prepared.

Apparatus

All absorption measurements were made on a Shimadzu UV-210A double-beam spectrophotometer and a Bausch & Lomb Spectronic 20 spectrophotometer, using matched 1-cm optical glass cells.

Procedure

An aliquot of the sample solution containing 10−600 μg cerium(IV) was transferred to a series of 10-ml volumetric flasks, and 1 ml of pH 3 buffer solution, 1 ml of 3×10^{-3} *M* iron(II) solution, and 2 ml of 7×10^{-3} *M* tiron solution were added, and the volume was completed to the mark with distilled water. The absorbance was measured against a reagent blank, prepared in the same manner but containing no cerium(IV), at 650 nm using 1-cm cells. The color develops immediately and is stable for a long time (>2 days). A rectilinear curve passing through the origin was obtained, indicating that Beer's law was followed over the concentration range 10−600 μg cerium(IV) in a final volume of 10 ml, i.e., 1−60 ppm. The apparent molar absorptivity (referred to cerium(IV)) in the region of least photometric error was 2.1×10^3 liters mol^{-1} cm^{-1} and the sensitivity index, according to Sandell (*4*), was 0.0667 μg cm^{-2}.

RESULTS AND DISCUSSION

When tiron solution was added to cerium(IV) solution, containing an excess of iron(II) in acidic buffer solution, a stable blue color developed immediately with maximum absorption at 650 nm. This wavelength, where the blank showed nearly negligible absorbance, was adapted in the subsequent experiments.

Effect of pH

It is known that the reaction between cerium(IV) and iron(II) and the formation (*5*) of the resulting blue tiron−iron(III)-colored complex ion are pH dependent. Therefore, the effect of pH on color formation and intensity was first established. A series of buffer (pH 1−6) solutions were tested. Buffers of pH 5 and 6 developed a violet color in the blank, while pH 1, 2, and 4 could not develop full color intensity with the latter, a faint blue color developed in the blank. The use of 0.5 – 1.5 ml of pH 3 buffer gave maximum constant absorbance and a colorless blank, and 1 ml was adapted for the procedure.

Effect of Iron(II) Solution

The results of the experiments showed that at least 0.5 ml of 3×10^{-3} *M* iron(II) solution should be present in the reaction mixture. In this study 1 ml of iron(II) at a prescribed concentration was used to ensure quantitative determination at the upper limit of the calibration curve.

Effect of Tiron Solution

The experimental data revealed that 1.5 – 2.5 ml of 7×10^{-3} *M* solutions were suitable for the optimal constant absorbance. A 2-ml volume of the reagent was selected for the procedure.

Standing Time and Stability Period

Under the optimized conditions, the color still developed instantaneously and remained stable for a long time (>2 days).

Accuracy and Precision of the Method

To test the reliability of the method, standard cerium(IV) samples were analyzed for cerium by the recommended procedure. The results (of five determinations) presented in Table 1 show that cerium(IV) can be determined precisely and accurately.

Interferences

The effect of some oxidizing, reducing, and complexing agents and a very few cations on the indirect spectrophotometric determination of cerium(IV) was considered in the present study. The tolerable amount of each, in the presence of 300 μg cerium(IV)/10 ml, is shown in Table 2. The figures in Table 2 correspond to an error of ±2% in the absorbance reading. However, the seriously interfering anions can simply be removed by the use of a cation exchanger. Thus, cerium(IV) will be trapped by the resin and the anions will pass; then it is eluted and determined.

TABLE 1
ANALYTICAL DATA ON THE DETERMINATION OF CERIUM(IV)

Cerium(IV) (μg)	Error (%)	Standard deviation (%)
10	+1.3	5.5
300	0.0	1.2
600	0.0	0.8

TABLE 2
INTERFERING EFFECT ON THE DETERMINATION OF CERIUM(IV)

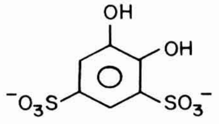
Interferent	Permissible amount (μg)
Al^{3+}	3800
Pb^{2+}	450
Mg^{2+}	400
As^{3+}	300
Hg^{2+}	50
NO_3^-	5500
SO_3^{2-}	5000
HCO_3^-	2000
PO_4^{3-}	1800
IO_3^-	1500
NH_3	1300
$S_2O_8^{2-}$	1100
CO_3^{2-}	800
CN^-	350
NO_2^-	250
SCN^-	50
EDTA (disodium salt)	40
MnO_4^-	30
$S_2O_3^{2-}$	20
$Cr_2O_7^{2-}$	15
$C_2O_4^{2-}$	15
CrO_4^{2-}	10
S^{2-}	10
F^-	10
I^-	10

Color Reaction Mechanism

The following pathway has been suggested for the formation of the blue chromophore:

$$Ce^{4+} + Fe^{2+} \longrightarrow Ce^{3+} + Fe^{3+}, \qquad (1)$$

Fe^{3+} + pH 3 +



$$\xrightarrow{(1)} Fe\left[C_6H_2O_2(SO_3)_2\right]^- . \qquad (2)$$

SUMMARY

A simple and very fast photometric method for the determination of trace levels of cerium(IV) has been developed. Use has been made of the reaction in which iron (II), on oxidation with cerium(IV), gives iron(III) which is complexed with tiron, in acidic medium, to form a stable blue color, the intensity of which is in direct proportion to the amount of cerium(IV) originally present in the sample solution. In addition, the method has a wide

range of determination and reasonable sensitivity, and it avoids both temperature control and the tedious extraction step.

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Fluorimetric Determination of Vanadium Based on Its Reaction with 1-Amino-4-hydroxyanthraquinone

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INTRODUCTION

The aminohydroxyanthraquinones have been used for the determination of nitrites (*14*), as photometric reagents (*6–9*) and as oxidation–reduction (*1*) and acid–base indicators in glacial acetic acid (*3*).

White and associates have studied 1-amino-4-hydroxyanthraquinone and proposed it as a reagent in the qualitative fluorimetric determination of beryllium and thorium (*15–17*), based on the red fluorescence it produces with beryllium(II) in an alkaline solution and with thorium(IV) in a weak acid solution. Dissolved in H_2SO_4, this reagent can be employed for the detection (*10*) and quantitative determination (*2*) of boron.

In this paper we describe the reaction and analytic properties of 1-amino-4-hydroxyanthraquinone by fluorimetric techniques and the spectrofluorimetric determination of traces of vanadium(V).

MATERIALS AND METHODS

Equipment. FIKA 55 MK II spectrofluorimeter. UVATOM-70 ultraviolet lamp providing excitation wavelengths of 254 and 360 nm. CRISON 501 digital pH measurer, fitted with a combined glass-calomel electrode.

Reagents and solutions. Solutions of 0.1% of EGA-CHEMIE 1-amino-4-hydroxyanthraquinone in ethanol and recrystallized by us in ethanol were used. A solution of NH_4VO_3 (Merck), containing 0.755 g/liter of vanadium, was standardized gravimetrically (*13*). A pH 1.2 (HCl–KCl) buffer solution was prepared according to Clark and Lubs (*5*).

Procedure for the determination of vanadium(V). Five milliliters of ethanol, 2 ml of a 4.2×10^{-4} *M* solution of 1-amino-4-hydroxyanthraquinone (28% ethanol), a quantity of vanadium(V) necessary to ensure a final concentration of 0.06 to 0.36 ppm, and 10 ml of pH 1.2 buffer solution were placed in a 25-ml volumetric flask and filled to the mark with deionized water. With an excitation wavelength of 480 nm, the

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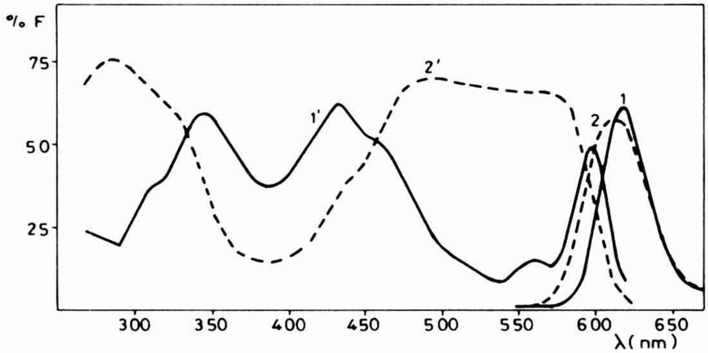

FIG. 1. Excitation (1',2') and emission (1,2) spectra of 1-amino-4-hydroxyanthraquinone. Curve (1',1) [R] = 5×10^{-4} *M* (λ_{ex} = 434 nm, λ_{em} = 620 nm); curve (2',2) [R] = 10^{-4} *M* (λ_{ex} = 530 nm, λ_{em} = 610 nm). Sensitivity ×40.

fluorescence emitted at 573 nm was measured, after 60 min, against a reagent blank.

RESULTS AND DISCUSSION

Reaction and Analytical Properties of the Reagent

The fluorescent reaction of the reagent with inorganic ions in differing media has been studied. In addition to the reactions with beryllium(II) and thorium(IV) already mentioned, it also reacts with gold(III), cerium(IV), and vanadium(V) in hydrochloric acid with sensibilities whose p*D*s are 6.0, 6.1, and 6.5, respectively. A variation is observed in the position of the peaks of the excitation spectra with reagent concentration (Fig. 1). This change is described in the literature (*11*) for some hydroxyanthraquinones and is attributable to the formation of molecular associations.

The acid–base dissociation constant was determined according to the Pease and Williams method. The p*K* value was found to be 10.6 and corresponds to the hydroxyl group (Fig. 2).

Moderate concentrations of oxidizing substances alter the fluorescence of the reagent and consequently its excitation and emission spectra.

Reaction between 1-Amino-4-hydroxyanthraquinone and Vanadium(V)

Excitation and emission spectra. Excitation and emission spectra of the oxidation product and the reagent in identical acidic conditions are shown in Fig. 3 where a shift in the emission peak can be observed from 620 to 573 nm (in the presence of vanadium) as well as an increase in the fluorescence intensity.

Factors influencing the fluorescence intensity. The fluorescence intensity of the oxidation product is greatest at pH 1.2 (Fig. 4).

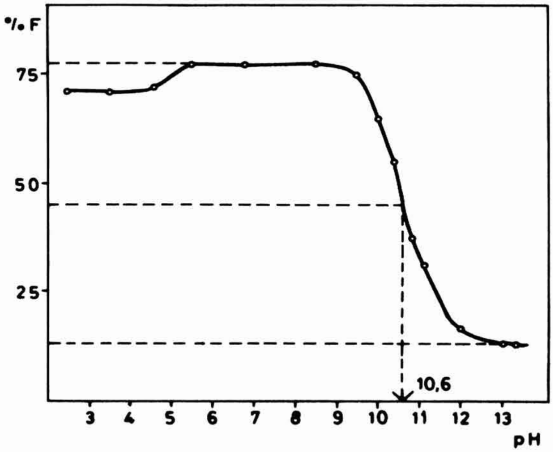

FIG. 2. Fluorimetric determination of the ionization constant of 1-amino-4-hydroxyanthraquinone. [R] = 5×10^{-5} *M*, 20% ethanol (λ_{ex} = 530 nm, λ_{ex} = 610 nm). Sensitivity ×100.

The amount of ethanol has a notable effect on the fluorescence intensity. When this is less than or equal to 20%, the reagent precipitates. Maximum fluorescence is obtained at an ethanol concentration of 28 to 40% and above this concentration fluorescence diminishes.

In 28% ethanol, the fluorescence intensity is maximum after 45 min after preparation of the sample and remains constant for at least 3 hr. When the ethanol concentration is 40%, however, 2.5 hr are required to attain maximum fluorescence.

For the cation concentrations studied, the optimum concentration of 1-amino-4-hydroxyanthraquinone is 3.4×10^{-5} *M*, emission fluorescence

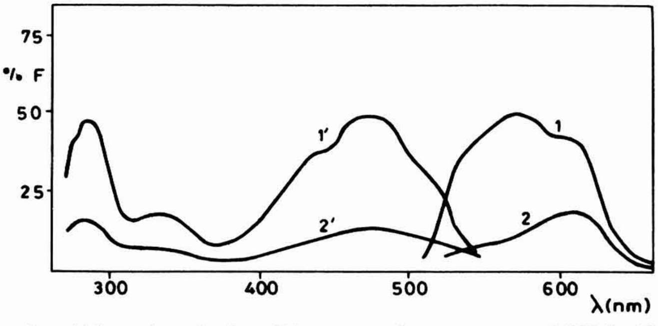

FIG. 3. Excitation (1′) and emission (1) spectra in presence of V(V). Excitation (2′) and emission (2) spectra of the reagent. [R] = 3.4×10^{-5} *M*, [V(V)] = 0.8 ppm, [HCl] = 0.5 *M*, 40% ethanol (λ_{ex} = 480 nm, λ_{em} = 573 nm). Sensitivity ×20.

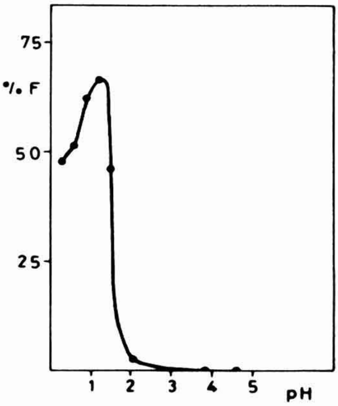

FIG. 4. pH influence on the oxidation product. $[V(V)] = 0.8$ ppm $[R] = 3.4 \times 10^{-5}$ *M*, 40% ethanol ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 573$ nm). Sensitivity ×20.

gradually diminishing at greater concentrations. Temperature does not affect the fluorescence intensity of the oxidation product, over the temperature range of 18 to 50°C.

Fluorimetric determination of vanadium(V). Fluorescence intensity is linearly proportional to the concentration of V(V) for concentrations of vanadium between 0.06 and 0.36 ppm. For 10 samples containing 0.24 ppm of V(V) a relative error with an average value of ±1.5% was found.

Table 1 shows the results of a study of the effect of foreign ions on the determination of V(V). Ions of the alkaline earth elements and the ions Zn(II), Mn(II), and Co(II) do not interfere even when their concentrations are the order of 100 times the concentration of vanadium. Interferences

TABLE 1

CONCENTRATIONS OF FOREIGN IONS TOLERATED (ERROR ≤1.5%) FOR 0.24 PPM VANADIUM (V)

Foreign ion	ppm
Ca(II), Sr(II), Ba(II), Mg(II), Be(II)	20
Zn(II), Mn(II), Co(II), Ni(II), Cd(II)	
Hg(II), Pt(IV), Al(III), Ga(III), UO_2^{2+}	
Ce(III), NO_3^-, SO_4^{2-}	
ZrO^{2+}, Cr(III), AsO_4H^{2-}, F^-, $C_2O_4^{2-}$	10
Cu(II), Th(IV)	5
Pd(II), Fe(III)	1
Au(III), Ce(IV)	<0.2

caused by Au(III) and Ce(IV) are notable even in concentrations equal to those of vanadium because of their reaction with the reagent.

Nature of the Reaction Product

In recent studies (*4, 12*) we have found that the yellow fluorescence observed in the presence of vanadium(V) is due to an oxidation product of the reagent 1-amino-4-hydroxyanthraquinone (probably 1,4-diketoanthraquinone) where vanadium acts a catalyst.

SUMMARY

A fluorimetric method for the determination of vanadium(V) (0.06−0.36 ppm) is described. The 1-amino-4-hydroxyanthraquinone is oxidized by this ion which yields a product exhibiting an intense yellow fluorescence in acidic solution. The experimental variables and interferences in this determination have been studied.

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Kinetic–Fluorimetric Determination of Cerium with 1-Amino-4-hydroxyanthraquinone

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INTRODUCTION

The only method for the fluorimetric determination of cerium(IV) found in the literature is based on the oxidative reaction between cerium(IV) and oxine-5-sulfonic acid (*2*). To date no kinetic–fluorimetric method for the determination of cerium has been reported. Owing to the simplicity, quickness and selectivity of these techniques we had thought of establishing a method for the fluorimetric kinetic determination of the above mentioned cation.

We have recently found that 1-amino-4-hydroxyanthraquinone reacts with certain cations (V(V), Ce(IV), Au(III)) producing a yellow fluorescent substance in an acid medium (*3*). The speed at which this oxidation takes place is slow enough to be measured easily and precisely which has allowed us to establish the determination proposed in this paper.

When the molar ratio of Ce(IV) to reagent is appropriate fluorescence appears preceded by a period of induction which is proportional to the concentration of catalyst. For this reason we have employed the induction period method (*4*) in addition to the three traditional methods of initial rate, fixed time, and fixed fluorescence (*1*).

In an attempt to improve the sensibility of the reaction we have employed an activator.

MATERIALS AND METHODS

Apparatus and measurement conditions. FIKA 55 MK II spectrofluorimeter. The intensity of fluorescence emitted-time curves has been obtained fixing the excitation and emission wavelengths and by constant movement of the needle of the recorder, whose speed was 60 sec/cm for all the experiments done.

Reagents and solutions. EGA-CHEMIE 1-amino-4-hydroxyanthraquinone, recrystallized by us in ethanol. It has been prepared to 0.05% (w/v) in ethanol, and the solutions of lower concentration were prepared by dilution in ethanol of the former.

Standard solution of Ce(IV) 7.1 × 10^{-3} *M*, prepared from Merck

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$(NH_4)_2Ce(NO_3)_6$; by dilution of the former that of 2.85×10^{-5} *M* concentration is prepared and renewed daily.

Solution of 0.01 *M* $KC10_3$.

Procedure for the determination of cerium(IV). In a 50-ml beaker 1 ml of 2.1×10^{-4} *M* reagent, 0.4 ml of 1:1 HCl, 0.5 ml of 0.01 *M* $KC10_3$, and the volume of cation solution necessary for the final concentration of Ce(IV) to be between 0.1 and 0.9 ppm are placed, the total final volume being 4 ml, this being achieved if necessary by addition of deionized water previous to the addition of the cation.

Thirty seconds after the addition of the Ce(IV) recording of the intensity of fluorescence emitted-time curve is started, the excitation wavelength being 480 nm and the emission wavelength 575 nm.

RESULTS AND DISCUSSION

Kinetic studies

The reactant presents a red fluorescence ($\lambda_{ex} = 530$ nm, $\lambda_{em} = 610$ nm), while the product obtained has a yellow fluorescence ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 575$ nm).

For the purpose of finding the optinum conditions for the determination of Ce(IV), the influence of the acidity and the concentration of the reagent on the reaction rate have been studied. An attempt has been made to increase the sensibility of the reaction by using an activator which would increase the catalytic activity.

From the analytical point of view the existence of a plateau in the curve of reaction rate vs concentration (Figs. 1 and 2) is very convenient as small variations in the concentrations in the plateau region practically do not influence the results. For this reason a HCl concentration of 0.63 *M* and a reagent concentration of 5.25×10^{-5} *M* are considered optimum.

In order to increase the catalytic activity, the use of moderate oxidizing

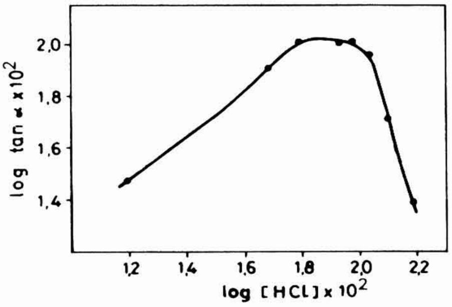

FIG. 1. Influence of the pH on the initial rate. [R] = 5.25×10^{-5} *M*, [Ce(IV)] = 0.5 ppm, [$KC10_3$] = 2.5×10^{-3} *M*. Sensibility ×20 ($\lambda_{ex} = 480$ nm, $\lambda_{em} = 575$ nm).

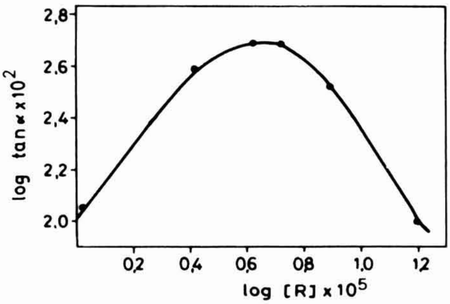

FIG. 2. Influence of the concentration of reagent on the initial rate. [Ce(IV)] = 0.25 ppm, [HCl] = 0.63 *M*, [KC10$_3$] = 2.5 × 10^{-3} *M*. Sensibility ×20 (λ_{ex} = 480 nm, λ_{em} = 575 nm).

agents capable of accelerating the regeneration of the catalysts has been tested. These include Fe(III), hydrogen peroxide, potassium chlorate, and citrate and sodium tartrate and arsenate. The only one to show an activation effect was potassium chlorate. The concentration of KC10$_3$ that does not appreciably alter the substrate when the catalyst is absent is considered optimum and corresponds to 1.25 × 10^{-3} *M* potassium chlorate.

Characteristics of the Analytical Methods

The fluorescence-time curves have been registered at the maximum excitation and emission wavelengths (λ_{ex} = 480 nm, λ_{ex} = 575 nm) for different amounts of Ce(IV), using optimum concentrations of reagent, KC10$_3$, and HCl. Such curves have been treated later using the kinetic methods (Fig. 3); the data thus obtained are shown in Table 1, from which it may be deduced that the highest sensitivity and precision correspond to the initial rate method.

The selectivity of the methods has been assayed studying the effects of foreign ions on the reaction rate and induction period. It has been found that the lowest level of interferences corresponds to the initial rate

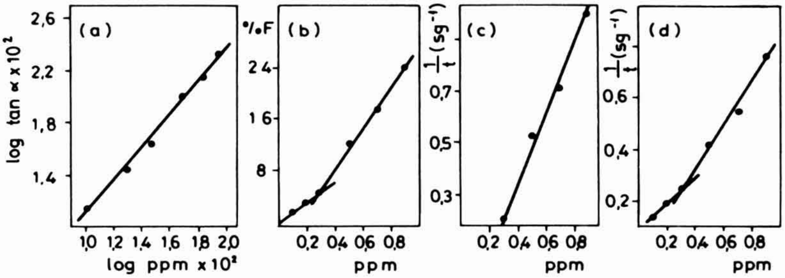

FIG. 3. Calibration curves for Ce(IV). (a) Tangent method, (b) fixed time method, (c) fixed fluorescence intensity method, (d) induction period method. [R] = 5.25 × 10^{-5} *M*, [HCl] = 0.63 *M*, [KC10$_3$] = 1.25 × 10^{-3} *M*. Sensibility ×20 (λ_{ex} = 480 nm, λ_{em} = 575 nm).

TABLE 1
CHARACTERISTICS OF THE KINETIC METHODS

Method	Range of applicability (ppm)	Percentage error
Initial rate	0.1–0.9	2.7
Fixed time	0.1–0.3	4.9
	0.3–0.9	2.7
Fixed fluorescence	0.3–0.9	3.3
Induction period	0.1–0.3	6.3
	0.3–0.9	3.4

method, for which only V(V) interferes at similar concentrations to those of cerium(IV) to be determined, as may be seen in Table 2.

Of the four methods studied, the one presenting better analytical characteristics (higher sensitivity and precision and lower number of interferences) is the initial rate method, therefore this is recommended.

Nature of the Reaction and the Product Obtained

The cations Ce(IV) and V(V) react with 1-amino-4-hydroxyanthraquinone producing substances of identical characteristics. The study of this product and the nature of the reaction have been presented in a previous paper (*3*) which shows the formation of an oxidation product of the substrate which probably corresponds to the diquinone which is

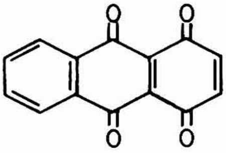

TABLE 2
CONCENTRATIONS OF FOREIGN IONS TOLERATED (ERROR $\leq$ 2.7%) FOR 0.5 PPM CERIUM(IV)

Ion	Amount tolerated (ppm)
Ca(II), Mg(II), Ba(II), Sr(II)	50
Be(II), Cd(II), Hg(II), Cu(II)	
Ni(II), Zn(II), Co(II), Pb(II)	
Mn(II), Al(III), Cr(III), PO_4^{3-}	
SO_4^{2-}, Br^-, Cl^-, NO_3^-	20
AsO_4^{3-}	10
Fe(III)	1
V(V)	0.01

in agreement with the constancy of the yellow color of the compound relative to the pH.

SUMMARY

A simple, sensitive, and selective method for the kinetic–fluorimetric determination of cerium (0.1–0.9 ppm), based on the oxidative reaction between cerium(IV) and 1-amino-4-hydroxyanthraquinone, has been developed. The reaction is followed by the measurement of the rate of appearance of yellow fluorescence (λ_{ex} = 480 nm, λ_{em} = 575 nm). About 20 cations and anions show negligible interference effects, even when present in large amounts. Only V(V) interferes at similar concentrations to those of Ce(IV) to be determined.

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MICROCHEMICAL JOURNAL **27**, 37–43 (1982)

Spectrophotometric Determination of Aluminum with Bromopyrogallol Red in the Presence of Surfactants

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INTRODUCTION

This paper constitutes a part of a continued investigation about the use of xanthene dyes as reagents in the presence of surfactants for very sensitive spectrophotometric determination of metal ions. The complexing properties of bromopyrogallol red have been used for the spectrophotometric determination of many ions (*3*). In a number of cases surface-active substances were used to increase the sensitivity of the determination (*1*, *2*, *4*–*6*).

In this work a sensitive method for the determination of aluminum, based on the ternary aluminium-bromopyrogallol red-cetyltrimethylammonium bromide or nonylphenol tetradecaethylene glycol ether system is reported.

EXPERIMENTAL

Reagents and Apparatus

Aluminum stock solution: 1 mg Al/ml. Dissolve 8.5900 g of aluminum sodium sulfate in water and 2 ml of concentrated sulfuric acid and dilute with water to 500 ml.

Aluminum dilute standard solution: 5 μg Al/ml. Dilute 1 ml of aluminum stock solution with 0.02 *M* sulfuric acid to 200 ml.

Bromopyrogallol red (BPR): 3.4×10^{-4} M. Dissolve 50 mg of the reagent in 125 ml of ethanol and dilute with water to 250 ml.

Cetyltrimethylammonium bromide (CTA): 2.6×10^{-3} M. Dissolve 0.2500 g of the reagent in water and dilute with water to 250 ml.

Nonylphenol tetradecaethylene glycol ether (NTGE): 1.8×10^{-3} M. Dissolve 0.3750 g of the reagent in water and dilute with water to 250 ml.

Buffer solutions (pH 5.5 and 7.0). An aqueous 10% hexamine solution, adjust the pH to 7.0 or 5.5 with dilute hydrochloric acid.

Apparatus. Spectrophotometer Varian Techtron Model 635, 10-mm cells, N-517 pH meter (Polish).

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Procedure I

Dilute a sample of test solution, containing 5 to 15 μg of aluminum in a beaker and add 2 ml of BPR solution. Adjust the pH to 3.5 with dilute ammonia. Transfer this solution to a 25-ml standard flask, fill with water to the mark, and mix. After 10 min, measure the absorbance of the solution at 530 nm against a reagent blank.

Procedure II

Dilute a sample of test solution, containing 2.5 to 10 μg of aluminum to about 10 ml in a beaker. Add 4 ml of BPR and 5 ml of CTA, and adjust the pH to approx 5.5 with dilute ammonia. Transfer this solution to a 25-ml standard flask, add 3 ml of buffer solution, fill with water to the mark, and mix. After 20 min, measure the absorbance of the solution at 627 nm against a reagent blank.

Procedure III

Add 3 ml of BPR solution and 3 ml of NTGE solution to acidic sample solution containing 2.5 to 12 μg of aluminum. Adjust the pH to approx 7.0 with dilute ammonia, and transfer this solution to a 25-ml standard flask. Add 3 ml of buffer solution, fill with water to the mark, and mix. After 10 min, measure the absorbance of the solution at 612 nm against a reagent blank.

RESULTS AND DISCUSSION

Binary System

Aluminum ions react with bromopyrogallol red in the acid range (pH 2.5−4.0) and form an orange-red complex. The optimum pH is 3.5 ± 0.1, at which $\lambda_{\max}$ of the complex is 530 nm.

The maximum absorbance was observed in the range $3.0 \times 10^{-5} - 4.0 \times 10^{-5}$ M of BPR. A spectrophotometric titration shows that the molar ratio of aluminium to BPR is 1:1. Under the conditions used in Procedure I, Beer's law is obeyed over the range 0.2−0.8 μg/ml of aluminum. The molar absorptivity at 530 nm is 1.4×10^4 liter mol^{-1} cm^{-1}.

Ternary System

An excess of surfactants causes a bathochromic shift of absorption maximum from 530 to 612 nm in the presence of NTGE, and 530 to 627 nm in the presence of CTA. The absorption spectra of BPR and the binary and ternary complexes are shown in Fig. 1.

Effect of pH

Dependence of the absorbance of the ternary complexes on pH was studied in the pH range 4.0−8.0. This is illustrated by the absorption

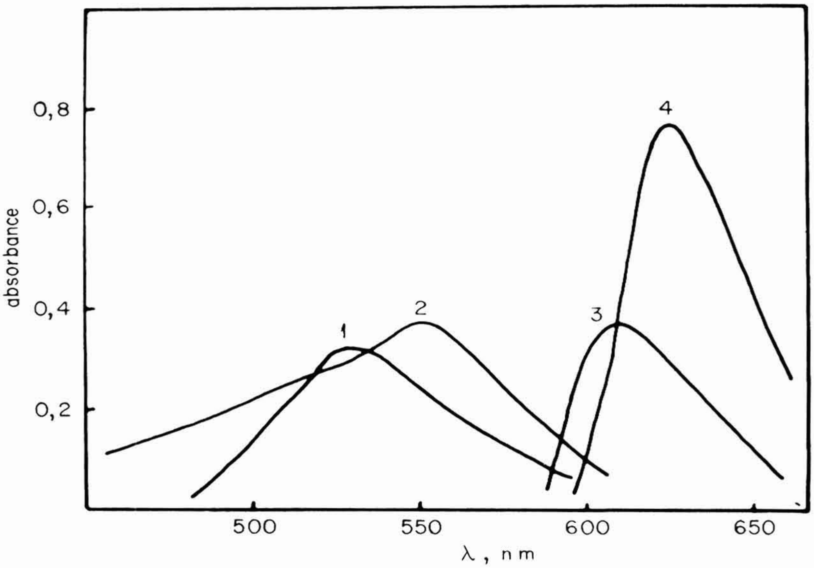

FIG. 1. Absorption spectra of: (1) Al–BPR complex, $C_{Al} = 1.5 \times 10^{-5}$ M, $C_{BPR} = 3.0 \times 10^{-5}$ M, pH 3.5, measured against a blank; (2) bromopyrogallol red, $C_{BPR} = 1.5 \times 10^{-5}$ M, pH 5.5, measured against water; (3) Al–BPR–NTGE complex, $C_{Al} = 1.5 \times 10^{-5}$ M, $C_{BPR} = 4.5 \times 10^{-5}$ M, $C_{NTGE} = 2.1 \times 10^{-4}$ M, pH 7.0, measured against a blank; (4) Al–BPR–CTA complex, $C_{Al} = 1.5 \times 10^{-5}$ M, $C_{BPR} = 5.9 \times 10^{-5}$ M, $C_{CTA} = 5.2 \times 10^{-4}$ M.

spectra of the Al–BPR–CTA complex shown in Fig. 2. Further measurements were carried out at 612 nm in the presence of NTGE and 627 nm in the presence of CTA. The results are shown in Fig. 3, in which it is shown that maximum intensity can be obtained in the pH range 5.3 to 5.7 for the Al–BPR–CTA complex and 6.8 to 7.5 for the Al–BPR–CTA complex. Hexamine buffer can be used.

Effect of BPR and Surfactants Concentration

Dependence of the absorbance of the ternary complexes on the BPR concentration was examined with a large excess of surfactants (40-fold ratio to Al). Concentration of the BPR varied in the range 4.5 to 7.5 × 10^{-5} M. The maximum absorbance was observed in the range 4.5 to 5.3 × 10^{-5} M for the Al–BPR–NTGE complex. Above a concentration of BPR equal to 5.5 × 10^{-5} M the absorbance decreases, whereas constant absorbance of the Al–BPR–CTA complex can be obtained over the range 5.0 to 7.0 × 10^{-5} M BPR. Above this concentration of BPR the absorbance insignificantly increases.

The effect of changes in the concentration of surfactants on the absorbance of the ternary complex was examined by measuring the absorbance. The molar ratio of metal ions to BPR was constant and equal to 1:4, while the molar excess of surfactants was variable. At low surfactant

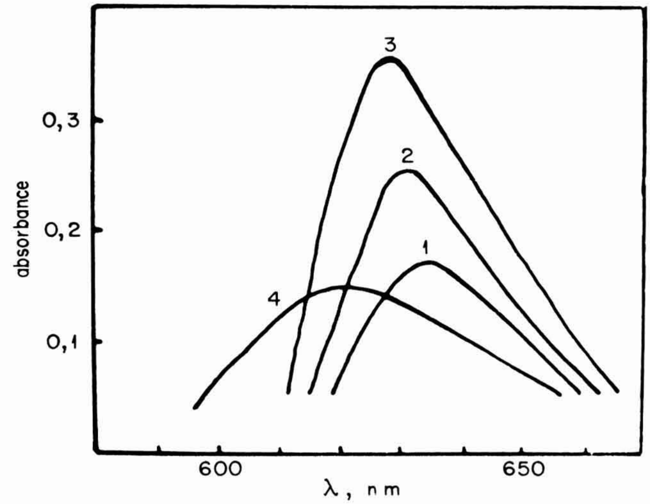

FIG. 2. Absorption spectra of aluminum complex with BPR and CTA in dependence on pH, $C_{Al} = 0.2$ μg/ml, $C_{BPR} = 5.4 \times 10^{-5}$ *M*, $C_{CTA} = 5.2 \times 10^{-4}$ *M;* (1) pH 3.5, (2) pH 4.5, (3) pH 5.5, (4) pH 7.5.

concentration the ternary complexes (Al−BPR−NTGE, λ_{max} 615 nm, and Al−BPR−CTA, λ_{max} 632 nm) are formed. As the molar excess of surfactants increases to 20 to 50-fold, a shift of the absorption maximum to 612 nm for Al−BPR−NTGE complex and 627 nm for Al−BPR−CTA com-

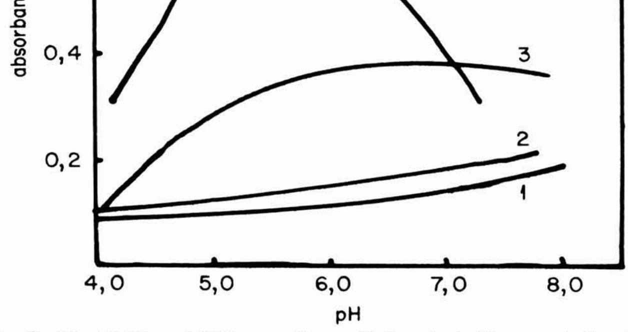

FIG. 3. Effect of pH at 612 and 627 nm, $C_{Al} = 0.4$ μg/ml, $C_{BPR} = 5.4 \times 10^{-5}$ *M*, $C_{CTA} = 5.2 \times 10^{-4}$ *M*, $C_{NTGE} = 2.1 \times 10^{-4}$ *M* of: (1) BPR + CTA at 627 nm, pH 5.5; (2) BPR + NTGE at 612 nm, pH 7.0, measured against water; (3) Al−BPR−NTGE at 612 nm, pH 7.0; (4) Al−BPR−CTA at 627 nm, pH 5.5, measured against a blank.

plex is observed. The absorbance is maximum and constant. Further increase in the molar excess of surfactants to 60 to 100-fold results in a shift of the absorption maximum toward shorter wavelengths (Al–BPR–NTGE, λ_{max} 610 nm; Al–BPR–CTA, λ_{max} 625 nm) while the absorbance decreases. The absorption spectra of the Al–BPR–CTA complex for various CTA concentrations are shown in Fig. 4. At low CTA concentration (fourfold ratio to Al) the solution becomes turbid. A 20-fold molar excess of NTGE or 45-fold molar excess of CTA to aluminum ensures the formation of the ternary complex with a maximum absorbance at λ_{max} 612 for Al–BPR–NTGE complex and at λ_{max} 627 nm for Al–BPR–CTA complex.

Also cetyldimethylbenzylammonium chloride has been used for the determination of aluminum with BPR. At low Al and high CDBA concentrations the solutions are not clear, and probably the ternary complex precipitates.

Beer's Law

Under the conditions used in Procedure II, Beer's law is obeyed over the range 0.1–0.4 μg/ml of aluminum. The molar absorptivity is 5.0×10^4 liter mol^{-1} cm^{-1}. However, according to Procedure III, Beer's law is obeyed over the range 0.1–0.46 μg/ml of aluminum and molar absorptivity is 2.2×10^4 liter mol^{-1} cm^{-1}. Sensitivity with this method (procedure

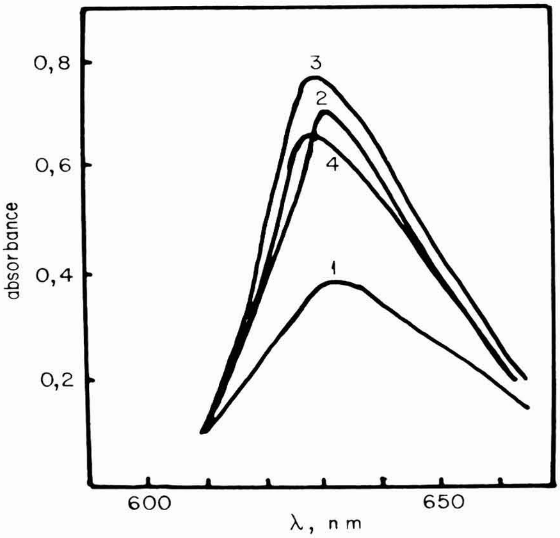

FIG. 4. Absorption spectra of the Al–BPR–CTA complex in dependence on CTA concentration, $C_{Al} = 1.5 \times 10^{-5}$ *M*, $C_{BPR} = 5.4 \times 10^{-4}$ *M*. (1) 5.0×10^{-5} *M*; (2) 4.0×10^{-4} *M*; (3) 5.2×10^{-4} *M*; (4) 1.0×10^{-3} *M*.

II) is similar to that of the method using pyrogallol red with CTA (molar absorptivity = 4.8 × 10^4) (*7*).

Effect of Foreign Ions

The effect of foreign ions was tested by determining 10 μg of aluminum in the presence of a large number of interfering ions. The most commonly encountered ions were added individually to a solution. Under the conditions used in procedure II aluminum can be determined in the presence of 500-fold weight excess: magnesium, copper, thallium, barium; 100-fold: cobalt, zinc, nickel, mercury, tin (IV), silver; and 50-fold: cadium, manganese, lead. Numerous metals interfere: iron (III), iron (II), bismuth, antimony (III), titanium, and vanadium. EDTA, oxolates, and phosphate also interfere. Citrates of concentration 1 mg, tartrates of 4 mg, and acetates of 10 mg can be accepted in determination of aluminum. These interfering ions can be easily removed by preliminary extraction of these metals with cupferron. This method was described previously (*7*).

Complex Formation

Job's method of continuous variations have been used to establish the molarity of the aluminum complex in the presence of surfactants. A Job plot shows that the molar ratio of aluminum to BPR is 1:2.

CONCLUSIONS

Aluminum forms violent ternary complexes with BPR in the presence of nonionic surfactant (NTGE) and ionic surfactant (CTA). These complexes are proposed for the spectrophotometric determination of microgram amounts of aluminum and they have absorbance maxima at 612 and 627 nm, respectively, with molar absorptivities of 2.2 × 10^4 and 5.0 × 10^4 liter mol^{-1} cm^{-1}.

The molar ratio of aluminum to BPR, which is 1:1 in the binary complex, increases to 1:2 in the ternary complex. The presence of surfactants changes the number of BPR molecules bonded to aluminum. On the basis of the obtained absorption spectra, it can be supposed that various complexes are formed in the ternary system as the surfactant concentration increases. These complexes show different absorption spectra and different numbers of surfactant molecules bonded. It has been suggested that the micelles of surfactants take part in the structure of the ternary complex. The sensitizing effect of nonionic surfactant on color reaction is less than that of CTA.

SUMMARY

Spectrophotometric methods are described for the determination of microgram amounts of aluminum based on the formation of a ternary complex between the metal,

bromopyrogallol red, and cetyltrimethylammonium bromide or nonylphenol tetradecaethylene glycol ether. The complexes have absorbance maxima at 627 and 612 nm, respectively, with molar absorptivities of 5.0×10^4 and 2.2×10^4 liter mol^{-1} cm^{-1}. Numerous metals interfere. Aluminum can be easily separated by extraction of interfering ions with cupferron.

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MICROCHEMICAL JOURNAL 27, 44–48 (1982)

Simultaneous Microdetermination of Sulfur and Phosphorus in Organic Compounds

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INTRODUCTION

Many methods describe the elemental microdetermination of sulfur while others deal with the estimation of phosphorus in organic compounds. However, the existence of both elements in the same compound impose certain restrictions, especially when sulfur is to be determined in organophosphorus compounds.

On the other hand, the only method reported for the simultaneous microdetermination of sulfur and phosphorus is that of Shah *et al.* (*4*) applicable for the analysis of phenothiaphosphine trioxides or compounds of the type containing a known ratio of sulfur to phosphorus. The present method is based upon our previous work for the microdetermination of sulfur (*2*) and phosphorus (*3*) after necessary modifications.

EXPERIMENTAL

Apparatus

A Sargent–Welch model XVI polarograph with accessories was used. The electrolytic vessel was that described by Akrawi (*1*) composed of two concentric tubes with a sintered-glass disc to allow contact between the electrolytic solution (inner compartment) and the anode (outer compartment). The dropping mercury electrode had a drop time of 3–4 sec under an open head of 85 cm of mercury. The anode was a saturated calomel electrode (SCE).

Reagents

All reagents used were of AR or MAR grade and double-distilled water was used.

Barium chloride. 0.2 *N* solution prepared by dissolving 11.7688 g of the dihydrate in 500 ml of water.

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Calcium chloride. 4 *M* solution.

Gelatin. 1% solution, prepared every week.

Quinoline hydrochloride. Double-distilled quinoline (14 ml) was dissolved in 300 ml of 1.5 *M* hydrochloric acid.

Sodium molybdate. Sodium molybdate (13.1613 g) was dissolved in water and 106 ml of conc HCl was added and the volume made up to 250 ml with distilled water (1 ml = 20.8744 mg of Mo). The solution was kept in a polythene bottle.

Nitrogen (O_2-free) was used for deaerating the solutions; the gas was purified by passing through a series of three bubblers, each containing vanadium(II) chloride solution and amalgamated zinc, followed by one containing water and one empty.

Procedure

Weigh exactly 3–8 mg of the organic sample and wrap it as usual with the oxygen-flask method. Charge a 250-ml conical flask with 10 ml of water, fill the flask with pure oxygen, and, just before starting combustion, introduce 1.5 ml of saturated bromine water. After combustion, shake for 7 min, rinse the stopper and platinum gauze out using about 10 ml of water. Boil gently until the volume of the solution is 5–10 ml.

Step I. Add 1 ml of $BaCl_2$ solution (total volume should not exceed 11 ml) and digest under reflux condenser for 20 min on a hot plate. Allow to cool for 15 min. Transfer quantitatively to a 25-ml measuring flask. Introduce 2.5 ml of $CaCl_2$ solution and 0.15 ml of gelatin solution followed by water up to the mark. Mix, transfer an aliquot to the cathode compartment of the cell, and bubble purified nitrogen gas for 2 min. Record the wave of Ba(II) starting at −1.8 V applied potential vs SCE; use damping 2, and a sensitivity of 0.15 μA/mm (37.5 μA full-scale deflection).

Step II. Remove the cathode compartment of the cell containing the electrolytic solution and filter it through a sintered-glass crucible (G3). Then filter the test solution (remaining in the 25-ml volumetric flask) through the same crucible and receive the filtrates and washings in a 100-ml conical flask. Evaporate until the solution volume is approximately 20 ml. While hot, add 1 ml of 5 *M* HCl solution followed by exactly 3 ml of sodium molybdate solution. Precipitate phosphate by introducing 3 ml of quinoline hydrochloride solution slowly with stirring. Boil for 1–2 min, then leave to cool. Transfer the solution, and precipitate, quantitatively to a 50-ml measuring flask. Add 0.6 ml of gelatin solution, then water up to the mark. Mix, transfer an aliquot to the electrolytic vessel, and bubble nitrogen gas for 2 min. Record the wave of Mo(VI) starting at +0.10 V vs SCE; use damping 2, and a sensitivity of 0.30 μA/mm (75 μA full-scale deflection).

Blank solutions. (i) In a 25-ml volumetric flask, prepare a solution by mixing 1 ml of $BaCl_2$ solution, 2.5 ml of $CaCl_2$ solution, 0.15 ml of gelatin solution, and water to volume. Record the wave of Ba(II) as described in Step I and calculate the percentage of sulfur. (ii) In a 50-ml volumetric flask introduce 1 ml of HCl solution, 3 ml of molybdate solution, 3 ml of quinoline hydrochloride solution, 0.75 ml of gelatin solution, and water to volume. Record the polarogram of Mo(VI) as given in Step II and calculate the percentage of phosphorus.

RESULTS AND DISCUSSION

The proposed method depends upon measuring the wave height corresponding to a known amount of Ba(II) before and after it has been used to precipitate "sulfate" present in a known weight of sample. The reaction has to be achieved in acidic medium to prevent coprecipitation of phosphate. In previous work (*2*), precipitation of sulfate was carried out from 0.002 *N* acetic acid medium. However, acetic acid shifts the half-wave potential of Ba(II) toward more negative values thus affecting slightly full development of the barium wave. Acids stronger than acetic acid obliterate the wave of Ba(II) due to discharge of the relatively higher concentration of hydronium ion. Study of the effect of the pH value of reaction medium on the precipitation of 3 mg phosphate (corresponding to about 1 mg P) revealed that even solutions of pH up to 5.7 could not cause any appreciable precipitation of phosphate. This has been achieved by introducing 0, 0.3, and 0.5 ml of 0.2 *N* acetic acid solution in three 100-ml conical flasks followed by 3 ml of 0.01 *M* K_2SO_4 solution (about 1 mg S), 2 ml of 0.015 *M* KH_2PO_4 solution (about 1 mg P), heating to boiling then adding 1 ml of $BaCl_2$ solution, digesting for 20 min, and cooling. The solutions were then transferred quantitatively to three 25-ml volumetric flasks to which was added 2.5 ml of $CaCl_2$ solution, 0.15 ml of gelatin solution, and water up to volume. The final pH values were 5.7, 3.9, and 3.6, respectively. The solutions when polarographed gave well-defined waves for Ba(II); the diffusion currents were constant for the three solutions and equal to that obtained for a solution containing the same ingredients except for phosphate. The pH value of sample solutions obtained after combustion of the available organic compounds and addition of reagents did not exceed 5.7. Consequently, precipitation of sulfate in the presence of a few milligrams of phosphate was achieved without addition of acetic acid.

Phosphorus was determined by the quinoline phosphomolybdate method (*5*) but using a polarographic finish. The wave height corresponding to a known amount of Mo(VI) is measured before and after precipitation of "phosphate" present in a weighed amount of sample. To maintain identical conditions for the sample solution and the blank, the latter was first prepared following the whole procedure. However, the

TABLE 1
SIMULTANEOUS MICRODETERMINATION OF SULFUR AND PHOSPHORUS

Sample	Sample wt (mg)	Percentage S			Percentage P		
		Theory	Found	Error	Theory	Found	Error
Triphenylphosphine sulfide	5.641	10.89	10.17	−0.72	10.52	10.10	−0.42
	7.385		10.98	+0.09		9.35	−1.17
	6.318		10.68	−0.21		10.66	+0.14
Dimethyldithiophosphoric acid ammonium salt	3.233	36.60	37.35	+0.75	17.68	16.99	−0.69
	3.081		36.67	+0.07		16.58	−1.10
Dibenzyldisulfide + Triphenylphosphine	(4.391+ 3.807)	26.03	26.48	+0.45	11.81	11.40	−0.41
	(3.610+ 3.021)		25.94	−0.09		11.98	+0.17
Toluene-*p*-sulfonic acid + Triphenylphosphine	(4.839+ 4.785)	16.86	16.43	−0.43	11.81	11.35	−0.46
	(4.232+ 3.239)		16.51	−0.35		11.92	+0.11
o-Mercaptobenzoic acid + Phenyldisodium orthophosphate	(3.168+ 4.829)	20.80	20.60	−0.20	12.19	11.47	−0.72
	(3.187+ 3.086)		20.76	−0.04		12.01	−0.18
[1-(1-Hydroxy-4-methyl-2-phenylazo)-2-naphthol-4-sulfonic acid] + Triphenylphosphine	(4.312+ 5.560)	8.95	8.02	−0.93	11.81	12.12	+0.31
	(5.743+ 4.711)		8.14	−0.81		11.03	−0.78

molybdenum wave so obtained had a height equal to that obtained for a solution prepared directly by mixing 1 ml of 5 *M* HCl, 3 ml of sodium molybdate, 3 ml of quinoline hydrochloride, 0.75 ml of gelatin, and water to 50 ml.

Only two organic compounds containing both elements were available. Mixtures were analyzed after mixing 3–5 mg each of organosulfur and organophosphorus compound (Table 1). The mean relative error for 13 determinations was 2.99% for sulfur and 4.07% for phosphorus; the mean relative standard deviation amounted to 1.43 and 4.19%, respectively. There seems to be no reason why the proposed method is not applicable to all types of organic compounds and thus overcomes the restriction reported previously (*4*).

SUMMARY

A simple method for the simultaneous determination of sulfur and phosphorus in 3–8 mg organic sample is presented. Following oxidative combustion by the oxygen flask method, sulfate is precipitated by addition of a known amount of Ba(II) and the excess of the latter is measured polarographically. After filtration, the quinoline phosphomolybdate method is used to precipitate phosphate; the unreacted Mo(VI) is determined polarographically. The mean relative error for 13 determinations was 2.99% for S and 4.07% for P; the mean relative standard deviation was 1.43 and 4.19%, respectively.

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The Use of Redox Reactions in the Analysis of Dyes and Dye Intermediates

XI. A Chromometric Determination of Some Azobenzene Derivatives in an Acetonitrile–Methanol–Water Mixed Medium[1]

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INTRODUCTION

The ever increasing manufacture of azo-dyes requires methods suitable for their determination in technological preparations (*8, 13*). If an azo-compound is a major component in a sample, reductometric methods are chiefly used for its determination (*1, 2, 8*). Chromium(II) ion is one of the most powerful reductants used for this purpose (*1, 2, 4, 7*).

Recently we have experienced a great development in the production of water-insoluble azo-dyes. Therefore, this work deals with a chromometric determination of some azobenzene derivatives in a medium in which these substances are sufficiently soluble. Acetonitrile was used for dissolution of the test substances and cheaper and more easily available methanol was employed as the titration medium.

Although standard solutions of chromium(II) ions in dioxan (*10–12*), dimethylformamide (*5*), or tetrahydrofuran (*9*) have been prepared and used, we have decided to use an aqueous solution of the reagent because of its simpler preparation.

The test substances are listed in Table 1 and are further denoted by the appropriate Roman numerals.

EXPERIMENTAL

Reagents

Solutions (0.005 *M*) of the test substances in acetonitrile were prepared by dissolving accurately weighed amounts of the pure substances (Research Institute for Organic Syntheses, Pardubice-Rybitví) in this solvent, purified according to Coetzee *et al.* (*6*). The purity of the preparations of azo-compounds was checked by thin-layer chromatography (*3*).

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[1] Part X: Polarographic and Constant-Potential Coulometric Determination of 4,4′-dihydroxyazobenzene and 4-nitro-4′-hydroxyazobenzene. *Microchem. J.* 26, 221–227 (1981).

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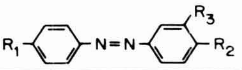
TABLE 1

A SURVEY OF THE STUDIED SUBSTANCES OF THE TYPE



Substance	R_1	R_2	R_3	General formula	Molecular weight
I	NH_2-	OH−	H−	$C_{12}H_{11}N_3O$	213.241
II	CH_3NH-	OH−	H−	$C_{13}H_{13}N_3O$	227.268
III	$(CH_3)_2N-$	OH−	H−	$C_{14}H_{15}N_3O$	241.295
IV	NO_2-	OH−	H−	$C_{12}H_9N_3O_3$	243.224
V	OH−	OH−	H−	$C_{12}H_{10}N_2O_2$	214.226
VI	$(CH_3)_2N-$	OH−	OH−	$C_{14}H_{15}N_3O_2$	257.295
VII	$(CH_3)_2N-$	NH_2-	H−	$C_{14}H_{16}N_4$	240.310
VIII	$(CH_3)_2N-$	CH_3CONH-	H−	$C_{16}H_{18}N_4O$	280.347
IX	$(CH_3)_2N-$	F−	H−	$C_{14}H_{14}N_3F$	243.286
X	$(CH_3)_2N-$	Cl−	H−	$C_{14}H_{14}N_3Cl$	259.741
XI	$(CH_3)_2N-$	Br−	H−	$C_{14}H_{14}N_3Br$	304.197
XII	$(CH_3)_2N-$	I−	H−	$C_{14}H_{14}N_3I$	351.192
XIII	$(CH_3)_2N-$	−COOH	H−	$C_{15}H_{15}N_3O$	269.300
XIV	$(CH_3)_2N-$	$-SO_3Na$	H−	$C_{14}H_{14}N_3SO_3Na$	327.340

A 0.01 *M* chromium(II) chloride solution was prepared (*7*) by dissolving 7.5 g of potassium dichromate in 500 ml of distilled water, adding 80 ml of concentrated hydrochloric acid, and then 20 ml of 30% hydrogen peroxide in small portions. The solution was heated to the boiling point for 20 min, allowed to cool, and transferred to a flask containing zinc amalgam. Here it was reduced for 3 days under an inert atmosphere. Its titer was determined before use as follows (*7*): An amount of 5.00 ml of a 0.01 *M* ferrous sulfate solution in 0.1 *M* hydrochloric acid, prereduced by zinc amalgam to negative reaction with thiocyanate, was measured into a titration vessel, 1.00 ml of 0.01 *N* potassium dichromate and 10 ml of methanol were added, nitrogen was passed through the solution, and then automatic titration was carried out with 0.01 *M* chromium(II) chloride to the endpoint potential of +100 mV.

The other chemicals used were of p.a. purity.

Apparatus

Potentiometric titrations were performed in a thermostatted titration vessel using an ABU 1 autoburette and a TTT 1 titrator (Radiometer, Copenhagen), with a bright platinum indicator and a saturated calomel reference electrode. A constant temperature was maintained using a U 3 thermostat (Mechanik Prüfgeräte, Medingen, German Democratic Republic).

Procedures

The procedure recommended for direct titration. Into a titration vessel, 1.00 ml of acetonitrile solution containing 0.5–2.0 mg of the studied substance is measured, 15 ml of methanol is added, the solution is freed of oxygen by bubbling with nitrogen for 5 min, and then titrated potentiometrically under an inert atmosphere with a standard solution of 0.01 *M* chromium(II) chloride.

The procedure recommended for an indirect determination. Into a titration vessel, 1.00 ml of acetonitrile solution containing 0.5–2.0 mg of the test substance is measured, 15 ml of methanol is added, and the solution is freed of oxygen by bubbling with nitrogen for 5 min. Then 5.00 ml of 0.01 *M* chromium(II) chloride is added from an autoburette and the solution is allowed to decolorize. After another 5 min 10.00 ml of a 0.01 *M* solution of ferric sulfate in 0.1 *M* hydrochloric acid is added and the unconsumed reagent is immediately back-titrated from the same autoburette with 0.01 *M* chromium(II) chloride to the end-point potential of +0.025 V. The blank is determined in parallel and the chromium(II) consumption is calculated from the difference.

RESULTS AND DISCUSSION

The chromium(II) consumption in the direct titration and the indirect determination is in accordance with the expected Eq. (1) describing the reduction of azogroups

$$R_1-N=N-R_2 + 4\ Cr^{2+} + 4\ H^+ \rightarrow R_1NH_2 + R_2NH_2 + 4\ Cr^{3+} \quad (1)$$

During the direct titration of substances I–III and V–VIII, the potential stabilization is rather slow at laboratory temperature, especially in the vicinity of the equivalence point. However, the titrations are feasible even though the time required is 30–45 min. Substances IV and IX–XIV are not amenable to this determination because of too slow potential stabilization.

For this reason a series of titrations were carried out at an elevated temperature (65°C). It has been found that potential stabilization is improved only slightly so that the time required for the titration of substances I–III and V–VIII is shortened to 15–20 min. However, the other substances are not determinable even at an elevated temperature. The results of the potentiometric titrations of the test substances by chromium(II) chloride are given in Table 2.

Because of the too slow potential stabilization in the direct titration, an indirect determination was also studied. It has been found that it is possible to determine substances I–III and V–VIII at laboratory temperature

TABLE 2
DIRECT POTENTIOMETRIC TITRATION OF THE TEST SUBSTANCES WITH 0.01 *M* CHROMIUM(II) CHLORIDE

Substance	Temperature (°C)	E^{f}(mV)[a]	ΔE(mV)/ 0.02 ml[b]	Taken (mg)	Found[c] (mg)	Standard deviation (mg)
I	20	+210	15	1.066	1.071	0.0005
I	65	+220	15	1.066	1.072	0.0005
II	20	+235	15	1.136	1.144	0.0012
II	65	+235	20	1.136	1.144	0.0005
III	20	+240	15	1.207	1.216	0.0010
III	65	+240	20	1.207	1.218	0.0009
IV	—	—	—	—	—	—
V	20	+460	25	1.071	1.079	0.0007
V	65	+475	25	1.071	1.080	0.0007
VI	20	+435	25	1.287	1.283	0.0008
VI	65	+440	30	1.287	1.283	0.0004
VII	20	+285	20	1.202	1.195	0.0005
VII	65	+285	25	1.202	1.197	0.0006
VIII	20	+275	20	1.402	1.405	0.0006
VIII	65	+275	30	1.402	1.407	0.0006

[a] These values represent the formal redox potentials of the test substances read out at half-equivalence.

[b] These values represent the change of potential in equivalence corresponding to addition of 0.02 ml of 0.01 *M* $CrCl_2$.

[c] These values represent the average of seven determinations, from which the standard deviation was found.

by the procedure described under Experimental. However, the reduction of substance IV is not complete within 1 hr even at an elevated temperature.

The results obtained are given in Table 3, together with the time required for the reduction of the corresponding azo-compound. (This time was estimated visually as the time required for the decoloration of the solution.) As mentioned in the procedure, the reduction is always carried out for 5 min longer than the time required for decoloration.

CONCLUSIONS

It can be seen that the indirect determination is less time consuming and has a broader scope than the direct titration because even carboxy, sulfo, and halogeno derivatives of azobenzene, not amenable to direct titration, can be determined. The fact that substance IV is not determinable even indirectly at an elevated temperature is probably connected with a more difficult reduction of the nitro-group present. However, the azo-group is probably reduced without problems even in this molecule. Because of

TABLE 3
THE ACCURACY AND REPRODUCIBILITY OF THE INDIRECT DETERMINATION OF THE STUDIED AZOBENZENE DERIVATIVES WITH CHROMIUM(II) CHLORIDE

Substance	Temperature (°C)	Time required for decoloration (min)	Taken (mg)	Found[a] (mg)	Standard deviation (mg)
I	20	8	1.066	1.073	0.0011
I	65	4	1.066	1.073	0.0013
II	20	6	1.136	1.144	0.0008
II	65	3	1.136	1.143	0.0009
III	20	10	1.207	1.220	0.0013
III	65	6	1.207	1.219	0.0011
IV	20	More than 60	—	—	—
IV	65	More than 60	—	—	—
V	20	5	1.071	1.073	0.0009
V	65	2	1.071	1.073	0.0013
VI	20	4	1.287	1.286	0.0011
VI	65	2	1.287	1.287	0.0014
VII	20	12	1.202	1.205	0.0015
VII	65	7	1.202	1.204	0.0017
VIII	20	10	1.402	1.404	0.0013
VIII	65	2	1.402	1.404	0.0017
IX	20	18	1.216	1.219	0.0014
IX	65	4	1.216	1.219	0.0023
X	20	22	1.299	1.300	0.0012
X	65	5	1.299	4.299	0.0023
XI	20	27	1.521	1.524	0.0008
XI	65	7	1.521	1.524	0.0020
XII	20	25	1.756	1.773	0.0012
XII	65	8	1.756	1.773	0.0018
XIII	20	28	1.347	1.351	0.0013
XIII	65	8	1.347	1.352	0.0036
XIV	20	25	1.637	1.647	0.0019
XIV	65	9	1.637	1.647	0.0020

[a] These values represent the average of seven determinations, from which the standard deviation was found.

frequent occurrence of nitro-groups in azo-dyes, attention has been devoted to the titanometric determination of these compounds, which will be dealt with in a future communication.

ACKNOWLEDGMENT

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SUMMARY

Optimal conditions for a direct and indirect determination of 14 azobenzene derivatives with chromium(II) chloride have been found.

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MICROCHEMICAL JOURNAL **27**, 55–61 (1982)

The Oxidation of Organic Substances with Compounds of Trivalent Manganese

XVIII. A Contribution to the Coulometric Generation of Manganese(III) Acetate in an Acetic Acid Medium[1]

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INTRODUCTION

Considerable attention has recently been devoted to the analytical use of redox reactions in nonaqueous media which enable determinations of samples insoluble in water or reacting with water through hydrolysis or redox reactions (*6, 7*). Limited stability of some redox reagents in nonaqueous solvents and their complicated chemical preparation have led to the study of coulometric titrations in nonaqueous media (*11*). Manganese(III) acetate is one of the redox reagents suitable for determinations of reducing substances in nonaqueous media of acetic acid. The use of this reagent is discussed in reviews (*1, 2*) and the coulometric generation of various forms of manganese(III) is described in Barek *et al.* (*3*). So far, manganese(III) acetate has been used for the coulometric titration of hydroquinone (*10*), hydrazine and its derivatives (*8*), and substances containing –SH groups (*9*).

Pastor and co-workers (*10*) noted that accurate results in the titration of hydroquinone can be attained in a medium of 0.6 *M* potassium acetate in glacial acetic acid saturated with manganese(II) acetate. The presence of potassium acetate increases the solubility of manganese(II) acetate in glacial acetic acid and simultaneously decreases the electrolyte resistance. In the coulometric titration of hydroquinone, the presence of water in the anolyte has no influence on the accuracy of the results provided that hydroquinone is present in the anolyte from the beginning of the generation of manganese(III) ions. If, however, hydroquinone is added after an amount of manganese(III) has been generated, the results are lower when there is more than 1% of water present in the anolyte. This fact suggests that the current efficiency in the coulometric generation of manganese(III)

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[1] Part XVII: Coulometric Generation of the Diphosphate Complex of Trivalent Manganese in Sulfuric Acid Medium and Hexaquomanganese(III) Ions in Perchloric Acid Medium. *Microchem. J.* **24**, 454–62 (1979).

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acetate decreases with an increasing content of water in the anolyte. Good results of the titration of hydroquinone in the presence of water can be explained by direct oxidation of this substance on the generating electrode. Therefore, the dependence of the current efficiency in the coulometric generation of manganese(III) acetate on the concentrations of water and manganese(II) acetate at various current densities was studied in this work.

These data are also important because of the well-known fact (*1*, *2*) that the rate of the oxidation of organic substances with manganese(III) compounds strongly depends on the concentration of manganese(II) ions and of complexing substances.

After finding the conditions under which the generation of manganese(III) acetate proceeds with 100% current efficiency, the possibility of direct and indirect coulometric determinations of organic substances with this reagent was investigated. Simultaneously, the applicability of various electrometric methods for the end-point determination was evaluated.

EXPERIMENTAL

Reagents. Hydroquinone, 0.01 N and 0.001 N solutions in glacial acetic acid, was prepared by dissolving accurately weighed amounts of the p.a. substance in glacial acetic acid and its titer was found daily by potentiometric titration of dichromate (*5*).

Ferrous sulfate, 0.01 M solution, was prepared by dissolving 2.8 g of the p.a. substance in 250 ml of 1 M sulfuric acid and diluting with water to 1 liter. Its titer was found daily using dichromate. The other chemicals used were of p.a. purity.

Apparatus. Manganese(III) acetate was generated using an OH 104 coulometric analyzer (Radelkis, Budapest) in a thermostatted EA 880 T-V vessel (Metrohm, Herisau). A platinum generating electrode with an active surface area of 5 cm^2 and a similar auxiliary electrode separated by a frit were used. A bright platinum indicating and a saturated calomel reference electrode were used for potentiometric indication. Two identical platinum wire electrodes were used for biamperometric or bipotenciometric indication. All the electrodes used were part of the analyzer accessories.

PROCEDURES AND RESULTS

Dependence of the Current Efficiency of the Coulometric Generation of Manganese(III) Acetate on the Concentration of Water

It followed from preliminary experiments that the current efficiency is decreased even by small amounts of water. Therefore, it was necessary to remove water from acetic acid by reaction with acetanhydride. The generation solution was prepared as follows: An amount of 800 ml of acetic acid was mixed with 200 ml of acetanhydride, 60 g of potassium acetate and 14.7 g of manganese(II) acetate were added. The solution thus prepared was set aside for 24 hr to complete the reaction of water with acetanhydride. In this solution the concentration of potassium acetate was 0.6 *M* and that of manganese(II) acetate, 0.06 *M*.

The dependence of the current efficiency on the concentration of water was measured as follows: An appropriate amount of water was added to 50 ml of the generation solution, resulting in the water concentrations of 1, 2, 5, or 10%. Then a charge of approximately 5000 mC was passed through the solution, the generation current used being 5 mA. The manganese(III) acetate thus formed was then titrated with 0.01 *M* ferrous sulfate using bipotentiometric indication with two identical platinum electrodes and an applied current of 2 μA. The current efficiency of the electrolysis was calculated from the consumption of this reagent. The results obtained are given in Table 1 from which it follows that 100% current efficiency can be achieved only in a completely anhydrous medium.

Therefore, all the other dependences were measured in a medium of acetic acid from which traces of water were removed by reaction with acetanhydride.

Dependence of the Current Efficiency of the Coulometric Generation of Manganese(III) Acetate on the Current Density

This dependence was investigated in a medium of anhydrous acetic acid, the concentration of potassium acetate being 0.6 *M* and that of manganese(II) acetate 0.06 *M*. A generation current of 1, 2, 3, and 5 mA was used. It was impossible to employ a higher current because of a too low conductivity of the generation solution. Otherwise, the procedure was analogous to that in the previous case. The results obtained are given in Table 2 from which it follows that under these conditions 100% current efficiency can be achieved at current densities of 0.2–1 mA/cm^2.

Dependence of the Current Efficiency of the Coulometric Generation of Manganese(III) Acetate on the Concentration of Manganese(II) Ions

The procedure was analogous to that described above, with the concentrations of potassium acetate and manganese(II) ions of 0.6 and 0.005

TABLE 1

DEPENDENCE OF THE CURRENT EFFICIENCY OF THE COULOMETRIC GENERATION OF MANGANESE(III) ACETATE ON THE CONCENTRATION OF WATER[a]

Concentration of water (%)	0	1	2	5	10
Current efficiency (%)	100.21	94.56	89.22	82.15	77.45

[a] Medium: 0.6 *M* potassium acetate and 0.06 *M* manganese(II) acetate in acetic acid, room temperature, current density 4 mA/cm^2.

TABLE 2

DEPENDENCE OF THE CURRENT EFFICIENCY OF THE COULOMETRIC GENERATION OF MANGANESE(III) ACETATE ON THE CURRENT DENSITY[a]

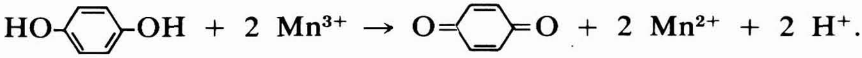
Current density (mA/cm²)	0.2	0.4	0.6	1.0
Current efficiency (%)	100.03	100.11	100.13	100.21

[a] Medium: 0.6 *M* potassium acetate and 0.06 *M* manganese(II) acetate in anhydrous acetic acid, room temperature.

to 0.06 *M*, respectively. A current density of 0.2 to 1 mA/cm² was used. The results obtained are given in Table 3 from which it follows that the higher the concentration of manganese(II) ions, the higher the current density that can be employed, maintaining the 100% current efficiency.

Direct Coulometric Titration with Manganese(III) Acetate

On the basis of the above results, a medium of anhydrous acetic acid with 0.6 *M* potassium acetate and 0.6 *M* manganese(II) acetate was chosen and the feasibility of a direct coulometric titration under the above conditions was verified. Hydroquinone was chosen as a model substance as it is known to be rapidly and quantitatively oxidized with trivalent manganese according to (*4*, *5*).



(1)

The following procedure was employed: An appropriate amount of hydroquinone (1.00 or 5.00 ml of 0.01 or 0.001 *N* solution) was added to 50 ml of the generation solution and a coulometric titration was carried out with a current density of 1 mA/cm². Various electrometric end-point detection methods were compared. The potentiometric indication was found to be unsuitable because of too slow potential stabilization, especially in the vicinity of the equivalence point. Better results were obtained with the biamperometric indication, and the bipotentiometric indication was found to be the most suitable. Biamperometric and bipotentiometric titration

TABLE 3

DEPENDENCE OF THE CURRENT EFFICIENCY OF THE COULOMETRIC GENERATION OF MANGANESE(III) ACETATE ON THE CONCENTRATION OF MANGANESE(II) ACETATE AT VARIOUS CURRENT DENSITIES[a]

Current density (mA/cm²)	Concentration Mn^{2+} (*M*)					
	0.06	0.04	0.02	0.01	0.005	0.001
1.0	100.21	99.95	99.83	94.27	81.98	70.12
0.6	100.13	99.89	100.03	100.13	92.22	82.03
0.2	100.03	100.13	100.08	99.91	98.64	89.06

[a] Medium: 0.6 *M* potassium acetate in anhydrous acetic acid, room temperature.

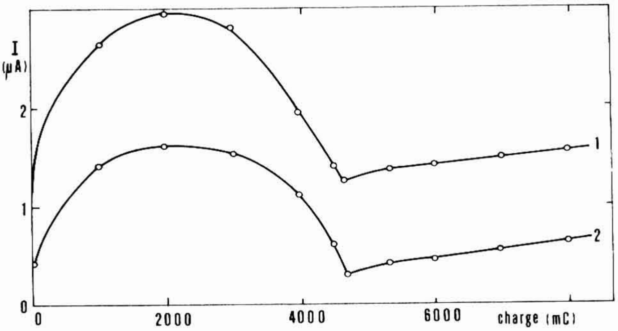


FIG. 1. Biamperometric titration curve for the coulometric titration of hydroquinone with manganese(III) acetate. Applied potential, 600 mV (2) and 800 mV (1).

curves are shown in Figs. 1 and 2, respectively. (In both cases, 5.00 ml of 0.01 *N* hydroquinone was titrated.) The accuracy and reproducibility of these determinations are apparent from the results given in Table 4. It follows from this table that the results obtained with the bipotentiometric indication show a better accuracy and reproducibility than those obtained with the biamperometric indication.

Indirect Coulometric Determination with Manganese(III) Acetate

The rate of the oxidation of some organic substances with trivalent manganese compounds is not sufficient for a direct coulometric determination. Thus attention has been paid to the possibility of an indirect coulometric determination with manganese(III) acetate.

First, it was necessary to find a suitable method for coulometric back-titration of an unreacted manganese(III) acetate. Therefore, the possibility of coulometric generation of ferrous ions was investigated. Because of the poor solubility of ferric salts in anhydrous acetic acid, it was necessary to carry out this generation in a mixture of acetic acid and water. It was found, however, that addition of water to the solution of man-

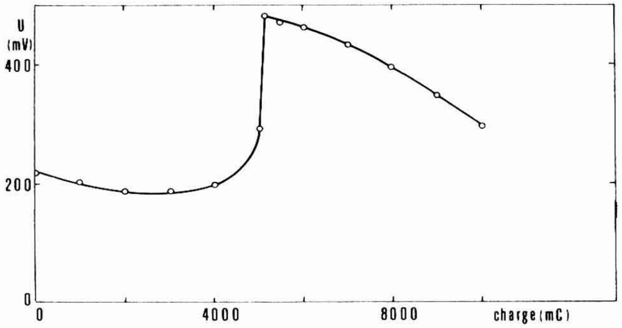


FIG. 2. Bipotentiometric titration curve for the coulometric titration of hydroquinone with manganese(III) acetate. Applied current, 2 μA.

TABLE 4

ACCURACY AND REPRODUCIBILITY OF THE DIRECT COULOMETRIC TITRATION OF HYDROQUINONE WITH MANGANESE(III) ACETATE

Taken (mg)	Found (mg)[a]	SD (mg)
2.846[b]	2.882	0.008
0.5226[b]	0.5132	0.007
0.2678[b]	0.2588	0.003
0.0536[b]	0.0510	0.004
2.812[c]	2.756	0.027

[a] Each value is the average of seven determinations, from which the standard deviation was calculated.

[b] Bipotentiometric indication with an applied current of 2 μA.

[c] Biamperometric indication with an applied potential of 600 mV.

ganese(III) acetate causes a decrease in its titer; this method of back-titration thus being practically inapplicable.

Therefore, the indirect coulometric determination of unconsumed manganese(III) acetate via its reaction with hydroquinone was further investigated. In this procedure, manganese(III) acetate is reduced with a known excess of hydroquinone, the unconsumed amount of which is then titrated with coulometrically generated manganese(III) acetate, using bipotentiometric indication. Thus it is unnecessary to change either the polarity of the generation electrode or the composition of the generation solution.

The applicability of this procedure was verified using hydroquinone as a model substance. The procedure was as follows: Into a generation vessel, 50 ml of acetic acid containing potassium acetate (0.6 *M*) and manganese(II) acetate (0.06 *M*) was measured and 5.00 ml of 0.005 N hydroquinone was added. Then the solution was electrolyzed by a current of 5 mA for 600 sec. The excess manganese(III) acetate thus generated was allowed to react with hydroquinone for time *t*. Afterward, another 5.00 ml of 0.005 *N* hydroquinone was added and the unconsumed hydroquinone was back-titrated with coulometrically generated manganese(III) acetate, using bipotentiometric indication. The consumption of the oxidizing agent in moles per mole of hydroquinone was calculated (see Table 5). It follows from Table 5 that under the given conditions the quinone formed does not undergo a further oxidation, even after 1 hr of the action of excess reagent. Further it follows from this table that electrochemically prepared manganese(III) acetate is sufficiently stable even for indirect determinations. The accuracy of the results obtained confirms the applicability of this method to the determination of an unconsumed excess of manganese(III) acetate.

SUMMARY

Conditions were found under which manganese(III) acetate can be electrochemically generated at a platinum anode with 100% current efficiency. It was found that even traces of

TABLE 5
TIME DEPENDENCE OF THE CONSUMPTION OF THE OXIDIZING REAGENT IN THE INDIRECT DETERMINATION OF HYDROQUINONE WITH COULOMETRIC GENERATED MANGANESE(III) ACETATE

Time (min)	0	5	10	30	60
Consumption of Mn(III) (mol/mol)	2.00	1.99	2.00	2.01	2.00

water decrease the current efficiency of the generation. The observed fact (*10*) that small amounts of water do not interfere in direct coulometric titrations of some substances can be explained by direct anodic oxidation of substances to be determined. The possibility of a direct coulometric titration of hydroquinone with manganese(III) acetate and an indirect coulometric determination of an unconsumed excess of manganese(III) acetate via its reaction with hydroquinone was further verified. It was confirmed that under the given conditions, the coulometrically prepared reagent is sufficiently stable even for indirect determinations.

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MICROCHEMICAL JOURNAL 27, 62–65 (1982)

The Oxidation of Organic Substances with Compounds of Trivalent Manganese

XXI. The Oxidation of Glycoaldehyde with Hexaquomanganese(III) Ions in a Noncomplexing Perchloric Acid Medium[1]

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INTRODUCTION

In the framework of the systematic study of the oxidation of organic substances by trivalent manganese compounds, the previous work was devoted to the oxidation of glycolaldehyde with manganese(III) sulfate (*3*). On the basis of a kinetic study of this reaction, an optimum procedure for its analytical use has been proposed. The procedure is based on the oxidation of glycolaldehyde with excess manganese(III) sulfate and back-titration of an unconsumed reagent with ferrous sulfate. This procedure enables the determination of 7–23 mg of glycolaldehyde. However, this reaction proceeds too slowly in dilute solutions for use on a micro level. For this reason the oxidation of glycolaldehyde with hexaquomanganese(III) ions in a noncomplexing perchloric acid medium was studied in this work. The reagent is substantially more reactive than manganese(III) sulfate whose reactivity is decreased by the formation of a sulfate complex of trivalent manganese (*2*). Thus it could be assumed that more reactive hexaquomanganese(III) ions would be applicable even for a microdetermination of glycolaldehyde.

EXPERIMENTAL

Reagents. A solution of hexaquomanganese(III) ions (0.005 *M*) in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate was prepared in the way already described (*6*). A standard solution of ferrous sulfate (0.01 *M*) in 0.5 *M* sulfuric acid was prepared in the usual way and its titer was found daily using dichromate. Glycolaldehyde solutions of the required molarity were prepared by dissolving accurately weighed amounts of the p.a. substance (Lachema, Brno) in redistilled water and diluting to the mark.

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[1] Part XX: The Oxidation of Galactose with manganese(III) sulphate. *Coll. Czech. Chem. Commun.*, submitted for publication.

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Their titer was checked by oxidation with sodium periodate (*9*) whose unconsumed excess was back-titrated with hydrazine sulfate (*8*). All the other chemicals used were of p.a. purity.

Apparatus. Potentiometric titrations were carried out using a TTT 1 titrator and an ABU 1 autoburette (Radiometer, Copenhagen). A bright platinum indicator and a saturated calomel reference electrode were used.

PROCEDURES AND RESULTS

Time Dependence of the Oxidation

Into a titration vessel, 20.00 ml of a 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate was measured and 5.00 ml of 0.005 *M* glycolaldehyde was added. The mixture was thoroughly stirred and allowed to stand at laboratory temperature. After time *t* the excess reagent was determined by potentiometric titration with 0.01 *M* ferrous sulfate. Simultaneously a blank was carried out and the consumption of the oxidizing agent in equivalents per mole of glycolaldehyde was calculated from the difference for the blank and the sample. The results obtained are given in Table 1. It follows from this table that under the given conditions the reaction proceeds nonstoichiometrically although the reagent consumption reaches its maximum value within 30 min and then is practically constant. This fact can be explained, similar to the oxidation of tartaric (*1*), citric (*7*), malonic (*4*), and pyruvic (*5*) acids, by a reaction of temporarily formed free radicals with the oxygen dissolved in the reaction mixture. This reaction leads to a lower oxidant consumption in comparison with that expected theoretically.

Therefore, the oxidation of glycolaldehyde with hexaquomanganese(III) ions was investigated under an inert atmosphere. The procedure was quite analogous to that described above, however, the solutions of the reagent and of the substance to be oxidized were freed from oxygen

TABLE 1
TIME DEPENDENCE OF THE OXIDATION OF GLYCOLALDEHYDE WITH HEXAQUOMANGANESE(III) IONS IN NONCOMPLEXING PERCHLORIC ACID MEDIUM IN THE PRESENCE (a) AND ABSENCE (b) OF ATMOSPHERIC OXYGEN

	Time (min)					
	5	30	60	120	240	360
Consumption of reagent (equiv/mol)						
(a)	1.58	1.80	1.81	1.82	1.84	1.83
(b)	1.66	1.89	1.92	1.96	1.98	1.97

by bubbling with nitrogen and an inert atmosphere was maintained during mixing of the solutions and the course of the reaction.

The results obtained are given in Table 1. It follows from this table that the reaction is quantitative and stoichiometric after 2 hr, the oxidant consumption being 2 equivalents per mole of glycolaldehyde. The reagent consumption does not increase after this time which proves that the reaction products do not undergo further oxidation. Similar to the oxidation of glycolaldehyde with manganese(III) sulfate (*3*) the consumption found corresponds to the reaction given by Eq. (1).

$$\begin{aligned} OH-CH_2-CHO + 2\ Mn^{3+} + H_2O \rightarrow CH_2O + HCOOH \\ + 2\ Mn^{2+} + 2\ H^+ \end{aligned} \tag{1}$$

Recommended Procedure

Into a titration vessel for titrations under an inert atmosphere, 20.00 ml of a 0.005 *M* solution of hexaquomanganese(III) ions in 6 *M* perchloric acid and 0.4 *M* manganese(II) perchlorate is measured. Nitrogen is passed through the solution for 15 min and 5.00 ml of a solution containing 0.7−2.3 mg of glycolaldehyde prebubbled with nitrogen is added. The inert atmosphere is maintained during the addition of this solution and during the oxidation. After 4 hr at laboratory temperature, the unconsumed oxidant is back-titrated potentiometrically with 0.01 *M* ferrous sulfate. A blank is carried out in parallel and the amount of glycolaldehyde is calculated from the difference. One millilitre of 0.01 *M* ferrous sulfate corresponds to 300.3 μg of glycolaldehyde. The accuracy and reproducibility of this determination follows from the results given in Table 2.

SUMMARY

The oxidation of glycolaldehyde with hexaquomanganese(III) ions in a noncomplexing perchloric acid medium was studied. The optimum conditions have been found for analytical use of the reaction. The recommended procedure is based on the oxidation of the test

TABLE 2

ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF GLYCOLALDEHYDE WITH HEXAQUOMANGANESE(III) IONS IN A NONCOMPLEXING PERCHLORIC ACID MEDIUM

Taken (μg)	Found (μg)[a]	Standard deviation (μg)
751	748	12
1501	1485	15
2252	2192	7

[a] The values are the average of seven determinations, from which the standard deviation value was calculated.

substance with the oxidant in the absence of atmospheric oxygen and back-titration of the unconsumed reagent with ferrous sulfate.

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The Oxidation of Organic Substances with Compounds of Trivalent Manganese

XXII. The Oxidation of Glyceraldehyde with Manganese(III) Sulfate[1]

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INTRODUCTION

Previous works in this series dealt with the oxidation of glycoaldehyde with manganese(III) sulfate (*1*) and hexaquomanganese(III) ions in a noncomplexing medium of perchloric acid (*2*). In the framework of a systematic study of the oxidation of organic substances with compounds of trivalent manganese, this work was devoted to the study of glyceraldehyde, which belongs to the same group of substances as those studied previously.

EXPERIMENTAL

Reagents. A solution of manganese(III) sulfate (0.05 *M*) in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate was prepared in the way already described (*4*). A standard solution of ferrous sulfate (0.1 *M*) in 0.5 *M* sulfuric acid was prepared in the usual way and its titer was found daily using dichromate. Glyceraldehyde solutions of the required molarity were prepared by dissolving a precisely weighed amount of the p.a. substance (Lachema, Brno) and diluting with redistilled water to the mark. Their titer was checked by oxidation with sodium periodate (*7*) and back-titration of the excess reagent with hydrazine sulfate (*5*). A saturated solution of 2,4-dinitrophenylhydrazine in 2 *M* hydrochloric acid and a 10% solution of chromotropic acid in distilled water were further used. A solution of 4.8 *M* sulfuric acid and 0.08 *M* manganese(II) sulfate containing 100 μg of formaldehyde per ml was prepared by adding the necessary amount of sulfuric acid and manganese(II) sulfate to a stock solution of formaldehyde in water and diluting with distilled water to the mark. The

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[1] Part XXI: The oxidation of glycolaldehyde with hexaquomanganese(III) ions in a noncomplexing perchloric acid medium. *Microchem. J.* 27, 62–65 (1982).

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titer of the stock solution of formaldehyde was determined according to Siggia and Maxcy (*8*). All chemicals used were of p.a. purity.

Apparatus. Potentiometric titrations were carried out using a TTT 1 titrator and an ABU 1 autoburette (Radiometer, Copenhagen). A bright platinum indicator and a saturated calomel reference electrode were used. Spectrophotometric measurements were carried out using a Specord UV VIS spectrophotometer (Zeiss, Jena) in 1-cm glass cuvettes.

PROCEDURES AND RESULTS

Time Dependence of the Oxidation

Into a titration vessel, 20.00 ml of a 0.05 *M* solution of manganese(III) sulfate in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate was measured and 5.00 ml of 0.025 *M* glyceraldehyde was added. The mixture was thoroughly stirred and allowed to stand at laboratory temperature. After time *t* the excess reagent was determined by potentiometric titration with 0.1 *M* ferrous sulfate. Simultaneously a blank was carried out and the consumption of the oxidant in equivalents per mole of glyceraldehyde was calculated from the difference for the blank and the sample. The results obtained are given in Table 1. It follows from this table that under the given conditions the reaction proceeds quantitatively and stoichiometrically within 2 min, the oxidant consumption being 4 equivalents per mole of glyceraldehyde. The reagent consumption does not increase even after 20 hrs, which indicates that the reaction products do not undergo further oxidation.

Detection of Formaldehyde Formed

2,4-Dinitrophenylhydrazine was used for this detection. The procedure was as follows: Into a beaker, 60 ml of 0.05 *M* solution of manganese(III) sulfate in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate was measured and 15 ml of 0.025 *M* glyceraldehyde was added. The mixture was thoroughly stirred and after 5 min the excess reagent was removed by adding of 2 g of solid oxalic acid. An amount of 80 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2 *M* hydrochloric acid was added, the precipitate formed filtered off, washed with distilled water, and

TABLE 1
TIME DEPENDENCE OF THE OXIDATION OF GLYCERALDEHYDE WITH MANGANESE(III) SULFATE

	Time (min)						
	1	2	5	30	60	240	1200
Reagent consumption (equiv/mol)	3.75	4.01	4.01	4.00	3.99	4.03	4.02

dried at 100°C. The melting point of the precipitate was found to be 166°C, which is consistent both with the melting point of an authentic formaldehyde derivative (167°C) and with the tabulated value (*9*).

The Determination of Formaldehyde Formed

The formaldehyde formed was determined by an adapted method of Bricker and Johnson (*6*).

Construction of the Calibration Curve

Amounts of 1.00, 1.20, 1.40, 1.60, 1.80, or 2.00 ml of 4.8 *M* sulfuric acid and 0.08 *M* manganese(II) sulfate solution containing 100 μg of formaldehyde per ml of the solution were measured into glass stoppered test tubes. Then 1.00 ml of 10% solution of chromotropic acid in water was added, the mixtures were thoroughly stirred, and 10 ml of concentrated sulfuric acid was added. The solutions thus formed were thoroughly agitated and then heated on a boiling water bath. After cooling to laboratory temperature, the solutions were quantitatively transferred into 100-ml volumetric flasks, diluted with distilled water to the mark, and their absorbances at 570 nm were immediately measured in 1-cm glass cuvettes.

The Sample Determination

An amount of 5.00 ml of 0.025 *M* solution of glyceraldehyde was added to 20.00 ml of 0.05 *M* solution of manganese(III) sulfate in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate. The reaction mixture was thoroughly stirred and after 5 min the excess reagent was removed by adding 2 g of solid oxalic acid. After another thorough mixing, 1.00 ml of the solution thus obtained was measured into a glass stoppered test tube. Further procedures were the same as that employed in the construction of the calibration curve. After measuring the absorbance at 570 nm, the amount of formaldehyde formed was found from the calibration curve constructed by the above described procedure. It was found that 1 ml of the solution obtained after the oxidation of glyceraldehyde contained 133 μg of formaldehyde. This amount roughly corresponds to 1 mol of formaldehyde arising from 1 mol of glyceraldehyde. (The value theoretically expected was 150 μg of formaldehyde.)

Reaction Stoichiometry

It can be assumed on the basis of the above results that the oxidation of glyceraldehyde with manganese(III) sulfate proceeds according to Eq. (1).

$$\begin{aligned}\mathrm{OHCH_2{-}CHOH{-}CHO} + 4\ \mathrm{Mn^{3+}} + 2\ \mathrm{H_2O} &\rightarrow \mathrm{CH_2O}\\ &+ 2\ \mathrm{HCOOH} + 4\ \mathrm{Mn^{2+}} + 4\ \mathrm{H^+}\end{aligned} \tag{1}$$

TABLE 2
ACCURACY AND REPRODUCIBILITY OF THE DETERMINATION OF GLYCERALDEHYDE WITH MANGANESE(III) SULFATE

Taken (mg)	Found (mg)[a]	SD (mg)
5.63	5.67	0.03
11.26	11.44	0.04
16.89	16.93	0.02

[a] Each value is the average of seven determinations, from which the standard deviation was calculated.

This equation is consistent with earlier findings on the oxidation of glycolaldehyde (*1*, *2*) and galactose (*3*) with various forms of trivalent manganese.

Recommended Procedure

Into a titration vessel, 20.00 ml of 0.05 *M* solution of manganese(III) sulfate in 6 *M* sulfuric acid and 0.1 *M* manganese(II) sulfate is measured and 5.00 ml of a solution containing 5–17 mg of glyceraldehyde is added. The reaction mixture is thoroughly stirred and allowed to stand for 5 min at laboratory temperature. The excess reagent is then potentiometrically back-titrated with 0.1 *M* ferrous sulfate. Simultaneously, a blank is carried out and an amount of the substance to be determined is calculated from the difference. One milliliter of 0.1 *M* ferrous sulfate corresponds to 2.252 mg of glyceraldehyde.

The accuracy and reproducibility of the determination of glyceraldehyde by the recommended procedure is given in Table 2.

SUMMARY

The oxidation of glyceraldehyde with manganese(III) sulfate was studied. The reaction stoichiometry was found and a procedure for an indirect determination of 5–17 mg of the test substance has been proposed. The recommended procedure is based on the oxidation of glyceraldehyde with the reagent, the excess of which is back-titrated with standard solution of ferrous sulfate.

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Extraction–Spectrophotometric Determination of Nickel with 2-Hydroxy-1-naphthaldoxime[1]

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INTRODUCTION

Salicylaldoxime has =NOH and –OH as functional groups, and has been recommended for the gravimetry (*2*), the solvent extraction–spectrophotometry (*3, 7, 10*), and the flame spectrophotometry (*4*) of nickel, copper, and palladium. Burger and Egyed (*1*) studied the complex formation of salicylaldoxime and its derivatives with some transition metal ions by spectrophotometry and potentiometry, and found that the electrophilic and nucleophilic substituents have an effect on the stability of these complexes. 2-Hydroxy-1-naphthaldoxime (HNA), one of these compounds, which was first used for the extraction–spectrophotometric determination of palladium (*9*), reacts with palladium in a strongly acidic solution.

This paper describes the extraction–spectrophotometric determination of nickel with HNA. HNA reacts with nickel(II) to form an insoluble complex, which can be extracted quantitatively into organic solvents, such as benzene, chloroform, and 1,2-dichloroethane. A method has been established for the direct spectrophotometric determination of nickel, after the extraction of nickel as its HNA complex into chloroform. The method is simple in that a single extraction suffices; common ions, except iron(III), cobalt(II), copper(II), and vanadium(V), do not interfere in the determination.

EXPERIMENTAL

Reagent and Apparatus

Standard nickel solution. A standard stock solution (100 μg Ni/ml) was prepared by dissolving 404.9 mg of nickel chloride hexahydrate in distilled water, adding small amounts of hydrochloric acid, and diluting the solution to 1 liter. The solution was standardized gravimetrically by the di-

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methylglyoxime method. The working solutions were made by suitable dilution of this stock solution as required.

HNA solution. The HNA was obtained by oximation of 2-hydroxy-1-naphthaldehyde in ethanol at a temperature of 0°C with hydroxylamine hydrochloride (*9*). The compound was recrystallized twice from a chloroform–petroleum mixture, giving a melting point of 159 to 160°C (found: C 70.44%, H 4.88%, N 7.31%; $C_{11}H_9NO_2$ requires C 70.58%, H 4.85%, N 7.48%). The compound was slightly soluble in water and very soluble in virtually all organic solvents. A solution of 2.0×10^{-2} mol/liter in chloroform was prepared and stored in the dark.

Buffer solution (pH 5.8). A buffer solution of pH 5.8 was prepared by mixing a 0.5 *M* potassium dihydrogen phosphate solution and a 0.5 *M* disodium hydrogen phosphate solution.

All other chemicals used were analytical reagent grade.

A Hitachi Model 624 automatic recording digital spectrophotometer was used to measure absorbances in a quartz cell of 1.00-cm path length. A Hitachi-Horiba Model F-7LC glass-electrode pH meter was used to measure pH values. An Iwaki Model KM shaker was used.

The Standard Procedure for Nickel Determination

Transfer the sample solution containing 5–50 μg of nickel to a 50-ml separatory funnel, add 5 ml of the phosphate buffer solution, and dilute to 20 ml with distilled water. Add 10 ml of the HNA–chloroform solution and shake the mixture for 10 min. Discard the aqueous phase and filter the organic phase through a dry filter paper. Measure the absorbance of the organic phase at 410 nm using a reagent blank as a reference.

RESULTS AND DISCUSSION

Absorption Spectra

Absorption spectra of HNA in chloroform and those of the extracted nickel(II)–HNA complex are shown in Fig. 1. The nickel complex has maximal absorbance at 410 nm.

Effect of Experimental Variables.

The effect of pH of the aqueous phase on the extraction of nickel complex was examined by measuring the absorbance of the organic phase at 410 nm. The final pH of each aqueous solution was measured after extraction. The results are shown in Fig. 2, from which it can be seen that maxmial absorbance can be obtained in the pH region between 5.0 and 6.4. The reagent blank curve was obtained in the same manner, using the same amounts of reagents. A phosphate buffer solution, pH 5.8, was found to be satisfactory for this purpose.

The effect of the concentration of HNA in chloroform on the extraction

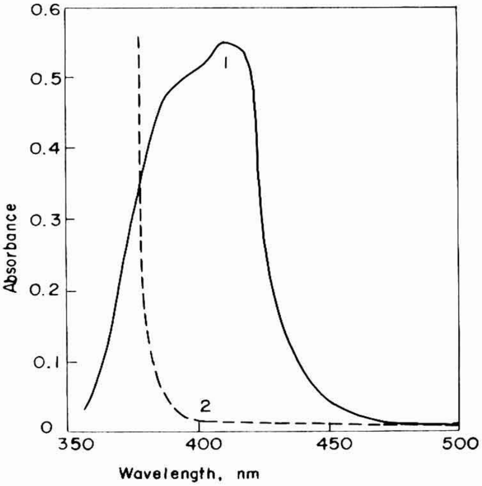

FIG. 1. Absorption spectra of HNA and Ni–HNA complex in chloroform. Aqueous phase: 20 ml of the solution containing 40 μg of nickel (pH = 5.8). Organic phase: 10 ml of 2×10^{-2} *M* HNA–chloroform solution. Shaking time: 10 min; (1) Ni–HNA complex; (2) blank.

of the complex was examined. It was found that the absorbance of the chloroform phase was essentially constant provided that the HNA concentration in the initial chloroform phase was more than 1.0×10^{-2} *M* for 50 μg of nickel.

Several kinds of organic solvents were tested in order to achieve a quantitative extraction of the complex. The complex can be extracted into

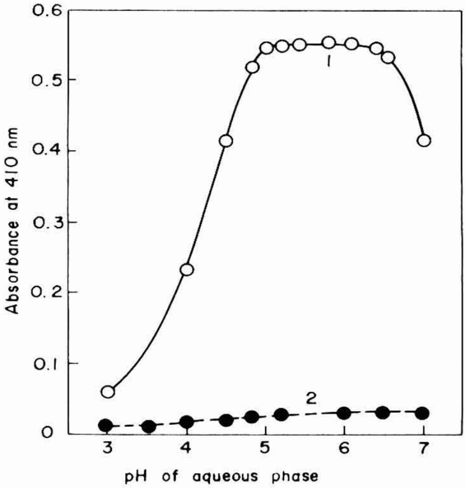

FIG. 2. Dependence of pH on the extraction of Ni–HNA complex in chloroform. Aqueous phase: 20 ml of the solution containing 40 μg of nickel. Organic phase: 10 ml of 2×10^{-2} *M* HNA–chloroform solution. Shaking time: 10 min; (1) Ni-HNA complex; (2) blank.

benzene ($\epsilon = 7.0 \times 10^3$, $\lambda_{max} = 412$ nm), chloroform ($\epsilon = 8.1 \times 10^3$, $\lambda_{max} = 410$ nm), and 1,2-dichloroethane ($\epsilon = 7.7 \times 10^3$, $\lambda_{max} = 410$ nm), but not into *n*-amyl acetate, sec-butyl acetate, isopropyl ether, and isobutyl methyl ketone. Toluene and xylene caused turbidity. Chloroform was chosen as the solvent which gave the highest absorbance.

Shaking for 10 min was found sufficient to ensure maximal absorbance. The organic extracts were found to be stable for at least 60 min.

The effect of volume of the aqueous phase was examined for the standard procedure. When the aqueous phase was varied from 10 to 100 ml, the absorbance of the complex in the chloroform phase was not affected, provided that the pH in the aqueous phase was maintained at about 5.8.

A calibration curve for the determination of nickel was prepared by the standard procedure. A good linear relationship was obtained over the concentration range from 5 to 50 μg of nickel per 10 ml of chloroform. The molar absorptivity of the extracted species was approximately 8.1×10^3 liters mol^{-1} cm^{-1} at 410 nm.

A standard solution containing 40 μg of nickel was analyzed eight times by the standard procedure. The relative standard deviation was 1.05%.

The proposed method is compared with a method using eight reagents of salicylaldoxime and its derivatives in Table 1. It can be seen that the proposed method is the most sensitive for nickel.

Effect of Diverse Ions

The effect of diverse ions on the determination of nickel was examined. The effect of metal ions is summarized in Table 2. It can be seen that copper(II) causes a high negative error, while cobalt(II), iron(III) and vanadium(V) give positive errors; in particular copper(II) interferes seri-

TABLE 1

EXTRACTION OF SOME NICKEL–SALICYLALDOXIME COMPLEXES

Reagent	λ_{max} (nm)	ϵ ($\times 10^{-3}$)	pH	Solvent
(1) Salicylaldoxime (SA)	387	5.7	7–8	Chloroform
(2) 5-Bromo-SA	395	5.7	8–9	Chloroform
(3) 3,5-Dibromo-SA	354	1.7	11	*tert*-Butyl acetate
(4) 5-Chloro-SA	397	5.9	7–9	Chloroform
(5) 3,5-Dichloro-SA	352	0.6	10	*tert*-Butyl acetate
(6) 3-Methoxy-SA	397	4.4	6–10	Chloroform
(7) *o*-Hydroxy acetophenoxime	376	5.0	7–9	Chloroform
(8) 2-Hydroxy propiophenoxime	378	5.0	7–9	Chloroform
(9) 2-Hydroxy-1-naphthaldoxime (HNA)	410	8.1	5.2–6.4	Chloroform

TABLE 2
EFFECT OF DIVERSE IONS[a]

Ion	Added (μg)	Ni found (μg)	Ion	Added (μg)	Ni found (μg)
Co(II)	40	41.0	Al(III)	200	40.0
			Cr(III)	200	
	100	42.8	Mo(VI)	200	
			Cd(II)	200	
Cu(II)	40	9.9			
Fe(III)	40	43.6	Mn(II)	200	39.6
			Pb(II)	200	
			Zn(II)	200	
	40	40.2[b]	Hg(II)	200	
	200	45.0			
			Cd(II)	200	39.4
	200	43.8[b]	Pb(II)	200	
			Mn(II)	200	
V(V)	40	42.7	Hg(II)	200	
	40	40.3[b]			
	100	48.8			
	100	43.2[b]			

[a] Nickel taken: 40.0 μg.

[b] 1 ml of NaF solution (0.1 *M*) was added.

ously even when present only at equal weight with nickel. The following metal ions do not interfere up to at least 1 mg: aluminum(III), barium(II), calcium(II), cadmium(II), lead(II), magnesium(II), manganese(II), mercury(II), molybdenum(VI), silver(I), strontium(II), and zinc(II). Of the anions tested, chloride, fluoride, perchlorate, phosphate, nitrate, and sulfate have no effect even in large amounts. Chelating agents such as EDTA and CyDTA, however, interfere seriously.

Sodium fluoride was investigated as a masking agent of the more seriously offending ions. Iron(III) and vanadium(V) of equal weight with nickel can be effectively masked by fluoride and can be tolerated without further separation. However, the interference by copper(II) and cobalt(II), and more than 50 μg of iron(III) and vanadium(V), cannot be eliminated by the addition of fluoride. Therefore, these ions must be separated by suitable procedures.

Several methods were tried to eliminate the interfering ions for the determination of nickel. The isobutyl methyl ketone extraction method for iron(III) (*6*), the dithizone extraction method for copper(II), (*5*), and the 2-nitroso-1-naphthol-4-sulfonic acid extraction for cobalt(II) (*8*), effected a good separation of these metal ions.

Composition of the Extracted Species

The composition of the nickel complex extracted into the chloroform phase was examined by Job's continuous variation method which gave a ratio of 1:2, nickel-to-HNA, and the ratio was verified by the mole ratio method.

SUMMARY

A method for the solvent extraction–spectrophotometric determination of nickel with 2-hydroxyl-1-naphthaldoxime (HNA) has been studied. The method is based upon the formation of a nickel–HNA complex which is extracted into chloroform from an aqueous solution of pH 5.8. The nickel–HNA complex in chloroform exhibits an absorption maximum at 410 nm with molar absorptivity of 8.1×10^3 liters mol^{-1} cm^{-1}. Beer's law is applicable in the range from 5 to 50 μg of nickel. The mole ratio of the complex and effect of interfering ions are described.

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Some Analytical Applications of Aromatic Sulfonyl Haloamines: Determination of Thiocyanate and Cyanide Ions in Metal Complexes and Salts with Bromamine-B and Dichloramine-B

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INTRODUCTION

As a part of our broad program of introducing new redox titrants in general and aromatic sulfonyl haloamines in particular, to develop rapid and accurate analytical procedures for determining variety of industrially and biologically important organic and inorganic substances in solution (*4–11*) we have introduced two new reagents, *N*,*N*′-dichlorobenzene sulfonamide or dichloramine-B ($C_6H_5SO_2NCl_2$, abbreviated as $RNCl_2$ or DCB) (*13*) and sodium *N*-bromobenzene sulfonamide or bromamine-B ($C_6H_5SO_2NBrNa \cdot 1.5\ H_2O$, abbreviated as RNBrNa or BAB) (*1*), as additions to the class of *N*-haloamines. Thiocyanates and cyanides have a number of industrial applications and the present paper reports the analytical procedures to determine these ions in metal salts and complexes with the new oxidants. The proposed procedures are simple, elegant, and reproducible under the experimental conditions described. An added advantage is that the technique can be employed for computing the number of CN^- and NCS^- ligands in metal complexes.

MATERIALS AND METHODS

Potassium thiocyanate (AR, E'Merck) was dried at 150°C and its purity was checked. Metal thiocyanates LiNCS, NaNCS, $Cd(NCS)_2$, $Zn(NCS)_2$,

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$Ni(NCS)_2 \cdot 0.5\ H_2O$, $Ba(NCS)_2 \cdot 2\ H_2O$, $Pb(NCS)_2$ and $UO_2(NCS)_2 \cdot 3\ H_2O$, and metal complexes $K_2Cd(NCS)_4 \cdot 2\ H_2O$, $K_2Zn(NCS)_4 \cdot 4\ H_2O$, $K_4Ni(NCS)_6 \cdot 4\ H_2O$, $KUO_2(NCS)_3 \cdot 2\ H_2O$, and $K_4Pb(NCS)_6$ were prepared (*4, 6, 11*) and their purity was checked by elemental analyses. Potassium cyanide (AR, Reanal, Hungary) and NaCN were used without further purification. The metal salts AgCN and $Zn(CN)_2$ and complexes $KAg(CN)_2$ and $K_2Zn(CN)_4$ were prepared (*5, 6, 8, 11*) and the purity was checked.

Reductant solutions. Aqueous solutions (~2 mg/ml) of soluble metal salts and complexes of thiocyanate and cyanide were prepared in triple distilled water. Lead thiocyanate, $Pb(NCS)_2$ was dissolved (~2 mg/ml) in 2 *N* acetic acid. Aqueous solution of KCN (0.025 *M* and previously standardized) was used as solvent (*5, 6, 11*) for the insoluble cyanides, AgCN and $Zn(CN)_2$.

Preparation of bromamine-B. This was prepared by the partial debromination of dibromamine-B (*1*) which in turn was prepared by the bromination of chloramine-B. Dibromamine-B (31.5 g) was added in small quantities at a time and with constant stirring to 50 ml of 4 *M* NaOH. The solution was cooled in ice. The crystals formed were filtered under suction and dried over anhydrous calcium chloride. The compound was recrystallized from hot water (50°C). The purity of BAB was checked iodometrically based on the active bromine present.

NMR spectrum of bromamine-B. The FT-NMR spectrum of BAB was obtained in D_2O solvent, using tetramethylsilane (TMS) as the internal standard. The ^{13}C spectrum (obtained on a Bruker WH 270-MHz nuclear magnetic resonance spectrometer) showed signals (ppm relative to TMS) at: 143.38 (C-1, carbon attached to S atom); 134.30 (C-4, para to the heteroatom); 131.26 (C-2,6), and 129.31 (C-3,5).

Bromamine-B solution. An approximately decinormal solution of BAB was prepared by dissolving 7.15 g of the solid in 500 ml of triple distilled water. The solution was then standardized by the iodometric method and stored in amber colored bottles.

Preparation of dichloramine-B. This was prepared (*13*) by passing chlorine gas through an aqueous solution of chloramine-B. About 30 g of chloramine-B were dissolved in 500 ml of water and pure chlorine gas was bubbled through the solution for about 2 hr. The white precipitate of DCB formed was filtered under suction, washed several times with water, and dried in a blackened vacuum desiccator. The purity of the compound was checked iodometrically.

NMR spectrum of dichloramine-B. The FT-NMR spectrum of DCB was obtained in $CDCl_3$ solvent, using tetramethylsilane (TMS) as the internal standard. The ^{13}C spectrum (obtained on a Bruker WH 270-MHz nuclear magnetic resonance spectrometer) showed signals (ppm relative to TMS) at: 136.16 (C-1, carbon attached to S atom); 129.09 (C-4, para to the heteroatom); 131.38 (C-2,6) and 129.37 (C-3,5).

Dichloramine-B solution. An approximately decinormal solution of DCB was prepared by dissolving 3 g of the solid in 500 ml of glacial acetic acid containing 10% (v/v) acetic anhydride. It was then standardized iodometrically and stored in amber colored bottles.

Buffer solutions. The following buffer solutions were prepared according to the standard methods reported in literature (*3*). pH 1 and 2 (HCl + KCl), pH 3 (citric acid + Na_2HPO_4), pH 4–6 (acetate + acetic acid), pH 7–9 (borax + boric acid + NaCl), and pH 10 ($NaHCO_3$ + Na_2CO_3).

Reagents of acceptable grades of purity were used in preparing other solutions. Experiments with KCN in acids and acid buffer media were conducted in a fume chamber.

Preliminary investigations. Known quantities of the reductant solutions in different solvent media were added to a known volume of oxidant (25 ml of 0.1 *N* solution) in an iodine flask (only aqueous solutions of reductants were used with DCB). The reaction mixtures were set aside for various intervals of time at room temperature (27 ± 3°C) with occasional shaking. The excess of the oxidant (BAB or DCB) left unconsumed was iodometrically determined by back titration with standard thiosulfate solution.

The results of some of these investigations with BAB are shown in Table 1, where the oxidation period is 5 min. It is seen that the oxidation of NCS^- ion is slow in $HClO_4$, HCl, and H_2SO_4 (0.1 *N*) and buffers of pH 1–10, but is rapid in presence of NaOH and an eight electron stoichiometry is noted in 0.05–0.20 *M* alkali. Slow oxidation of KCN took place in acid and buffer media and a two electron stoichiometry was noted in 0.05–0.20 *M* NaOH. The rate of oxidation of aqueous KNCS and KCN solutions with DCB is shown in Table 2. It is seen that the same two and eight electron stoichiometries respectively per mole of thiocyanate and cyanide were observed with DCB in partially aqueous medium in about 30 min. Similar observations were noted with other metal salts and complexes of thiocyanate and cyanide.

Further studies have revealed that stoichiometric oxidation of the reductants takes place within the specified time period even with 50–60% excess of the oxidant.

Recommended procedures. (*i*) *With bromamine-B.* Add aliquots of NCS^- or CN^- (salt or complex) solutions to a known volume (50–60% excess) of ~0.1 *N* BAB in an iodine flask, containing enough NaOH to maintain an overall concentration of 0.05–0.20 *N* alkali. Set aside the reaction mixtures for about 5 min with occasional shaking. Add 10 ml of 2 *N* H_2SO_4 and 20 ml of 10% KI and dilute to 150 ml. Titrate the liberated

TABLE 1

OBSERVED STOICHIOMETRY OF OXIDATION OF POTASSIUM THIOCYANATE AND POTASSIUM CYANIDE IN DIFFERENT BUFFER AND SOLVENT MEDIA WITH BROMAMINE-B (BAB)

Medium	μmol of BAB consumed[a]/ μmol of KNCS taken[b]	Number of electrons changing per mol of KNCS	μmol of BAB consumed[a]/ μmol of KCN taken[c]	Number of electrons changing per mol of KCN
0.1 *N* $HClO_4$	3.900	7.80	0.045	0.09
0.1 *N* HCl	3.930	7.86	0.055	0.11
0.1 *N* H_2SO_4	3.790	7.58	0.045	0.09
pH 1	3.450	6.90	0.065	0.13
pH 2	3.495	6.99	0.060	0.12
pH 3	3.435	6.87	0.040	0.08
pH 4	3.315	6.63	0.060	0.12
pH 5	3.350	6.70	0.055	0.11
pH 6	3.450	6.90	0.070	0.14
pH 7	3.295	6.59	0.070	0.14
pH 8	3.230	6.46	0.095	0.19
pH 9	3.200	6.40	0.080	0.16
pH 10	3.510	7.02	0.445	0.89
0.002 *N* NaOH	3.650	7.30	0.710	1.42
0.02 *N* NaOH	3.745	7.49	0.740	1.48
0.05 *N* NaOH	4.005	8.01	1.000	2.00
0.10 *N* NaOH	4.005	8.01	1.000	2.00
0.20 *N* NaOH	4.004	8.01	1.000	2.00

[a] Time, 5 min; BAB taken, 1250 μmol.
[b] KNCS taken, 94 μmol.
[c] KCN taken, 146.5 μmol.

TABLE 2

EXTENT OF OXIDATION OF POTASSIUM THIOCYANATE AND POTASSIUM CYANIDE WITH DICHLORAMINE-B (DCB) IN PARTIALLY AQUEOUS MEDIUM

Time (min)	μmol of DCB consumed[a]/ μmol of KNCS taken[b]	Number of electrons changing per mol of KNCS	μmol of DCB consumed[a]/ μmol of KCN taken[c]	Number of electrons changing per mol of KCN
1	1.545	6.18	0.335	1.33
5	1.790	7.16	0.350	1.40
15	1.970	7.88	0.400	1.60
30	1.995	7.98	0.503	2.01
45	2.005	8.02	0.502	2.01
60	1.999	8.00	0.508	2.03

[a] DCB taken, 625 μmol.
[b] KNCS taken, 94 μmol.
[c] KCN taken, 146.5 μmol.

iodine against 0.05 N sodium thiosulfate to a starch end point (V_1ml). Run a blank with BAB solution alone (V_2ml).

(*ii*) *With dichloramine-B*. Add aliquots of reductant solutions to a known volume (50–60% excess) of ~0.1 N DCB and set the reaction mixture aside for about 30 min. Then add 20 ml of 10% KI and titrate the liberated iodine with 0.05 N sodium thiosulfate (V_1 ml) using starch indicator. Run a blank with DCB solution alone (V_2 ml).

The amount of the reductant (X, micromoles) in the sample solution is given by $X = 10^3N(V_2 - V_1)/\mathrm{E}$, where N is the normality of thiosulfate and E is the number of electrons changing per molecule of the reductant. For $KAg(CN)_2$ and $K_2Zn(CN)_4$, $E = 4$ and 8, respectively. For Cd, Ni, Pb, Zn, Ba, and UO_2 salts of thiocyanate $E = 16$, while for complex thiocyanates of U, Zn, Cd, Ni, and Pb $E = 24$, 32, 32, 48, and 48, respectively.

Calculation of recovery with insoluble cyanides. Add known aliquots (a, ml) of insoluble cyanides in 0.025 M KCN to a known excessive (50–60% excess) volume of BAB or DCB (V, ml). Maintain an overall NaOH concentration of 0.05–0.2 M with BAB. Set aside the reaction mixtures for about 5 min with BAB and 30 min with DCB, with occasional shaking. Determine the unconsumed oxidant (V_3, ml). Add a ml of 0.025 M KCN to V ml of BAB or DCB under the same conditions as above. Estimate the unreacted oxidant by iodometric titration with thiosulfate (V_4 ml). Then the amount (Y, micromoles) of insoluble cyanide in the sample solution is given by $Y = 10^3N(V_4 - V_3)/E$ where $E = 2$ and 4 for AgCN and $Zn(CN)_2$, respectively, and N is the normality of thiosulfate.

RESULTS AND DISCUSSION

Table 1 shows the time dependence of the oxidation of NCS^- and CN^- ions by BAB in various buffer and solvent media. It follows from this table that the studied reactions with BAB proceed quantitatively and stoichiometrically with the consumption of eight and two equivalents of the oxidant per mole of NCS^- and CN^- ions, respectively. The stoichiometric oxidation of thiocyanate ion in metal salts and complexes with BAB is shown in Eqs. (1) and (2), respectively.

$$\begin{aligned} M(NCS)_x &+ 4x\,RNBrNa + 2x\,OH^- + 3x\,H_2O \rightarrow M^{x+} + x\,CNO^- \\ &+ x\,SO_4^{2-} + 4x\,RNH_2 + 4x\,Na^+ + 4x\,Br^- \end{aligned} \tag{1}$$

For the oxidation of lithium, sodium, and potassium salts of thiocyanate, $x = 1$; while $x = 2$ for Cd, Zn, Ni, UO_2, Ba, and Pb salts.

$$\begin{aligned} MyM_1(NCS)_x &+ 4x\,RBNrNa + 2x\,OH^- + 3x\,H_2O \rightarrow yM^+ + M_1^{2+} \\ &+ x\,CNO^- + x\,SO_4^{2-} + 4x\,RNH_2 \\ &+ 4x\,Na^+ + 4x\,Br^- \end{aligned} \tag{2}$$

For the cadmium and zinc complexes, $y = 2$ and $x = 4$, and $y = 4$ and $x = 6$ for Ni and Pb complexes, while for potassium uranyl thiocyanate $y = 1$ and $x = 3$.

Oxidation of cyanide ion in salts and complexes is represented by Eqs. (3)–(5).

$$\begin{aligned} \mathrm{M(CN)}_x &+ x\ \mathrm{RNBrNa} + x\ \mathrm{H_2O} \rightarrow \mathrm{M}^{x+} + x\ \mathrm{CNO}^- \\ &+ x\ \mathrm{RNH_2}\ x\ \mathrm{Na}^+ + x\ \mathrm{Br}^- \end{aligned} \tag{3}$$

$$\begin{aligned} \mathrm{KAg(CN)_2} &+ 2\ \mathrm{RNBrNa} + 2\ \mathrm{H_2O} \rightarrow \mathrm{K}^+ + \mathrm{Ag}^+ \\ &+ 2\ \mathrm{CNO}^- + 2\ \mathrm{RNH_2} + 2\ \mathrm{Na}^+ + 2\ \mathrm{Br}^- \end{aligned} \tag{4}$$

$$\begin{aligned} \mathrm{K_2Zn(CN)_4} &+ 4\ \mathrm{RNBrNa} + 4\ \mathrm{H_2O} \rightarrow 2\ \mathrm{K}^+ + \mathrm{Zn}^{2+} \\ &+ 4\ \mathrm{CNO}^- + 4\ \mathrm{RNH_2} + 4\ \mathrm{Na}^+ + 4\ \mathrm{Br}^- \end{aligned} \tag{5}$$

Similarly, the stoichiometry observed with DCB may be represented as in Eqs. (6)–(10).

$$\begin{aligned} \mathrm{M(NCS)}_x &+ 2\,x\ \mathrm{RNCl_2} + 5\,x\ \mathrm{H_2O} \rightarrow \mathrm{M}^{x+} + x\ \mathrm{CNO}^- \\ &+ 2\,x\ \mathrm{RNH_2} + 6\,x\ \mathrm{H}^+ + x\ \mathrm{SO_4^{2-}} + 4\,x\ \mathrm{Cl}^- \end{aligned} \tag{6}$$

$$\begin{aligned} \mathrm{MyM_1(NCS)}_x &+ 2\,x\ \mathrm{RNCl_2} + 5\,x\ \mathrm{H_2O} \rightarrow y\ \mathrm{M}^+ + \mathrm{M_1^{2+}} + 2\,x\ \mathrm{RNH_2} \\ &+ 6\,x\ \mathrm{H}^+ + x\ \mathrm{CNO}^- + x\ \mathrm{SO_4^{2-}} + 4\,x\ \mathrm{Cl}^- \end{aligned} \tag{7}$$

$$\begin{aligned} \mathrm{M(CN)}_x &+ (x/2)\ \mathrm{RNCl_2} + x\ \mathrm{H_2O} \rightarrow \mathrm{M}^{x+} + x\ \mathrm{CNO}^- + (x/2)\ \mathrm{RNH_2} \\ &+ x\ \mathrm{H}^+ + x\ \mathrm{Cl}^- \end{aligned} \tag{8}$$

$$\begin{aligned} \mathrm{KAg(CN)_2} &+ \mathrm{RNCl_2} + 2\ \mathrm{H_2O} \rightarrow \mathrm{K}^+ + \mathrm{Ag}^+ + 2\ \mathrm{H}^+ + 2\ \mathrm{CNO}^- \\ &+ 2\ \mathrm{Cl}^- + \mathrm{RNH_2} \end{aligned} \tag{9}$$

$$\begin{aligned} \mathrm{K_2Zn(CN)_4} &+ 2\ \mathrm{RNCl_2} + 4\ \mathrm{H_2O} \rightarrow 2\mathrm{K}^+ + \mathrm{Zn}^{2+} + 4\ \mathrm{H}^+ + 4\ \mathrm{CNO}^- \\ &+ 4\ \mathrm{Cl}^- + 2\ \mathrm{RNH_2} \end{aligned} \tag{10}$$

where $\mathrm{R} = \mathrm{C_6H_5SO_2}-$.

The oxidation of NCS^- and CN^- ions present in metal salts and complexes is in agreement with the stoichiometry reported earlier (*4–6, 8, 11*).

In reactions (1) to (5) the oxidant (BAB) undergoes a two electron change while in reactions (6) to (10) DCB undergoes a four electron change. The products formed in the reactions do not undergo further oxidation. Some typical results of analysis of thiocyanate and cyanide ions present in salts and complexes are given in Tables 3–6.

The tables show the range of reductants employed, standard deviation, percentage coefficient of variance, and percentage error in recovery. Each range covers the sample sizes present in 8–10 different aliquots of the reductant solutions. It is seen that the results are accurate within an error of about 0.5% in the case of BAB and about 1% with DCB.

Interference

Common anions such as $\mathrm{SO_4^{2-}}$, $\mathrm{PO_4^{3-}}$, $\mathrm{NO_3^-}$, $\mathrm{ClO_4^-}$, F^-, Cl^-, and Br^- do not interfere but hydrazine, urea, and thiourea in any amount interfere in the estimation.

TABLE 3

ACCURACY AND REPRODUCIBILITY OF DETERMINATION OF THIOCYANATE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-B AND DICHLORAMINE-B

Reductant	Bromamine-B			Dichloramine-B		
	Amount taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)	Amount taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)
LiNCS	117.7	0.016	0.20	118.0	0.017	0.23
KNCS	102.9	0.031	0.31	98.5	0.042	0.43
NaNCS	140.0	0.021	0.19	140.0	0.021	0.19
$Cd(NCS)_2$	43.8	0.006	0.06	43.5	0.036	0.37
$Ni(NCS)_2 \cdot 0.5\ H_2O$	53.7	0.021	0.21	53.7	0.031	0.32
$Pb(NCS)_2$	27.5	0.016	0.18	27.5	0.052	0.58
$Zn(NCS)_2$	52.6	0.000	0.00	52.6	0.036	0.38
$Ba(NCS)_2 \cdot 2\ H_2O$	48.4	0.036	0.26	48.4	0.042	0.30
$UO_2(NCS)_2 \cdot 3\ H_2O$	26.7	0.028	0.24	26.7	0.072	0.62
$K_2Zn(NCS)_4 \cdot 4\ H_2O$	25.0	0.026	0.23	25.0	0.036	0.32
$K_2Cd(NCS)_4 \cdot 2\ H_2O$	25.1	0.036	0.32	25.1	0.036	0.32
$K_4Ni(NCS)_6 \cdot 4\ H_2O$	15.0	0.011	0.12	15.0	0.031	0.33
$K_4Pb(NCS)_6$	15.0	0.007	0.06	15.0	0.036	0.34
$KUO_2(NCS)_3 \cdot 2\ H_2O$	24.9	0.036	0.28	24.9	0.052	0.40

[a] Calculated for six trials.

TABLE 4
DETERMINATION OF THIOCYANATE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-B AND DICHLORAMINE-B

Reductant	Bromamine-B		Dichloramine-B	
	Range studied (μmol)	Error in recovery (%)	Range studied (μmol)	Error in recovery (%)
LiNCS	23.53–588.30	0.26–0.00	23.53–589.70	0.43–0.00
KNCS	20.60–514.51	0.50–0.03	19.65–492.39	0.84–0.01
NaNCS	28.00–700.01	0.44–0.00	28.00–700.01	0.88–0.00
$Cd(NCS)_2$	8.75–218.76	0.50–0.02	8.71–217.66	0.84–0.00
$Ni(NCS)_2 \cdot 0.5\ H_2O$	10.74–268.58	0.50–0.00	10.74–268.58	0.81–0.00
$Pb(NCS)_2$	5.51–137.62	0.50–0.02	5.51–137.62	0.67–0.02
$Zn(NCS)_2$	10.52–263.04	0.17–0.00	10.52–263.04	0.42–0.00
$Ba(NCS)_2 \cdot 2\ H_2O$	9.67–241.80	0.36–0.02	9.67–241.80	0.60–0.09
$UO_2(NCS)_2 \cdot 3\ H_2O$	5.34–133.46	0.43–0.00	5.34–133.46	0.77–0.17
$K_2Zn(NCS)_4 \cdot 4\ H_2O$	5.00–125.03	0.45–0.00	5.00–125.03	0.54–0.05
$K_2Cd(NCS)_4 \cdot 2\ H_2O$	5.01–125.29	0.49–0.00	5.01–125.29	0.87–0.04
$K_4Ni(NCS)_6 \cdot 4\ H_2O$	2.99–74.73	0.50–0.01	2.99–74.73	0.63–0.00
$K_4Pb(NCS)_6$	2.99–74.78	0.47–0.00	2.99–74.78	0.66–0.01
$KUO_2(NCS)_3 \cdot 2\ H_2O$	4.99–124.67	0.39–0.00	4.99–124.67	0.77–0.02

Product Analyses

The presence of sulfate ion in the reaction mixture was detected (*2*) using sodium rhodizonate and barium chloride. Cyanate was identified by Werner test (*12*) as follows: a few drops of pyridine and 2–3 drops of 1% solution of copper sulfate was added to 10 ml of water. Then 2 ml of chloroform was added followed by the test solution. On shaking the mixture briskly, a lilac blue color appeared in the chloroform layer due to the

TABLE 5
ACCURACY AND REPRODUCIBILITY OF DETERMINATION OF CYANIDE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-B AND DICHLORAMINE-B

Reductant	Bromamine-B			Dichloramine-B		
	Amount taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)	Amount taken (μmol)	Standard deviation[a]	Coefficient of variance[a] (%)
KCN	155.1	0.036	0.36	147.0	0.042	0.43
NaCN	240.8	0.000	0.00	240.8	0.062	0.53
AgCN	99.7	0.011	0.08	99.7	0.083	0.62
$Zn(CN)_2$	87.3	0.042	0.41	100.1	0.072	0.62
$KAg(CN)_2$	51.4	0.011	0.11	51.4	0.044	0.43
$K_2Zn(CN)_4$	39.5	0.017	0.18	39.5	0.036	0.37

[a] Calculated for six trials.

TABLE 6
DETERMINATION OF CYANIDE ION IN METAL SALTS AND COMPLEXES WITH BROMAMINE-B AND DICHLORAMINE-B

	Bromamine-B		Dichloramine-B	
Reductant	Range studied (μmol)	Error in recovery (%)	Range studied (μmol)	Error in recovery (%)
KCN	31.0–775.5	0.29–0.00	29.3–734.8	0.84–0.01
NaCN	48.2–1204.1	0.00–0.00	48.2–1204.1	0.68–0.07
AgCN	19.9–498.6	0.37–0.07	59.8–498.6	0.49–0.12
$Zn(CN)_2$	17.5–436.5	0.49–0.02	60.1–500.4	0.68–0.00
$KAg(CN)_2$	10.3–257.1	0.49–0.00	10.3–257.1	0.98–0.03
$K_2Zn(CN)_4$	7.9–197.3	0.50–0.01	7.9–197.3	1.00–0.02

formation of the complex, $Cu(CNO)_2(C_5H_5N)_2$. The reduction product of the oxidants, benzene sulfonamide was detected (*13*) by thin-layer chromatography. A mixture of petroleum ether, chloroform, and *n*-butanol (2:2:1 v/v) was used as the solvent (R_f = 0.88). Iodine was employed as the detection reagent.

It can be concluded that the proposed analytical techniques are simple, rapid, and reproducible and are useful for estimating the reductants in solution and for computing the number of ligands present in the cyanide and thiocyanate complexes.

SUMMARY

Simple, rapid, and reproducible methods for the determination of thiocyanate and cyanide ions in metal salts and complexes with bromamine-B (BAB) and dichloramine-B (DCB) have been developed. The oxidation involves eight and two electron changes, respectively, with NCS^- and CN^- ions in 0.05–0.20 *N* NaOH medium in the case of BAB and in partially aqueous medium with DCB. The proposed methods could be employed for computing the number of thiocyanate and cyanide ligands present in the respective metal complexes.

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Sodium *N*-Chloro-*p*-Nitrobenzenesulfonamide (Chloramine-N) as a New Redox Titrant

I. The Status of Chloramine-N as a Titrimetric Reagent

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INTRODUCTION

Analytical applications of chloramine-T have been well explored by Bishop and Jennings (*5*). Oxidimetric reactions of aromatic sulfonyl haloamines such as chloramine-T, dichloramine-T, bromamine-T, dibromamine-T, chloramine-B, dichloramine-B, bromamine-B, and dibromamine-B have been extensively studied by several workers (*1, 7, 10–12, 14, 15, 18, 19*). Among these aromatic sulfonyl haloamines, the monohalocompounds such as chloramine-T and bromamine-T are soluble in water, while the dihalocompounds such as dichloramine-T and dibromamine-T are soluble in nonaqueous solvents and hence can be employed as redox titrants in nonaqueous or partially aqueous media. Ultraviolet and visible spectrophotometric methods for the assay of chloramine-T and chloramine-B in aqueous media have recently been developed in our laboratories (*9, 17*).

As a part of our investigations of potential oxidimetric reagents we have recently sythesized a new oxidant, dichloramine-N, and studied its utility as an analytical reagent in acetic acid medium (*8*). In the present paper we report the preparation of a new member of the class of aromatic sulfonyl haloamines, namely, chloramine-N (sodium salt of *N*-chloro-*p*-nitrobenzenesulfonamide, $p\text{-}O_2N-C_6H_4-SO_2NClNa\cdot 3H_2O$; hereafter abbreviated as CAN), which can be used as a potential redox titrant in aqueous medium. The CAN prepared was characterized by its uv, ir, and FT ^{1}H- and ^{13}C-NMR spectral data. The status of this titrant as a new

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titrimetric reagent was examined by determining some typical, yet diverse, reducing agents such as glutathione, thioglycolic acid, thiourea, ascorbic acid, methionine, sodium sulfite, and sodium arsenite by direct titration methods. Advantages of the proposed analytical methods include rapidity and reproducibility. Another significant feature of this novel oxidant, CAN, is that the presence of the electron-withdrawing nitro group in the *para* position of the benzene ring could influence the redox reactions involving electron-deficient transition states.

MATERIALS AND METHODS

Chloramine-N

CAN was prepared by the chlorination of *p*-nitrobenzenesulfonamide in alkaline medium. Pure chlorine gas was bubbled through an aqueous solution of *p*-nitrobenzenesulfonamide (10 g) containing sodium hydroxide (4*N*, 100 ml) at 65°C with constant stirring of the solution until a slight yellow precipitate of dichloramine-N appeared. The solution was filtered under suction and the mother liquor obtained was shaken well three times with CCl_4 (~50 ml each time) to remove the dissolved contaminants, dichloramine-N and *p*-nitrobenzenesulfonamide. The aqueous layer separated was cooled in a freezer (0°C) overnight. The yellow crystals of CAN formed were filtered, washed carefully with a minimum amount of ice cold water (~5 ml) and then twice with CCl_4 (~20 ml each time), and dried. The yield of the compound was 1.0 g. The solid was stored in amber-colored bottles. The dry sample melted at 188–190°C with decomposition.

Elemental analyses of CAN for carbon, hydrogen, nitrogen, sulfur, chlorine, and sodium were performed by Huffman Laboratories, Inc. The chlorine content in CAN was also checked iodometrically based on the active chlorine present.

Found: carbon 23.01, hydrogen 3.12, nitrogen 8.70, sulfur 10.29, chlorine 11.50, and sodium 7.16%.

Calculated: carbon 23.07, hydrogen 3.22, nitrogen 8.96, sulfur 10.23, chlorine 11.34, and sodium 7.36%.

The compound was also characterized by its uv and ir spectral data and by recording its Fourier transform ^1H- and ^{13}C-NMR spectra.

Spectral Characteristics of Chloramine-N

Ultraviolet. The ultraviolet spectrum of CAN was obtained in ethanol with a Beckman DK-2A ratio-recording dual-beam spectrophotometer (Fig. 1). CAN has two λ_{max} at 215 (ϵ_{max} = 4531) and 260 nm (ϵ_{max} = 6275).

Infrared. The infrared spectrum of CAN (in KBr) was recorded on a Perkin–Elmer grating infrared spectrophotometer. The ir spectrum (*2–4,*

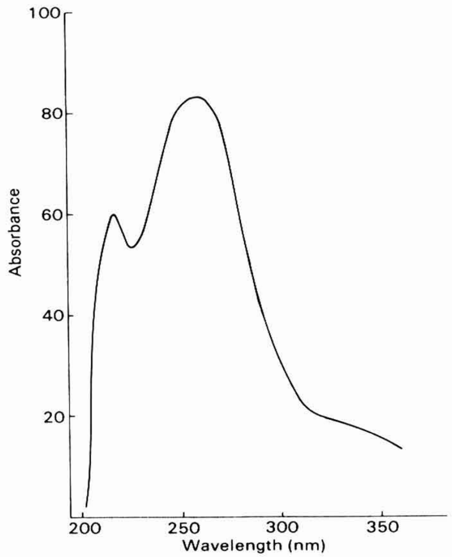


FIG. 1. Ultraviolet spectrum of sodium *N*-chloro-*p*-nitrobenzenesulfonamide (chloramine-N) (20 μg/ml) in ethanol at room temperature.

6, 13) of the compound (Fig. 2) shows bands (in cm^{-1}) at 3550–3100 (broad, O–H stretching), 2035 (weak, phenyl ring vibrations), 1930 (weak, υ-NCl), 1600 (strong, phenyl ring), 1510 (weak and broad, υ_{sym}-NCl; υ_{asym}-NO$_2$), 1345 (strong, υ_{sym}-NO$_2$), 1240 (strong, υ_{asym}-SO$_2$), 1125 (υ_{sym}-SO$_2$), 1080 (medium doublet, aromatic CH in-plane bending), 925 (very strong, S–N stretching), 848 (strong, υC–N), 725 (medium, C–N–O bending and out-of-plane CH bending of two adjacent aromatic H atoms), and 674 (medium, in-ring plane deformation).

Nuclear magnetic resonance. The nuclear magnetic resonance (NMR) spectra of CAN were carried out in D_2O solvent with dioxane as the internal standard.

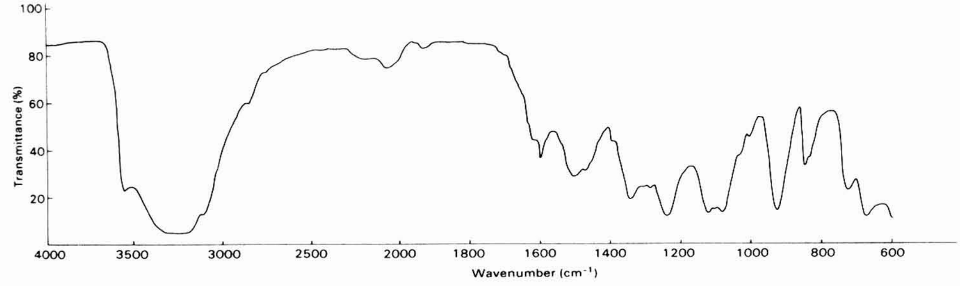


FIG. 2. Infrared spectrum of sodium *N*-chloro-*p*-nitrobenzenesulfonamide (chloramine-N) in KBr at room temperature.

^1H spectrum. The spectrum obtained on a Varian 60-MHz nuclear magnetic resonance spectrometer shows a quadruplet centered around 8.0δ with coupling constant $J_{o,m} = 8.0$ Hz, indicating the presence of *ortho* and *meta* protons. However, the Fourier transform ^{1}H spectrum (Varian 80-MHz FT-80A nuclear magnetic resonance spectrometer) shows a fine structure indicative of an A_2B_2 pattern.

^{13}C spectrum. The ^{13}C FT NMR spectrum (Varian 80-MHz FT-80A nuclear magnetic resonance spectrometer) shows signals (ppm) at 148.98 (C-1 atom attached to S atom), 128.8 (C-2,6), 146.36 (C-4 atom attached to N atom of the NO_2 group), and 124.08 (C-3,5). In this spectrum, the deshielding effect of the substituent nitro group is evident because all carbon resonances occur at lower fields when compared to other organic sulfonyl haloamines.

Preparation and Standardization of Stock Solutions of Chloramine-N

CAN is slightly soluble in common organic solvents such as ethanol, carbon tetrachloride, and acetic acid. Solubilities of the compound in water and alcohol were 194.8 and 0.21 g per kilogram of the solvent at 30°C, respectively.

An approximately 0.05*M* (~0.1*N*) solution of CAN was prepared by dissolving 15.6 g of the sample in a liter of distilled water and was preserved in an amber-colored bottle. The strength of the solution kept in a colorless bottle was found to decrease by about 2% over a period of 2 months, whereas that of a solution kept in an amber-colored bottle showed no change. An approximately 0.005*M* (0.01*N*) aqueous solution of CAN employed in the analytical procedures was prepared by appropriate dilution of the above stock solution. The CAN solution was standardized by the iodometric method suggested for chloramine-T (*5*) by adding 10 ml 2*N* H_2SO_4 and excess amount of 10% aqueous KI solution to a known volume of the oxidant and titrating the liberated iodine with a standard thiosulfate solution to a starch endpoint.

Reductant Solutions

The compounds used were of acceptable analytical reagent grade. Solutions of reduced glutathione (Aldrich Chemical Co.) *d*-isoascorbic acid (Eastman–Kodak Co.), and thiourea (Fisher Scientific Co.) (~4 mg/ml), and DL-methionine (Pfaltz and Bauer, Inc.) (~3 mg/ml) were prepared by dissolving weighed amounts of the solids in distilled water. Solutions (~4 mg/ml) of sodium sulfite (Baker Chemical Co.) and sodium arsenite (Fisher Scientific Co.) were prepared in deaerated (saturated with nitrogen) distilled water. Aqueous solution of thioglycolic acid (~4.0 mg/ml; Eastman Kodak Co.) was prepared and its stength checked by the chloramine-T method (*11*).

Aqueous solutions of $NaHCO_3$ (1 *M*), KI (1%;10%), KBr (10%) indigo carmine (0.05%), H_2SO_4 (2*N*;3*N*), and starch (1%) were prepared from reagents of acceptable grades of purity.

Recommended Determination Procedures

1. Oxidation of ascorbic acid, glutathione, and thiourea. To a measured volume (1–25 ml) of the reductant (ascorbic acid, glutathione, or thiourea) solution, 5–10 ml of 2 *N* H_2SO_4, 0.1–0.5 ml of KI solution (1%), and 1 ml of starch (1%) were added. The solution was diluted to 50 ml with distilled water and titrated with 0.01 *N* CAN to the appearance of a permanent pale blue color.

This procedure was modified appropriately for the determination of sulfite.

2. Oxidation of sulfite. The procedure was completely analogous to procedure 1, except that the solution of sulfite was saturated with nitrogen gas and the titration was carried out in an atmosphere of nitrogen.

3. Oxidation of arsenite. To an aliquot (1–25 ml) of sodium arsenite solution, 0.5–1.0 ml of 3 *N* H_2SO_4, 0.1–0.5 ml of KI solution (1%), 1 ml of starch (1%), and 50 ml of 1 *M* $NaHCO_3$ were added. The resultant solution was titrated under nitrogen with 0.01 *N* CAN to a permanent pale blue color. The presence of $NaHCO_3$ seems to have a buffering action by maintaining a favorable range of pH (4–6) during the course of the reaction, permitting a stoichiometric oxidation of the arsenite.

4. Oxidation of thioglycolic acid. To an aliquot (1–25 ml) of thioglycolic acid (mercaptoacetic acid) solution, 0.05–1.00 g of KI and 1 ml of starch (1%) were added. The solution was diluted to 50 ml and titrated with 0.01 *N* CAN to the appearance of a pale blue color.

5. Oxidation of methionine. To an aliquot (1–25 ml) of methionine solution, 5–10 ml of 2 N H_2SO_4, 1 ml of 10% potassium bromide, 0.1–0.2 ml of indigo carmine indicator (0.05%), and enough distilled water to give a total volume of 50 ml were added. The resultant blue solution was titrated with 0.01 *N* CAN to the appearance of a pale yellow color. A blank was run concurrently with the same amount of the indicator solution.

The amount (X μmol) of the reductant in the sample solution is given by $X = 10^3 N\ V/E$, where N and V are the normality and volume of CAN solution, respectively, and E is the electron-change per mole of the reductant ($E = 1$ for glutathione, thioglycolic acid, and thiourea and 2 for ascorbic acid, methionine, sulfite, and arsenite).

Product Analyses

The reaction mixture of CAN and each of the reductants (ascorbic acid, glutathione, and methionine) was analyzed for the products.

TABLE 1
OXIDATION OF SOME REDUCTANTS WITH CHLORAMINE-N (CAN)

Reductant	Reductant taken (mmol)	CAN consumed[a] (meq)	Reductant found[b] (mmol)	Standard error[c] (μmol)
Glutathione	0.1305	0.1309	0.1309	0.42
Thioglycolic acid	0.0889	0.0892	0.0892	0.51
Thiourea	0.1172	0.1180	0.1180	0.82
Ascorbic acid	0.0941	0.1888	0.0944	0.33
Methionine	0.0925	0.1852	0.0926	0.50
Sulfite	0.1271	0.2546	0.1273	0.50
Arsenite	0.0934	0.1872	0.0936	0.23

[a] Calculated from normality × volume of CAN (in ml).

[b] Calculated on the assumption that the number of equivalents of CAN consumed per mole of the reductant is 1 for each of glutathione, thioglycolic acid, and thiourea and 2 for each of ascorbic acid, methionine, sulfite, and arsenite, averages of four replicates.

[c] Calculated for four replicates.

The reduction product of CAN, *p*-nitrobenzenesulfonamide ($R_f = 0.37$), was identified by thin-layer chromatography (TLC) (*8, 16*) in silica gel plates (Uniplate, Analtech, Inc., Newark, Del.) using the solvent system petroleum ether:chloroform:*n*-butanol = 1:1:0.5 (v/v).

In the case of ascorbic acid ($R_f = 0.55$), the reaction products, dehydroascorbic acid ($R_f = 0.72$) and *p*-nitrobenzenesulfonamide ($R_f = 0.89$), were identified by TLC (*8*) using ethanol:10% acetic acid = 90:10 (v/v) as the solvent system.

Paper chromatography was employed to detect the presence of oxidized glutathione (disulfide) (*10*) and methionine sulfoxide (*7*) in the

TABLE 2
DETERMINATION OF SOME REDUCTANTS WITH CHLORAMINE-N

Reductant	Range studied[a] (μmol)	Error in recovery (%)
Glutathione	13.1–326.4	0.60–0.13
Thioglycolic acid	44.3–1107.5	0.60–0.16
Thiourea	58.6–1464.8	0.75–0.26
Ascorbic acid	23.5–587.7	0.50–0.02
Methionine	23.1–578.1	0.37–0.04
Sulfite	31.8–794.6	0.48–0.18
Arsenite	15.6–778.2	0.50–0.20

[a] About eight aliquots of the reductant solution were taken in the range.

reaction mixtures from glutathione and methionine, respectively, after titration. Phenol saturated with water was used as the solvent system for the identification of oxidized glutathione ($R_f = 0.088$), while benzyl alcohol saturated with water served as the solvent system for methionine sulfoxide ($R_f = 0.67$). The spray reagent used was ninhydrin.

RESULTS AND DISCUSSION

Table 1 presents some typical data showing the amount of CAN consumed per mole of the reductant and the standard error calculated from four replicates for each determination. The results of the reductant range studied and percentage error in the recovery are presented in Table 2.

In all the reactions studied chloramine-N undergoes a two-electron change (Table 1) resulting in the formation of *p*-nitrobenzenesulfonamide as the reduction product. The reduction half-reactions of chloramine-N in acid and alkaline media can be represented as follows:

$$\mathrm{RNCl^- + 2\,H^+ + 2}e^- \rightarrow \mathrm{RNH_2 + Cl^-} \qquad \text{(acid medium)}, \tag{1}$$

$$\mathrm{RNCl^- + 2\,H_2O + 2}e^- \rightarrow \mathrm{RNH_2 + Cl^- + 2\,OH^-} \qquad \text{(alkaline medium)}, \tag{2}$$

where $\mathrm{R} = p\text{-}\mathrm{O_2N{-}C_6H_4{-}SO_2{-}}$.

It follows from Tables 1 and 2 that the oxidations proceed quantitatively and stoichiometrically with the consumption of one equivalent of CAN per mole of each of glutathione, thioglycolic acid, and thiourea, giving the respective disulfides, namely, oxidized glutathione, dithioglycolic acid, and dithiourea. The observed stoichiometries of these reducing agents are in agreement with those reported in the literature (*7, 8, 18*) in titrations with other oxidants. These reactions are represented by the equations

$$\mathrm{2\,GSH + RNClNa \rightarrow GS{-}SG + RNH_2 + Na^+ + Cl^-}, \tag{3}$$

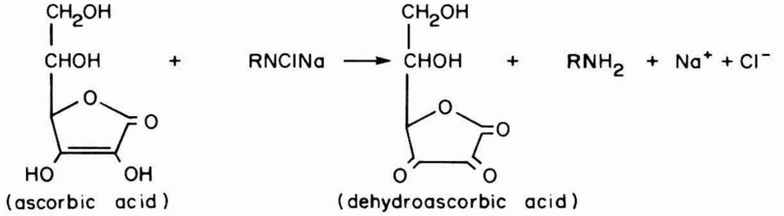
$$\mathrm{2\,HSCH_2COOH + RNClNa \rightarrow HOOCCH_2S{-}SCH_2COOH + RNH_2 + Na^+ + Cl^-}, \tag{4}$$

$$\mathrm{2\,H_2N\underset{\underset{NH}{\|}}{C}SH + RNClNa \rightarrow H_2N\underset{\underset{NH}{\|}}{C}S{-}S\underset{\underset{NH}{\|}}{C}NH_2 + RNH_2 + Na^+ + Cl^-}, \tag{5}$$

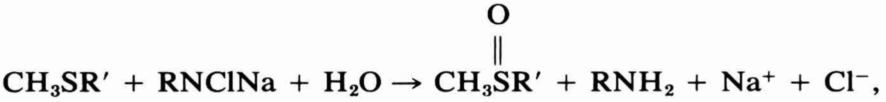
where

$$\mathrm{G = HOOC{-}\underset{\underset{NH_2}{|}}{CH}{-}CH_2{-}CH_2{-}CONH{-}\underset{\underset{CH_2-}{|}}{CH}{-}CONH{-}CH_2COOH.}$$

The oxidation of ascorbic acid, methionine, sulfite, and arsenite under these conditions involves a two-electron change giving dehydroascorbic acid, methionine sulfoxide, sulfate, and arsenate, respectively. The oxidation of these compounds is in agreement with that reported earlier (*7, 8*). These reactions are stoichiometrically represented by the equations



(6)

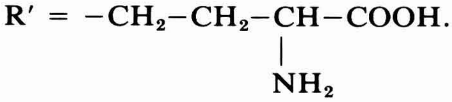


$$CH_3SR' + RNClNa + H_2O \rightarrow CH_3\overset{\overset{\displaystyle O}{\|}}{S}R' + RNH_2 + Na^+ + Cl^-, \qquad (7)$$

$$Na_2SO_3 + RNClNa + H_2O \rightarrow Na_2SO_4 + RNH_2 + Na^+ + Cl^-, \qquad (8)$$

$$Na_3AsO_3 + RNClNa + H_2O \rightarrow Na_3AsO_4 + RNH_2 + Na^+ + Cl^-, \qquad (9)$$

where



$$R' = -CH_2-CH_2-\underset{\underset{\displaystyle NH_2}{|}}{CH}-COOH.$$

In the case of sulfite and arsenite it is necessary to carry out the reaction in an inert atmosphere, as these compounds are susceptible to aerial oxidation which results in a decrease in the amount of the compound recovered. In addition, as the endpoint in the arsenite titration is not sharp, addition of a large excess of $NaHCO_3$ becomes necessary to get a clear endpoint. Addition of $NaHCO_3$ seems to create a buffer medium (pH 4–6) suitable for the reaction to proceed instantaneously. Presence of an optimal amount (0.05–1.00 g) of KI is necessary in order to avoid premature endpoints in the thioglycolic acid titrations.

Although CAN directly reacts with glutathione, ascorbic acid, thioglycolic acid, thiourea, sulfite, and arsenite, it is likely that in the case of methionine the actual oxidizing species is bromine formed *in situ* by the reaction of added bromide with the oxidant.

$$RNClNa + 2\ H^+ + 2\ Br^- \rightarrow RNH_2 + Br_2 + Na^+ + Cl^-. \qquad (10)$$

Further investigations are under way to explore the analytical applications and biocidal effects of CAN.

SUMMARY

The status of sodium *N*-chloro-*p*-nitrobenzenesulfonamide (chloramine-N) as a new redox titrant is examined. Analytical applications of CAN as a potential titrimetric reagent in the direct determination of diverse reducing agents such as glutathione, thioglycolic acid, thiourea, ascorbic acid, methionine, sulfite, and arsenite have been described. CAN has been synthesized and its structure elucidated from the spectral data and elemental analyses. The ultraviolet and infrared spectra and Fourier transform ^1H and ^{13}C nuclear magnetic resonance spectral data have been presented.

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MICROCHEMICAL JOURNAL 27, 97–101 (1982)

Spectrochemical Determination of Tungsten and Boron in Mineral Matrix

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INTRODUCTION

The two elements tungsten and boron cannot be determined easily either by chemical or by spectrochemical methods. Their spectrochemical properties prevent a sensitive spectrochemical detection. Both elements are nonvolatile; they have a tendency to form carbides, the decomposition of which is difficult and which possess high boiling points. The oxides of these elements have lower boiling points; therefore we have tried to determine them in the oxidic form in the present work. Moreover tungsten and boron have higher ionization potentials (W, 7.98 eV; B, 8.29 eV), and for their excitation, higher intensity of arcing is required.

The last difficulty is the lack of convenient sensitive lines. Boron has only two sensitive lines (B I 249.6 nm and I 249.7 nm), the second being interfered. Tungsten has more lines but less sensitivity. The most intense line in the visible region (W I 400.8 nm, I 407.4 nm, and I 426.9 nm) is covered by CN bands. In the ultraviolet region there are two lines (W I 289.6 nm and I 294.7 nm), mostly used by spectrochemists, that are evaluated in this work.

Unfortunately the amounts of tungsten and boron in silicate mineral matrix are very low, mostly at the parts per million level. Therefore it is necessary to be extremely careful in choosing the excitation conditions.

EXPERIMENTAL

Optimization of Operating Conditions

The volatilization and excitation of tungsten and boron can be improved by adding appropriate buffers. Especially oxygen-carrier buffers are efficient as they prevent the tendency toward carbide formation and make possible the oxide formation that is very advantageous. Sulfidization and halogenation processes are recommended also, since they enable volatilization at lower temperature (*1*, *4*).

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All these types of buffers were added to the samples of silicate rocks containing tungsten and boron and the moving plate studies were performed. The admixtures used were LiF^3, $CdCl_2$, $Sb_2S_3{}^2$, Li_2CO_3, $CaCO_3$, $SrSO_4$, CuO, PbO, and Sb_2O_3.

The lines measured were W I 289.6 nm, W I 294.7 nm, and B I 249.6 nm. The volatilization curves for tungsten and boron demonstrated the optimal influence of the CuO and Sb_2S_3 addition: the highest intensity of the line blackening and the shortest time of the quantitative evaporation. Furthermore, the detection limit and the reproducibility were taken into account and the results found proved the optimal influence of the CuO addition; nevertheless the differences among the two buffers were slight.

The first results were performed by measuring the line blackening against the background blackening. To improve the precision several reference elements were added to the CuO buffer—palladium, niobium, thorium—that have spectrochemical properties similar to those of the analysis elements. Palladium was found to give the best results and its line Pd I 276.3 nm was used as a reference line for both tungsten and boron analysis lines. The optimal exposure time was chosen according to the volatilization curves and the higher intensity (12 A) ac arc was used for excitation. The final operating conditions are given in Table 1. The analysis lines and the reference line, as well as the detection limits, are given in Table 2.

Standardization of the Method

The use of reliable standard reference samples appears to be very critical in every spectrochemical method. The use of natural reference materials of the same type is usually impossible and therefore it is more common to use synthetic reference samples. In this case the reference materi-

TABLE 1
OPERATING CONDITIONS FOR THE DETERMINATION OF TUNGSTEN AND BORON

Spectrograph	— Grating, PGS 2, Zeiss-Jena
Wavelength range	— 230–380 nm, first order
Slit width	— 20 μm
Diaphragm	— Round
Exposure time	— 100 sec
Analytical gap	— 4 mm
Power source	— 12 A ac arc, FES 270, RSV Hechendorf
Electrodes	— SU 306, SU 202 Elektrokarbon-Topolčany
Emulsion	— ORWO blau extra hart
Buffer	— 5 g CuO + 0.02 g Pd
Mixing ratio	— 1:2 (buffer:sample)
Densitometer	— MD 100 Zeiss-Jena

TABLE 2
ANALYSIS AND REFERENCE LINES AND THE DETECTION LIMITS

Element	Analysis line (nm)	Excitation potential (eV)	Detection limit (ppm)
Tungsten	W I 289.6	4.63	8
	W I 294.6	4.57	
Boron	B I 249.6	4.96	9
	Reference line (nm)		
Palladium	Pd I 276.3	4.48	—

als were prepared by mixing the oxides of major elements of the matrix analyzed—silicate rock—with oxides of tungsten and boron in serially diminishing amounts. These synthetic standards were tested by using the international natural rock standard samples. Unfortunately there are few international standard samples with recommended values for tungsten and boron, mostly with very low concentration content.

The analytical calibration curves plotted by means of synthetic reference samples have nearly unit slope and a good agreement of points for both elements.

Precision and Accuracy

The precision of this method was expressed as the relative standard deviation for both elements determined and was calculated from 30 parallel results (on one plate). As seen in Table 3, deviation is very low for tungsten with Pd reference line. The accuracy was tested by comparison of results with the recommended values of international standard samples and some results of the chemical determination. The agreement is good, especially for lower contents (Table 4).

TABLE 3
PRECISION OF THE METHOD

Element	Line measured	Standard deviation ($S_r\%$)
Tungsten	W I 289 nm/background	10.2
	W I 289 nm/Pd	2.9
	W I 294 nm/background	6.5
	W I 294 nm/Pd	3.1
Boron	B I 249 nm/background	6.6
	B I 249 nm/Pd	9.6

TABLE 4
COMPARISON OF RESULTS

Standard (sample)	Concentration (ppm) W (recommended value) SPA method	Chemical result	B (recommended value) SPA method	Chemical result
Greisen	480	530		
Skarn	9	10		
Feldspat-schiefer	13	15		
Granit	20	23		
JB 1	21	20	10	10
JG 1			8	8
NIM S			8	10
Kaolin			39	37
Schwärz-schiefer			42	56
1796			175	190
1797			128	140
754	500	490		
762	260	230		
771	240	220		

RESULTS AND DISCUSSION

The goal of this method was the reaching of low detection limits for tungsten and boron in the silicate mineral matrix. In this point it was successful and the CuO buffer proved to be a very efficient oxidizing agent. It influenced positively the reproducibility of the method as well by stabilizing the excitation conditions in the burning plasma, i.e., the temperature, the particle density, and the effective ionization potential (Cu ionization potential—7.72 eV). The detection limit could probably be even lower by using the direct current arc excitation, but the reproducibility would be a little worse.

The accuracy that was tested by comparison with the recommended values of some international standards is good also, especially in very low concentrations.

The use of the reference element palladium gives very low standard deviation for tungsten, but the results gained with the background reference values are sometimes more accurate in higher concentrations. Therefore for higher amounts of tungsten both reference values should be used.

The method is very simple and can be applied very easily for different mineral materials with appropriate standard samples.

CONCLUSIONS

A spectrochemical method for the determination of small amounts of tungsten and boron in mineral matrix was presented. The oxidizing influence of CuO buffer was utilized to prevent the carbide formation and to increase the volatilization rate of the analysis elements. The background blackening and the palladium line were used as the reference values and the grating spectrograph and ac arc excitation provided precise and accurate results with low detection limits.

SUMMARY

The spectrochemical behavior of nonvolatile tungsten and boron was tested by adding different buffers and using 12 A ac arc excitation. The oxidization and sulfidization processes were found to be most effective for facilitating the volatilization. The optimal admixture was the copper oxide buffer for both the detection limit and the precision of the method. The reference values of the background or a palladium line blackening were used.

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Microdetermination of Aliphatic Diamines and Polyamines with Cupric Ion[1,2]

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INTRODUCTION

We previously reported a novel analytical method for the determination of 1,2-diaminoethane dinitrate (*14*). This method was based on potentiometric titration with standard cupric solution near pH 9. Electromotive forces were monitored with a copper ion-selective indicator electrode and a single-junction reference electrode. In this paper we have extended this work to other diamines, their salts, and polyamines.

EXPERIMENTAL

The titrant was 0.01 *M* aqueous cupric sulfate. It was standardized vs aliquots of a 0.01 *M* disodium EDTA solution, prepared by dilution of 0.1 *M* P-H TAMM solution. The pH of the aliquots was adjusted to 9.5–10 with 1:9 ammonia, using a pH meter. They were then titrated potentiometrically using the system previously described (*13*). Electromotive forces were monitored with a cupric ion-selective electrode (ISE) and a single-junction reference electrode. The amines and amine salts were of the highest purity available commercially. They were used without additional purification. Samples containing approximately 0.04 mmol were pipetted into a 50-ml beaker containing a Teflon-coated stirring bar. They

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were diluted to 25 ml with distilled water and the pH was adjusted with dilute ammonia to ~9, using a pH meter.

Some titrations were performed without added ammonia, as discussed below. Liquid samples were dispensed from a weight burette into a volumetric flask containing some distilled water and then diluted to volume. Aliquots were withdrawn immediately prior to pH adjustment and titration, because on standing the material oxidized, yielding lower titration values.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooling plate and an aluminum plate connected to ground. Titrations were performed at ambient temperature (23 ± 1°C). All titrations were carried out at 0.5 ml/min. Titration endpoints were calculated as previously described (*13*).

RESULTS AND DISCUSSION

We recently reported a new method for the potentiometric titration of some aliphatic amines with cupric ion, using a cupric ISE as endpoint detector (*4*). In the course of this investigation we found that 1,2-diaminoethane (EN) and 1,2-diaminopropane reacted differently from the other amines and diamines examined:

(1) The *vic*-diamines could be titrated with cupric ion in the presence of an excess of ammonia and even sodium hydroxide.

(2) The *vic*-diamines yielded a pink/purple color upon titration, while the other amines always gave a blue color.

As a continuation of this work, we now examined a large number of diamines and polyamines. The results for compounds which could be titrated in the presence of ammonia are presented in Table 1; standard deviations, mean recoveries, and optimum pH, as well as the feasible pH range, are given. The number of moles of amine titrated per mole of cupric ion is also shown. Some representative titration curves are shown in Figs. 1 and 2.

We previously found that diamines which are separated by no more than three methylene groups react as a single amino group, with 2 mol of the amine reacting per mole of Cu(II) (*4*). If the amino moieties are separated by four or more methylene groups they react as two separate amino groups, and the compound reacts on an equimolar basis with Cu(II). Thus, 1,3-diaminopropane reacts on a 2:1 basis, while 1,4-diaminobutane reacts on an equimolar basis. In general, this rule still applies, with the following exceptions:

(1) Most tetraaza compounds reacted with Cu(II) on an equimolar basis. However, 1.5 mol of TRIEN (also named 1,4,7,10-tetraazadecane) reacted with each mole of Cu(II). Nevertheless, TRIEN disulfate reacted like other tetraaza compounds, on an equimolar basis. Decahydropyra-

TABLE 1

STATISTICS FOR RESULTS OF TITRATION OF DIAMINES AND POLYAMINES WITH CUPRIC ION (FEASIBLE IN PRESENCE OF AMMONIA)

Compound	Mean recovery (%)	Standard deviation	Number of replicates	Optimum pH	Feasible pH	Moles of amine per Cu(II)
1,2-Diaminoethane	94.66	0.17	5	10.5	≥10.35	2
1,2-Diaminoethane sulfate	98.00	0.13	7	9.0	8.85–9.4	2
1,2-Diaminoethane dihydrochloride	98.30	0.10	6	9.15	9.0–9.35	2
1,2-Diaminoethane dinitrate[a]	43.04	0.06	6	9.0	8.2–9.7	2
1,2-Diaminoethane tartrate	97.82	0.31	6	9.5	9.3–10.2	2
1,2-Diaminopropane[b]	94.66	0.18	6	9.75	≥ 9.75	2
1,2-Diaminopropane dihydrochloride	97.56	0.22	7	9.55	≥ 9.2	2
1,2-Diamino-2-methylpropane	96.08	0.11	3	10.2	≥ 9.9	2
1,2-Diaminocyclohexane	93.50	0.21	7	10.65	≥10.65	2
1,3-Diamino-2-propanol[c,d]	96.28	0.46	4	10.35	≥10.35	1
2,4-Diaminobutyric acid dihydrochloride[c]	98.06	0.22	8	9.5	9.3–10.0	2
2,3-Diaminopropionic acid hydrochloride	99.35	0.21	5	9.5	≥ 9.15	2
Decahydropyrazino[2,3-*b*]pyrazine	36.27	0.23	4	10.2		2
1,4,8,11-Tetraazacyclotetradecane	96.73	0.43	4	10.5	≥10.25	1
1,4,8,11-Tetraazaundecane	96.42	0.19	4	10.5	≥10.35	1
1,4,8,12-Tetraazacyclopentadecane	95.44	0.11	3	10.8	≥10.8	1
1,5,8,12-Tetraazadodecane	97.58	0.08	3	11.0	≥10.6	1
1,5,9,13-Tetraazatridecane	95.35	0.27	4	10.9	≥10.9	1
Diethylenetriamine (DIEN)	96.93	0.29	7	10.5	≥10.5	(2)[e], 1
Triethylenetetramine (TRIEN)	94.40	0.16	6	10.25	≥10.25	1.5, (1)[e]
TRIEN disulfate	95.37	0.31	4	9.6	≥ 9.2	1
Tetraethylenepentamine (TETRAEN)	100.22	0.57	5	10.45	≥10.45	2, (1)[e]

[a] Previously reported in Ref. (*14*).

[b] Previously reported in Ref. (*4*).

[c] Compounds do not exhibit purple/pink color on addition of ammonia.

[d] Reacts on an equimolar basis only in the presence of ammonia; in its absence it requires 1.5 mol amine per Cu.

[e] Numbers in parentheses are for potentiometric breaks which are not analytically useful (variable).

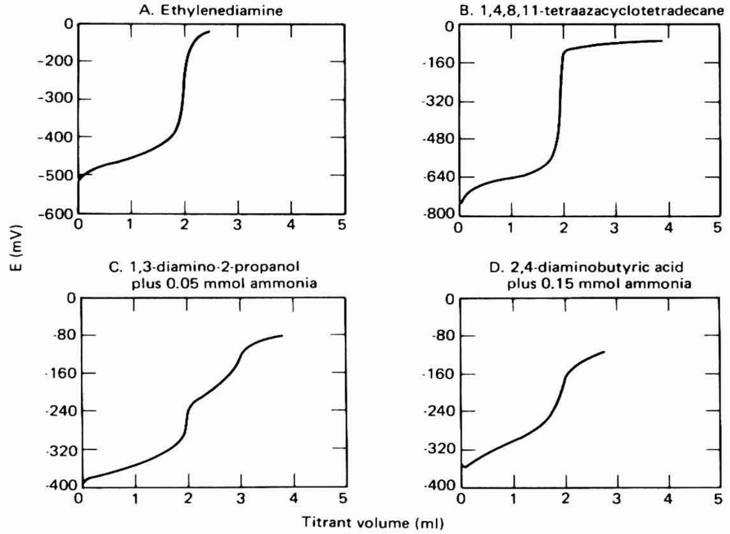


FIG. 1. Titration curves for some diamines and polyamines vs cupric ion.

zino[2,3-*b*]pyrazine (scheme 1) reacted like *vic*-1,2-diamines (two amines per Cu(II)). This compound may be considered as two fused piperazine molecules. Piperazine (discussed below) reacts, as expected, on a 2:1 basis and cannot be titrated in the presence of ammonia.

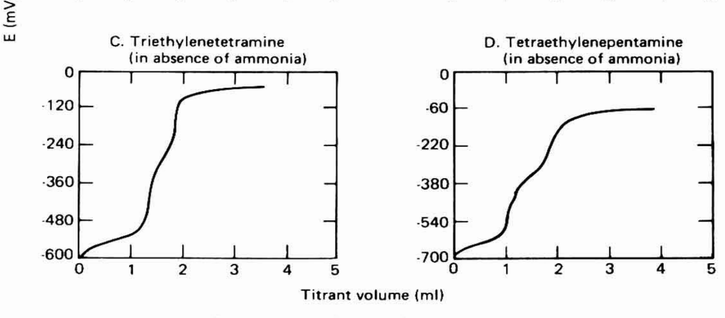
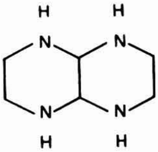


FIG. 2. Titration curves for ammonia and some polyamines vs cupric ion.



SCHEME 1. Decahydropyrazino[2,3-*b*]pyrazine.

(2) DIEN is a triaza compound which, like the tetraaza compounds, reacts with Cu(II) on an equimolar basis.

(3) TETRAEN is a pentaaza compound which exhibited several potentiometric breaks although the most reliable one was at 2:1 ratio of amine to copper. It can be seen that, in general, the polyaza compounds react with cupric ion on an equimolar basis.

(4) 1,3-Diamino-2-propanol was expected to react like 1,3-diaminopropane, requiring 2 mol of the amine per Cu(II) because it is not a *vic*-diamine. However, in the presence of ammonia, it reacted on an equimolar basis, while in the absence of ammonia 1.5 mol of the amine reacted per Cu(II). It seems that the *vic*-hydroxyl group takes part in the complexation reaction with Cu(II).

(5) 2,4-Diaminobutyric acid was not expected to be titratable in the presence of ammonia. It appears, however, that amino acids represent a different category of compounds which can be titrated with Cu(II) if the pH is accurately adjusted with ammonia.

It is also noteworthy that with only a few exceptions the di- and polyamines listed in Table 1 yield, upon titration with Cu(II) in ammoniacal solution, a violet/pink color. This was noted previously in the analysis of 1,2-diaminoethane dinitrate (*14*). While there are many photometric methods for variety of amines (*2, 3, 6, 9*), only Kuba and Banic (*8*) noted that EN had a violet color with a λ_{max} at 550 nm, while DIEN had a blue color with a λ_{max} at 620 nm. Although we have made no attempt to utilize this color photometrically, it may well be possible to use it as the basis for such methods. Exceptions to the general color-forming behavior were 1,3-diamino-2-propanol and 2,4-diaminobutyric acid hydrochloride which, although titratable in the presence of ammonia, did not yield the purple/violet color. These compounds are discussed above. An exception of the opposite kind was the hydrochloride of 1,3-diaminopropane. As expected, this compound could not be determined with Cu(II) because it is not a *vic*-diamine. Nevertheless, in the presence of ammonia a purple color was observed.

Table 2 presents statistics for the cupric ion titration of those diamines which could not be determined in the presence of ammonia and which did

TABLE 2

STATISTICS FOR RESULTS OF TITRATION OF DIAMINES WITH CUPRIC ION (NOT FEASIBLE IN PRESENCE OF AMMONIA)

Compound	Mean recovery (%)	Standard deviation	Number of replicates	Initial pH	Moles of amine per Cu(II)
1,8-Diaminooctane	104.1		2	9.75	1
1,9-Diaminononane	105.2	0.75	3	10.0	1
1,10-Diaminodecane with Cu ISE	105.7	0.32	3	10.75	1
1,10-Diaminodecane with Pt electrode	108.5	0.77	4		
1,12-Diaminododecane	105.0	0.40	4	10.0	1
N,*N*′-Dimethyl-2-butene-1,4-diamine	105.5	0.31	4	10.8	1
Piperazine hexahydrate	108.5	0.77	4	9.85	2
sym-Dimethylethylenediamine	93.14	0.39	3	10.65	2
unsym-Dimethylethylenediamine	95.90	0.06	3	10.5	2
N,*N*,*N*′-Trimethylethylenediamine	92.88	0.27	3	10.3	2
Tetramethylethylenediamine	95.12	0.27	3	9.25	2
Tetramethyl-1,2-diaminocyclohexane	93.52	0.14	3	11.55	2
Triethylenediamine	111.9	0.7	4	9.8	2
1,2′-Ethylenedipiperidine	97.64	0.22	3	10.55	2, 1
3,3′-Methylimino-bis(*N*-methylpropylamine)	110.8		2	10.1	2, 1
N,*N*′-Ethylenedimorpholine	not feasible			8.65	

not yield the characteristic violet/purple color of *vic*-diamines. Representative titration curves for these compounds are presented in Fig. 3. For many of these compounds recoveries were high, as were the standard deviations. The latter resulted from the fairly shallow potentiometric curves, and the former may be caused by the presence of lower-molecular-weight impurities. It is noteworthy that *N,N'*-ethylenedimorpholine, which has an initial pH of 8.65, could not be determined by titration with Cu(II). It seems, therefore, that an initial minimum pH of ~9 for a 10^{-3} *M* solution is necessary for amines to be titratable with cupric ion. This was also found elsewhere (*4*).

It is of interest that although EN can be titrated in the presence of ammonia, if any of the nitrogens are substituted with methyl groups, this is no longer true. It seems that for formation of the violet copper complex a five-membered ring is required in which neither nitrogen is substituted. This reasoning also applies to tetramethyl-1,2-diaminocyclohexane and piperazine, which may be considered the cyclic analog of *sym*-dimethylethylenediamine.

Rossotti (*11*) has stated that five-membered ring metal chelates are usually more stable in solution than analogous species with six-membered rings. Thus many EN and 1,2-diaminopropane complexes are more stable than those of 1,3-diaminopropane. Perhaps it is this additional stability that results in the characteristic violet color.

Compounds which could not be titrated with Cu(II) were the hydro-

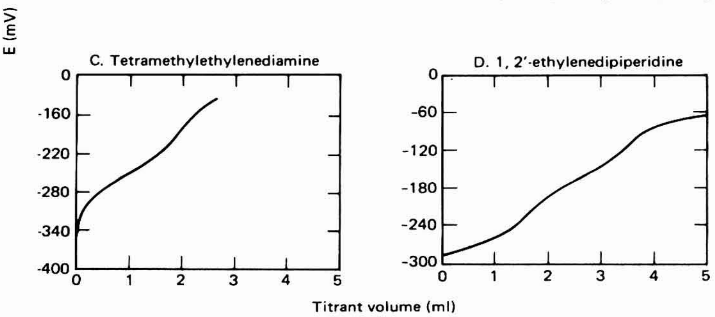


FIG. 3. Titration curves for some diamines not feasible in presence of ammonia.

chlorides of 1,3-diaminopropane, 1,4-diaminobutane, and 1,5-diaminopentane. Even adjustment to a pH of ≥9 did not render these amine salts titratable, unlike the *vic*-diamine salts reported in Table 1.

We have stated in a previous paper (*4*) that titrations are feasible in a partially nonaqueous medium such as methanol. This was confirmed with 1,3-diaminocyclohexane. Fairly large amounts of ammonia can be tolerated with the compounds listed in Table 1. However, as the amount of ammonia is increased, both the magnitude and the steepness of the titration curves decrease. The excess ammonia (on a molar basis) that could be tolerated was: TRIEN, 16×; 1,4,8,11-tetraazaundecane, 80×; and EN, 120×.

1,3-Diamino-2-propanol, as well as some of the *vic*-diamines, shows, in the presence of ammonia and in some cases dilute sodium hydroxide, two potentiometric breaks. The second of these breaks can be attributed to the amount of base present. This break is rather shallow and was not evaluated analytically. For 1,3-diamino-2-propanol the two breaks are shown in Fig. 1C. Dilute ammonia alone also reacts with cupric ion, each Cu(II) requiring two ammonia molecules as shown in Fig. 2A.

Table 3 presents a list of the compounds which yielded multiple breaks, even though not all of these breaks were analytically useful. The important compounds giving more than one break upon titration with cupric ion were DIEN, TRIEN, and TETRAEN (see Fig. 2). Several of these polyamines were previously used in the titration of metal ions such as Cu(II) (*1, 7, 10*). Reilly and Vavoulis (*10*) used TETRAEN as a selective

TABLE 3

COMPOUNDS YIELDING MULTIPLE BREAKS UPON TITRATION WITH CUPRIC ION

Compound	Amine to cupric ion ratio	
	At 1st break	At 2nd break
Diethylenetriamine (DIEN)[a]	2	1
Triethylenetetramine (TRIEN)[b]	1.5	1
Tetraethylenepentamine (TETRAEN)[c]	2	1
3,3′-Methylimino-bis(*N*-methylpropylamine)[d]	2	1
1,2′-Ethylenedipiperidine[e]	2	1

[a] First break is extremely shallow and present only when ammonia is absent. The second break only is useable for analytical purposes.

[b] On addition of ammonia the first break stays analytically reliable while the second break decreases in volume and size. If the disulfate is titrated it yields only a single break after the required pH adjustment.

[c] There are at least three breaks, though only the first one is analytically reliable at a 2:1 ratio.

[d] Neither break is analytically useful, and both are extremely shallow.

[e] Both breaks are quite shallow, and titration is feasible only in absence of ammonia.

TABLE 4
COPPER:AMINE STOICHIOMETRY FOUND BY VARIOUS WORKERS WITH POLYAMINES

Compound	Reilly and Vavoulis[a]	Al-Daher and Kratochvil[b]	This work
DIEN	2:1		2:1
TRIEN	1:1	1:1	1.5:1 and 1:1
TETRAEN	1:1		2:1 and 1:1

[a] With mercury electrode at pH 5 (*10*).

[b] With platinum electrode in acetonitrile (*1*).

titrant for metal ions with potentiometric endpoint detection and found it superior to TRIEN. The indicator electrode was a mercury electrode at pH 5. They found that TRIEN and TETRAEN yielded stable equimolar complexes with Cu(II). Our findings were obtained under different conditions with a copper ISE at pH 9.

Al-Daher and Kratochvil (*1*) investigated EN, DIEN, and TRIEN as titrants for metal cations in acetonitrile. The sharpest inflections were obtained by these workers with a platinum electrode. We have attempted to use such an electrode in lieu of the copper ISE in the titrations of TRIEN and TETRAEN. The latter did not yield any titration curve while the former yielded titration curves which were not reproducible.

A comparison of our results with those of Reilly and Vavoulis (*10*) and Al-Daher and Kratochvil (*1*) with respect to the stoichiometry of the reaction of Cu(II) with polyamines is presented in Table 4.

1,2-Diaminoethan tartrate did not yield a steep titration curve but it could still be analyzed. However, we were not able to obtain reliable results with 1,2-diaminoethane disalicylate; this compound was extremely sensitive to pH changes and we surmise that complications arose from the complexation of cupric ion by salicylic acid.

Hassan and Zaki (*5*) previously reported a microdetermination for hydrazines using cupric ion and the copper ISE. Private communication with the senior author led us to agree that this method needs further investigation in order to yield reproducible results. Our preliminary experiments have shown that at pH 9−10 cupric ion is probably reduced to metallic copper by hydrazine. According to the literature (*12*) hydrazine has the ability to reduce many metal salts, including copper, to the metallic state.

The relationship between log K_1 of cupric−amine complexes and the feasibility of their titration with cupric ion will be the topic of further study.

SUMMARY

The reactions of diamines and polyamines with cupric ion were investigated potentiometrically, using a copper ion-selective indicator electrode and a single-junction reference elec-

trode. In general, cupric ion reacted with 2 mol of *vic*-diamines or their salts. Tetraaza compounds reacted on an equimolar basis with Cu(II). Both *vic*-diamines and their salts, as well as tetraaza compounds, could be titrated in the presence of excess ammonia. With cupric ion in ammoniacal solution these compounds yield a characteristic purple/violet color. Diamines separated by more than two methylene groups cannot be determined in the presence of ammonia and yield a blue color. Diamines with more than four methylene groups react with cupric ion on an equimolar basis, also yielding a blue color.

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Mercuric Chloranilate as Solid Colorimetric Reagent in Microanalysis of Complex Ions

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INTRODUCTION

Recently, inorganic solid surfaces have been employed for preconcentration of species of interest (*1*). However, many such surfaces have also found specific analytical uses in the determination of cations and anions. Insoluble metal chloranilates have been successfully employed in the exchange colorimetric determination of simple and complex anions such as halides, SO_4^{2-}, SO_3^{2-}, S^{2-}, CN^-, $(C_6H_5)_4B^-$, $S_2O_3^{2-}$, and PO_4^{3-} (*2, 3, 5–7, 13*). Lambert and Menzo (*8, 9*) have shown that the solid ion-associated species tris (*o*-phenanthroline)ferrous triiodide is selective toward CN^-, while crystal violet mercuric tetraiodide is selective toward S^{2-} ions. Mehra and co-workers (*4, 10, 12*) have reported on the analytical use of solid surfaces encompassing tetraphenylborate ion and the cations brilliant green, tris(2,2′-bipyridine)Fe^{2+}, and bis(2,4,6-tri(2-pyridyl)-*S*-triazine)Fe^{2+} in the colorimetric analysis of Ag^{2+}, Hg^{2+}, and Tl^+ ions in solution.

In the course of our investigations, we felt the need to quantatize the complex ions triphenylcyanoborate ($(C_6H_5)_3CNB^-$; TPCB), azide (N_3^-; Az), and monothiophosphate (PO_3S^{3-}; MTP) in solution. Colorimetric reactions with Fe^{3+} are known only for N_3^- ion (*14*), and more recently it has been analyzed through its exchange reaction with $(Fe(CN)_5NH_3)^{3-}$ ion (*11*). There are hardly any colorimetric procedures reported for the other two species.

We have observed that solid reagent mercuric chloranilate exchanges rapidly with these complex ions to release the colored chloranilate ion in solutin (λ_{max} = 530 nm). The exchange occurs quantitatively in acidic medium with microgram amounts of the complex species. Thus a simple, but an indirect, colorimetric procedure for the analysis of these colorless ions is now available.

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EXPERIMENTAL

Reagents and chemicals. The reagents used in this study were of analytical-grade quality. Mercuric chloranilate was procured from K and K Chemicals, U.S.A. Double-distilled deionized water was employed in all solution preparations. Bausch and Lomb spectrophotometer Spectronic-70 with matched 1-cm rectangular cells was employed for absorbance measurement.

Procedure. In 50-ml standard flasks place solution of an anion to yield an overall concentration varying from 0 to 300 ppm. Add the requisite amount of 1 *N* H_2SO_4 and 1 *N* CH_3COONa to obtain the desired pH (N_3^- ~ 2.8; $(C_6H_5)_3CNB^-$ ~ 1.6; PO_3S^{3-} ~ 1.5). Complete the volume and mix solutions thoroughly. Add 50 mg of solid mercuric chloranilate in each flask and shake vigorously on a magnetic stirrer for 15 min following which set aside for 1 hr to attain equilibrium.

Prepare a blank in a similar manner in the absence of the anion of interest in solution.

In each case filter the equilibrated solution through a double layer of Whatmann-42, measure the absorbance at 530 nm against a blank, and obtain a calibration curve for each anion of interest.

RESULTS AND DISCUSSION

The analytical data (Table 1) show that TPCB (8.5–268 ppm), Az (3–100 ppm), and MTP (3–100 ppm) can be successfully analyzed through this colorimetric exchange reaction on solid mercuric chloranilate. The sensitivity appears to follow the order

$$\text{MTP} > \text{Az} > \text{TPCB}.$$

The exchange reaction, though pH dependent, can be envisaged to occur through ligand displacement; $HgCh_{(s)} + L \rightleftharpoons HgL_{(s)} + Ch^{2-}$. The order of sensitivity can be rationalized on the basis of relative affinities of the complex species. In the case of MTP, it is the strong affinity between $Hg-S^{2-}$ atoms on MTP which renders this exchange efficacious, while N atom on Az and bound CN^- on TPCB have relatively weaker affinities for Hg with the solid surface. Some evidence to this effect is found in the period of exchange, where one finds that MTP exchange is complete in a few minutes, while 30 and 60 min are required for complete exchange of N_3^- and $(C_6H_5)_3CNB^-$ ions, respectively.

The acidity of the medium apparently is critical since at elevated pH, mercuric chloranilate has a tendency to decompose, while at lower pH the reaction is partially blocked. It has been established experimentally that for MTP and TPCB, pH in the range 1.5–1.7 is necessary, while for N_3^- it should be in the range 2.8–3.0.

TABLE 1

ABSORBANCE DATA FOR PO_3S^{3-}, N_3^-, AND $(C_6H_5)_3BCN^-$ IONS AT 530 nm

Concn (ppm)	Absorbance PO_3S^{3-}	Absorbance N_3^-	Concn (ppm)	Absorbance $(C_6H_5)_3BCN^-$
3	0.009	0.010	8.5	0.002
5	0.021	0.016	17.0	0.005
10	0.046	0.028	33.5	0.073
15	0.073	0.043	67.0	0.033
20	0.095	0.057	134	0.068
25	0.118	0.072	200	0.092
30	0.142	0.082	268	0.121
40	0.186	0.110		
50	0.232	0.136		
100	0.459	0.259		

Note. HG chloranilate—50 mg, contact period 30–60 min volume 50 ml.

The reaction obviously is nonselective since a host of other ions react in a similar manner. However, the aim of this work was to investigate if these three complex ions can be quantatized in a simple manner. The results show that this reaction can be employed for standardization work or for equilibration studies dealing with an individual ion in this series.

SUMMARY

The colorless complex anions triphenylcyanoborate ($(C_6H_5)_3CNB^-$), azide (N_3^-), and monothiophosphate (PO_3S^{3-}) have been determined in an acidic aqueous medium through colorimetric exchange on solid mercuric chloranilate surface. The released colored chloranilate ion absorbing at 530 nm responds linearly in the range 3–100 ppm for PO_3S^{3-} and N_3^- ions and in the range 8–200 ppm for $(C_6H_5)_3CNB^-$ ion. The reaction is pH dependent and equilibration is achieved within an hour.

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Determination of Carbon Monoxide with Palladium Chloride

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INTRODUCTION

The commonly used method for source measurement of carbon monoxide is nondispersive infrared (NDIR). However, a need for an accurate and reliable wet chemical method for the range of 100 to 1000 ppm CO (v/v) has been indicated. The wet chemical method would be used to check the accuracy of the NDIR instruments and for certain specific stationary source CO emission measurements. A recent literature search revealed that most classical wet chemical methods for CO determinations were developed in the 1930s for other than pollution measurements. The later developments and improvements were for ambient levels of CO (<50 ppm) (*4*–*6*, *8*). Therefore, work on a source method was indicated.

The reaction of I_2O_5 with CO (*1*) is the currently accepted method for (manual) chemical CO determination, but due to the time-consuming purification and preparation of the I_2O_5 reagent, in addition to the required reaction temperature of 150°C (*1*, *3*, *7*), the I_2O_5 method was rejected. The reduction of $PdCl_2$ by CO was the method chosen based on its overall suitability, namely, no requirement for a temperature-controlled, high-temperature reaction vessel. Conventional gas sampling techniques were utilized in the development of this procedure, which facilitated the transfer of gas mixtures from sampling containers to the reaction vessel in a quantitative manner.

This technique, based on chemical reduction of a metal by CO (*2*),

$$PdCl_2 + CO + H_2O \rightarrow Pd^{\circ} + CO + 2HCl,$$

exposes a known volume of palladium chloride solution to the gas sample containing carbon monoxide. After the reaction is complete (4 hr), the excess $PdCl_2$ is separated from the palladium metal by filtration. Potassium iodide, used in excess, initially precipitates the $PdCl_2$ as PdI_2, then dissolves the PdI_2 to yield a pinkish red solution ($PdI_4^{2=6}$). The $PdCl_2$ is

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determined in an indirect manner by spectrometric comparison with standard solutions.

MATERIALS AND METHODS

Apparatus and Reagents

Spectrophotometric measurements were performed using a Bausch & Lomb Spectronic 20 spectrophotometer, with Hycel-certified 13 × 100-mm round cuvettes.

The sampling system, as shown in Fig. 1, allows leak-free system evacuation and transfer of gas sample from Tedlar bag to reaction vessel.

Several glass stopcocks were made, as shown in Fig. 2, to allow leak-free evacuation, as well as an airtight reaction flask.

Reaction vessels were 125-ml flasks fitted with standard 24/40 ground glass joints to fit the glass stopcocks. The volumetric capacity of each of the reaction flasks/stopcocks was computed from the weight and temperature of the distilled deaerated water required to fill the flask and stopcock.

Tedlar sample bags with quick connects and TFE housings were used to hold gas mixtures for sampling.

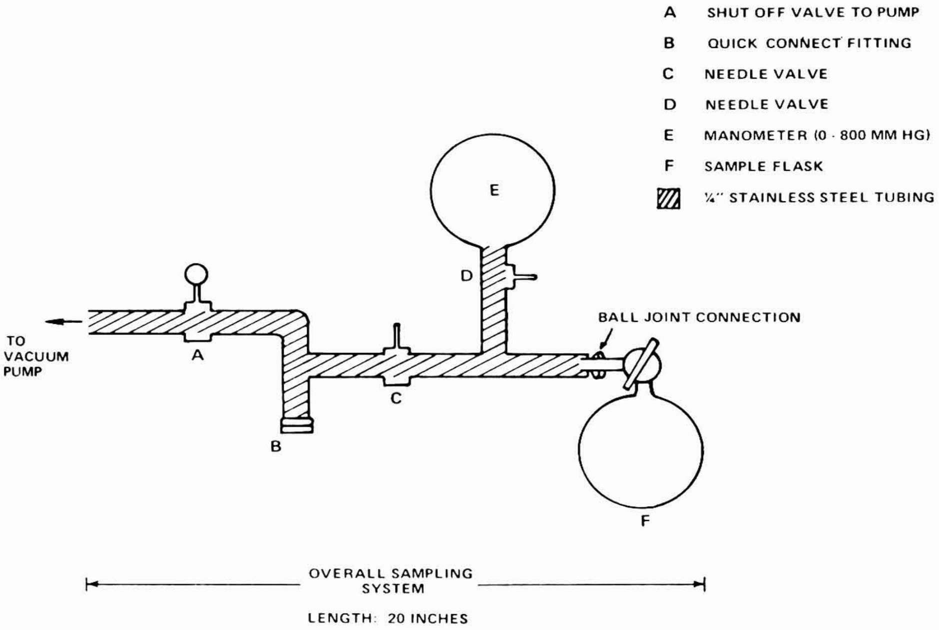

FIG. 1. Carbon monoxide sampling system design.

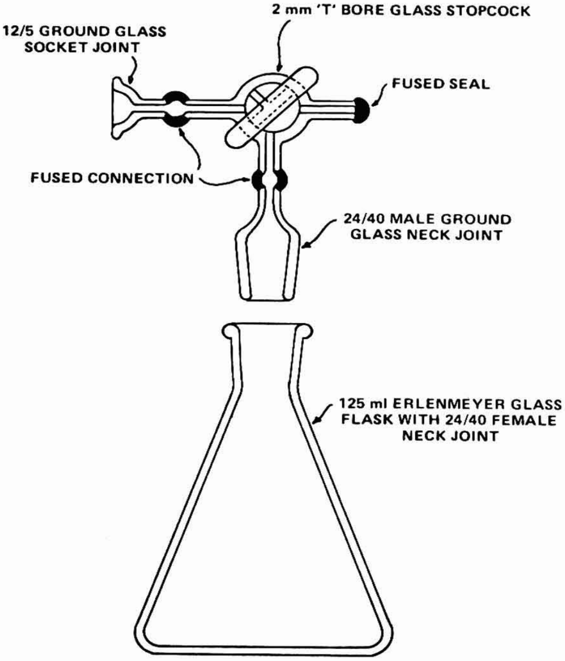

FIG. 2. Carbon monoxide reaction vessel.

Vacuum pump, capable of evacuating sample flask to approximately 25 mm Hg, absolute.

Differential pressure gauge, graduated in 1-mm divisions (0–800 mm Hg).

Wrist-action shaker.

Coarse-fritted filter funnels, 30-ml capacity.

Repipet, capable of delivery within ±2% precision with a reproducibility of 0.5%.

Palladium chloride ($PdCl_2$) 5% (w/v) stock solution was analyzed for Pd content by the standard gravimetric procedure which consists of the precipitation of Pd in acid solution as the salt of dimethylglyoxime.

Palladium chloride ($PdCl_2$) working solution (approximately 0.25 mg $PdCl_2$/ml) was made by diluting the 5% $PdCl_2$ stock solution, 200:1, with distilled–deionized H_2O.

Potassium iodide (KI) 15% (w/v) solution was prepared from certified reagent-grade KI with distilled, deionized H_2O just prior to each group of determinations.

Aluminum sulfate [$Al_2(SO_4)_3$] 10% (w/v) solution of reagent-grade $Al_2(SO_4)_3$ in distilled, deionized H_2O was prepared (to facilitate the flocculation of the colloidal palladium precipitate).

Pretest Procedure

Prior to the actual determinations, each reaction flask with its companion stopcock was calibrated to determine its volumetric capacity. The 5% (w/v) $PdCl_2$ solution was checked for palladium content with dimethylglyoxime.

$PdCl_2$ Manual Method for the Determination of Carbon Monoxide Content

The following description covers the step-by-step procedure applicable to a single analysis for CO by the subject method:

(a) Fill Tedlar sample bag with gas to be analyzed, flush out with vacuum pump, and refill to desired level.

(b) Using ±0.5% pipet or repipet, place 4 ml solution (0.25 mg Pd/ml) into sample flask (item F in Fig. 1) and add two drops of 10% aluminum sulfate solution.

(c) Insert stopcock into flask and place stopcock valve into open position.

(d) Connect flask assembly to gas sampling manifold and evacuate flask slowly until pressure gauge registers a value equivalent to the vapor pressure of water (about 25 mm Hg absolute at normal room temperature). When boiling commences in flask, close valve at inlet of vacuum pump and record evacuated flask pressure.

(e) Check the flask and interconnecting manifold for leaks by allowing the system to sit undisturbed for 3 min. A change in pressure of no more than 5 mm Hg is acceptable.

(f) Close valve between sample bag connection and flask, then attach sample bag via quick connect fitting.

(g) Start vacuum pump and open inlet valve of vacuum pump for 5 sec to purge adjacent tubing with sample gas; then close inlet valve.

(h) Gradually open valve between sample bag and flask to allow sample gas to flow into flask until desired pressure is reached, then close valve.

(i) After equilibrating for 15 sec, record pressure gauge reading and close stopcock and pressure gauge valve (to protect gauge).

(j) Disconnect flask at ball/socket joint and place on wrist-action shaker for 4 hr.

(k) Transfer flask contents to a glass coarse-fritted filter funnel and collect the filtrate in a 100-ml volumetric flask containing approximately 25 ml distilled, deionized water.

(l) From a 20-ml pipet containing 15% potassium iodide solution, place about 8 ml on the filter and the remainder directly into the volumetric flask.

(m) Wash filter several times (with distilled deionized water) into volumetric and dilute to the mark.

(n) Make duplicate standard solutions in 100-ml volumetric flasks by adding the working solution $PdCl_2$, $Al_2(SO_4)_3$, and KI and diluting to the mark.

(o) Place test solutions and standard solutions in matched cuvettes and read against a distilled deionized water blank at 490 nm.

(p) Calculate results as shown below.

The volume of the gas sample (STP, dry) drawn into the reaction flask is calculated as

$$V_g = (C - V) \times \frac{\Delta P}{P} \times \frac{P - p}{760} \times \frac{273}{273 + T},$$

where

C = measured ml capacity of the reaction flask (including the volume of stopcock apparatus),
V = ml volume of reagent solution in flask,
ΔP = pressure increase of sample gas added, in mm Hg,
P = atmospheric pressure, in mm Hg,
p = vapor pressure of H_2O at room temperature, in mm Hg,
T = room and reaction flask temperature, in °C.

The volume of CO (STP, dry) in a test is calculated as

$$V_{\mathrm{CO}} = Q - \left[\frac{OD_{\mathrm{t}}}{OD_{\mathrm{std}}} \times Q\right] \times 0.1261,$$

where

Q = mg $PdCl_2$ originally present,
OD_{t} = optical density of test solution,
OD_{std} = mean optical density of two standard solutions.

Then (ml CO/total gas (V_g) tested) $\times 10^6$ = ppm CO. Note that 0.1261 ml CO (STP, dry) is equivalent to 1.00 mg $PdCl_2$.

RESULTS AND DISCUSSION

Tests were conducted to assess the performance of the proposed manual method on samples of cylinder gases at the levels of 1000, 500, and 100 ppm carbon monoxide in nitrogen. The gas mixtures used in the program had been blended in aluminum cylinders and analyzed to ±1% by either IR (1000 and 500 ppm levels) or GC (100 ppm level) and were traceable to National Bureau of Standards (NBS) standard gases.

Initial analyses using the method were performed on samples of cylinder gas containing 1100 ppm carbon monoxide in nitrogen. Subsequently, CO mixtures of 473 and 93 ppm were obtained to evaluate the method at lower concentrations. Results from these tests are given in Table 1.

TABLE 1

SUMMARY OF CARBON MONOXIDE DETERMINATIONS

	Carbon Monoxide Concentration (ppm)								
	1100	1100 + AA[a]	999[b]	503[b]	473	473 + AA[a]	100[b]	93	93 + AA[a]
n^c	17	10	10	10	8	6	10	9	8
$\bar{X}$ (ppm)	1057	1036	959	477	455	444	93	87	84
Std. dev.	25.6	39.6	11.8	15.8	18.6	17.6	5.5	11.8	11.5
% RSD	2.4	3.8	1.2	3.3	4.1	4.0	5.9	13.6	13.7

[a] Sample gas mixtures containing ambient air.

[b] Data collected at a later date.

[c] Number of analyses.

In 1937, Christman and co-workers (*4*) theorized that the presence of oxygen within the gas sample might contribute to substantial interferences in the $PdCl_2$ reduction method, based on the reaction

$$Pd + 1/2O_2 + 2HCl \rightarrow PdCl_2 + H_2O.$$

If oxygen were an interference, the method would be of no value since samples would be collected from stationary sources which normally contain from 3 to 12% (v/v) oxygen. To determine the level of oxygen interference, if any, at each concentration level (1100, 473, and 93 ppm CO), the test procedure required the introduction of a known volume of CO into the sample flask, plus a known volume of ambient air ($\sim$21% O_2). Care was taken to prevent the vessel from reaching ambient pressure, to prevent backflushing. Once the ambient air had been added to the flask which contained the carbon monoxide plus reagents, the 4-hr mixing period began, after which time the CO concentration was determined in the normal manner. The presence of oxygen up to 10% (v/v) in the reaction flask had a negligible effect [Table 1 (a)] on the recovery of CO.

Additional data were collected at a later date, using 999, 503, and 100 ppm CO in nitrogen [Table 1 (b)]. Precision and accuracy were comparable to those of the previous runs.

SUMMARY

The gas sample transfer apparatus, as shown in Fig. 1, was designed to facilitate the analysis of several carbon monoxide samples during a normal work day. With this apparatus, gas samples were accurately transferred from the Tedlar bags leak free through the system to the reaction flask. Changing from one gas concentration to another was greatly simplified by using the quick-connect fittings; gas volumes were readily calculated from direct readings taken from the differential pressure gauge.

Tests were conducted to assess the performance of the $PdCl_2$ method and the sampling system with various gas mixtures. The proposed $PdCl_2$ method utilizing this particular sampling scheme is capable of successfully measuring carbon monoxide in nitrogen cylinder gas at levels of 0.1, 0.05, and 0.01% with expected average recoveries of 96, 96, and 93%, respectively.

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A Microcell for Small-Volume Dialysis

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Advancements in technology have emphasized adaption of laboratory methods to microsystems which conserve materials, reagents, and sample volumes. However, reports of reliable methods for dialysis of microliter fractions prior to further analysis have been scarce (*2*).

In this paper, we report an easily constructed microcell which not only provides a simple, rapid method for small-volume dialysis, but also allows for total protein recovery with little risk of sample loss.

MATERIALS AND METHODS

The microcell was constructed from a 200-μl disposable pipet tip which was cut 23 mm from the delivery end (Fig. 1a). The cut end was gently flamed and, when malleable, was pushed against a glass plate to create a flanged end (Fig. 1b). A single layer of wet dialysis tubing (2 × 2 cm) was placed over the flanged end and held in place by an O ring having an inside diameter of 0.11 in. and a thickness of 0.07 in. (Fig. 1c).

The sample was placed in the open end of the microcell, making certain that no air bubbles formed near the membrane surface. The microcell was then taped to the inside wall of a 500-ml beaker and the dialysis solution was added until a level 2–4 mm above the O ring was reached. Following dialysis, the sample was recovered through the open end with a Pasteur pipet.

The efficiency of the microcell was tested by dialyzing a series of 25-μl samples, each containing 1 mg/ml bovine serum albumin (BSA) and either 40% w/w sucrose or 2 *M* NaCl.

The concentrations of sucrose and NaCl were determined by refractive index.

Protein concentrations were determined by the spectrophotometric method of Groves *et al.* (*1*).

RESULTS

The solutions to be dialyzed were added in 25-μl aliquots to a series of 10 microcells. The microcells were attached to the inside of a 500-ml

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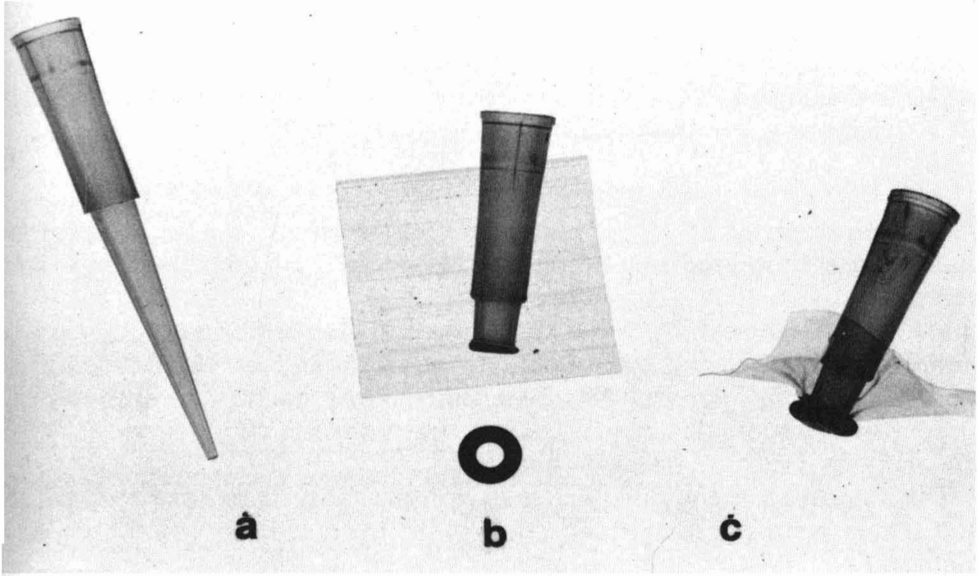

FIG. 1. Construction of a microcell for small-volume dialysis. (a) A 200-μl pipet tip; (b) a heat-flanged tip with wet dialysis membrane and O ring; and (c) an assembled microcell ready for sample dialysis.

beaker and water was added to a height of 2 mm above the O ring. At time zero and 10-min intervals thereafter, the contents of one microcell were removed and assayed for concentration of reactants. As shown in Fig. 2, there is a rapid removal of NaCl from the sample during the first 20 min of

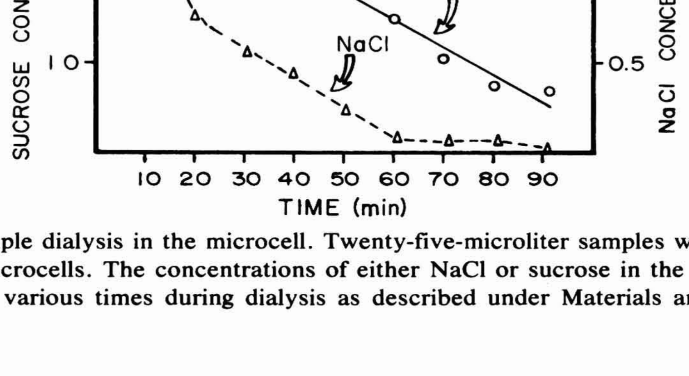

FIG. 2. Sample dialysis in the microcell. Twenty-five-microliter samples were added to each of ten microcells. The concentrations of either NaCl or sucrose in the samples was determined at various times during dialysis as described under Materials and Methods.

dialysis with total removal within 1 hr. The sucrose was removed from the sample in a similar manner, however, as expected, the time required for exchange was considerably longer. Although the final protein concentration of the samples decreased, total protein recovery was demonstrated in all experimental conditions.

DISCUSSION

The requirement of an apparatus to contain small-volume samples for dialysis has prompted our laboratory to design the microcell described herein.

We have successfully used the microcell for buffer equilibration of samples prior to rocket immunoelectrophoresis and acrylamide gel electrophoresis. The microcell has been particularly useful for removing sucrose from aliquots of density gradient fractions which allowed for activity analysis prior to fraction pooling.

The microcell technique for small-volume dialysis is easy to perform and it has been our experience that, with the exception of the dialysis membrane, all parts are reusable.

SUMMARY

We have designed a microcell for use in the dialysis of microliter sample volumes. The microcell was easily constructed from inexpensive laboratory materials and allowed for a rapid buffer exchange with total protein recovery.

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Spectrophotometric Determination of Cadmium with Uramyldiacetic Acid

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INTRODUCTION

Cadmium is closely related to zinc. The cadmium to zinc ratios in nature are changeable. In most minerals and soils, ratios of 1:100 to 1:1000 were found (*2*). Zinc is an essential metal for most life forms (*18*).

Cadmium is obtained as a by-product in the refining of zinc and other metals. However, it is difficult to separate zinc and cadmium. The latter will often be found in small amounts in commercially available zinc compounds (*15*).

Though cadmium has been recognized for only a relatively short period of time, copper, lead, zinc, and some other metals have been used for several thousand years. Thus, as soon as man started to produce metals, he also started to pollute the environment with cadmium. In this century cadmium and cadmium compound have been used increasingly by industries, causing a sharp increase in environmental contamination. Cadmium will be emitted to air and water mines, by metal smelteries, especially lead, copper, and zinc smelteries, and by industries using cadmium in alkaline accumulators, alloys, paints, and plastics. The burning of oil and waste and scrap metal treatment will also contribute. The use in agriculture of fertilizers, either as chemicals or as sludge from sewage plants, and the use of cadmium-containing pesticides might also contribute to the contamination.

Some of the cadmium emitted to the air will be inhaled by people and animals, but most of it will be deposited in soil or water. The cadmium deposited in water may then increase the concentrations of cadmium in edible water organisms. In the event of flooding or irrigation, cadmium in water might also increase the concentrations in soil, in turn causing an increase of cadmium in agricultural products, such as rice and wheat.

The behavior of cadmium in nature has not been extensively studied; it is only recently that systematic investigations have begun on the transfer of cadmium between different compartments of the ecosystem (*2*). It has

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been suspected that organic cadmium compounds similar to the ones described for mercury could exist, but it has not been possible to prove the existence of such compounds (*17*).

A larger number of organisms are poisoned by cadmium. For example, it is known that human renal function is inhibited, calcium loss is increased, and competition with zinc ions about certain sites of complexes containing proteins occurs. Moreover, the devastating effects of cadmium are noted by recent papers; it is involved in the case of Itai–Itai Japanese disease, a situation which needs cadmium control in the environment.

A new procedure for spectrophotometric determination of cadmium with uramyldiacetic acid is described; from previous studies of uramyldiacetic acid in this department, it was found that it can be applied for photometric determinations of other metals (*6–14*).

MATERIALS AND METHODS

Reagents

Standard cadmium(II) solution. This solution was prepared from $CdSO_4 \cdot 8H_2O$ R.A. The concentration was volumetrically determined with EDTA, using a neT indicator, resulting in 0.001052 *F* with 115.26 μg Cd(II)/ml from another solution prepared by dilution (*1*).

Standard uramyldiacetic acid (UDA) solution. A standard aqueous solution of UDA was prepared and titrated with standard zinc(II) solution and neT indicator, resulting in 0.0002613 *F* (*1*).

Uramyldiacetic acid (UDA) solution at 0.02%. The necessary amount of UDA was weighed so that a 0.02% solution was obtained by dissolving in distilled water to 100 ml.

Sodium hydroxide solution 0.1 F.

Hydrochloric acid solution 0.1 F.

Apparatus

Beckman Model DU spectrophotometer. The spectrophotometer was equipped with 10-mm quartz cells.

Beckman Model Expandomatic pH meter. The pH meter was equipped with glass and calomel electrodes (sensitivity ± 0.01 pH).

Procedure

To a series of 25-ml volumetric flasks transfer aliquots of a standard cadmium(II) solution, add 1.5 ml of 0.02% UDA solution, the necessary drops of 0.1 *F* hydrochloric acid or sodium hydroxide solution to approximate the pH to 6, and dilute to the mark with distilled water. The absorbance measurements are carried out at 265 nm (or the scanning was made) using 10-mm quartz cells and a solution on 1.5 ml of 0.02% UDA solution diluted to 25 ml as a blank.

RESULTS AND DISCUSSION

Absorption Spectra

In order to fix the optimum working wavelength, the spectral characteristics of the cadmium(II)–UDA system at various pH values were studied. Figure 1 shows the results obtained at pH 2.6, 6.2, and 9.9, from which the identities of the spectra are determined at the given pH values, exhibiting a maxima at 265 nm in all cases.

Effect of pH

The absorbance constant in the pH interval 5.0–7.0 was found. Figure 2 illustrates the results obtained when using a cadmium concentration of 2.36 μg Cd(II)/ml. Further measures were made to pH 6.0.

Effect of Reagent Amount

Once the pH optimum had been fixed, the effect of the amount of reagent was studied. It was found that 1 ml of 0.02% UDA solution was enough to form the complex and further additions of reagent did not appreciably affect the absorbance of the system.

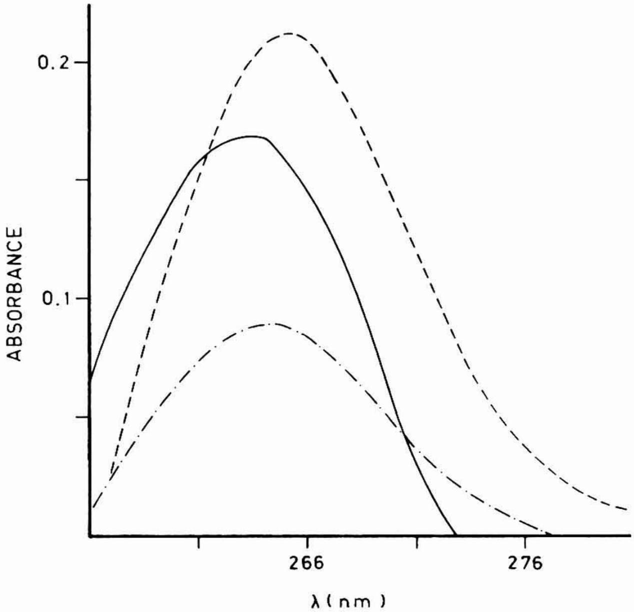

FIG. 1. Absorption spectra of cadmium(II)–UDA complex solutions at pH 2.6, – · –; 6.2, – – –; 9.9,——, and concentration of 2.36 μg Cd(II)/ml.

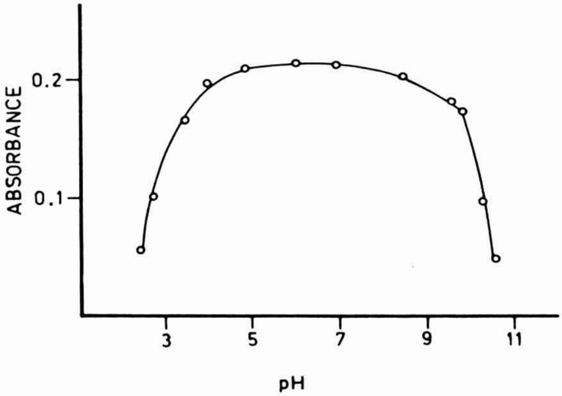

FIG. 2. Effect of pH variations on the absorbance of the cadmium(II)–UDA complex solutions. Cadmium(II) concentration was 2.36 μg/ml.

Time and Temperature Effects

The effects of time and temperature on the stability of the cadmium(II)–UDA system were also studied. Cadmium reacts instantly with uramyldiacetic acid; the absorbance of the formed complex solution was unchanged for up to 8 hr up to 100°C.

Beer's Law, Ringbom's Interval, and Molar Absorptivity

To verify Beer's law a series of solutions was prepared following the usual procedure, the cadmium concentration being between 0.12 and 3.6 μg/ml. In the absence of interfering ions, cadmium concentration is proportional to the radiant energy absorbed between the limits already mentioned at 265 nm.

From the results obtained, the molar absorptivity of the system was calculated as 1.0×10^4 liters mol^{-1} cm^{-1}. The sensibility, according to Sandell's expression, was 0.011 μg cm^{-2} and Ringbom's optimum interval is between 0.6 and 3.3 μg Cd(II)/ml.

Reproducibility and Precision

To carry out these studies four series of different-concentration solutions were prepared following the previously described procedure. The results obtained for concentrations of 1.48, 2.36, 2.66, and 3.55 μg Cd(II)/ml are 0.0024, 0.0018, 0.0024, and 0.0003 of standard deviation.

The relative error on the mean value is $(\sigma_m \cdot t/\bar{x} \times 100$ of 1.28, 0.03, 0.74, and 0.57% at the same concentrations.

Stoichiometry of the Reaction

To establish the composition of the cadmium(II)–UDA chelate three methods were followed: (a) "molar ratio" of Yoe and Jones (*19*), (b) "continuous variations" of Job (*4*), and (c) "slope ratio" of Harvey and Manning (*3*). The results are illustrated in Figs. 3–5, from which it is inferred that the formation of the complex takes place in a cadmium(II) to UDA ratio of 1:1.

Interferences from the Various Ions

The effects on the absorbance of the system under study of the most common ions were investigated, finding that, for a concentration of 2.36 μg Cd(II)/ml, the Cd(II)–UDA system permits the presence of all anions studied, except chromate and vanadate. Also it was found that all cations studied except chromium interfere.

Application of the Method for the Determination of Cadmium in Biological Samples

The method described above was used for the determination of the cadmium content in *Sepia officinalis* hepatopancreas.

After the grease is taken out, a sulfonitric and a nitroperchloric mixture are added to the sample until a clear yellow liquid is obtained.

The subcommittee of Metallic Impurities in Organic Matter (*16*) proposed a method for the cadmium extraction of compounds like ditizonate in a strong alkaline medium with cyanide, and it was elected to separate the cadmium of the resulting solution.

Once cadmium is separated, the organic matter is eliminated by using the upper procedure. The resulting solution is neutralized with ammonium hydroxide and the excess is eliminated by ebullition; afterward the re-

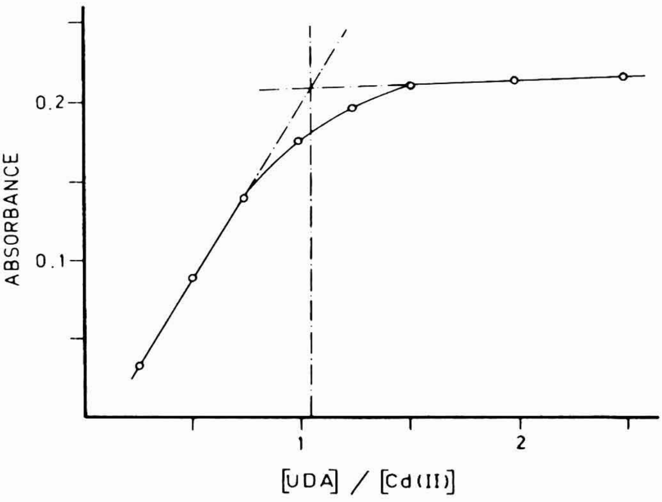

FIG. 3. Application of the molar ratio method for determination of cadmium(II)–UDA complex composition. pH = 6.

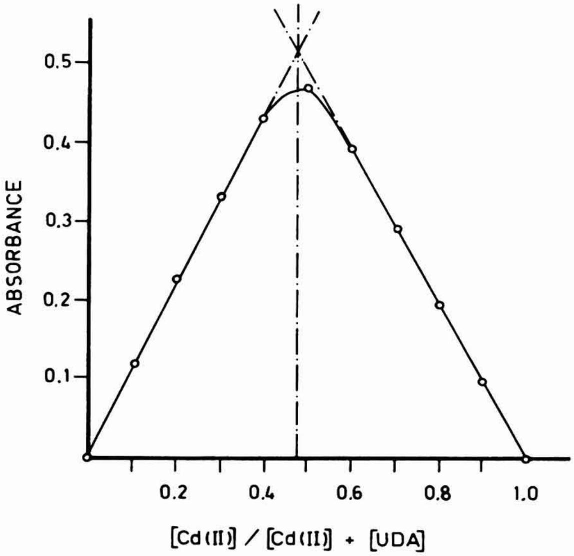

FIG. 4. Application of the continuous variations for determination of cadmium(II)–UDA complex composition. pH = 6.

sulting solution cools, and finally, distilled water is added up to a volume of 100 ml, from which an aliquot is transferred to a 25-ml volumetric flask and treated as in the general procedure previously described.

A cadmium content of 1.9 ppm was found in *Sepia officinalis* hepatopancreas.

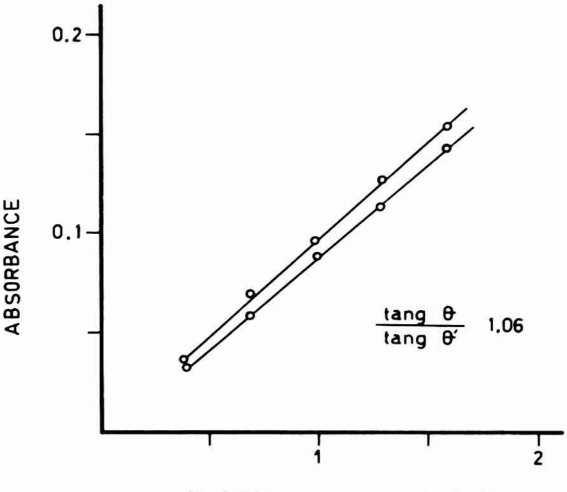

FIG. 5. Application of the slope–ratio method for determination of cadmium(II)–UDA complex composition. pH = 6.

Application for the Determination of Cadmium in White Metal

The method is applied to the determination of the cadmium content in a British Chemical Standard alloy; the composition is the following: Sn 86.2%, Sb 6.72%, Pb 3.23%, Cu 3.03%, Fe 0.072%, Zn 0.042%, Cd 0.29%, Al 0.03%, As 0.16%, and Bi 0.08%.

The sample is treated with 5 ml of concentrated sulfuric acid and 0.5 ml of concentrated nitric acid until it is dissolved. After evaporation up to dryness 30 ml of 2% sulfuric acid solution is added and warmed in order to dissolve the sulfates. After cooling, it is filtrated, washing the precipitate with 2% sulfuric acid solution. After 6 *M* yodhidric acid solution is added to the filtrate, the cadmium is then separated.

The sample solution is transferred through a column that contains 10 g of Amberlite IR-120 resin, which previously was treated with water and 2 *N* hydrochloric acid and was washed with the effluent liquid used. The sample is transferred with a velocity of 7 ml/min, after which the eluate is received in a glass and returned through the column. Finally, 3 vol of effluent liquid is transferred through the column (*5*).

The total eluate is evaporated up to dryness for the yodhidric acid elimination and hydrogen peroxide is added. The solution is transferred to a 25-ml volumetric flask and distilled water is added to the mark. An aliquot of the solution is transferred to a 25-ml volumetric flask and treated as in the general procedure previously described.

The analysis of several samples of the indicated alloy was realized and a 0.28% average concentration was found, a relative error of 3% being acceptable in the spectrophotometric methods.

The recuperation of procedure was also determined, being a 97.6% average value.

CONCLUSIONS

The above investigation has led to the development of a method for determination of minute amounts of cadmium after forming the cadmium(II)–uramyldiacetic chelate at pH 6 and measuring the absorbance of the complex at 265 nm.

Those ions which interfere in the procedure can readily be eliminated by using some methods already described. Although the method was applied to the determination of cadmium in biological samples and white metal, it can be used with a variety of materials.

SUMMARY

A procedure for the determination of cadmium(II) by uv spectrophotometry is proposed. This metal reacts at pH 6 with uramyldiacetic acid (UDA) to give a complex with a maximal absorbance at 265 nm and follows Beer's law in the range 0.12–3.6 μg Cd(II)/ml, the optimal interval being from 0.6 to 3.3 μg Cd(II)/ml. The sensitivity is 0.011 μg cm^{-2}. Through this

study the stoichiometry of the chelate compound, interfering elements, and the reproducibility of the method were evaluated. The method has been applied to the determination of cadmium in biological samples and a white metal.

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Kinetic Determination of Microquantities of D(−)-Arabinose

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INTRODUCTION

In our previous paper (*1*) dealing with a kinetic method for the determination of some biologically active substances, on the basis of their reaction with molybdenum(VI) and hydrogen peroxide, we found that *D*(+) glucose affects these reactions when present in concentrations considerably higher than those of the substances which were determined.

In the course of further investigations we observed that *D*(−) arabinose reacts with molybdenum(VI) and hydrogen peroxide as well, and that the latter reaction is more sensitive in solutions containing acetonitrile. A linear dependence between the concentration of D(−)-arabinose and the rate of the colored product formation was established, thus rendering possible a kinetic determination of D(−)-arabinose.

EXPERIMENTAL

Apparatus. The reaction rate was followed photometrically with a Zeiss Specol by measuring the solution absorbance at the wavelength of 350 nm, in 5-cm cells, every 30 sec in the course of 5 min from the onset of the reaction. Thermostating was carried out by means of an "Ultratermostat nach Höppler" type NBE (VEB Prüfgeräte-Werk, Medingen). The pH measurements were performed by the use of a Radiometer type PHM 4C pH meter.

Reagents. The solutions were prepared from reagent-grade chemicals and bidistilled water. Concentrations of the stock solutions of D(−)-arabinose (Merck), ammonium molybdate (Merck), and hydrogen peroxide (Merck) were 1×10^{-2}, 1×10^{-1}, and 9.8 *M*, respectively. Carbonate buffer was made by mixing 1 *M* sodium carbonate solution (Merck) with 1 *M* sodium hydrogen carbonate solution (Merck).

Procedure. The reaction was carried out as described in our previous

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paper (1). Molybdenum(VI), D(−)-arabinose, and acetonitrile were placed into one compartment of a special vessel, buffer was placed into the second compartment, and hydrogen peroxide and water were added up to 25 ml into the third compartment of the same vessel.

All the experiments were performed at 25 ± 0.1°C.

RESULTS AND DISCUSSION

Since acetonitrile was shown to affect the rate of this reaction, it was necessary to determine its optimum concentration in the reaction mixture, as well as the other experimental conditions for the determination of D(−)-arabinose. The optimum conditions were determined on the basis of data obtained by kinetic investigations of the reaction between molybdenum(VI) and hydrogen peroxide in the presence, as well as in the absence, of D(−)-arabinose.

Figure 1 shows the dependence of these reaction rates on acetonitrile concentration. It may be seen that the difference in rates between the reactions studied is greatest in solutions containing 50 vol% of acetonitrile; therefore, further investigations were carried out under these conditions.

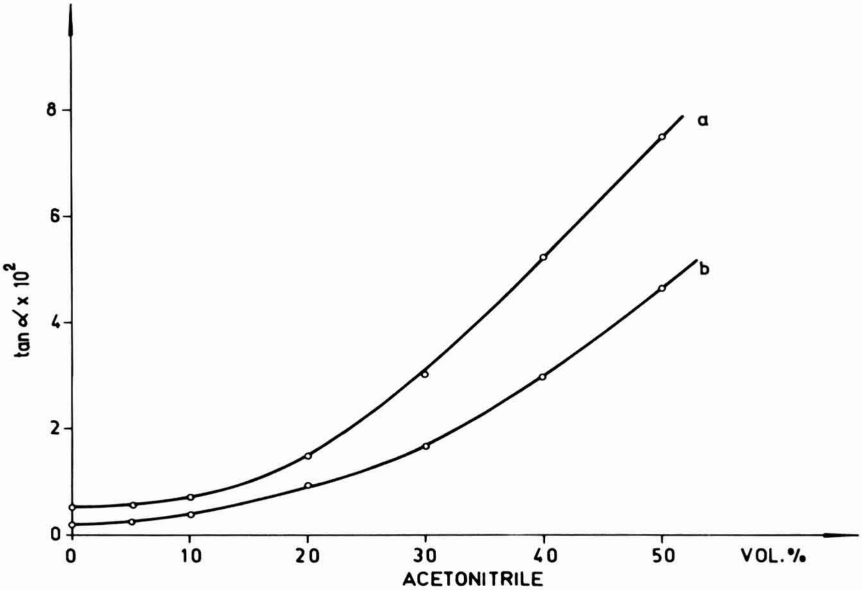

FIG. 1. Dependence of tan α on acetonitrile concentration. (a) Reaction with $D(-)$ arabinose, (b) reaction without $D(-)$ arabinose. Initial concentrations: hydrogen peroxide, 4.0×10^{-2} M; molybdenum(VI), 1.1×10^{-3} M; carbonate buffer, 1.6×10^{-1} M, pH(R) 9.50; $D(-)$ arabinose, 7.0×10^{-4} M.

The dependence of tan α on hydrogen peroxide concentrations for both reactions is given in Fig. 2, which shows that the reaction rate with D(−)-arabinose reaches its maximum value at the hydrogen peroxide concentration of 5.88×10^{-2} *M;* at this concentration the difference in rates between the two reactions studied is greatest. Both reactions are of the first order with respect to the carbonate buffer, whereas the dependence of reaction rates on pH(R) value is shown in Fig. 3. It was established that the rates of both investigated reactions increase linearly with the increase of molybdenum(VI) concentration to a definite value for each reaction, and that with further increase of molybdenum(VI) concentration the rates of these reactions decrease abruptly (Fig. 4). Figure 4 shows that the rate of the reaction in the presence of D(−)-arabinose increases linearly until the molybdenum(VI) concentration becomes equivalent to D(−)-arabinose concentration in solution. As it was found that the rate of the reaction studied increases also linearly with increasing concentrations of D(−)-arabinose, up to a concentration of 10×10^{-4} *M* (Fig. 5), it might be concluded that in this case, similar to earlier investigated reactions (*1*), a catalytically active monocomplex of D(−)-arabinose and molybdenum(VI) is most probably formed.

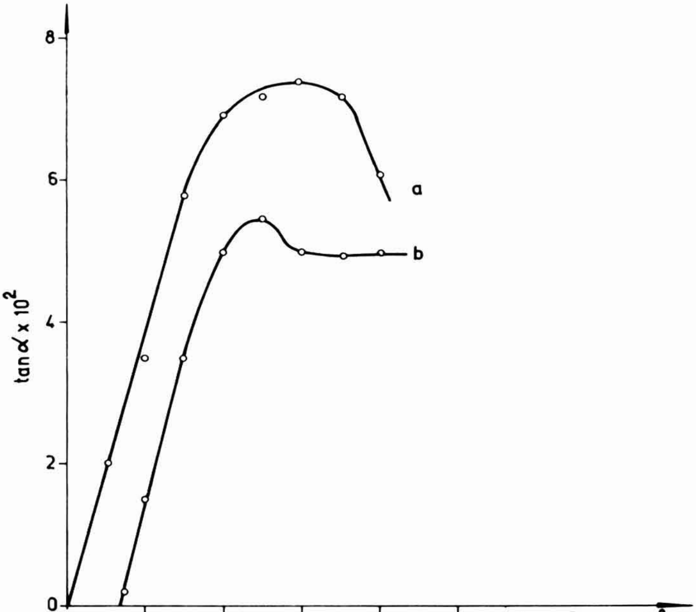

FIG. 2. Dependence of tan α on hydrogen peroxide concentration. (a) Reaction with *D*(−) arabinose, (b) reaction without *D*(−) arabinose. Initial concentrations: molybdenum(VI), 1.1×10^{-3} *M;* carbonate buffer, 1.6×10^{-1} *M*, pH(R) 9.50; *D*(−) arabinose, 7.0×10^{-4} *M*

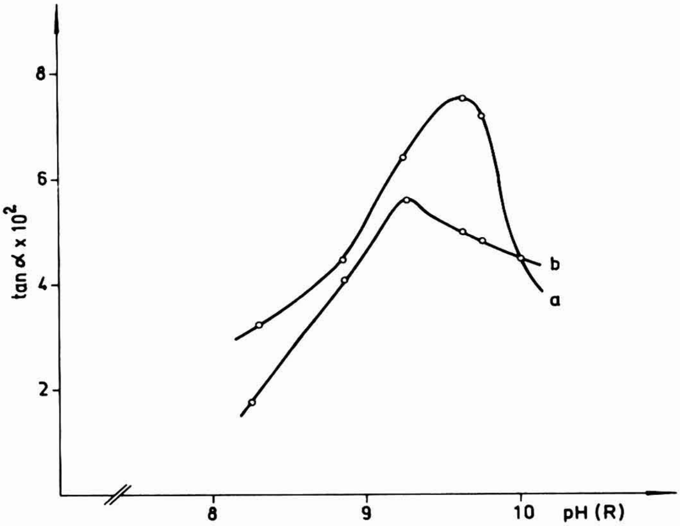

FIG. 3. Dependence of tan α on pH(R). (a) Reaction with $D(-)$ arabinose, (b) reaction without $D(-)$ arabinose. Initial concentrations: hydrogen peroxide, 5.88×10^{-2} M; molybdenum(VI), 1.1×10^{-3} M; carbonate buffer, 1.6×10^{-1} M; $D(-)$ arabinose, 7.0×10^{-4} M.

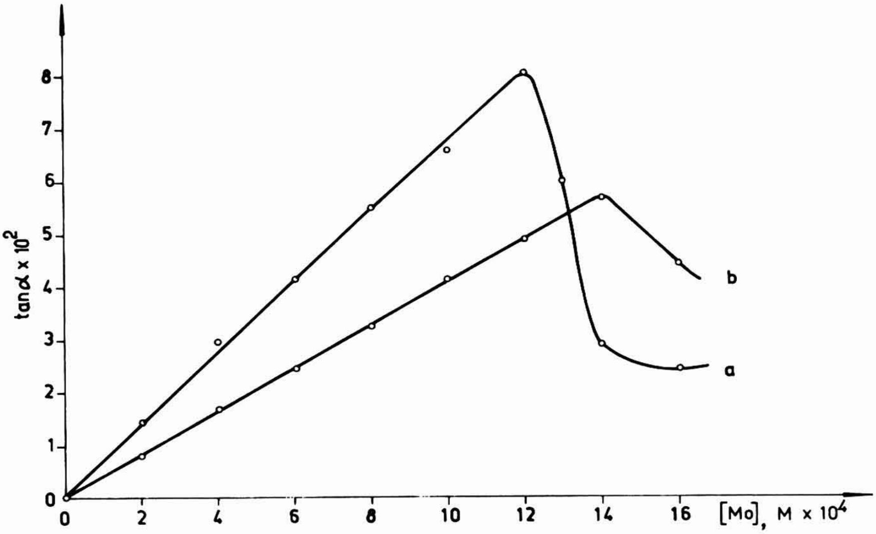

FIG. 4. Dependence of tan α on molybdenum(VI) concentration. (a) Reaction with $D(-)$ arabinose, (b) reaction without $D(-)$ arabinose. Initial concentrations: hydrogen peroxide, 5.88×10^{-2} M; carbonate buffer, 1.6×10^{-1} M, pH(R) 9.64; $D(-)$ arabinose, 12.0×10^{-4} M.

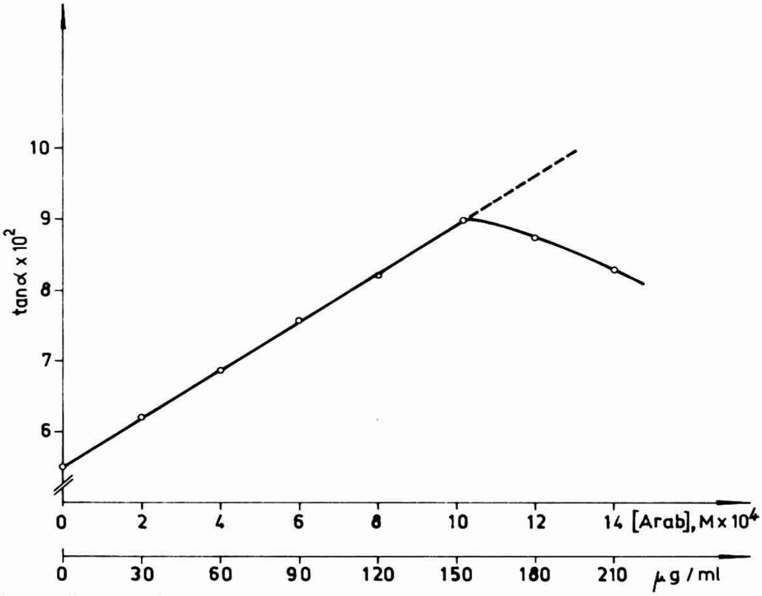

FIG. 5. Dependence of tan α on *D*(−) arabinose concentration. Initial concentrations: hydrogen peroxide, 5.88×10^{-2} *M*; molybdenum(VI), 1.2×10^{-3} *M*; carbonate buffer, 1.6×10^{-1} *M*, pH(R) 9.64.

On the basis of the results obtained, optimum concentrations of all the components taking part in the reaction were determined: hydrogen peroxide, 5.88×10^{-2} *M*; molybdenum(VI), 1.2×10^{-3} *M*; carbonate buffer, 1.6×10^{-1} *M*, pH(R), 9.64; acetonitrile, 50 vol%. The determinations were performed by the use of the differential form of the tangent method, and the corresponding calibration curve is given in Fig. 5. The results of these determinations are shown in Table 1, from which it might be seen that the proposed kinetic method renders possible the determination of D(−)-arabinose in amounts from 46 to 135 μg/ml with a maximum standard deviation of 10%.

D(+)-Glucose interferes with the determination when present in equivalent concentrations.

TABLE 1
KINETIC DETERMINATION OF D(−)-ARABINOSE

Taken (μg/ml)	Found (μg/ml)	Number of determinations	Relative standard deviation (%)
46	47 ± 4.5	5	9.6
90	88 ± 3.0	5	3.4
135	135 ± 1.5	5	1.1

SUMMARY

A kinetic method for the determination of microquantities of D(−)-arabinose is presented. The method is based on the accelerating effect of D(−)-arabinose on the reaction between molybdenum(VI) and hydrogen peroxide in solution containing 50 vol% of acetonitrile.

In order to find optimum experimental conditions for the determination of D(−)-arabinose, the kinetics of the reaction between molybdenum(VI) and hydrogen peroxide in the presence, as well as in the absence, of D(−)-arabinose was studied. D(−)-Arabinose was determined photometrically by following the rate of the colored reaction product formation. The concentrations of D(−)-arabinose which were determined ranged from 46 to 135 μg/ml, and the standard deviation was lower than 10%.

ACKNOWLEDGMENT

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Microdetermination of Magnesium in Biological Fluids[1]

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INTRODUCTION

The determination of magnesium in blood serum by means of atomic absorption spectrophotometry is considered by some investigators to be the method of choice (*1, 6, 11, 14, 18*). However, because the instrument is not readily available in many clinical laboratories, an alternative approach is required, such as a simple, rapid, and reliable manual method. The need appears more urgent in view of growing evidence indicating the importance of monitoring serum magnesium under various clinical conditions (*2, 5, 7, 8, 13, 17*).

In the search for a method that could meet the necessary criteria, we have experimented with a wide range of techniques based on colorimetric approaches. Of these, the compound Magon, sodium 1-azo-2-hydroxy-3-(1,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate), first proposed by Mann and Yoe (*9*), appeared most promising as a reactor for magnesium. However, the technique as originally described suffered from serious drawbacks (*15*). The reaction was slow and subject to interferences by calcium. Furthermore, because the reaction was sensitive to the water content of the reaction mixture, it had to be carried out in a medium containing a large proportion of ethanol. Subsequently, the method was modified by employing the easier to synthesize unsulfonated Magon (*10*). As a result, the reaction was claimed to be more rapid, in that a greater tolerance for water was allowed while interference from calcium was negligible.

The method was adapted to the determination of magnesium in biological fluids by several investigators (*3, 4, 12, 16*) who employed different amounts of ethanol and used borax to buffer the medium to various pH values. The optimal pH and the amount of ethanol needed in the reaction

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were investigated by Svoboda and Chromy (*15*). They concluded that a buffer solution at a pH of 9.5 and the presence of at least 50% ethanol in the final mixture should be employed.

In the method described here, ethanol is eliminated from the system and replaced by dimethyl sulfoxide; potassium hydroxide replaces borax, and calcium interference is circumvented by incorporating EGTA into the system, while the interference of other trace metals apt to be present in serum is masked by cyanide. Only 10 μl of specimen are used per milliliter of the reagents and, importantly, from a simplicity point of view there is no need for either a separation or a deproteinization step.

EXPERIMENTAL

Reagents

Color reagent. Transfer 200 ml of dimethyl sulfoxide into a 5-dl glass beaker. Place a magnetic stirring bar inside the beaker and while stirring add 100 mg of Magon sulfonate, 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate) sodium salt (ICN–K&K Laboratories, Inc., Plainview, N.Y.).

Transfer 800 ml of water into a 2-liter glass beaker. Place a magnetic stirring bar inside the beaker and while stirring add 100 mg of EGTA, ethylene-bis(oxyethylenenitrilo)-tetraacetic acid, followed by two pellets of potassium hydroxide to aid in solubilizing the EGTA. When all dissolves, transfer the Magon solution prepared above into the beaker containing the EGTA. Mix well and store this final stable solution in a polyethylene bottle.

Base solution. Transfer 16.0 g of KOH pellets and 0.5 g of KCN into a graduated polypropylene beaker containing about 800 ml of metal-free water. Stir until all dissolves, dilute to 1 liter with water, and transfer the solution into a polyethylene bottle, where it is stable.

Magnesium stock standard. This standard was obtained from Fisher Scientific or Gallard–Schlesinger as a solution containing 1 mg of magnesium per milliliter (82.28 meq/liter).

Magnesium working standard (2.0 meq/liter). Dilute 2.43 ml of the stock magnesium standard to 1 dl with water.

METHOD

Procedure

1. Use one 4-ml polystyrene cup (Technicon Analyzer) with polyethylene caps for each specimen, one for the working magnesium standard, and one for the reagent blank.
2. Pipet 10 μl of the specimen into the bottom of the cup. Pipet 10 μl of magnesium working standard (2.0 meq/liter) into the cup to be used for the

standard, and 10 μl of distilled water into another cup to be used for the reagent blank.

3. Pipet 0.5 ml of the color reagent solution into each cup. Swirl to mix the solutions and then add 0.5 ml of the base solution to each cup. Cap and mix the solutions by inversion.

4. After about 5 min transfer the reaction mixture into clean cuvettes and determine the absorbances of the samples and the standard at 548 nm against the reagent blank.

RESULTS AND DISCUSSION

The optimal Magon concentration for the reaction was investigated by preparing standards containing 4.0 and 6.0 meq of magnesium per liter, using varying amounts of Magon in the reagent solution. The results are illustrated in Fig. 1. It can be seen that the peaks from left to right, representing the two standards, increase in intensity with an increase of the reagent concentration from 50 through 75 to 100 mg of Magon per liter, indicating that at least 100 mg of Magon per liter of the reagent solution is needed to achieve optimal absorbance. One can also see from Fig. 2 that when spectra of standards containing 2.0, 4.0, and 6.0 meq/liter of magnesium are determined using the optimal reagent concentration, Beer's law is obeyed across the entire range. From a practical standpoint the range covered in Fig. 2 is more than adequate because it includes both

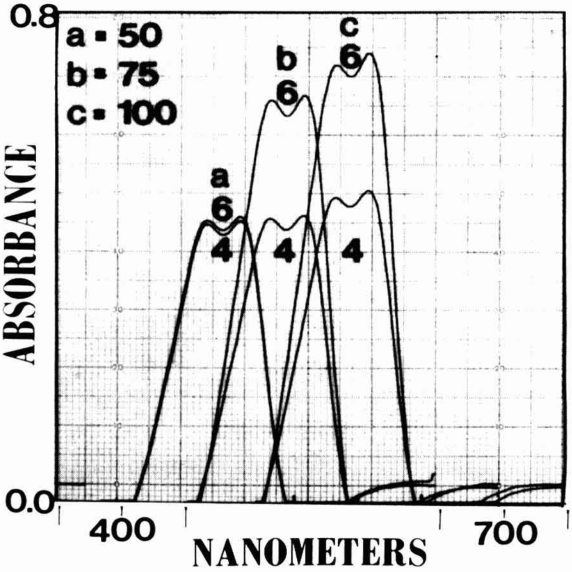

FIG. 1. The effect of varying the concentration of Magon by testing at 50, 75, and 100 mg/liter when reacted with 4 and 6 meq/liter of magnesium.

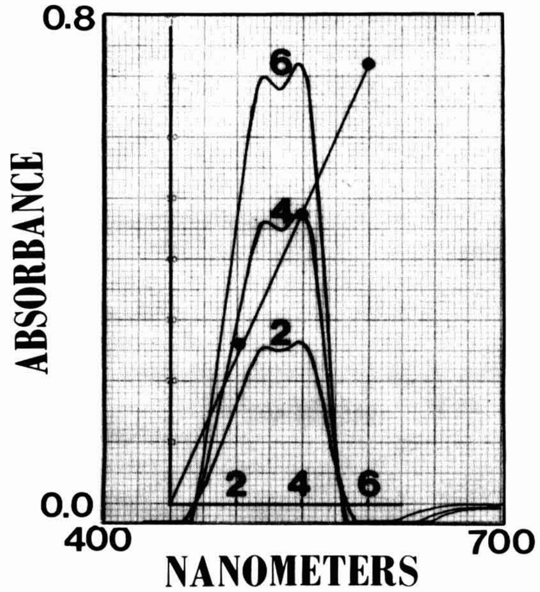

FIG. 2. Spectra and calibration slope of Magon–Mg (II) reaction demonstrates linearity across the range of 0–6 meq/liter of magnesium at optimum concentration of Magon.

normal and rare pathological cases. It is obvious that since the 6.0 meq/liter concentration appears to be at the upper edge of the limit of linearity, if one encounters a serum specimen containing magnesium concentration above that range, it should either be diluted before analysis, the sample size should be decreased from 10 μl to 5 μl, or the amount of reagents should be doubled. It must be remembered, however, that the serum magnesium in many pathological cases is found to be below 1.5 meq/liter.

One of the features involved in using Magon as a metal chelator is the difference in spectral characteristics encountered between the Magon itself (reagent blank), the Magon–magnesium complex, and the subtraction of reagent blank spectrum from the spectrum of the magnesium–Magon complex, containing residual Magon. This aspect was investigated next and the spectrophotometric results are illustrated in Fig. 3. Graph *A* is that of a spectrum obtained for a reagent blank. Graph *B* is the spectrum prepared with reagents containing 2.0 meq/liter magnesium standard, and graph *B-A* is the spectrum for *B* determined against *A* as the reference. It can be seen that graph *A* is broad and actually appears to contain two peaks, one at the same wavelength as the peak of graph *B* and the other peak at a shorter wavelength. When *B* is scanned against *A* as a reference solution, graph *B-A* is obtained, consisting of two small peaks which become more pronounced when the magnesium concentration increases (see Fig. 2). It can also be seen from the latter figure that the choice of

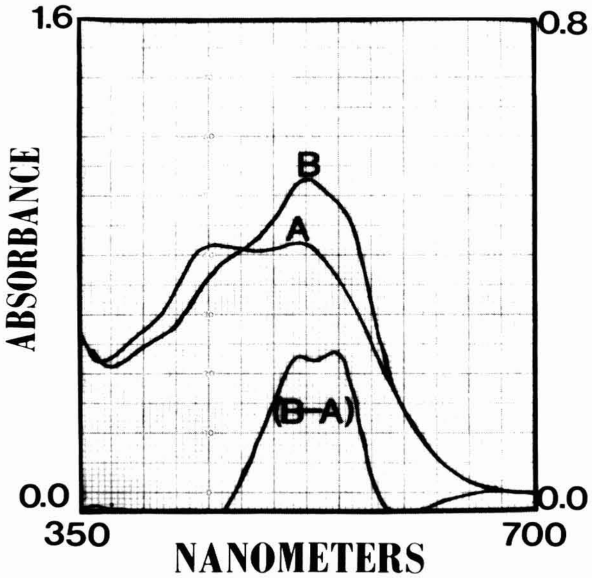

FIG. 3. Spectra of Magon against a water reference (*A*), Magon – Mg (II) against a water reference (*B*), and Magon – Mg (II) against a Magon reference (*B-A*). Spectra *A* and *B* are graphed using the 0.0 – 1.6 A ordinate at the left, while spectrum *B-A* is graphed on the 0.0 – 0.8 A ordinate on the right.

measurements at wavelengths at either peak results in linearity. However, the peak at the longer wavelength (548 nm) is more favorable for measurement and it is recommended for the method.

It can be noticed in both Figs. 2 and 3 that when the spectra of magnesium standards are determined against a reagent blank, the tracings fall below the base line at both sides of the spectrum. This results from the fact that some of the Magon in the sample tube is used up in the process of forming the Magon – magnesium complex so the resulting mixture exhibits a lower absorbance at these wavelengths than the Magon of the reagent blank that is used as a reference, because the latter is obviously at full strength. In connection with this observation, it was interesting to determine whether the Magon itself obeys Beer's law as it must if the end calibration is to be linear as shown in Fig. 2. Magon solutions were prepared as described under Reagents except the amounts were varied to contain 25, 50, 75, and 100 mg of Magon per liter of solution. These were then mixed with the base solution as described under Procedure and the spectra were determined against a water reference between 700 and 350 nm. The spectra are shown in Fig. 4. It can be seen that Magon itself does obey Beer's law under the conditions of the reaction. It also appears clear that when increasing amounts of magnesium are added to Magon, the latter must be consumed proportionately and linearly. Therefore, the re-

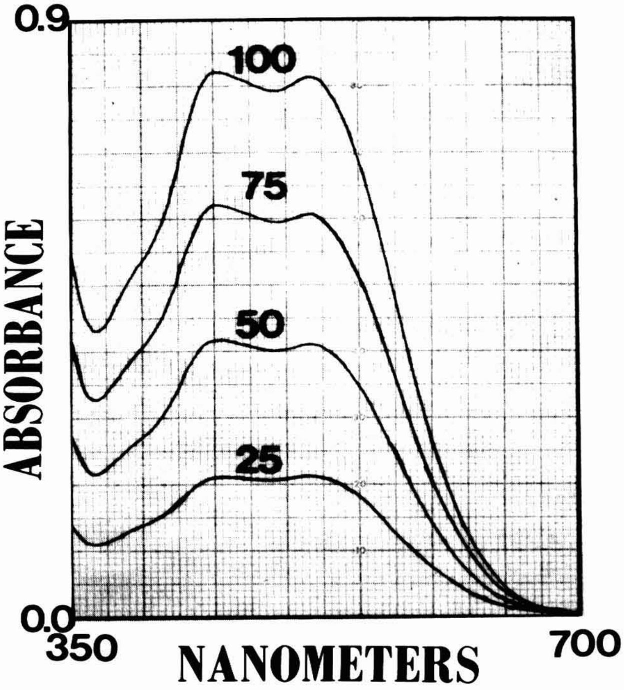

FIG. 4. Spectra of Magon concentrations (25–100 mg) against a water reference.

sulting mixtures contain increasing concentrations of the Magon–Mg (II) complex and decreasing amounts of the free Magon. Because of these linearity characteristics for both Magon as well as Magon–Mg (II) complex, there is a resultant linear relationship obtained between absorbance and magnesium concentration, as indicated in Fig. 2. But, in order to maintain linearity in the range considered, at least 100 mg of Magon per liter of the reagent solution appears to be needed (see Fig. 1).

Figure 5 shows the spectra of magnesium standards determined against water as the blank, where it can be observed that the reagent blank exhibits considerable absorbance. This is not a serious drawback and the spectrophotometers in most clinical laboratories can be used in the method. The Magon concentration chosen here represents the necessary compromise for including the higher ranges of magnesium concentrations that may be encountered in severe pathological circumstances. However, in considering the spectra of Fig. 1, it appears clear that the use of a lower Magon concentration may not result in the linearity needed even at the lower range of magnesium concentrations measured.

Serum magnesium concentrations were then determined on serum specimens from 57 patients by comparing the proposed method with

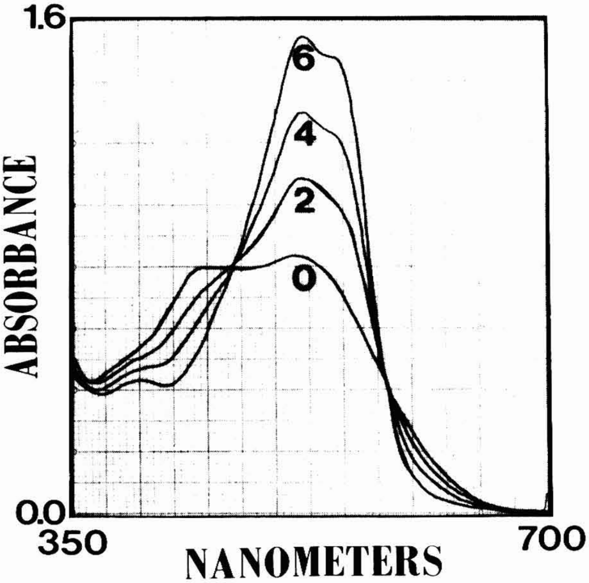

FIG. 5. Spectra of calibration standards (2, 4, and 6) and Magon (0) against a water reference.

atomic absorption spectrometry. The studies were carried out blind in two different laboratories (E.S.B. and R.E.K.) and they included a series of specimens containing normal magnesium concentrations with some specimens spiked with magnesium up to levels of 5.0 meq/liter and other diluted to levels below 1.0 meq/liter. Because the procedure is a direct one with none of the serum removed, four specimens containing highly elevated bilirubin concentrations were also included in the group to see if this potential perturbance was a viable one. The atomic absorption spectrophotometer was Instrumentation Laboratories Model 353 and the procedure for magnesium was that recommended by the manufacturer. It included a 1:50 dilution of each sample with 1% lanthanum diluent with each test carried out in duplicate, and the result were based on a calibration curve prepared using magnesium standards containing 140 meq/liter of sodium and 5.0 meq/liter of potassium.

The correlation between results obtained by the Magon method and atomic absorption spectrometry is illustrated in Fig. 6. The regression equation, $Y = 1.003X + 0.023$, and the correlation coefficient of 0.990 indicate the excellent agreement between the two methods. The solid line of the plot represents the line of identity, while the open circles are the results obtained with the icteric speciments. Additional comparison statistics for the two methods are presented in Table I. No significant

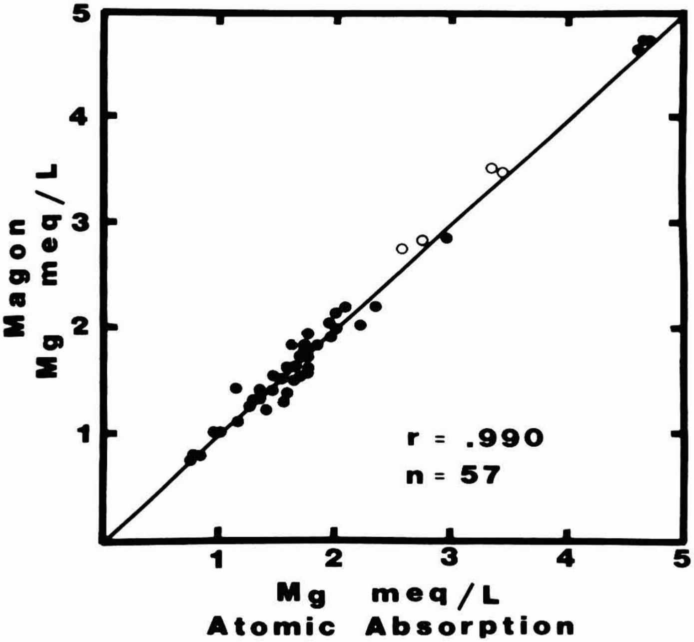

FIG. 6. Regression plot of the results by the Magon method versus those by atomic absorption. The solid line represents $Y = X$ and the actual regression equation was $Y = 1.003X + 0.023$. Open circles represent data from high bilirubin specimens.

difference was noted in either the mean values of the two distributions as judged by the *t* test, or in the precision of the two methods as judged by the *F* test.

The ions of serum that have been demonstrated to be major interferences are calcium, copper, iron, and phosphate (*16*). Calcium can be masked by EGTA, and copper and iron by cyanide. Phosphate has been the more difficult to deal with as a problem ion. It was shown to be an important interference in the determination of magnesium with Magon,

TABLE 1
METHOD COMPARISON STATISTICS

	Atomic absorption	Magon
$\bar{X}$	1.88	1.91
SEM	0.111	0.112
S (within day)	0.020	0.021
n	*t*	*F*
57	1.95 $(0.1 > P > 0.05)$	1.10 $(F_{0.05} = 3.19)$

but a precipitation step with aluminum chloride plus boiling was able to eliminate the problem *(4)*. However, others have indicated that when Magon is reacted with magnesium prior to alkalinization, phosphate will not form the insoluble magnesium phosphate it normally does at high pH and therefore it will be obviated as an interference *(16)*. This is the reason why the procedure is performed using two additions of reagent in two steps as described, rather than by confining them into a one-piece reagent.

SUMMARY

A modified procedure for the spectrophotometric determination of serum magnesium is described in which a direct addition of serum is made to a two-piece color reagent system. One solution contains an optimized concentration of the color reagent Magon, 1-azo-2-hydroxy-3-2-(2,4-dimethylcarboxanilido)-naphthalene-1′-(2-hydroxybenzene-5-sulfonate) sodium salt, in a dimethyl sulfoxide medium for binding magnesium and also EGTA, ethylene-bis(oxyethylenenitrilo)-tetraacetic acid, for binding calcium. The other is an alkaline solution of dilute cyanide to bind copper and iron and provide the optimum reaction pH. By means of these ingredients the simple procedure involves a reaction with serum magnesium while masking the interference of calcium and trace metals and allowing the reaction to take place without either separation or deproteinization steps. Optimized characteristics are shown for a linear reaction involving a variable reaction blank in the procedure where calcium is measured against a blank that is constant in its makeup. The linearity of the procedural reagent blank, with respect to its own concentration, helps attain the required linear results for the overall system and this is described by spectral studies.

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