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Microchemical Journal

devoted to the application of microtechniques in all branches of science

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Cell Separation:
METHODS IN HEMATOLOGY

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

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

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

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CONTENTS

JOHN T. STOCK AND ROBERT D. BRAUN. Dissimilar-Metal and "Titrant-Stream" Electrode Systems in the Potentiometric Titration of Millimolar Concentrations of Copper(II) in Dimethylformamide with Titanium(III) 519

PAUL CARTER. Ultramicroestimation of Human Serum Albumin: Binding of the Cationic Dye, 5,5′-Dibromo-o-cresolusulfonphthalein 531

MRINAL K. DAS AND A. K. MAJUMDAR. Spectrophotometric Determination of Copper(II) with β-Benzoyl-α-pyridyl Thiourea 540

ZBIGNIEW GREGOROWICZ AND JÒZEF ŚLIWIOK. Application of New Fuchsine for Quantitative Determination of Organic Substances in Thin-Layer Chromatography 545

VASANT L. SHAH AND SATENDRA P. SANGAL. Composition and Stability Constants of Cerium-Chromotrope 2R Chelate and the Spectrophotometric Determination of Cerium 548

DONALD E. OKEN. Quantitation of Picogram Quantities of Serum Albumin by Ultramicrodisc Electrophoresis and Direct Densitometry 557

JOHN T. STOCK. Electrode Deactivation in the Anodic Voltammetry of Some Phenolic Derivatives of Isoquinoline 564

B. L. GOYDISH. The Quantitative Determination of Boron in Glasses Used as Encapsulants for Electronic Devices 572

M. EDRISSI, A. MASSOUMI AND J. A. W. DALZIEL. A Selective Differential Spectrophotometric Method for the Determination of Mercury(II) Using Tris(2-thiopyridine-1-oxide)-iron(III) as Reagent 579

G. S. R. KRISHNA MURTI, A. V. MOHARIR, AND V. A. K. SARMA. Spectrophotometric Determination of Iron with Orthophenanthroline 585

C. E. CHILDS AND E. B. HENNER. A Direct Comparison of the Pregl, Dumas, Perkin-Elmer, and Hewlett-Packard(F&M) Carbon–Hydrogen–Nitrogen Procedures 590

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J. A. Howell, G. E. Linington, and D. F. Boltz. The Preparation and Indirect Spectrophotometric Determination of Total Oxidizing Capacity of Chlorine Dioxide in Acidic Solution 598

Jui-Chang Wang and K. L. Cheng. Precipitation of Tellurium with Bismuthiol II 607

F. Bermejo-Martinez and J. A. Rodriguez Campos. Analytical Applications of Chelons. LVII. Chelometric Determination of Bismuth with DTPA and Reciprocally 622

K. Genest and Lorna J. Lowry. Microcrystalloptic Tests for Some Lupine and Ormosia Alkaloids 625

Joseph K. Samuels III and D. F. Boltz. Near-Infrared Spectrophotometric Determination of Germanium by Modified Heteropoly Blue Method .. 638

K. N. Johri and Harish C. Mehra. Trace Metal Analysis by Combined Thin-Layer Chromatography Incorporating Fluorescent Support and Ring Oven Colorimetry 642

K. N. Johri, Harish C. Mehra, and N. K. Kaushik. Suggested Procedure for Microdetermination of Arsenic in Arsenical Animal Feed 649

H. Flaschka and R. Weiss. The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Lead (Mercury, Copper, or Silver) Using 1-(2-Pyridylazo),2-naphthol (PAN) and Employing Iodide Masking 653

Benjamin W. Grunbaum and Nello Pace. Microchemical Urinalysis. VI. Determination of Sodium, Potassium, Calcium, and Magnesium by Atomic Absorption Spectrophotometry in the Microliter Range of Urine 666

Benjamin W. Grunbaum and Nello Pace. Microchemical Urinalysis. VII. Determination of Citric Acid in Microliter Quantities of Urine .. 673

David B. Sabine. Photomicrographs of Metallo Amino Acid Complexes .. 677

Benjamin W. Grunbaum. Reliability of the Grunbaum Pipet as a Self-Cleaning Device 680

Geoffrey Gordon and George M. Malacinski. An Improved Microinjection Apparatus for Biochemical Embryology 685

Book Reviews 692

Author Index 698

Microchemical Journal, Vol. 15, No. 4

Briefs

Dissimilar-Metal and "Titrant-Stream" Electrode Systems in the Potentiometric Titration of Millimolar Concentrations of Copper(II) in Dimethylformamide with Titanium(III). JOHN T. STOCK AND ROBERT D. BRAUN, *Department of Chemistry, University of Connecticut, Storrs, Connecticut 06268.*

Thirty platinum-nonplatinum electrode couples have been examined as possible end point indicators in the potentiometric titration of millimolar concentrations of copper(II) in *N,N*-dimethylformamide with titanium(III) in the same solvent. Molybdenum, titanium, or stainless steel gave standard deviations of less than 1%. Preconditioned gold, tantalum, and tungsten gave similar results. Other systems were used, but were found to be less satisfactory.

Microchem. J. **15,** 519 (1970).

Ultramicroestimation of Human Serum Albumin: Binding of the Cationic Dye, 5,5′-Dibromo-o-cresolsulfonphthalein. PAUL CARTER, *Research Laboratory, The Memorial Division of The Wilmington Medical Center, Wilmington, Delaware 19899.*

An ultramicrotechnique for estimating human albumin directly in a buffered sulfonphthalein dye solution is described. The method, which is extremely sensitive, requires only 10 μl of specimen and can be accomplished with equipment available in most routine clinical chemistry laboratories.

Microchem. J. **15,** 531 (1970).

Spectrophotometric Determination of Copper(II) with β-Benzoyl-α-pyridyl Thiourea. MRINAL K. DAS AND A. K. MAJUMDAR, *Department of Chemistry, Jadavpur University, Calcutta 32, India.*

A complex is formed between the metal and the reagent and the color development was found to be completed within the pH range 2 to 6. Copper was determined in the presence of a number of other ions.

Microchem. J. **15,** 540 (1970).

Application of New Fuchsine for Quantitative Determination of Organic Substances in Thin-Layer Chromatography. ZBIGNIEW GREGOROWICZ AND JÒZEF ŚLIWIOK, *Department of Analytical and General Chemistry, Silesian Technical University, Gliwice; and Department of Organic Chemistry, Silesian University, Katowice, Poland.*

Research on quantitative determination of substances, developed with fuchsine dyes, based on the relation between the number micrograms of substances analyzed and the size of the surface of the chromatographic spot is presented.

Microchem. J. **15,** 545 (1970).

Composition and Stability Constants of Cerium-Chromotrope 2R Chelate and the Spectrophotometric Determination of Cerium. VASANT L. SHAH AND SATENDRA

P. SANGAL, *Chemical Laboratories, Laxminarayan Institute of Technology, Nagpur University, Nagpur, India.*

The composition and stability of the pinkish violet chelate of cerium–CTR (λ_{max} 550 mμ) have been established by (i) the method of continuous varation, and (ii) mole ratio method. The stoichiometric ratio was found to be 1:1 (metal: ligand) at pH 6.0.

Microchem. J. **15,** 548 (1970).

Quantitation of Picogram Quantities of Serum Albumin By Ultramicrodisc Electrophoresis and Direct Densitometry. DONALD E. OKEN, *Department of Medicine, Peter Bent Brigham Hospital; and Harvard Medical School, Boston, Massachusetts 02115.*

Disc electrophoresis has been used for the measurement of serum albumin in picogram amounts and concentrations as low as 0.1 mg/100 ml. The method lends itself to the determination of picogram quantities of albumin in nanoliter samples of renal tubule fluid.

Microchem. J. **15,** 557 (1970).

Electrode Deactivation in the Anodic Voltammetry of Some Phenolic Derivatives of Isoquinoline. JOHN T. STOCK, *Department of Chemistry, University of Connecticut, Storrs, Connecticut 06268.*

The anodic voltammetry of submillimolar concentrations of 7-hydroxy-1,2,3,4,-tetrahydroisoquinoline, and of the related compounds corypalline and coclaurine, has been examined at the rotating platinum microelectrode (RPE) and at the drum-activated platinum electrode (DAPE) of Berge and Strübing. The electrolysis of these compounds markedly deactivates the RPE, but has a much smaller effect upon the performance of the DAPE.

Microchem. J. **15,** 564 (1970).

The Quantitative Determination of Boron in Glasses Used as Encapsulants for Electronic Devices. B. L. GOYDISH, *RCA Laboratories, Princeton, New Jersey 08540.*

N-Methylthione (Azure C) reacts with BF_4- to form a colored complex in a 0.5 *N* sulfuric acid medium. This colored complex is extractable with dichloroethane or a mixture of dichloroethane–dichloropropane, with a maximum absorbance at 660 mμ.

Microchem. J. **15,** 572 (1970).

A Selective Differential Spectrophotometric Method for the Determination of Mercury(II) Using Tris(2-thiopyridine-1-oxide)-iron(III) as Reagent. M. EDRISSI, A. MASSOUMI, AND J. A. W. DALZIEL, *Department of Chemistry, Tehran Polytechnic, Tehran, Iran; Department of Chemistry, Pahlavi University, Shiraz, Iran; Chelsea College of Science and Technology, London S.W. 3, England.*

A very selective spectrophotometric method is described for the determination of mercury(II) using iron(III) thione as reagent. Selectivity is achieved by taking advantage of the difference in the stabilities of metal–thione chelates and the use of masking agents.

The sensitivity of the method depends on the concentration of reagent used and the volume of sample. A 5×10^{-5} *M* reagent is applicable to the determination of 0.02–0.1 mg of Hg(II) in 100 ml of sample solutions.

Microchem. J. **15,** 579 (1970).

Spectrophotometric Determination of Iron with Orthophenanthroline. G. S. R. KRISHNA MURTI, A. V. MOHARIR, AND V. A. K. SARMA, *Division of Agricultural Physics, Indian Agricultural Research Institute, New Delhi, India.*

A modified procedure is presented. Reduction of the iron prior to development of the color is accomplished with thioglycolic acid at room temperature. The system is buffered to avoid precipitation of hydroxides and phosphates. Copper and nickel cause the most interference.

Microchem. J. **15,** 585 (1970).

A Direct Comparison of the Pregl, Dumas, Perkin-Elmer, and Hewlett-Packard (F&M) Carbon–Hydrogen–Nitrogen Procedures. C. E. CHILDS AND E. B. HENNER, *Parke, Davis and Company, Ann Arbor and Holland, Michigan.*

The data indicate that the classical Pregl and Dumas procedures, although slower, give the most reliable results followed by the Perkin-Elmer and the Hewlett-Packard in that order. Acceptable results can be obtained with any of the four procedures. From an operation standpoint, the Pregl and Dumas were the most trouble-free, the Hewlett-Packard next, and the Perkin-Elmer last in this respect, having shown a considerable amount of down time. Catalyst was the cause of most of the trouble with the Hewlett-Packard. Under normal conditions, it was easier to get acceptable results with the Perkin-Elmer than with the Hewlett-Packard.

Microchem. J. **15,** 590 (1970).

The Preparation and Indirect Spectrophotometric Determination of Total Oxidizing Capacity of Chlorine Dioxide in Acidic Solution. J. A. HOWELL, *Western Michigan University, Kalamazoo, Michigan 49001;* AND G. E. LININGTON AND D. F. BOLTZ, *Wayne State University, Detroit, Michigan 48202.*

Electrolytic generation of chlorine dioxide, free from extraneous oxidants, is described. An indirect spectrophotometric method for the determination of trace quantities of chlorine dioxide in acidic solution is proposed. The method is based on measuring the diminution of absorbance of the tris-1,10-phenanthroline-iron(II) complex relative to a reagent blank by a differential spectrophotometric technique.

Microchem. J. **15,** 598 (1970).

Precipitation of Tellurium with Bismuthiol II. JUI-CHANG WANG AND K. L. CHENG, *Department of Chemistry, University of Missouri-Kansas City, Kansas City, Missouri 64110.*

A study has been made of the precipitation of tellurium with the organic reagent Bismuthiol II. The gravimetric factor is very favorable. The effect of other ions, time of precipitation, pH, etc., have been studied.

Microchem. J. **15,** 607 (1970).

Analytical Applications of Chelons. LVII. Chelometric Determination of Bismuth with DTPA and Reciprocally. F. BERMEJO-MARTINEZ AND J. A. RODRIGUEZ CAMPOS, *Department of Analytical Chemistry of High Council Scientific Research, Santiago de Compostela, Spain.*

Pyrocatechol violet is used as an indicator for the chelometric determination of bismuth with DTPA. Determinations were done in the range of 1 μg to 0.125 mg of material to be determined.

Microchem. J. **15,** 622 (1970).

Microcrystalloptic Tests for Some Lupine and Ormosia Alkaloids. K. GENEST AND LORNA J. LOWRY, *Research Laboratories, Food and Drug Directorate, Ottawa, Ontario, Canada.*

Microcrystal tests for lupanine, isolupanine, hydroxylupanine, epihydroxylupanine, angustifoline, ormosanine, ormojanine, and panamine are described. Microoptical data for the crystals are reported.

Microchem. J. **15,** 625 (1970).

Near-Infrared Spectrophotometric Determination of Germanium by Modified Heteropoly Blue Method. JOSEPH K. SAMUELS III AND D. F. BOLTZ, *Department of Chemistry, Wayne State University, Detroit, Michigan 48202.*

A near-infrared spectrophotometric method based on the initial formation of a stable form of molybdogermanic acid and the heteropoly blue reaction is proposed.

Microchem. J. **15,** 638 (1970).

Trace Metal Analysis by Combined Thin-Layer Chromatography Incorporating Fluorescent Support and Ring Oven Colorimetry. K. N. JOHRI AND HARISH C. MEHRA, *Department of Chemistry, University of Delhi, Delhi-7, India.*

Clean and speedy analytical separation of microgram quantities of Pb(II), Bi(III), U(VI), and Th(IV); and Cu(II), Ni(II), Co(II), and Cd(II) from their mixed solutions has been made possible by ascending thin-layer chromatography.

Microchem J. **15,** 642 (1970).

Suggested Procedure for Microdetermination of Arsenic in Arsenical Animal Feed. K. N. JOHRI, HARISH C. MEHRA, AND N. K. KAUSHIK, *Department of Chemistry, University of Delhi, Delhi-7, India.*

The ring oven is used for the microdetermination of arsenic as arsenite using potassium thiocarbonate. As little as 0.04 μg/μl is determined within 6–8% error.

Microchem. J. **15,** 649 (1970).

The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Lead (Mercury, Copper, or Silver) Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking. H. FLASCHKA, *School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332;* AND R. WEISS, *Chemistry Department, Humboldt State College, Arcata, California 95521.*

Zinc is determined photometrically as the PAN complex after being extracted with chloroform. Potassium iodide is added to the sample to mask the lead, as well as, cadmium, copper, mercury, and silver. Cyanide is used to mask nickel and cobalt. The results are reliable to several tenths of a microgram of zinc.

Microchem. J. **15,** 653 (1970).

Microchemical Urinalysis. VI. Determination of Sodium, Potassium, Calcium, and Magnesium by Atomic Absorption Spectrophotometry in the Microliter Range of Urine. BENJAMIN W. GRUNBAUM AND NELLO PACE, *Department of Physiology-Anatomy, University of California, Berkeley, California 94720.*

A procedure is described for the microdetermination of urinary sodium, potassium, calcium, and magnesium. The method utilizes a single diluted sample for multiple determinations of a number of additional elements. For the calcium and magnesium determinations, a combination lamp is used, which is time saving.

Microchem. J. **15,** 666 (1970).

Microchemical Urinalysis. VII. Determination of Citric Acid in Microliter Quantities of Urine. BENJAMIN W. GRUNBAUM AND NELLO PACE, *Department of Physiology-Anatomy, University of California, Berkeley, California 94720.*

Thiourea–borax solution is used in a spectrophotometric method with which reliable results are obtained in the 5- to 50-μg range.

Microchem. J. **15,** 673 (1970).

Photomicrographs of Metallo Amino Acid Complexes. DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705.*

Photomicrographs are shown of copper monoglutamate, copper diglutamate, copper methionine, and barium aspartate.

Microchem. J. **16,** 677 (1971).

Reliability of the Grunbaum Pipet as a Self-Cleaning Device. B. W. GRUNBAUM, *Department of Physiology-Anatomy, University of California, Berkeley, California 94720.*

Data are presented to show that the Grunbaum pipet is self-cleaning and as such is a useful analytical instrument. It contributes to speed and economy in volumetric quantitative measurement. It is also a precision pipet because the same pipet is used for aliquoting blanks, standards, and unknowns.

Microchem. J. **15,** 680 (1970).

An Improved Microinjection Apparatus for Biochemical Embryology. GEOFFREY GORDON AND GEORGE M. MALACINSKI, *Department of Zoology, Indiana University, Bloomington, Indiana 47401.*

The design characteristics of a new syringe control and micropipette holder are described. The large volume of the syringe control permits rapid control and the leak-free design of the pipette holder facilitates injection of large numbers of amphibian embryos. A test of the pipetting precision of the instrument is presented.

Microchem. J. **15,** 685 (1970).

MICROCHEMICAL JOURNAL 15, 519–530 (1970)

Dissimilar-Metal and "Titrant-Stream" Electrode Systems in the Potentiometric Titration of Millimolar Concentrations of Copper(II) in Dimethylformamide with Titanium(III)

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Although acid–base titrimetry in nonaqueous solvents is a common operation, oxidation–reduction (redox) titrimetry in such solvents has received little attention until comparatively recently (*1–4*). Of the solvents commonly employed in nonaqueous titrimetry, acetonitrile, either alone or admixed with acetic acid, has received the most attention as a medium for redox titrimetry. The utility of *N,N*-dimethylformamide (DMF) in redox titrimetry was first demonstrated by Hinton and Tomlinson (*5*). More recently, Hladky (*6, 7*) has used this solvent in the titration of organic thiocompounds with copper(II) or iron(III). These studies have been extended by Hladky and Vrestal (*8, 9*).

Most of the reported redox titrations have involved titrand concentrations of 0.01 *M* or greater. The aims of the present work were (i) to demonstrate that nonaqueous redox titrimetry is possible at millimolar or even lower titrand concentrations, and (ii) to study the utility of bimetallic "zero current" potentiometric electrode systems in such titrations. Such systems are simple and compact and do not involve the variable liquid-junction potentials that are sometimes encountered with reference electrodes of the usual type. The copper(II)–titanium(III) titration reaction in DMF was chosen for further study. In their experiments at an apparent titrand concentration of approximately 0.07 *M*, Hinton and Tomlinson (*5*) found that this reaction was stoichiometric and the titration was precise and accurate.

The utility of two dissimilar metal electrodes in aqueous redox titrimetry has long been known (*10, 11*) and the underlying theory has been examined (*12–14*). No such studies concerning nonaqueous redox titrimetry have been reported.

MATERIALS AND METHODS

Equipment. Emf measurements were made with a Leeds & Northrup model 7664 pH meter. Several meters were used when various electrode

systems in the same solution were being compared. The titration cell was a 100-ml tall-form beaker that was closed by a tightly-fitting cover. Electrodes, the jet of a 2-ml Gilmont microburet, and nitrogen inlet and escape tubes fitted snugly in holes in the cover. Stirring was by means of a Teflon-coated bar that was rotated magnetically.

Most of the electrodes were of wire form, with a wetted area of approximately 1 cm^2. Stainless steel electrodes were merely 22-gauge $\times$ 1.5 inch hypodermic needles (Becton, Dickinson & Co.). Platinum and stainless steel were also used as "buret electrodes" (*15*) or "titrant stream" reference electrodes that were mounted in the jet of the microburet as shown in Fig. 1. In the form shown at (a) 28-gauge platinum wire was sealed into the wall of a small bulb on the jet tube. To construct form (b), the jet tube was cleanly cut and the portions were then rejoined by sealing in a 12-mm length of 1-mm bore platinum tubing. A hypodermic needle cemented into a glass jet made from melting-point tubing, as shown at (c), was used as a third type of "titrant-stream" electrode. This type can be directly attached to a microburet with a standard Luer outlet. In all cases, connection to the metal electrode was made by a small alligator clip.

Reagents. Standard copper(II) chloride in DMF solution, approximately 0.01 *M*; prepared by weighing copper(II) chloride dihydrate (J. T. Baker Chemical Co.) that had been checked iodometrically. Stored in the dark under nitrogen. More dilute solutions that were used as titrands were prepared in small batches by accurate dilution with DMF (Matheson, Coleman and Bell, DX1730) under nitrogen.

Titanium(III) chloride in DMF solution, 0.1 to 0.2 *M*. Prepared in 25-ml batches in a nitrogen-filled glove bag from solid titanium(III) chloride (K. & K. Laboratories, Inc.) and stored under nitrogen.

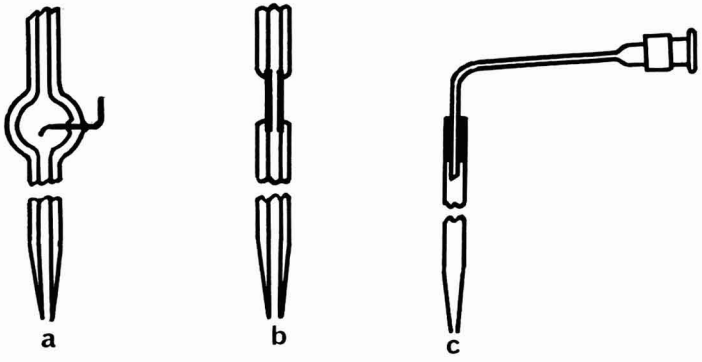

FIG. 1. Titrant stream electrodes: (a) platinum wire; (b) platinum tube; (c) stainless steel.

General procedure. Unless otherwise specified, one member of any electrode pair was a platinum wire that dipped into the solution in the titration cell. This platinum wire was connected to the "reference electrode" socket of the pH meter. After the dried cell had been assembled and flushed with nitrogen, copper(II) chloride in DMF solution was measured in. A nitrogen-flushed glove bag was used in the filling of the microburet with titanium(III) chloride solution. The microburet was then rapidly mounted on the cell cover so that the tip of the jet was just under the surface of the titrand and the titration was begun.

RESULTS AND DISCUSSION

Preliminary experiments gave promising results in the titration of copper(II) at a concentration of 1 m*M*. Unless otherwise specified, all of the reported titrations were carried out at this concentration of copper(II). Except in cases where the emf was obviously drifting rapidly, readings were taken within 1 minute after each addition of titrant.

Titrimetry with platinum–metal X electrode systems. Thirty different single metals and alloys were examined as counterelectrode to a platinum wire. The results are summarized in Table 1. In most cases, the end point was located on the falling portion of the titration curve. The end point signal given in Table 1 is the approximate change in emf in going from 1% before the end point to 1% beyond this point. End points taken at the peak of the curve are indicated by the suffix "P." The signal is then defined as the approximate peak height when measured from a line that represents the mean of the emf reading taken 2% before the end point and the reading taken 2% after the end point.

Gold, molybdenum, tantalum, titanium, tungsten, zirconium, and stainless steel are noteworthy in giving standard deviations of less than 1%. Typical peaked curves given by molybdenum and stainless steel are shown in Fig. 2, at A and B, respectively. The same titrant solution was used in the obtaining of these curves. In most other cases, a different batch of titrant was used for each set of runs. In the case of molybdenum, the peak is so abrupt that the end point can easily be missed.

In the first run made with the tungsten electrode, the emf rose to a peak after remaining at essentially zero during most of the titration, as shown by curve A, Fig. 3. The emf rise that caused the peak became successively smaller in subsequent runs, so that the peak finally changed into a point beyond which a sharp drop in emf occurred (curve B). Tantalum behaved in a generally similar manner.

An initial titration with the gold electrode gave curve C, Fig. 3. The peak became progressively smaller in subsequent runs and finally dis-

TABLE 1

END POINT CHARACTERISTICS IN THE TITRATION OF 1 m*M* COPPER(II) WITH TITANIUM(III)

Electrode system, platinum–metal X.

Metal X	End point signal (mV) [a]	No. of runs	SD (%)
Aluminum	120	4	7.4
Beryllium	340	5	10.3
Cadmium	380	1	—
Copper	180	8	1.59
Gold [b,f]	25	8	0.54
Hafnium [c]	150	5	2.9
Indium	600	2	2.8 [d]
Iron	270	8	1.82
Lead	240	2	4.0 [d]
Magnesium	—	1	—
Mercury [e]	400	2	10.8 [d]
Molybdenum	45(P)	7	0.85
Nickel	60	6	18.1
Niobium	100	2	19.1 [d]
Palladium	70 [f]	5	4.4
Rhodium	50	7	4.4
Silver	390	3	28.4
Tantalum [b]	90	5	0.80
Thallium	160	1	—
Tin	300	1	—
Titanium	200	8	0.36
Tungsten [b]	50	8	0.61
Vanadium	250	3	31.7
Zinc	360	1	—
Zirconium [c]	270	12	0.42
Alumel	100	2	101 [d]
Chromel P	60	3	52
Chromel X	50	2	11.4 [d]
Copel X	100	7	1.61
Stainless steel	90(P)	9	0.43

[a] See text.
[b] After preconditioning.
[c] Response sluggish.
[d] Average deviation.
[e] Pool approximately 10-mm diameter in J-tube.
[f] End point on rising portion of curve.

appeared. Although the disappearance is partially due to the decrease in the rise of emf from essentially zero, the main cause is the cessation of the postrise emf drop and its changeover to a small rate of rise (curve D, Fig. 3). The peak on curve C occurs well before the commencement of the rising portion of curve D, so that the standard deviation for this preliminary set of seven titrations was almost 10%.

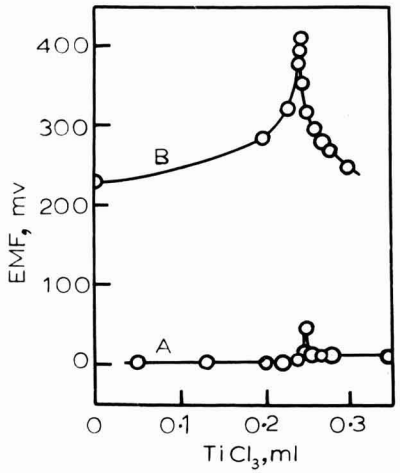

FIG. 2. Titration curves of 1 m*M* copper(II) with the platinum and (A) molydenum; (B) stainless steel electrode systems.

In an attempt to stabilize the gold electrode, it was immersed in 1 m*M* titrand solution and left overnight. This "conditioning" caused the dulling of the electrode surface. A further set of titrations was then made with the conditioned electrode. The first run gave a curve with a valley, as shown at E in Fig. 3. This valley diminished during the performance of subsequent titrations and had vanished completely by the end of the fifth titration. This gave a curve essentially similar to the curve obtained late in the previous set of titrations (curve D, Fig. 3). In subsequent titrations with the conditioned electrode, the rising portion of the curve (F, Fig. 3) showed a small inflection. However, the end point taken as indicated by the arrow was surprisingly precise (Table 1).

Titrations with the aid of a titanium electrode were characterized by an emf that started at approximately 450 mV and decreased only slightly during most of the titration. The emf then rose slightly before a very sharp drop, as shown by curve A, Fig. 4. In titrations with a zirconium electrode, the emf started at approximately 680 mV, rose progressively during the titration to approximately 850 mV, and fell abruptly, as shown by curve B, Fig. 4.

Titrations that were monitored by copper (480 mV), iron (500 mV), and Copel X (370 mV) electrodes exhibited standard deviations of between 1% and 2%. (The values in parentheses are the approximate emf readings at the beginning of titrations carried out with the respective electrodes.) These metals gave titration curves characterized by an emf which is quite high initially, and which rises to an obtuse peak before exhibiting a rapid drop. The curve obtained with a platinum–copper couple is shown at C in Fig. 4.

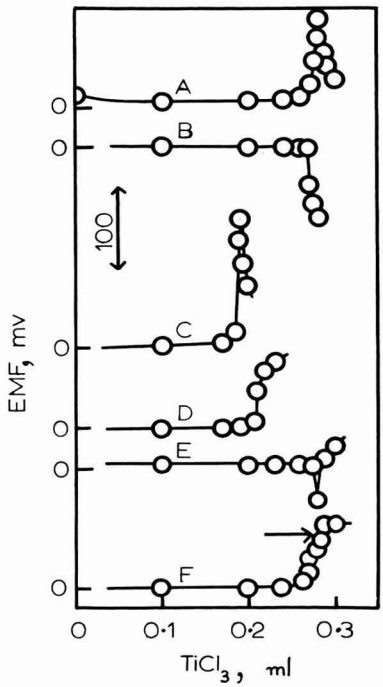

FIG. 3. Effect of electrode conditioning on the shape of the titration curves of 1 m*M* copper(II) obtained with platinum–metal X electrode systems: (A) X = tungsten, first titration; (B) tungsten, fifth titration; (C) X = gold, first titration; (D) gold, seventh titration; E gold, first titration after conditioning; F gold, eighth titration after conditioning. Different batches of titrant were used in the various sets of titrations.

All of the other electrodes gave unacceptably large standard deviations, or were rejected for other reasons. Copper visibly plated out on lead and zinc electrodes, which gave titration curves of form approximating that given by copper (curve C, Fig. 4). Surface effects were also observed on the submerged portions of the indium (700 mV), magnesium (>1400 mV), silver (250 mV), thallium (1000 mV), tin (550 mV), and vanadium (300 mV) electrodes. The silver surface became dull, while indium and magnesium were blackened. Thallium acquired a white deposit. The surfaces of tin and vanadium became dark grey and light grey, respectively. Magnesium gave a drifting emf and soon began to destroy the yellow color of the titrand. Titration curves roughly similar in general shape to that given by copper (curve C, Fig. 4) were obtained by use of the other five metals.

No deposits were observed on aluminum (900 mV), beryllium (900 mV), hafnium (200 mV), mercury (270 mV), nickel (350 mV),

niobium (150 mV), palladium (zero), rhodium (zero), Alumel (270 mV), Chromel P (300 mV), or Chromel X (200 mV). All except hafnium, palladium, and rhodium gave titration curves of shape roughly similar to that given by copper (curve C, Fig. 4). The curve given by hafnium rose to a quite sharp peak approximately 300 mV above the start of the curve and then dropped off as the titration was continued. In the case of palladium, the emf remained essentially zero during most of the titration and then underwent a sharp rise of approximately 120 mV. The behavior is similar to that of a gold electrode after use in several titrations (curve D, Fig. 3). Rhodium likewise gave an emf that remained at zero during most of the titration. This constant emf region was followed by an abrupt drop of approximately 50 mV. The behavior is similar to that exhibited by tungsten after use in several titrations (curve B, Fig. 3).

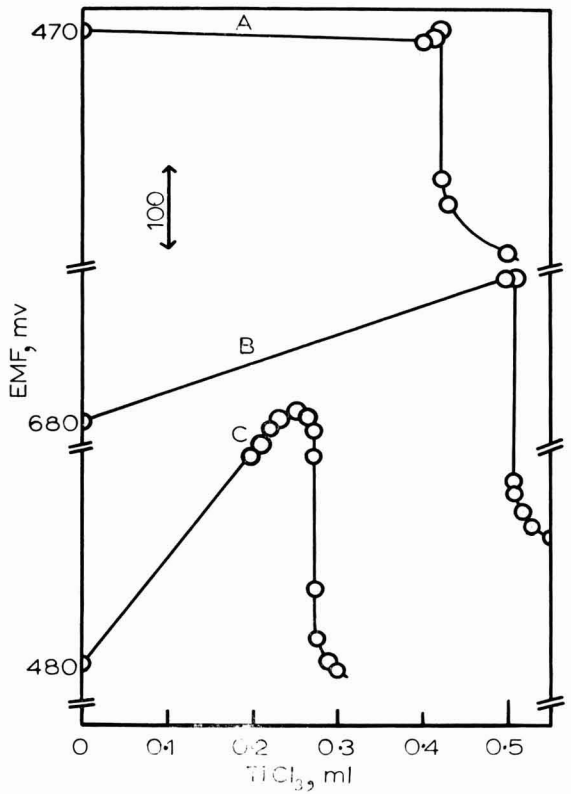

FIG. 4. Titration curves of 1 m*M* copper(II) obtained with platinum in conjunction with (A) titanium; (B) zirconium; (C) copper. These curves were obtained with differing batches of titrant.

Emf instability of certain platinum–metal X electrode systems. The emf drift with time of certain of the platinum–metal X couples that behaved poorly in titrimetry was examined. Each couple was immersed in a separate portion of 1 m*M* copper(II) chloride in DMF solution and the resulting emf was observed for periods of up to approximately 1 hour. Some of the emf–time curves are shown in Fig. 5.

With beryllium, the very rapid emf rise in the first 10 minutes was followed by a smoothly continued rise at a lower rate (curve A). The rapid initial rise caused by nickel was followed by a region of very erratic emf (curve B). This type of behavior was also shown by Alumel. An emf that first rose rapidly, remained roughly constant for approximately 10 minutes and then began a slow descent, was obtained with Chromel P and Chromel X. Indium behaved somewhat similarly, but

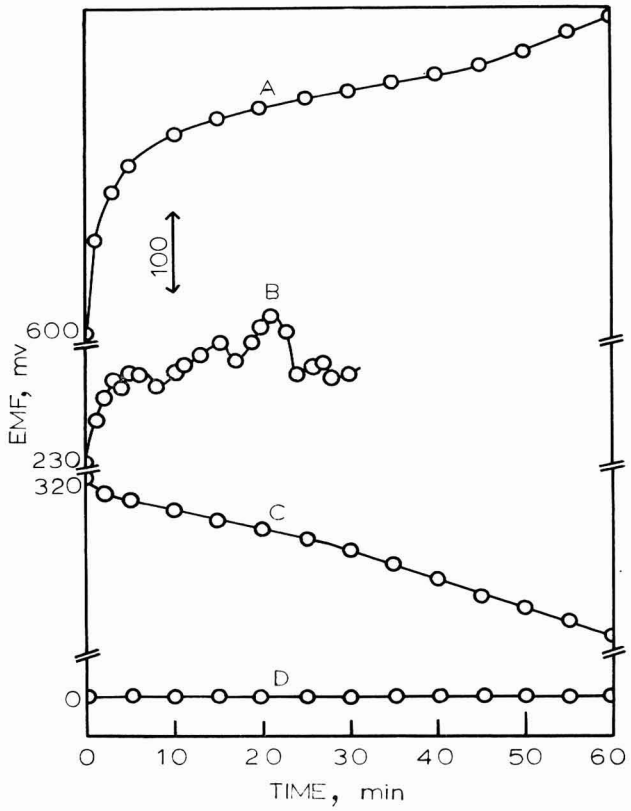

FIG. 5. Emf drift with time with platinum–metal X electrodes in 1m*M* copper-(II): (A) X = beryllium; (B) X = nickel; (C) X = vanadium; (D) X = rhodium.

the emf fall began almost immediately after completion of the initial emf rise.

The emf–time curve given by vanadium fell continuously and almost linearly (curve C). Mercury gave a similar but flatter curve; the emf fell by only 36 mV in 60 minutes. In the case of thallium, the emf dropped by more than 200 mV in the first 5 minutes, and then continued to fall at a lower rate. The general behavior of cadmium and of niobium was roughly intermediate between that of vanadium and thallium.

The emf exhibited by the platinum–rhodium couple remained at or near zero up to the termination of the observation (curve D). In this case, the unacceptably large standard deviation in titrimetry (Table 1) cannot be attributed to emf instability with respect to the titrand.

Effect of added water. The 1 m*M* titrand solutions used in most of this work contained approximately 0.1% w/v of water. To ascertain the effect of larger percentages of this contaminant, 1 m*M* titrand solutions containing up to 40% w/v of water were prepared and titrated by use of the platinum–titanium couple.

The most noticeable effect of added water was the marked decrease of the starting emf to values less than 100 mV. Although some distortion occurred, the titration curve retained its general outline (curve A, Fig. 4) with water concentrations as high as 20%. In the titration of the solution containing 40% of water, the starting emf was zero. This value remained essentially constant until the onset of a sudden *rise* to approximately 300 mV. Although end points could be located on all of the titration curves, these points gave positive errors (with respect to the titration of a solution containing no added water) that increased with the water content, as indicated in Table 2.

Table 2 also summarizes the results of similar titrations that were carried out with the platinum–stainless steel couple. Up to 10% of added water caused a rise in the starting emf, but the peak of the curve remained visible. The rapid postpeak fall of emf was retained with a water content as high as 20%. No end point indication was obtained in

TABLE 2

EFFECT OF ADDED WATER IN THE TITRATION OF 1 m*M* COPPER(II) WITH TITANIUM(III)

Added water (%):	2	10	20	40
Error (%) [a] (Pt–Ti)	+2.3	+8	+11	+23
(Pt–SS)	−1.1	+8	+14	—

[a] With respect to zero added water.

the titration of the solution containing 40% of water. During this titration, the emf first rose somewhat and then declined erratically.

Titrimetry with "titrant stream" reference electrodes. Hinton and Tomlinson (*5*) have shown that the precise and accurate titration of moderately high concentrations of copper(II) in DMF with titanium(III) is possible with the aid of the platinum (titrand)–platinum (titrant stream) electrode system. The utility of this system at much lower concentrations of copper(II) was, therefore, examined. Because of the useful performance of stainless steel in bimetallic dissimilar electrode titrimetry (Table 1), the behavior of this metal in "titrant-stream" titrimetry was also examined.

Seven titrations of 50.0-ml portions of 1 m*M* copper(II) were carried out with the platinum wire (titrant stream)–platinum (titrand) couple. The standard deviation was 1.89%. A similar set of 8 runs, in which the platinum tube (titrant stream)–platinum (titrand) couple was used, gave a standard deviation of 1.60%. Lower concentrations of copper(II) can be titrated, but less precisely. The standard deviation in the titration of 0.2 m*M* copper(II) was 22%. No end point could be detected when the concentration was lowered to 0.1 m*M*.

The composite titration curve A shown in Fig. 6 was obtained with a single platinum wire in the titrand. Two burets were used, the first of which carried a "titrant-stream" electrode of stainless steel and the second a "titrant-stream" electrode of platinum wire. The zeroing control of the pH meter used to measure the emf developed by the stainless steel (titrant stream)–platinum (titrand) couple was deliberately offset to bring the initial reading to that shown by the other meter that monitored the platinum (titrant stream)–platinum (titrand) emf. When the difference in initial emf is allowed for, the response of the two electrode couples is essentially identical.

Curve B, Fig. 6, is a composite obtained by use of the same two burets, but with a platinum wire electrode and a stainless steel electrode immersed in the titrand. In this case the emfs of platinum (titrant stream)–platinum (titrand) and stainless steel (titrant stream)–stainless steel (titrand) were measured. Before beginning the titration, the meter connected to the two stainless steel electrodes was adjusted so that its reading was the same as that shown on the other meter. As indicated by curve B, the two emf readings then remained essentially the same throughout the titration. In practice, "titrant-stream" titrations using either two stainless steel electrodes or one stainless steel electrode and one platinum electrode are somewhat less precise than titrations run with the aid of an all-platinum electrode system.

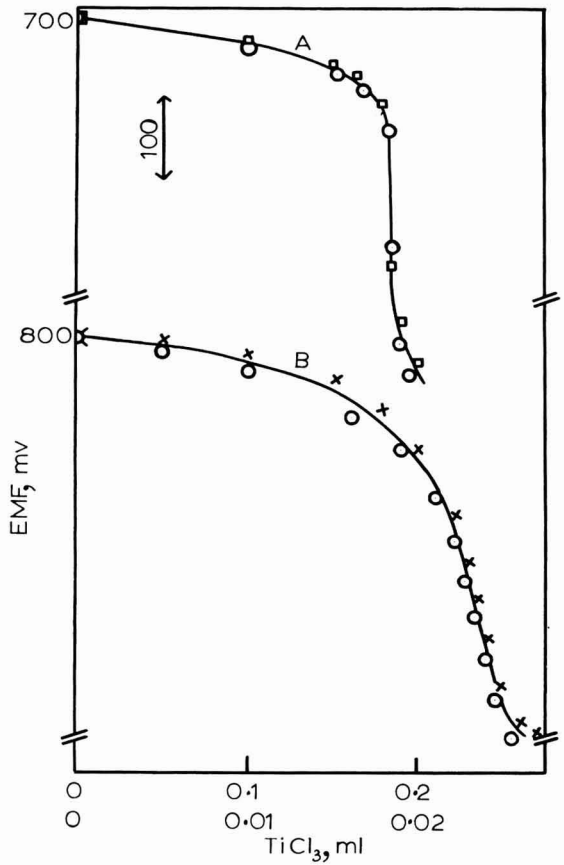

FIG. 6. Composite titration curves obtained by simultaneous use of two titrant-stream and one or two titrand electrodes: copper(II) concentration, 0.4 m*M*; (A) platinum wire (titrant stream)–platinum wire (titrand) (○—), and stainless steel (titrant stream)– platinum wire (titrand) (□—); (B) platinum wire (titrant stream)–platinum wire (titrand) (○—), and stainless steel (titrant stream)–stainless steel (titrand) (X–). Zero control of meter connected to stainless steel electrode(s) adjusted to bring initial reading to that of meter connected to platinum electrodes.

SUMMARY

Thirty platinum-nonplatinum electrode couples have been examined as possible end point indicators in the potentiometric titration of millimolar concentrations of copper(II) in *N*,*N*-dimethylformamide with titanium(III) in the same solvent. Molybdenum, titanium, or stainless steel gave standard deviations of less than 1%. If preconditioned, gold, tantalum, or tungsten gave similar standard deviations. Several other platinum–nonplatinum electrode couples are useful but less satisfactory. Titrations carried out with the platinum (titrant stream)–platinum

(titrand) couple gave a standard deviation of less than 2%. The stainless steel (titrant stream)–stainless steel (titrand) couple is also useful but is somewhat less precise.

ACKNOWLEDGMENT

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Ultramicroestimation of Human Serum Albumin: Binding of the Cationic Dye, 5,5′-Dibromo-o-cresolsulfonphthalein

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INTRODUCTION

The possibility that certain pH indicators might undergo reaction with solutions of protein was hinted indirectly 60 years ago when Soerensen (*9*) observed that a number of dyes were unsuitable for monitoring the acidity of biological specimens because of an unexpected change in color and therefore some compensation for this "protein error" had to be taken into account. Today an interest in the protein binding capacity of the sulfonphthalein dyes is being revived leading to the discovery that some of these dyes are highly selective, sensitive reagents for the measurement of albumin in human serum.

In a recent communication from this laboratory (*2*) it has been reported that the cationic form of 5,5′-dibromo-*o*-cresolsulfonphthalein (bromocresol purple) is (a) a specific direct reagent for the measurement of serum albumin, unaffected by the presence of abnormal quantities of gamma globulin, myelomatous globulins, lipids, sodium salicylate, and other compounds; and (b) an extremely sensitive reagent for the measurement of serum albumin as indicated by the increased spectral absorbance observed when a microgram quantity of serum albumin is subjected and compared to the milieu of three popular dye binding methods presently in use (*1, 5, 6*). These properties have led us to develop an ultramicromethod requiring only 10 μl of human serum, especially adaptable to infants and victims of severe skin lesions where large specimen quantities normally obtained by venipuncture must be avoided. We have found that variation in absorbance is less affected by a sodium acetate buffering system than phosphate buffer as used in the original method. A wetting agent has been employed also which extends the sensitivity and prevents turbidity in the reaction mixture.

EXPERIMENTAL METHODS

Equipment

1. A Beckman DU spectrophotometer equipped with micro absorption cells having a 1-cm light path and a volume capacity of 1.2 ml.
2. Disposable plastic test tubes with caps, 12 × 75 mm.

Reagents

1. Stock bromocresol purple reagent (0.01 M sodium bromocresol purple). Saturate about 10 g of technical bromocresol purple[1] with diethyl ether containing a small amount of activated charcoal. Filter the solution and allow the filtrate to crystallize in the refrigerator overnight. Dissolve 540 mg of dry recrystallized bromocresol purple in a 100-ml volumetric flask containing 9.9 ml of 0.1 *N* sodium hydroxide. This reagent is stable for several months at 4°C.

2. 25% v/v BRIJ. Pipet 2.5 ml of BRIJ 35[2] into a 10-ml volumetric flask and dilute to volume with water. This reagent is stable for at least one day at 4°C.

3. Working buffer (sodium acetate, pH 5.6, I = 0.050). Pipet 5.6 ml of 1 *M* acetic acid, 5.0 ml of 1.0 *M* sodium acetate, and 0.1 ml of 25% v/v BRIJ into a 100-ml volumetric flask and dilute to volume with water.

4. Buffered bromocresol purple reagent (0.05-I sodium acetate buffer, pH 5.6, containing 9.9 × 10⁻⁵ M sodium bromocresol purple). Add 1.0 ml of stock bromocresol purple to 100.0 ml of working buffer. This reagent is stable for at least 1 day at 4°C.

5. Standard human albumin solution (purified fraction V albumin containing about 5 g/dl as assayed by macro-Kjeldahl analysis). Dilute 0.5 ml of Mann assayed albumin 10%[3] with 0.5 ml of isotonic saline. This solution is stable for several months at 4°C.

Procedure

In a disposable plastic test tube (12 × 75 mm[4] labeled "test"), add 2.0 ml of buffered bromocresol purple to 0.010 ml of clear, centrifuged human serum. In a second tube (labeled "blank") add 2.0 ml of buffered bromocresol purple to 0.010 ml of isotonic saline. With each set of unknowns prepare a standard by adding 2.0 ml of buffered bromocresol purple to 0.010 ml of standard human albumin. Mix all tubes by buzzing on a vortex mixer. Transfer the solution to a micro absorption

[1] Obtainable from Fischer Scientific Co., King of Prussia, Pa.
[2] Obtainable from Atlas Chemical Co., Wilmington, Del.
[3] Mann Research Laboratories, Orangeburg, N.Y.
[4] Falcon Plastics, Los Angeles, Calif.

cell and measure the increase in absorbance by reading the test against the reagent blank set at zero absorbance at 605 mμ on the Beckman DU spectrophotometer. The absorbance difference is directly proportional to the albumin concentration up to at least 8 g/dl. If the test specimen appears visibly lipemic correction can be made by using a test blank. In this case add 0.010 ml of serum to a test tube (labeled "test blank") followed by 2.0 ml of working buffer. Read the test blank against working buffer set at zero absorbance at 605 mμ. The absorbance difference between the test and the test blank is the corrected absorbance for lipemic sera. Albumin in specimens which are grossly icteric is underestimated by this method and should be avoided or corrected for as described in a previous communication (*2*).

Calculation

$$\frac{A_u}{A_s} \times C_s = \text{g of albumin/dl of serum},$$

where A_u and A_s are the absorbance or corrected absorbance of the unknown and standard, respectively, and C_s is the concentration of albumin in the standard; the reaction mixture does not change its absorption for several hours when maintained at room temperature.

RESULTS

The effect of various buffers on the absorbance maxima of albumin bound bromocresol purple is illustrated in Fig. 1. Recrystallized human albumin[5] was used as the source with five common systems (i) sodium acetate, (ii) Tris acid maleate, (iii) sodium citrate, (iv) universal buffer, (v) phosphate. Although phosphate buffer yields the largest absorbance per microgram of albumin of any other buffering system tested, measurements in the acetate system appear to be less affected by small changes in pH at the plateau maximum with only slight diminution in the peak absorbance. Buffering capacity of sodium acetate is sufficient to withstand 4.8 μmoles of strong base, more than 20 times the quantity ordinarily found in 10 μl of serum, which is the amount of specimen used in this test.

Ness and co-workers (*6*) who investigated the binding of albumin with an azo-benzoic dye occasionally encountered turbidity in the presence of acetate buffer. While this has not been our experience with bromocresol purple we have attempted to safeguard against this situation by the addition of a wetting agent, BRIJ, at a concentration of 2.5 μl/ml of the reaction mixture. This solution has been used successfully by others

[5] Dade Products, Miami, Fla.

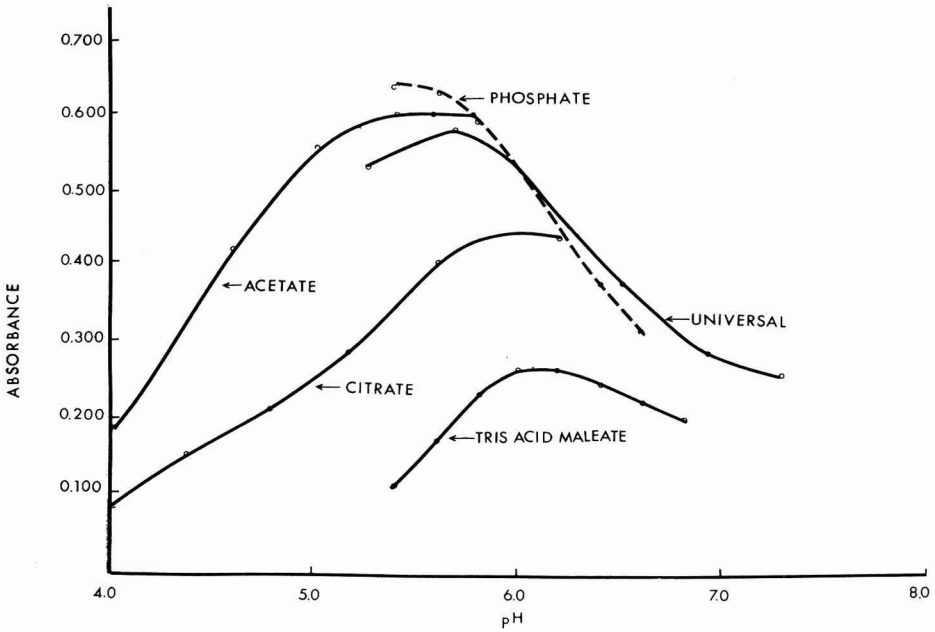

FIG. 1. Absorbance of human recrystallized albumin bound bromocresol purple as a function of pH at 605 mμ in a variety of buffers.

(*7*) as a clearing agent for turbidity observed when albumin is bound to bromocresol green. Figure 2 illustrates the gain in sensitivity obtained when wetting agent has been added to a buffered acetate solution of the dye.

A number of commercial sources of human albumin have been studied for suitability as standards with respect to their binding capacity with bromocresol purple. These compounds were analyzed for their protein content by microbiuret (*3*) and/or electrophoresis on cellulose acetate (*4*) as well as by proposed method. Mann assayed fraction V purified human albumin was selected as a primary standard because it is relatively inexpensive and has been used successfully to standardize other dye binding techniques (*8*). Hyland purified fraction V[6] human albumin as well as Dade[5] recrystallized human albumin are also proposed as acceptable standards for the ultramicroassay. Monitrol I,[5] a lyophylized human serum pool is a suitable secondary standard. Serial dilutions of the standards containing 100 to 800 μ/ml of the reaction mixture all showed a linear response to absorbance which was almost identical to the primary standard as indicated by the cluster of points around the straight line in Fig. 2, which exemplifies obedience to Beer's Law.

[6] Hyland Division, Travenol Lab., Los Angeles, Calif.

Convential techniques for the separation of albumin from other proteins depend upon the physical characteristics of the molecule, for example the solubility in a solution of high salt concentration (the salting-out effect) or the net charge on the molecule and its ability to migrate through an electric field (electrophoresis). Dye binding techniques rest on the principle that numerous repetitive chemical binding sites on the albumin molecule are available for potential combination with azo or

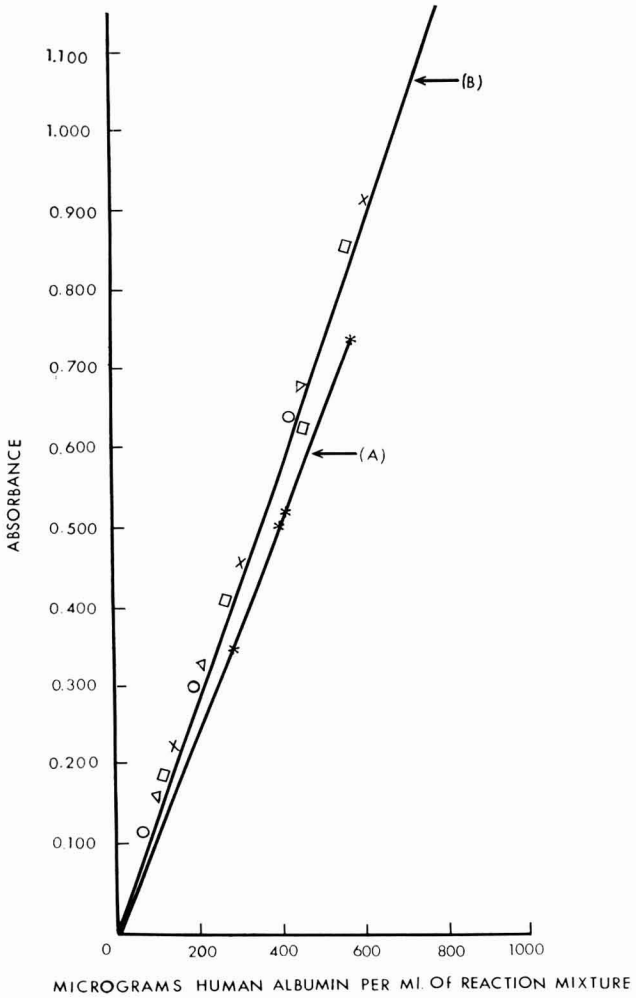

FIG. 2. The effect of commercial sources of human albumin on the binding of bromocresol purple as a function of spectral absorbance (A) Mann assayed fraction V *without* BRIJ; (B) *with* BRIJ; fraction V Mann (□); fraction V Hyland (×); recrystallized human albumin, Dade (○); reconstituted human serum pool, Monitrol I (▽).

TABLE 1

SERUM ALBUMIN VALUES OBTAINED BY CELLULOSE ACETATE ELECTROPHORESIS AND BROMOCRESOL PURPLE DYE BINDING FROM 9 "CLINICALLY HEALTHY" HOSPITAL PATIENTS

Specimen no.	Total protein (g/dl)	Albumin (g/dl)		Difference (g/dl)
		Elect.	BCP dye binding	
1	6.0	2.94	3.21	−0.27
2	7.4	3.85	3.99	−0.14
3	7.1	4.06	4.20	−0.14
4	7.7	4.08	4.33	−0.25
5	6.3	3.59	3.75	−0.16
6	7.7	3.24	3.48	−0.24
7	7.8	4.74	4.41	+0.33
8	7.1	3.98	4.16	−0.18
9	7.2	4.41	4.06	+0.35

sulfonphthalein dyes. Since it is tempting to believe that these sites may be altered or even destroyed in the disease state, we felt obliged to perform our method on a number of serum specimens obtained from "healthy" and "unhealthy" hospital patients and compare the results with a well established microelectrophoretic technique in which serum proteins are separated on a membrane coated with cellulose acetate in a carefully controlled pH buffer and quantitated after staining in a scanning densitometer (*4*). Total protein was determined by measuring the refractive index of the serum; wherever this was not possible due to the presence of turbidity or interfering chromogens a biuret method (*3*) was employed.

The data in Table 1 show the comparison between the reference and proposed technique when serum albumin from a group of "healthy" hospital patients was determined by both methods. These specimens had been screened previously for abnormal quantities of 12 chemical constituents[7] and found to be negative. The mean absolute difference in serum albumin concentration from this group is 0.23 g/dl, indicating that no appreciable clinical difference in the estimation of albumin by the two methods exists. Values obtained by the dye binding technique generally will be slightly higher than those obtained by electrophoresis. Data from 25 hospital patients who were being diagnosed and treated

[7] SMA 12/60 Analysis (Technicon Corp., Tarrytown, N.Y.), yields a chemical "profile" which includes determination in serum for (1) glutamic oxalacetic acid transaminase, (2) alkaline phosphatase, (3) lactic dehydrogenase, (4) cholesterol, (5) total bilirubin, (6) total protein, (7) albumin, (8) urea nitrogen, (9) glucose, (10) calcium, (11) phosphorus, and (12) uric acid.

for a variety of diseases is illustrated in Table 2. Medication had been administered to some patients during the period when serum specimens had been collected for analysis. All analyses were performed in duplicate within 4 days from the time they were received in the laboratory. Mean absolute difference in albumin concentration between the dye method and the reference method is 0.22 g/dl, which is almost identical to the value found for the "healthy" group with certain exceptional differences.

Specimens containing large quantities of conjugated bilirubin are underestimated by the dye technique (see specimen No. 7) whereas specimens containing free bilirubin have more binding sites available for combination with the dye (see specimen No. 8) and will yield low but clinically acceptable values in the presence of at least 20 mg of bilirubin/dl of serum. Other appreciable differences between specimens assayed by the dye and cellulose acetate electrophoresis method occur in the presence of metanephrine (see specimen No. 10) and alkaline phosphatase induced by cholecystitis (see specimen No. 6). Whether these disparities are directly due to competitive or additional reaction with foreign substances normally absent in healthy serum or are the effect of medication administered during therapy, it is difficult at this writing to determine. Figure 3 is a graphical representation of comparative data

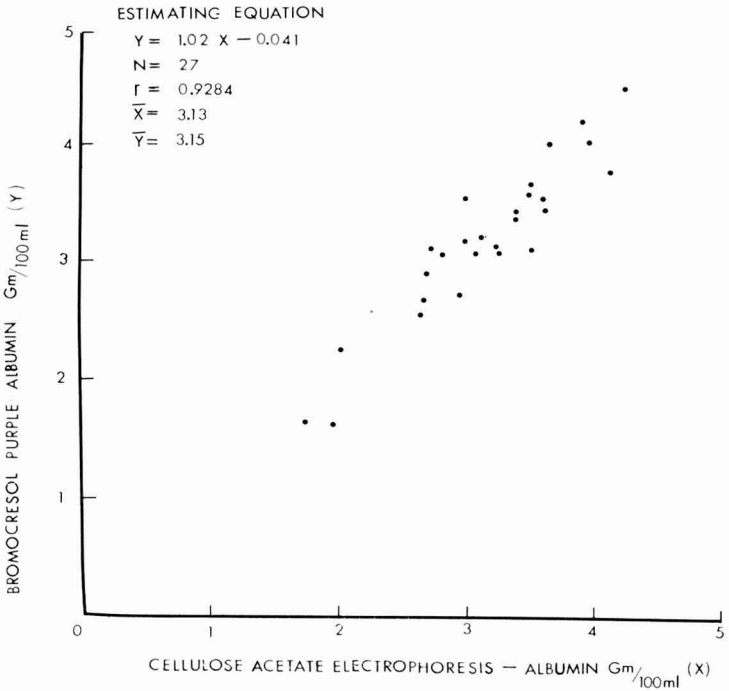

FIG. 3. Comparison of serum albumin concentration obtained by bromocresol purple measurement and cellulose acetate electrophoresis.

TABLE 2

SERUM ALBUMIN VALUES OBTAINED BY CELLULOSE ACETATE ELECTROPHORESIS AND BROMOCRESOL PURPLE DYE BINDING FROM 24 HOSPITAL PATIENTS WITH CLINICALLY ABNORMAL DIAGNOSIS

Spec. no. Remarks	Total protein (g/dl)	Albumin (g/dl)		Difference (g/dl)
		Elect.	BCP dye binding	
1. Elevated cholesterol, 385 mg/dl	7.57	4.25	4.45	−0.20
2. Elevated cholesterol, 355 mg/dl	7.17	3.92	3.92	0.00
3. Elevated α_2 globulin, 1.9 g/dl	7.96	3.10	3.20	−0.10
4. Myeloma γ globulin, 3.5 g/dl	8.00	3.03	3.16	−0.13
5. Lipemic serum, chylomicra, and elevated β lipoproteins present	7.90	4.13	3.76	+0.37
6. Cholecystitis; alk. phos., 63 IU	5.93	3.55	3.10	+0.45
7. Bleeding ulcer, bilirubin total, 32 g/dl; conjugated, 27 g/dl	6.07	1.98	1.56	+0.42
8. Versatol Pediatric,[a] 20 mg/dl free bilirubin	5.82	3.72	3.44	+0.28
9. Hydronephrosis; urea nitrogen, 120 mg/dl	6.55	2.80	3.07	−0.27
10. Pheochromocytoma; elevated metanephrine	6.93	2.94	3.54	−0.60
11. Diabetes mellitus; glucose, 321 mg/dl	5.59	2.66	2.87	−0.21
12. Congestive failure; glucose, 246 mg/dl	5.40	2.05	2.23	−0.18
13. Elevated uric acid, 10.1 mg/dl	7.26	3.50	3.73	−0.23
14. Depressed uric acid, 2.2 mg/dl	7.30	3.50	3.66	−0.16
15. Ca of prostate; acid phosphatase, 320 IU	6.82	2.94	2.63	+0.31
16. Elevated alkaline phosphatase, 207 IU	6.45	2.62	2.52	+0.10
17. Elevated glucose, 474 mg/dl	6.30	3.08	3.00	+0.08
18. Elevated urea nitrogen, 72 mg/dl	7.4	2.64	2.60	+0.04
19. Aortic stenosis	7.0	3.65	3.97	+0.32
20. Metastatic cancer of breast	6.8	3.25	3.04	+0.21
21. Myocardial ischemia	9.2	3.90	4.13	−0.23
22. Glucose, 414 mg/dl	5.0	1.79	1.63	+0.16
23. Glomerulonephritis	7.5	3.36	3.45	−0.09
24. Chronic leukemia	5.3	3.20	3.06	+0.14
25. Bronchogenic cancer	6.67	2.78	3.08	−0.30
26. Monitrol II [a]	5.48	2.75	2.76	−0.01
27. Hyland Special [a]	5.96	3.47	3.50	−0.03

[a] Reconstituted lypohylized pooled serum containing assayed constituents at the abnormal level; Versatol Pediatric, Warner-Chilcott, Morris Plains, N. J.; Monitrol II, Dade, Miami, Fla.; Hyland Special, Travenol Lab., Los Angeles, Calif.

illustrated in Table 1 indicating a high degree of correlation between the two distinctively different techniques.

Precision of the ultramicrotechnique was determined by analyzing 10 specimens in duplicate. The coefficient of variation is 3.64% and is somewhat larger than the value found by the micro method (*2*) but lies well within the range of acceptance for analytical procedures.

SUMMARY

An ultramicrotechnique for estimating human albumin directly in a buffered sulfonphthalein dye solution is described. The method which is extremely sensitive requires only 10 μl of specimen and can be accomplished with equipment generally available in most routine clinical chemistry laboratories. The integrity of the method is exemplified in the high degree of correlation to values obtained by electrophoresis on cellulose acetate.

ACKNOWLEDGMENTS

Technical assistance of Peter Hsu and Kathryn Adams is gratefully acknowledged.

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MICROCHEMICAL JOURNAL 15, 540–544 (1970)

Spectrophotometric Determination of Copper(II) with β-Benzoyl-α-pyridyl Thiourea

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INTRODUCTION

Many organic reagents containing sulfur and nitrogen in the functional groups have been found to be good spectrophotometric reagents for copper(II) (*5*). Copper(II) ion can act both as a soft and hard acid and it has a tendency to form strong complexes with soft bases like sulfur ligands as well as hard bases like nitrogen ligands (*1, 4*). In this paper is reported the use of a new thiourea derivative for the spectrophotometric determination of copper(II). This thiourea derivative, β-benzoyl-α-pyridyl thiourea, has not been previously explored as an analytical reagent. Combined with copper it produced a very stable orange-yellow color, the intensity of which remained unchanged even after 24 hours of its formation. The pH range for maximum color intensity was 2 to 6 and the system obeyed Beer's law over the range of 2 to 20 ppm at the wavelength of maximum absorption, 430 mμ. Copper can be determined in the presence of a large number of diverse ions.

EXPERIMENTAL METHODS

Apparatus and reagents. A Unicam SP 600 spectrophotometer was used with 10-mm Corex glass cells for absorbance measurements. A Cambridge pH indicator was used for all the pH determinations.

A standard solution of copper(II) was prepared by dissolving reagent grade cupric sulfate in doubly distilled water containing few drops of sulfuric acid and estimated by usual methods. A stock solution of 0.1 mg of copper/ml was prepared by proper dilution.

Standard solutions of other cations were prepared from their chlorides, nitrates, or sulfates; and of the anions from ammonium or sodium salts.

The reagent, β-benzoyl-α-pyridyl thiourea, was prepared according to the method described by Douglass and Dains (*2*). A 1% (w/v) solution of the reagent in 95% ethanol was used.

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All chemicals used were reagent grade quality.

General procedure. An aliquot of the copper solution was transferred to a small beaker, 4 ml of the 1% reagent solution was added and the mixture was diluted to about 15 ml. The pH of the solution was adjusted within 2 to 6 and the solution was transferred to a 25-ml volumetric flask, rinsing and diluting to the mark with 95% ethanol. The absorbance was measured against a reagent blank.

RESULTS AND DISCUSSION

Absorbance curve. The absorption spectrum of a copper-complex solution containing 8 ppm copper is shown in Fig. 1. The complex was found to have a maximum absorption at 430 mμ and all other measurements were made at this wavelength.

Effect of pH, reagent concentration, and time. A mixture of definite quantity of copper solution and 4 ml of 1% reagent solution was diluted to about 15 ml in a beaker and the pH was adjusted with the help of a pH meter. The absorbance of the solution was measured at 430 mμ against a reagent blank after diluting it to proper volume. The absorbance of the complex was constant in the pH range 2 to 6 and decreased rapidly outside this range. For all other studies, the pH was adjusted by using pH indicator paper.

The effect of reagent concentration on the color of the complex, formed with 8 ppm of copper and different amounts of reagent varying from 0.5 to 4 ml reagent, was studied within the pH range 2 to 6. It was found that 1 ml of 1% reagent was sufficient for color development and excess reagent had no adverse effect.

The color development was instantaneous and the absorbance remained constant for at least 24 hours.

Beer's law, sensitivity and molar absorptivity. Beer's law was obeyed by the colored system from 2 to 20 ppm when 4 ml of the reagent solution was used. Spectrophotometric sensitivity, calculated by Sandell's method (*5*) was found to be 0.02 μg of Cu/cm^2 and the molar absorptivity was 3098.

Composition of the complex. The composition of the colored complex was determined by Job's method of continuous variation (*3*) and the mole ratio method (*6*).

For continuous variation method, equimolar solutions ($2.5 \times 10^{-3}\ M$) of copper and reagent were mixed in different proportions (12 ml of total mixture). The pH of the mixture was adjusted by pH meter or pH indicator paper and then diluted to 25 ml in a volumetric flask. At lower reagent concentrations the color development was slow. Therefore, all color measurements were made 1.5 hours after the time of mixing.

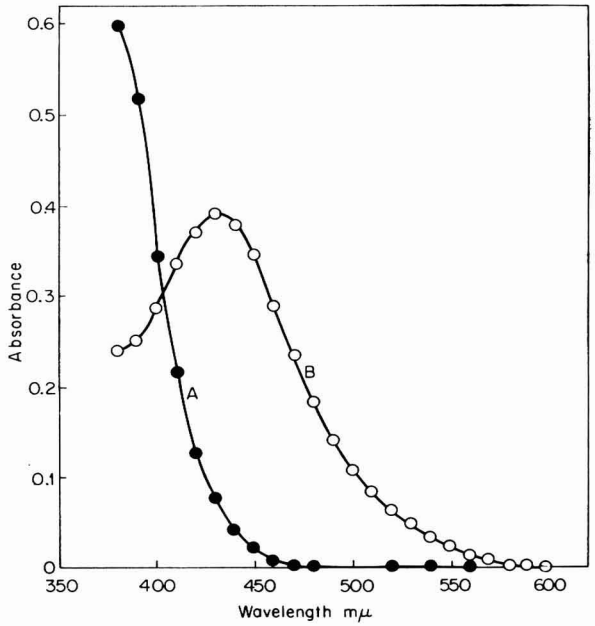
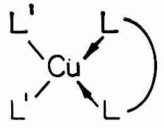


FIG. 1. Absorption spectra of β-benzoyl-α-pyridyl thiourea and its copper(II) complex; (A) Reagent (6.2×10^{-3} M) vs water; (B) copper complex (8 ppm Cu) vs a reagent blank.

The data, presented in Fig. 2, indicated that the metal and reagent combined in the ratio of 1:3. The composition determined at two other concentrations namely 1×10^{-3} and 1.5×10^{-3} M also indicated the presence of a 1:3 complex in solution.

For the mole ratio method, equimolar solutions (2.5×10^{-3} M) of the metal and the reagent were mixed in varying proportions. After adjustment of pH within the optimum range, the mixtures were diluted to 25 ml and allowed to stand for 1.5 hours and the absorbances were measured at 430 mμ. The plot (Fig. 3) again showed the formation of a 1:3 (Cu:reagent) complex. An explanation for the formation of 1:3 complex is not readily available but one possibility is the presence of the complex of the type



where L and L′ are nitrogen and sulfur ends of the reagent, respectively.

Stability constant. The conditional stability constant of the complex was calculated from the absorbance data by two methods: (a) mole

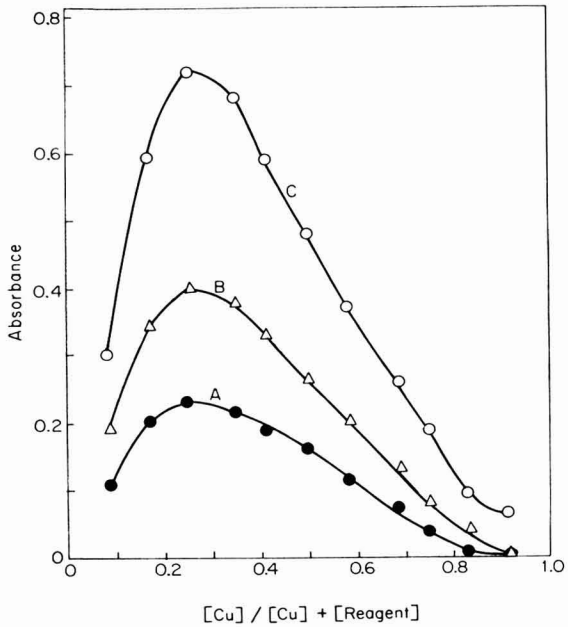


FIG. 2. Job's method of composition: (A) copper and reagent: 1.0×10^{-3} *M*; (B) copper and reagent: 1.5×10^{-3} *M*; (C) copper and reagent: 2.5×10^{-3} *M*.

ratio method, and (b) method of continuous variations using non-equimolar solutions, and was found to be 7.75×10^{11} and 2.02×10^{11}, respectively.

The complex could be extracted into a number of organic solvents including benzene, isopropyl ether, amyl acetate, methyl isobutyl ketone, and chloroform. The absorbance decreased in all solvents except chloroform and the wavelength of maximum absorption shifted to a lower value. When reagent grade chloroform (further purified by washing

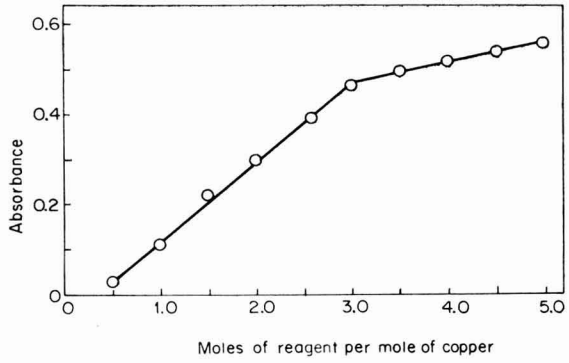


FIG. 3. Mole ratio method of composition: copper and reagent: 2.5×10^{-3} *M*.

with sulfuric acid and then repeatedly with water) was used for extraction, the color intensity increased even slightly above that in aqueous-alcoholic solution, but the absorption maximum was shifted to 395 mμ. Although the color was stable for 45 minutes and obeyed Beer's law from 2 to 16 ppm, the measurements were not further carried out at that wavelength because of the possibilities of more interferences at shorter wavelengths. On the other hand the color system in aqueous-alcoholic medium is stable and highly reproducible.

Effect of diverse ions. All experiments on the effect of diverse ions were carried out with 8 ppm copper. The ions Cl^-, Br^-, I^-, SO_4^{2-}, NO_3^-, ClO_4^- (all in large excess), Cd^{2+}, Be^{2+}, Cr^{3+}, Th^{4+}, UO_2^{2+} (all in 100-fold excess), Mn^{2+}, Zn^{2+} (both in 50-fold excess) did not interfere. The ions Al^{3+}, Fe^{2+}, Fe^{3+}, rare earth metal(III) ions, Ti^{4+}, Zr^{4+}, As^{3+}, Sb^{3+}, Co^{2+}, Ni^{2+} when present in the ratio of 1:1 or less did not interfere. The ions Hg^{2+}, Pb^{2+}, Bi^{3+}, Ag^+, Au^{3+}, Sn^{2+}, Sn^{4+}, WO_4^{2-}, MoO_4^{2-}, VO_3^- interfered and must be removed before the analysis. Acetate, citrate, tartrate, and fluoride tended to slow down the color development and reduced the absorbance slightly. EDTA and cyanide interfered.

SUMMARY

A method for the determination of copper(II) with a new spectrophotometric reagent β-benzoyl-α-pyridyl thiourea is described. The conditional stability constant and the composition of the complex are reported. The color development was found to be completed within the pH range 2 to 6. Copper can be determined in presence of many foreign ions.

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MICROCHEMICAL JOURNAL 15, 545–547 (1970)

Application of New Fuchsine for Quantitative Determination of Organic Substances in Thin-Layer Chromatography

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The problem of quantitative determination of chemical compounds analyzed by thin-layer chromatography is analogous in technique as in tissue-paper chromatography. Quantitative determination of substances distributed on a thin layer can be carried out as follows:

(a) Direct way, e.g., by estimating the relation between the number of micrograms of the analyzed substance and the size of the surface of the chromatographic spot, or by densitometric measurement, i.e., measuring the absorption of light penetrating the chromatogram;

(b) Indirect way, by eluting out the substance from the carrier and determining it by another technique, i.e., the colorimetric, polarographic, etc.

An essential matter in quantitative determinations carried out by means of thin-layer chromatography is the application of an appropriate developing reagent to ensure obtaining durable chromatographic spots, having sharp outlines.

In this work the authors did research on quantitative determination of substances, developed with fuchsine dyes, based on the relation between the number micrograms of substances analyzed and the size of the surface of the chromatographic spot.

Quantitative determinations were made for selected substances which were detected by means of new fuchsine as well as basic fuchsine. Quantitative determinations were made on cholesterol, stearic acid, and stearil alcohol.

EXPERIMENTAL PART

The chromatographic analysis was carried out under the following conditions: Kieselgel G in a 0.5-mm thick layer was used as adsorbent and activated for 45 minutes in a temperature of 110°C. Mobile phase: benzene plus methanol, 9:1. Water solutions (0.005%) of new fuchsine were used for developing.

TABLE 1

QUANTITATIVE DETERMINATION OF CHOLESTEROL BY MEANS OF PLANIMETRICAL TECHNICS

Quantity of cholesterol (μg)	No. of determinations	Surface of a spot (mm^2) X	SD (mm^2) S	Relative SD ($S \times 100/X$)	Dispersion of the spot surfaces (mm^2)
10	20	43	2.26	5.26	38–47
20	20	54	2.13	3.94	50–57
30	20	63	2.67	4.24	60–69
40	20	71.5	1.63	2.28	70–76
50	8	83	2.17	2.61	80–86

The cholesterol analyzed had a R_f value equal to 0.53. Determination of cholesterol was based on the relation between the size of the surface of the chromatographic spot and the number of the substance having been chromatographed Table 1.

The surface sizes of chromatographic spots for identical numbers of micrograms of the substance determined are included in a definite interval (Figs. 1 and 2). There was a direct relation between the size of the chromatographic spot and the number of micrograms of analyzed cholesterol in a concentration range of 10 to 50 μg. A substantial difference exists between the detectivity minimum of a substance on the

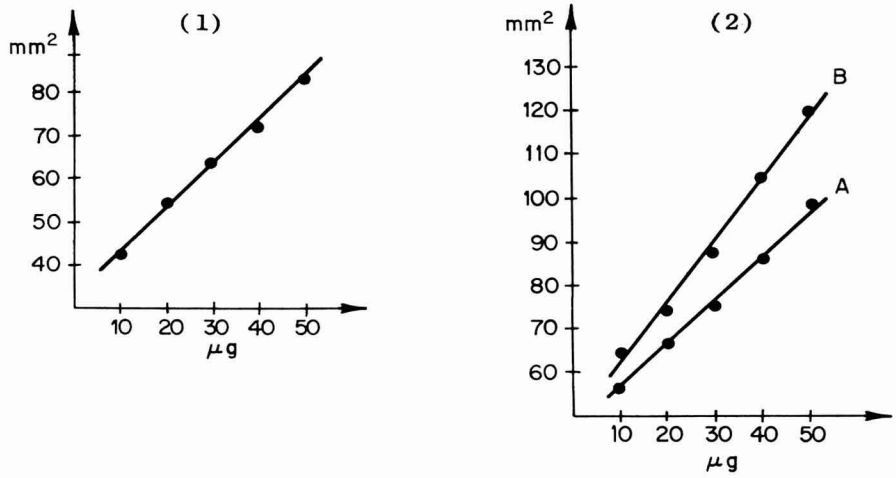

FIG. 1. Relation of the spot surface area to the number of micrograms of cholesterol analyzed.

FIG. 2. Relation of the spot surface area to the number of micrograms of analyzed stearil alcohol (A) and stearic acid (B).

carrier and the determinability minimum when applying planimetric technique.

Cholesterol was detected in a quantity of 2 μg, where as the aforesaid substance can be quantitatively determined by planimetric technique in a range of 10 to 50 μg. For the chromatographic conditions worked out, a direct relation between the surface area of the chromatographic spot and the number of micrograms of the substance tested has been stated.

The quantitative determinations were worked out for alcohol and stearic acid.

Stearic acid and stearil alcohol were detected on a thin layer in a quantity of 3 μg, however, the aforesaid substances can be quantitatively determined in a range of 10 to 50 μg.

The examples of quantitative determinations carried out have proved, that the detectability minimum is far smaller than the determinability minimum of the aforesaid substances. New fuchsine as developing reagent can be applied not only for the purpose of qualitative detection, but also for quantitative determinations of substances like higher aliphatic monohydrooxygen alcohols, higher aliphatic monocarboxyl acids, cholesterol esters, phthalic acid esters, barbiturans, naphthalene derivatives. These are substances which can be detected by means of fuchsine dyes (*1*).

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MICROCHEMICAL JOURNAL 15,548–556 (1970)

Composition and Stability Constants of Cerium–Chromotrope 2R Chelate and the Spectrophotometric Determination of Cerium

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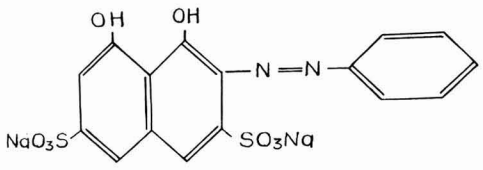
4,5-dihydroxy-3-phenylazo-2,7-naphthalene disulfonic acid di-Na Salt, commonly known as chromotrope 2R (CTR) which is designated by the following structure,



has been found to form colored chelates with various inorganic ions (*6*). In an earlier publication the formation of 1:1 (metal:ligand) chelates with scandium, yttrium (*1*), praseodymium, neodymium, samarium, and europium (*7*) have been reported from these laboratories. This communication reports the results on the composition, stability, and other characteristics of the chelate, formed between CTR and cerium. The application of this dye has also been reported for the colorimetric determination of cerium.

EXPERIMENTAL METHODS

Instruments

Beckman model B spectrophotometer was used for absorbance measurements. It was operated on 110 volts AC mains stabilized by a constant voltage transformer. All absorbance measurements were noted against distilled water blank using 1-cm glass cells.

The hydrogen ion concentration of the solutions was measured on a Toshniwal (Type CL41) pH meter, standardized with the pH solu-

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tions supplied along with the instrument. The electrodes used were combined glass calomel type.

Reagents

Solution of cerium chloride. Standard solution of cerium was prepared by dissolving cerium chloride in distilled water containing dilute hydrochloric acid. It was standardized by the usual method and then diluted suitably to obtain solutions of different concentrations.

Solutions of the ligand. Standard solution of chromotrope 2R was prepared by dissolving a spot-test reagent in distilled water. Every time freshly prepared solutions were used.

Solutions of other cation and anions. Standard solutions of various cations and anions were prepared either by direct weighing or by standardization. All reagents used were of AnalaR grade.

Conditions of Study

All experiments were carried out at room temperature ranging from 28–30°C. The pH of the solution was adjusted by addition of hydrochloric acid or sodium hydroxide, keeping the total volume 25 ml. in each case. The observations were recorded at 590 and 600 mμ and not at the λ_{max} of the chelate because the difference in absorbances of the chelate and dye was very small.

RESULTS AND DISCUSSION

Effect of time on the color of the chelate. The color formation of the chelate was found to be instantaneous and the absorbance value remains constant even after 12 hours, a stability quite adequate for spectrophotometric determination. However, the solutions were kept for 1 hour before taking the readings so that the equilibrium conditions of the mixture were reached.

Order of addition of the reagent. No significant change was observed to occur when the order of addition of reagent was altered. In the present work, however, the metal solution was added to the reagent.

Nature of the complexes formed. The method of Vosburgh and Cooper (*8*) was employed to determine the nature of the complexes formed in solution between cerium and CTR. Observations show that the maximum absorption of the reagent lies at 530 mμ, whereas that of the mixture at 550 mμ at pH 6. From this it is evident that only one complex having its λ_{max} 550 mμ is formed under the conditions of study (Fig. 1).

Effect of pH on the stability of the chelate. Several mixtures containing 0.5×10^{-4} *M* CTR and cerium in 1:1 equimolar proportion

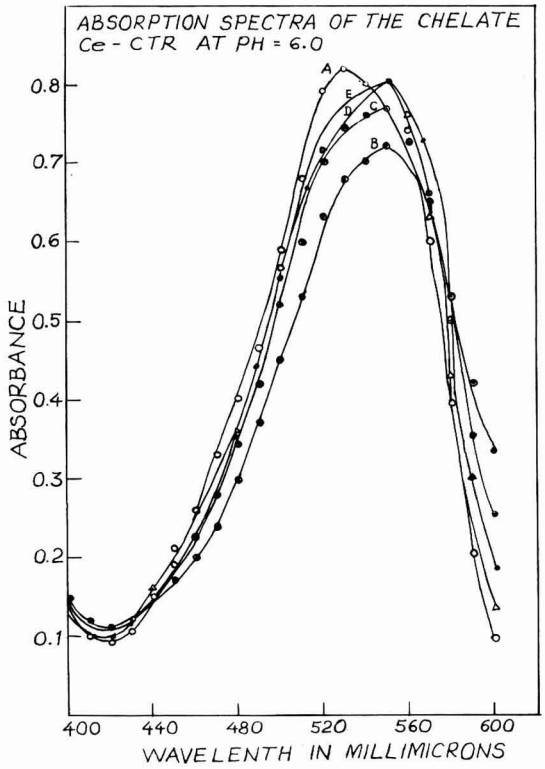

FIG. 1. Absorption spectra of the Cerium–CTR chelate at pH 6.0: concentratoin of the reagents (M): (A) CTR, 0.5×10^{-4}; (B) CTR, 0.5×10^{-4}; $CeCl_3$, 1.0×10^{-4}; (C) CTR, 0.5×10^{-4}; $CelCl_3$, 0.5×10^{-4}; (D) CTR, 0.5×10^{-4}; $CeCl_3$, 0.25×10^{-4}; and (E) CTR 0.5×10^{-4}; $CeCl_3$, 0.167×10^{-4}.

were prepared at different pH, and their absorption spectra were studied from 400 to 600 mμ. It was found that λ_{max} of the chelate, i.e., 550 mμ holds good between pH 5.1–6.7 showing that the chelate is stable within this pH range (Fig. 2).

Stoichiometry of the components. The ratio of the metal:ligand has been established by two different methods, (i) the method of continuous variations (*2*), and (ii) the mole ratio method (*9*). In both the methods the absorbances were noted at 590 and 600 mμ. The results of both the methods show that the stoichiometric ratios of the metal and CTR are 1:1 (Figs. 3 and 4).

Evaluation of the conditional stability constant. The evaluation of the stability constant offers a better understanding of chelate forming reactions. The stability constants are concerned with the reaction between different solvated reactants and products. From a precise knowledge of the stability constants, thermodynamic constants may be evaluated.

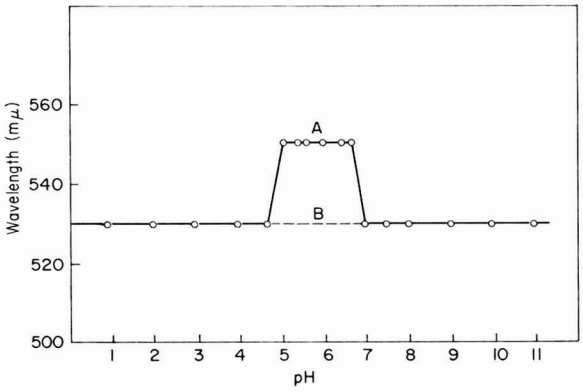

FIG. 2. Variation of λ_{max} of Ce–CTR chelate with pH: final concentration of the reagents, 0.5×10^{-4} *M*; (A) CTR; and (B) Ce–CTR chelate.

However, the method is accompanied by many difficulties and it is doubtful whether the thermodynamic quantities of chelation can be determined except in very simple cases.

The value of stoichiometric constants are reliable under a given set of experimental conditions and are useful for practical purposes. In the present study the constants determined are those obtained at fixed temperature and pH as mentioned. This constant has been termed con-

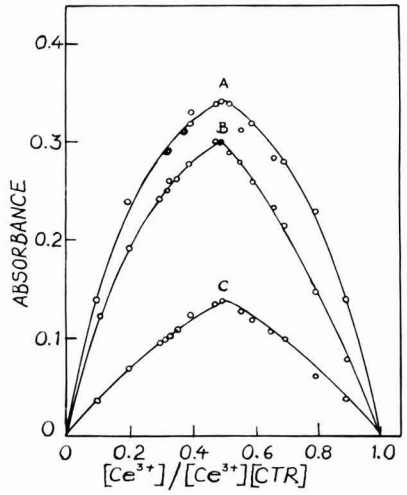

FIG. 3. Composition of Ce–CTR chelate by the method of continuous variation at pH 6.0 and at 600 mμ: concentration of the reagents (*M*): (A) 1.33×10^{-4}; (B) 1.00×10^{-4}; and (C) 0.66×10^{-4}.

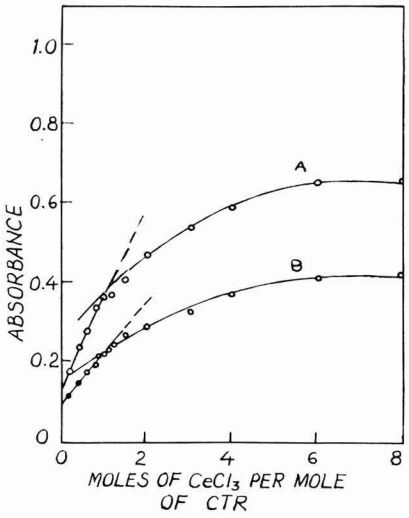

FIG. 4. Determination of the composition of Ce–CTR chelate by moleratio method at pH 6.0 and at 600 mμ: final concentration of CTR (A) 0.66×10^{-4} *M*; and (B) 0.50×10^{-4} M.

ditional stability constant in the present work. The values were determined by two different methods, i.e., the method of Mukherji and Dey (*3*) and the mole ratio method (*9*). The free energy change of formation of the chelate has also been calculated with the help of the following expression,

$$\Delta F^0 = -RT \ln K,$$

the terms having their usual importance. The results of stability constant and free energy change of formation of the chelate, are shown in Table 1.

Tentative suggestion on the structure of the chelate. The study of the chelate in the ion-exchange resins show that the chelate is anionic in character and thus is absorbed completely by the anion exchange resin [Amberlite IR 40 (OH)]. On this basis position of the chelation in the reagent can be shown by the following structure.

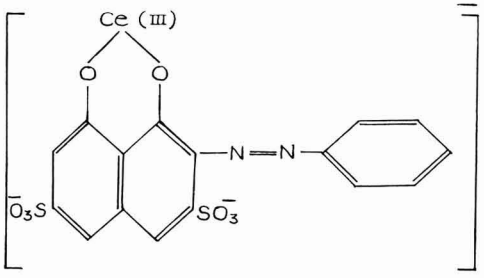

TABLE 1

STABILITY CONSTANT AND FREE ENERGY CHANGE OF Ce–CTR CHELATE AT pH 6.0

Method	Log K at 28°	ΔF^0 at 28° (kcal)
Mukherji and Dey	3.8 ± 0	−5.2
Mole ratio	4.8 ± 0.1	−6.6

Colorimetric Determination of Cerium

Beer's Law. Varying quantities (1, 2, 3 . . . 8, 9, 10 ml) of cerium chloride were added to an excess of the reagent and the volume was raised to 25 ml (pH maintained at 6.0). The color intensity was measured with Beckman model B spectrophotometer at 590 and 600 mμ. It was found that the system adheres to Beer's Law in the range from 0.56 to 10.16 ppm of cerium (Fig. 5).

Ringbom's curve (*4*) as obtained by plotting absorbance at 590 mμ against log concentration (Fig. 6) reveal that the optimum concentration ranges where the curve has the steepest slope is 2.18 to 12.92 ppm of cerium with CTR.

Influence of pH on absorbance. A series of mixtures containing CTR and metal ion in the ratio of 1:1 were prepared at different pH values.

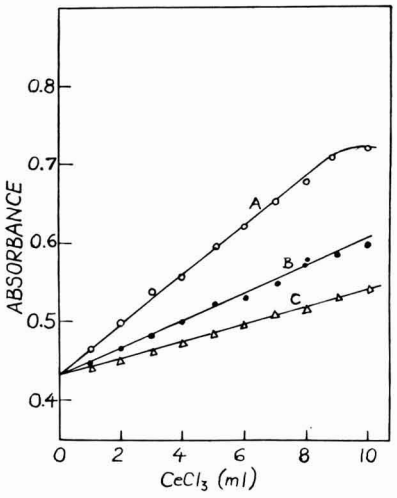

FIG. 5. Adherence to Beer's Law: (A) 5 ml of 0.5 × 10^{-3} M CTR + x ml of 0.2 × 10^{-3} M $CeCl_3$ + (20 − x) ml of H_2O; (B) 5 ml of 0.5 × 10^{-3} M CTR + x ml of 0.125 × 10^{-3} M $CeCl_3$ + (20 − x) ml of H_2O; and (C) 5 ml of 0.5 × 10^{-3} M CTR + x ml of 0.1 × 10^{-3} M $CeCl_3$ + (20 − x) ml of H_2O.

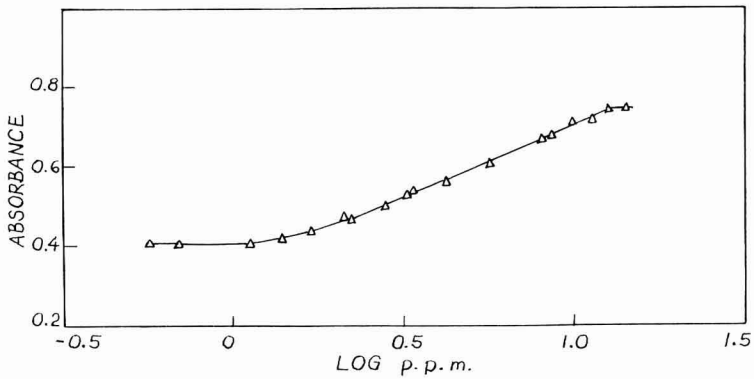

FIG. 6. Ringbom plot for Ce–CTR system.

The absorbance values were measured at 590 mμ and the results are graphically shown in Fig. 7.

Molar absorption coefficient. The average value of molar absorption coefficient calculated at 590 mμ and at pH 6.0 was 6650 litres/mole centimeter.

Sensitivity. The sensitivity, as defined by Sandell (*5*), was found to be 0.141 μg/cm^2. The practical sensitivity based upon the absorbance of 0.01 unit was found to be 1.41 μg/cm^2 at 590 mμ.

Effect of added foreign ions. The influence of various cations and anions was studied. It was found that copper, beryllium, aluminum, gallium, indium, zirconium, tungsten, nickel, cobalt, platinum, palladium, lanthanum, yttrium, praseodymium, neodymium, samarium, europium, tellurate, citrate, tartrate, oxalate, phthalate interfere at all concentra-

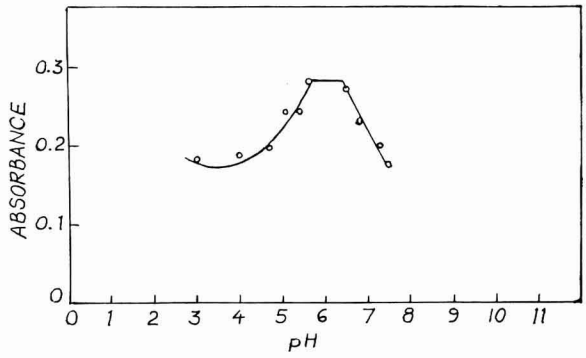

FIG. 7. Variation of absorbance of Ce–CTR chelate with pH at 590 mμ: final concentrations of the reagent and metal solutions, 0.5 $\times$ 10^{-4} *M*.

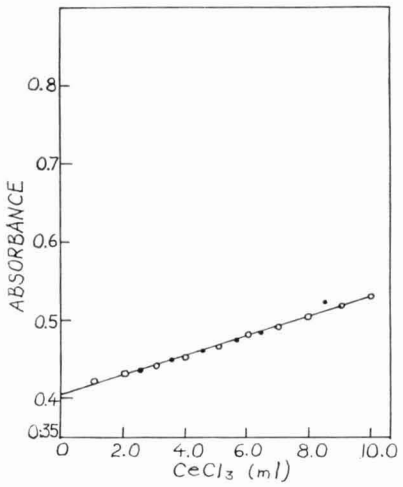

FIG. 8. Calibration curve for the spectrophotometric determination of cerium(III) at 590 mμ, pH 6.0.

tions. Barium, strontium, calcium, bromide, iodide, do not interfere even if present in excess.

Recommended procedure for spectrophotometric determination of cerium. To an aliquote of 5 ml of reagent solution (0.5×10^{-3} *M*) add the sample (so that the amount of cerium is less than 5.6 ppm and the volume not to exceed 15 ml). Adjust the pH of the solution to 6.0 after making the volume up to 25 ml. Measure absorption at 590 mμ. Determine the ppm of Ce from the standardized calibrated curve similar to that given in Fig. 8.

SUMMARY

The composition and stability of the pinkish violet chelate of cerium–CTR (λ_{max} 550 mμ) have been established by (i) the method of continuous variation, and (ii) mole ratio method. The stoichiometric ratio was found to be 1:1 (metal: ligand) at pH 6.0. The conditional stability constant of the chelate is determined by the method of Mukherji and Dey and the mole ratio method at pH 6.0 and at 28°C. The value of log *K* by the method of Mukherji and by mole ratio method are 3.81 and 4.8, respectively. Suitable conditions for the determination of the metal in micro amounts have been worked out. The range for adherence to Beer's law is 0.56–10.16 ppm. The molar absorption coefficient is 6650. Sensitivity is 1.41 γ/cm². The pH range suitable for determination of metal ion is 5.6–6.4. Effect of the various forms of ions on the chelate has also been studied.

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MICROCHEMICAL JOURNAL 15, 557–563 (1970)

Quantitation of Picogram Quantities of Serum Albumin by Ultramicrodisc Electrophoresis and Direct Densitometry [1]

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Disc electrophoresis, developed by Ornstein (*7*), has been applied to the quantitation of protein in amounts as small as 10^{-9} g by Grossbach (*2*) and Neuhoff (*6*), and even lesser amounts by Hydén and associates (*3, 4*). The method has been used here for the measurement of serum albumin in picogram amounts and concentrations as low as 0.1 mg/100 ml. Although exacting, the method is simple, gives highly reproducible results when carefully executed, and lends itself well to the determination of picogram quantities of albumin in nanoliter samples of renal tubule fluid.

MATERIALS AND METHODS

Methods. The columns are prepared in 3-cm long constant bore capillary tubes with an internal diameter of 140 μ (Drummond Scientific Company, Broomall, Pennsylvania). The capillaries and all glassware used are soaked in concentrated nitric acid for 24 hours prior to use and rinsed repeatedly in distilled water. Rinsing is best achieved by aspiration. The capillary tubes are filled with 2% Siliclid by aspiration, rinsed thoroughly, and dried in an oven at 100°C for 20 minutes. Siliconizing is repeated if necessary to give minimal capillarity and the flattest possible meniscus when the tubes are partially filled with water. After drying, they are stored in tightly stoppered vials until used.

Apparatus. A mounting device greatly facilitates the filling of the capillaries. This comprises a circular turntable of Lucite, 20 cm in diameter and 5 cm thick, around the periphery of which are bored 12 equally spaced vertical holes to accommodate the pin vises (Starret No. 197B) which hold the capillary tubes during filling. This turntable is mounted over a ballrace spindle and attached to a 25 cm square, 5 cm thick Lucite block on which it turns freely. A ballbearing detent is set at each pin vise position to accurately place each column in front of the

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viewing microscope. The base of this equipment is milled to fit over a laboratory jack with which its height can be easily changed. A dissecting microscope with a micrometer eyepiece (Ealing No. 8620) is set at a fixed distance from the mounting device.

The electrophoresis apparatus is constructed to permit the simultaneous electrophoresis of 6 columns in parallel. Its lower chamber is a 30 × 8 × 4-cm Lucite block into which six 2-cm diameter wells are milled equidistantly to within 1 cm of the bottom. A 5-cm length of 16-gauge platinum wire is sealed through a tightly fitting hole in the base of each well with epoxy resin. The wires are joined to a conducting lead in a groove milled in the base. This wire leads to an amphenol plug by way of a microswitch which is closed only when the upper chamber is in place. The base of the block is covered with a Lucite plate to isolate the electrical wiring. Plates of 1-cm Lucite, 8 × 8 cm, are cemented to the ends of the lower electrophoresis chamber to support the rack which holds the upper electrophoresis chambers. This rack, when in place, closes the lower microswitch. The upper electrophoresis chambers are 2.5-cm diameter wells milled to 0.5 cm from the bottom of 4-cm Lucite cubes. Channels (0.5 × 0.5 cm) are milled on opposite sides of these cubes and contain tightly fitting Teflon slides which permit positioning of the height of these chambers in the holding rack. One-mm holes are drilled vertically through the center of the bottom of each chamber. Five-mm diameter, 1-mm deep recesses are milled concentrically around these holes, and contain 1 mm thick, solf rubber gaskets with a hole just large enough to accommodate the 140 μ electrophoresis columns without leakage. The uppermost compartment is a Lucite box, 30 × 8 × 8 cm, with an amphenol plug connected to 5 cm long, 16-gauge platinum wires in parallel centered over each electrophoresis cell. An ammeter (Simpson No. 1212T) is placed in series with each platinum wire. An interlock switch permits current to flow only when this chamber is in place. Electrophoresis is accomplished with a Kepco (Kepco, Inc., Flushing, N. Y.) 1500M High Voltage Power Supply.

Preparation of the electrophoresis columns. After exhaustive testing with small pore gels of various compositions, a 22% solution of recrystallized (*5*) acrylamide (Eastman Kodak Co., Rochester, N. Y.) was adapted. As suggested by Neuhoff (*6*) and by Hydén and Lange (*4*), the addition of 0.5% hydantoin to such a high concentration of polyacrylamide stabilizes the gelation and provides greater consistency of pore size. Gel of this composition gives far more consistent results in albumin quantitation than the usual 7% separative gel used for macrodisc electrophoresis of plasma. Apart from this change and the ommission of sucrose from the large pore gel, the gels and running buffers are made

up as described by Ornstein (*7*). To achieve uniform results, it is important that the purest available chemicals be used, pH be precisely controlled, and that the conditions of gel preparation are kept constant. Stock solutions are made fresh every 2 weeks and stored in dark bottles at 4°C. In preparing the gels, small aliquots of stock solutions are poured into covered beakers and warmed in constant light to 32°C for 30 minutes prior to use.

A 2-day supply of gel tubes can be prepared simultaneously; the small pore gel retains its suitability for up to 72 hours when kept in a water saturated atmosphere. Under microscopic visualization, a small dot of red nail polish is applied with a fine piece of glass exactly 2 cm from the end of the capillary tubes. Small pore gel, pH 6.7, is aspirated precisely to this mark. The gel is then degassed for 15 seconds in a desiccator attached to an evacuation pump. This step is essential to even gelation, but should not be prolonged or excessive drying of the gels occurs. For the purposes of this study, no improvement in gel properties resulted from water layering of the gel surface, permitting gelation to proceed in a nitrogen atmosphere, or of adding tris-thioglycolate, maneuvers reported by Hydén and Lange (*4*) to be helpful in the electrophoretic separation of brain proteins.

Filling capillaries are made from 1-mm o.d. soft glass capillary tubing on a pipet puller similar to that described by Alexander and Nastuk (*1*) to have a length of 2 cm and an outside diameter of 30–50 μ. When larger diameter capillaries are used, there is a tendency for fluid transferred to the electrophoresis column to layer on the walls of the capillary tube and to give incorrect sample volumes.

Gelation of the small pore gel is allowed to continue for a minimum of 16 hours in a water saturated atmosphere. Thereafter, the columns are placed in the mounting device and the large pore gel is applied from above with a filling capillary. Although this can be achieved by hand, it is accomplished more readily with a micromanipulator. A precisely measured 3 mm length of large pore gel is layered onto the small pore gel under microscopic visualization with a micrometer eyepiece. Great care is taken in applying the large pore gel that the junction between the gels is not disturbed. Gelation of the large pore gel usually is complete within 10 minutes. After 20 minutes, the protein sample may be applied, again taking care not to distort the gel surface. The height of the protein sample is measured precisely with a micrometer eyepiece and the volume derived. Ten replicate 16-nl volumes of ^{14}C labeled inulin solution (30 μCi/ml) transferred in this manner to empty 140-μ diameter capillary tubes, rinsed into a butyl PBD fluor solution and counted in a Nuclear Chicago liquid scintillation counter gave a standard deviation of

4.6%. Volumes of 6 nl gave a standard deviation of 10.3%. A second stacking gel (7) is not mixed with the sample. To prevent loss of the sample into the upper buffer reservoir by diffusion, the end of the capillary is sealed with large pore gel prior to electrophoresis. After this has set, the tips of the capillary are crushed as close to the end as possible to assure good electrical contact with the electrophoresis buffers. The capillary tubes are then mounted in their holders, ready for electrophoresis.

Electrophoresis is performed in front of the outlet of an air conditioner to minimize ohmic heating of the samples. At a constant potential of 75 V, an initial current of approximately 7 μA is achieved and, with careful preparation of the gels, there is very little difference in current from column to column. As electrophoresis proceeds, there is a tendency for the current to fall, finally reaching approximately 1 μA/tube when the protein disc passes the junction between the large and small pore gels. Electrophoresis is stopped when the bromphenol blue band is 2 mm beyond this junction, total electrophoresis time being approximately 25 minutes. After electrophoresis, the gel columns are extruded from their capillary tubes as quickly as possible by inserting a plunger made of 0.005-inch diameter music wire into the upper end of each column, as suggested by Hydén *et al.* (3). The gels are fixed for 20 minutes in a drop of 7% acetic acid on 1 mm thick optically plane, CO quartz slides, (ESCO Products, Oak Ridge, New Jersey). The gels then are washed in water for 10 minutes and stained with a filtered, aqueous solution of Coomassie blue for an additional 20 minutes. Excess dye is eluted with water until the gel between discs is colorless. The gel is straightened and oriented as nearly parallel with the edges of the glass slides as possible. This is best accomplished by holding the slide vertically and gently flowing water over the lower half of the gel. Further adjustment can be made by touching the end of the gel with a wisp of absorbent paper, to which it adheres weakly, and gently drawing the gel into the correct position. The slide is placed on the revolving stage of the microdensitometer where the gel is more precisely oriented for scanning if necessary.

Densitometry. The optical density of the Coomassie blue stained discs is determined directly on an ultramicrodensitometer specifically designed and built for this purpose by Canalco (Canal Industrial Corporation, Rockville, Maryland) and subsequently modified. For reproducible results, the entire optical system must be kept exactly focused and aligned. Monochromatic light, 560 mμ is used as the energy source and the sample is read with the exit slit of the monochrometer exactly focused at the specimen plane (critical illumination). The sample is scanned on a constant speed, motor driven stage at a rate of 170 μ/min,

care being taken to scan through the center of the gel and to orient the gel so that the direction of scanning is exactly perpendicular to the protein discs. The densitometer is set to scan a 6-μ diameter spot at the specimen plane. Each gel is scanned three times. The photomultiplier output is fed to a logarithmic conversion amplifier, integrated, and recorded on a chart recorder. A digital readout gives the integrator value.

RESULTS

This method is capable of reproducibly measuring serum albumin in amounts as small as 3×10^{-11} g and concentrations as low as 0.1 mg/100 ml (Table 1). To measure the reproducibility of densitometric measurement, 10 scans were performed sequentially on each of three gels containing 1.38×10^{-10} g of rat serum albumin. Mean integrator output values of 149 $\pm$ 2.6 (SD), 150 $\pm$ 4.0, and 139 $\pm$ 2.4 were obtained.

The reproducibility of values obtained in replicate samples of the same solutions electrophoresed in parallel and analyzed sequentially is shown in Table 1. Standard rat serum samples used in this portion of the study were diluted in 140 m*M* saline to contain 0.1 to 3.1 mg/100 ml of albumin and total amounts of albumin ranging from 3.1×10^{-11} to 3.9×10^{-10} g. Five or six replicate samples of each solution were run at each volume. Standard deviations obtained on the replicate samples whose volume was 9 nl or more, were 5.4 to 12.0% of the mean values. Greatly higher standard deviations were obtained with the 6-nl samples reflecting, at least in part, the inaccuracy of delivery of these smallest samples (see Methods). The wide standard deviation values obtained for

TABLE 1

REPLICATE DETERMINATIONS OF ALBUMIN AT VARYING CONCENTRATIONS AND SAMPLE VOLUMES

	Albumin conc (mg %)	Vol (nl)	Total albumin (g $\times 10^{10}$)	Mean value (i.U/U)	Replicates	SD/mean (%)
A.	3.1	6.25	1.93	38.5 $\pm$ 5.8 [a]	6	15.0
	3.1	9.35	2.90	36.8 $\pm$ 2.0	6	5.5
	3.1	12.5	3.86	36.7 $\pm$ 2.7	6	7.4
B.	1.03	6.25	0.64	8.6 $\pm$ 3.6	5	42.0
	1.03	9.35	0.96	10.5 $\pm$ 1.2	5	12.0
	1.03	12.5	1.28	10.5 $\pm$ 0.8	5	7.6
C.	0.10	30.8	0.31	1.1 $\pm$ 0.06	6	5.4
	0.10	61.6	0.62	1.2 $\pm$ 0.07	6	8.2

[a] All values are given as integrator units/unit volume $\pm$ 1 SD.

the smallest samples presumably was not related to limitation of the quantity of albumin present, since even smaller amounts measured in Group C produced standard deviations of 5.4 and 8.2% of the mean integrator output values.

This method was developed specifically for the measurement of albumin concentration in renal tubule fluid. Because tubule fluid in the various segments of the nephron differs in sodium concentration, osmolality, and pH, it was necessary to determine the effect of variation in these parameters on the quantitation of albumin. Accordingly, electrophoresis was performed on fresh rat serum albumin diluted in either 50 m*M* NaCl, 200 m*M* NaCl, or Ringer's solution to give an albumin concentration of 3.1 mg/100 ml. The densitometric pattern obtained with 5.1×10^{-10} g of each solution is shown in Fig. 1. As expected, no effect of variation in electrolyte composition within these limits was discernible. Alteration of sample pH between 5.65 and 7.44 by the addition of 0.01 *M* phosphate buffer also made no appreciable difference to the measured albumin content.

DISCUSSION

As shown earlier by Grossbach (*2*), Hydén and associates (*3, 4*), and Neuhoff (*6*), disc electrophoresis can be readily adapted to the measurement of very small samples of protein. Neuhoff has measured

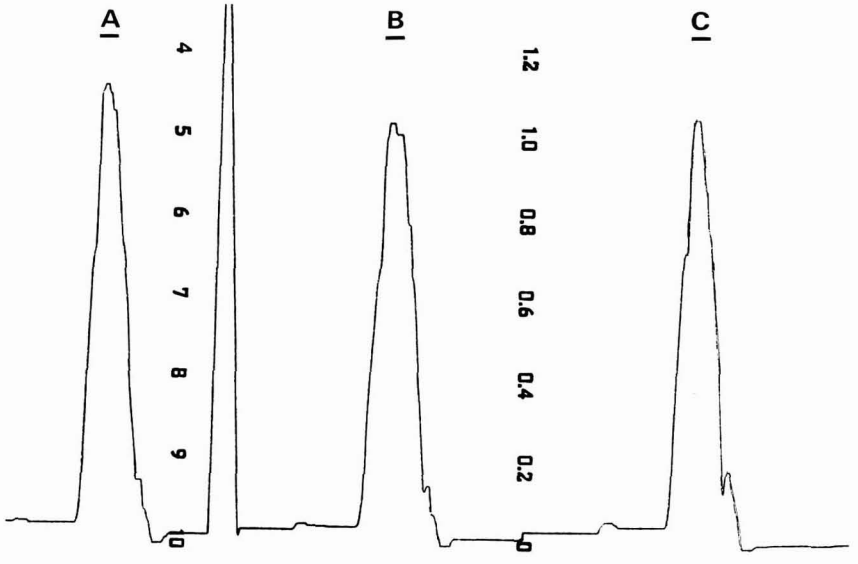

FIG. 1. The densitometer reading of 3 gels normally containing 5.1×10^{-10} g of rat serum albumin diluted in (A) 50 m*M* NaCl, (B) Ringer's solution, (C) 200 m*M* NaCl.

less than 10^{-9}-g quantities of albumin by direct densitometry of amido black stained protein discs using the Joyce Loebl microdensitometer (*6*), and Hydén *et al.* using radiometric analysis of labeled protein have measured 3.6×10^{-11} g of protein. Still lower amounts were "detectable by their ability to scatter incident light." The limit of measurement by interference microscopy was calculated by Hydén and Lange (*4*) to be 2×10^{-10} g of protein.

In the study of albumin transport by the renal tubule, only 0.01- to 0.1-μl volumes of fluid are easily obtained for analysis by micropuncture. Since tubule fluid samples contain little protein, it is necessary to have a method which will permit the ready quantitation of 10^{-10} g of albumin or less. Within defined limits, this method must be independent of sample pH and electrolyte concentrations, since tubule fluid composition varies in different segments of the nephron. These criteria are met by disc electrophoresis. As little as 3×10^{-11} g of albumin can be measured reproducibly and rapidly. Once the small pore gel has set, six samples can be prepared and analyzed within 2 hours. Double the number of samples can be prepared and run using a similar electrophoresis apparatus with 12 chambers instead of 6.

Direct densitometry has proven emminently satisfactory for the measurement of albumin in acrylamide gels of the dimensions used.

ACKNOWLEDGMENTS

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MICROCHEMICAL JOURNAL 15, 564–571 (1970)

Electrode Deactivation in the Anodic Voltammetry of Some Phenolic Derivatives of Isoquinoline

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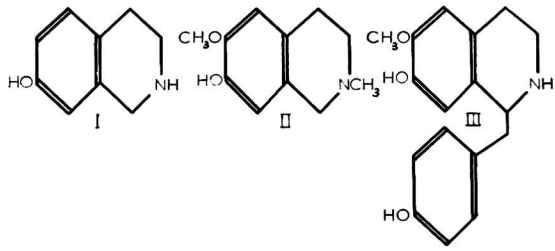
A problem that is sometimes encountered in voltammetry at a solid microelectrode is electrode deactivation. This gives rise to currents that decay with time and to current–voltage curves of abnormal shape. A common cause is the adsorption, or actual deposition, upon the electrode surface of the products of the electrolysis. Methods that can be used to overcome or alleviate this problem have been known for more than 20 years. One approach is intermittent polarization, with or without reversal of polarity, of the electrode (*11*). A recent example of this approach is amperometric titration with sodium tetraphenylborate (*14*). Another method relies upon the continuous mechanical cleaning of the electrode surface by rubbing or even by mild abrasion. The continuous monitoring of chlorine in water supplies is an early example of this type of technique (*1, 10*). More recently, Berge and Strübing (*2*) have described a simple and ingenious device for continuous mechanical activation. The electrode presses lightly upon the periphery of a nonconducting horizontal drum, the lower one-third of which is submerged in the solution to be examined. Rotation of the drum continuously activates the electrode and, at the same time, carries a film of solution past the electrode. The applications reported by Berge and Strübing (*2*) are to ferricyanide–ferrocyanide and other well-known inorganic systems for which continuous electrode activation is usually unnecessary.

As part of a program aimed at the synthesis of alkaloids and related compounds by electrolytic oxidative coupling (*7, 9, 13*), numerous isoquinoline derivatives have been prepared and examined by conventional rotating platinum microelectrode anodic voltammetry. The results demonstrate the strong electrode deactivating properties of many of these compounds. It seemed that the real efficacy of the drum-activated platinum electrode might be assessed by application to compounds of this type. 7-Hydroxy-1,2,3,4-tetrahydroisoquinoline (I), 7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (corypalline) (II), and 7-hydroxy-1-(4-hydroxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (coclaurine) (III) were chosen as test compounds.



MATERIALS AND METHODS

Equipment. The drum-activated platinum electrode (DAPE) was constructed on the lines of that described by Berge and Strübing (*2*). Most of the runs were made with a 25-mm diameter hardwood drum that had received two sprayings of Krylon No. 1301 Crystal Clear Acrylic Coating (Borden Chemical Co.). The hooklike platinum wire electrode was imbedded in a pendant drop of epoxy cement. When the drop had hardened, the bottom was carefully abraded with extra fine sandpaper until an approximately 1-mm length of the wire was exposed. To minimize local wear, the electrode was moved about 2 mm across the length of the drum after each hour of total running time.

A belt and pulley system driven by a Sargent Synchronous Rotator caused the drum to rotate at a constant rate of 190 rpm. Direct 600 rpm drive by a second Rotator was used for the rotating platinum electrode (RPE). This was of the axial type (*12a*) with the projecting platinum wire cut down until almost flush with the glass, so that the sensitivity approximated to that of the DAPE. A troughlike cell made from Lucite sheet was used to permit runs with the RPE and the DAPE to be made with the same portion of solution. The arrangement is shown schematically in Fig. 1.

Current–voltage curves were recorded at a scan rate of 0.24 V/minute by means of a Beckman Electroscan. Since the operation of the DAPE caused slight pen "quiver," all recordings were made with the damping switch in the "0.5-second" position. A simple changeover switch enabled either the RPE or the DAPE to be connected to the recorder. The large bottle-type saturated calomel electrode (SCE) with flexible salt bridge and agar-filled bridge tip (*12b*) made contact with the solution being examined by means of a junction tube with a medium-porosity fritted-glass disk (*12c*). All potentials are with respect to the SCE.

Reagents. The test compounds were employed as 0.025 *M* solutions of their hydrochlorides. 7-Hydroxy-1,2,3,4-tetrahydroisoquinoline (I) was prepared (*3*) by methods described by Bobbitt *et al.* (*5*). Corypalline (II) was prepared from isovanillin as described by Bobbitt *et al.*

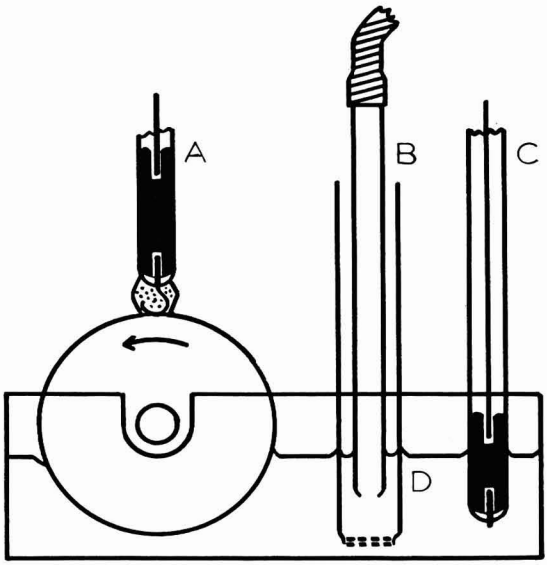


FIG. 1. Schematic arrangement of voltammetric cell: (A), DAPE; (B), salt bridge tip and junction tube; (C), RPE; (D), test solution.

(*6*). Coclaurine (III) was synthesized by a modification (*4*) of the procedure of Finkelstein (*8*). All other reagents were of analytical grade.

Procedure for RPE conditioning. Unless otherwise specified, the RPE was conditioned before each run. Conditioning involved rotation for 1 minute in concentrated nitric acid, rinsing with water, then anodization for 1 minute in the appropriate supporting solution at approximately 0.05 V more positive than the decomposition potential of the solution. The potential was then scanned downwards until the current just changed sign. Runs were started at this point. The DAPE required no conditioning.

Procedure for recording. Current–voltage curves were recorded at room temperature (25 to 27°C). Fifty ml of supporting solution was pipetted into the cell and residual current curves at the RPE and the DAPE, respectively, were recorded in succession. The 0.025 *M* solution of the test compound was then introduced from a microburet and current–voltage curves at the two electrodes were recorded in succession. In some cases, the potential was held at a fixed value and current–time curves were either recorded (for runs not exceeding 5 minutes) or constructed by taking readings at suitable intervals.

Determination of electrode sensitivities. Electrode sensitivities were determined by measurement of the limiting current of potassium fer-

rocyanide in 0.01 *M* perchloric acid at +0.90 V, with due correction for the residual current. Sensitivities (μA/m*M* $K_4Fe(CN)_6$/liter) at 26°C found were RPE, 6.26; DAPE, 5.06.

RESULTS

7-Hydroxy-1,2,3,4-tetrahydroisoquinoline(HTQ). A preliminary study of the voltammetry of HTQ at the RPE has shown that quite large but unstable currents can be obtained in sodium bicarbonate or carbonate media (*3*). Curve B, Fig. 2, shows the typical peaked curve obtained with HTQ in 0.1 *M* sodium carbonate. A repeat of the scan without reconditioning the electrode caused the peak to disappear (curve C). The current up to the decomposition potential of the medium was then only slightly greater than the residual current. No peak was observed with curves obtained at the DAPE (curve E). Repeated scans with this electrode gave virtually identical curves. Results for HTQ solutions in 0.1 *M* sodium bicarbonate were qualitatively similar to those obtained in carbonate medium.

Table 1 summarizes the effect of time upon the current when the electrode is held at a fixed potential *E*. The contrast between the behavior of the RPE and that of the DAPE is striking. Rapid deactivation of the RPE soon after onset of the oxidation of the test compound is almost

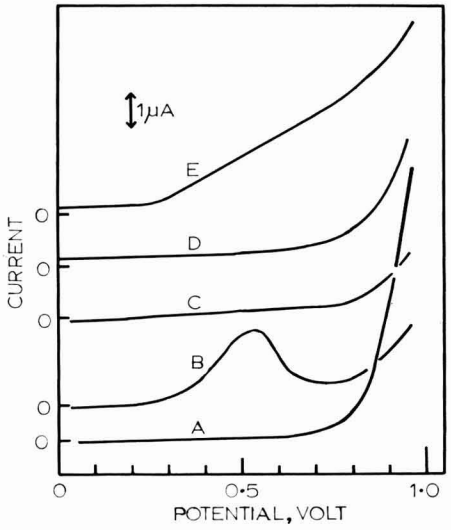


FIG. 2. Current–voltage curves of 5×10^{-4} *M* HTQ in 0.1 *M* Na_2CO_3: (A), RPE, residual current; (B), RPE, first scan; (C), RPE, second scan; (D), DAPE, residual current; (E), DAPE, first and second scans.

TABLE 1

CURRENT (μA)–TIME RESPONSE AT $E = +0.80$ V OF ELECTRODES IN 5×10^{-4} M HTQ

Time (min)	0.1 M $NaHCO_3$		0.1 M Na_2CO_3	
	RPE	DAPE	RPE	DAPE [a]
0	~4	~3.5	~4	~3.5
0.5	1.1	3.40	0.9	3.48
1	0.86	3.38	0.74	3.47
3	0.57	3.40	0.50	3.49
5	0.47	3.39	0.41	3.49
10	—	3.40	—	3.52
20	0.34	3.39	0.30	3.53
30	—	3.39	—	3.55

[a] Temperature rise of ~1° during run.

certainly the cause of the peaked current-voltage curves obtained at the RPE.

Corypalline. The voltammetry of corypalline (II) at the RPE has been described by Kirkbright *et al.* (*9*). In 0.1 M sodium bicarbonate, the current–voltage curve of this compound exhibits a quite well-defined wave, as shown at B in Fig. 3. However, the smaller wave obtained upon

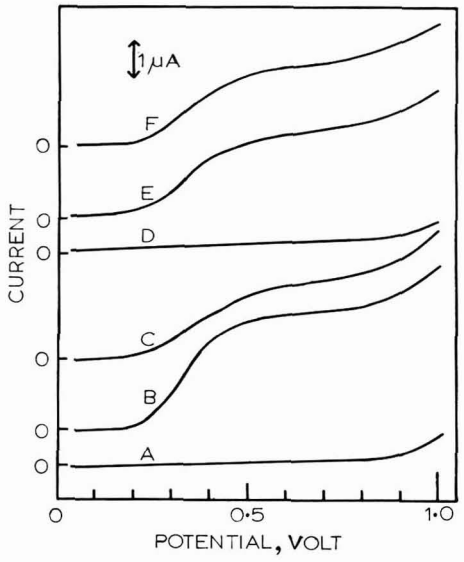


FIG. 3. Current–voltage curves of 5×10^{-4} M corypalline in 0.1 M $NaHCO_3$: (A), RPE, residual current; (B), RPE, first scan; (C), RPE, second scan; (D), DAPE, residual current; (E), DAPE, first scan; (F), DAPE, second scan.

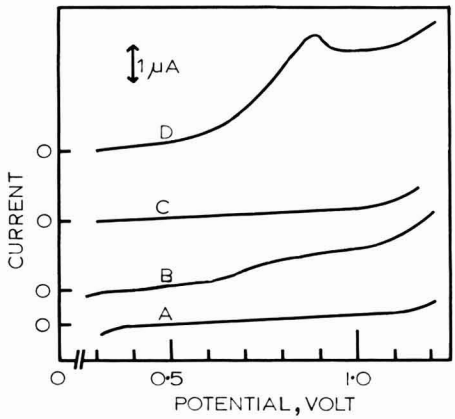


FIG. 4. Current-voltage curves of 5×10^{-4} *M* coclaurine in 0.01 *M* $HClO_4$: (A), RPE, residual current; (B), RPE, first scan; (C), DAPE, residual current; (D), DAPE, first scan.

repeating the scan without reconditioning the electrode indicates the occurrence of significant electrode deactivation (curve C). Similar runs at the DAPE gave the nearly identical pair of curves E and F. The slightly smaller wave height of curve F is a real effect. A current–time plot at a potential of +0.75 V indicated that the current had fallen to 80% of the initial value after approximately 30 minutes. At the RPE, current decrease to the 80% point required only about 3 minutes.

Coclaurine. The current–voltage curve of coclaurine (III) in 0.01 *M* perchloric acid is shown at B in Fig. 4. The drawn-out wave system is abnormally small, in contrast to the result obtained at the DAPE (curve D). Table 2 summarizes the current–time behavior at the respective electrodes.

DISCUSSION

At the quite conventional scan rate used in these experiments, the recording of a current–voltage curve takes approximately 4 minutes. Any electrode deactivation will be confined to the shorter time interval in

TABLE 2

CURRENT (μA)–TIME RESPONSE AT $E = 1.00$ V OF ELECTRODES IN 5×10^{-4} *M* COCLAURINE IN 0.01 *M* $HClO_4$

Time (min)	0	2	5	7	10	12
RPE	~2.5	1.10	0.65	0.50	0.42	0.38
DAPE	~3.2	2.22	1.66	1.59	1.54	1.52

which electrolysis of the test compound is actually proceeding. As a reasonable estimate, a deactivation of up to approximately 20% in 3 minutes should not prevent the recorded wave from resembling fairly closely the true shape of the wave. Even with a 50% deactivation in the same time, the record may be qualitatively useful. Application of these criteria to the results obtained at the RPE in the media specified shows that this electrode is of little use with HTQ or coclaurine, but is definitely applicable to studies with corypalline. Modifications such as change of drum material, drum rotation rate, and pressure of the electrode upon the drum may further minimize any deactivation of the DAPE. However, even in its present state, this electrode should give a picture of the voltammetry of these compounds that is much nearer to the truth than recordings obtained at the RPE or analogous electrode systems.

Although the DAPE is less readily deactivated than the RPE, the relative rates of deactivation obviously depend upon the test compound or, more specifically, upon the nature of the oxidation products that collect upon the electrode surface. Corypalline, which deactivates the RPE comparatively slowly, also produces definite deactivation of the DAPE. However HTQ, which is an intense deactivator of the RPE, is without obvious effect upon the DAPE.

SUMMARY

The anodic voltammetry of submillimolar concentrations of 7-hydroxy-1,2,3,4-tetrahydroisoquinoline, and of the related compounds corypalline and coclaurine, has been examined at the rotating platinum microelectrode (RPE) and at the drum-activated platinum electrode (DAPE) of Berge and Strübing. The electrolysis of these compounds markedly deactivates the RPE, but has a much smaller effect upon the performance of the DAPE.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 15, 572–578 (1970)

The Quantitative Determination of Boron in Glasses Used as Encapsulants for Electronic Devices

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INTRODUCTION

Glasses of varying compositions are now being investigated at encapsulants for silicon devices; some of these glasses contain boron. We were asked to develop a chemical method suitable for determining small percentages of boron (1 to 8%) in these materials: sample sizes being 0.1 to 2 mg.

Hydrofluoric acid is usually required for dissolution of borosilicate glasses; hence, our search for possible methods was narrowed to those that were feasible in the presence of fluoride. Several methods have been described in the literature wherein borofluoride is reacted with derivatives of thionine to form colored complexes that are extractable with organic solvents (*1–3*). Pasztor and Bode (*4*) have made a comprehensive study of thionine derivatives and various solvents and Skaar (*5*) has studied the effects of various ions when methylene blue (tetramethyl thionine) is used as the complexant. After some preliminary study, we decided to use *N*-methylthionine (Azure C) as the complexant, and dichloroethane, or a mixture of dichloroethane and dichloropropane, as the organic extractant. In addition, we have found that optimally the aqueous phase should be 0.5 *N* in sulfuric acid and 0.5 *M* in hydrofluoric acid.

EXPERIMENTAL METHODS

Reagents and Apparatus

Platinum dishes and polyethylene labware or boron-free glassware were used.

Standard boron solution containing 10 μg of boron was prepared by dissolving analytical grade boric acid in distilled water and diluting appropriately. The solution was stored in a polyethylene bottle.

Standard boron solutions containing 1 μg of boron/ml of BF_4^- were prepared by adding 5 ml of 5% hydrofluoric acid to 50 ml of the standard boron solution (10 μg/ml); after 24 hours, the solution was

diluted to 500 ml with distilled water and stored in a polyethylene bottle.

Sulfuric acid, 0.5 *N*.

Azure C, 0.005 M (Eastman Organic Chemical).

Solvents, 1,2-dichloroethane, 1,2-dichloropropane (Eastman Organic Chemical).

For the solvent mixture, equal volumes of the two solvents were mixed together. All other chemicals were of reagent grade.

Beckman DU, spectrophotometer, using 1-cm silica cells.

Nalgene Squibb separatory funnels, polypropylene (cap., 125 ml) were used for extraction.

PROCEDURE

A. Preparation of Calibrations Curves

1. For boron, 0–15.0 μg. Transfer various amounts of boron (0-15 μg) to 125-ml Squibb polypropylene separatory funnels. Add 10 ml of 0.5 *N* sulfuric acid and 10 ml of 0.005 *M* Azure C. Mix and let stand for 5 minutes, then add 10 ml of a 1:1 mixture of dichloroethane–dichloropropane and extract for 1 minute. Measure at 660 mμ against a reagent blank; see Fig. 1, Table 1.

2. For boron, 0-3.0 μg. Proceed as described under A-1 but replacing the solvent mixture with 10 ml dichloroethane; see Fig. 2, Table 1.

B. Determination of Boron in Borosilicate Glass

Weigh the sample on a microbalance, place in a platinum dish, and etch with 2.0 ml of 1:1 HF solution. When the glass film is completely etched, the silicon substrate will repel water. Remove the substrate using platinum forceps, wash, dry, and reweigh to obtain the weight of the film. Let the sample solutions stand at least 2 hours. Dilute to appropriate volume and proceed as described under A1 or A2; see Table 2.

TABLE 1

SUGGESTED SOLVENT MIXTURES FOR DIFFERENT CONCENTRATION RANGES

Boron (range, μg)	Solvent
0–3	1, 2-Dichloroethane, 10 ml
3–15	1, 2-Dichloroethane–dichloropropane, (1:1), 10 ml
15–50	1, 2-Dichloroethane–dichloropropane, (1:1), 25 ml

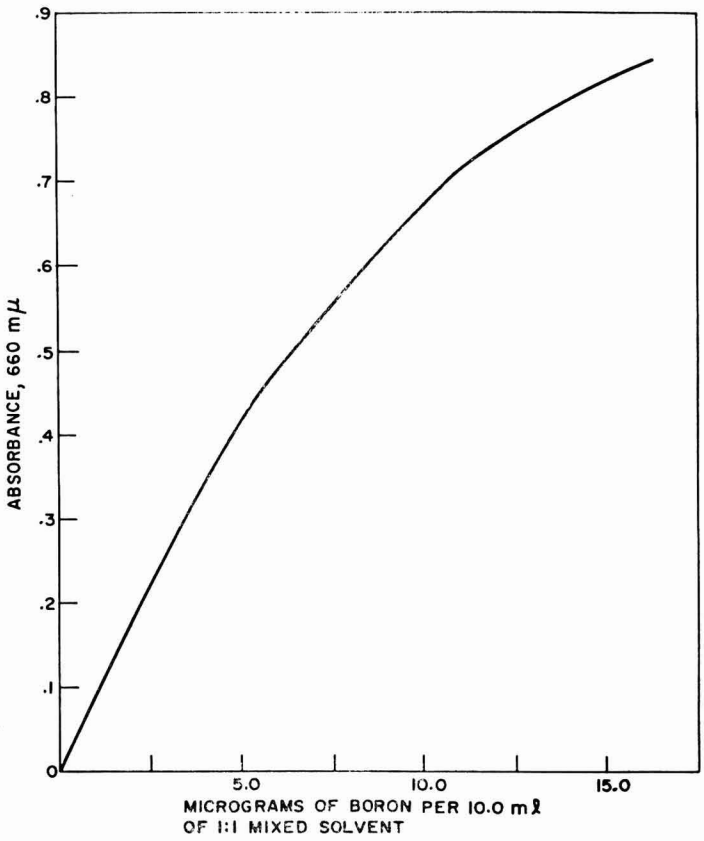


FIG. 1. Calibration curve.

RESULTS AND DISCUSSIONS

A. Absorption Spectra

As indicated in Fig. 3, the BF_4^- Azure C complex extracted with the mixed solvent or the dichloroethane show identical absorption maxima at 660 mμ. The sensitivity for the BF_4^- extracted with dichloroethane is more than three times that of the mixed solvent extraction; see Fig. 3.

B. Beer's Law

The BF_4^- azure C complex follows Beer's law between 0–3.0 μg with dichloroethane as the extractant; however, Beer's law is not followed in the 3–15.0 μg range, with the mixed solvent. If the conditions are carefully controlled, excellent reproducibility can be obtained (Figs. 1 and 2).

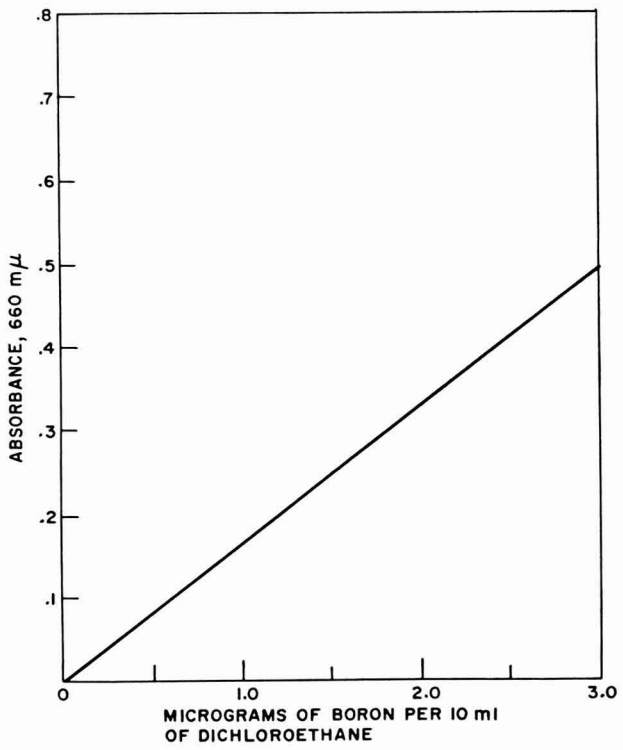
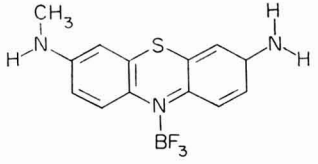


FIG. 2. Calibration curve.

C. Complex Formation

In the presence of excess fluoride, it is difficult to establish the empirical formula of the extractable boron complex. The method of continuous variation was employed and a Job's curve was constructed; it appears as if the BF_4^- forms a 1:1 complex with Azure C. To insure complete complex formation, a fourfold excess of Azure C should be used. (see Fig. 4). The following structure is proposed for the colored boron complex in solution, based on the information obtained from the Job's Curve.



D. Effect of Acid Concentration

The work reported in this paper was carried out using dilute sulfuric

TABLE 2

Sample identification	Sample wt (mg)	Boron (%)	
		Theoretical	Found
	1.178	7.66	7.42
Standard	1.062	7.66	7.62
Borosilicate	1.132	7.66	7.33
7740	1.519	Unknown	3.79
7740	1.001	Unknown	3.85
30	0.211	Unknown	3.91 ± 0.11
40	0.931	Unknown	2.55 ± 0.13
30329–7–2	0.150	Unknown	3.85 ± 0.15
30329–7–4	0.146	Unknown	2.40 ± 0.15
30329–7–1	0.091	0.00	0.13

acid as the diluent for the aqueous phase. Pasztor has reported the use of water as a diluent (*1*) but however, we had difficulty obtaining consistent results with water. Organic acids, nitric, and hydrochloric acid interfere. Good reproducibility can be obtained if the concentration of hydrofluoric acid in the aqueous phase is kept below 0.30 *M*; the amount of fluoride in our method did not exceed 0.05 *M*. We have attempted to

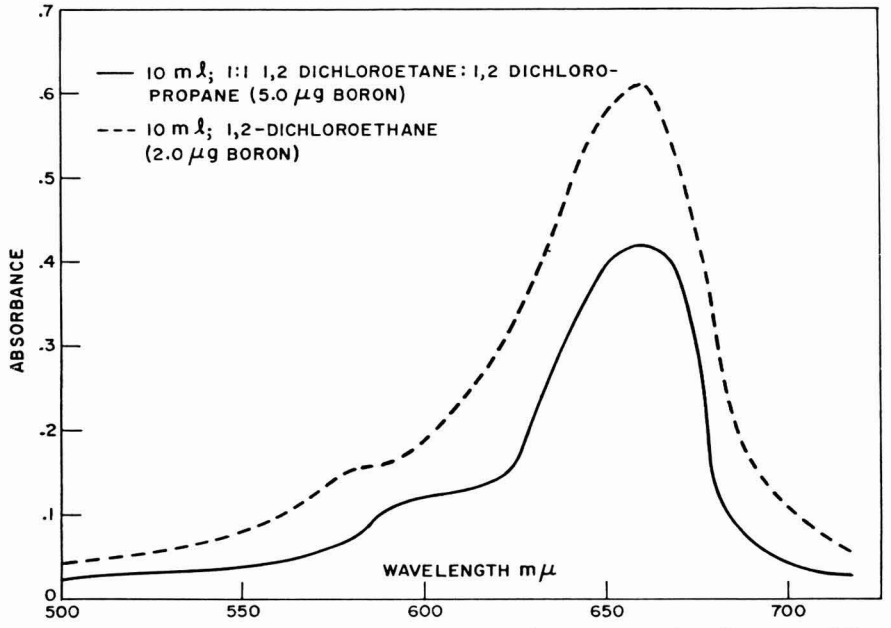


FIG. 3. Absorption spectra of the azure C-boron complex in two different solvents.

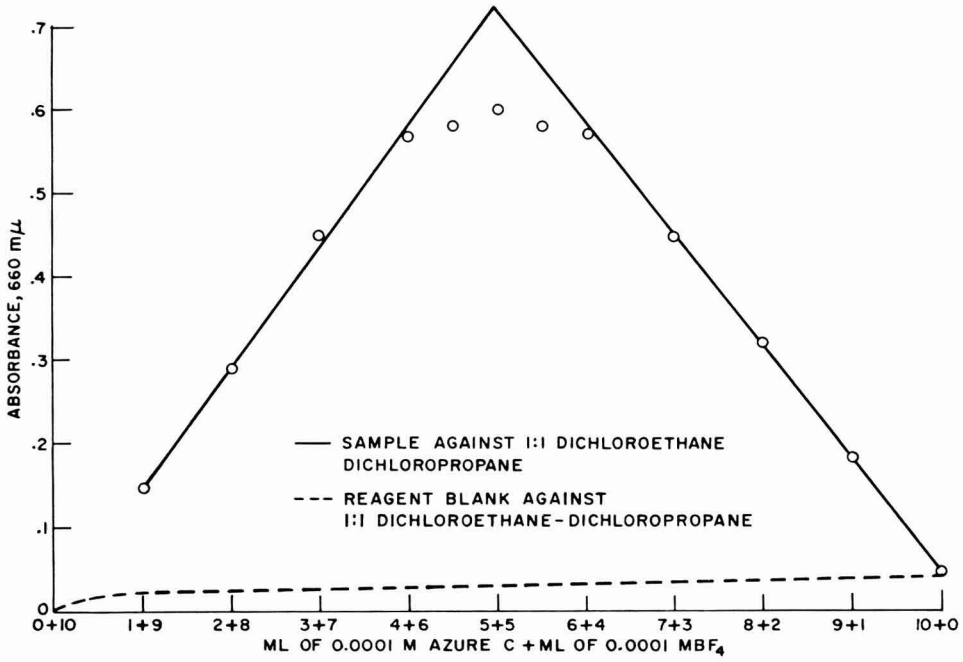


FIG. 4. Job's curve.

complex excess fluoride with beryllium, in line with the attempts of others to use aluminum for the same purpose (*2*). However, this approch led to inconsistent results, possibly due to competition between the formation of BF_4^- and fluoride complexes involving aluminum and beryllium.

E. Effect of Various Organic Solvents

Pasztor (*2*) has discussed various organic solvents used in extracting the Azure C BF_4^- complex; by using various chlorinated or brominated solvents, we found that the sensitivity of the method could be altered. We selected 1,2-dichloroethane, and a mixture of 1,2-dichlorothane and 1,2-dichloropropane as our extractants, since these solvents are effective in extracting 0-15 μg of boron. Purity of the solvent is important, since impure solvents can cause high reagent blanks. The Eastman organic solvents used in our work did not contain any impurities that affected our blanks.

The described procedure offers a rapid, reliable, and accurate method for the determination of boron in borosilicate thin films. Boron contents

CONCLUSIONS

from 0.5 to 15.0 μg may be determined with an average deviation of ± 0.2 μg.

Because the borosilicate films must be etched away from the silicon substrates with HF, the BF_4^- complex is easily formed, thus permitting a direct determination of boron without prior separation. Other methods would require such separation from fluoride, thereby increasing the possibility of loss of boron or contamination from other sources.

The simplicity of the method permits many samples to be processed at the same time; hence, the method can be used routinely.

SUMMARY

N-Methylthione (Azure C) reacts with BF_4- to form a colored complex in a 0.5 *N* sulfuric acid medium. This colored complex is extractable with dichloroethane or a mixture of dichloroethane–dichloropropane, with a maximum absorbance at 660 mμ. Since it is necessary to dissolve the borosilicate glass with HF, the presence of fluoride creates a problem when attempting to utilize other spectrophotometric methods for boron, e.g., hydoxyanthraquione and anthraquinonylamine reagents. In the proposed method, fluoride is necessary because Azure C reacts only with the BF_4- complex; this makes the method very selective for the determination of boron in the presence of fluoride without prior separation. This method has been successfully applied to the determination of boron in glasses used as encapsulants.

ACKNOWLEDGMENT

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MICROCHEMICAL JOURNAL 15, 579–584 (1970)

A Selective Differential Spectrophotometric Method for the Determination of Mercury(II) Using Tris(2-thiopyridine-1-oxide)-iron(III) as Reagent

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INTRODUCTION

2-Mercaptopyridine-1-oxide or 1-hydroxy-2-pyridine-thione, subsequently referred to as "thione" (I) gives 1:3 chelate with iron(III) which has been used for the gravimetric and absorptiometric determination of iron (*2, 3*). The Iron(III)–thione or tris(2-thiopyridine-1-oxide)–iron(III) is a black crystalline precipitate, stable towards light and atmospheric oxidation. It is soluble in chloroform resulting in a violet coloration. The visible spectra of this solution against chloroform has a broad absorption with two bands of maximum at (519 and 588 mμ) having equal extinction coefficients of $\varepsilon = 3550$ (*4*).

In this work the chloroform solution of iron(III)–thione chelate is used for a selective absorptiometric determination of mercury(II). The procedure is based on the measurement of the decolorization of reagent in chloroform layer by mercury(II) ion in the aqueous layer of pH 4.0. The bleaching of the reagent is measured by a differential absorptiometric method using a "trace analysis procedure" (*9*).

Formation of Mercury Complex

The displacement reaction can be represented as:

$$2Fe(C_5H_4NOS)_3 + 3Hg^{2+} \rightleftharpoons 3Hg(C_5H_4NOS)_2 + 2Fe^{3+}.$$

The more stable 1:2 chelate of mercury(II) is colorless in chloroform and has no absorption at 325–750 mμ. The recovered product from the

colorless chloroform phase was a white powder (dec, 214–216°C). The analysis of the product gave:

Element:	C (%)	H (%)	N (%)	S (%)	Hg–
Found	26.7	1.66	6.36	14.16	44.1
$Hg(C_5H_4NOS)_2$ requires	26.5	1.78	6.18	14.26	44.3

The percentage of mercury in the complex was determined by oxygen flask combustion (*5*) using concentrated nitric acid as oxidizing absorbant and then Volhard's titration with standard potassium thiocyanate (*7*).

MATERIALS AND METHODS

Reagents

8×10^{-5} *M* iron(III)–thione was prepared by diluting 80 ml of 10^{-3} *M* (0.4343 g/liter) iron(III)–thione in chloroform and making it up to 1 liter by the addition of chloroform. The solid reagent was prepared in a pure form by the addition of 10% excess 0.01 *M* aqueous solution of sodium salt of 2-mercaptopyridine-1-oxide (purchased from Aldrich INC.) to 0.01 *M*, A.R. ferric chloride at pH 2–4. The precipitate was recrystallized from acetone–water and dried at 110°C in an air oven (dec, 193–195°C).

A 0.001% solution of mercury(II) in 0.01 *N* sulfuric acid was prepared by dilution of a 0.100% mercury(II) solution (0.1354 g of A.R. mercuric chloride/100 ml of *N* sulfuric acid) (*8*).

Technique

The instrument (a Unicam SP 600 spectrophotometer) was set to read 100% transmission with pure chloroform and to read zero with unbleached reagent as follows:

(a) The unbleached reagent was put in the light path, the transmission scale was set at zero, the instrument was switched to test and the dark current control was adjusted to balance the needle.

(b) The pure solvent was set in the light path and the needle was balanced with the slitwidth control to a 100% transmission.

(c) Operations (a) and (b) were repeated until the galvanometer balance was maintained with the reagent in the beam and zero transmission, pure solvent in the beam and a 100% transmission.

(d) The reagent was put in the light path after shaking with a standard mercury(II) solution with switch to "test" and the needle balanced with transmission control. A calibration curve of transmittance against concentration was prepared using the above technique.

Procedure

A suitable aliquot of a sample (25–100 ml) containing 0.05–0.2 mg of mercury(II) was taken in a separating funnel. The pH was adjusted to about 4 by the addition of dilute solutions of sodium hydroxide or acetic acid using universal indicator paper. Five ml of potassium hydrogen phthalate buffer (0.05 *M* solution) and 10 ml of 8×10^{-5} *M* solution of iron(III)–thione in chloroform was added and shaken for 30 seconds.

The phases were left to separate and some of the chloroform layer was run off through a plug of glass-wool in the stem of the funnel into a 1-cm glass cell. The bleaching of the organic layer was measured and the concentration of mercury was calculated by reference to the calibration curve which was prepared in the same way as the sample solution.

Calibration Curve

The data given in Table 1 were obtained for the calibration curve (Fig. 1) using standard mercury(II) solutions and 10 ml of 8×10^{-5} *M* reagent. This concentration represented the upper limit of the range of adjustment allowed by the dark current of the instrument. A reagent solution of 5×10^{-5} *M* was applicable to the determination of 0.02–0.1 mg of mercury under the same conditions.

Investigation of the optimum pH

The determination of 0.100 mg of Hg^{2+}/25 ml at different pH values (0.7–7) was carried out using sodium acetate–hydrochloric acid, pH 0.7–4.2 (*6*), potassium hydrogen phthalate, pH 4.0, and disodium hydrogen phosphate–citric acid buffers, pH 4.2–7. It was found that at pH range 2–6 the displacement reaction was fast and quantitative. The

TABLE 1

CALIBRATION DATA FOR MERCURY(II)

Mercury(II) taken (mg)	Transmittance (%)
0.025	5
0.050	12.6
0.075	22.5
0.100	30.0
0.125	39.0
0.150	52.0
0.175	61.5
0.200	76.5
0.225	85.5

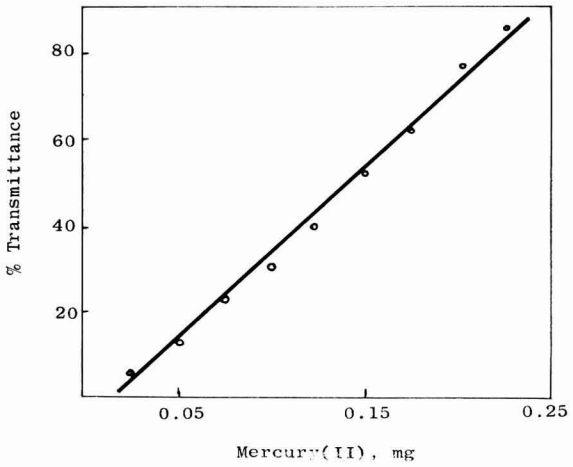

FIG. 1. Calibration curve for mercury(II), using a "trace analysis method."

% transmittance values were 30 ± 0.3. Therefore a pH value of 4.0 which is the middle of the working pH range was chosen as the optimum for the displacement reaction. At pH less than 2 the reaction was slow and the aqueous phase became blue (this may be due to the formation of $Fe^{+}(C_6H_4NOS)_2$, as the same blue color can be seen when a portion of reagent is diluted with concentrated HCl or H_2SO_4) and transmittance values were low. At pH 6–7 the iron(III)–thione is partly hydrolyzed and the transmittance values were high.

Interferences

The interfering effect of a combined total of 38 cations and anions were investigated on the determination of 0.100 mg of Hg^{2+}/25 ml of a sample solution (molarity of reagent 8×11^{-5}). It was found that the following ions: Mg(II), Ca(II), Sn(II), Ba(II), Al(III), Y(III), Ce(IV), Ti(IV), Zr(IV), Th(IV), V(V), Cr(III), Mn(II), Fe(II), Co(II), Ni(II), Pt(IV), Zn(II), Cd(II), Ga(III), Tl(III), Pb(II), As(III), Os(VI), W(VI), F^-, SO_4^{2-} PO_4^{3-}, Cl^-, did not displace iron(III) from Fe(III)–thione solution even when increased 1000-fold. The following seven metal ions: Hg(I), Cu(II), Bi(III), Sn(IV), Ag(I), Pd(II), Mo(VI) displace iron(III) and decreases the color of organic layer, i.e., the thione complexes of these metals are more stable than iron(III)–thione complex. In the presence of 0.1 *M* EDTA up to 20-fold excess of Cu(II) and Bi(III) had no effect. Sn(II) was masked with sodium fluoride and silver with chloride. In the presence of EDTA the tolerable weight ratios of Mo(VI) and Pd(II) to mercury ion,

TABLE 2

REPRODUCIBILITY OF THE DETERMINATION OF MERCURY

Mercury(II) (mg)	Transmittance (%)	Av	Av deviation
0.100	29.5, 30.0, 29.4, 30.0	29.72	±0.3
0.125	39.0, 38.6, 39.0, 38.8	38.85	±0.2

which gave an error of about 2%, were 2 and 0.6, respectively. Mercury(I), if present, can be oxidized to mercury(II) by the addition of nitric acid. Nitrite reduces the color of the reagent and thiocyanate masks the mercury and must be absent.

Reproducibility

Four identical determinations were carried out using 100 ml of standard mercury solutions containing 0.100 mg and 0.125 mg of mercury(II) and 10 ml of 8×10^{-5} *M* reagent according to the recommended procedure. Results are presented in Table 2.

Application of the method to the determination of the solubility product of mercurous chloride. About 1 g of A.R. Hg_2Cl_2 was shaken with 300 ml of distilled water for 1 hour; the container was left for 2 hours in a thermostat tank at 25 ±0.5°C. The solution was filtered through a Whatman No. 42 filter paper and a few drops of concentrated nitric acid were added to the filtrate to oxidize Hg_2^{2+} to Hg^{2+} ion. A duplicate determination was carried out according to the recommended procedure using 25 ml of 5×10^{-5} *M* reagent and 100 ml of filtrate. The concentration of Hg^{2+} ion was found to be 0.24 mg/liter or 1.2×10^{-8} mole/liter. Therefore, (Hg_2^{2+}), 6×10^{-7} and the solubility product of mercuric chloride becomes 8.6×10^{-19} (lit. value 1.1×10^{-18}) (*1*).

DISCUSSION

Most of the available absorption spectrophotometric reagents for the determination of mercury are not sufficently selective, e.g., diphenylcarbazone and dithizone (*7*). However, dithizone is probably the most useful reagent for the determination of mercury because of its high sensitivity. One objection to the dithizone method is the instability of the reagent solution. The present method is highly selective but less sensitive than the dithizone method. The reagent solution is stable. The method is applicable for the determination of traces of mercury in colored solutions and commercial acetic acid.

SUMMARY

A very selective spectrophotometric method is described for the determination of mercury(II) using iron(III)–thione as reagent. Selectivity is achieved by taking

advantage of the difference in the stabilities of metal–thione chelates and the use of masking agents.

The sensitivity of the method depends on the concentration of reagent used and the volume of sample. A 5×10^{-5} *M* reagent is applicable to the determination of 0.02–0.1 mg of Hg(II) in 100 ml of sample solutions.

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Spectrophotometric Determination of Iron with Orthophenanthroline

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Orthophenanthroline forms with ferrous iron in aqueous solution a soluble orange-red complex, stable between pH 2 and 9 (*10*). Measurement of the color intensity at 515–540 mμ has been widely used for the determination of traces of iron in reagent chemicals (*11*), biological materials (*5*), plant extracts (*1, 3*), and citric acid extracts from soils (*7*). Several reagents have been suggested for the reduction of ferric iron prior to formation of the complex. Of these, hydroxylamine hydrochloride has been considered to be the most efficient (*2*). However, for systems containing citrate, hydroxylamine hydrochloride has been variously reported to be unsuitable (*1*) and quite suitable (*7*).

The accuracy of the colorimetric determination of iron with orthophenanthroline depends on (a) completeness of reduction of Fe^{3+} to Fe^{2+}, (b) pH of iron solution before complex formation, (c) stability of the colored complex, and (d) interference of other ions in the formation of the color.

Several reagents have been tried as reductants. Sodium dithionite, though reducing iron completely, introduces turbidity in the test solution within a few minutes (*5*). Sodium sulfite and formaldehyde complex with Fe^{3+} and thus cause considerable error in the determination (*2*). Sodium and potassium formates lead to error due to formic acid complexing with Fe^{3+} (*2*).

The ferrous iron–orthophenanthroline complex, once formed, is stable even up to 15 days, and has constant color intensity between pH 2 and 9 (*10, 9*). It has been found that the intensity of the color increases with time if the pH of the iron solution is adjusted to a value >4 before reduction at room temperature with hydroxylamine hydrochloride, indicating incompleteness of reduction (*7*). Complete reduction of the iron can, however, be achieved in such solutions by heating the solution with the reductant and a pH 3.5 acetate buffer for 1 hour at 65°C (*7*).

It has been reported that most anions and cations, up to a concentra-

tion 250 times that of iron, do not interfere with iron determination by orthophenanthroline (*2*). No common soil ions except orthophosphate are considered to interfere in the determination (*6*).

The methods recommended by earlier workers are time consuming and difficult to apply when the number of samples is large. It was, therefore, considered necessary to develop a rapid and reliable modification of the method. Thioglycolic acid has been successfully used to reduce iron to eliminate interference in the colorimetric determination of zirconium (*4*). It was thought worthwhile to investigate the suitability of thioglycolic acid as reductant in the spectrophotometric determination of iron with orthophenanthroline. The procedure so developed is presented below.

MATERIALS AND METHODS

Iron standards were obtained by dilution of 100 ppm iron stock solution prepared from spectroscopically pure ferric oxide. A Lumetron Model 402-E photoelectric colorimeter with a narrow band 515 mμ monochromatic filter was used for measurement of transmittance of the solutions after development of color.

Proposed method. A suitable aliquot (<150 μg of Fe) of the solution is taken in a 50-ml volumetric flask and 10 ml of (pH 3.5) *M* sodium citrate–citric acid buffer, followed by 1 ml of 4% thioglycolic acid, is added. The contents of the flask are mixed well, 2 ml of 0.4% orthophenanthroline are added, and the volume is made up to 50 ml. Transmittance of the solution is measured at 515 mμ after 5 minutes. A standard curve in the range 0–150 μg of Fe is prepared in the same way using aliquots of standard iron solution, and the iron content of the test solution is read from the curve.

RESULTS AND DISCUSSION

The optical densities obtained with different amounts of iron are presented in Table 1 and show that Beer's law is obeyed in the range 0–3 ppm Fe. Development of color was instantaneous, and the color of the complex was stable for 72 hours.

Effect of pH

Aliquots of standard iron solution representing 62.5 μg of Fe were buffered with 15 ml of citrate–citric acid buffers of different pH values, and the amount of iron was determined by the proposed method. Table 2 gives the amount of iron recovered from each solution and the final pH of the solution. Complete recovery in all cases showed that reduction by thioglycolic acid was complete at all the pH values studied. In

TABLE 1

OPTICAL DENSITIES OF STANDARD IRON SOLUTIONS AT 515 mμ USING THE PROPOSED METHOD

No.	Final iron conc (ppm)	Optical density
1	0.25	0.042
2	0.50	0.084
3	1.00	0.168
4	1.50	0.248
5	2.00	0.333
6	2.50	0.415
7	3.00	0.495

TABLE 2

EFFECT OF FINAL pH OF SOLUTION ON THE DETERMINATION OF IRON

No.	Amount of iron taken (μg)	pH of buffer added	Final pH of solution	Amount of iron found (μg)
1	62.5	3.1	3.2	63.0
2	62.5	4.1	4.3	63.0
3	62.5	5.3	5.5	62.5
4	62.5	6.0	6.2	63.0
5	62.5	7.3	7.0	63.0

the proposed method, however, addition of pH 3.5 buffer is recommended to prevent the possible precipitation of phosphates from test solutions.

Aliquots of standard iron solution representing 50 μg of Fe were adjusted to different pH values between 2 and 9 by predetermined aliquots of 0.1 *N* NaOH and 0.1 *N* HCl. The iron in the solutions was then determined by the present method and the methods of Olson (*9*) and Krishna Murti *et al.* (*7*). The results, given in Table 3, show that complete recovery of iron was obtained using the method of Krishna Murti *et al.* (*7*) and the present method. Olson's (*9*) method gave extremely low values for iron when the pH of the solution was greater than 2. In a solution of pH higher than 4, the iron forms hydroxylated complexes which are difficult to reduce (*7*). Hydroxylamine hydrochloride is capable of reducing the iron only with heating at 65°C for 1 hour (*7*). Thioglycolic acid, however, reduced the iron instantly at room temperature at any pH.

Interference of Other Ions

Suitable aliquots representing various concentrations of several other ions in the final solution were taken with 50 μg of iron and the amount of iron was determined by the proposed method. No interference of Ca (10,000 ppm), Mg (1200 ppm), Al (150 ppm), Ti (80 ppm), Zr (50 ppm), PO_4 (30 ppm), Mn (20 ppm), Ba (20 ppm), Zn (20 ppm), Cu (2 ppm), and Ni (0.6 ppm) was found in the determination of iron with the proposed method.

Free Iron Oxides Extracted from Soil Clay

The free iron oxide content in the clay fraction (<2 μ of two soils from Madhya Pradesh, India, were extracted by the dithionite–citrate–bicarbonate method of Mehra and Jackson (*8*). Suitable aliquots of the extract were taken and the iron was determined by the present method and the method of Krishna Murti *et al.* (*7*). The amounts of iron obtained are presented in Table 4. The closeness of the values show that the proposed method is reliable and accurate for such determinations.

TABLE 3

EFFECT OF INITIAL pH OF IRON SOLUTION ON THE DETERMINATION OF IRON BY VARIOUS METHODS

No.	Amount of iron taken (μg)	Initial pH of iron solution [a]	Amount of iron found (μg)		
			Olson (1965)	Krishna Murti *et al.* (1966)	Proposed method
1	50.0	2.0	50.0	50.0	50.0
2	50.0	4.0	20.5	49.5	50.0
3	50.0	6.0	15.0	50.0	50.0
4	50.0	9.0	0	49.5	50.0

[a] pH adjusted by 0.1 *N* HCl or 0.1 *N* NaOH.

TABLE 4

DETERMINATION OF IRON IN EXTRACTS OF FREE IRON OXIDES FROM CLAY FRACTIONS OF SOILS

No.	Clay sample	Amount of iron found (μg)	
		Proposed method	Krishna Murti *et al.*
1	Umaria	97.5	96.5
2	Raogarh	64.5	64.5

SUMMARY

A modified procedure is presented for the spectrophotometric determination of iron by orthophenanthroline. Reduction of iron prior to development of color is accomplished by thioglycolic acid, and is instantaneous at room temperature. The system is buffered at pH 3.5 with sodium citrate–citric acid to avoid precipitation of hydroxides and phosphates from the test solution. Moderate amounts of Ca, Mg, Al, Ti, Zr, PO_4, Mn, Ba, Zn are permissible but more than 2 ppm of Cu and 0.6 ppm of Ni interfere.

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A Direct Comparison of the Pregl, Dumas, Perkin-Elmer, and Hewlett-Packard(F&M) Carbon–Hydrogen–Nitrogen Procedures [1]

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INTRODUCTION

About 8 years ago there appeared on the market a number of automatic instruments capable of determining the amount of carbon, hydrogen, and nitrogen in organic compounds. These instruments were greeted with a great deal of interest, and some skepticism, by microanalysts because if they proved to be effective they would ease the burdening work load that most analysts were feeling. Up until this time all carbon, hydrogen, and nitrogen determinations had to be made "by hand," a rather tedious procedure involving hot furnaces, gas burners, etc. Within a span of a few years about a half-dozen instruments were introduced which included the Fisher Scientific Company carbon–hydrogen–nitrogen analyzer based on the work of Sundberg and Maresh (*9*), the F&M 180 put out by the F&M Corporation on the work of Hinsvark and Beltz (*6*), the Aminco Carbon and Hydrogen Analyzer from the American Instrument Company, the Technicon C-H-Nalyzer based on the work of Walisch (*10*), the Perkin-Elmer 240 from the work of Simon *et al.* (*8*), and the F&M 185 from the F&M Scientific Corporation. Of these only the Perkin-Elmer 240 and the F&M 185, which came out in 1965, were fully accepted.

MATERIALS AND METHODS

Since we were fortunate enough to have both of these instruments on hand we thought it might be of interest to run a comparative study between these instruments and the older established procedures. Consequently, a number of rather typical compounds were analyzed by the classical Pregl carbon–hydrogen method, the Dumas nitrogen method using the Coleman nitrogen analyzer, the F&M 185 now known as the Hewlett-Packard CHN Analyzer, and the Perkin-Elmer 240 CHN Analyzer. Each sample was submitted as an unknown at random times

[1] Presented at the Pittsburgh Conference of Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 7, 1969.

to each procedure until at least three analyses were accomplished. The samples were mixed in with the daily work but every effort was made to insure that each instrument was operating properly. The entire program lasted about 6 months and included the work of eight analysts. The results both good and bad are presented just as we found them.

RESULTS

As can be seen from the raw data all the results (Table 1) are comparable with one exception which is quite significant. The compound naphthalene, which has a low melting point (80°C) and a high vapor pressure volatilizes from the open boat during the purge or flush cycle of the F&M and Perkin-Elmer procedures. Consequently some of the sample is lost prior to the actual combustion leading to low and erratic results. The same thing will happen in a slightly different manner to hydrates, that is, the water will be driven off leading to low hydrogen results. Anyone operating these instruments should keep this in mind and make all allowances for such.

In a study of precision (Table 2) it appears from the standard deviation that all the results are within the 0.3% allowable error which is usually accepted in organic microanalysis. On a comparative basis the Perkin-Elmer appears slightly more precise on carbon and hydrogen than the other procedures but the other methods do a little better on nitrogen. As a point of interest, the standard deviations found for the Perkin-Elmer agree very closely to those calculated by Clerc and Simon (*2*). The range (the highest reading above average minus the lowest reading below average) gives an indication of the dispersion, and of course the smaller the dispersion the better. Again the Perkin-Elmer seems to do better on carbon and hydrogen while not so well for nitrogen. In a comparison of accuracy (Table 3) one can see than percentage-wise the Pregl method checks more of the samples within 0.3% than the other procedures. However, a look at the average deviation or mean error (ignoring the sign) shows that the Perkin-Elmer is slightly more accurate than the other methods. (Allowances were made here for naphthalene since it is not really a typical sample.) Again all the results are well within limits and are really quite acceptable. Statistically, and generally speaking, it appears that the Perkin-Elmer 240 is somewhat better for carbon and hydrogen while the Coleman Dumas procedure is slightly better for nitrogens. However, when one considers the fact that neither the Perkin-Elmer or F&M analyzed naphthalene properly, and would do the same with similar compounds, one must conclude that the Pregl–Dumas procedures are really the most reliable. However, it must be pointed out that both of the instruments will do at least twice the work

Table 1 COMPARATIVE RESULTS OF A NUMBER OF TYPICAL ORGANIC COMPOUNDS

Sample		Pregl-Dumas			Perkin-Elmer			Hewlett-Packard(F&M)		
		C	H	N	C	H	N	C	H	N
p-Chlorobenzoic Acid		53.74	3.37	0	53.59	3.24	0	53.57	3.04	0
		53.24	3.27	0	53.78	3.30	0	53.80	3.08	0
		53.60	3.52	0	53.80	3.29	0	53.86	3.34	0
	Average	53.53	3.39		53.79	3.28		53.74	3.15	
	Calcd	53.69	3.22		53.69	3.22		53.69	3.22	
Phenacetin		67.11	7.63	7.90	67.12	7.25	7.95	66.93	7.36	7.88
		67.45	7.46	7.68	66.82	7.19	7.80	66.93	7.51	7.88
		67.35	7.51	7.69	67.35	7.35	7.89	66.65	7.32	7.88
		67.39	7.59							
	Average	67.33	7.55	7.78	67.10	7.26	7.88	66.84	7.40	7.88
	Calcd	67.01	7.30	7.81	67.01	7.30	7.81	67.01	7.30	7.81
Cystine		30.04	4.91	11.75	30.26	4.92	11.41	29.85	5.17	11.65
		30.01	5.10	11.49	30.24	5.03	11.60	30.05	5.30	11.41
		30.07	5.12	11.70	30.06	4.97	11.56	29.99	4.99	11.41
	Average	30.04	5.04	11.65	30.19	4.97	11.52	29.96	5.15	11.49
	Calcd	29.99	5.03	11.66	29.99	5.03	11.66	29.99	5.03	11.66
Anthracene		93.67	5.77	0	94.70	5.81	0	94.17	5.86	0
		93.79	5.77	0	94.46	5.72	0	94.73	5.75	0
		94.13	5.82	0	94.46	5.79	0	94.45	5.46	0
		94.53	6.08	0						
	Average	94.03	5.86		94.54	5.77		94.45	5.69	
	Calcd	94.34	5.66		94.34	5.66		94.34	5.66	
8-Hydroxyquinoline		74.13	4.89	9.84	74.78	4.96	9.96	74.16	4.99	9.69
		74.20	5.01	9.71	74.82	4.95	9.64	74.16	4.84	9.69
		74.20	4.85	9.50	74.58	5.01	9.50	74.44	4.69	9.69
		74.45	5.03		74.50	4.97	9.77			
	Average	74.25	4.95	9.68	74.67	4.97	9.72	74.25	4.84	9.69
	Calcd	74.46	4.86	9.65	74.46	4.86	9.65	74.46	4.86	9.65
p-Fluorobenzoic Acid		59.79	3.66	0	60.04	3.68	0	60.32	4.29	0
		60.69	3.85	0	59.84	3.50	0	60.21	3.63	0
		60.18	3.77	0	59.90	3.70	0	59.93	4.04	0
	Average	60.22	3.76		59.93	3.63		60.15	3.99	
	Calcd	60.01	3.60		60.01	3.60		60.01	3.60	

in the same time span as the older procedures and will use less than one-fifth the sample. This is a very important feature and one of the main advantages of the instrumented methods.

DISCUSSION

In comparing the two instruments there are a number of points to consider. The F&M is a much simpler instrument than the Perkin-Elmer

Table 1 - Page 2

Sample	Pregl-Dumas			Perkin-Elmer			Hewlett-Packard(F&M)		
	C	H	N	C	H	N	C	H	N
Naphthalene	93.65	6.66	0	91.98	6.33	0	88.13	5.54	0
	93.26	6.57	0	91.74	6.23	0	88.83	5.37	0
	93.92	6.52	0	92.16	6.33	0	89.61	5.32	0
Average	93.61	6.52		91.96	6.30		89.52	5.41	
Calcd	93.71	6.29		93.71	6.29		93.71	6.29	
Cyclohexanone-2,4-dinitrophenyl hydrazone	51.89	4.99	20.08	51.78	5.14	20.42	51.81	5.79	19.93
	51.90	4.87	19.84	51.81	5.10	20.63	51.77	4.73	19.83
	51.89	4.98	20.13	51.79	5.15	20.14	51.61	5.30	19.83
Average	51.89	4.95	20.02	51.79	5.13	20.40	51.73	5.27	19.86
Calcd	51.79	5.07	20.14	51.79	5.07	20.14	51.79	5.07	20.14
Acetanilide	71.18	6.55	10.41	71.27	6.80	10.45	70.54	6.64	10.26
	71.12	6.91	10.37	71.04	6.76	10.41	70.70	6.75	10.36
	71.08	6.73	10.46	70.70	6.73	10.33	70.54	6.93	10.36
Average	71.13	6.73	10.41	71.00	6.76	10.40	70.59	6.77	10.33
Calcd	71.09	6.70	10.36	71.09	6.70	10.36	71.09	6.70	10.36
Nicotinic Acid	58.60	4.19	11.43	58.48	4.07	11.56	58.24	3.83	11.25
	58.54	4.22	11.57	58.54	4.17	11.23	58.24	4.05	11.25
	58.25	4.06	11.75	58.74	4.21	11.34	58.41	3.68	11.41
Average	58.46	4.16	11.58	58.58	4.15	11.38	58.30	3.85	11.30
Calcd	58.53	4.09	11.38	58.53	4.09	11.38	58.53	4.09	11.38
S-Benzyl-thiuronium HCl	47.60	5.60	14.08	47.19	5.40	14.06	47.32	5.36	13.96
	47.14	5.34	13.89	47.40	5.41	13.92	47.58	5.83	13.67
	47.51	5.27	14.21	47.37	5.48	13.72	47.31	5.87	13.67
Average	47.41	5.40	14.06	47.32	5.43	13.93	47.40	5.69	13.77
Calcd	47.40	5.47	13.82	47.40	5.47	13.82	47.40	5.47	13.82
House Standard CHNO	71.36	6.01	8.42	70.93	5.92	8.05	71.10	6.12	8.21
	71.70	5.92	8.59	71.16	6.00	8.23	71.66	6.23	8.37
	71.83	5.90	8.36	71.39	6.05	8.51	71.10	5.97	8.21
Average	71.63	5.94	8.46	71.16	5.99	8.26	71.41	6.11	8.32
Calcd	71.41	5.99	8.32	71.41	5.99	8.32	71.41	5.99	8.32
Phenyl-thiourea	55.41	5.45	18.65	54.59	5.24	18.41	55.28	5.33	18.04
	55.42	5.34	18.56	55.47	5.34	18.71	55.39	5.23	18.01
	55.30	5.30	18.42	55.24	5.32	18.55	55.57	5.57	18.07
Average	55.38	5.36	18.51	55.35	5.30	18.56	55.41	5.38	18.04
Calcd	55.23	5.29	18.41	55.23	5.29	18.41	55.23	5.29	18.41

but the very nature of the latter makes it complicated. As usual, every advantage is offset to some degree by a disadvantage. One advantage of the F&M is its simplicity but this is offset by its readout system. An

Table 1 - Page 3

Sample	Pregl-Dumas C	Pregl-Dumas H	Pregl-Dumas N	Perkin-Elmer C	Perkin-Elmer H	Perkin-Elmer N	Hewlett-Packard(F&M) C	Hewlett-Packard(F&M) H	Hewlett-Packard(F&M) N
House Standard CHN	54.56	5.02	40.32	54.52	5.14	39.80	54.52	4.93	39.78
	54.51	5.30	40.52	55.01	5.25	40.73	55.08	5.16	40.50
	55.01	5.27	40.58	54.98	5.13	40.60	54.97	5.36	40.11
	55.02	5.35	40.46	54.70	5.10	40.63			
			40.38						
Average	54.78	5.24	40.45	54.80	5.16	40.44	54.86	5.15	40.13
Calcd	54.84	5.18	39.98	54.84	5.18	39.98	54.84	5.18	39.98
House Standard CHNOSCl	34.89	4.19	9.06	34.39	4.04	8.89	34.59	4.51	8.79
	34.69	4.16	9.20	34.57	4.15	8.96	34.42	4.50	8.79
	34.46	4.21	8.96	34.60	4.04	8.88	34.31	3.98	8.72
Average	34.68	4.19	9.07	34.52	4.08	8.91	34.44	4.33	8.77
Calcd	34.55	4.19	8.95	34.55	4.19	8.95	34.55	4.19	8.95
House Standard CHNOSCl	33.33	2.06	2.49	33.13	1.93	2.51	33.62	2.19	2.62
	33.38	1.73	2.57	33.38	1.92	2.48	33.57	2.37	2.62
	33.14	2.04		33.38	1.96	2.58	33.17	1.99	2.62
Average	33.28	1.94	2.53	33.30	1.94	2.52	33.45	2.18	2.62
Calcd	33.27	1.86	2.59	33.27	1.86	2.59	33.27	1.86	2.59
House Standard CHNO	55.24	4.56	18.73	55.09	4.76	18.91	55.53	4.93	18.66
	55.58	4.53	18.71	55.49	4.83	18.84	55.94	4.80	18.74
	55.58	4.61	18.70	55.20	4.75	18.75	55.94	5.08	18.60
Average	55.47	4.57	18.71	55.26	4.78	18.83	55.80	4.94	18.67
Calcd	55.62	4.67	18.54	55.62	4.67	18.54	55.62	4.67	18.54
House Standard CHNO	43.90	2.97	41.58	(43.72)	3.02	41.66	43.88	2.70	40.09
	44.24	3.12	41.62	44.42	3.15	41.63	43.88	2.77	40.11
	44.13	2.96	41.76	44.47	3.14	41.87	44.00	2.89	40.57
				44.18	2.85	41.87			
Average	44.09	3.02	41.65	44.36	3.04	41.76	43.92	2.79	40.26
Calcd	44.12	2.96	41.17	44.12	2.96	41.17	44.12	2.96	41.17
House Standard CHNO	60.26	5.89	14.58	59.51	6.01	14.40	60.31	5.94	14.27
	59.77	5.70	14.47	59.86	5.95	14.15	60.87	6.08	14.27
	60.32	6.19	14.12	59.52	5.99	14.06	60.31	6.08	14.27
	59.96	5.88	14.25						
Average	60.08	5.92	14.36	59.63	5.98	14.20	60.50	6.03	14.27
Calcd	59.99	6.04	13.99	59.99	6.04	13.99	59.99	6.04	13.99

advantage of the Perkin-Elmer is its readout system which is offset by its complexity. In order to produce a more accurate readout Simon *et al.* (*8*) developed what they called a self-integrating system which im-

TABLE 2

PRECISION

	Standard Deviations		
	Pregl–Dumas	Perkin–Elmer	Hewlett–Packard (F&M)
Carbon	0.1854	0.1486	0.1742
Hydrogen	0.0999	0.0523	0.1823
Nitrogen	0.1099	0.1691	0.1177
	Range (max X − min X)		
Carbon	0.93	0.57	0.71
Hydrogen	0.49	0.30	0.84
Nitrogen	0.46	0.93	0.72

TABLE 3

ACCURACY

	Checks within 0.3% calc values (%)		
	Pregl–Dumas	Perkin–Elmer	Hewlett–Packard (F&M)
Carbon	78	72	67
Hydrogen	95	95	75
Nitrogen	76	77	80
	Av deviation from calc values		
Carbon	0.2000	0.1756	0.2203
Hydrogen	0.1376	0.0788	0.2016
Nitrogen	0.2090	0.2206	0.2028

proved the linear range but necessitated all the valves, detectors, traps, etc. This is described very nicely by Condon (*1*). Here the plateau readings are easily taken directly from the chart to within 1 μV. On the other hand with the F&M the peak heights are measured with a ruler, which leaves something to be desired. For the best results the peaks have to be measured within 0.1 mm and if, for example, the base line is questionable it is very difficult to get a meaningful measurement. The F&M tends to get around this by use of what they call ratio recording. This is a clever arrangement in which the balance is tied in with the recorder so that the weight dial modifies the recorder response to compensate for the sample weight. In this way the unknowns are calculated on the peak height ratios between the standard and the unknown providing the proper sample size is used. The advantage is that the sample weights do not have to be recorded, but the disadvantage lies with the

accuracy of the standard. If that is wrong all of the unknowns will be wrong. Most analysts run several standards and average the peak heights, but the most accurate procedure is to compare peak areas since the peak height becomes unlinear above 0.6 mg of carbon which of course presents serious limitations. The Perkin-Elmer for its calculations uses sensitivity factors based on the total signals and the theoretical weights of the three elements. This had the advantage of consistency, that is, the factors can be checked from day to day to see if there is any change. In our experience the factors are usually so consistent any change indicates there is something wrong with the instruments. This makes the total calculations slightly more complicated but the accuracy seems to be better. Of course the same procedure can be used on the F&M, that is, by using the peak area and the calculated weight of the element, but this is quite slow and time consuming. Gustin and Tefft (*5*) and Graham (*4*) have eliminated most of these problems by electronically integrating the signals from both of these instruments with excellent results.

Another point to consider is the sample size. Generally speaking the larger the sample the more accurate the results. There are several reasons for this. The larger sample is liable to represent a better cross section of the material to be analyzed, and the weighing error is reduced. The same holds true, of course, in microanalysis. The recommended sample size for the F&M is about 0.6 to 0.8 mg while for the Perkin-Elmer the best size is around 1.5 to 2.0 mg. Here, then, is a comparative source of error. Besides a possible sampling error there is a factor of two in the weighing error. Some microanalysts grind each sample to reduce the sampling error, but even under good conditions the Cahn gram electrobalance cannot be expected to give weighings closer than ± 3 μg (in these laboratories we find it closer to ± 5 μg) so there is a built in error with either procedure. For example, if we consider an organic compound with a value of 50% carbon, which is the most significant determination, with the Perkin-Elmer using a sample weight of 1.500 mg a total weighing error of just 6 μg would result in a carbon error of 0.20%. While with the F&M using the same sample but a weight of 0.700 mg the same weighing error would result in a carbon error of 0.43% (using the carbon calibration curve).

Since the two instruments are of entirely different design, the sample combustion problems are different also. The Perkin-Elmer provides for a static combustion under pure oxygen, which is very effective for most compounds (*3*) while the F&M uses an oxygen donor catalyst such as MnO_2 and WO_3. The catalyst has been one of the main stumbling blocks in the successful use of the F&M, in fact, it has been found that most of the troubles with this instrument can be attributed to this source. Scheidl

(7) and others have made many improvements here, and this point cannot be overemphasized, to get good results with the F&M one must use a good catalyst.

SUMMARY

In summary, the data gathered at these laboratories indicate that the classical Pregl and Dumas procedures, although slower, give the most reliable results followed by Perkin-Elmer and the F&M (Hewlett-Packard) in that order. Acceptable results, however, can be achieved by any of the four procedures. Operationally the Pregl and Dumas have been trouble-free, the F&M nearly so except for the catalyst, while the Perkin-Elmer has shown a considerable amount of down time. Under normal conditions, however, it is easier to get acceptable results from the Perkin-Elmer than it is from the F&M.

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The Preparation and Indirect Spectrophotometric Determination of Total Oxidizing Capacity of Chlorine Dioxide in Acidic Solution

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INTRODUCTION

Most methods of preparing chlorine dioxide yield a product contaminated with chlorine and other undesirable oxidants (*1*). Consequently, before serious investigations of the kinetics, reactions, and properties of chlorine dioxide can be undertaken, tedious and time-consuming separations are required to commence the actual studies. A rapid and simple laboratory scale electrolytic method of preparing chlorine dioxide, free from other oxidants, has been developed.

Spectrophotometric methods for the determination of chlorine dioxide are frequently limited by low sensitivity or nonspecificity. In many applications, both sensitivity and specificity must be compromised. Several methods have been based on the direct measurement of the ultraviolet absorbance of chlorine dioxide (*2–5*) but for many applications the sensitivity of these methods is inadequate. In the field of water treatment a number of methods have been based on the production of a blue-green product resulting from the reaction of chlorine dioxide and *o*-tolidine (*6–10*) while still other methods have been based on the interaction of chlorine dioxide and tyrosine (*11, 12*). The oxidation of 8-napthol-3,6-disulfonic acid has also been utilized in the determination of chlorine dioxide (*13*). A method based on the oxidation of acid chrome violet K with chlorine dioxide has been reported (*14*). A very sensitive, but not highly specific, indirect spectrophotometric method for the determination of chlorine dioxide in acidic solution based on its

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reaction with excess iron(II) ion, followed by the development of the tris-1, 10-phenanthroline iron(II) complex and its subsequent differential absorbance measurement is described in this paper.

MATERIALS AND METHODS

Reagents

Iron(II) perchlorate solution. Dissolve 326 mg of $Fe(ClO_4)_2 \cdot 6H_2O$ in 300 to 400 ml of distilled water, add approximately 1.5 ml of concentrated sulfuric acid and dilute to 1 liter.

1,10-phenanthroline solution. Dissolve 1.0 g of 1,10-phenanthroline monohydrate in 300 ml of water, heating if necessary to effect solution, and dilute to 1 liter.

Sulfuric acid solution. Dilute 33.3 ml of concentrated sulfuric acid (sp gr 1.84; 95.5% H_2SO_4 by wt) to 1 liter with distilled water.

Saturated sodium acetate solution. Add 1200 g of anhydrous sodium acetate to 500 ml of distilled water, dilute to 1 liter and mix thoroughly to insure saturation.

Sodium chlorite solution. Dissolve approximately 9.05 g of "Analytical Reagent" sodium chlorite (Matheson) in 500 ml of distilled water and dilute to 1 liter. Do not attempt to oven dry this reagent since it may undergo violent decomposition.

Standard chlorine dioxide solution. This solution is prepared by the electrolysis of the sodium chlorite solution. It should be stored in an amber bottle at approximately 4°C. Standardization of this reagent may be effected by acidification with 3–5 ml of glacial acetic acid in the presence of excess potassium iodide followed by titration with 0.1 *N* thiosulfate solution to the disappearance of the starch–iodine color. Due to the instability and volatility of the chlorine dioxide solution, standardization should be made at least once a day or oftener depending upon the temperature of the solution and the frequency of its use. When stored at 4°C the main loss of titer is attributed to the volatility of the chlorine dioxide.

Apparatus

A diagram of the chlorine dioxide generation apparatus is shown in Fig. 1. The electrolysis cell consists of a 250-ml bottle with a T 34/28 ground glass neck into which the electrolysis and gas purging systems are inserted. The insert contains an 8-mm o.d. gas dispersion tube in the center, which is encompassed by a platinum gauze electrode sealed through the top of the insert. To one side of the gas dispersion tube is an isolated cell compartment constructed by sealing to the insert a 13-

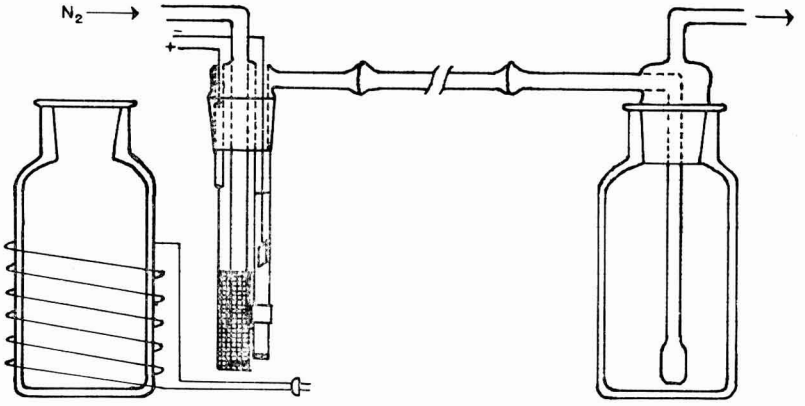

FIG. 1. Chlorine dioxide generation apparatus.

mm o.d. sealing tube which has had its lower half removed just below the sintered glass disk. A salt bridge, made from a 1 inch section of 13-mm o.d. sealing tube, is connected to the isolated cell compartment by means of a short section of Tygon tubing. In addition to the gas inlet, the anode connection, and the opening to the isolated cathode cell in the top of the insert, a gas outlet opening is made with 10-mm o.d. Pyrex tubing. This gas outlet is connected to the scrubbing bottle assembly by means of two S 18/7 ground glass ball joints. The anode is simply a platinum gauze electrode while the cathode was constructed from a 0.25 cm^2 platinum foil which is inserted into the cathode cell compartment containing a saturated potassium chloride solution. The salt bridge is filled with 5% agar gel saturated with potassium chloride. A Leeds and Northrup Co. Coulometric Analyzer, Catalog No. 4960, was used as the current source. A heating mantel was constructed around the lower half of the generation cell in order to decrease the solubility of the generated chlorine dioxide. This is accomplished by wrapping the bottle with 5.5 ft of chromel wire, 2.52 ohms/ft, and covering the wire with asbestos tape. Temperature control of the generation cell is maintained by means of a Variac. The scrubbing bottle is immersed in an ice bath in order to increase the solubility of the chlorine dioxide. A cylinder of "High Purity" nitrogen (General Dynamics) was used as the purging gas. A two-stage reducing valve was used to reduce the tank pressure to approximately 10 psi and a needle valve was used to control the flow rate of the sweep gas.

Spectrophotometric measurements were made using either a Cary Model 14 or a Beckman Model DU spectrophotometer. All measurements were made in 1.000-cm silica cells.

General Procedure

Preparation of standard chlorine dioxide solution. Aqueous solutions of chlorine dioxide were prepared by placing approximately 200 ml of a 0.1 *M* solution of sodium chlorite in the generation cell and an equal volume of 0.1 to 0.09 *M* sulfuric acid in the scrubbing bottle. With one of the ball joints open, nitrogen is purged through the system to remove any possible volatile decomposition products of sodium chlorite. During the prepurging operation the temperature controls on both the generation system and the scrubbing bottle should be allowed to equilibrate for approximately 30 minutes. The temperature of the generating cell should be maintained between 75 and 80°C. After the preconditioning procedures have been completed, the system is made air tight and the coulometer may then be turned on. Passage of a constant current of 64.3 mA for approximately 30 minutes produces solutions of approximately 150 ppm of chlorine dioxide. Higher efficiencies can be achieved by using more efficient scrubbing systems. A controlled potential coulometric electrolysis in conjunction with quantitative absorption could permit generation and standardization in a single step.

Determination of chlorine dioxide. To a 100-ml volumetric flask containing 30 ml of 0.6 *M* sulfuric acid solution, pipet 10.00 ml of 0.0009 *M* iron(II) perchlorate solution. Transfer an aliquot of chlorine dioxide solution containing from 0.7 to 70 μg of chlorine dioxide to the flask containing the sulfuric acid and iron(II) perchlorate solutions. Mix thoroughly and allow 2 minutes for the reaction to reach completion; then add 10 ml of saturated sodium acetate solution and 10 ml of 0.1% 1,10-phenanthroline solution. Mix thoroughly and dilute to volume. Prepare a reagent blank in a similar manner omitting the aliquot of chlorine dioxide solution.

Measure the differential absorbance at 510 nm by placing the reagent blank in the sample beam and the sample containing the aliquot of chlorine dioxide in the reference beam. The concentration of chlorine dioxide may be read from a calibration graph, previously constructed from data obtained from the standard chlorine dioxide solution, or by computation from the Beer-Lambert relationship. It should be noted, however, that the quantity measured and plotted is the differential absorbance, ΔA, and is directly proportional to the chlorine dioxide concentration.

RESULTS

Reactions

The reaction of chlorine dioxide with iron(II) produces a fivefold molar enhancement due to the particular oxidation states of the reac-

tants. This reaction was found to be rapid and stoichiometric when carried out in solutions of sulfuric acid which were 0.1 *M* or greater. The fivefold molar enhancement in conjunction with the high molar absorptivity of the tris-1,10-phenanthroline iron(II) complex produces absorbance measurements which are very sensitive to variations in chlorine dioxide concentrations. The direct reaction of chlorine dioxide with the complexed iron(II) species was found to be too slow to be of any practical value. The variables affecting the colored species have long been established (*15*). Several advantages of the differential absorption measurement have been illustrated (*16*).

Chlorine Dioxide Concentration

The optimum concentration range, as determined from a Ringbom plot, was found to be from 135 to 700 ppb. Conformity to Beer's law was observed from 0 to 700 ppb (Fig. 2). The effective molar absorptivity for chlorine dioxide at 510 nm was found to be 5.5×10^4 liters/mole-cm.

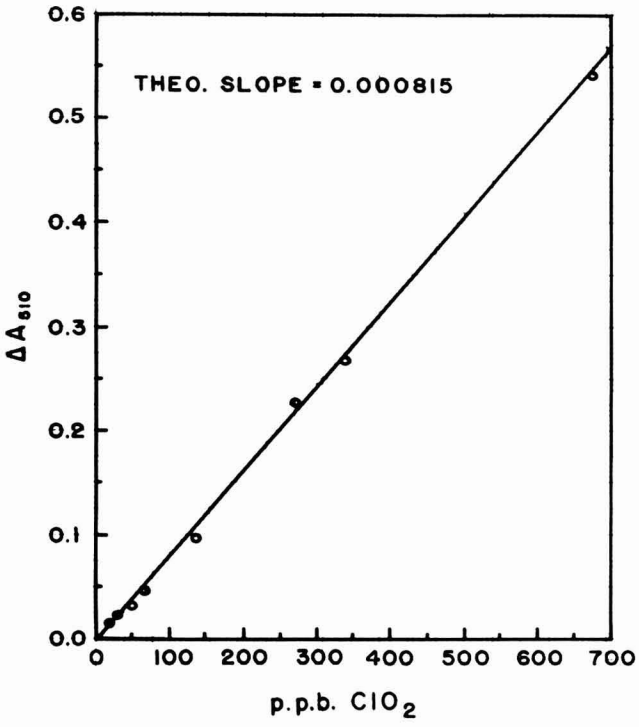

FIG. 2. Calibration graph for the determination of chlorine dioxide.

Effects of pH

The adjustment of pH for the chlorine dioxide–iron(II) reaction was not found to be critical provided the hydrogen ion concentration was in excess of 0.2 *M*. Lower hydrogen ion concentrations exhibited slower reaction rates. Excessive hydrogen ion concentrations are to be avoided in order to prevent the use of large volumes of saturated sodium acetate solution for buffering the tris-1,10-phenanthroline iron(II) solution.

The color intensity of the tris-1,10-phenanthroline–iron(II) complex has been found to be independent of pH in the range of 2 to 9 (*17*). Consequently, the amount of saturated sodium acetate added for buffering was not found to be critical.

Reagent Concentration

It was found that if the iron(II) concentration exceeded 5.0 ppm in the reagent blank, relatively large slit widths were encountered, unless the chlorine dioxide concentration was significantly greater than 500 ppb in the sample. It is therefore necessary to maintain the relative amounts of chlorine dioxide and iron(II) in the reagent blank and the sample in such a ratio as to be consistent with reasonable slit widths.

Precision

A summary of a statistical study of typical data obtained from the proposed method may be found in Table 1. The principle restrictions on the degree of precision obtained appear to arise from two sources. The high degree of volatility of chlorine dioxide and its lack of stability tend to give rise to errors in both the calibration procedure as well as the analysis itself. More effective solution transfer techniques would obviously tend to minimize such errors.

Effect of Diverse Ions

One thousand ppm of the following ions did not cause interference in the determination of 120 ppb of chlorine dioxide: sodium, potassium, ammonium, magnesium, calcium, aluminum, chloride, perchlorate, acetate, nitrate, dihydrogen phosphate, sulfate, and boric acid. Those ions which caused interference are listed in Table 2 with their permissible concentration limits.

As can be seen from Table 2 chlorine exhibits a significant interference. However, malonic acid has been reported to be an effective masking agent for chlorine while in a 20-fold excess of chlorine (*13*). It was found that the chlorine interference could be significantly reduced, if not completely eliminated by the addition of malonic acid to the sample

TABLE 1

STATISTICAL STUDY OF THE FERROIN METHOD FOR THE DETERMINATION OF CHLORINE DIOXIDE

Detn. No.	No. of detns.	ClO_2 Added (ppb)	ClO_2 Found (ppb)	Deviation (ppb)	Rel. SD (%)
1	9	13.5	16.6	3.1	10.0
2	9	27.0	27.3	0.3	7.0
3	9	40.5	37.6	−2.9	4.1
4	9	67.5	54.8	−12.7	5.8
5	7	135	119	−16	1.6
6	10	270	280	10	0.4
7	10	338	329	−9	0.6
8	4	675	664	11	0.5

TABLE 2

INTERFERING IONS

Ion	Added as	Amount added (ppm)	Relative error (%)	Permissible amount[a] (ppm)
Cu^{2+}	$CuCl_2$	10	+6	1
Pb^{2+}	$Pb(C_2H_3O_2)_2$	10	+11	1
Cr^{3+}	$CrCl_3$	1	−12	0
Fe^{3+}	$FeNH_4(SO_4)_2$	10	−7	1
ClO_3^-	$NaClO_3$	50	+4	10
$EDTA^{4-}$	Na_2H_2EDTA	10	+100	1
$H_2C_3H_2O_4$[b]	$H_2C_3H_2O_4$[b]	300	+8	200
Cl_2	Cl_2	1	+100	0

[a] Causes less than 2.5% relative error using 120 ppb chlorine dioxide.
[b] Malonic acid.

followed by approximately 3 minutes reaction time prior to adding the sample to the iron(II) perchlorate solution. An excess of malonic acid, up to approximately 200 ppm, can be tolerated without significant interference in the determination of chlorine dioxide. It should also be noted that determination of both chlorine and chlorine dioxide in mixtures of the two can be effected simply from the differences in absorbance observed between two samples, one sample containing malonic acid and the other sample untreated with malonic acid.

The effect of chlorite was not investigated because chlorite is not stable in a 0.2 *M* acid solution. This instability results from the disproportionation of chlorite to form chlorine dioxide in all but slightly

acidic, neutral, and basic solutions. Therefore, solutions containing chlorite and chlorine dioxide cannot be analyzed by the procedure as described. Prior to the analysis of such systems, masking or separation of the two species must be carried out (*18*).

SUMMARY

A rapid and simple method of preparing electrolytically generated chlorine dioxide free from extraneous oxidants has been described. Also an indirect spectrophotometric method for the determination of trace quantities of chlorine dioxide in acidic solution is proposed. The method is based on measuring the diminution of absorbance of the tris-1, 10-phenanthroline-iron(II) complex relative to a reagent blank by a differential spectrophotometric technique. The effect of solution variables and numerous diverse ions has been investigated.

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Precipitation of Tellurium with Bismuthiol II

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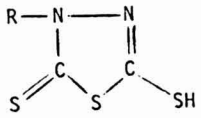
I. INTRODUCTION

Many organic complexing agents have recently been used for the determination and detection of tellurium. Of them, the thio compounds such as dithiocarbamates (*2*), thioureas (*26, 27*), tetramethylthiuram disulfide (*25*), Bismuthiols (*4*), mercaptoacetic acid (*11*), dithizone (*20*), and their derivatives are particularly important.

The spectrophotometric methods have been commonly used for determining tellurium. Fluorometric (*10*) and indirect complexometric methods (*22*) have been reported. However, no organic compound has been used for the gravimetric determination of tellurium.

Gravimetric determination of tellurium is conventionally carried out by weighing the precipitate either as its element or as its dioxide (*12*). Tellurium is best reduced from a dilute hydrochloric acid solution of Te(IV) or Te(VI) with a mixture of sulfur dioxide and hydrazine. With other reducing agents such as stannous chloride, titanous chloride, hypophosphorous acid, or metallic zinc, results are often high because the precipitate may be contaminated by some of the reducing agent or its oxidation product. Tellurium can also be weighed as dioxide; this is obtained by the addition of a suitable base (ammonia, pyridine, or hexamethylenetetramine) to an aqueous solution of Te(IV). The procedure is only applicable in the absence of highly charged metals which are hydrolyzed under the same condition. A highly selective method was reported by Cheng (*5*) that tellurium is precipitated as tellurous acid at pH 4.5–5.5 in the presence of EDTA. The weighing of precipitate as tellurium dioxide instead of elemental tellurium offers a slightly improved gravimetric factor.

Among the organic reagents used for the determination of tellurium, one group of compounds, namely Bismuthiols, have been widely used for the determination of other metals. The Bismuthiols have the following general formula:



(I) R = H; 2,5-dimercapto-1,3,4-thiadiazole; (Bismuthiol I).

(II) R = phenyl; 5-mercapto-3-phenyl-1,3,4-thiadiazole-2-thione; (Bismuthiol II).

(III) R = 2-naphthyl; 5-mercapto-3-(2-naphthyl)-1,3,4-thiadiazole-2-thione; (Bismuthiol III).

(IV) R = 2,3-dimethoxyphenyl; 3-(2,3-dimethoxyphenyl-5-mercapto-1,3,4,-thiadiazole-2-thione; (Bismuthiol IV).

Bismuthiol II is used for the gravimetric determination of Ag, As, Au, Bi, Cd, Cu, Hg, Ir, Os, Pb, Pd, Pt, Rh, Ru, Sb, Sn, and Tl (*13–15, 24*); the titrimetric determination of Ag, Bi Cd, and Pb (*16–18*); the amperometric determination of Ag, Bi, Cd, Cu, Hg, and Pb (*8*); and the spectrophotometric determination of Bi, Pd, and Te (*7, 19*). Bismuthiol I is less intensively studied than Bismuthiol II. Both Bismuthiol III and Bismuthiol IV were recently synthesized and studied by Busev and his co-workers for the extraction-photometric determination of tellurium in semiconductor after preliminary separation (*4*) and in lead concentrates and sulfur (*3*). All the Bismuthiols form with tellurium yellow compounds which are sparingly soluble in water, readily extractable by organic solvents.

An attempt was undertaken to explore the possibility of determining tellurium gravimetrically with Bismuthiol II. It has been reported that tellurium reacts with Bismuthiol II and forms a 1 to 4 complex (*6, 28*). It would be interesting to develop an analytical method for tellurium with a much more favorable gravimetric factor over the existing gravimetric methods which weigh the precipitate either as elemental tellurium or as tellurium dioxide. Consequently, a highly accurate method will be available for determing either macro or micro amounts of tellurium, particularly when the supply of sample is limited.

II. EXPERIMENTAL METHODS

Apparatus and Reagents

Bismuthiol II (potassium salt of 5-mercapto-3-phenyl-1,3,4-thiadiazole-2-thione) was obtained from Aldrich Chemical Co., Inc.; a 2% aqueous solution was freshly prepared.

Standard tellurium solution, 0.02 *M*, was prepared by dissolving 2.5523 g of tellurium (semiconductor grade 99.999%, American Smelting and Refining Company) in 20 ml of concentrated sulfuric acid

by heating. The solution was fumed to drive off most of the acid. Then it was cooled and 200 ml of water were carefully added. The tellurous acid precipitate was dissolved by adding a sufficient amount of 4 *M* potassium hydroxide. The solution was cooled again and then made up to 1 liter with water.

Other chemicals were analytical reagent grade.

A Radiometer pH meter model 26 was used for pH measurements.

The infrared spectra were obtained with a Perkin-Elmer 621 grating IR spectrophotometer in KBr disks and in nujul mulls.

A vacuum desiccator with phosphorus pentoxide as drying agent was used.

A Fisher-Johns melting point apparatus and a Thomas Hoover capillary melting point apparatus were used.

Several small samples ranging from 10 to 50 mg of tellurium were weighed with a Cahn electrobalance.

Procedure for Quantitative Precipitation of Tellurium with Bismuthiol II

To a solution containing 0.1 to 0.4 mmole of tellurium, add 15 ml of 2% tartaric acid and an appropriate amount of EDTA. Adjust the solution to pH 10 with 6 *M* ammonium hydroxide and readjust to pH 3.0–3.5 with acetic acid. Precipitate the tellurium with 25 ml of freshly prepared 2% Bismuthiol II solution. Let the solution stand for at least 1 hour with occasional stirring. Adjust the solution to pH 6.5–7.0 with 6 *M* ammonium hydroxide and acetic acid to coagulate the precipitate. Do not allow the temperature of the precipitate solution to raise over 45° during the neutralization, cool the solution with ice water bath if necessary. Filter the precipitate through a fine sintered glass crucible. Dry the precipitate in a vacuum desiccator for about 1 to 2 hours until constant weight is obtained. Weigh as $Te(C_8H_5N_2S_3)_4$, the gravimetric factor being 0.1240.

Qualitative Reactions of Metals Ions with Bismuthiol II

Qualitative tests were made in order to determine the selectivity of Bismuthiol II as a reagent for tellurium. They were carried out in the presence and absence of tartaric acid or EDTA.

Two drops of 0.1 *M* metal ion solution to be tested were mixed with 4 drops of water or 0.1 *M* masking agent (tartaric acid or EDTA) and followed by 5 drops of pH 3.2 ammonium acetate–acetic acid buffer solution. Four drops of 2% Bismuthiol II solution were then added.

The results are shown in Table 1. The metal ions of Zn(II), Cr(III), Co(III), Ni(II), Al(III), As(III), and Fe(III) did not form

TABLE 1

QUALITATIVE REACTIONS OF METAL WITH BISMUTHIOL II

Foreign ion	Observation after Bismuthiol II added		
	Without masking	With EDTA	With tartaric acid
Hg(II)	Pale yellow ppt.	Pale yellow ppt.	Pale yellow ppt.
Zn(II)	—[a]	—	—
Cu(II)	Brown ppt.	Yellow ppt.	Brown ppt.
Co(II)	—	—	—
Cr(III)	—	—	—
Ni(II)	—	—	—
Al(III)	—	—	—
As(III)	—	—	White ppt.
Se(IV)	Light yellow ppt.	Light yellow ppt.	Light yellow ppt.
Ag(I)	Light yellow ppt.	Light yellow ppt.	Light yellow ppt.
Fe(III)	—	—	—
Bi(III)	Brown ppt.	—	Brown ppt.
Pb(II)	Yellow ppt.	—	Yellow ppt.

[a] No significant precipitation.

precipitates with Bismuthiol II. As expected (*6*), Hg(II), Cu(II), Se(IV), Ag(I), Pb(II), and Bi(III) gave precipitates. The interference of bismuth and lead could be eliminated by EDTA. Certain highly charged metal ions do not react with Bismuthiol II, but are easily hydrolyzed at pH 3 to 7. Addition of tartaric acid can prevent them from hydrolyzing.

Physical Properties of Tellurium Bismuthiol II Precipitate

Tellurium(IV) reacted with Bismuthiol II to form a bright yellow precipitate. The precipitate decomposed with evolving hydrogen sulfide odor at 72–74° and melted at 84–86°. But it gradually decomposed at about 45° after a lengthy heating. The precipitate was very stable at room temperature. Upon heating, the color of the precipitate changed from yellow to orange, then to brick-red at high temperature (100°).

At room temperature, the precipitate was slightly soluble in water and pentane; relatively soluble in most alcohols such as methanol, ethanol, *n*-propanol, isopropanol, and *n*-butanol; soluble in acetone, chloroform, carbon tetrachloride, ether, toluene, and benzene; and very soluble in concentrated nitric acid, pyridine, and *N,N*-dimethylformamide.

The yellow precipitate dissolved in 2 *M* sodium hydroxide formed a black precipitate. This might be due to the reduction of tellurium(IV) to

the elemental tellurium. It seemd that some sulfur was liberated from the precipitate when it was dissolved in carbon disulfide. The color of precipitate changed from yellow to pale yellow in 1,4-dioxane. Apparently some of these solvents reacted with the precipitate.

The solubility of the precipitate in water increased with increasing temperature, being less than 1×10^{-6} *M* at below 40°. Also, the precipitate is more soluble in higher concentrations of ammonium chloride or ammonium sulfate.

All this information is summarized in Table 2. The increase in solubility in a concentrated solution of ammonium chloride or ammonium sulfate may be due to the complexation of tellurium with chloride or sulfate.

Preparation of Metal Bismuthiol II Complexes

Silver Bismuthiol II complex. To a hot solution of 450 ml of water containing 0.1513 g of silver nitrate and 50 ml of 1 *N* nitric acid, 50 ml of 1% Bismuthiol II solution were added. The solution was heated on a hot plate until the pale yellow precipitate was coagulated. It was filtered, washed with hot water, and dried at 100° for 2 hours.

Bismuth Bismuthiol II complex. The above mentioned procedure was used to obtain the bismuth precipitate by using 0.2532 g of bismuth nitrate pentahydrate. A brick-red precipitate was obtained.

TABLE 2

PHYSICAL PROPERTIES OF PRECIPITATE

1. Fine bright yellow powder
2. Decomposition temperature 72–74° (Gradually decomposes at about 45° after heating a long time and becomes orange, then turns to brick-red solid.)
3. Melting point 84–86°
4. Solubility test (room temp.)
   (a) Relatively insoluble in water, pentane
   (b) Slightly soluble in most alcohols, e.g., methanol, ethanol, *n*-propanol, isopropanol, butanol
   (c) Soluble in concentrated HNO_3 (very soluble)
   2 *M* NaOH[a] (immediately dissolves and forms black precipitate) acetone, $CHCl_3$, CCl_4
   CS_2[a] (dissolves, then forms bright yellow precipitate) ether, toluene, benzene, pyridine (very soluble)
   1,4-dioxane[a] (quite soluble, then precipitate becomes pale yellow)
   N,*N*-dimethylformamide (very soluble)

[a] The precipitate reacts with solvent and the precipitate changes the color.

III. RESULTS AND DISCUSSION

Effect of pH on Precipitation

By qualitative test, no precipitation was found of tellurium with Bismuthiol II above pH 6. This is due to the fact that in a neutral or alkaline medium, tellurium exists as tellurite and an acidic medium favors the formation of tellurium(IV) ion.

$$TeO_2 + 2OH^- = TeO_3^{2-} + H_2O, \quad (1)$$

$$TeO_2 + 2H^+ = H_2TeO_2^{2+}, \quad (2)$$

$$H_2TeO_3 + 4\,H^+ = Te^{4+} + 3\,H_2O \quad (3)$$

The presence of Te^{4+} makes the precipitation of tellurium with Bismuthiol II possible, though its process is relatively slow. At pH 4 to 6, which is an optimum condition for the hydrolysis of tellurium(IV), the precipitation of tellurium with Bismuthiol II was particularly slow and difficult to obtain a quantitative precipitation.

According to Eq. (3), the production of Te^{4+} is favored in a higher acid concentration; however, high acidities tend to decompose Bismuthiol II, forming hydrogen sulfide which precipitates tellurium as tellurium sulfides with no definite composition. Consequently, there would be a compromise pH which allows a quantitative precipitation of tellurium as tellurium Bismuthiol II having a reasonable speed and free from contamination. Such an optimum condition has been found at pH 3.0 to 3.5 Figure 1 also indicates that at pH below 2.5, the results were higher.

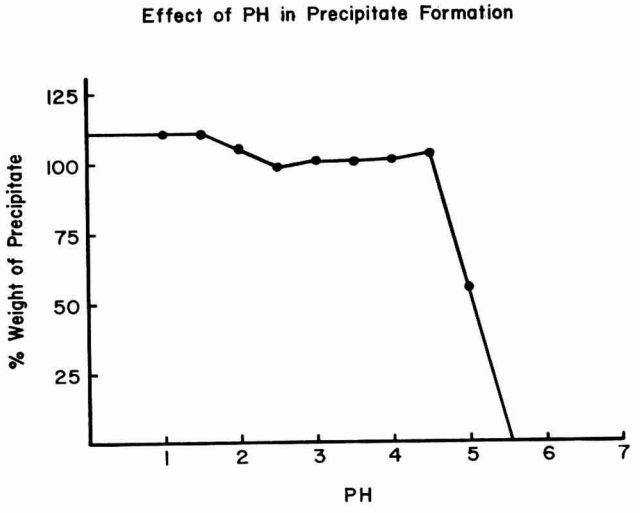


FIG. 1. Effect of pH on precipitate formation.

Effect of Amount of Bismuthiol II

The curve shown in Fig. 2 indicates the formation of a 1 to 4 tellurium Bismuthiol II complex. At least 10% excess of Bismuthiol II is required to achieve a complete precipitation. But a large excess of the reagent should be avoided, to eliminate the contamination.

Effect of Time on Precipitation

The reaction of tellurium with Bismuthiol II at pH 3.0 to 3.5 is a relatively slow process. The time required for the quantitative precipitation of milligram amounts of tellurium is at least 30 minutes as shown in Fig. 3. Heating could speed up the reaction, but heating at the same time causes the decomposition of the reagent.

Effect of Tartaric Acid

In the study of the effect of tartaric acid on the precipitation of tellurium with Bismuthiol II, various amounts of tartaric acid were added

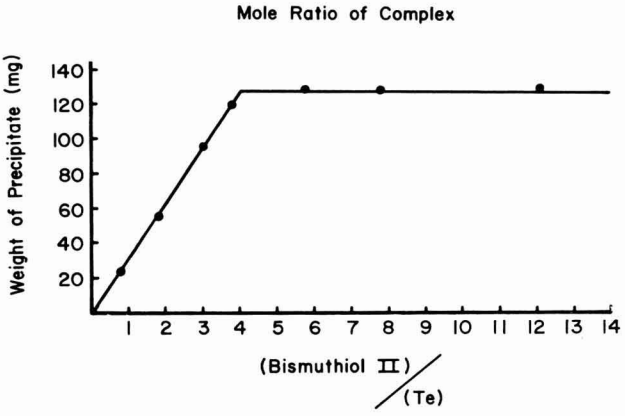


FIG. 2. Mole ratio of complex.

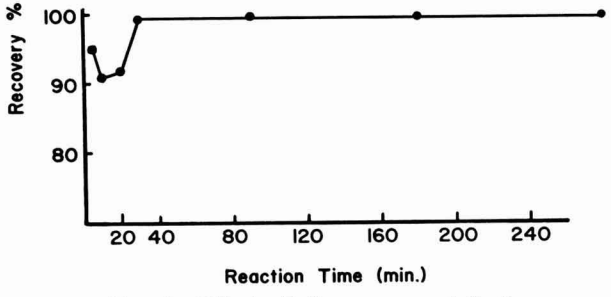


FIG. 3. Effect of time on precipitation.

into each tellurium solution of the same concentration before adding Bismuthiol II. When the tartaric acid solution and the tellurium solution were mixed at mole ratios of tartaric acid to tellurium from 4 to 16, the pH being 4.6 to 2.7, precipitation or turbidity occurred after standing overnight. The white precipitate could be dissolved by adjusting the pH to 10 with ammonia. No precipitation took place when the solution was readjusted to pH 3.5 from pH 10 with acetic acid. This interesting phenomenon is probably related to the formation of a stronger tellurium tartrate complex in an alkaline medium. In a weakly acidic medium, the slow reaction of tellurium with tartaric acid permits partial hydrolysis of tellurium. The reaction of tellurium with tartaric acid appears to be a fast process in an alkaline medium. Once the tellurium tartrate complex is formed, it will not be decomposed by acidification.

Tartaric acid not only is a desirable complexing agent to prevent tellurium and other highly charged metal ions from hydrolyzing, but it also serves as a buffer. Too much excess of tartaric acid should be avoided on account of its relatively low solubility at pH 3 to 5.

A large excess of tartaric acid could be tolerated if the temperature of the solution is kept at not higher than 45° during the pH adjustment. The heat produced from neutralization caused a partial hydrolysis of tellurium even in the presence of tartaric acid. This will result later in formation of an impure tellurium Bismuthiol II precipitate contaminated by tellurium dioxide. The more the free tartaric acid is present, the more ammonia needed to adjust the pH of solution. As a result, generation of more heat causes the hydrolysis of tellurium and lower results.

It seems that since tartaric acid forms a much weaker complex with tellurium than Bismuthiol II, tellurium can be quantitatively precipitated with Bismuthiol II. However, this will not be so when citric acid is used in place of tartaric acid. It is known that citric acid is generally a stronger complexing agent than tartaric acid for metal ions.

The results shown in Table 3 indicate that the optimum ratios of tartaric acid to tellurium were in the range from 4 to 6.

Drying Precipitate

The tellurium Bismuthiol II precipitate is stable at room temperature but decomposes at high temperature. The precipitate may be dried in a vacuum desiccator with phosphorus pentoxide as drying agent. Sometimes it could be air dried in a well-ventilated hood overnight. But the drying in a vacuum desiccator for a least 1 hour is preferred. Larger amount of precipitate requires longer drying time as shown in Table 4.

TABLE 3

EFFECT OF TARTARIC ACID ON TELLURIUM PRECIPITATION

Te taken	Tartaric acid (2%) added (ml)	[Tartaric acid]/[Te]	Te found (mg)	Recovery (%)
16.0	1.0	1	15.1	94.4
16.0	1.9	2	15.6	97.5
16.0	3.8	4	15.7	98.1
16.0	5.6	6	15.8	98.8
16.0	7.5	8	15.3	95.6
16.0	15.0	16	15.0	93.8
16.0	47.0	50	13.3	83.1
16.0	93.7	100	7.2	45.0

TABLE 4

DRYING TIME OF PRECIPITATE

Te taken (mg)	Wt (g) of precipitate after vacuum suction (min): 30	60	90	120	150	Recovery (%) after 150
64.0	1.2488	0.8282	0.5670	0.5048	0.5050	97.9
38.4	0.7115	0.4028	0.3060	0.3030	0.3027	97.8
25.6	0.3458	0.2057	0.2057	0.2057	0.2057	99.7
10.2	0.0882	0.0828	0.0824	0.0824	0.0824	99.9

Composition of Tellurium Bismuthiol II Complex

In order to determine the composition of the tellurium Bismuthiol II complex by a mole ratio method, a known amount of tellurium was precipitated by varying quantities of Bismuthiol II.

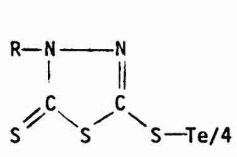
The curve shown in Fig. 2 indicates that a complex of 1 tellurium to 4 Bismuthiol II was formed. The composition of this complex was found to be the same as that reported by Cheng (*6*) and Yoshida *et al.* (*28*) based on the photometric study.

Based on the empirical formula of the complex as $Te(C_8H_5N_2S_3)_4$, the gravimetric factor for tellurium is 0.1240.

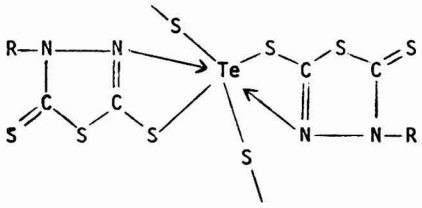
Structure of Tellurium Bismuthiol II Complex

After knowing the composition of tellurium Bismuthiol II complex, one would like to find out how tellurium is bonded to the ligand. Cheng (*6*) postulated the complex where Bismuthiol II acts as an unidentate ligand (see structure A). But there are several other possibilities (see

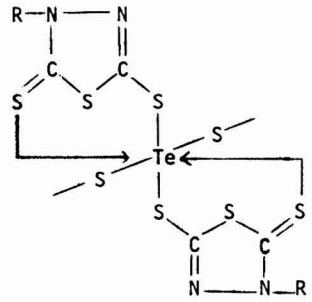
structure B–E). For testing these possibilities, the infrared spectra of Bismuthiol II complexes of potassium, silver, bismuth, and tellurium were examined.



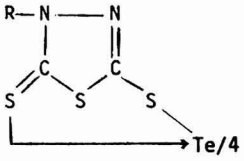
(A)



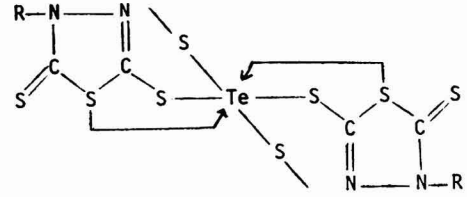
(B)



(C)



(D)



(E)

R=phenyl group

By comparison of the infrared spectra of the Bismuthiol II complexes (Fig. 4), it is noticed that the spectrum of the tellurium Bismuthiol II is significantly different from that of the Bismuthiol II complex of potassium or silver, but close to that of the bismuth Bismuthiol II complex in the range of 1400–1290 cm^{-1} and 1200–1000 cm^{-1}. It is also noted that a strong absorption peak at 1423 cm^{-1} in potassium complex which is absent from the other complexes. The variation of the absorption position in the range of 1400–1290 cm^{-1} could arise from the C–N vibration of the =N—C=S group under the influence of the different metals.

The structures (C) and (D) are postulated based on the chelated structure of bismuth Bismuthiol II proposed by Dubsky *et al.* (*9*). They are unlikely because the heterocyclic ring being planar, two sulfurs are so far apart that it probably could not form a ring structure containing tellurium. Furthermore, the structure (D) required a coordination number 8 for tellurium, which has only been postulated in unusual cases (*21*).

The usual coordination number in tellurium complexes is six (*23*). If it is so in tellurium Bismuthiol II complex, part of the tellurium, besides being bound to the mercapto sulfur, should coordinate also with either the neighboring nitrogen (B) or cyclic sulfur (E) to form a 4-member ring. None of these types of linkage are known so far. Also, it is very unfavorable for tellurium to have a 4-member ring in a nonterminal dithio or thioamide system. Since there are not enough infrared

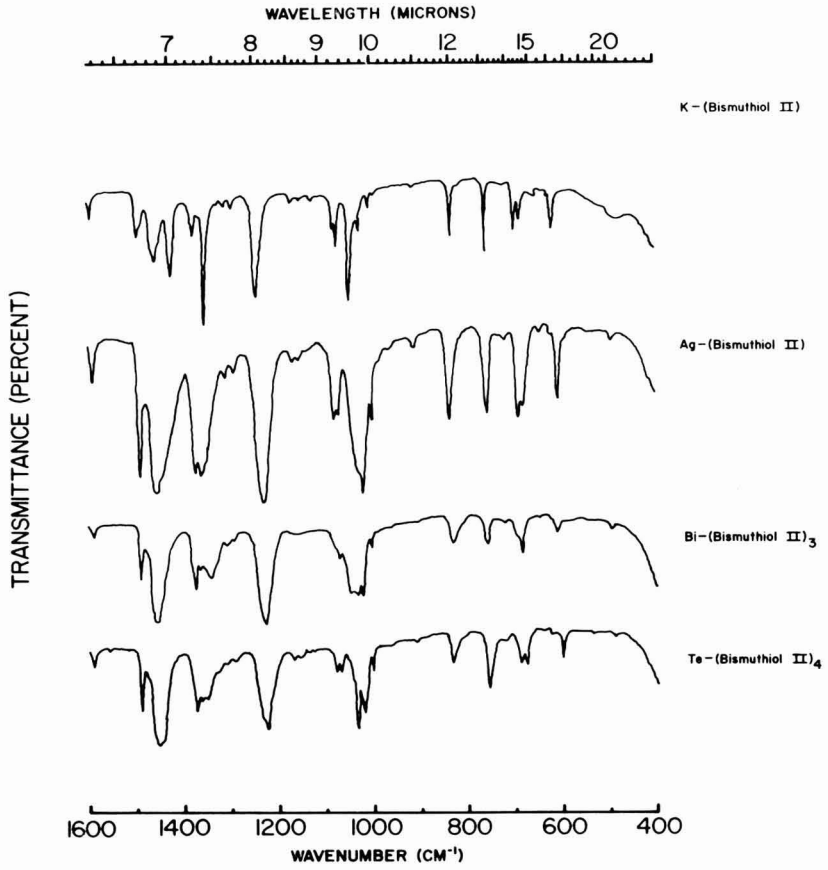


FIG. 4. Infrared spectra of metal Bismuthiol II complexes.

data available to identify individual groups, it is very difficult to give a clear picture of the structure from the infrared spectra obtained.

The tellurium Bismuthiol II complex is very soluble in many organic solvents; it is very unlikely that the complex has a polymeric structure.

The infrared spectra are not ideal for studying the thiocarbonyl group because of their weak absorption. Their Raman spectra in which thio group vibration appears as an intense band may offer better data and information (*1*). Therefore, it is suggested that the structure of the thio complexes should be carefully studied by other techniques such as Raman spectroscopy, X-ray diffraction, and photoelectron spectroscopy so that their structures can be better understood.

Precision and Accuracy

Gravimetric determination is relatively time consuming; however, it offers an attractive feature of high precision and accuracy as demonstrated in Table 5.

The higher results obtained from air drying (Table 5A) are probably due to the precipitates not being completely dried.

Some samples were weighed out directly with the microbalance (Table 5B). It seems that they give better results than using pipets (Table 5A and C).

When high accuracy is desired, pipets and flasks must be calibrated. If possible, the sample is weighed in its solid form, and the weight of precipitate is obtained right after vacuum drying.

CONCLUSION

A gravimetric method for determining tellurium using Bismuthiol II as a precipitant has been developed. This method may be the first one using an organic reagent for the quantitative precipitation of tellurium.

The accuracy of the method is demonstrated by the results obtained for samples of 10 to 50 mg of tellurium. Precision of the method was tested by running several 16-mg tellurium solutions with a standard deviation of about ± 0.22. An average of 99.0% (range 97.8–100.0) recovery was always obtained with pure tellurium (semiconductor grade) by the proposed procedure. The tellurium samples containing the foreign ions which can be masked by tartaric acid and EDTA under the reaction condition should give as good results as pure tellurium. If interfering ions are present, their preliminary separation by appropriate means such as ion exchange, volatilization, etc., should be made before the precipitation of tellurium.

The infrared spectra of the Bismuthiol II complexes do not offer

TABLE 5

PRECISION AND ACCURACY OF TELLURIUM DETERMINATION BY THE DEVELOPED METHOD

Te taken (mg)	pH	Reaction time (min)	Wt of ppt. (g)	Te found (mg)	Recovery (%)
(A) Dried in air inside a ventilated hood					
16.0	2.5	80	0.1269	15.7	98.1
16.0	3.0	58	0.1300	16.1	100.6
16.0	3.5	60	0.1295	16.1	100.6
16.0	4.0	60	0.1304	16.2	101.2
SD 0.22					
Relative SD 1.37					
(B) Dried in a vacuum desiccator					
18.60[a]	3.2		0.1499	18.59	99.93
17.52[a]	3.2		0.1404	17.41	99.37
18.44	3.2		0.1480	18.35	99.52
(C) Dried in a vacuum desiccator					
64.0				62.6	97.8
38.4				37.6	97.9
38.4				37.8	98.4
25.6				25.5	99.6
25.6				25.4	99.2
16.0				15.8	98.8
10.2				10.2	100.0

[a] The samples were weighed by a microbalance.

enough information to solve their structure problems. Further study is necessary to find out their structures.

Because of the very favorable gravimetric factor, this method should be useful in the accurate analysis of tellurium compounds, particularly in the stoichiometric determination of tellurides.

SUMMARY

A gravimetric method using Bismuthiol II (potassium salt of 5-mercapto-3-phenyl-1,3,4-thiadiazole-2-thione) as a reagent for determining tellurium has been developed with a very favorable gravimetric factor of 0.1240.

Tellurium(IV) is quantitatively precipitated in the presence of tartaric acid and (ethylenedinitrilo) tetraacetic acid (EDTA) by Bismuthiol II at pH 3.0–3.5. The yellow precipitate decomposes on heating, but it can be vacuum-dried at room temperature at $Te(C_8H_5N_2S_3)_4$. The structure of the precipitate is discussed based on its infrared spectrum.

The solubility of the precipitate in various solvents and other factors affecting the precipitation have been studied.

A selective precipitation can be made in the presence of tartaric acid and EDTA. Many interfering ions such as Ni(II), Al(III), Cr(III), Fe(III), Bi(III), PbII, and As(III) can be masked by EDTA, but Hg(II), Cu(II), Se(IV), and Ag(I) still interfere.

The samples containing 10 to 50 mg of tellurium were analyzed. An accuracy of ±0.5% or better was obtained. The proposed method for tellurium will be valuable for the stoichiometric analysis of telluride compounds where accuracy is of prime concern.

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MICROCHEMICAL JOURNAL 15, 622–624 (1970)

Analytical Applications of Chelons

LVII. Chelometric Determination of Bismuth with DTPA and Reciprocally

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Pyrocatechol violet has been used by Malat and others (*2*) for the chelometric determination of bismuth with EDTA. This indicator forms with bismuth ion, in nitric acid solution to pH 2, a blue complex that changes sharply at the equivalence point to yellow color of the single indicator.

The present paper describes a procedure for the chelometric determination of DTPA with pyrocatechol violet as an indicator, useful also with an ultramicroquantity of bismuth. The procedure can be reversed for DTPA determination with standard bismuth solution, using the same indicator.

The property of the pyrocatechol violet as a tricolored acid–base indicator is used: red color with a strongly-acid solution, yellow with a weak acid or neutral one, and red violet in alkaline medium.

MATERIALS AND METHODS

Reagents

DTPA. Dissolve a convenient quantity of DTPA (diethylenetriaminepentaacetic acid) in a minimum volume of 10% sodium hydroxide aqueous solution, and dilute with distilled water to obtain solutions 0.05 or 0.01 *F*. Titrate this solution as described in the procedure.

Indicator. Disolve 0.1 g of pyrocatechol violet in 100 ml of distilled water. This solution is stable for a long time.

Standard bismuth solution. Make in diluted nitric acid from pentahydrated bismuth nitrate, p.a., and volumetrically check the concentration (*1*). Alternatively, dissolve directly in the same acid, metallic bismuth or bismuth oxide obtained by calcination of the carbonate.

Procedure

(a) *Titration of the DTPA solution.* Add to an aliquot of the bismuth nitrate nitric solution, 2–4 drops of indicator by 100 ml of unknown solution. A violet color is produced, which gives evidence that

the solution is acid. Neutralize carefully with diluted ammonia until appearance of a dark blue color (pH 2–3).

Add now, DTPA solution until blue color changes to yellow. Before the end point, the solution becomes violet transitorily.

Known the necessary volume of the DTPA solution to react with the known volume of the aliquot part of the standard bismuth solution, it is possible to obtain the normality of this DTPA solution.

(b) *Chelometric determination of bismuth with DPTA.* Proceed with the bismuth unknown solution exactly as in (a); only at the end it is necessary to observe that now it is the concentration of the DTPA solution that is known; and then it is necessary to obtain the quantity of bismuth in the test solution.

Titration must always be done in nitric medium in order to reduce to the minimum the danger of bismuth salts hydrolysis.

For very little bismuth concentrations, and because the high equivalent weight of this metal, diluted solutions of DTPA, 0.05 or 0.01 *F* for instance, must be utilized.

The results obtained in determination of giving quantities of bismuth between the approximated limits 1 μg to 0.125 mg are given in Table 1.

Application for bismuth determination in pharmaceuticals: Bismuth–guanidine salycilate samples were analyzed. The organic matter had been destroyed by careful calcination, the residue was taken with 4 ml of concentrated nitric acid, evaporate the solution and the residue was dissolved in 3 ml of dilute nitric acid (1:1). The bismuth chelometric determination in this solution was then made by procedure (b).

The determination of bismuth with EDTA and thiourea as indicator was done in other samples. The comparative results are given in Table 2.

TABLE 1

CHELOMETRIC DETERMINATION OF BISMUTH WITH DTPA

Bi taken (μg)	DTPA vol		Bi found (μg)	Error (%)	Observations
	Theoretical	Used			
1.0	0.50	0.50	1.0	0	—[a]
2.0	1.00	1.03	2.1	5	—[a]
4.1	2.00	2.03	4.2	0	—[a]
12.5	6.00	6.00	12.5	0	—[a]
20.9	10.00	10.00	20.9	0	—[a]
29.3	14.00	13.95	29.3	0	—[a]
41.1	20.00	20.00	41.1	0	—[a]
125.8	10.00	12.03	126.1	0.2	—[b]

[a] DTPA 0.01 *F* solution.

[b] DTPA 0.05 *F* solution.

TABLE 2

CHELOMETRIC DETERMINATION OF BISMUTH IN BISMUTH–GUANIDINE SALICYLATE

Sample	Bismuth (%)		Differences
	EDTA detn	DTPA detn	
1	4.13	4.11	−0.02
2	4.10	4.12	+0.02

Interferences are the same as those describe in (*1*) but the method is available for determination of smaller quantities of bismuth.

SUMMARY

A new method is proposed for the chelometric determination of bismuth with DTPA using pyrocatechol violet as an indicator. The procedure has been used for determination 1 μg to 0.125 mg of bismuth, and is also useful for determination of DTPA with standard solution of bismuth.

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Microcrystalloptic Tests for Some Lupine and Ormosia Alkaloids

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Lupine alkaloids occur widely in many species of the Leguminosae family, subfamily Faboideae. The toxicity of many of these alkaloids presents an economic problem because of the use of the leaves and seeds of *Lupinus* species as cattle fodder. Medicinal use of lupine alkaloids covered by this study appears still to be in an experimental stage (*15*). The occurrence of most *Ormosia* alkaloids is restricted to that leguminous genus while some lupine alkaloids have also been detected in *Ormosia* species. The chemistry of the lupine and *Ormosia* alkaloids has been thoroughly reviewed (*1, 13, 14*). The importance of having sensitive and specific tests available became evident recently when novelty jewelry made from toxic seeds was discovered on the American market. Although the toxic material was soon identified to be due to seeds of *Abrus precatorius* (Leguminosae) (*12*), necklaces, etc., made from *Ormosia* seeds, which could possibly be confused with the highly toxic material, are marketed as well, and chemical tests had to be developed to distinguish the two types of seeds (*10*). Another example for the need of analytical control in this area arose from problems in the production of edible flour from detoxified seeds of *Lupinus* species which were thought to be due to ill effects of small amounts of alkaloids (*16*). The usefulness of microcrystal tests, in particular when coupled with microoptical measurements, has been discussed before (*4, 11*). These tests are most effective when used in conjunction with other chromatographic and spectroscopic procedures as confirmatory tests (*3, 5–9*). The alkaloids selected for this investigation include both those which are relatively widely distributed in the plant world and also some which, until now, have only been found in a limited number of plant species. Thus, lupine occurs in at least 20 species of *Lupinus,* several species of *Cytisus* and *Podolyria* and in at least one species each of *Virgilia, Baptisia, Leontice* and *Solanum.* Isolupanine and hydroxylupanine have been detected in several species of *Lupinus.* Angustifoline has been found in three species of *Lupinus* and also in two species of *Ormosia.* Epihydroxylupanine is a minor alkaloid in *O. jamaicensis,*

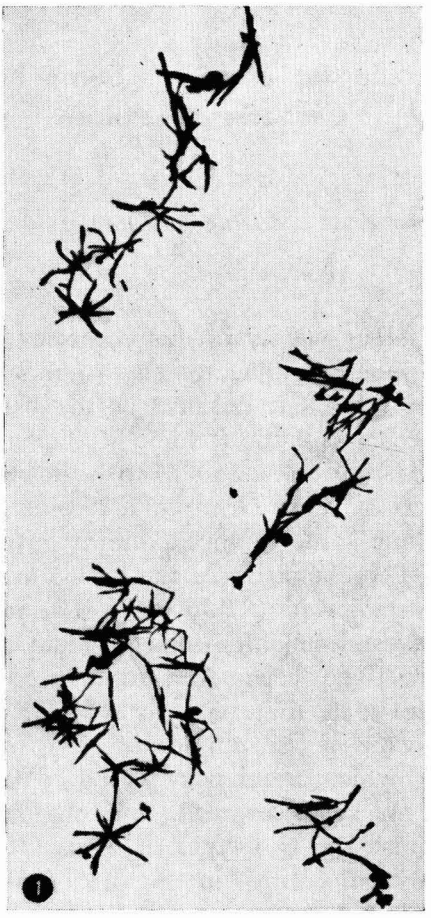

FIG. 1. Microcrystals of D-lupanine with potassium triiodide; crystals after 30 min; 80×.

while ormosanine, ormojanine, and panamine are typical *Ormosia alkaloids.* No microcrystal tests for any of the investigated alkaloids have been reported, except lupanine for which two tests but no microoptical data have been given (*2*).

METHODS

Microtechniques and equipment were the same as described in several previous publications (*4–11*). Solutions of alkaloids (1 μg/μl) were prepared in 1% acetic acid and applied to cover glass slides with 1 μl disposable microcaps (Drummond Sci. Co.) together with 1 μl of reagent. Observations were made by the hanging drop method on ped-

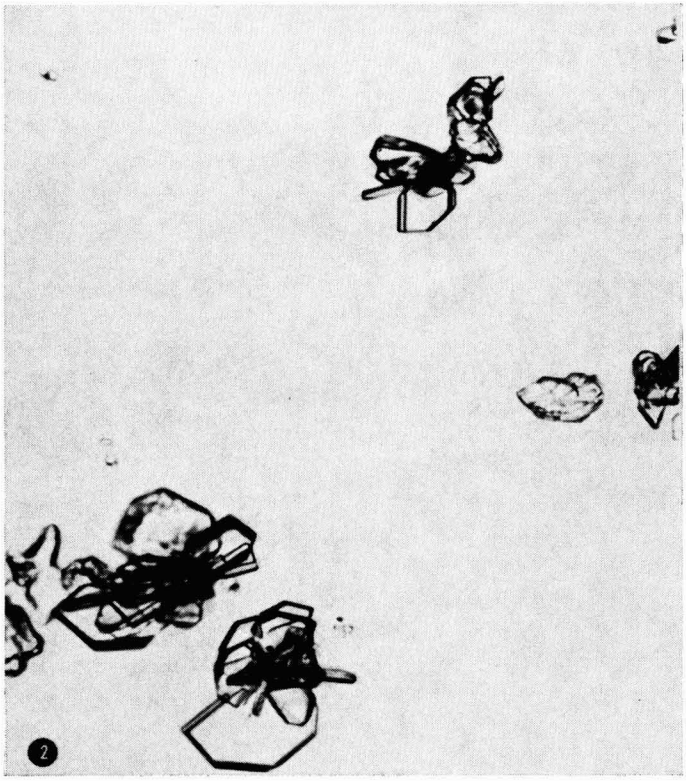

FIG. 2. Microcrystals of D-α-isolupanine with mercuric chloride; crystals after 5 min; 200×.

estal slides. Blank tests with reagent and solvent only were always carried out.

RESULTS

D-*Lupanine*

1. Potassium triiodide (2 g of iodine and 4 g of KI in 100 ml of water). Needles in branched aggregates, Class 9 g; 1 μg; dim birefringence, second order; inclined extinction; angle of extinction 8°; indifferent sign of elongation (Fig. 1).

D-α-*Isolupanine*

1. Mercuric chloride (5% solution in water). Plates, in clusters, Class 7a; 1 μg; dim birefringence, first order; parallel extinction; positive sign of elongation (Fig. 2).

2. Gold chloride (5% solution in water). Hairs, radiating, Class 4;

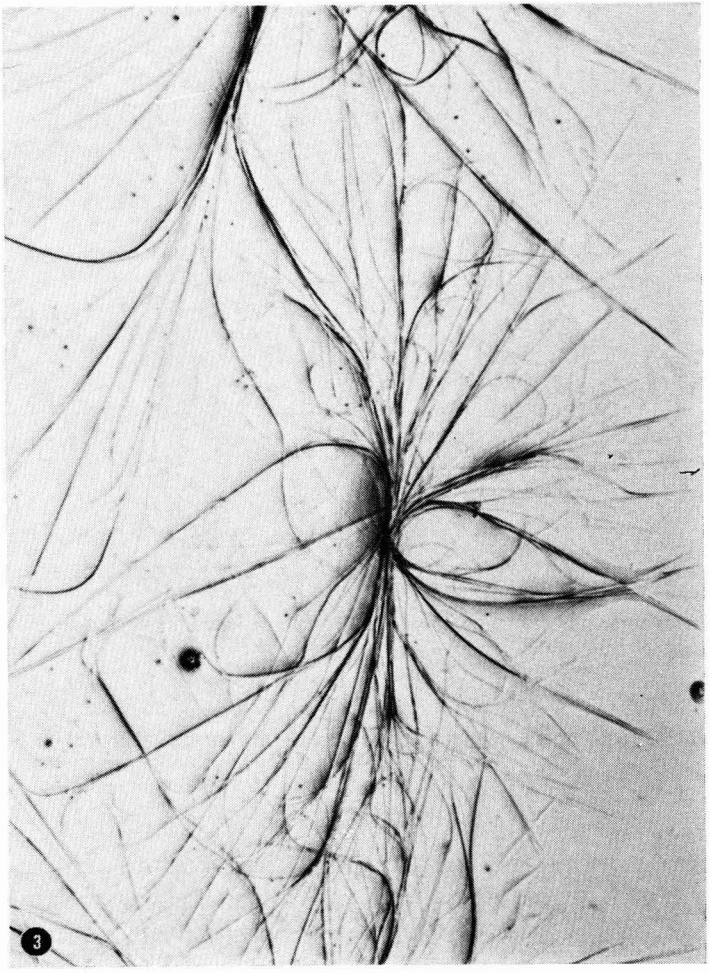

FIG. 3. Microcrystals of D-α-isolupanine with gold chloride crystals after 10 min; 80×.

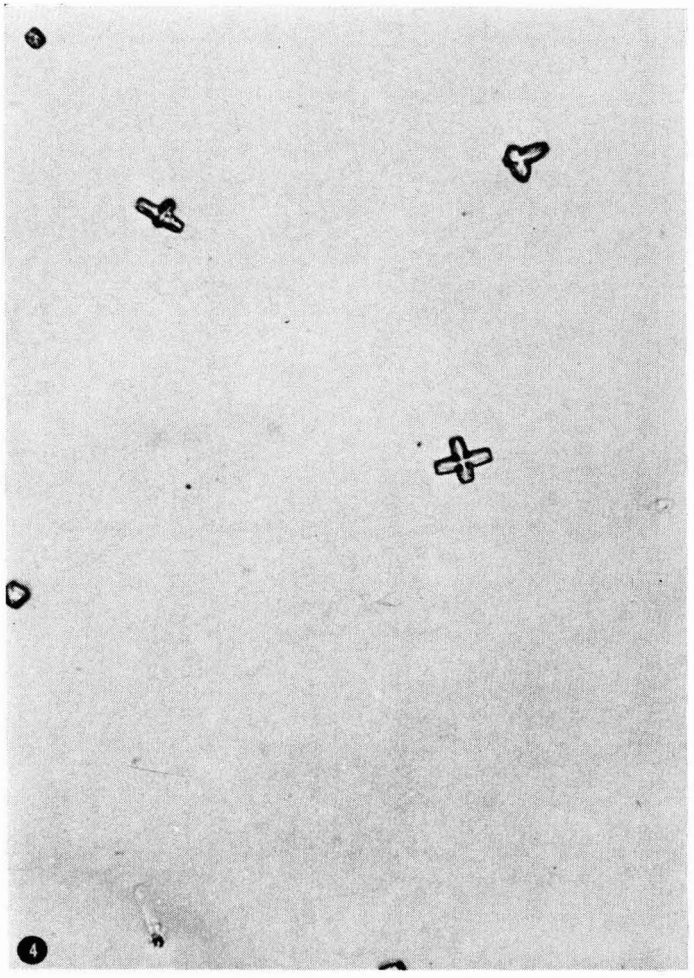

FIG. 4. Microcrystals of D-α-isolupanine with platinum chloride; crystals after 1 hour; 200×.

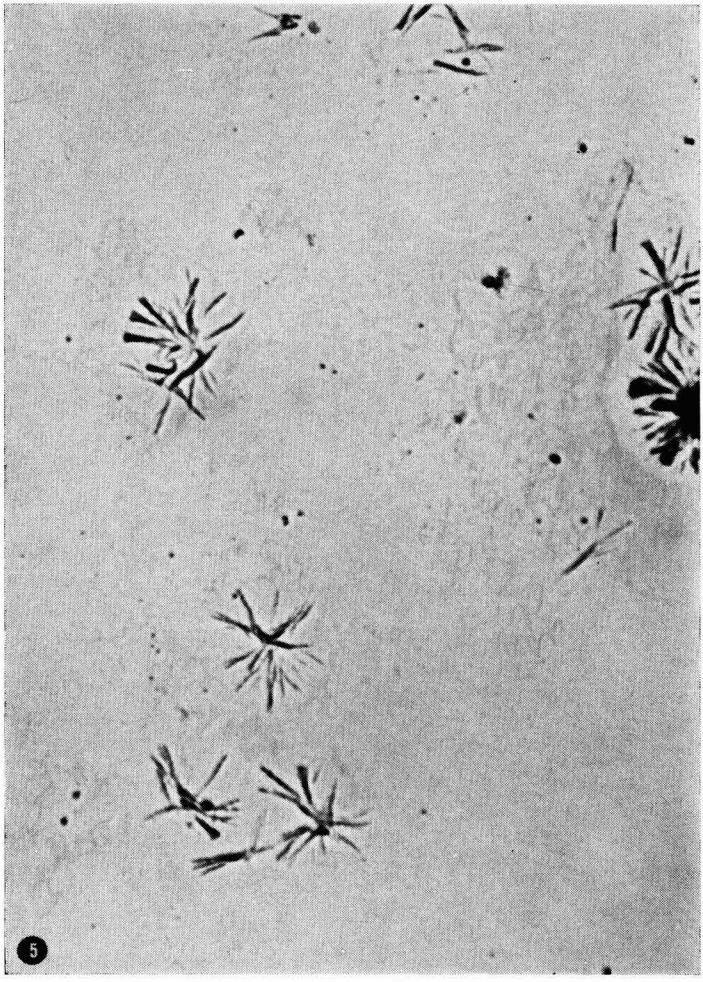

FIG. 5. Microcrystals of D-hydroxylupanine with picrolonic acid; crystals after 1 hour; 200×.

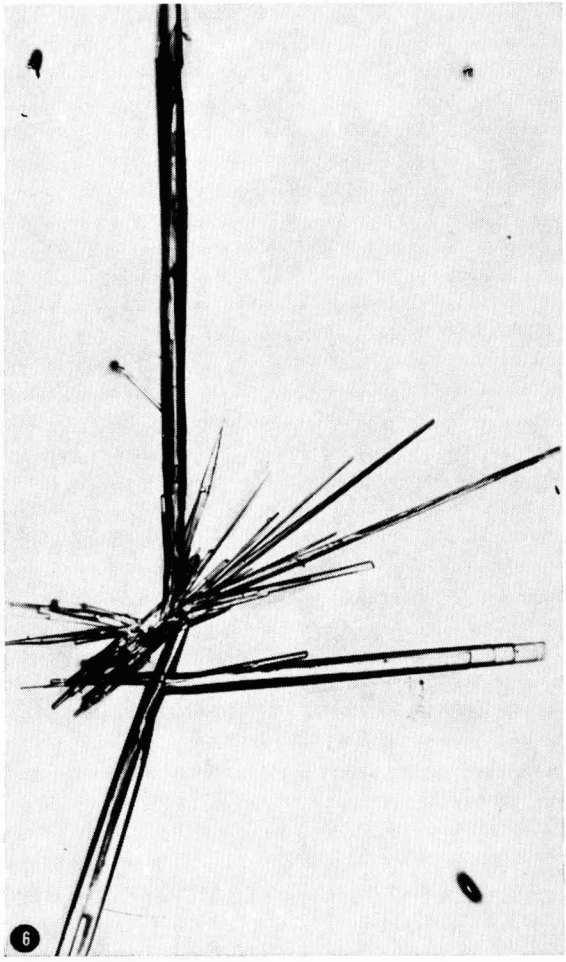

FIG. 6. Microcrystals of D-hydroxylupanine with potassium cadmium iodide; crystals after 15 min; 80×.

1 μg; moderate birefringence; first order; parallel extinction; negative sign of elongation (Fig. 3).

3. Platinum chloride (5% solution in water). Plates, some crossed, Class 7a; 1 μg; no birefringence (Fig. 4).

D-Hydroxylupanine

1. Picrolonic acid (saturated solution in water). Aggregates, branching needles, some rosettes, Class 9 g; 1 μg; dim birefringence, first order; parallel extinction; positive sign of elongation (Fig. 5).

FIG. 7. Microcrystals of epihydroxylupanine with potassium cadmium iodide; crystals after 30 min; 80×.

2. Potassium cadmium iodide (1 g of CdI_2 and 2 g of KI in 100 ml of water). Rods, long, in aggregates, Class 5; 1 μg; dim birefringence, second order; inclined extinction; angle of extinction 10°; negative sign of elongation (Fig. 6).

Epihydroxylupanine

1. Potassium cadmium iodide. Sheaves of rods; Class 9b; 4 μg; dim birefringence, second order; inclined extinction; angle of extinction 9°; negative sign of elongation (Fig. 7).

2. Potassium mercuric iodide (1.5 g of $HgCl_2$ and 5 g of KI in 100 ml of water). Rosettes of blades, Class 9c; 10 μg; dim birefringence, second order; inclined extinction; angle of extinction 9°; negative sign of elongation (Fig. 8).

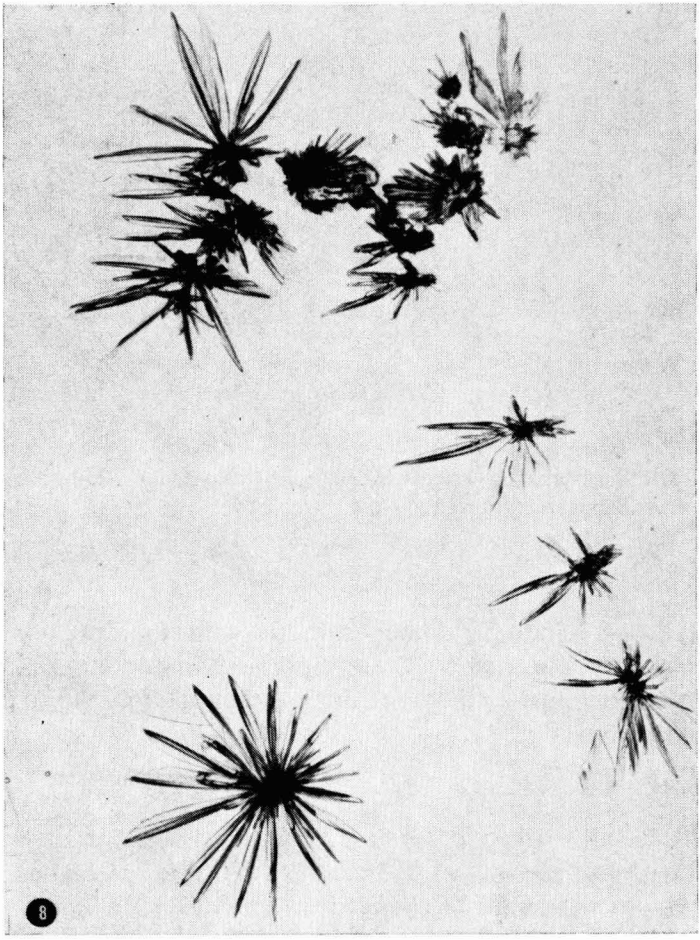

FIG. 8. Microcrystals of epihydroxylupanine with potassium mercuric iodide; crystals after 20 min; 80×.

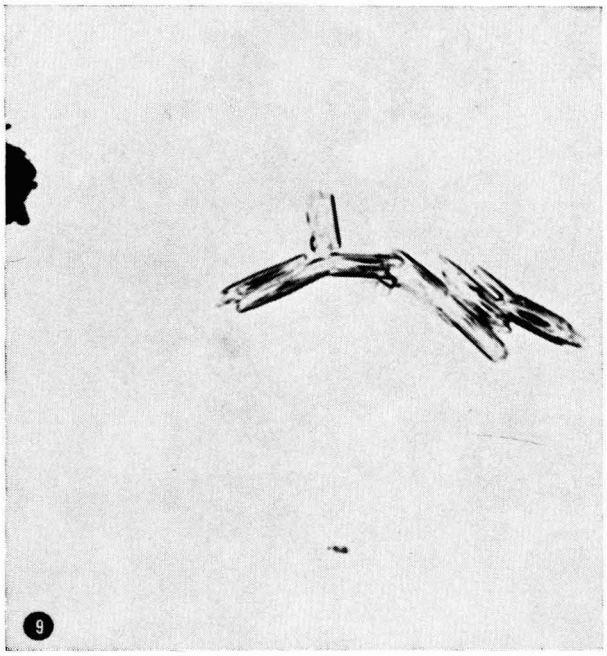

FIG. 9. Microcrystals of angustifoline with 8-hydroxy-7-iodo-5-quinolinesulfonic acid; crystals after 10 min; 200×.

Angustiofoline

1. 8-Hydroxy-7-iodo-5-quinolinesulfonic acid (saturated solution in water). Rods, in aggregates, Class 5; 1 μg; moderate birefringence, second order; inclined extinction; angle of extinction 9.5°; indifferent sign of elongation (Fig. 9).

Ormosanine

1. Gold cyanide (5 g of gold chloride in 100 ml of water with NaCN added until precipitate redissolves). Rosettes of blades and rods, Class 9c; 1 μg; moderate birefringence, first order; inclined extinction; angle of extinction 18°; negative sign of elongation (Fig. 10).
2. Picrolonic acid (see *10*).

Ormojanine

1. Gold chloride (see *10*).

Panamine

1. Potassium chromate (5% in water). Rods, in radiating aggre-

FIG. 10. Microcrystals of ormosanine with gold cyanide; crystals after 20 min; 80×.

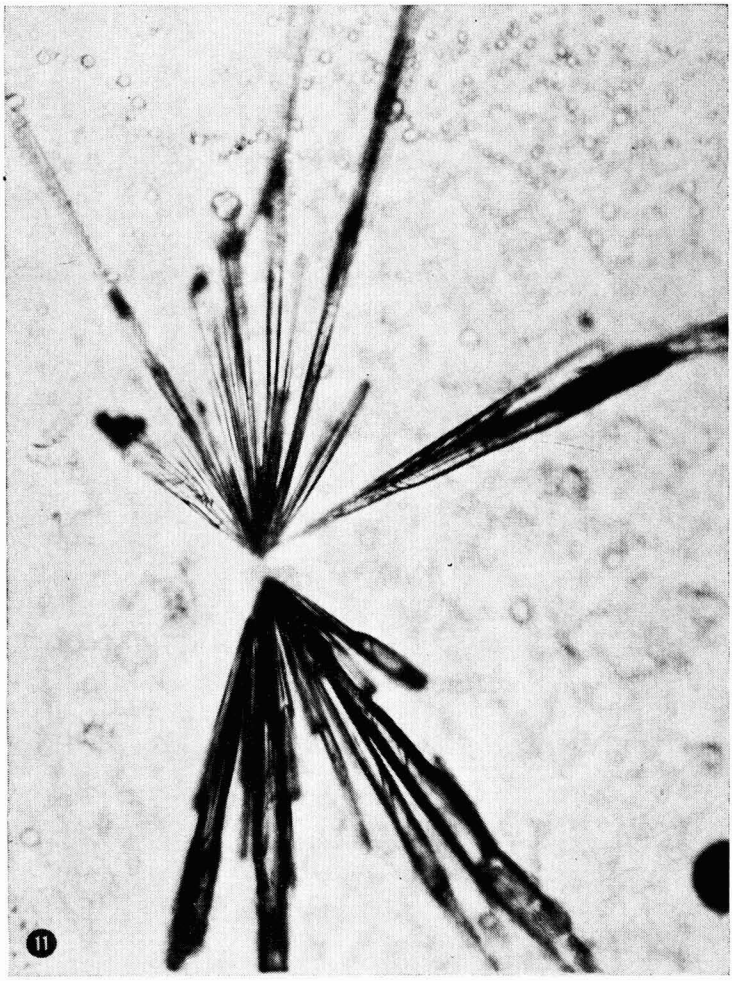

FIG. 11. Microcrystals of panamine with potassium chromate; crystals after 30 min; 80×.

gates, Class 5; 1 μg; dim birefringence, second order; inclined extinction; angle of extinction 12.5°; indifferent sign of elongation (Fig. 11).

SUMMARY

Microcrystal tests for lupanine, isolupanine, hydroxylupanine, epihydroxylupanine, angustifoline, ormosanine, ormojanine, and panamine are described. Microoptical data for the microcrystals are reported.

ACKNOWLEDGMENTS

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Near-Infrared Spectrophotometric Determination of Germanium by Modified Heteropoly Blue Method

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INTRODUCTION

Germanium(IV) and molybdenum(VI) in acidic solution form 12-molybdogermanic acid which has an intense yellow hue and has been used in the spectrophotometric determination of germanium (*2, 6*). The intensity of the color and stability of the heteropoly complex have been reported to be dependent on absolute and relative concentrations of germanium, molybdate, and acid; and the sequence of mixing reagents. A more sensitive spectrophotometric method for the determination of germanium has been based on the reduction of the molybdogermanic acid to give a blue reduction product (*1, 3, 5, 7*). Although the heteropoly blue method is intrinsically more sensitive it lacked precision because of the instability of the molybdogermanic acid and the critical nature of the time interval between the formation of the molybdogermanic acid and the addition of the reductant (*8*). Recently, the conditions with a stable form of molybdogermanic acid were delineated (*4*). This paper reports on a further study of the heteropoly blue method for germanium based on the initial formation of a stable 12-molybdogermanic acid which resulted in the development of a sensitive, precise, and convenient near-infrared spectrophotometric method for the determination of small amounts of germanium.

MATERIALS AND METHODS

Reagents

Standard germanium solution (25 μg of Ge/ml). Transfer 0.1803 g of reagent grade GeO_2 to a platinum crucible, and mix thoroughly with 1 g of Na_2CO_3. Fuse for 10 minutes or until a clear melt is obtained. After cooling, dissolve in 10 ml of distilled water. Adjust to pH 2 to 3 with 1:1 sulfuric acid and dilute to 500 ml with distilled water. Dilute a 50-ml aliquot to 500 ml with distilled water in a volumetric flask. The pH should be approximately 3 to 4. Store in polyethylene bottle.

Dilute molybdate solution (0.13%). Dissolve 2.60 g of reagent grade ammonium molybdate in 100 ml of distilled water. Store this

stock solution in a polyethylene bottle. Transfer a 5.0-ml aliquot to a 100-ml volumetric flask and dilute to volume with distilled water. Prepare this dilute solution daily.

Dilute hydrochloric acid solution (1.0 *N*). Store in a polyethylene bottle.

l-Ascorbic acid (0.3%). Dissolve 0.7500 g of *l*-ascorbic acid in distilled water and dilute to 250 ml in a volumetric flask. Store in a polyethylene bottle. Solution can be used for 2 weeks without appreciable loss in reducing strength.

Diverse ion solutions. Prepare all diverse ion solutions from reagent grade chemicals dissolved in distilled water.

Apparatus

Spectrophotometric measurements were made with a Cary 14 spectrophotometer operated in near-infrared mode and using 1.000-cm silica cells.

General Procedure

Transfer a 10.0-ml aliquot of the dilute molybdate solution to a 25-ml volumetric flask having a very low graduation mark. Add an amount of sample containing not more than 100 μg of soluble germanium. Use a buret to add 2.75 ml of dilute (1.0 *N*) hydrochloric acid solution, and dilute to volume with distilled water. Add immediately from buret exactly 1.0 ml of ascorbic acid solution and mix thoroughly the contents of flask. Place the volumetric flask in boiling water bath for 25 minutes for maximum color development. Remove flask and place it in a cold water bath. After 6 minutes, remove the flask from the water bath. Wait 6 minutes for the contents to reach room temperature. Prepare a reagent blank solution by this same procedure. Read the absorbance of the sample solution versus the reagent blank solution at 825 nm.

RESULTS AND DISCUSSION

Near-infrared absorption spectrum. The typical near-infrared absorption spectrum for the heteropoly blue of germanium is shown in Fig. 1. The maximum absorbance occurs at 825 nm.

Germanium concentration. The optimum concentration range based on a Ringbom plot is 0.7 to 2.9 ppm of germanium. Conformity to Beer's law was observed up to 4.8 ppm of germanium. The molar absorptivity is 3.58×10^4 liter mole $^{-1}$ cm^{-1}.

Effect of acid concentration. In studying the effect of various concentrations of hydrochloric acid on the formation and subsequent reduction

of molybdogermanic acid, it was found that the optimum final acidity was 0.08 to 0.12 *N* in respect to hydrochloric acid.

Reagent concentration. For a maximum concentration of 5 ppm of germanium no change in absorbance was found when 0.75 to 1.25 ml of a 0.3% ascorbic acid solution was used.

Development of heteropoly blue complex. The effect of the time allowed to elapse between formation of the molybdogermanic acid and the addition of reductant was investigated inasmuch as a 30-minute equilibration period had been recommended for maximum color development *(8)*. Although there was no appreciable difference in absorbance obtained when reductant is added immediately and 30 minutes after the formation of molybdogermanic acid, it was found that a shorter heating time was required for formation of the heteropoly blue when the ascorbic acid reagent was added immediately and that conformity to Beer's law was observed for higher concentrations of germanium. Hence, the immediate addition of the ascorbic acid reagent following addition of the sample solution to the molybdate reagent is recommended. In a series of determinations about 50 minutes is required for each sample.

Stability. The heteropoly blue of germanium is very stable. No appreciable change in absorbance was observed between 18 minutes after cooling in water and 12 hours later.

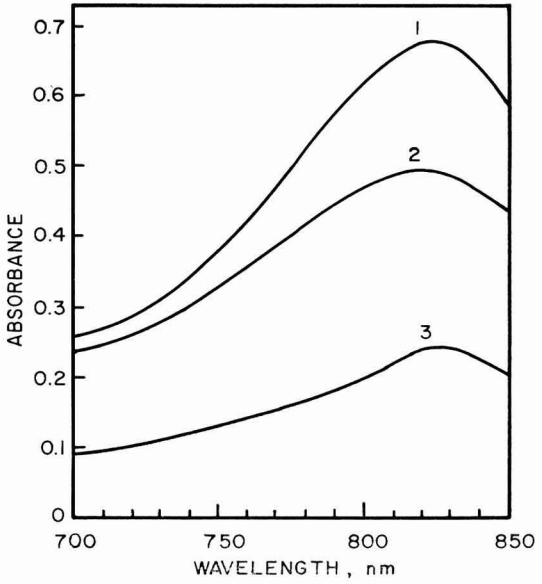

FIG. 1. Typical near-infrared absorption spectra for heteropoly blue of germanium: (1) 1.92 ppm Ge; (2) 1.44 ppm Ge; and (3) 0.72 ppm Ge.

Diverse ions. The effect of several cations and anions was studied using 1.44 ppm of germanium. Arsenate, arsenite, phosphate, silicate, and tungstate must be absent as these ions cause serious interference. The tolerances for several other ions are as follows (ppm): bismuth(III), 40; iron(III), 40; lead(II), 10; copper(II), 400; and vanadate, 40.

Precision. An indication of the precision obtainable by this modified heteropoly blue method for germanium was obtained by analyzing a series of 7 samples containing 1.44 ppm of germanium. A mean absorbance of 0.493 was obtained with a standard deviation of 0.006 absorbance unit, or a relative standard deviation of 1.2%.

SUMMARY

A near-infrared spectrophotometric method based on the initial formation of a stable form of molybdogermanic acid and the heteropoly blue reaction is proposed. The molar absorptivity at 825 nm is 3.58×10^4 liter mole^{-1} cm^{-1}.

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Trace Metal Analysis by Combined Thin-Layer Chromatography Incorporating Fluorescent Support and Ring Oven Colorimetry

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INTRODUCTION

Thin-layer chromatography (*1*) has the advantage over paper chromatography of affording highly delineated spots for different constituents with enhanced rapidity and sensitivity, while permitting a wide range of supports. Furthermore, the evaluation (*9*) of materials separated by TLC has a great potential so far as the reproducibility and precision of results are concerned. However, very few papers incorporating different convenient yet efficient quantitative analytical techniques with TLC separation are available for the microdetermination of inorganic materials.

Present studies have been made to explore this possiblity for the separation of Pb(II), Bi (III), U(VI), Th(IV) and Ni(II), Cu(II), Co(II), Cd(II) from their mixed solutions, using silica gel support and their evaluation by ring oven technique, using potassium thiocarbonate (*3–6*) (PTC) and other chromogenic reagents.

TLC SEPARATION

Experimental Methods

MATERIALS

Solvent systems. (a) *tert*-Butyl alcohol-acetic acid (3:2, v/v); (b) isobutyl alcohol–5 N HCl–ethyl methyl ketone (8:3:1, v/v).

Standard metal solutions. Pb(II), Bi(III), U(VI), Th(IV), Cu(II), Ni(II), Co(II) and Cd(II)—1 mg/ml of these metals were prepared by dissolving (A.R.) samples.

Visualization. The spots were visualized with sprays of 0.05 *M* aqueous potassium thiocarbonate (PTC) reagent, 3% $K_4Fe(CN)_6$ and 0.1% aqueous thoron solution.

Apparatus. Glass plates (20 × 6 × 0.5 cm) coated with a layer of silica gel G (E. Merck) and silica gel G containing 2% fluorescent indicator green (M. Woelm) were used. Impurity of iron was first removed by developing each plate in CH_3OH:HCl (9:1, v/v) after which activated by heating at 120° for 1 hour.

PROCEDURE

Separation of Pb(II), Bi(III), U(VI), and Th(IV). The glass plates coated with thin layer of silica gel G, 1–5 μl each of Pb(II), Bi(III), U(VI), and Th(IV) salt solution were applied separately from one end along the base line as a reference with the help of a microsyringe. At the other end of the base line, 1–5 μl each of the sample solutions were applied on one and the same spot, so as to produce a mixed sample of known composition. Each plate was developed within 45 minutes by the ascending technique, using solvent system (a), thereby achieving a clean separation of the constituent metal ions. The plate was then removed from the jar, well air-dried, and the resulting spots were visualized with sprays of 0.05 *M* PTC, 3% $K_4Fe(CN)_6$, and 0.1% thoron. For their evaluation, separation of the constituents was repeated on the plates of silica gel G containing fluorescent indicator; and the resulting spots were marked out by viewing under ultraviolet light. It is found that with solvent system (a), Pb(II) did not move at all; the order of movement of the constituent ions was:

$$U > Bi > Th > Pb.$$

Separation of Cu(II), Ni(II), Co(II), and Cd(II). The procedure followed was the same as earlier except that the solvent system (b) was used instead, permitting development within 60 minutes. Spots were visualized with sprays of 0.05 *M* PTC.

The order of movement in this case was found as:

$$Cd > Cu > Ni > Co.$$

Limits of identifications of the metal ions and the characteristic colors of the spots are given in Table 1.

RING OVEN COLORIMETRIC (*10*) DETERMINATION OF THE CHROMATOGRAPHED CONSTITUENTS

The Weisz ring oven technique is an elegant method for the determination of chemical constituents at submicro level. In this method, different rings after development with suitable chromogenic reagents are compared with the rings forming the standard scale.

Ring oven in conjunction with thin-layer chromatography has already applied by Ottendrofer (*7*) and Scherz *et al.* (*8*) in the determination of few ions using chromogenic reagents.

Experimental Methods

MATERIALS

Weisz ring oven. (National Appliance Co., Portland, Oregon).

Mottier gadget (*2*). It was used for removing the thin-layer chromato-

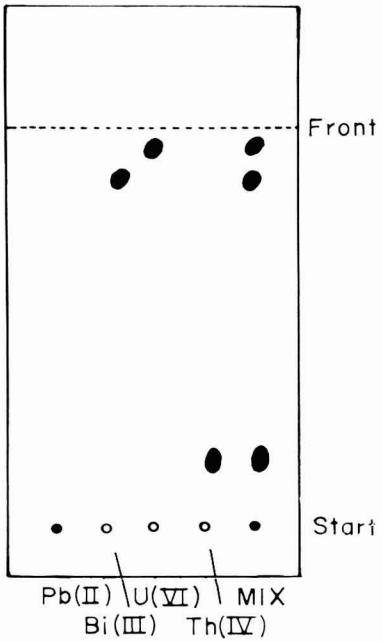

FIG. 1. Separation of Pb/Bi/U/Th; solvent: *tert*-butyl alcohol: acetic acid (3:2; v/v); spray reagents: PTC, $K_4Fe(CN)_6$ and thoron.

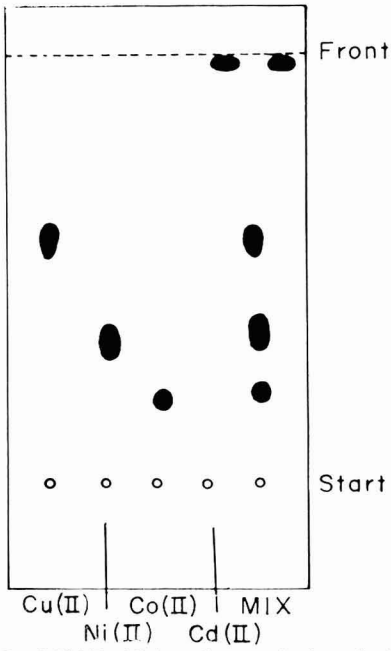

FIG. 2. Separation of Cu/Ni/Co/Cd; solvent: isobutyl alcohol–5 *N* HCl–mehyl ethyl ketone (8:3:1; v/v); spray reagent: PTC.

TABLE 1
RESULTS OF TLC SEPARATION

Ions	Visualizing agent	Color	$R_f \times 100$	Limits of identification (μg)
Pb(II)	PTC	Pinkish red	0	3.00
Bi(III)	PTC	Dark brown	90.8	3.20
U(VI)	$K_4Fe(CN)_6$	Dark brown	96.9	1.20
Th(IV)	Thoron	Crimson pink	15.1	1.00
Cu(II)	PTC	Brown	60.5	1.30
Ni(II)	PTC	Yellow	33.9	2.20
Co(II)	PTC	Black	20.1	2.80
Cd(II)	PTC	Yellow	98.4	2.00

grams. This is preferable to the direct scooping method as it does not allow any loss of chromatographed samples during their transfer to the ring oven.

Hydrochloric acid (1*N*), prepared from B.D.H. AnalaR sample.

Silver nitrate 1% aqueous solution of AnalaR grade material was used.

PROCEDURE

The marked out portions of the silica gel G fluorescent absorbent layer were sucked out one by one by means of Mottier gadget, as based upon the principle of a vacuum cleaner. A circle of filter paper (Whatman No. 41) of 55-mm diameter was placed on the oven set at 100°C. The collected material containing the individual constituent was placed at the marked center of the filter paper circle and washed by means of a self-filling capillary pipette. Each ring corresponding to Pb(II), Bi(III) was developed separately by applying PTC (*5, 6*) solution with the help of a capillary pipette at the center and washing the same into the ring. After drying, subsequent washing was done with 1*N* HCl to fix the metal ions as sulfides and to remove excess of PTC from the ring zone. Final washing was done with distilled water.

For uranium, the ring was developed with a 3% solution of $K_4Fe(CN)_6$ while the ring due to the Th(IV) was developed with aqueous solution of thoron.

Different rings were thus obtained for different ionic constituents.

Similarly, standard rings were prepared with 2, 4, 6, 8, 10, and 12 drops each of the standard ion solutions, taken separately. These standard rings were finally compared with the rings obtained for the different test samples and thus the amounts were computed in each case.

Determination of Cu(II), Ni(II), Co(II), and Cd(II). Visualized spots on the thin-layer of silica gel G were first marked out and fumed with bromine to oxidize the thiocarbonate/sulfide to sulfate form. The delineated portions of the adsorbent layer were then sucked by means of Mottier gadget one by one.

Next, rings due to Ni(II), Co(II), Cu(II) and Cd(II) were obtained by developing with PTC solution. The standard rings for the different ions were then prepared and compared with the rings obtained for Ni(II), Co(II), Cu(II), and Cd(II). Results of a few representative experiments are given in Table 2.

Silver scale method. In the case of CdS ring the color being light yellow, efficient comparison of the same for direct evaluation was not

TABLE 2
REPRESENTATIVE RESULTS OF DETERMINATION

Metal ion	Metal ion (mg/ml)		
	Taken	Found	Error (%)
Pb(II)	1.80	1.94	+7.77
	2.40	2.20	−8.33
	2.14	2.32	+8.41
Bi(III)	1.70	1.85	+8.82
	2.30	2.10	−8.80
	2.80	2.62	−6.42
U(VI)	2.20	2.00	−9.09
	1.90	2.05	+7.89
	1.80	1.96	+8.44
Th(IV)	0.84	0.92	+9.52
	0.96	1.03	+7.29
	1.04	0.96	−7.67
Cu(II)	1.40	1.52	+8.56
	2.52	2.30	−8.73
	1.88	1.72	−8.51
Co(II)	3.64	3.36	−7.69
	4.24	3.90	−8.01
	2.80	3.00	+7.14
Ni(II)	2.90	3.12	+7.58
	3.32	3.10	−6.62
	3.82	3.56	−6.80
Cd(II)	2.70	2.90	+7.4
	3.46	3.18	−8.92
	3.80	3.50	−7.99

possible. Determination of cadmium, therefore, was made possible by treatment with 1% silver nitrate solution to obtain a corresponding amount of Ag_2S. Each ring was dried and compared with the standard rings, prepared similarly.

DISCUSSION

For the separation of Pb(II), Bi(III), U(VI), Th(IV), the presence of ethyl methyl ketone in the solvent system is essential as it prevents tailing of the spots. However, Pb(II) did not form a complex and hence remained at the origin. But for the separation of Cu(II), Ni(II), Co(II), and Cd(II) the presence of acetic acid in the solvent system serves the same purpose. Table 2 reveals that the results are accurate and that the errors lie within permissible limits of semiquantitative procedures.

SUMMARY

Clean and speedy analytical separation of microgram quantities of Pb(II), Bi(III), U(VI), and Th(IV); and Cu(II), Ni(II), Co(II), and Cd(II) from their mixed solutions has been made possible by ascending thin-layer chromatography. For the evaluation of different metal ions, the resulting delineated spots were scooped out with the help of a Mottier gadget and the collected material was transferred to the paper set on the ring oven. Separate rings were obtained for the individual metal ions and the computation of results was made possible by ring colorimetry, using PTC and other chromogenic reagents.

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Suggested Procedure For Microdetermination of Arsenic In Arsenical Animal Feed

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INTRODUCTION

Due to the highly poisonous nature of arsenic, much attention has been given by analytical chemists to the detection and determination of even trace amounts of it, especially when suspected to be present in foodstuffs. Consequently, from time to time various analytical methods for its identification and estimation have been proposed. The earliest though sensitive but inconvenient method is by Gutzeit (*1*). Lately, numerous colorimetric (*1*) and paper chromatographic (*2*) methods have been reported. More recently, a review (*2*) and a method by Wilson and Lewis (*3*) discussing the estimation of arsenic have been reported. It is gathered that the methods reported so far are either qualitative in scope, or if quantitative, are inconvenient and not so sensitive as sometime desired.

Present studies relate to the problem which the authors had received from the United States Department of Agriculture, Agriculture Research, Animal Health Division, Beltsville, Maryland. This problem requires a sensitive and convenient analytical method of determining as low an amount as 0.2% arsenite (as As_2O_3) present in arsenical animal feed. For this study, the starting material required is the muriatic acid extract of the cattle feed. The presently proposed method for the detection and determination of trace amounts of arsenite is based on the ring oven technique using potassium thiocarbonate (PTC) reagent. PTC (*4–6*) has already been reported as a good source of thiocarbonate and sulfide ions for efficient precipitation of various cations and as well as for complexation of those cations which yield soluble thiocarbonate products which are characteristically colored.

Weisz (*7*) ring oven technique is a versatile and elegant method for increasing the sensitivity of spot test on microscale, and for the development of semiquantitative and quantitative procedure at the submicro level. By this technique, a single drop of a test solution is applied to the center of filter paper placed on an electrically heated metallic ring. One or more of the components of a test sample are fixed (precipitated)

with one or more suitable reagents, others are washed out to the ring zone. For the semiquantitative analysis the rings after development with specific reagents are compared with the rings forming the standard scale. The standard scale in a particular case is prepared by developing known amounts of metal ions in the ring with the same reagents.

Standard scale (7) procedure of Semiquantitative Analysis by Ring Colorimetry

The standard scale rings are prepared with 1, 3, 5, 7, and 9 drops using self-filling pipette and marked as I, III, V, VII, and IX. During the application of several drops great care is taken to avoid enlargement of the spots. This is done by drying the previously spotted drop before applying the new ones. Generally, the standard scale is prepared with not more than 9 to 10 drops of the standard solution. This is, because with large number of drops the intensity of the color is too high to be distinguished. A single standard solution is sufficient for preparing one standard scale.

EXPERIMENTAL METHOD

Instrument. Weisz Ring Oven used for this study was obtained from National Appliance Co., Portland, Oregon.

Potassium thiocarbonate (PTC) aqueous solution, prepared fresh by suitable dilution of stock solution (1 *M*) with distilled water.

Standard Metal Solution. 0.1% solution of sodium arsenite (A.R.) was prepared as stock solution and was used after suitable dilution to work out the standard scale.

Silver nitrate. 1% aqueous solution of AnalaR grade material was used.

Hydrochloric acid. 1 *N* prepared from B.D.H., AnalaR sample.

PROCEDURE

A circle of filter paper (Whatman No. 41) of 55-mm diameter was placed on the oven set at 100°C. A drop (1.5 μl) of diluted test solution containing microgram quantities was spotted at the marked center of the filter paper circle using self-filling capillary pipette and was washed out into the ring with distilled water, taking 10 μl of it at a time. The ring was developed by applying PTC solution 2 to 3 times with the help of capillary pipette at the center and washing the same into the ring. After drying, HCl was applied to fix As_2S_3 and to remove any excess of PTC from the ring. The filter paper was finally washed with excess of distilled water.

Determination by Silver Scale (7) Method

Arsenite being present in micro amounts and the color of As_2S_3 being light yellow, efficient comparison of the same for direct evaluation is not possible. Determination of arsenic, therefore, is made possible by transforming As_2S_3 into equivalent amount of silver sulfide. For this, rings comprising the standard scale as well as the unknown (after repeatedly washing with distilled water) were finally developed by dipping into a bath of 1% silver nitrate solution. Each ring was dried and compared with standard scale rings, prepared similarly.

Represented results of a few experiments are given in Table 1.

DISCUSSION

Detection and determination of arsenic in muriatic acid extract of animal feed is possible down to the 0.04 $\mu g/\mu l$ present. The presence of calcium, iron, and other metal ions usually present in animal feed does not interfere in this estimation of arsenic. Computation of results as based on the silver scale method allows the determination of arsenic within 6–8% error.

SUMMARY

The ring oven technique has been proposed for the microdetermination of arsenic present as arsenite in arsenical animal feed using potassium thiocarbonate (PTC) reagent as the principal reagent. It is possible to estimate as low as 0.04 $\mu g/\mu l$ of arsenic within 6–8% error.

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TABLE 1

RESULTS OF THE STANDARDIZATION PROCEDURE FOR THE DETERMINATION OF As(III)

Amount of Arsenic ($\mu g/\mu l$)		
Present	Found	Error
0.06	0.055	−8.33
0.50	0.53	+6.00
0.14	0.15	+7.14

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The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Lead (Mercury, Copper, or Silver) Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking

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INTRODUCTION

In a previous paper (*1*) the extraction and photometric determination of zinc with 1-(2-pyridylazo)-2-naphthol (PAN) has been described. That paper dealt with the application of the method in the presence of great excesses of cadmium using iodide ion as an effective masking agent. The influence of several parameters including pH, type of buffer, kinetic factors in extraction and color development and the effectiveness of the masking have been studied. For details concerning these aspects as well as the optical properties of the system the paper mentioned should be consulted.

During studies of interference due to the presence of other metals likely to be found in systems high in cadmium content it was observed that the iodide is effective in masking significant amounts of lead, copper, mercury, and silver. Since zinc is a commonly occurring impurity in lead and lead salts, a method for determining zinc in this matrix would be of special practical interest. These facts resulted in the initiation of a detailed investigation of the adaptability of the zinc in cadmium method to the determination of zinc in the presence of large amounts of lead. Preliminary studies were also made of the applicability of the method to the determination of zinc in the presence of large amounts of copper, mercury, or silver with emphasis on finding the maximum permissible matrix metal to zinc ratio.

EXPERIMENTAL METHODS

Apparatus

The analytical measurements were made with a Bausch and Lomb 505 or Spectronic 20 spectrophotometer. The former instrument was also employed to record the absorbance curves.

Reagents

Zinc solutions. A 0.01 *F* stock solution was prepared by dissolving "Baker Analyzed" reagent zinc nitrate hexahydrate. Solutions 10^{-3} and 10^{-4} *F* were prepared by appropriate dilution of the stock solution. The exact zinc concentrations in the soltutions were established by EDTA titrations.

Lead nitrate (0.50 *F*), *copper(II) nitrate* (0.50 *F*), *mercury(II) nitrate* (0.25 *F*) and *silver nitrate* (1.0 *F*) *stock solutions* and *working solutions of other metals* for the interference studies were prepared from purest available material.

Potassium iodide. "Baker Analyzed" reagent, Baker USP and Curtin USP grade potassium iodide were used interchangeably. No differences were noted.

Maleate buffer (pH 6.8). An approximately 1 *F* solution of maleic acid was prepared and neutralized with 50% sodium hydroxide solution. After cooling the pH was adjusted to about 7. Metal impurities were removed by dithizone extraction and then the solution was diluted to a concentration of about 0.5 *F* in maleate. Hydrochloric acid or sodium hydroxide was added to bring the pH to 6.8. The buffer solution is usable for a few days up to a few weeks depending on the experimental conditions of its use, temperature, storage, etc. Attempts to increase the useful life of the buffers by adding thymol as a bactericide were unsuccessful. The problems associated with the use of aged buffers, and the remedies are discussed in the preceding paper (*1*).

Tartrate solution (pH 6.8). "Baker Analyzed" reagent *d*-tartaric acid was employed to prepare a 1 *F* solution which was then brought to pH 6.8 with sodium hydroxide.

Backwash solution. The solution was prepared by placing 50 g of potassium iodide, 18 ml of maleate buffer, 5 ml of tartrate solution, pH 6.8, 5 ml of 1 *F* sodium thiosulfate and some water in a beaker. After dissolution was complete, water was added to bring the volume to about 100 ml.

PAN solution. Fisher Certified PAN (0.62 g) was dissolved in 95% ethanol (250 ml) to yield a 0.01 *F* solution.

Chloroform. NF grade was used after extractive washing with dilute aqueous hydrochloric acid to remove metal traces.

Water. Doubly de-ionized water was used exclusively.

PROCEDURE 1

Limitations for the application of the procedure are as follows: The lead content of the sample must not exceed 0.5 g and the zinc content must not exceed 25 μg. The molar lead:zinc ratio should not exceed 50,000:1 (corresponding weight ratio 160,000:1).

For samples with higher ratios minor procedural changes are necessary as described in Procedure 2.

Interferences can be handled according to type and tolerance limits as shown in Table 4.

Simplifications are possible in many instances. If metals (other than lead and zinc) that react with PAN are present in negligible amounts, addition of tartrate solution in Step ii and of thiosulfate solution in Step iv can be omitted. Both of these solutions can also be omitted in the preparation of the backwash solution employed in Step x. Several other simplifications are mentioned in the notes to the procedure.

Procedure

(i) Dissolve the sample in water or the minimum amount of nitric acid required, avoiding too great a dilution. Transfer to a volumetric flask and dilute with water to mark. Pipet an aliquot of appropriate size into a 100-ml beaker (Note 1).

(ii) Add 1 ml of tartrate solution of pH 6.8. For each 0.1 g of lead present, add 10 g of potassium iodide (Note 2). Then add sufficient water to dissolve all solids (Note 3).

(iii) Immerse the electrodes of a pH meter in the solution and add sodium hydroxide or nitric acid as necessary until a meter reading of about 7.1–7.3 is obtained (Note 4).

(iv) Add 2 ml of 1 *F* sodium thiosulfate, a spatula tip (2–4 mg) of ascorbic acid and 1 drop of 1 *F* potassium cyanide. Now proceed without undue delay until Step ix is completed.

(v) Transfer the solution to a 60- or 125-ml separatory funnel equipped with a Teflon stopcock.

(vi) Add 1.4 ml of 0.01 *F* PAN in 95% ethanol in increments of 0.2, 0.2, 0.2, 0.2, and 0.6 ml. Shake for 2 to 5 seconds after each 0.2 ml increment and 45 seconds after the final addition.

(vii) Add 3 to 4 ml of chloroform and shake for 20 seconds. Add

water to almost fill the separatory funnel (Note 5) and shake briefly and allow the phases to separate.

(viii) Drain the organic layer into a second separatory funnel the stem and stopcock bore of which must be free of water.

(ix) Extract the aqueous layer remaining in the first separatory funnel with five 1- to 2-ml portions of chloroform (Note 6) and collect these extracts in the second funnel too.

(x) To the combined chloroform extracts add 10 ml of backwash solution and a spatula tip (2–4 mg) of ascorbic acid. Add water to aid the separation of the phases, shake briefly, and allow phase separation.

(xi) Drain the organic layer into a dry 25-ml volumetric flask and wash the agueous layer with two 2- to 3-ml portions of chloroform.

(xii) To the combined extract and washings add 5 ml of 95% ethanol, allow the system to attain room temperature and then fill with chloroform to mark (Note 7).

(xiii) Measure the absorbance of the chloroform–ethanol solution against water as reference at the wavelength of the absorbance maximum (about 550 μm) (Note 8).

(xiv) Correct the absorbance for that of a blank containing the same amount of lead as does the sample (Note 9).

(xv) Obtain the zinc content of the sample from a calibration curve (Note 10).

Notes

(1) If the composition of the sample and its mode of dissolution are such that neutralization is unnecessary, omit Step iii and transfer the solution directly to the separatory funnel and make the additions mentioned in step ii and in step iv into that funnel.

(2) If the sample is free of extraneous iodide-consuming interferences the amount of potassium iodide may be decreased to 7 g/0.1 g of lead.

(3) There is indication that marginally better results are obtained if the solution cooled by dissolution of the salt is not allowed to warm to ambient temperature before proceeding. There is certainly no reason to lengthen the time required to finish the determination by allowing the solution to warm.

(4) If the electrodes of the pH meter are immersed in the cold solution on hand at this point, the pH should be as indicated. The discrepancy between this value and the nominal value of the buffer pH 6.8 stems from the grossly changed conditions (temperature and ionic strength). It is therefore appropriate to make reference to "meter reading" instead of pH.

(5) If no water were added, organic and aqueous phase would show almost identical densities and phase separation would be extremely slow.

(6) If the organic extract is colorless after less than the specified number of chloroform portions, discontinue washing and proceed to the next step.

(7) A negative error not exceeding 3% is encountered when the solution is brought to mark without temperature equilibration.

(8) The exact wavelength of the absorbance maximum of the species of interest, $Zn(PAN)_2$, is 556 μm. This wavelength may be employed for routine work. However, the band pass of the monochromator and the absorbance of excess PAN influence the absorbance values obtained. It is therefore recommended that the maximum be empirically established for the particular instrument employed.

(9) For samples containing 0.2 g of lead or less the absorbance of the blank is negligible and step xiv may be safely omitted. For samples with about 0.5 g of lead the blank corresponds to about 2 μg of zinc. The standard lead nitrate solutions should be prepared from high purity material to assure negligible amounts of zinc.

(10) In preparation of the calibration curve, not more than 10 g of potassium iodide need be added in Step ii and the backwash operation in step x may be omitted. The extract and washings (Steps vii, viii, and ix) are drained directly into the volumetric flask (Step xi).

PROCEDURE 2

Limitations of the method have not been determined; however, the procedure has been tested up to 1 g of lead and a lead:zinc molar ratio of 250,000:1 (corresponding to a weight ratio of 800,000:1). There is every reason to expect that higher amounts can be processed; however, with 10 g of potassium iodide being required for each 0.1 g of lead, economic considerations become important.

Procedure

(i) Dissolve an appropriate amount of sample in water or the minimum amount of nitric acid in a 100-ml beaker.

(ii) through (ix) as in procedure 1.

(A) To the combined extract and washings in the second funnel add 2.00 ml of 0.100 *F* hydrochloric acid (Note 1) and shake. Allow phase separation and discard the organic layer (Note 2).

(B) Add 2.00 ml of 0.100 *F* sodium hydroxide (Note 1), 2 ml of the maleate buffer and 5 g of potassium iodide and shake.

(C) Carry out step vi of Procedure 1.

(D) Shake with 2 ml of chloroform, add water, allow phase separation, and drain the organic layer into a 10-ml volumetric flask. Wash the organic layer with five 1-ml portions of chloroform.

(E) To the combined extract and washings in the volumetric flask, add 2 ml of 95% ethanol, allow to attain room temperature and fill with chloroform to mark.

(xiii) through (xv) of Procedure 1.

Notes

(1) Neither volume nor concentration of the hydrochloric acid and sodium hydroxide in Steps (A) and (B), respectively, are critical as long as nearly equivalent amounts are employed.

(2) Care should be taken to remove all chloroform droplets.

DISCUSSION AND RESULTS

Many of the findings made during the investigation of the zinc–cadmium system could be applied to the zinc–lead system because of some close similarities in the behavior of cadmium and lead. Both cadmium and lead form PAN complexes in weakly acidic or neutral media. The stabilities of the complexes are of approximately the same order as can be concluded from percentage extraction *versus* pH plots (*2, 3, 4*). Furthermore, the stepwise formation constants of the iodo complexes of cadmium and lead are approximately equal (*5*).

However, there are a few differences which, although they appear to be of no great consequence, require that the procedure developed for the zinc–cadmium system be modified significantly for the determination of zinc in the presence of a large excess of lead.

In the zinc–cadmium system a mere partial masking of the cadmium is sufficient because of the kinetics of the extraction process. In order to guarantee the complete extraction of zinc it is enough that a situation prevail that allows the zinc to compete successfully with the cadmium for some of the PAN introduced. Under partial masking conditions, cadmium–PAN complexes are formed also that are coextracted. However, these complexes rapidly decompose in the chloroform phase and the cadmium returns to the aqueous phase. To assure full removal of the cadmium from the organic phase a backwash step is incorporated in the procedure (*1*).

The lead–PAN complexes are not decomposed upon extraction, or at least not at a sufficiently high rate and thus their formation has to be prevented *a priori*. That means for the practice of the procedure that a sufficiently large amount of potassium iodide must be added to fully mask lead against reaction with PAN. It was expected that this disad-

vantage would in part at least be balanced by the advantage of the backwash step becoming unnecessary. However, such expectation did not materialize. The chloroform layer always contains a haze of minute droplets of aqueous phase. These droplets contain highly concentrated lead solution and must be removed by a backwash step. If no such step is applied the lead becomes available for reaction with PAN when alcohol is added in a later step of the procedure. Another difference between the systems containing cadmium and lead is that the iodide of the former metal is soluble while that of the latter forms a precipitate. However, no practical difficulty arises from this fact because the amount of iodide required to fully mask the lead toward PAN is more than sufficient to completely redissolve the lead iodide with formation of soluble iodo complexes.

Results with solutions containing only zinc and lead. Some artificial "unknowns" were prepared by taking known amounts of the lead nitrate stock solution and adding measured quantities of a dilute zinc nitrate solution. Results obtained from application of Procedure 1 are shown in Table 1. At a lead content of about 0.5 g the blank correction (Step xiv) is equivalent to 1.8 $\pm$ 0.2 μg of zinc. The correction is sufficiently small and reproducible to allow good results to be obtained with a lead content at that level. However, at a lead content of about 1 g the zinc equivalent is 6 $\pm$ 1 μg. This blank correction is so large and so variable in comparison with the quantity of zinc sought that good results are no longer obtained. Furthermore, it was observed that this large and variable blank correction persists even when potassium iodide is added in an amount 50% greater than specified in Procedure 1. Thus the applicability of Procedure 1 is limited to samples with a lead content not exceeding about 0.5 g.

For "unknowns" with a lead content in excess of 0.5 g, Procedure 2 was applied. Results are shown in Table 2.

The data in Tables 1 and 2 show the method to yield results that are reliable within some tenths of a microgram of zinc. The standard deviation is 0.7 μg. It has been observed throughout the investigation that the magnitude of the absolute error is essentially independent of the zinc content of the sample. Hence, the larger the zinc content, the smaller the relative error.

Interference studies in the zinc–lead system. Only a few anions were considered. These were the counter ions present in some commercially important lead salts. A number of cations were investigated and particular attention was paid to those likely to be found in high purity lead. Representative results are shown in Table 3.

For practical purposes it is of value to establish tolerance limits or

TABLE 1

RESULTS OF THE DETERMINATION OF ZINC IN LEAD USING PROCEDURE 1

Lead taken (g)	Zinc (μg)		Error (μg)	Lead:zinc molar ratio
	Taken	Found		
0.21	25.2	25.6	+0.4	2,500
		23.9	−1.3	
		24.6	−0.6	
		24.1	−1.1	
0.21	12.6	12.2	−0.4	5,000
		12.3	−0.3	
0.21	6.3	5.7	−0.6	10,000
		5.9	−0.4	
0.21	3.2	2.9	−0.3	20,000
		3.5	+0.3	
		4.0	+0.8	
0.53	25.2	24.0	−1.2	6,200
		26.0	+0.8	
		26.0	+0.8	
0.53	9.4	9.6	+0.2	17,000
		9.4	0	
0.53	3.1	2.7	−0.4	50,000
		2.5	−0.6	
		3.1	0	

interference thresholds. Since the error increases with increasing amounts of interference, it is necessary to define the maximum error permissible. For the present purpose the interference threshold is arbi-

TABLE 2

RESULTS OF THE DETERMINATION OF ZINC IN LEAD USING PROCEDURE 2

In each run the quantity of zinc taken was 1.3 μg. The lead content of each sample was 1.06 g, thus the lead:zinc molar ratio was 250,000:1 and the weight ratio was 800,000:1.

Run No.	Quantity of zinc found (μg)	Error (μg)
1	1.6	+0.3
2	1.8	+0.5

TABLE 3

REPRESENTATIVE RESULTS OF THE DETERMINATION OF ZINC IN LEAD SAMPLES CONTAINING ADDITIONAL INTERFERENCES

Procedure 1 was used unless noted otherwise. Each sample contained 4.0 μg of zinc. The lead content of each sample was 0.25 g, thus the lead:zinc molar ratio was 20,000:1.

Interference	Quantity of interference present (mg)	Interference:zinc molar ratio	Quantity of zinc found, (μg)	Error (μg)
Acetate	540	150,000	3.8	−0.2
Sulfate	260	44,000	3.6	−0.4
Chloride	190	130,000	3.6	−0.4
Cd	25	3600	4.4	+0.4
Cu	3.0	750	4.3	+0.3
Hg(II)	19	1600	4.3	+0.3
Ag	7.2	1100	3.9	−0.1
Au(III)	5.2	430	4.3	+0.3
Al	1.3	750	4.1	+0.1
Ga	0.085	20	4.3	+0.3
In	0.035	5	4.4	+0.4
Tl(I)	1.7	140	3.8	−0.2
Sb	0.150	20	4.1	+0.1
Bi	0.380	30	3.9	−0.1
Fe(III)[a]	0.085	25	3.9	−0.1
Fe(III)	0.035	10	3.7	−0.3
Fe(II)	0.035	10	3.8	−0.2
Co	0.140	40	4.4	+0.4
Ni	0.230	65	3.9	−0.1
Mn	0.020	6	4.2	+0.2
Sn(II)[b]	0.060	8	4.1	+0.1
Sn(IV)	0.012	2	3.5	−0.5
Cd	24	3600		
Cu	1.6	400	5.0	+1.0
Hg(II)	4	320		
Ag	1.8	280		
Cd	25	3600		
Cu	6	1500		
Hg(II)	19	1600		
Ag	7.2	1100	4.8	+0.8
Co	1.7	500		
Ni	0.7	200		

[a] The addition of ascorbic acid was omitted in Step iv.

[b] PAN was added dropwise until color persisted for 20 to 30 seconds prior to the addition of the specified increments of PAN stated in Step vi. This is required as PAN is almost instantaneously decomposed by Sn(II).

[c] Using the approach of the additional cyanide masking previously described (*1*) in conjunction with Procedure 1 of this paper.

trarily defined as the amount of interfering substance that causes an error equivalent to 0.4 μg of zinc. The approximate tolerance limits for some interferences are listed in Table 4.

The cations listed other than tin and thallium introduce a positive error. Tin introduces a negative error and thallium, as well as the sulfate anion, interfere by formation of a precipitate. Those cations which form relatively stable iodo complexes, namely cadmium, copper, mercury, and silver, interfere in an indirect fashion by demasking lead. The remaining cations interfere by formation of PAN complexes which absorb in the vicinity of 550 μm.

For all cations other than thallium the interference thresholds are considerably lower in the lead system than in the cadmium system. This

TABLE 4

TOLERANCE LIMITS OF VARIOUS SPECIES IN THE DETERMINATION OF ZINC IN 0.25-g LEAD SAMPLES USING PROCEDURE 1

Interference	Tolerance limit (mg)
Acetate	500
Sulfate	250
Chloride	180
Cd[a]	25
Cu[a]	4
Hg(II)[a]	30
Ag[a]	10
Au(III)	6
Al	3
Ga	0.1
In	0.03
Tl(I)	1.5
Sb	0.2
Bi	0.5
Fe(III)[b]	0.1
Fe(II)	0.05
Co[a]	0.12
Ni[a]	0.25
Mn	0.02
Sn(II)[c]	0.06
Sn(IV)	<0.01

[a] Moderate increases in tolerance limits are possible by use of the approach of additional cyanide masking previously described (*1*) in conjunction with Procedure 1 of this paper.

[b] Omitting the addition of ascorbic acid in Step iv.

[c] Adding PAN dropwise until color persists for 20 to 30 seconds prior to the addition of the specified increments of PAN in Step vi. This is required as PAN is almost instantaneously decomposed by Sn(II).

significant difference can be explained as follows. Because the lead–PAN complexes do not decompose to an appreciable extent during the extraction or the backwashing, lead has to be fully masked by iodide against PAN. Thus, unlike cadmium, lead fails to act as a PAN "sink" thereby allowing the ready formation of PAN complexes of many extraneous metals. However, the most striking differences occur with those cations previously mentioned that form relatively stable iodo complexes and thus prevent the complete masking of lead.

Only very slight increases in interference thresholds result from the use of the approach of additional cyanide masking described in the preceding paper (*1*) in conjunction with Procedure 1.

As is commonly the case in systems containing more than one interference, tolerance limits are not additive.

Although the tolerance limits are lower here than in the cadmium system they are sufficiently high to readily permit the determination of zinc in high purity lead.

Determination of zinc in copper, mercury, or silver systems. Only superficial observations were made of the copper, mercury, and silver systems. These observations were sufficient to establish the feasibility of iodide masking in these systems, and to estimate the limiting metal to zinc ratios. The values shown in Table 5 are the result of the application of the essence of Procedure 1 with the modifications noted below to the various systems. In Step ii in each case the quantity of potassium iodide used was restricted to 20 g. In Step iv, in the case of copper, sufficient sodium thiosulfate was used to reduce all the iodine produced in the reaction between copper(II) and iodide and, in addition, considerably more than 2–4 mg of ascorbic acid were employed.

Since mercury and silver form iodo complexes of great stability, it is expected that the addition of more than 20 g of potassium iodide would permit the use of larger samples of the metal and would not affect the

TABLE 5

APPROXIMATE LIMITING RATIOS FOR THE DETERMINATION OF ZINC IN THE PRESENCE OF GREAT EXCESSES OF COPPER, MERCURY, OR SILVER

Matrix metal	Maximum permissible amount of matrix metal (g)	Limiting metal:zinc	
		Molar ratio	Weight ratio
Copper	0.3	50,000:1	50,000:1
Mercury	10	500,000:1	1,500,000:1
Silver	3	300,000:1	500,000:1

sensitivity. Thus it would be readily possible to increase the metal:zinc ratios above those shown.

Although copper(I) also forms a very stable iodo complex the addition of more potassium iodide does not increase the permissible copper:zinc ratio above the indicated level. When a sufficiently large quantity of copper is present the various reducing agents either fail to completely reduce the last traces of copper(II) or, alternatively, some copper(II) is produced instantaneously by air oxidation during the shaking operation required to form the zinc–PAN complexes. In any event some copper–PAN is formed and this complex is sufficiently robust as to not decompose during the course of the extraction and backwashing operations. To increase the permissible copper:zinc ratio would require the acid decomposition of the extracted complexes, followed by neutralization and reextraction as in Procedure 2 for the lead system, or some other similar approach.

Silver does not form PAN complexes and thus would not require any masking. However, the addition of iodide is desirable in order to mask other impurities that do form PAN complexes. The addition of only moderate amounts of iodide would result in the formation of a silver iodide precipitate which would interfere with the extraction. Thus, iodide in high concentrations is employed and masks silver against the precipitation by formation of the soluble silver–iodo complex.

No investigations were made of the tolerance of the copper, mercury, or silver systems for extraneous interferences. Since these cations all form high stability iodide complexes the tolerance limits would be expected to be no greater than those of the lead system, and might well be even lower.

SUMMARY

The zinc in high purity lead is determined photometrically as the PAN complex after extraction into chloroform. Massive amounts of potassium iodide are added to the sample solution to mask lead. Cadmium, copper, mercury, and silver are also masked by the iodide. Some cyanide is added which effects masking of nickel and cobalt. Tolerance limits for these and several other interferences are given. The results are reliable to several tenths of a microgram of zinc. Samples containing up to about 0.5 g of lead can be handled. Using a simple procedure, zinc can be determined at a lead:zinc molar ratio of 50,000:1; with additional operations incorporated even at ratios of 250,000:1. Preliminary studies indicate this approach is also applicable to the determination of zinc in copper, mercury, or silver at respective matrix metal:zinc molar ratios of 50,000:1, 500,000:1, and 300,000:1.

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MICROCHEMICAL JOURNAL 15, 666–672 (1970)

Microchemical Urinalysis

VI. Determination of Sodium, Potassium, Calcium, and Magnesium by Atomic Absorption Spectrophotometry in the Microliter Range of Urine [1]

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INTRODUCTION

In line with our aims set forth in the first paper (*1*) of this series, this laboratory has developed a simplified procedure for the determination of sodium, potassium, calcium, and magnesium in microliter quantities of urine, using atomic absorption spectrophotometry (AA).

Previously (*1*) calcium and magnesium were determined by titration to a colorimetric end point. This procedure is useful when only a few samples are available for analysis and only these two elements are desired. However, AA offers a number of distinct advantages over titrations. It is faster, more sensitive, specific; has a larger dynamic range of concentrations, uses one sampling for all elements; and is not subject to an analyst's individual variations in colorimetric end point determinations.

By using the appropriate hollow-cathode tube, AA is theoretically specific for a given element. However, after extensive investigations in this laboratory (*3, 4*), it has been found that the analyses for sodium, potassium, calcium, and magnesium are subject to interferences by other elements. This, as will be seen, is very easily compensated. Blank solutions are prepared that suppress the interfering elements, and since 1 part sample is diluted with 99 parts blank the accuracy of the method is very much enhanced.

Because this is a continuing series of publications of microchemical urinalyses, it is important that a description be given at this time of the urine sample treatment prior to analysis.

A good and reliable analysis is one that utilizes a procedure in which the measured quantity of a constituent represents the true amount present at the time a sample is collected. The time interval between

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obtaining the sample from its source to the time it is analyzed is very critical. Urine specimens, depending on their nature, source, and composition, are subject to an unpredictable degree of deterioration on standing. Factors such as urine pH, temperature changes, and bacterial action can cause secondary alterations, both physical and chemical, compared to the original state of the specimen. A shift in pH induced by bacterial action may cause precipitates to form. Uric acid may precipitate out of acid urines, while in alkaline urine the phosphates may precipitate and thus occlude calcium and magesium. Urines that contain particulate matter are difficult to aliquot, and care must be taken in drawing a representative sample. While analyses ideally should be performed on freshly voided urine, this is not always feasible because either there are too many samples to analyze or too many different constituents are desired per specimen, or both.

At present there is no physical or chemical means of treating urinary specimens that preserves the inherent chemical integrity of all desired constituents. This laboratory is aware of these difficulties, and until better and proven methods of urine preservation become available, uses the following procedure.

1. Record date and precise start time of urine collection period.
2. Record date and precise end time of urine collection period.
3. Measure collected urine volume to the nearest 0.1 ml.
4. Measure specific gravity using a calibrated refractometer.
5. Measure pH to the nearest 0.1 unit with pH meter.
6. Whatever volume it is desired to preserve is then placed into a clean polyethylene bottle which was previously charged with a lentil-sized crystal of thymol. This has the effect of keeping the urine bacteriostatic.
7. Aliquots are drawn for whatever analytical determinations can be conveniently performed in a reasonably short time.
8. The bottle is stoppered with a screw cap with a polyethylene liner and frozen in a storage freezer maintained at $-20°C$.
9. In order to prevent possible sample identification errors, the bottle containing the urine is marked with indelible ink with the following information on it:

a) Name or code number of subject.
b) Consecutive specimen number (if successive samples were taken).
c) Date of collection.
d) Actual start and end time of collection.
e) Total period of collection (to nearest 0.1 hour).
f) Total volume.

g) Specific gravity.
h) pH.

Considerable errors may result, especially in the determination of calcium and magnesium, if the urine sample has a precipitate. To prevent errors from this source, an aliquot of fresh urine should be taken for calcium determination. However, if a precipitate has formed already, the sample must be shaken vigorously and an aliquot drawn while the sample is mixed and the particles are uniformly suspended. In case of aliquoting suspensions, it is best to perform duplicate sampling to achieve reliability of results.

MATERIALS AND METHODS

Equipment

1. Atomic absorption spectrophotometer, Perkin-Elmer Model 290-B with the following hollow-cathode lamps: (a) combination calcium-magnesium; (b) Sodium; (c) Potassium.

The model 290-B is relatively small, simple to operate, and has the advantage of reading out directly and linearly in concentration. The direct concentration readout is important when a large number of samples are being analyzed for a limited number of elements. This model is also provided with a two-position burner mount, the conventional parallel one and one perpendicular to it. In the latter position the sensitivity is reduced 10 to 20 times, thus lowering the analytical sensitivity and increasing the dynamic range of concentration for a particular element.

2. Disposable plastic test tubes with caps, 17 × 100 mm. (Falcon Plastics Co., 5500 West 83rd St., Los Angeles, California 90045).

3. Grunbaum(R) pipettes (*2*); an assortment of a 10, 25, 50, 100, 500, and 1000 μl pipettes is desirable, as it permits sample dilutions to be performed between 1:1000 and 1:10.

4. "Repipet," 10 ml, graduated in 0.1-ml divisions. Items 3 and 4 are available from Labindustries, 1802 2nd St., Berkeley, California 94710.

5. Plastic bottles (Nalgene, Boston) round, polyethylene with polypropylene screw closure. It was found that unless standards were prepared in this type bottle, they would change in concentration. This is due either to extraction of the elements under investigation, or to loss by ion exchange if kept in glass containers or plastic containers with screw caps with other liners. The particular combination of bottle and screw cap recommended here preserved the originally prepared standard and blanks for many months with no noticeable change.

Reagents

1. Sodium chloride; analytical grade, J. T. Baker.
2. Potassium chloride; analytical grade, J. T. Baker.
3. Magnesium metal; analytical grade, J. T. Baker.
4. Calcium carbonate; NBS standard reference material (U.S. Department of Commerce, National Bureau of Standards, Office of Standard Reference Materials, Washington, D. C. 20234).
5. Lanthanum oxide; American Potash and Chemical Corp. Code 528, Lot 2167.
6. Cesium chloride; American Potash and Chemical Corp., Lot 12902.
7. Hydrochloric acid, reagent grade.
8. Water. The water used to make up standards must be free of the ions to be measured. This takes high quality de-ionized water. Such water can be obtained by repeated efficient distillations from sulfuric acid or a chelating agent such as disodium ethylene diamine tetraacetic acid. In this laboratory the "distilled" water used was that obtained from a Super-Q System, manufactured by the Millipore Corp. of Bedford, Mass. In this system the water is free from ionic contamination up to an 18 megohm level.

Preparation of Reagents

1. Lanthanum chloride, 1 *M* stock solution. Disslove 162.93 g of lanthanum oxide with approximately 320 ml of concentrated HCl and dilute to 1 liter with distilled water.
2. Cesium chloride, 160 m*M* stock solution. Dissolve 26.94 g with distilled water and dilute to 1 liter.
3. Blank reagent. This is prepared by mixing 25 ml of 1 *M* lanthanum chloride and 25 ml of 160 m*M* cesium chloride and diluting with distilled water to 1 liter. This results in final concentrations of 25 m*M* lanthanum and 4 m*M* cesium, and is used as a blank for setting the zero on the AA.
4. Sodium chloride. Dry crystals overnight at 100°C and prepare a 100 m*M* stock solution by weighing 5.845 g and diluting it with water to 1 liter.
5. Potassium chloride. Dry crystals overnight at 100°C and prepare a 100 m*M* stock solution by weighing 7.455 g and diluting to 1 liter with distilled water.
6. Calcium chloride. Dry calcium carbonate powder overnight at 100°C and prepare a 100 m*M* stock solution by dissolving 100.009 g in

a minimal amount of concentrated hydrochloric acid, then diluting it to 1 liter with distilled water. An additional dilution was made to obtain a 10 m*M* solution by diluting 1 part stock standard with 9 parts water.

7. Magnesium chloride. Weigh 2.432 g of the metal and dissolve in a minimal amount of concentrated hydrochloric acid and dilute to 1 liter with distilled water. This stock solution was diluted 1 to 10 to yield a 10 m*M* solution.

To calibrate the AA, the diluted standards were prepared from the stock solutions (Table 1).

Procedure

Normally 100 μl urine was transferred with a Grunbaum(R) pipette into a 17 $\times$ 100-mm plastic tube. A 9.9-ml aliquot of blank reagent, containing lanthanum and cesium, was then added with a Repipet. The

TABLE 1

CALIBRATION STANDARDS [a]

Element	Desired final conc. (m*M*)	Stock solution added (ml)
Sodium	1.00	10
(100 m*M* stock solution)	0.80	8
	0.60	6
	0.40	4
	0.20	2
	0.10	1
Potassium	1.00	10
(100 m*M* stock solution)	0.75	7.5
	0.50	5.0
	0.25	2.5
	0.10	1.0
Calcium	0.30	30
(10 m*M* stock solution)	0.20	20
	0.15	15
	0.10	10
	0.05	5
	0.03	3
Magnesium	0.10	10
(10 m*M* stock solution)	0.08	8
	0.06	6
	0.04	4
	0.02	2
	0.01	1

[a] To each one of above was added in succession 25 ml of 1 *M* lanthanum chloride, 25 ml of 160 m*M* cesium chloride, and the balance of distilled water to make 1 liter.

test tube was stoppered, and mixed by inversion. This resulted in 1:100 dilution of the urine. It was convenient to prepare many samples (if available) for analysis at the same time. First, the blank reagent acts as a sort of preservative in which the urinary sodium, potassium, calcium, and magnesium concentrations will not be altered. Secondly, the same Grunbaum(R) pipette used for the first urine sample is used for successive urine samples. This is time saving and avoids errors in sampling due to use of different pipettes (*2*). In addition, the use of the same Repipet, dispensing reproducible volumes, makes any additional volumetric equipment unnecessary, as the final volume is always identical.

The standards for the four elements were prepared to cover only a tenfold increase in concentration. This was very convenient for calibrating the AA. The blank reagent was used to set the scale at zero, while the highest standard was used to set the scale at 100. In this manner, the ten-fold dilution of this standard read 10 on the meter scale, and so on. If the standards were properly prepared, that is by careful quantitative dilutions, a set of standards for each of the four elements produced a linear function over the whole scale.

The particular set of standards chosen for a given element was adequate for the majority of urines analyzed. If a urine specimen had a reading of more than 90 or less than 10 on the AA meter scale, a new urine dilution was prepared. It was found more convenient and more accurate to maintain a limited concentration range of standards rather than maintain too many dilutions to test every concentration range of urine. With the set of pipettes (from 10 to 1000 μl volumes) it was simple and fast to prepare a fresh urine specimen for analysis with a dilution factor up to one decade in either direction. If the original preparation was too concentrated, the latter could be diluted up to tenfold by using the 500 or 1000-μl pipettes and 9.5 ml or 9.0 ml of blank reagent, respectively.

After the AA was adjusted for optimum performance and calibrated with a set of standards, the reading of samples took only seconds. With a linear response to the standards the meter reading was therefore directly in terms of concentration. Thus, by including the dilution factor, the calculation of the undiluted urine sample became a simple matter.

RESULTS AND DISCUSSION

The method described was used for quantifying sodium, potassium, calcium, and magnesium in human and monkey urines. Even though only four urinary elements were estimated, almost every other element could be determined on the same urine dilution, provided the appropriate hollow-cathode lamp was available. The AA was adjusted to spray the

diluted urine sample into the burner, at a rate equivalent to 2 to 3 ml/minute. Since only about 10 seconds were required for the meter needle to stabilize, very little of the 10 ml of urine dilution was used up. Thus there was sufficient quantity of fluid for the determination of additional elements.

The most time-consuming part in the determination of more than one element is the time required to change hollow-cathode lamps. For the calcium and magnesium determinations, a combination lamp was used, which was convenient and time saving. Some instruments have provisions for preheating the lamps, which cuts the time considerably. Good, stable, and reproducible readings are obtained routinely when the AA burner is maintained in clean condition. A clean flame without ripples yields a nonfluctuating meter reading. Lastly, it is important that the room housing the AA be clean. The AA requires a hood for removing heat and exhaust gases. This produces a draft of air, and unless the air in the room is dust free, the flame will burn the dust passing through it and cause rapid and irregular oscillations in meter readings, or so-called meter noise.

In summary, a procedure is described for the microdetermination of urinary sodium, potassium, calcium, and magnesium. The method utilizes a single diluted sample for multiple determinations of a number of additional elements.

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Microchemical Urinalysis
VII. Determination of Citric Acid in Microliter Quantities of Urine [1]

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Citric acid is an important urinary constituent. Its quantity in the urine, or its excretion rate, is a reflection of a varied number of metabolic activities (*4, 5, 6*), as well as exogenous sources. In a diet free of citric acid, an enzyme, citrogenase, present in heart, liver, and kidney will catalyze the condensation of pyruvic and oxalacetric acid (*3*), suggesting an endogenous origin. It is not known to what degree synthesis of citric acid will occur in an organism on a diet plentiful in citric acid. In our own studies, urinary citrate contributes substantially as a negative ion in the overall electrolyte balance, as well as to the general physiological profile.

Citric acid excretion is closely related to calcium excretion. A large part of total body citrate is present in bones as a superficial deposit on the crystal lattice of apatite. It forms soluble and poorly ionized complexes with calcium and can effectively remove calcium ions from solution. A local increase in citric acid concentration as a result of cellular activity might possibly be a factor in promoting dissolution of bone salt.

Since our last paper in this series (*2*) dealt with quantitation of calcium in urine, it is appropriate to consider now the quantitation of urinary citric acid. In this communication a simple and convenient procedure is described for the quantitative estimation of urinary citric acid in both man and monkeys. Our method is a modification of that described by Beutler and Yeh (*1*), who made a thorough investigation of the various steps involved in the analysis, as previously published by others.

MATERIALS AND METHODS

Reagents

1. *Citric acid.* Monohydrate crystals (mol wt, 210.14) from Baker

[1] This study was supported by NASA Grant NGL-05-003-024.

and Adamson. Weigh 100.0 mg and transfer into 100-ml volumetric flask. Dissolve and adjust volume with 1 *N* sulfuric acid. This standard stock solution is then diluted 1:10, 1:4, and 1:2 to yield three working standards of 10, 25, and 50 μg/100 μl, respectively. These standards were kept in the refrigerator at 4°C and were found to be stable for many months.

2. *Sulfuric acid.* Reagent grade, Baker and Adamson. Prepare 50% solution by slowly adding while mixing 1 vol of concentrated sulfuric acid to 1 vol of water.

3. *Potassium bromide.* Analytical grade (mol wt, 119.02) from Baker and Adamson. Prepare 1 *M* solution, by dissolving 11.9 g with water in a 100-ml volumetric flask.

4. *Potassium permanganate.* Saturated and weak solutions. J. T. Baker crystals (mol wt, 158.03) were used to prepare a saturated solution (approx 12 g/100 ml of water). The weak potassium permanganate was prepared by mixing 1 ml of saturated solution with about 30 ml of water.

5. *Hydrogen peroxide.* A 2% solution is prepared just before use from a 30% solution (J. T. Baker); i.e., a 1:15 dilution.

6. *n-Heptane.* Eastman-Kodak Catalog No. 2215.

7. *Thiourea–borax.* Sodium borate, decahydrate crystals (mol wt, 381.42) J. T. Baker. Thiourea (mol wt, 76.12) Eastman-Kodak. Two g of sodium borate were dissolved with water in a 100-ml volumetric flask. The dissolution can be aided through use of warm water or shaking in a water bath. Four g of thiourea are then added, dissolved in more water up to the 100-ml mark. Thiourea–borax should be prepared on the day of intended use.

Equipment

1. Pyrex screw-cap test tubes, 16 × 100 mm.
2. Repipets, 1 and 5 ml.
3. Grunbaum(R) pipette, 100 μl. Items 2 and 3 are available from Labindustries, 1802—2nd Street, Berkeley, California 94710.
4. Disposable glass transfer pipettes, 5¾ inches long.
5. Vortex mixer.
6. Mechanical shaker.
7. Volumetric pipettes, 2 ml.
8. Spectrophotometer with a capability of registering absorbance in cuvettes of 10-mm light path and less than 2-ml volume.

Procedure

1. *Oxidation.* Into a 16 × 100-mm test tube, place 100 μl of either water, working standards, citric acid, or urine sample. Then add in

succession 100 μl of 50% sulfuric acid and 1 ml of water, and mix contents. Next add 50 μl of 1 *M* potassium bromide and follow with 0.2 ml of saturated potassium permanganate. After mixing, allow to stand for 5 minutes. Then chill the samples in an ice water bath. Decolorize the permanganate by adding, dropwise, 2% hydrogen peroxide while mixing the tubes on a vortex mixer. Avoid adding excess of hydrogen peroxide. Again, while mixing on the vortex mixer, add, dropwise, weak potassium permanganate solution until a faint violet color appears and persists for more than 5 seconds.

2. *Extraction.* Add exactly 3.0 ml of *n*-heptane with a Repipet. Screw tops on tightly and shake on a mechanical shaker for 5 minutes. Transfer 2 ml of upper layer, which is the *n*-heptane extract, into a clean 16 × 100-mm screw-cap tube.

3. *Color development.* To the 2 ml of *n*-heptane extract, add 2.0 ml of freshly prepared thiourea–borax solution. Shake the tubes on a mechanical shaker for 5 minutes. Color development is complete at this time and remains stable for several hours. The *n*-heptane phase is aspirated with water suction. The chromophore is formed in the thiourea–borax phase. The latter is transferred into an appropriate cuvette with a 10-mm light path and the absorbance is measured at 445 mμ.

A blank and three different concentrations of standard citric acid were prepared by the same procedure as outlined above. The blanks should have a negligible absorbance.

4. *Calculation.*

$$\frac{\text{OD unknown}}{\text{OD standard}} \times \text{concentration of standard} = \mu\text{g of citric acid/100 } \mu\text{l}$$

RESULTS AND DISCUSSION

The absorbance was a linear function only up to 50 μg of citric acid/100-μl sample. Under the conditions of the above described procedure, the range of linearity extended to about 0.3 optical density.

The citric acid concentration in either human or monkey urine rarely exceeded an absorbance of 0.3 when 100 μl of urine was used. Human urines generally had a considerably higher citric acid content, compared to monkey urine. Perhaps this is a reflection of a higher intake of exogenous citrate by humans.

By the method described, the reliable range for citric acid determination is between 5 and 50 μg. However, it is possible to quantitate 1 μg of citric acid with an absorbance of 0.03 if instead of 2 ml of thiourea–borax only 0.4 ml are used in a 10-mm light path cuvette with a 100- to 200-μl total volume requirement. Table 1 shows the reproducibility achieved from day to day and the absorbance of about 0.3 at

TABLE 1

REPLICATE DETERMINATIONS OF STANDARD CITRIC ACID IN 2 CONSECUTIVE DAYS

Citric acid (μg/100 μl)	First day OD [a]		Second day OD [a]	
10	0.059	0.060	0.050	0.063
25	0.148	0.148	0.151	0.155
50	0.285	0.281	0.287	0.286
75	0.428	0.428	0.428	0.434
100	0.552	0.548	0.555	0.565

[a] Absorbance of duplicate samples.

which the relationship of concentration to optical density begins to deviate from a straight line. In this laboratory, 30 urine specimens were conveniently analyzed by 1 technician in 1 day's work.

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Photomicrographs of Metallo Amino Acid Complexes

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Complexes of glutamic acid and methionine with copper and of aspartic acid with barium were prepared as previously described (*1*). The complexes were recrystallized from aqueous solution by "layering" on acetone and letting stand at room temperature (*2*). The crystals were collected on a sintered glass filter, mounted on slides, and photographed with a 35-mm SLR camera (*3*). These are shown in Figs. 1–4.

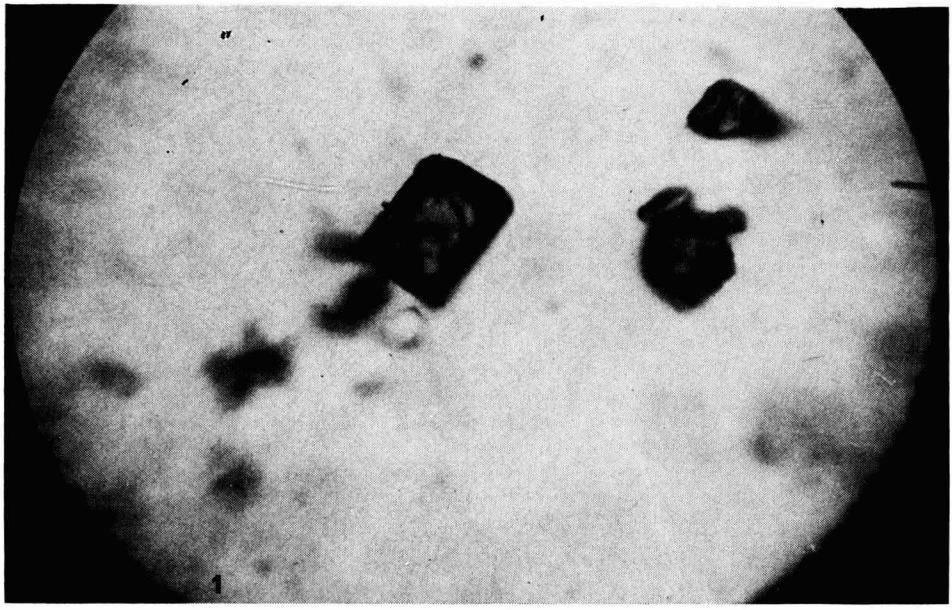

FIG. 1. Copper monoglutamate (×400).

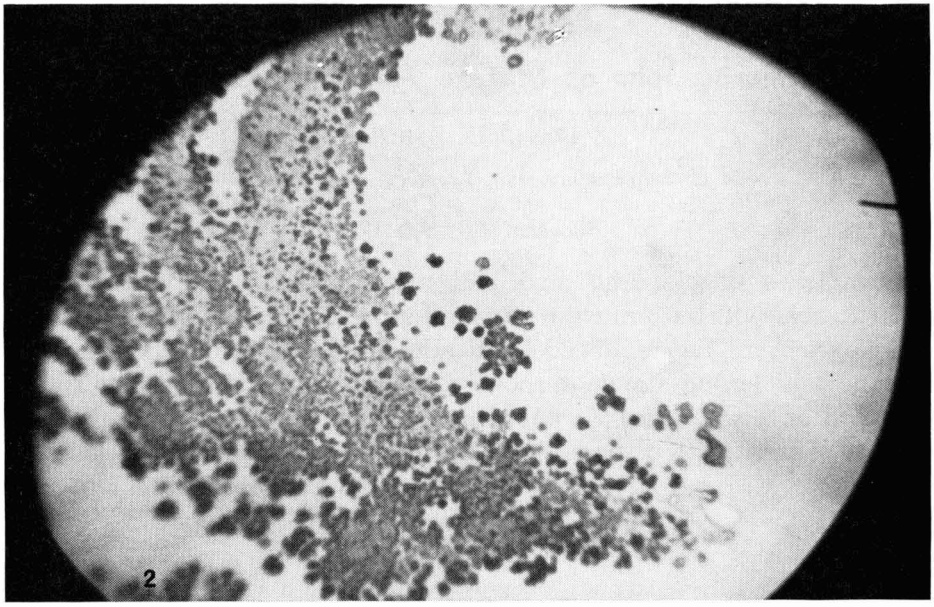

FIG. 2. Copper diglutamate (×400).

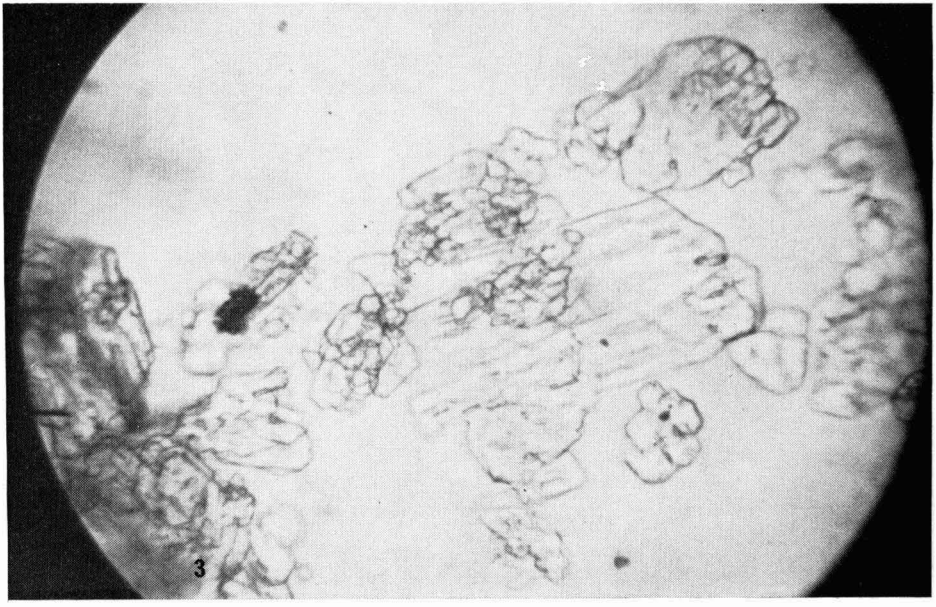

FIG. 3. Copper methionine (×400).

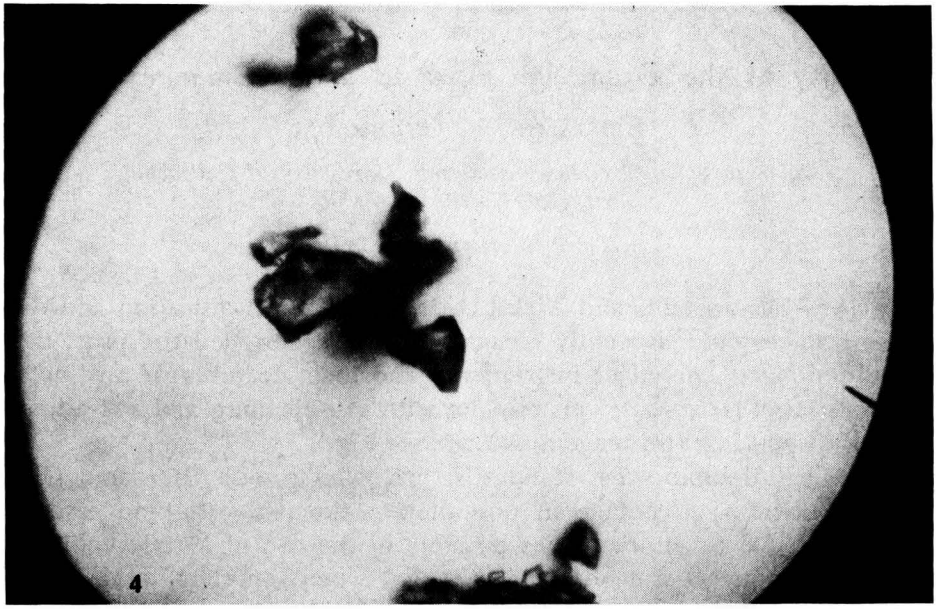

FIG. 4. Barium aspartate (×400).

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Reliability of the Grunbaum Pipet as a Self-Cleaning Device[1]

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In 1955, Grunbaum and Kirk (*1*) described a self-adjusting and dispensing micropipet. Recently Grunbaum (*2*) redesigned the pipet into a self-contained analytical instrument. The main features of any given pipet size are: (i) successive sampling with self-cleaning and self-adjusting; (ii) dispensing; (iii) reagent storage, see Fig. 1.

Sampling of unknowns, standards, and blanks with the same pipet has the obvious advantage in quantitative chemistry that no error is introduced due to discrepancies possible in the use of a different pipet for each aliquot. It was suggested (*2*) that when sampling similar solutions, a small quantity of the second sample be drawn through the lumen of the pipet and dumped into the reservoir, thus leaving a pipet full of the desired pure sample. The question remained, however: how can an analyst be certain that no contamination due to carry-over from the preceding aliquot was included? This concern of the analyst was indeed important, especially when the methodological sensitivity for a given constituent was very high. In this communication data are presented to define the extent of the self-cleaning ability of the whole range of sizes of the Grunbaum pipet.

EXPERIMENTAL METHODS

In order to determine the extent of carry-over quantitatively, the following experiment was set up. Three pipets (available from Labindustries, 1802 2nd Street, Berkeley, California) were used, 10, 100, and 500 μl. (The useful range of the Grunbaum pipet is between 1 and 1000 μl.)

Each pipet was filled with radioactive carbon (^{14}C), and transferred into a vial to which a scintillation fluid was added. After emptying the radioactive fluid, water was drawn into each pipet and care was taken to fill the pipet just to the very inside tip without allowing it to overflow. This first rinsing fluid was added to a separate vial and treated similarly to the aliquot of the original radioactive fluid. The rinsing was then repeated three more times in a similar manner.

[1] This study was supported by NASA Grant NGL-05-003-024.

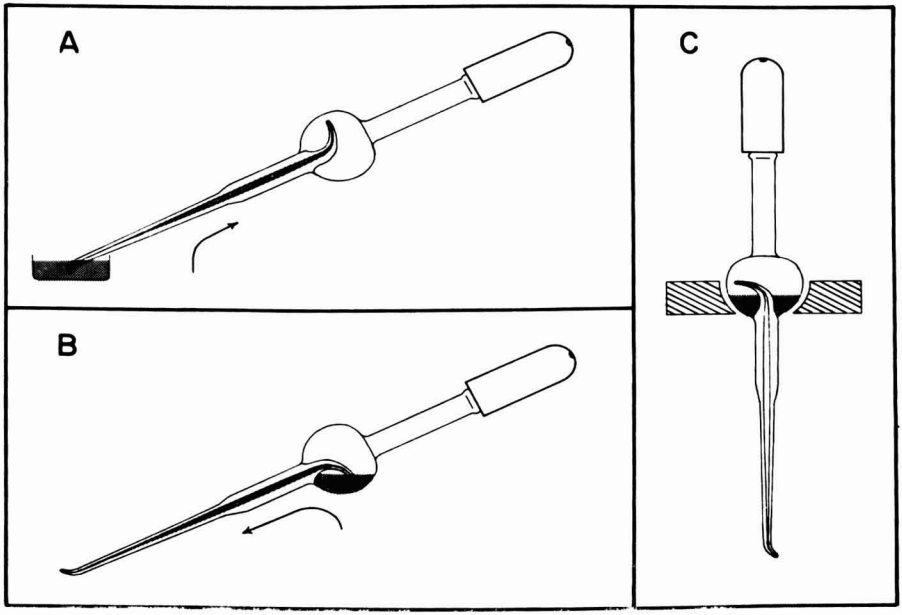

FIG. 1. Functional modes of the Grunbaum pipet: (A) sampling; (B) dispensing; and (C) reagent storage

The three pipets were then thoroughly washed in alcoholic potassium hydroxide followed by dilute nitric acid and many rinses of distilled water. While the pipets were still wet, a solution of approximately 8% Siliclad, water soluble silicone concentrate (available from Clay-Adams, Inc.) [also Desicote (available from Beckman Co.) can be similarly used] was drawn in and left inside the pipet for about 10 minutes. Care was taken to have at least 1 inch of the outer tip of the pipet immersed in the Siliclad fluid. The Siliclad was then expelled and the pipet was rinsed many times with distilled water. This is best done by attaching water suction to the stem of the pipet. The pipets were then placed with the tips upright in an oven at 150°C and dried overnight. This method of treating the pipets renders the glass surface hydrophobic (that is, water repellent).

These pipets were then used for aliquoting the radioactive carbon in the same manner as the nontreated pipets. All vials were then placed in a Packard Tri-Carb scintillation counter and counted for 10 minutes.

RESULTS

1. Untreated pipets retained a radioactive count in the first rinse equivalent to approximately 1% for the 500- and 100-μl sizes and approximately 0.2% for the 10-μl size. This is consistent for pipets

which are calibrated "to deliver" as the Grunbaum pipet normally is.

2. The second rinse for the 500- and 100-μl sizes had a carry-over of 0.04 and 0% for the 10-μl pipet.

3. The third and fourth rinses in all pipets were almost 0%.

4. The treated pipets (the water repellent pipets) showed a retention of radioactivity of 0.07% in the first rinse in the 10-μl pipet, 0.009% in the 100-μl pipet, and 0.007% in the 500-μl pipet. The subsequent rinses had practically no radioactivity left.

DISCUSSION

It appears from Table 1, that if the Grunbaum pipet were never cleaned between two successive samples, the maximum carry-over would be less than 1% in the nontreated pipets (calibrated "to deliver"), and about one to two orders of magnitude less in hydrophobic pipets (calibrated "to contain"). However, if the pipet is rinsed with

TABLE 1

CARRY-OVER OR SELF-CLEANING CHARACTERISTICS OF GRUNBAUM PIPETS

	Pipets with hydrophilic surface		Pipets with hydrophobic surface	
	(cpm)	Carry-over (%)	(cpm)	Carry-over (%)
10 μl				
Blank	22		22	
^{14}C (0.016 μCi)	34,588	100	34,623	100
1st Rinse	69	0.2	46	0.07
2nd Rinse	22	0	24	0
3rd Rinse	24	0	20	0
4th Rinse	22	0	23	0
100 μl				
Blank	23		24	
^{14}C (0.16 μCi)	331,743	100	337,079	100
1st Rinse	2,982	0.9	54	0.009
2nd Rinse	133	0.03	29	0
3rd Rinse	25	0	29	0
4th Rinse	22	0	26	0
500 μl				
Blank	23		24	
^{14}C (0.80 μCi)	1,764,706	100	1,800,000	100
1st Rinse	16,638	0.9	104	0.004
2nd Rinse	593	0.03	41	0
3rd Rinse	60	0.002	34	0
4th Rinse	31	0	22	0

a volume of fluid equal to its capacity, the carry-over, or contaminant, is further reduced substantially in the "to deliver" pipets and practically 0% in the "to contain" pipets. In the nontreated pipets as normally used, the second sample drawn into the pipet displaces the first one by carrying it into the pipet reservoir. Thus, by dumping any amount of the second sample as rinsing fluid into the reservoir, the pipet can be considered self-cleaned, because the dumped droplet of fluid as a result of the concentration gradient created by sweeping the lumen with a second fluid is richest in carry-over. In sampling similar solutions such as blood, serum, urine, acids, bases, etc. for the routine analytical procedures, errors due to carry-over would be undetectable. Based on the data presented in Table 1, an analyst should find it very helpful to decide how much self-cleaning of the Grunbaum pipet is required. This, of course, would depend on the sensitivity of the method used and to what accuracy a particular analysis is required.

The extent of carry-over is also a function of the size of the pipet. In both the hydrophobic and hydrophilic pipets the percentage carry-over is inversely proportional to pipet volume. The percentage carry-over decreases with increase in pipet size.

Since the treated water-repellent pipets have negligible carry-over with minimal self-cleaning, the use of such treated pipets is very useful for successive sampling in immunological analyses and in the use of radioactive materials. There the sensitivity of detection is very great and the effort spent in treating the glass is well worth it. Also, since there is practically no residue in water repellent pipets, the volume delivered is slightly higher. For measurement of absolute volumes, the pipets should be recalibrated.

In instances where there just is not enough sample for complete rinsing of the Grunbaum pipet to avoid carry-over, the analyst can do one of two things: (i) after sampling he can rinse the pipet with a pure solvent and then proceed with the next sample; or (ii) he can treat the pipet to make it hydrophobic. The treated pipet is especially useful in aliquoting successive samples of whole blood. After transfer of an aliquot of whole blood the lumen of the pipet remains dry and clear, with no visible red tinge. However, when aliquoting a sample of centrifuged packed red blood cells, which is extremely viscous, some red cells do stick to the inner surface of the capillary. If an absolute transfer is required, it is best to rinse the lumen of the capillary from the reservoir with an appropriate solvent such as physiological saline or water, depending on the intended analysis. The rinsing solution is placed inside the reservoir prior to sampling. Quantitative transfer of all viscous solutions could be handled similarly.

SUMMARY

Data are presented to show that the Grunbaum pipet is self-cleaning and as such is a useful analytical instrument. It contributes to speed and economy in volumetric quantitative measurement. It is also a precision pipet because the same pipet is used for aliquoting blanks, standards, and unknowns.

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An Improved Microinjection Apparatus for Biochemical Embryology

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INTRODUCTION

The analysis of the biochemical features of the early events of embryogenesis and differentiation are easily carried out on single amphibian eggs and embryos. A micromanipulation technology is currently available and has provided useful information in experimental embryology (*1*). Commercially available components are usually employed and have generally proved satisfactory for most applications. The use of such techniques has recently been extended to include the microinjection of radioactive isotopes and the mass injection of large numbers of amphibian eggs with cytoplasmic substances and protein solutions (*2, 3*). Presently available equipment has proven, however, to be somewhat unsatisfactory for routine applications in which rapid, large-scale microinjection of amphibian eggs with precise and accurate volumes of radioactive isotopes or crude preparations of protein solutions is desired.

Various substances, which have an affinity to precipitate, frequently clog the micropipette and strain the capacity of commercially available 2-ml syringe controls and pipette holders, resulting in severe syringe leakage problems. The small size of such syringe controls also does not allow for the rapid and convenient injection of several hundred embryos.

This communication presents a description of new microinjection components which incorporate several advances in design characteristics. A large heavy-duty syringe control which is capable of withstanding the enormous pressure required to "unclog" precipitated material in micropipettes is described. The large volume of the syringe facilitates rapid control. A leak-free micropipette holder is also described, along with an examination of the pipetting precision obtainable with these components and an application of this methodology to biochemical embryology.

[1] To whom reprint requests should be addressed.

METHODS AND MATERIALS

A syringe control was machined from 0.065-inch wall welded seam 1.0-inch o.d. type 304 stainless steel tubing and fit with a Teflon (Dupont) plunger. A photograph of the syringe control is presented in Fig. 1. Each of the components is readily available from commerical supply houses. The syringe barrel is first honed on the inside and then the Teflon plunger is carefully machined to fit tightly within the barrel.

The capacity of the syringe barrel is approximately 20 ml. The Teflon plunger is greased with silicone high-vacuum grease and placed

FIG. 1. The large capacity stainless steel syringe control: photograph of the unit mounted in an inclined position; schematic diagram showing (A) aluminum knob; (B) knob bonded to brass screw by epoxy cement; (C) ½ inch–13 N.C. threaded brass screw; (D) aluminum end-cap, ½ inch–13 N.C. inside, ⅞ inch–32 outside; (E) stainless steel tube polished inside, ⅞ inch i.d. × 1.0 inch o.d.; (F) water reservoir above plunger; (G) tripod clamp support; (H) plunger fastened to brass screw by binding of threads; (I) baffle groove filled with grease; (J) Teflon plunger; (K) threads sealed with epoxy cement; (L) Luer-Lok connection (male), (M) aluminum end-cap, ⅞ inch–32 outside.

in the water filled barrel. The space above the plunger is then filled with approximately 10 ml of water and the unit is mounted at a 60° angle with a ball joint clamp.

A leak-free pipette holder was designed to accommodate the large pressure produced by the syringe control during the course of unclogging a micropipette. The unit contains a Tygon tube which can be tightly sealed and also easily loosened for quick pipette replacement. Commercially available pin vises (Starrett Co., Athol, Mass.) were modified by de-tempering the jaws, drilling them out so as to accommodate the tubing gasket, and re-tempering them. Two pin vises thus modified were inserted back to back in an aluminum tube and glued in place with epoxy cement. An 18-gauge needle is inserted into one end of the tubing gasket and the tubing is inserted through the length of the pin vises. A photograph of the micropipette holder is shown in Fig. 2.

One pin vise serves to clamp the needle tightly and provides a Luer-Lok connection to the syringe control. The pin vise at the other end clamps a micropipette in place.

The syringe control was mounted onto an aluminum plate and the micropipette holder was held in a micromanipulator which was

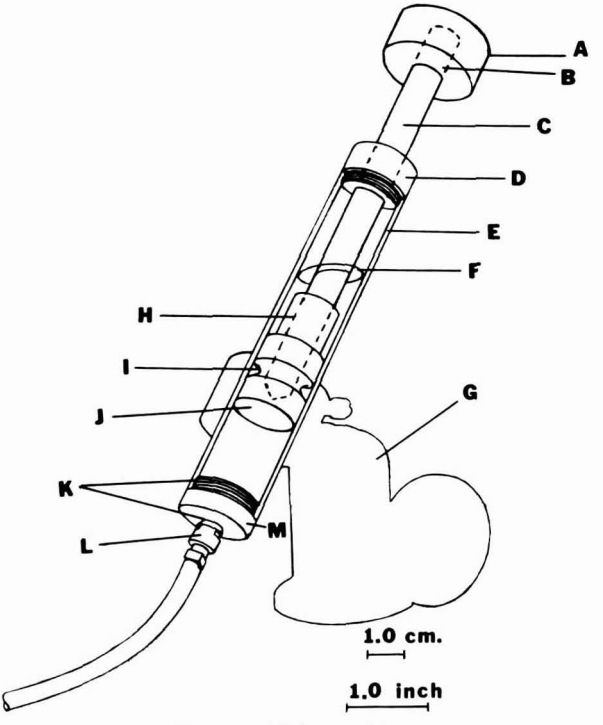

FIG. 1. (Schematic)

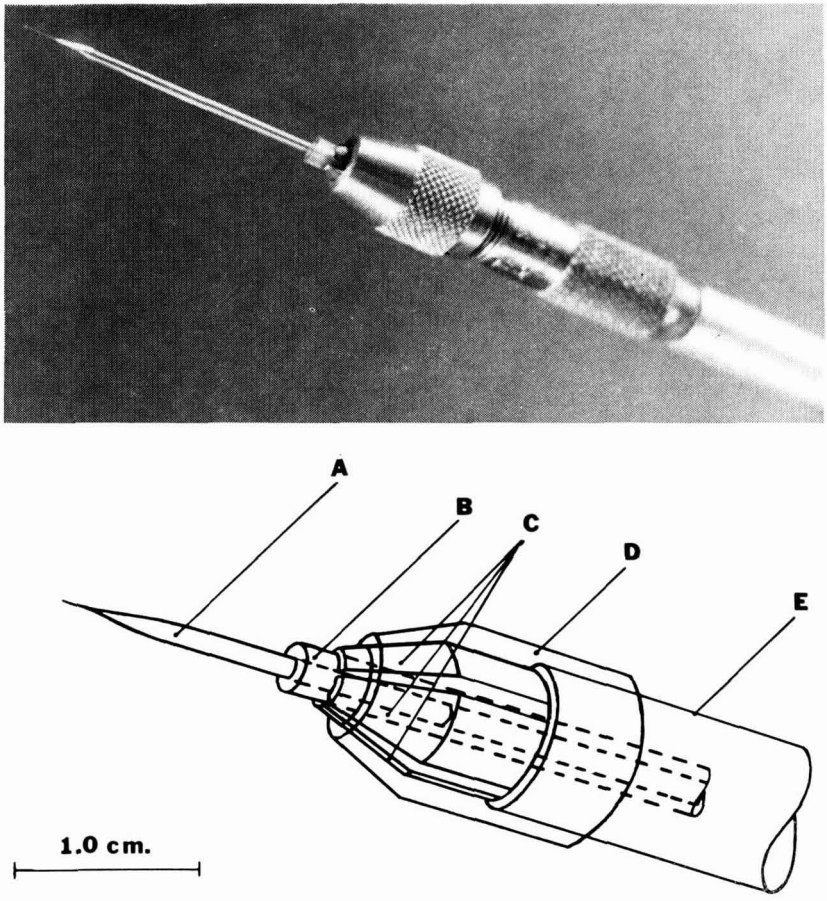

FIG. 2. Micropipette holder with positive seal mechanism: (upper) photograph showing holder with micropipette in position; (lower) schematic diagram showing: (A) micropipette; (B) tubing gasket Technicon Corp., Tarrytown, N.Y., (cat. no. 116-0532-10); (C) drilled out jaws; (D) threaded closing ring; (E) body of pin vise.

mounted in a similar manner to the aluminum plate. The entire microinjection apparatus, including the microscope and lamps is shown in Fig. 3.

RESULTS AND DISCUSSION

This syringe control has been employed extensively for the injection of crude amphibian egg homogenates and partially purified protein solutions into eggs of the Mexican axolotl (*Ambystoma mexicanum*). The powerful control of the syringe has virtually eliminated pipette clogging. The water reservoir above the Teflon plunger has effectively compen-

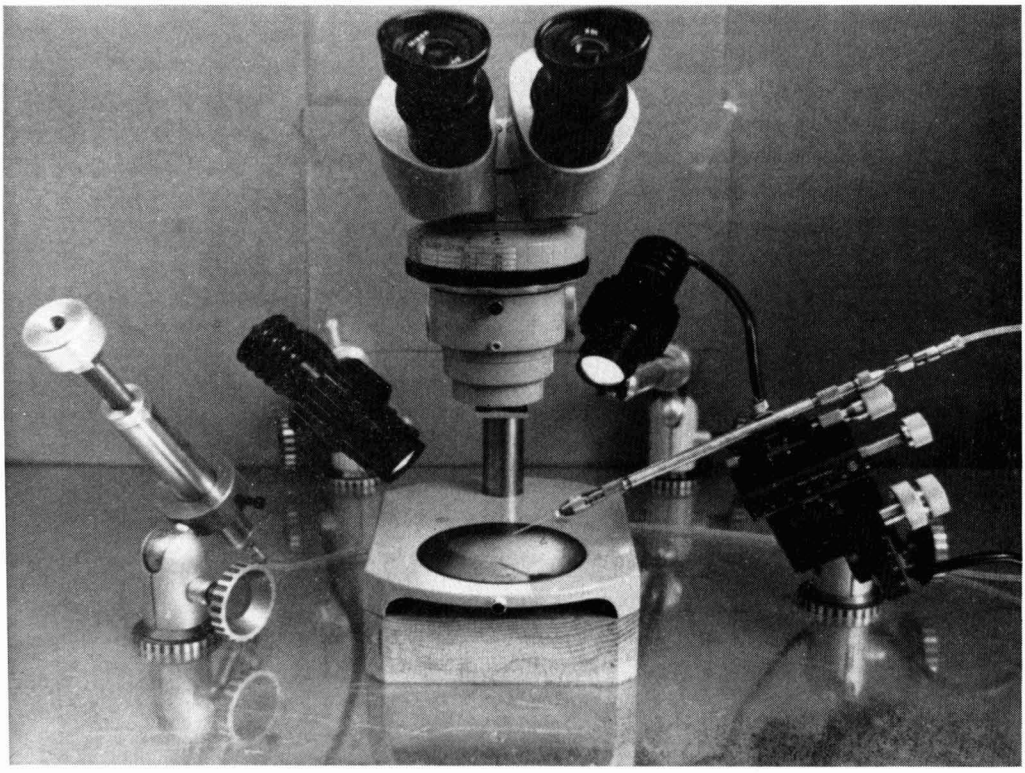

FIG. 3. The complete microinjection assembly, including syringe control, micropipette holder, microscope, and lights.

sated for any minor volume exchanges which may occur during a rapid upward stroke of the plunger.

The degree of precision obtainable during repetitive pipetting operations was examined by serially pipetting aliquots of a radioactive isotope solution (^{3}H uridine) into the pipette and discharging those aliquots into individual liquid scintillation counting vials. A single pipette was calibrated to deliver two different aliquots. Assay of the dispensed radioisotope provided a critical analysis of the pipetting precision. As the data in Fig. 4 reveal, the pipetting control was very precise and at the volumes tested the accuracy was within $\pm 5\%$.

This microinjection system has been used for the routine injection of radioisotope into amphibian eggs and allows for the injection of 100–200 eggs/hour. The rate of protein synthesis has been measured by following the incorporation of injected radioisotope (^{3}H leucine) into acid insoluble material. The data presented in Fig. 5 are an example of the type of

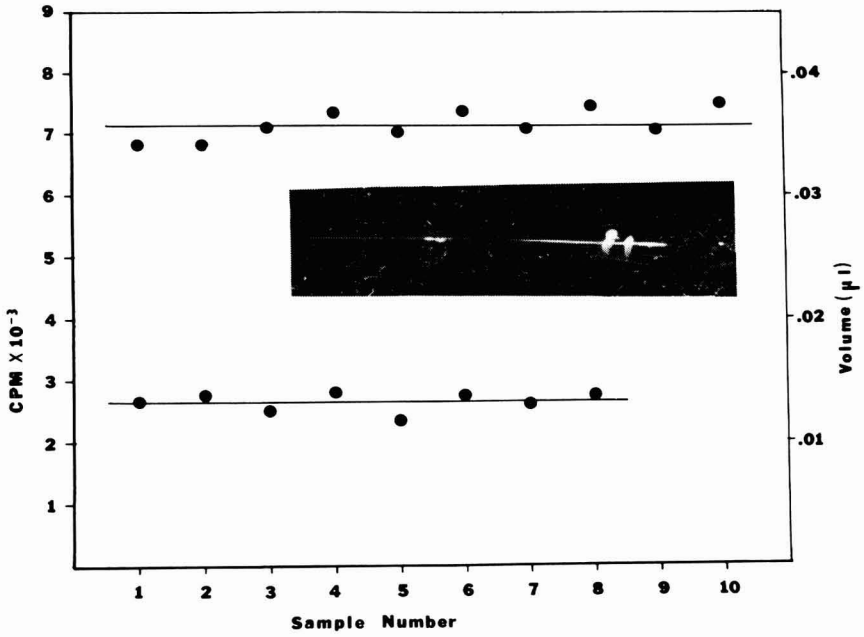

FIG. 4. Pipette accuracy at 2 different volumes as measured by repetitive pipetting; inserts shows calibrated micropipette.

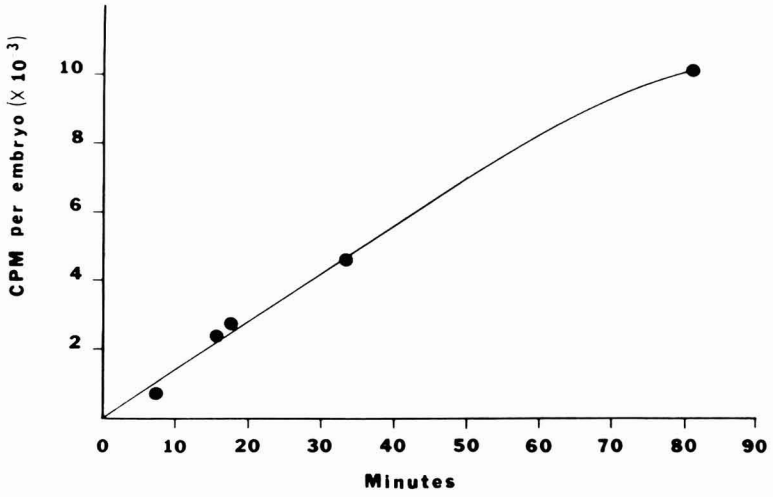

FIG. 5. Time dependent incorporation of ^{3}H leucine into stage 1 axolotl embryos: 0.04-ml aliquots of isotope were injected into 24 embryos. At time intervals during incubation at 18°C, 3 egg samples were homogenized and precipitated with 10% trichloroacetic acid. Radioactivity incorporation was measured by liquid scintillation counting of the acid precipitate.

information which can be derived with the components described in this report.

It is expected that useful application of this micromanipulation assembly will be readily extended to other operations in biochemical embryology which employ similar equipment. This instrumentation should facilitate, for example, performing such operations as nuclear transplantation, microsurgery, and ultramicro fluid transfers.

SUMMARY

The design characteristics of a new syringe control and micropipette holder are described. The large volume of the syringe control permits rapid control and the leak-free design of the pipette holder facilitates injection of large numbers of amphibian embryos. A test of the pipetting precision of the instrument is presented.

ACKNOWLEDGMENT

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Book Reviews

Monographs in Organic Functional Group Analysis. General editors: R. BELCHER AND D. M. W. ANDERSON. **Volume 3. The Determination of Carboxylic Functional Groups.** By R. D. TIWARI AND J. P. SHARMA. Pergamon, Oxford, New York, Toronto, 1970. vii + 132 pp. $8.00; 60 s.

This monograph deals with the determination of carboxylic acids, acid anhydrides, acid chlorides, esters, amides, and binary and ternary mixtures. About one-half of the book is devoted to the first mentioned and the rest of the book is divided, more or less, equally among the others.

Included are macro-, semimicro-, micro-, and submicromethods. A large variety of methods are included, such as, titrimetric (conductometric, high-frequency, nonaqueous, potentiometric, etc.), various chromatographic, spectrophotometric, etc. Some methods are described in detail while others are briefly discussed. In general, comprehensive theoretical details have been omitted. Also, the authors made no attempt to present a complete bibliography on the subject, but to put emphasis on what they believed to be the key methods useful in general analytical work. In so doing, in the opinion of the reviewer, some important references have been omitted.

Generally speaking, this monograph will make a valuable addition to the library of anyone involved in organic analytical work.

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Instrumental Methods of Chemical Analysis. By GALEN W. EWING. McGraw-Hill, New York, 1969. × + 627 pp. $12.50; 3rd edition.

This book provides an excellent survey of instrumental methods of analysis. Included in the 26 chapters are the "classical" methods of optical and electrochemical analysis (UV-visible spectrophotometry, emission spectroscopy, fluorimetry, phosphorimetry, flame emission spectroscopy, infrared analysis, polarimetry, potentiometry, polarography, voltammetry, coulometry, electrodeposition, conductimetry, nephelometry) as well as the newer techniques such as atomic absorption spectroscopy, mass spectrometry, X-ray, microprobe, NMR, chronopotentiometry, radiation measurements, optical rotary dispersion, circular dichroism, thermometric, and separations methods. To the microchemist this section on separation methods that includes gas chromatography, the ramifications of liquid chromatography (column, molecular sieve, ion-exchange, paper and TLC), solvent extraction (Craig, bubble separation methods, and foam fractionation), electrophoresis, and electrochromatography may be of considerable interest. The microchemist too will be interested in the concluding chapter of the text that compares different methods and approaches to analysis and considers the question of the techniques to employ, the sensitivities of the different methods, and the anticipated accuracy. One separate chapter on electronic circuitry introduces both the concepts of tube and solid state devices.

The reviewer used the text in his course in instrumental analysis this past semester. He found that he was able to cover a wider range of material than in

past years. The book surveys the field well; it is not exhaustive in detail and with the rapidity with which instrumental analysis is changing, quite up-to-date. The index is comprehensive. He asked the class their thoughts on the text and they suggested that more illustrative examples of problem calculations, answer to problems, and more problems at the end of the chapters would have further enriched it as a text.

The book is generally free of errors and is quite complete as a text. Illustrations of instruments, diagrams of components, and a selection of well chosen experiments are included. As with any instrumental text, in the opinion of the reviewer it would be advisable to test an experiment before it is assigned to a class as adjustments in concentrations may have to be made to make the experiment compatible with the equipment at hand.

The book introduces the topics from the basic physicochemical principles involved in the measurements. Although primarily a text, to the practicing chemist it will serve as a rapid reference source for general information on instrumentation.

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Vth International Congress on X-Ray Optics and Microanalysis. Edited by MOLLENSTEDT, G., AND GAUKLER, K. H., Springer-Verlag, New York, 1969. xii + 612 pp. $54.50.

This text is a collection of the papers presented at the Fifth International Congress on X-ray optics and microanalysis held at Tübingen University, in Western Germany. By microanalysis is meant analysis by means of the electron microprobe.

The book is divided into seven sections. In the first section, the newer developments in X-ray optics are presented. Worthy of special mention are the papers on X-ray interferometery, X-ray optical methods in solar astronomy and X-ray reflection optics. The practical application of X-ray interferometery to the measurement of refractive index, lattice defects in crystals and angstrom-scale length measurements suggest the potential value of this technique.

The use of the X-rays emitted from the sun, in the wavelength range of 10–250 Å as a means of imaging the sun, is also discussed in this section. The technique uses rocket born spectrometers and plots the X-ray spectrum, correlating the emission with a particular area of the sun's disc.

The X-ray microscopes of the reflection type have been handicapped because of geometrical aberration which limited their resolution. Mathematical analysis has led to the discovery of an equation describing an aspherical surface for which spherical aberration is reduced to a negligible amount. An account of the testing of such a surface is given and the practice bears out the theory.

The second and third sections are directed to studies on electron probe microanalysis. The general principles and limitations of the electron probe are first discussed. The various techniques for correction for background, and variation in the matrix are also discussed in a series of over 20 papers presented by different authors. The objective is to present the reader with the theory and techniques for the use of the microprobe in quantitative analysis for the elements, with a high degree of accuracy and precision.

A section on metallurgy and minerals concerns itself mainly with the application of the electron probe to these problems. Typical applications are to the iden-

tification of precipitates and inclusions in various alloys, especially steel, the study of the structure of alloys as a function of treatment, studies of oxidation and corrosion, analysis of defects in metals and the composition of surface coatings.

A section on instrumentation also concerns itself mainly with the electron probe. The construction of models, currently available commercially, are discussed in detail. Recent developments in the components of the electron probe are also discussed. These start with the electron gun and eventually go on to the X-ray detection systems. The newer developments in the electron probe instrumentation have taken four main directions; improvement in the curved crystal spectrometer construction, the use of grating spectrometers for the soft X-rays emitted by the light elements, non-dispersive detection, using the newly developed solid state detectors and automation. Thirty six papers are presented on this topic covering the various aspects of the field.

Microdiffraction, using the electron probe, is represented by six papers in a separate section. The collection of crystallographic data from a microcrystal, by using Kossel patterns generated by the electron probe, is the technique generally used. The orientation of crystals of the order of 10 μ in size is practicable. Instrumentation and results obtainable with this technique are illustrated by the contributors. A typical application is to the precise determination of lattice spacings in selected grains in a steel sample.

The application of X-ray optics and the microprobe to biological problems is the subject of the last section. Eleven contributors scan the various areas where the different techniques have had application. Techniques for assaying for potassium in cells using X-ray spectrometry and the microprobe are described in detail. The X-ray projection microscope is described and the histochemistry of single cells has been studied with this technique. Microcirculation studies in tissues, and studies of the fine structure of neuronal areas also find application with this technique.

The book is printed on heavy glossy paper which brings out the numerous half tones and line drawings illustrating the text.

Over 100 groups have contributed papers to form this volume. The main impact of the text is on the most recent developments taking place in the now widely used electron probe. This text is of greatest value to those either using or designing improvements in this instrument. It is also useful as a sourcebook for the use of X-rays in general, in analytical chemistry, especially in microanalysis.

The application of the instrumentation to biological systems is limited in scope but of value to those interested in the fine structure of tissues and their composition. For this reason the book should be of interest to the pathologists and chemists employed in the hospital laboratories. The text should be made available to the graduate student in the university library as a reference book on X-ray techniques.

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Methods of Biochemical Analysis. Volume 18. Edited by D. GLICK. Wiley (Interscience), New York, 1970. vi + 421 pp. $16.50.

The Eighteenth volume in this series designed to keep analytical biochemistry abreast of developments in the field maintains the high standards of the previous issues. The reviews, wide in scope and range of subjects, are by invitation ex-

tended to those who are experts in their fields. The authors have summarized and developed in single surveys their own ideas and expressed opinions of various facets or trends in research putting their subject matter in perspective.

The current volume containing six reviews, each with an extensive bibliography, are written by eminently qualified scientists from the U. S., Germany, England, and Sweden. The topics covered include the estimation of molecular size and molecular weights of biological compounds by gel filtration, application of free zone electrophoresis, application of optical rotary dispersion (ORD), and circular dichroism (CD) to the study of biopolymers, automatic peptide chromatography, use of the dansyl reaction in biochemical analysis, and recent developments in the analysis of steroids by gas chromatography. Of special interest in this reviewer's opinion are two reviews, the first one dealing with ORD and CD as applied to the study of biopolymers. At the present state of the art, ORD and CD measurements offer several substantial advantages over optical rotation measurements for most optically active systems. On the basis of these conclusions the authors predict that the virtual monopoly of the optical rotation technique in the published literature is not likely to be maintained and this contention is borne out by the fact that with the advent of commercially available automatic recording instruments the popularity of ORD and CD techniques has mushroomed as evidenced from published papers in *Chemical Abstracts* rising from 42 in 1958 to 499 in 1966. The other one deals with automatic peptide chromatography; the advantages over manual procedures are convenience and efficiency of automation, greater reproducibility, higher sensitivity, better definition of chromatographic resolution of zones, more reliable quantitation and ready application to both analytical and preparative purposes. Two of the other reviews—the use of the dansyl reaction in biochemical analysis, and recent developments in the analysis of steroids by gas chromatography—deal with well-established methods or techniques which have undergone sufficient improvement to merit recapitulation, reappraisal, and new recommendations.

Each review starts with an introduction or a discussion of the background and previous work, a critical evaluation of the various approaches, and a presentation of the procedural details of the methods recommended by the author. The presentation of the experimental detail is given in a manner that will furnish the laboratory worker with the complete information required to carry out the analyses.

The whole volume is well written and reads easily—obviously the result of good editorial work. A complete index—both author and subject—enhances the usefulness, and a cumulative index for all 18 volumes ties in with the preceding volumes. The book can be highly recommended as a worthy companion to its predecessors, and for the expert each volume should be required reading.

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

Instrumental Analysis Manual: Modern Experiments for the Laboratory. By GEORGE G. GUILBAULT AND LARRY G. HARGIS. Dekker, New York, 1970. xi + 444 pp. $7.75.

Although this book does not deal directly with microtechniques, the subject of instrumental analysis is so closely allied to microchemistry that any book of this nature is of interest to the practicing chemist. As indicated by the title, the book is a laboratory manual. It is designed to complement the available texts in instrumental analysis and to provide supplementary experiments. Each of the 18 chapters is introduced by a short resume of the theory pertaining to the subject

and each of the experiments has a short discussion of the basic principles pertaining to the experiment. The experiments were selected to intrigue the student while illustrating the method of analysis. For example, in spectrophotometry one of the experiments is an analysis of phosphate in blood serum using a kinetic method. Another experiment in the field of atomic absorption and emission is entitled "Trace Analysis of Metals by Flame Emission and Atomic Absorption."

Experiments are included on fluorescence (both molecular and atomic), phosphorescence, ESR and NMR, potentiometry (including a nonaqueous titration and the evaluation of a selective ion electrode), conductance measurements, polarography, and amperometry. The section on separation methods includes experiments on gas chromatography (including the use of an internal standard for quantitative measurements), electrophoresis, ion-exchange, thin-layer chromatography, and gel filtration. Experiments are also included on mass spectrometry, radiochemistry, and thermometric titrations. The discussion of operational amplifiers and electronics, totaling 60 pages, is very good. Three of the experiments in the text are computer oriented: the simultaneous UV analysis of a three-component mixture, a least-squares analysis of ESR data, and the use of a computerized library for IR data. No information is given on programming; an IBM 1620 program is given in the appendix for the IR experiment. The appendix also contains such information as the potentials of electrodes, ionization and formation constants, physical constants, a tabulation of dipole moments, the selection of filters for fluorometric measurements, a table of equivalent conductances, and the principle emission lines of the elements.

The book covers a wide range of experiments. By and large the directions are good. The authors have tried to make the directions general enough so that the laboratory portion is not limited or designed for any specific model or make of instruments. However, the success of the book in teaching instrumental analysis will depend just as much on the experience and skill of the laboratory instructor. It does not include all the hints and tricks. For example, one experiment requires the use of a KNO_3–agar salt bridge, but directions are not given for its preparation. The experiments using more sophisticated equipment such as the NMR experiments are, by their nature, very brief in operational details. As stated in the preface, this soft cover book is printed by photo-offset of typewritten pages at this time in a preliminary edition, to allow a more thorough evaluation. Some errors are present; an erratum insert is enclosed. No experiments appear to be included on the use of the emission spectrograph or on X-ray equipment. According to the preface, there are 50 experiments in the book; the table of contents lists 49. There is no index, which makes referral to any one particular point difficult. As the book had a large number of untrimmed leaves, possibly experiment 50, which has to be performed first, consists of cutting free the pages.

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Quantitative Analysis of Gaseous Pollutants. By WALTER E. RUCH. Ann Arbor-Humphrey, Ann Arbor, Michigan, 1970. x + 241 pp. $18.75.

Considering the none-too-soon interest recently shown in air quality control and air pollution in general, this book is extremely pertinent and will no doubt be well received by personnel who are suddenly faced with the demanding prospect of becoming instant experts in air pollution. They may be somewhat disap-

pointed to find that the book is a collection of annotated abstracts of chemical methods rather than a broad, comprehensive survey of gaseous pollutant analysis and associated problems, but the publication is still rather useful. Dr. Ruch, who is an industrial hygenist at Lawrence Radiation Laboratory, has collected 376 methods relating to the analysis of 176 different pollutants. Hopefully, even in New York City or Steubenville, one would be forced to analytically confront only a few of these simultaneously, but it is comforting to know that methods exist for such potential pollutants as 4, 4′–diisocyanatodiphenylmethane and polymethylenepolyphenyl isocyanate. Of course, methods for the more common oxides of carbon, nitrogen, and sulfur are also detailed.

This book is designed as a "quick reference source in the area of microchemistry of airborne gases and vapors." The pollutants are covered in alphabetical order with a variable number of abstracts under each. This may run 5–10 abstracts for certain gases down to a single abstract for many of those which are less common. The author specifies the original reference from which the abstract was prepared, outlines the method used for the quantitative analysis of the gas, and lists the sampling procedure. Interferences and analysis times are also described. The reader is expected to recognize the most pertinent method to his particular problem. No summary value judgements are made concerning a group of methods for one particular pollutant as to which method may be more reliable, trouble free, etc.

Used within the context in which the book is intended, it can be a very valuable reference guide to analytical methods and may save the analyst many hours in the library. The author would no doubt be the first to suggest that the information in this book should only act as a first guide to the methods. Original literature should certainly be consulted for additional details. It could be argued that the role of this book is also served by the readily available *Chemical Abstracts*. This is true to some extent, but $18.75 to have someone do a good bit of prior literature searching does not seem unreasonable. The coverage in Dr. Ruch's book is not exhaustive, however, and the analyst would generally want to augment his references with a more thorough survey.

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Author Index, Volume 15

Abdel-Ghany, Magda: *see* Barakat, M. F., 184

Alicino, Joseph F.: Assay for Hydroxyurea, 83

Autian, John: *see* Nematollahi, Jay, 53

Barakat, M. F.: Differential Estimation of Low Energy Beta-Emitters, ^{3}H, ^{14}C, and ^{35}S in Multilabeled Organic Samples, 184

Barakat, Mohamed Zaki: The Microdetermination of Salicylic Acid and Certain Derivatives, 10

Barsoum, B. N.: *see* Khalifi, H., 224

Bassette, R.: Body Fluid Ketones, Related to Fasting, Diabetes, and Tests of Blood Glucose Tolerance, 42

Bermejo-Martinez, F.: Analytical Applications of Chelons. LVII. Chelometric Determination of Bismuth with DTPA and Reciprocally, 622

Bishara, Safwat W.: Indirect Polarographic Method for the Microdetermination of Sulfur in Organic Compounds, 211

Boltz, D. F.: *see* Howell, J. A., 598

——: *see* Samuels, Joseph K., III, 638

Braun, Robert D.: *see* Stock, John T., 519

Burton, W. B.: Synthesis of 2-Imidazolidinone-^{14}C, 161

Campbell, D. E.: *see* Sugawara, K. F., 173

Campos, J. A. Rodriguez: *see* Bermejo-Martinez, F., 622

Carter, Paul: Ultramicroestimation of Human Serum Albumin: Binding of the Cationic Dye, 5,5′-Dibromo-o-cresolsulfonphthalein, 531

Chandra, S.: Oxidation of Some Phenolic Compounds with Cu(III), 78

Cheng, K. L.: *see* Wang, Jui-Chang, 607

Childs, C. E.: A Direct Comparison of the Pregl, Dumas, Perkin-Elmer, and Hewlett-Packard(F&M) Carbon–Hydrogen–Nitrogen Procedures, 590

Dalziel, J. A. W.: *see* Edrissi, M., 579

Das, Mrinal K.: Spectrophotometric Determination of Copper(II) with β-Benzoyl-α-pyridyl Thiourea, 540

DeThomas, Anthony V.: A Modified "Sandwich" Microapparatus for Descending Thin-Layer Chromatography, 71

——: A Simple Pre-equilibrium Apparatus for Thin-Layer Chromatography, 74

Edrissi, M.: A Selective Differential Spectrophotometric Method for the Determination of Mercury(II) Using Tris(2-thiopyridine-1-oxide)-iron(III) as Reagent, 579

El Aassar, Shaker Talkhan: *see* Barakat, Mohamed Zaki, 10

Fano, V.: A Versatile Automatic Polarograph with Hanging Mercury Drop Electrode, 97

——: Submicrogram Determination of Manganese with Other Elements by Polarography, 422

Fauth, Mae I.: *see* Houser, Marilyn E., 399

Fayzalla, Ahmed Shawki: *see* Barakat, Mohamed Zaki, 10

Fentiman, A. F.: *see* Poziomek, E. J., 475

Flaschka, H.: The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Lead (Mercury, Copper, or Silver) Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking, 653

Genest, K.: Microcrystalloptic Tests for Some Lupine and Ormosia Akaloids, 625

Gerarde, H. W.: *see* Walters, Martha I., 231

Golden, C. C.: *see* Sawicki, E., 25

Gordon, Geoffrey: An Improved Microinjection Apparatus for Biochemical Embryology, 685

Goydish, B. L.: The Quantitative Determination of Boron in Glasses Used as Encapsulants for Electronic Devices, 572

Gregorowicz, Zabigniew: Indexes for Estimation of Developing Regeants in Thin-Layer Chromatography, 60

——: Application of New Fuchsine for Quantitative Determination of Or-

ganic Substances in Thin-Layer Chromatography, 545
Grunbaum, Benjamin W.: Microchemical Urinanalysis. V. Quantitative Analysis of Individual Urinary 17-Ketosteroids by Thin-Layer Chromatography, 103
——: Microchemical Urinalysis. VI. Determination of Sodium, Potassium, Calcium, and Magnesium by Atomic Absorption Spectrophotometry in the Microliter Range of Urine, 666
——: Microchemical Urinalysis. VII. Determination of Citric Acid in Microliter Quantities of Urine, 673
——: Reliability of the Grunbaum Pipet as a Self-Cleaning Device, 680
Guess, Wallace: *see* Nematollahi, Jay, 53
Hanson, O. L.: *see* Bassette, R., 42
Hassan, S. S. M.: Microdetermination of the Hydrazine Function: A New Gasometric Method Based on Oxidation with Benzoquinone, 470
Haux, Peter: Identification of Renal Calculi on Micro Samples by Infrared Analysis, 126
Henner, E. B.: *see* Childs, C. E., 590
Houser, Marilyn E.: Indirect Determination of Nitrate, Nitrite, and Nitro Groups by Atomic Absorption Spectrophotometry, 399
Howell, J. A.: The Preparation and Indirect Spectrophotometric Determination of Total Oxidizing Capacity of Chlorine Dioxide in Acidic Solution, 598
Hozumi, Keiichiro: An Automatic Microdetermination of Carbon, Hydrogen, and Nitrogen in Organic Compounds Using Data Printing System, 481
Issa, Y. M.: *see* Khalifa, H., 415
Jaiswal, P. K.: Oxidation of Some Carbohydrates with Ag(III), 122
——: Oxidation of Ferrous Sulfate with Ag(III), 205
——: Tripositive Copper as a Titrant: Determination of Some Sugars, 434
Johri, K. N.: Trace Metal Analysis by Combined Thin-Layer Chromatography Incorporating Fluorescent Support and Ring Oven Colorimetry, 642
——: Suggested Procedure for Microdetermination of Arsenic in Arsenical Animal Feed, 649
Kaushik, N. K.: *see* Johri, K. N., 649
Kawada, Katsuro: *see* Nakamura, Kanichi, 364
Khalifa, H.: Applications Involving the Iodide Ion. V. Determination of Small Amounts of Platinum and Analysis of Its Mixtures with Some Metal Ions, 224
——: Applications Involving the Iodide Ion. VI. Determination of Thallium(I) and Analysis of Its Mixtures with Some Metal Ions, 415
Kober, T.: *see* Sawicki, E., 25
Krishna Murti, G. S. R.: Spectrophotometric Determination of Iron with Orthophenanthroline, 585
Kuebler, R.: *see* Pavel, J. 192
Kundiger, M.: *see* Bassette, R., 42
Kushima, Hideya: *see* Hozumi, Keiichiro, 481
Laessig, Ronald H.: Simultaneous Automated Submicrodetermination of Glucose, Blood Urea Nitrogen, and Uric Acid on 25 Microliters of Capillary Blood, 138
Linington, G. E.: *see* Howell, J. A., 598
Lowry, Lorna J.: *see* Genest, K., 625
Majumdar, A. K.: *see* Das, Mrinal K., 540
Malacinski, George M.: *see* Gordon, Geoffrey, 685
Massoumi, A.: *see* Edrissi, M., 579
Mehra, Harish C.: *see* Johri, K. N., 642
——: *see* Johri, K. N., 649
Méndez, J.: Ultraviolet Spectral Study of Indoles, 1
Mitsui, Tetsuo: *see* Nakamura, Kanichi, 461
Moharir, A. V.: *see* Krishna Murti, G. S. R., 585
Morris, G. F.: *see* Shearer, D. A., 199

Mueller, J. Robert: *see* Munson, Alan K., 95

Munson, Alan K.: An Inexpensive Sample Applicator for Thin-Layer Chromatography, 95

Nakamura, Kan-ichi: Simultaneous Determination of Hydrogen and Nitrogen in Organic Compounds by a Gas-Volumetric Method Using a Newly Designed Pt-P_2O_5 Electrolytic Cell, 364

——: Coulometric Microdetermination of Oxygen in Organic Compounds, 461

Natelson, Samuel: *see* Haux, Peter, 126

Nematollahi, Jay: Pyrolytic Characterization of Some Plastics by a Modified Gas Chromatography, 53

Nishimura, Masa-aki: *see* Nakamura, Kan-ichi, 461

Oken, Donald E.: Quantitation of Picogram Quantities of Serum Albumin by Ultramicrodisc Electrophoresis and Direct Densitometry, 557

Ono, Kikushige: *see* Nakamura, Kan-ichi, 364

Pace, Nello: *see* Grunbaum, Benjamin W., 103

——: *see* Grunbaum, Benjamin W., 666

——: *see* Grunbaum, Benjamin W., 673

Pande, S.: Microdetermination of L-Leucine Separately and in a Mixture of Amino Acids, 6

Paul, J.: *see* Sorrentinto, F. A., 441

——: *see* Sorrentino, F. A., 446

Pavel, J.: Microdetermination of Fluorine in Organic Compounds by Direct Measurement with a Fluoride Electrode, 192

Poirer, R. H.: *see* Poziomek, E. J., 475

Poziomek, E. J.: A Microchemical Study of the Photodegradation of 4,4′-Bis(diethylamino)benzophenone Oxime on Silica Gel, 475

Raković-Tresić, Z.: *see* Stefanac, Z., 218

Ramírez-Muñoz, J.: Relationship Between Sensitivity and Precision in Atomic-Absorption Flame Photometry, 244

——: Chemico-analytical Selectivity and Sensitivity in Atomic-Absorption Flame Photometry, 253

——: The Dilution Process in Sample Preparation for Atomic-Absorption Flame Photometry, 271

——: The Concentration Process in Sample Preparation for Atomic-Absorption Flame Photometry, 277

Rezl, Vlastimil: Simultaneous Determination of Carbon, Hydrogen, and Nitrogen by Means of Gas Chromatography, 381

Ruan, H. T.: *see* Wong, Tao, 88

Sabine, David B.: Photomicrographs of Metallo Amino Acid Complexes, 677

Samuels, Joseph K., III: Near-Infrared Spectrophotometric Determination of Germanium by Modified Heteropoly Blue Method, 638

Sangal, Satendra P.: *see* Shah, Vasant L., 548

Sarma, V. A. K.: *see* Krishna Murti, G. S. R., 585

Sass, Samuel: *see* Yurow, Harvey W., 285

——: *see* Yurow, Harvey W., 428

Sawicki, C. R.: *see* Sawicki, E., 25

——: *see* Sawicki, E., 294

Sawicki, E.: Fluorimetric and Colorimetric Methods of Analysis for Histamine, 25

Sawicki, W.: Solvent Effects in Photometric Analysis, 294

Saxena, A. K.: Use of Microcosmic Salt as a New Titrant for the Microdetermination of Iodoacetic and Oxalic Acids, 64

——: Use of Microcosmic Salt as a New Titrant for the Microdetermination of Acetic and Glycolic Acids, 171

——: Microdetermination of Glycolic Acid with Guanidine Carbonate as a Titrant, 459

Saxena, O. C.: Direct Titrimetric Determination of Samarium and Neodymium, 38

——: *see* Sinha, P. C., 92
——: Microdetermination of Vitamin B_6: Gold Chloride as Oxidizing Agent, 281
——: Direct Titrimetric Microdetermination of L-Arginine. I. Direct Estimations of L-Arginine and DL-Valine; L-Arginine and DL-Alanine; and L-Argine, DL-Valine Together in One Solution Without Separating, 391
Scalvini, M.: *see* Fano, V., 97
Schaad, R. E.: Thin-Layer-Chromatographic Separation of Some Polynuclear Hydrocarbons, 208
Secor, G. E.: The Coulometric Microdetermination of Iodide After Oxygen Flask Combustion of Organic Compounds. The Use of Iodate, Biiodate, and Iodide as Primary Standards, 409
Selig, Walter: Semimicrodetermination of Oxalate with a Lead-Specific Electrode, 452
Shah, Vasant L.: Composition and Stability Constants of Cerium-Chromotrope 2R Chelate and the Spectrophotometric Determination of Cerium, 548
Shearer, D. A.: Microdetermination of Fluorine in Organic Compounds with a Fluoride Ion Electrode Following an Oxygen Flask Combustion, 199
Sinha, P. C.: Titrimetric Microdetermination of Berylium, 92
Sliepčević, Z.: *see* Štefanac, Z., 218
Sliwiok, Jòsef: *see* Gregorowicz, Zbigniew, 60
——: *see* Gregorowicz, Zbigniew, 545
Sorrentino, F. A.: Spectrophotometric Determination of Silicon in the Presence of Germanium, 441
——: Simultaneous Determination of Arsenic, Germanium, Phosphorus, and Silicon, 446
Štefanac, Z.: Direct Microdetermination of Oxygen by Static Flash Combustion Pyrolysis, 218
Stock, John T.: Dissimilar-Metal and "Titrant-Stream" Electrode Systems in the Potentiometric Titration of Millimolar Concentrations of Copper(II) in Dimethylformamide with Titanium(III), 519
——: Electrode Deactivation in the Anodic Voltammetry of Some Phenolic Derivatives of Isoquinoline, 564
Sugawara, K. F.: The Analysis of Binary and Ternary Thin Alloy Films of Tantalum, Titanium Niobium, Zirconium, Molybdenum, and Beryllium, 173
Szekeres, László: New Data on the Determination of Calcium, Strontium and Barium Ions in the Presence of Each Other, 66
Tsuji, Osamu: *see* Hozumi, Keiichiro, 481
Ulrich, W. F.: *see* Ramírez, Muñoz, J., 244
Verma, M. L.: *see* Pande, S., 6
Wagner, H.: *see* Pavel, J., 192
Walters, Martha I.: An Ultramicromethod for the Determination of Conjugated and Total Bilirubin in Serum or Plasma, 231
Wang, Jui-Chang: Precipitation of Tellurium with Bismuthiol II, 607
Weiss, R.: *see* Flaschka, H., 653
White, L. M.: *see* Secor, G. E., 409
Winfield, T. W.: *see* Sawicki, E., 294
Witiak, Nancy J.: *see* Sorrentino, F. A., 441
Witkovic, David: *see* DeThomas, Anthony V., 71
Wong, Tao: Phosphocreatine. III. A Potential Error in the Determination of Phosphate Moiety of Phosphocreatine, 88
Yadava, K. L.: *see* Chandra, S., 78
Yannone, Michael: *see* Munson, Alan K., 95
Yurow, Harvey W.: Analytical Reactions in Trifluoroacetic Acid. II. Organic Spot Test Parameters, 285
——: Detection of Various Alicyclic Compounds Via the Komarowsky Reaction, 428

Zaki, M. T. N.: *see* Hassan, S. S. M., 470
Zdankiewicz, Mark: *see* DeThomas, Anthony V., 71
——: *see* DeThomas, Anthony V., 74
Zydeck, Frederick A.: Determination of the Minimal Amount of Antigen Detected by the Electroprecipitation Test on Cellulose Acetate, 438

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CONTENTS OF VOLUME 15

NUMBER 1, MARCH 1970

J. MÉNDEZ. Ultraviolet Spectral Study of Indoles 1

S. PANDE AND M. L. VERMA. Microdetermination of L-Leucine Separately and in a Mixture of Amino Acids 6

MOHAMED ZAKI BARAKAT, AHMED SHAWKI FAYZALLA, AND SHAKER TALKHAN EL AASSAR. The Microdetermination of Salicylic Acid and Certain Derivatives 10

E. SAWICKI, C. R. SAWICKI, C. C. GOLDEN, AND T. KOBER. Fluorimetric and Colorimetric Methods of Analysis for Histamine 25

O. C. SAXENA. Direct Titrimetric Determination of Samarium and Neodymium 38

R. BASSETTE, M. KUNDIGER, AND O. L. HANSON. Body Fluid Ketones Related to Fasting, Diabetes, and Tests of Blood Glucose Tolerance ... 42

JAY NEMATOLLAHI, WALLACE GUESS, AND JOHN AUTIAN. Pyrolytic Characterization of Some Plastics by a Modified Gas Chromatography 53

ZBIGNIEW GREGOROWICZ AND JÓSEF SLIWIOK. Indexes for Estimation of Developing Reagents in Thin-Layer Chromatography 60

A. K. SAXENA. Use of Microcosmic Salt as a new Titrant for the Microdetermination of Iodoacetic and Oxalic Acids 64

LÁSZLÓ SZEKERES. New Data on the Determination of Calcium, Strontium and Barium Ions in the Presence of Each Other 66

ANTHONY V. DETHOMAS AND MARK ZDANKIEWICZ, AND DAVID WITKOVIC. A Modified "Sandwich" Microapparatus for Descending Thin-Layer Chromatography 71

ANTHONY V. DETHOMAS AND MARK ZDANKIEWICZ. A Simple Pre-equilibrium Apparatus for Thin-Layer Chromatography 74

S. CHANDRA AND K. L. YADAVA. Oxidation of Some Phenolic Compounds with Cu(III) 78

JOSEPH F. ALICINO. Assay for Hydroxyurea 83

TAO WONG AND H. T. RUAN. Phosphocreatine. III. A Potential Error in the Determination of Phosphate Moiety of Phosphocreatine 88

P. C. SINHA AND O. C. SAXENA. Titrimetric Microdetermination of Beryllium 92

ALAN K. MUNSON, J. ROBERT MUELLER, AND MICHAEL YANNONE. An Inexpensive Sample Applicator for Thin-Layer Chromatography 95

V. FANO AND M. SCALVINI. A Versatile Automatic Polarograph with Hanging Mercury Drop Electrode 97

BENJAMIN W. GRUNBAUM AND NELLO PACE. Microchemical Urinalysis. V. Quantitative Analysis of Individual Urinary 17-Ketosteroids by Thin-Layer Chromatography 103

CONTENTS OF VOLUME 15

P. K. JAISWAL. Oxidation of Some Carbohydrates with Ag(III) 122

PETER HAUX AND SAMUEL NATELSON. Identification of Renal Calculi on Micro Samples by Infrared Analysis 126

RONALD H. LAESSIG. Simultaneous Automated Submicrodetermination of Glucose, Blood Urea Nitrogen, and Uric Acid on 25 Microliters of Capillary Blood 138

BOOK REVIEWS 147

NUMBER 2, JUNE 1970

W. B. BURTON. Synthesis of 2-Imidazolidinone-^{14}C 161

A. K. SAXENA. Use of Microcosmic Salt as a New Titrant for the Microdetermination of Acetic and Glycolic Acids 171

K. F. SUGAWARA AND D. E. CAMPBELL. The Analysis of Binary and Ternary Thin Alloy Films of Tantalum, Titanium, Niobium, Zirconium, Molybdenum, and Beryllium 173

M. F. BARAKAT AND MAGDA ABDEL-GHANY. Differential Estimation of Low Energy Beta-Emitters, ^{3}H, ^{14}C, and ^{35}S in Multilabeled Organic Samples 184

J. PAVEL, R. KUEBLER, AND H. WAGNER. Microdetermination of Fluorine in Organic Compounds by Direct Measurement with a Fluoride Electrode 192

D. A. SHEARER AND G. F. MORRIS. Microdetermination of Fluorine in Organic Compounds with a Fluoride Ion Electrode Following an Oxygen Flask Combustion 199

P. K. JAISWAL. Oxidation of Ferrous Sulfate with Ag(III) 205

R. E. SCHAAD. Thin-Layer-Chromatographic Separation of Some Polynuclear Hydrocarbons 208

SAFWAT W. BISHARA. Indirect Polarographic Method for the Microdetermination of Sulfur in Organic Compounds 211

Z. STEFANAC, Z. SLIEPČEVIĆ, AND Z. RAKOVIĆ-TRESIĆ. Direct Microdetermination of Oxygen by Static Flash Combustion Pyrolysis 218

H. KHALIFA AND B. N. BARSOUM. Applications Involving the Iodide Ion. V. Determination of Small Amounts of Platinum and Analysis of Its Mixtures with Some Metal Ions 224

MARTHA I. WALTERS AND H. W. GERARDE. An Ultramicromethod for the Determination of Conjugated and Total Bilirubin in Serum or Plasma 231

J. RAMÍREZ-MUÑOZ AND W. F. ULRICH. Relationship Between Sensitivity and Precision in Atomic-Absorption Flame Photometry 244

CONTENTS OF VOLUME 15

J. RAMÍREZ-MUÑOZ. Chemico-analytical Selectivity and Sensitivity in Atomic-Absorption Flame Photometry 253

J. RAMÍREZ-MUÑOZ. The Dilution Process in Sample Preparation for Atomic-Absorption Flame Photometry 271

J. RAMÍREZ-MUÑOZ. The Concentration Process in Sample Preparation for Atomic-Absorption Flame Photometry 277

O. C. SAXENA. Microdetermination of Vitamin B_6: Gold Chloride as Oxidizing Agent 281

HARVEY W. YUROW AND SAMUEL SASS. Analytical Reactions in Trifluoroacetic Acid. II. Organic Spot Test Parameters 285

E. SAWICKI, T. W. WINFIELD, AND C. R. SAWICKI. Solvent Effects in Photometric Analysis 294

KAN-ICHI NAKAMURA, KIKUSHIGE ONO, AND KATSURO KAWADA. Simultaneous Determination of Hydrogen, and Nitrogen in Organic Compounds by a Gas-Volumetric Method Using a Newly Designed Pt-P_2O_5 Electrolytic Cell 364

BOOK REVIEWS 374

NUMBER 3, SEPTEMBER 1970

VLASTIMIL REZL. Simultaneous Determination of Carbon, Hydrogen, and Nitrogen by Means of Gas Chromatography 381

O. C. SAXENA. Direct Titrimetric Microdetermination of L-Arginine. I. Direct Estimations of L-Arginine and DL-Valine, and L-Arginine and DL-Alanine; and L-Arginine, DL-Valine, and DL-Alanine Together in One Solution Without Separating 391

MARILYN E. HOUSER AND MAE I. FAUTH. Indirect Determination of Nitrate, Nitrite, and Nitro Groups by Atomic Absorption Spectrophotometry .. 399

G. E. SECOR AND L. M. WHITE. The Coulometric Microdetermination of Iodide After Oxygen Flask Combustion of Organic Compounds. The Use of Iodate, Biiodate, and Iodide as Primary Standards 409

H. KHALIFA AND Y. M. ISSA. Applications Involving the Iodide Ion. VI. Determination of Thallium (I) and Analysis of Its Mixtures with Some Metal Ions 415

V. FANO. Submicrogram Determination of Manganese with Other Elements by Polarography 422

HARVEY W. YUROW AND SAMUEL SASS. Detection of Various Alicyclic Compounds Via the Komarowsky Reaction 428

P. K. JAISWAL. Tripositive Copper as a Titrant: Determination of Some Sugars 434

CONTENTS OF VOLUME 15

FREDERICK A. ZYDECK. Determination of the Minimal Amount of Antigen Detected by the Electroprecipitin Test on Cellulose Acetate 438

F. A. SORRENTINO, NANCY J. WITIAK, AND J. PAUL. Spectrophotometric Determination of Silicon in the Presence of Germanium 441

F. A. SORRENTINO AND J. PAUL. Simultaneous Determination of Arsenic, Germanium, Phosphorus, and Silicon 446

WALTER SELIG. Semimicrodetermination of Oxalate with a Lead-Specific Electrode 452

A. K. SAXENA. Microdetermination of Glycolic Acid with Guanidine Carbonate as a Titrant 459

KAN-ICHI NAKAMURA, MASA-AKI NISHIMURA, AND TETSUO MITSUI. Coulometric Microdetermination of Oxygen in Organic Compounds. 461

S. S. M. HASSAN AND M. T. M. ZAKI. Microdetermination of the Hydrazine Function: A New Gasometric Method Based on Oxidation with Benzoquinone 470

E. J. POZIOMEK. A. F. FENTIMAN, AND R. H. POIRIER. A Microchemical Study of the Photodegradation of 4,4′-Bis(diethylamino)benzophenone Oxime on Silica Gel 475

KEIICHIRO HOZUMI, OSAMU TSUJI, AND HIDEYA KUSHIMA. An Automatic Microdetermination of Carbon, Hydrogen, and Nitrogen in Organic Compounds Using Data Printing System 481

BOOK REVIEWS 498

ANNOUNCEMENT 508

ERRATUM 517

NUMBER 4, DECEMBER 1970

JOHN T. STOCK AND ROBERT D. BRAUN. Dissimilar-Metal and "Titrant-Stream" Electrode Systems in the Potentiometric Titration of Millimolar Concentrations of Copper(II) in Dimethylformamide with Titanium (III) 519

PAUL CARTER. Ultramicroestimation of Human Serum Albumin: Binding of the Cationic Dye, 5,5′-Dibromo-o-cresolsulfonphthalein 531

MRINAL K. DAS AND A. K. MAJUMDAR. Spectrophotometric Determination of Copper(II) with β-Benzoyl-α-pyridyl Thiourea 540

ZBIGNIEW GREGOROWICZ AND JÒZEF ŚLIWIOK. Application of New Fuchsine for Quantitative Determination of Organic Substances in Thin-Layer Chromatography 545

VASANT L. SHAH AND SATENDRA P. SANGAL. Composition and Stability Constants of Cerium-Chromotrope 2R Chelate and the Spectrophotometric Determination of Cerium 548

DONALD E. OKEN. Quantitation of Picogram Quantities of Serum Albumin by Ultramicrodisc Electrophoresis and Direct Densitometry 557

JOHN T. STOCK. Electrode Deactivation in the Anodic Voltammetry of Some Phenolic Derivatives of Isoquinoline 564

B. L. GOYDISH. The Quantitative Determination of Boron in Glasses Used as Encapsulants for Electronic Devices 572

M. EDRISSI, A MASSOUMI, AND J. A. W. DALZIEL. A Selective Differential Spectrophotometric Method for the Determination of Mercury(II) Using Tris(2-thiopyridine-1-oxide)-iron(III) as Reagent 579

G. S. R. KRISHNA MURTI, A. V. MOHARIR, AND V. A. K. SARMA. Spectrophotometric Determination of Iron with Orthophenanthroline 585

C. E. CHILDS AND E. B. HENNER. A Direct Comparison of the Pregl, Dumas, Perkin-Elmer, and Hewlett-Packard(F&M) Carbon–Hydrogen–Nitrogen Procedures 590

J. A. HOWELL, G. E. LININGTON, AND D. F. BOLTZ. The Preparation and Indirect Spectrophotometric Determination of Total Oxidizing Capacity of Chlorine Dioxide in Acidic Solution 598

JUI-CHANG WANG AND K. L. CHENG. Precipitation of Tellurium with Bismuthiol II 607

F. BERMEJO-MARTINEZ AND J. A. RODRIGUEZ CAMPOS. Analytical Applications of Chelons. LVII. Chelometric Determination of Bismuth with DTPA and Reciprocally 622

K. GENEST AND LORNA J. LOWRY. Microcrystalloptic Tests for Some Lupine and Ormosia Alkaloids 625

JOSEPH K. SAMUELS III AND D. F. BOLTZ. Near-Infrared Spectrophotometric Determination of Germanium by Modified Heteropoly Blue Method .. 638

K. N. JOHRI AND HARISH C. MEHRA. Trace Metal Analysis by Combined Thin-Layer Chromatography Incorporating Fluorescent Support and Ring Oven Colorimetry 642

K. N. JOHRI, HARISH C. MEHRA, AND N. K. KAUSHIK. Suggested Procedure for Microdetermination of Arsenic in Arsenical Animal Feed 649

H. FLASCHKA AND R. WEISS. The Extraction and Photometric Determination of Zinc in the Presence of Large Amounts of Lead (Mercury, Copper, or Silver) Using 1-(2-Pyridylazo)-2-naphthol (PAN) and Employing Iodide Masking 653

BENJAMIN W. GRUNBAUM AND NELLO PACE. Microchemical Urinalysis. VI. Determination of Sodium, Potassium, Calcium, and Magnesium by Atomic Absorption Spectrophotometry in the Microliter Range of Urine. 666

BENJAMIN W. GRUNBAUM AND NELLO PACE. Microchemical Urinalysis. VII. Determination of Citric Acid in Microliter Quantities of Urine .. 673

CONTENTS OF VOLUME 15

DAVID B. SABINE. Photomicrographs of Metallo Amino Acid Complexes .. 677

BENJAMIN W. GRUNBAUM. Reliability of the Grunbaum Pipet as a Self-Cleaning Device 680

GEOFFREY GORDON AND GEORGE M. MALACINSKI. An Improved Microinjection Apparatus for Biochemical Embryology 685

BOOK REVIEWS 692

AUTHOR INDEX 698

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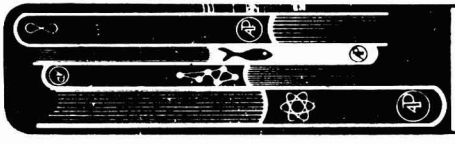

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