ISSN 0026-265X

Volume 24, Number 1, March 1979

Microchemical Journal

devoted to the application of microtechniques in all branches of science

Editor: Al Steyermark

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS
New York and London

Microchemical Journal

devoted to the application of microtechniques in all branches of science

Volume 24, Number 1, March 1979

Published under the auspices of the American Microchemical Society by

ACADEMIC PRESS

New York and London

Microchemical Journal

Volume 24, Number 1, March 1979

Microchemical Journal
Volume 24, Number 1, March 1979

CONTENTS

S. NIKDEL, A. MASSOUMI, AND J. D. WINEFORDNER. Detection Limits of Rare Earths by Inductively Coupled Plasma Atomic Emission Spectroscopy. . . . 1
M. MOHAMMADI AND H. MODARESS. Gas Phase Pyrolysis of Some Cyclic Dithioketals. . . . 8
H. KHALIFA AND I. A. ISMAIL. Analysis of Tungsten-Containing Mixtures and Some Alloy Steels by Potentiometric Titration Methods. . . . 16
C. PAPADOPOULOS, V. VASILIADIS, AND G. S. VASILIKIOTIS. Determination of Iron by Catalytic Oxidation of *p*-Aminophenol by Hydrogen Peroxide. . . . 23
JÓZEF SLIWIOK, ANTONINA MACIOSZCZYK, AND BOŻENA KÓCJAN. Intermolecular Interactions between the Mobile Phase Components and Isomeric Naphthols in a Thin-Layer Chromatography. . . . 33
H. SANKE GOWDA AND B. NARAYANA ACHAR. Spectrophotometric Determination of Vanadium(V) and Its Application to Vanadium Steel Containing Chronium, Molybdenum, Manganese, and Nickel. . . . 40
H. MODARESS AND M. MOHAMMADI. Correlation of NMR and UV Results to Evaluate the Stability Constants of Carbon Tetrachloride Complexes. . . . 46
CHONG W. CHANG. Determination of α-Amylase Activity from Cotton Leaves with Amylopectin-Azure. . . . 50
RICHARD R. SWAIN AND STEPHEN L. BRIGGS. Measurement of Total Protein in Urine: Comparison of Two Dye-Binding Procedures with a Gel Filtration/Modified Biuret Method. . . . 56
WILLIAM F. TULLY AND WILLIAM C. KURYLA. Suggestions for Improved Performance of the Thermal Energy Analyzer. . . . 62
G. M. MACIAK, P. W. LANDIS, AND E. KOZLOWSKI. Computerized Technique in Organic Microelemental Analysis. Part II. Automatic Determination of Sulfur in Organic Compounds. . . . 64
WALTER SELIG. Potentiometric Microdetermination of 1,3,5-Trinitro-1,3,5-triazacyclohexane and 1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane in Several Solvents. . . . 73
B. W. BUDESINSKY. Arsenic Colorimetry with Silver Diethyldithiocarbamate. . . . 80
L. J. PRIESTLEY, JR., AND B. E. WILKES. A Simple and Easily Fabricated Device for Desorbing Volatiles from Porous Polymers and Other Adsorbents. . . . 88
RAY E. HUMPHREY, GEORGE S. INGRAM, AND DAVID A. WAAK. Indirect Ultraviolet Determination of Cyanide with Mercury Complexes. . . . 92
H. M. STAHR, M. GAUL, W. HYDE, AND R. MOORE. A Cellulose Column Cleanup for Organophosphorus Pesticides. . . . 97
M. TADDIA, M. T. LIPPOLIS, AND L. PASTORELLI. Potentiometric Determination of EDTA and NTA in Detergents. . . . 102
PAUL BAILY, ELSA NORVAL, TERENCE A. KILROE-SMITH, MAURICE I. SKIKNE, AND HELEN B. RÖLLIN. The Application of Metal-Coated Graphite Tubes to the Determination of Trace Metals in Biological Materials. 1. The Determination of Lead in Blood Using a Tungsten-Coated Graphite Tube. . . . 107

M. MOJSKI AND M. PLESIŃSKA. Extraction–Spectrophotometric Determination of Palladium with Triphenylphosphine (TPP). 117
V. K. S. SHUKLA, C. K. KOKATE, AND K. C. SRIVASTAVA. Spectrophotometric Determination of Ascorbic Acid. 124
BOOK REVIEWS. 127
ANNOUNCEMENTS. 135

Published quarterly by Academic Press, Inc.,
111 Fifth Avenue, New York, New York 10003.
1979: Volume 24. Price: $64.50 U.S.A.;
$75.00 outside U.S.A. All prices include
postage and handling.
(Information about reduced price for personal subscriptions placed
by members is available from the American Microchemical Society.)
All correspondence and subscription orders should be sent to the office of the
Publishers at 111 Fifth Avenue, New York, N.Y. 10003.
Send notices of change of address to the office of the Publishers at least 6–8 weeks
in advance. Please include both old and new addresses.
Second class postage paid at New York, N.Y. and at additional mailing offices.

Microchemical Journal, Volume 24, Number 1, March 1979

Briefs

Detection Limits of Rare Earths by Inductively Coupled Plasma Atomic Emission Spectroscopy. S. NIKDEL, A. MASSOUMI, AND J. D. WINEFORDNER, *Department of Chemistry, University of Florida, Gainesville, Florida 32611.*

Using a medium power (1.25–1.50 kW) ICP as an excitation source and a glass concentric nebulizer, aqueous solutions of rare earth elements are measured. Limits of detection for all the rare earth elements in both pure solution and in a mixture of all the rare earth elements combined are measured for Pm. Analytical lines and ICP characteristics for such measurements are given.

Microchem. J. **24**, 1–7 (1979).

Gas Phase Pyrolysis of Some Cyclic Dithioketals. M. MOHAMMADI AND H. MODARESS, *Department of Chemistry, The Tehran Polytechnic, Tehran 5, Iran.*

Pyrolysis resulted primarily in cleavage of the molecules to thiocyclopentanone and thiocyclohexanone.

Microchem. J. **24**, 8–15 (1979).

Analysis of Tungsten-Containing Mixtures and Some Alloy Steels by Potentiometric Titration Methods. H. KHALIFA AND I. A. ISMAIL, *Faculty of Science, Zagazig University, Zagazig, Egypt.*

Tungsten is separated as Hg_2WO_4 and unreacted Hg(I) is titrated with KBr. The techniques of oxidation with I_2 or reduction with KI, and use of the selective arsenite reduction of Cr(VI) and/or Mn(IV) in the presence of V(V) or Fe(III) make it possible to determine alloy components without tedious separations.

Microchem. J. **24**, 16–22 (1979).

Determination of Iron by Catalytic Oxidation of *p*-Aminophenol by Hydrogen Peroxide. C. PAPADOPOULOS, V. VASILIADIS, AND G. S. VASILIKIOTIS, *Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece.*

The oxidation reaction of *p*-aminophenol by hydrogen peroxide in the presence of ferric ions was studied. A method for the ultramicrodetermination of ferric ions was developed as a result. The influence of other ions was also investigated.

Microchem. J. **24**, 23–32 (1979).

Intermolecular Interactions between the Mobile Phase Components and Isomeric Naphthols in Thin-Layer Chromatography. JÓZEF SLIWIOK, ANTONINA MACIOSZCZYK, AND BOŻENA KOCJAN, *Institute of Chemistry, Silesian University, 40-006 Katowice, Poland.*

The purpose of the study was to investigate intermolecular interactions between the components of a mobile phase (acetone–benzene) and the isomeric naphthols.

Microchem. J. **24**, 33–39 (1979).

Spectrophotometric Determination of Vanadium(V) and Its Application to Vanadium Steel Containing Chronium, Molybdenum, Manganese, and Nickel. H. SANKE GOWDA AND B. NARAYANA ACHAR, *Department of Post-graduate Studies and Research in Chemistry, University of Mysore, Manasa Gangotri, Mysore 570 006, India.*

Promethazine hydrochloride forms a red-colored complex with vanadium(V). Beer's law is valid over the concentration range of 0.1–7.0 ppm.

Microchem. J. **24**, 40–45 (1979).

Correlation of NMR and UV Results to Evaluate the Stability Constants of Carbon Tetrachloride Complexes. H. MODARESS AND M. MOHAMMADI, *Department of Chemistry, Tehran Polytechnic, Tehran, Iran.*

An equation has been suggested which correlates the NMR and UV results to evaluate the stability constant of electron donor acceptor complexes. Using this equation, the stability constant of complexation between carbon tetrachloride and hexamethylbenzene has been studied.

Microchem. J. **24**, 46–49 (1979).

Determination of α-Amylase Activity from Cotton Leaves with Amylopectin-Azure. CHONG W. CHANG, *Federal Research, Science and Education Administration, USDA, Western Cotton Research Laboratory, Phoenix, Arizona 85040.*

α-Amylase activity from phenolic-rich plant tissues is determined with an amylopectin-azure substrate. The procedure overcomes the effects of the interfering substances firmly associated with enzyme preparations.

Microchem. J. **24**, 50–55 (1979).

Measurement of Total Protein in Urine: Comparison of Two Dye-Binding Procedures with a Gel Filtration/Modified Biuret Method. RICHARD R. SWAIN AND STEPHEN L. BRIGGS, *Lilly Laboratory for Clinical Research, Wishard Memorial Hospital, Indianapolis, Indiana 46202.*

In contrast to the biuret-based procedure, it was found that the dye-binding methods are free from interferences by compounds known to appear in urine in high concentrations.

Microchem. J. **24**, 56–61 (1979).

Suggestions for Improved Performance of the Thermal Energy Analyzer. WILLIAM F. TULLY AND WILLIAM C. KURYLA, *Research and Development Department, Union Carbide Corporation, South Charleston, West Virginia 25303.*

Improvements were made by staging the temperatures of the traps and installation of a diverting valve after the liquid chromatograph analytical column.

Microchem. J. **24**, 62–63 (1979).

Computerized Technique in Organic Microelemental Analysis. Part II. Automatic Determination of Sulfur in Organic Compounds. G. M. MACIAK, P. W. LANDIS, AND E. KOZLOWSKI, *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206.*

A computerized rapid microanalytical determination of sulfur in organic compounds is described. Combustion is done in an empty tube, the products are transferred to a titration vessel, and the sulfuric acid is titrated with barium perchlorate using dimethylsulfonazo III. The determination requires 6 min.

Microchem. J. **24**, 64–72 (1979).

Potentiometric Microdetermination of 1,3,5-Trinitro-1,3,5-triazacyclohexane and 1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane in Several Solvents. WALTER SELIG, *Lawrence Livermore Laboratory, University of California, Livermore, California 94550.*

Pyridine, tetramethylguanidine, and hexamethylphosphoramide were evaluated as solvents for the microdetermination of two weakly acidic compounds of ordnance interest. The titrant was tetrabutylammonium hydroxide in methanol/toluene.

Microchem. J. **24**, 73–79 (1979).

Arsenic Colorimetry with Silver Diethyldithiocarbamate. B. W. Budesinsky, *Phelps Dodge Corporation, Morenci, Arizona 95540.*

The effect of 22 interferences has been studied. Sulfur(II) must be oxidized by nitric acid; germanium, nitric acid, and perchloric acid must be removed by repeated evaporation with hydrochloric acid. Conditions are given for the elimination of interference by the rest of the list of elements.

Microchem. J. **24,** 80–87 (1979).

A Simple and Easily Fabricated Device for Desorbing Volatiles from Porous Polymers and Other Adsorbents. L. J. Priestley, Jr., and B. E. Wilkes, *Union Carbide Corporation, Chemicals and Plastics Division, Research and Development Department, Technical Center, P.O. Box 8361, South Charleston, West Virginia 25303.*

An advantage over other devices includes its ease of fabrication from readily inexpensive materials. The device is useful for head space analysis, volatile organics analysis in aqueous solutions, and impurities in gaseous feed streams.

Microchem. J. **24,** 88–91 (1979).

Indirect Ultraviolet Determination of Cyanide with Mercury Complexes. Ray E. Humphrey, George S. Ingram, and David A. Waak, *Department of Chemistry, Sam Houston State University, Huntsville, Texas 77340.*

Cyanide ion can be determined in the range of 0.50 to 10 parts per million by the decrease in ultraviolet absorption of the mercury complexes $HgBr_4^{2-}$, $HgCl_4^{2-}$, HgI_4^{2-}, $Hg(SCN)_4^{2-}$, and $Hg(SO_3)_2^{2-}$. The decrease in absorbance is linear with cyanide concentration.

Microchem. J. **24,** 92–96 (1979).

A Cellulose Column Cleanup for Organophosphorus Pesticides. H. M. Stahr, M. Gaul, W. Hyde, and R. Moore, *Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa 50011.*

The column is composed of vacuum-activated cellulose. The eluting solvent is benzene and hexane. The extracts are more suitable for analysis of various types.

Microchem. J. **24,** 97–101 (1979).

BRIEFS

Potentiometric Determination of EDTA and NTA in Detergents. M. TADDIA, M. T. LIPPOLIS, AND L. PASTORELLI, *"G. Ciamician" Chemical Institute of the University, Bologna 40126, Italy.*

A method is described for the potentiometric titration with iron(III) of EDTA and NTA in detergents. Extraction is done at pH 9 in the presence of magnesium.

Microchem. J. **24**, 102–106 (1979).

The Application of Metal-Coated Graphite Tubes to the Determination of Trace Metals in Biological Materials. I. The Determination of Lead in Blood Using a Tungsten-Coated Graphite Tube. PAUL BAILY,[1] ELSA NORVAL,[2] TERENCE A. KILROE-SMITH,[1] MAURICE I. SKIKNE,[1] AND HELEN B. RÖLLIN.[1] [1]*National Research Institute for Occupational Diseases of the South African Medical Research Council, P.O. Box 4788, Johannesburg, 2000, South Africa, and* [2]*National Physical Research Laboratory of the Council for Scientific and Industrial Research, P.O. Box 395, Pretoria, 0001, South Africa.*

A new technique is described for trace metal determinations in biological materials by coating the graphite tube with tungsten. The coating is responsible for the prevention of residue formation that occurs in untreated tubes when lead in blood is analyzed.

Microchem. J. **24**, 107–116 (1979).

Extraction–Spectrophotometric Determination of Palladium with Triphenylphosphine (TTP). M. MOJSKI AND M. PLESIŃSKA, *Department of Analytical Chemistry, Warsaw Technical University, Noakowskiego 3, 00–664 Warsaw, Poland.*

Palladium is extracted as the $PdCl_2 \cdot 2TPP$ complex which shows a maximum absorption at 346 nm. A 20-fold excess of other platinum and transition metals has no effect on the extraction.

Microchem. J. **24**, 117–123 (1979).

Spectrophotometric Determination of Ascorbic Acid. V. K. S. SHUKLA,[1] C. K. KOKATE,[1] AND K. C. SRIVASTAVA,[2] [1]*Federal Center of Lipid Research, Piusalle 68, D-4400 Münster, West Germany, and* [2]*Department of Community Health and Environmental Medicine, Odense University, J. B. Winsløwsvej 19, DK-5000 Odense C, Denmark.*

Ascorbic acid readily forms a blue-colored complex with Folin–Ciocalteu reagent, which is measured spectrophotometrically. The procedure is accurate and in the microgram range.

Microchem. J. **24**, 124–126 (1979).

MICROCHEMICAL JOURNAL 24, 1–7 (1979)

Detection Limits of Rare Earths by Inductively Coupled Plasma Atomic Emission Spectroscopy[1]

S. NIKDEL, A. MASSOUMI,[2] AND J. D. WINEFORDNER[3]

Department of Chemistry, University of Florida, Gainesville, Florida 32611

Received July 25, 1978

INTRODUCTION

The analysis of rare earths in materials by chemical methods is plagued with separation and detection difficulties, and so most workers have resorted to spectroscopic measurement techniques. Fassel (*5*) has reviewed spectroscopic methods which have been extensively used for the quantitative determination of rare earth metals. Flame spectra of rare earth elements were observed by Rains *et al.* (*11*) who aspirated nonaqueous solutions of these elements into an oxy-hydrogen flame. Reducing flames to aid in atom production were used by Fassel *et al.* (*6*) who were successful in obtaining analytically useful line spectra of these elements in fuel-rich flames by introducing ethanol solution of the elements.

Further studies by Mossotti, Fassel, and others (*7, 8, 10*) on these elements provided over 1000 absorption lines in the optical region which were successfully used for quantitative determination of rare earth elements by atomic absorption spectrometry.

Amos and Willis (*1*) in their study observed that when higher temperature flames were employed as an absorption cell, the degree of ionization of rare earths became significant. Determination of traces of rare earths by atomic absorption with electrothermal atomizer and by dc arc emission spectroscopy was discussed by Dittrich and Borzym (*4*).

Dickinson and Fassel (*3*) with an ultrasonic aerosal generator and desolvation facility in their ICP determined detection limits for La and Ce. Later Fassel and Kniseley (*9*) reported ICP detection limits of rare earths which were superior to those from flames. Souilliart and Robin (*12*) used a highpower (6.6 kW) ICP and ultrasonic nebulizer in the study of rare earth metals. In this study, we have used a conventional moderate power (1.25–1.5 kW) ICP with a glass concentric pneumatic nebulizer for the determination of detection limits (LOD) and linear dynamic ranges (LDR)

[1] Research supported by AF-AFOSR-F44620-76-C-0005.

[2] On leave from: Department of Chemistry, Pahlavi University, Shiraz, Iran.

[3] Author to whom all reprint requests should be sent.

0026-265X/79/010001-07$01.00/0

of rare earth elements in pure solution and in a mixture of all the rare earth elements.

EXPERIMENTAL

Preparation of standards. Individual stock solutions of 1000 μg/ml of each rare earth element as well as their mixture were prepared by dissolving reagent grade pure oxides (ignited at 600°C for 4 hr) in hot 3 *M* HCl. Successive dilutions were made before each determination using distilled-deionized water. A solution of hydrochloric acid in deionized water was used as the blank.

Apparatus. A 2-kW inductively coupled plasma (Plasma Therm Inc., Kresson, NJ) with a 27-MHz radiofrequency generator was used in conjunction with a glass concentric nebulizer. The nebulizer solution flow rate was controlled with a syringe pump (Sage Instruments, Div. of Orion Research Inc., Cambridge, MA). A list of major instrumental components of the ICP system used in this study is given in Table 1.

Limits of detection (LOD) and linear dynamic ranges (LDR) were measured by integrating the electrometer signal for 10 sec. The LOD was taken to be that concentration equivalent to a signal three times that of the standard deviation of 16 consecutive, integrated blank readings.

Experimental conditions. The plasma system was operated at argon flows specified by the manufacturer. The ICP power was maintained at 1.25 to 1.5 kW throughout all measurements; the nebulizer solution flow rate was maintained at 2.2 ml min^{-1} and the cooling gas was controlled to 16 to 20 liters min^{-1}. A spherical lens (image 1:1), placed in a 3″ metal tube, was used to transfer the emission beam to the monochromator entrance slit. The entrance slit of the monochromator was set at 30 μm in width and 2 cm in height; the exit slit was adjusted to a width of 16 μm.

TABLE 1
SPECIFIC COMPONENTS OF EXPERIMENTAL SYSTEM

Component	Model number	Company
ICP		
Torch assembly	PT 1500	Plasma Therm Inc., Kresson, NJ
RF Generator	HFP-1500D	
Nebulizer	T-220-A2	JE Meinhard Associates, Santa Anna, CA
Monochromator	1870	SPEX, Metuchen, NJ
Photomultiplier	R-818	Hamamatsu Corp., Middlesex, NJ
High voltage power supply	224	Keithly Instruments, Cleveland, OH
Current/voltage convertor	601	Keithly Instruments, Cleveland, OH
Integrator		Lab Constructed

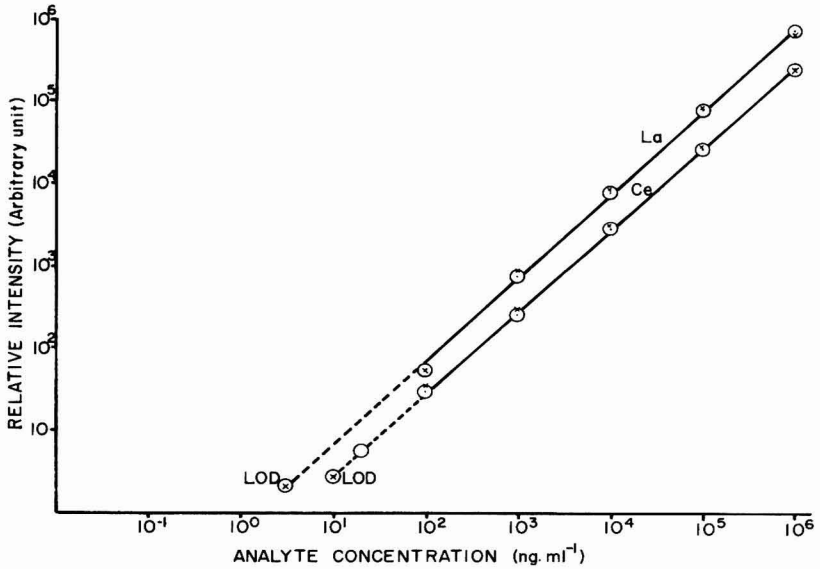


FIG. 1. Analytical calibration curves for lanthanum and cerium: (·) single element; (×) in mixture.

The ICP was mounted on an adjustable (x-y-z) table so that the observation height could be adjusted with respect to the monochromator. The optimum plasma observation height was found to be 12 mm above the load coil of the ICP.

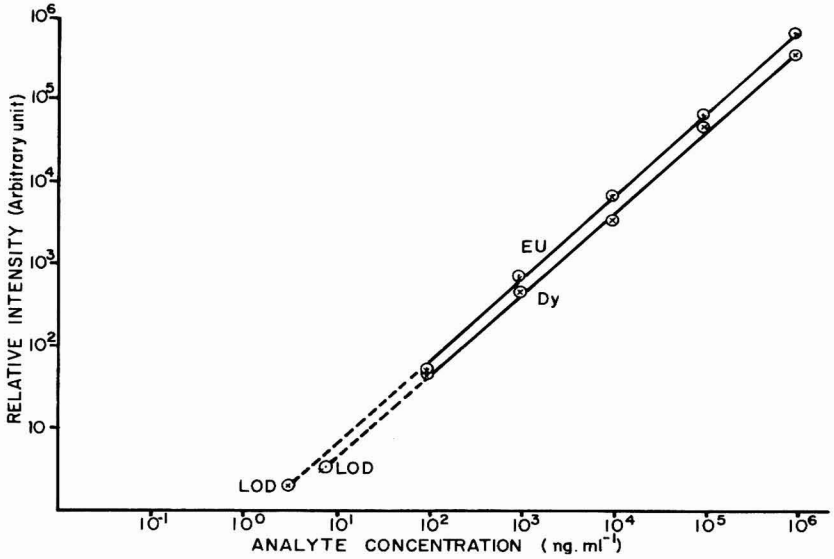


FIG. 2. Analytical calibration curves for europium and dysprosium: (·) single element; (×) in mixture.

TABLE 2
LIMITS OF DETECTION AND LINEAR DYNAMIC RANGES OF RARE EARTH METALS

Element	Wavelength (nm)	Energy levels[a] (K)	gA $\times 10^8$ (sec^{-1})	Limits of detection (ng/ml)				Linear dynamic range (dimensionless) this work
				This work—ICP		Previous works—ICP		
				Pure soln.	Mixture	Ref. (9)	Ref. (12)	
La_{II}	394.91	3250–28565	5.0	5	5			$>2 \times 10^5$
	379.48	1971–28315	2.3			3	6	
Ce_{II}	394.27	0–25360	2.0	20	10	7	30	$>10^5$
Pr_{II}	391.89	2998–28509	2.0	20	20			$>5 \times 10^4$
	390.8	4437–30018	1.9			60	30	
Nd_{II}	401.22	5086–30002	5.2	10	10	50	10	$>10^5$
Sm_{II}	360.95	2238–29935	3.9	20	20			$>5 \times 10^4$
	442.43	3910–26540	1.3			20	30	
Eu_{II}	420.50	0–23774	3.2	3	4			$>3 \times 10^5$
	412.97	0–24208	1.9			1	3	
	381.97	0–26173	4.8				3	

Gd_{II}	376.84	633–27162	8.3	10	7			$>10^5$
	342.25	1935–31146	19.0			7	10	
	336.22	633–30367	12.0				20	
Tb_{II}	350.92	NR[b]	NR	9	10	200	20	$>10^5$
Dy_{II}	353.17	0–28307	19.0	8	7	4	9	$>10^5$
Ho_{II}	345.6	NR[b]	NR	8	7	10	10	$>10^5$
Er_{II}	349.91	440–29011	9.9	10	10			$>10^5$
	337.28	0–29641	13.0			1	10	
Tm_{II}	384.80	0–25980	1.1	6	7	7	10	$>10^5$
Yb_{II}	369.42	0–27062	0.74	2	4			$>5 \times 10^5$
	328.94	0–30392	1.4			0.9	5	
Lu_{II}	350.74	0–28503	0.20	10	10	8	10	$>10^5$

[a] See Ref. (*2*).
[b] NR = not recorded.

RESULTS AND DISCUSSION

The observed detection limits and linear dynamic ranges of rare earth elements with the lines used for measurement (also energies of levels and gA values are given) are summarized in Table 2. The linear dynamic ranges were obtained by measuring the relative emission signals of selected analysis lines vs concentration of metal ions in solution. The analytical calibration curves are linear over a concentration range of ~5 orders of magnitude for all of the elements being studied. As representative examples, calibration curves for La, Ce, Eu, Dy, Ho, and Lu are presented in Figs. 1, 2, and 3.

Comparison of the results of this work with that of other authors (*9, 12*) indicates that the conditions employed in this study have resulted in similar or better limits of detection as those of others. It is also clear from the LODs obtained in the mixture of elements that matrix interferences are negligible in the ICP.

In the present studies, only ion lines were found to be sufficiently intense for analytical measurements of rare earth metals. Ion lines (see Table 2) were selected on the basis of maximization of intensities and minimization of spectral interferences and background. The detection limits obtained in this study in pure aqueous solutions or rare earth mixtures are similar to or superior to those reported in two previous studies of ICP excitation of rare earths (*9, 12*). The excellent linear dynamic ranges

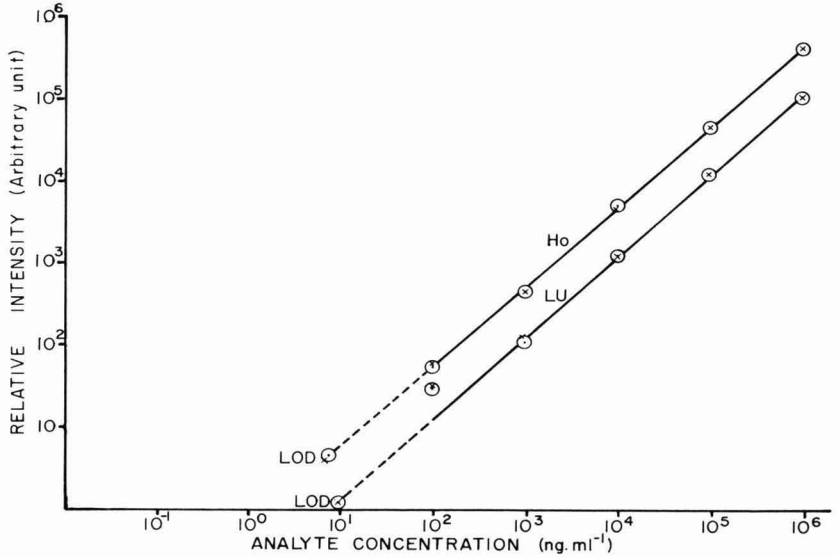


FIG. 3. Analytical calibration curves for holmium and lutetium: (·) single element; (×) in mixture.

in this study should be stressed; unfortunately, LDRs were not obtained in any of the previous studies.

SUMMARY

Using a medium power (1.25–1.50 kW) ICP as an excitation source and a glass concentric nebulizer, aqueous solutions of rare earth elements are measured. Limits of detection for all the rare earth elements in both pure solution and in a mixture of all the rare earth elements combined are measured except for Pm. Analytical lines and ICP characteristics for such measurements are given.

ACKNOWLEDGMENTS

One of the authors (A.M.) wishes to thank the Ministry of Science and Higher Education for the grant to perform these studies.

REFERENCES

1. Amos, M. D., and Willis, J. B., Use of higher temperature pre-mixed flames in atomic absorption spectroscopy. *Spectrochim. Acta* **22,** 1325–1343 (1966).
2. Corliss, C. H., and Bozman, W. R., Experimental transition probabilities for spectral lines of seventy elements. *NBS Monograph* **53,** July 20 (1962).
3. Dickinson, G. W., and Fassel, V. A., Emission spectrometric detection of the elements at the nanogram per milliliter level using induction-coupled plasma excitation. *Anal. Chem.* **41,** 1021–1025 (1969).
4. Dittrich, K., and Borzym, K., Bestimmung von Supren Seltener Erden in Anderen Seltenen Erden Durch Atom Absorption mit Elektrothermischer Atomisierung und Durch Emissionsspektrographie mit Dem Gleichstrodauerborgen. *Anal. Chim. Acta* **94,** 83–90 (1977).
5. Fassel, V. A., Analytical spectroscopy of the rare earths. *Anal. Chem.* **32,** 19A–47A (1960).
6. Fassel, V. A., Curry, R. H., and Kniseley, R. N., Flame spectra of the rare earth elements. *Spectrochim. Acta* **18,** 1127–1155 (1962).
7. Fassel, V. A., and Mossotti, V. G., Atomic absorption spectra of vanadium, titanium, niobium, scandium, yttrium, and rhenium. *Anal. Chem.* **35,** 252–253 (1963).
8. Fassel, V. A., Mossotti, V. G., Grossman, W. E. L., and Kniseley, R. N., Evaluation of spectral continua as primary sources in atomic absorption spectroscopy. *Spectrochim. Acta* **22,** 347–359 (1966).
9. Fassel, V. A., and Kniseley, R. N., Inductively coupled plasma-optical emission spectroscopy. *Anal. Chem.* **46,** 1110A–1121A (1974).
10. Mossotti, V. G., and Fassel, V. A., The atomic absorption spectra of the lanthanide elements. *Spectrochim. Acta* **20,** 1117–1129 (1964).
11. Raines, T. C., Hause, H. P., and Menis, O., Flame spectra of Sc, Y, and rare earth elements. *Anal. Chim. Acta* **22,** 315–328 (1960).
12. Souilliart, J. C., and Robin, J. P., Quelques Exemples d'applications du Chalumeau à Plasma Inductif à l'analyse élémentaire. *Analusis* **1,** 427–434 (1972).

MICROCHEMICAL JOURNAL 24, 8–15 (1979)

Gas Phase Pyrolysis of Some Cyclic Dithioketals

M. MOHAMMADI AND H. MODARESS

Department of Chemistry, Tehran Polytechnic, Tehran, Iran

Received April 11, 1978

INTRODUCTION

It has been observed in the dithioketals that variations in ring size bring about changes in their ultraviolet spectra (*14*). It is known that the ultraviolet absorption of these systems is due to interaction of the geminal sulfur atoms, and presumably involves the 3d-orbitals (*6*). From the photochemical behavior of cyclic dithioketals, it appears as if the course of the photolysis is governed by the size of the ring containing the two sulfur atoms (*12, 13*). Examination of the mass spectra of a series of cyclic dithioketals has indicated pronounced variations in the fragmentation pathways with changes in ring size (*8*).

The present work was invoked because further investigations were necessary in order to elucidate the role of the 3d-orbital interaction of sulfurs in dithioketals.

The pyrolysis of 1,5-dithiaspiro(5.4)decane and 1,5-dithiaspiro (5.5)undecane was studied to determine the effect, if any, of varying the size of the ring containing the two sulfur atoms. Pyrolysis of 1,4-dithiaspiro(4.4)nonane and 1,4-dithiaspiro(4.5)decane was likewise studied to determine any effect that changes in the hydrocarbon or the sulfur ring might have on the pyrolysis products.

EXPERIMENTAL

The compounds used were:

1,4-dithiaspiro(4.4)nonane	(I)
1,5-dithiaspiro(5.4)decane	(II)
1,4-dithiaspiro(4.5)decane	(III)
1,5-dithiaspiro(5.5)undecane	(IV)

which were synthesized as described in Refs. (*5*) and (*9*).

The pyrolysis apparatus was similar to that used by Cava and Spangler (*3*).

Samples were introduced into the pyrolysis chamber by flash vaporization from the sublimation chamber. The pressure of the system reduced to the minimum attainable (0.1 m). Dry nitrogen was then admitted to the

0026-265X/79/010008-08$01.00/0

system to increase the pressure to 0.4 mm. Pyrolysis was allowed to proceed for 2.5 to 7 hr.

The collected mixture of nongaseous products except CS_2 was passed through a semi-micro column[1] of a Woelm aluminum oxide (anionotropic) eluting with spectroquality methylene chloride.

In the cases where the isolated compound was known, its mass and infrared spectra with ge-retention time were compared to that of the authentic material. A mixed melting point was obtained when the material was a solid.

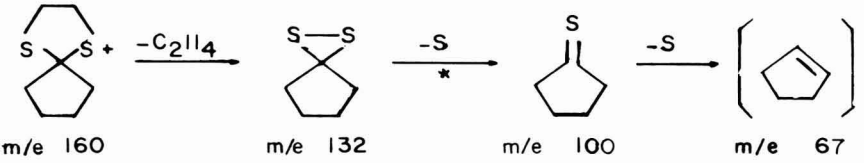
All compounds analyzed by mass and infrared spectroscopy were purified and checked for purity using columns of different polarity.

Mass spectra were determined at 70 eV with a Hitachi–Perkin–Elmer Model RMU-6 E single-focusing mass spectrometer. GC/MS were obtained with a Finnigan Model 1015 C gc/ms spectrometer.

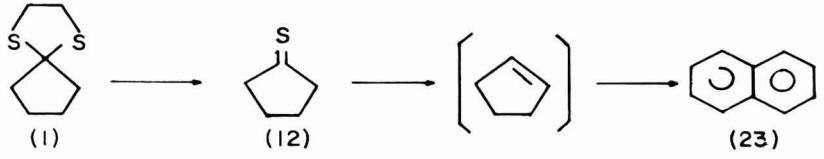
RESULTS AND DISCUSSION

The yields as a function of pyrolysis temperatures are given in Table 1.

The partial 70-eV mass spectrum of (i) shows *m/e* (relative abundance): 160 (79), 132 (100), 100 (26), 67 (67). These peaks account for 36% of the total ion current. The 25-eV spectrum consists of only these four peaks: 160 (100), 132 (50), 100 (7), and 67 (4) which correspond to the following fragmentation (*7*).



Pyrolysis of (I) resulted primarily in cleavage to thiocyclopentanone (*12*) and follows the electron-impact-induced fragmentation in the following manner:

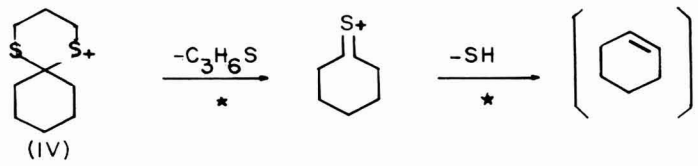


Thiocyclopentanone was synthesized (*1, 2, 11*) and pyrolyzed as an aid in analyzing the decomposition results.

The 70-eV mass spectrum of (IV) shows ions with *m/e* (relative abundance): 188 (91), 114 (84), and 81 (100). These peaks account for 30% of

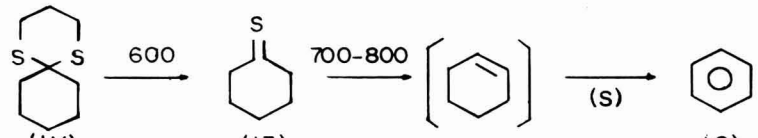
[1] 9″ L × 7.0 to 7.4-mm-o.d. disposable capillary pipets, filled with 3 ml $6Al_2O_3$.

the total ion current. The 15-eV spectrum consists of the same three peaks: 188 (100) 114 (17) and 81 (2) (65% total ion current).



(IV)

The pyrolysis of (IV) is related to the electron-impact-induced fragmentation in the following manner:



(IV) (13) (6)

From the experiments run, it is readily seen that at lower temperature the first products which were formed and were detectable by gc/ms were the thiocycloketones (12 and 13). At 600°C most of the starting materials were recovered unreacted. Higher temperatures decreased the percentage recovery of starting material, and at these temperatures no thiocycloketones were detected. It was also seen that with increasing temperature the yields of naphthalene and benzene were decreased for compounds (12) and (13), respectively. This behavior indicates that the thiocycloketones are undergoing further pyrolysis.

The results also show that major dissociation in the sulfur-containing ring occurs at lower temperature (500–600°C) and at higher temperature (700–800°C), dissociation extends to the alkane ring in a fashion analogous to their electron-impact behavior at 15 and 70 eV, respectively. This provides a parallel between the electron impact and thermal fragmentation of this type of molecule. The results correspond to the following mechanism which is expected for the pyrolysis of compounds (I) to (IV):

n=0 (I)
n=1 (II)

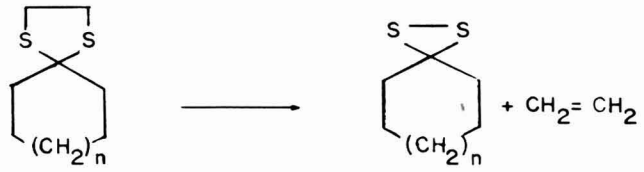


TABLE 1
ABSOLUTE YIELD PERCENTAGE OF PYROLYSIS PRODUCTS OF DITHIOKETALS

Number	Name	I (°C)			II (°C)			III (°C)			IV (°C)		
		600	700	800	600	700	800	600	700	800	600	700	800
1	Hydrogen sulfide	1.0	4.0	2.5	10.6	21.0	21.6	1.2	5.1	4.7	7.5	30.0	3.0
2	Ethylene	2.0	10.0	5.3	—	—	—	2.6	10.0	10.0	—	—	—
3	Propylene	—	—	—	6.9	11.3	13.5	—	—	—	3.4	10.0	15.0
4	Carbon disulfide	0.9	1.2	1.4	2.0	12.3	11.0	3.0	9.0	10.0	5.0	10.0	10.0
5	1,3-Cyclohexadiene							2.59					
6	Benzene	0.17	10.0	12.6	5.25	12.53	18.42	3.22	32.19	20.35	6.8	25.2	21.11
7	Thiophene	0.87	5.57	9.5	1.14	3.5	4.3	0.12	7.18	5.1	1.3	2.55	4.69
8	Toluene	—	2.1	2.2	3.78	2.5	4.13	v.s.[a]	0.74	2.76	0.54	1.19	1.54
9	2-Methyl thiophene	0.77	5.22	6.8	2.4	4.14	2.3	v.s.	0.34	1.81	0.24	0.25	0.77
10	3-Methyl thiophene	—	—	0.63	—	—	0.17	—	—	1.58	0.25	v.s.	—
11	Cyclopentyl mercaptan		0.05		0.28								
12	Thiocyclopentanone	4.84	—	—	4.57								
13	Thiocyclohexanone	1.02						5.43			5.49		
14	2-Ethyl thiophene									1.39			
15	3-Ethyl thiophene									0.14			
16	2,3-Dimethyl thiophene					0.16	0.08	0.12	—	0.12		v.s.	
17	Cyclooctatetraene	—	—	0.5	0.94	1.88	2.5	—	0.39	1.78	0.4	2.1	1.03

(Continued)

TABLE 1—*(Continued)*

Number	Name	I (°C)			II (°C)			III (°C)			IV (°C)		
		600	700	800	600	700	800	600	700	800	600	700	800
18	2-Vinylthiophene	—	—	0.2	0.23	0.12	0.5	0.19	1.23	3.1	0.36	0.95	1.55
19	Indene	0.2	—	0.35	0.23	2.24	0.04	—	0.25	1.2	0.76	0.25	0.17
20	2-Methyl-3-vinyl thiophene	1.17	0.65		1.8	0.96	0.12						
21	1-Methyl-1-vinyl thiophene	—	—	0.35	0.06	0.57	—						
22	Naphthalene	—	6.7	10.25	0.46	7.26	1.16	—	1.47	1.58	0.51	0.73	0.68
23	Thianaphthene	—	0.67	0.85	0.24	1.0	0.2	—	0.25	3.05	0.31	0.8	0.85
24	Trithiane				0.144			1.55					
25	Biphenyl	—	v.s.	0.53	—	v.s.	v.s.						
26	2-Naphthalenethiol	—	v.s.	1.47	—	v.s.	v.s.						
27	Acenaphthylene	—	3.4	3.2	—	0.72	0.37	—	v.s.				v.s.
28	1,6-Dehydro-2,5-dithia-bicyclo [4.3.0]nonane	0.10											
29	1,6-Dehydro-2,5-dithia-bicyclo [4.4.0]decane							v.s.	v.s.				
30	*trans*-2,5-dithiabicyclo-[4.3.0]nonane	0.05											

31	*cis*-2,5-dithiabicyclo-[4.3.0]nonane	0.05				
32	$C_{10}H_{12}S$	0.10				
33	$C_{10}H_{12}S_2$	0.10				
34	Dicyclopentyl disulfide	0.15				
35	Dicyclohexyldisulfide			v.s.		
36	*trans*-1,4-dithiaoctahydro naphthalene			v.s.		
37	*trans*-1,4-dithiaoctahydro naphthalene			v.s.		
38	1,2,3,5,6,7,8-Octahydro-9-thiafluorene			v.s.	0.31	
39	7,14-Dithiadispiro[5.1.5.1]tetradecane			v.s.		
40	1,7-Dehydro-2,6-dithia-bicyclo[5.3.0]decane		0.06			
41	5,5-Dimethyl-1,3-cyclo-pentadiene		0.86			

[a] v.s., very small.

The cyclic disulfides are not stable under the conditions of pyrolysis (*10, 4*) and either cleave to carbon disulfide and cyclobutane ($n = 0$), cyclopentane ($n = 1$), or to other hydrocarbons. The results and mechanism suggested for the pyrolysis of compounds (I) to (IV) indicated that for dithioketals with different ring sizes different thermal and fragmentation pathways are obtained. This lends support to the idea that to some extent the 3d-orbital interaction is involved in these systems and affects the consistency of the pyrolysis products.

This entire matter deserves further study. We are particularly interested in conducting appearance potential measurements to determine in more detail the exact energetics of these processes.

SUMMARY

A detailed analysis of the gas phase pyrolysis of 1,4-dithiaspiro[4.4]nonane(I), 1,5-dithiaspiro(5.4)decane(II), 1,4-dithiaspiro(4.5)decane(III), and 1,5-(5,5)undecane(IV) is presented. Pyrolysis resulted primarily in cleavage of the molecules to thiocyclopentanone and thiocyclohexanone. The products observed are discussed in relation to the effect of ring size in cyclic dithioketals. A definite correlation was noted between the thermal and their electron-impact-induced fragmentations of these materials.

REFERENCES

1. Cairns, T. L., Evans, G. L., Larchar, A. W., and Mckusick, B. C., *gem*-Dithiols. *J. Amer. Chem. Soc.* **74**, 3982–3989 (1952).
2. Campaigne, E., and Edwards, B. E., Thiocarbonyls. VIII. The question of *gem*-dithiol formation in aralkylketones. *J. Org. Chem.* **27**, 3760–3764 (1962).
3. Cava, M. P., and Spangler, R. J., 2-Cabomethoxybenzocyclobutenone. Synthesis of a photochemically sensitive small-ring system by a pyrolytic Wolf-rearrangement. *J. Amer. Chem. Soc.* **89**, 4550–4551 (1967).
4. Foss, O., Johnson, K., and Reisted, T., The crystal and molecular structure of the 3,6-dicarboxylic acid of 1,2-dithiane and 1,2-diselenane. *Acta Chem. Scand.* **18**, 2345–2354 (1964).
5. Fuhrer, H., and Gunthard, Hs. H., 242. Schwingungsspektren der Äthylen-di-thioketale Von Cyclanonen Partielle zuordnung fur den thioketalring. *Helv. Chim. Acta* **45**, 2036–2042 (1962).
6. Jaffe, H. H., and Orchin, M., "Theory and Applications of Ultraviolet Spectroscopy," pp. 475–476. John Wiley, New York, 1962.
7. Mohammadi, M., Electron-impact-induced fragmentation of cyclic dithioketals. M. S. Thesis, University of Idaho, 1971.

8. Mohammadi, M., and Willett, J. D., Electron-impact-induced fragmentation of cyclic dithioketals. I. A comparison with photochemistry. 161st Annual National Meeting of American Chemical Society, Los Angeles, California, 1971.
9. Reid, E. E., and Jelinek, A., Some mercaptols of 1,2-ethanedithiol. *J. Org. Chem.* **15,** 448–449 (1950).
10. Schonberg, A., Schutz, O., Bruckner, V., and Peter, J., Über thermolabile thio-ather (15 mitteilung:über organishe Schwefelverbindungen). *Chem. Ber.* **62,** 2550–2562 (1929).
11. Sen, D. C., Cyclic thioketones. I. Synthesis of non-polymerized thiocyclohexanone, thiocyclopentanone and their derivatives. *J. Ind. Chem. Soc.* **13,** 268–272 (1936).
12. Willett, J. D., The photochemistry of some cyclic dithioketals. Ph.D. Thesis, M.I.T., 1965.
13. Willett, J. D., Grunwell, J. R., and Berchtold, G. A., Photolysis of cyclic mercaptoles. *J. Org. Chem.* **33,** 2297–2302 (1968).
14. Yano, Y., and Oae, S., "Mechanisms of Reactions of Sulfur Compounds," Vol. 4, pp. 167–174. Intra-Science, New York, 1969.

MICROCHEMICAL JOURNAL 24, 16–22 (1979)

Analysis of Tungsten-Containing Mixtures and Some Alloy Steels by Potentiometric Titration Methods

H. KHALIFA AND I. A. ISMAIL

Faculty of Science, Zagazig University, Zagazig, Egypt

Received September 23, 1977

INTRODUCTION

Tungsten is among those elements that produces alloy steels that retain their hardness even at red heat, and interest continues in the determination of this metal when associated with the other components of technologically important alloys.

The well-known cinchonine and mercury(I) tungstate methods (*1*, *6*) are based on ignition of WO_3H_2O or Hg_2WO_3 and weighing as WO_3. After separation of vanadium as V_2O_5, Martinez *et al.* (*5*) precipitated WO_3H_2O with excess perchloric acid, separated it, and ignited to WO_3. Polyakov and Rumyantesev (*7*) stated that the precipitation of WO_3H_2O is quantitative in the presence of Fe(III), aluminuim, and lead, but is slightly affected by mineral acids or aqua regia. Varughese and Rao (*9*) determined tungsten as silver tungstate, in the absence of sulfate, acetate, arsenate, vanadate, and chloride. Shevahere (*8*) titrated tungstate with silver nitrate potentiometrically using silver and calomel electrodes. Ismail and coworkers (*2*) determined various constituents of vanadium and chromium steels by the back titration of EDTA, CDTA, KI, or KBr with mercury (II) or mercury (I) to a potentiometric end point. The present paper describes the application of this approach to the analysis of tungsten mixtures.

EXPERIMENTAL METHODS

Deionized water was used. The chemicals were of the highest purity available.

Solutions. The mercury (I) nitrate solution was prepared by reducing the calculated amount of $Hg(NO_3)_2$ previously dissolved in the least amount of HNO_3 with mercury metal, separating excess mercury by filtration, and diluting to volume with water. This solution was standardized against standard iron (III) alum solution in the presence of NH_4SCN (*3*). The arsenite solution was made by dissolving As_2O_3 in NaOH, slightly acidifying the solution with 10% H_2SO_4, and diluting to volume with water. This solution was standardized iodometri-

0026-265X/79/010016-07$01.00/0

cally. The 0.2 and 0.7% alcoholic iodine solutions were prepared from sublimed iodine and absolute ethanol. Synthetic mixtures were prepared in such a manner that they contained the metal components in the appropriate ratios of some alloy steels. These mixtures were standardized by recommended procedures.

Apparatus. The titration assembly was that described (*2*) except that a stream of oxygen-free nitrogen was allowed to pass into the solution, whenever tungsten (VI) and iron (III) were to be determined. The titration cell consisted of silver amalgam and calomel electrodes.

Procedures

(1) Analysis of a Mixture of W(VI) + Mn(II) + Fe(III) (Low-Alloy Tap Steel)

This mixture was prepared by mixing the following three solutions and diluting the resulting solution to 500 ml with water: (a) 0.0152 g of sodium tungstate dihydrate was dissolved in 20 ml of water and acidified with 75 ml of glacial acetic acid; (b) 0.484 g of iron metal was dissolved in 50 ml of 1:2 sulfuric acid; the iron (II) was oxidized with 1 ml of 30% hydrogen peroxide; the solution was boiled to decompose excess of the latter and was then cooled; (c) 0.0078 g of manganese (II) sulfate monohydrate was dissolved in 20 ml of water and acidified with 1 ml of 10% H_2SO_4. A 10-ml volume of this mixture contained 0.170 mg of W, 0.051 mg of Mn, and 9.68 mg of Fe.

(1a) For Mn. To a 10-ml portion of the mixture (aliquot A), cautiously add sodium peroxide to the appearance of a brown precipitate of hydrous iron (III) oxide, add an excess, boil for 20 min to oxidize Mn(II) to Mn(IV) and to decompose excess of Na_2O_2, cool, acidify with 5 ml of glacial acetic acid and 1 ml of 10% H_2SO_4, heat to dissolve iron oxide (WO_3H_2O does not precipitate in the presence of glacial acetic acid even with moderate boiling), cool, reduce the brown precipitated Mn(IV) with 5 ml of 0.01 *M* arsenite, set aside for 5 min, oxidize the excess arsenite with 5 ml of 0.2% alcoholic I_2 solution, and titrate the iodide formed with 0.01 *M* Hg(II). Find out As^{3+} = Mn by subtracting the iodide equivalent to excess arsenite from total arsenite to get the amount of the latter equivalent to manganese. Compute EDTA = Mn.

(1b) For Fe + Mn. To aliquot A, after the determination of Mn, add 8 ml of 0.05 *M* EDTA, 8 ml of 10% hexamethylene tetramine, and at pH 6.5 titrate the unreacted EDTA with 0.05 *M* Hg(II) (there is no interference from W or As). The unreacted EDTA is subtracted from the total added to give EDTA that reacted with iron (III) and Mn(II), that is, EDTA = Fe + Mn. Find EDTA = Fe by difference.

(1c) For W. In a second 10-ml aliquot, mask Fe + Mn with EDTA (4 ml of 0.05 *M* as calculated from 1b to be in slight excess); mask the excess

EDTA with $ZnSO_4$ (0.5 ml of 0.05 *M* as calculated to be sufficient for a slight excess) because EDTA has a reducing effect on mercury (I); heat for 2 min; upon cooling add 5 ml of 0.01 *M* mercury (I) solution, adjust to pH 6 with ammonia, stir for 15 min, pass nitrogen gas at a moderate rate to displace oxygen from the beaker, then close the holes of the rubber stopper and set aside in a dark place overnight. Now remove the stopper, separate the Hg_2WO_4 by filtration with 542 Whatman paper, wash thoroughly with 0.2% HNO_3, and titrate the unreacted Hg(I) in the filtrate with 0.01 *M* KBr. Subtract the unreacted Hg(I) from the total added to get the combined amount in Hg_2WO_4, that is, Hg(I) = W(VI).

(2) Analysis of a Mixture of W(VI) + Fe(III) + Mn(II) + Cr(III) (Tungsten Chisel Steel)

This mixture was prepared by mixing the three solutions obtained by dissolving separately 0.0101 g of $Na_2WO_42H_2O$, 0.484 g of Fe metal, and 0.0078 g of $MnSO_4H_2O$ in the same manner as described in procedure 1, with a Cr solution (prepared by dissolving 0.0547 g of $KCr(SO_4)_212H_2O$ in 50 ml of H_2O, acidifying the solution with 10 ml 1:2 H_2SO_4, and diluting to 500 ml with water). A 10-ml volume of this mixture contained 0.113 mg of W + 9.68 mg of Fe + 0.051 mg of Mn + 0.113 mg of Cr.

(2a) For Cr. Boil 10 ml of the mixture (aliquot B) with Na_2O_2 for 20 min to oxidize Cr(III) and Mn(II) and to destroy excess Na_2O_2. Acidify the mixture with 5 ml of glacial acetic acid and a few drops of 1:3 HCl; heat for 10 min to dissolve $Fe(OH)_3$ and to reduce Mn^{4+} to Mn^{2+} while passing N_2 to expel liberated Cl_2. Upon cooling, add 5 ml of 0.01 *M* arsenite to reduce Cr(VI), heat for 2 min to enhance the reduction (but avoiding boiling and possible volatilization of $AsCl_3$), add 25 ml of 0.2% I_2 solution, and proceed according to 1a). Find out As^{3+} = Cr by subtracting the iodide equivalent to excess arsenite from total arsenite to get the amount of the latter equivalent to chromium.

(2b) For Fe + Mn + Cr. To aliquot B, after determining Cr, add 8 ml of 0.05 *M* EDTA and proceed as described in 1b to obtain EDTA that combined with the three cations, that is, EDTA = sum of Fe + MN + Cr.

(2c) For Fe. To a second 10-ml portion of the mixture add a few drops of lactic acid (~ 90%) to keep W(VI) in solution during prolonged boiling, 10 ml of 0.1 *M* KI, boil for 20 min to reduce Fe^{3+} to Fe^{2+} while passing nitrogen gas at a moderate rate to expel the liberated I_2. Cool the reaction mixture in ice water to 5 to 10°C and titrate the unreacted KI with 0.05 *M* Hg(II). Subtract excess KI from the total to get KI = Fe, compute EDTA = Fe, and subtract it from EDTA = sum of Fe + Mn + Cr in 2b to get EDTA = Mn + Cr, then compute EDTA = Cr from 2a and find EDTA = Mn by difference.

(2d) For W. Boil a third 10-ml portion of the mixture with 4 ml of 0.05 *M*

EDTA for 15 min to mask Fe + Mn + Cr; mask free EDTA with $ZnSO_4$, and continue as described in 1c to obtain Hg(I) = W(VI).

(3) Analysis of a Mixture of W(VI) + Fe(III) + Cr(III) + V(IV) (High Speed Steels)

This mixture was prepared by mixing the three solutions obtained by dissolving separately 0.1525 g of $Na_2WO_42H_2O$, 0.4145 g of Fe metal, and 0.1622 g of $KCr(SO_4)_212H_2O$ as described in procedures 1 and 2, with a vanadium solution (prepared by dissolving 0.0246 g of NH_4VO_3 in 50 ml of hot water, acidifying the solution with 5 ml 1:2 H_2SO_4, reducing V(V) to V(IV) with SO_2 water, boiling the solution to remove excess SO_2, and then allowing it to cool). Dilute the solution to 500 ml with water. A 10-ml volume of this mixture contained 1.7 mg of W + 8.29 mg of Fe + 0.338 mg of Cr + 0.212 mg of V.

(3a) For Cr, Cr + V, and Fe + Cr + V. Boil separately, two 10-ml aliquots of the mixture with Na_2O_2, make both solutions acidic with 5 ml of glacial acetic acid and 1 ml of 10% H_2SO_4, heat for 10 min to dissolve $Fe(OH)_3$, reduce Cr(VI) in only one mixture with arsenite (5 ml of 0.01 *M*), and proceed according to 2a to obtain As^{3+} = Cr. Compute EDTA = Cr. To the second mixture add 5 ml of 0.01 *M* arsenite and 8 ml of 0.05 *M* EDTA (which catalyzes the arsenite reduction of V(V) to V(IV) through formation of the more stable V(IV)–EDTA complex), boil for 15 min to reduce V(V) to V(IV) and to ensure complete complexation of Cr(III), cool, add 25 ml of 0.2% I_2 solution, and titrate iodide equivalent to excess arsenite with 0.01 *M* Hg(II). Find out As^{3+} = Cr + V and EDTA = Cr + V as illustrated in 1a for Mn; add 8 ml of 10% hexamethylene tetramine solution and at pH 6.5 titrate unreacted EDTA with Hg(II) to find EDTA that combined with Fe, Cr, and V. Compute EDTA = Fe and EDTA = V by difference.

(3b) For W. Use a third identical mixture and proceed as described in 2d.

(4) Analysis of High Speed Steel containing W + Cr + V and Tungsten Chisel Steel Containing W + Mn + Cr

Treat a 200-mg sample with 15 ml HCl. Warm gently to decompose the steel, whereby W is separated as black particles. Stir and rub with a policeman to bring W into suspension; gradually add 10 ml 1:1 HNO_3 and digest at 100°C with occasional stirring until bright yellow WO_3H_2O is no longer formed. Cool, add 10 ml of conc H_2SO_4, heat to incipient white fumes of SO_3. After cooling in ice water, dilute with 10 ml of H_2O, add 2 ml of H_2O_2, and boil for 15 min to obtain Fe^{3+}, Mn^{2+}, Cr^{3+}, and V^{4+} and to destroy H_2O_2. Cool, add 10 ml of H_2O, carefully neutralize with NaOH, and add more caustic to dissolve tungstic acid. Acidify with

30 ml of glacial acetic acid and heat while stirring with dropwise addition of 10% H_2SO_4 to dissolve the hydroxides of Fe, Cr, and V (H_2WO_4 does not precipitate). Filter the clear solution from insoluble silica (542 Whatman), wash the filter thoroughly with 10% acetic acid, and dilute the filtrate and washings to 250 ml with water. Take 5-ml aliquots of this solution and proceed exactly as described in 3a, 3b, 2a, 2b, 2c, and 2d to obtain EDTA equivalent to each component. Calculate the molarity of the solution in respect of each component and the percentage of each component.

RESULTS AND DISCUSSION

Tables 1 and 2 list the results obtained by the present and classical methods (*10*), with three synthetic tungsten-containing mixtures as well as one sample each of high speed steel and tungsten chisel steel. The agreement is good and indicates that the present methods are reliable. The end point potential breaks are quite sharp and large in magnitude. For example, in the titration of excess EDTA and KI with Hg(II), in the absence of chloride, the potential breaks averaged 150 and 380 mV per 0.1 ml of titrant, respectively. In the presence of chloride and in the titration of Hg(I) with KBr the potential breaks averaged 85 and 170 mV per 0.1 ml of titrant, respectively.

Remarks

Acetic acid or preferably lactic acid (procedures 1a and 2c) prevents precipitation of tungstic acid during heating or boiling the mixture for a considerable period of time, even in the presence of low concentra-

TABLE 1
ANALYSIS OF SYNTHETIC TUNGESTEN MIXTURES BY THE NEW METHODS

Number		1	2	3
mg W	taken	0.170	0.113	1.70
	found	0.167	0.111	1.68
mg Fe	taken	9.68	9.67	8.29
	found	9.71	9.69	8.31
mg Mn	taken	0.051	0.051	—
	found	0.048	0.048	—
mg Cr	taken	—	0.113	0.338
	found	—	0.115	0.341
mg V	taken	—	—	0.212
	found	—	—	0.214
mg Co	taken	—	—	—
	found	—	—	—

TABLE 2
ANALYSIS OF (I) HIGH SPEED STEEL AND (II) TUNGSTEN CHISEL STEEL

	W(%)	Mn(%)	Cr(%)	V(%)
Ia	18.33	—	3.81	1.20
Ib	18.21	—	3.78	1.18
IIa	2.15	0.23	1.53	—
IIb	2.11	0.25	1.56	—

a, by the classical method; b, by the new method.

tions of mineral acids. The addition of EDTA masks possible hydrolysis of cations other than tungsten and thereby occlusion with Hg(I) during precipitation of Hg_2WO_4 at relatively low acidity and long standing. Arsenite reduces both Mn(IV) and Cr(VI) to the intermediate oxidation states rather than to their lower states Mn(II) and Cr(III). However, in the presence of traces of iodide produced by oxidation of excess arsenite with I_2, the process of reduction is catalyzed toward the quantitative formation of Mn(II) and Cr(III). On the other hand, arsenite has no reducing effect on V(V) or Fe(III), but, in the presence of EDTA, reduction of V(V) to V(IV) is accomplished on heating via formation of the highly stable V(IV)–EDTA complex. Reduction of Mn(IV) and Cr(VI) in acid medium with arsenite should proceed quantitatively due to the high values of the standard potentials of the systems Mn(IV)/Mn(II) (+1.23 V) and Cr(VI)/Cr(III) (+1.33 V), in comparison with that of the arsenate/arsenite system (+0.56 V). Oxidation of arsenite with iodine in alcoholic solution proceeds quantiatively, in spite of the low value for the standard potential of the iodine/iodide system (+0.54 V). This is attributed to the fact that Hg(II) ions of the titrant not only increase the oxidation potential of iodine (*9*) but also remove the iodide set free in the equilibrium

$$AsO_3^{3-} + I_2 + H_2O \rightleftharpoons AsO_4^{3-} + 2H^+ + 2I^-$$

as the sparingly soluble red HgI_2, and, thereby, continually shifts this equilibrium to the right. However, this oxidation process is not applicable to Mn(II), Cr(III), or V(IV). Acetate ions hinder the precipitation of W(VI) as Ag_2WO_4 with silver nitrate, even at elevated temperature and in slight acid medium, which is not the case with mercurous nitrate. The presence of HCl lowers the end point potential break when KI is titrated with Hg(II) due to formation of the stable $HgCl^{2-}$ ion (log K = 17.6). On adding 0.05 ml of Hg(II) just beyond the end point, the free Hg^{2+} concentration, to which is attributed a large change in the potential set by the silver amalgam, and

consequently large portion of the end point break, is substantially suppressed. However, the potential break is still of a good order of magnitude (85 in comparison with 350 mV/0.1 ml in the absence of the chloride). Na_2O_2 oxidizes Mn(II), Cr(III), and V(IV) according to the reactions:

$$Mn^{2+} + H_2O_2 + 2OH^- = MnO(OH)_2 + H_2O$$
$$2CrO_2^- + 3H_2O_2 + 2OH^- = 2CrO_4^{2-} + 4H_2O$$
$$2VO^{2+} + H_2O_2 + 6OH^- = 2VO_3^- + 4H_2O$$

SUMMARY

Reliable potentiometric titration methods are adopted for the analysis of tungsten mixtures analogous to some industrially important alloys such as low alloy tap steel, tungsten chisel, and high speed steels. Tungsten is separated as Hg_2WO_4 and unreacted Hg(I) is titrated with KBr. The techniques of oxidation with I_2 or reduction with KI and use of the selective arsenite reduction of Cr(VI) and/or Mn(IV) in the presence of V(V) or Fe(III) make it possible to determine alloy components without tedious separations. In all titrations the potential break is sharp enough for the precise establishment of the end point.

REFERENCES

1. Furman, N. H., "Standard Methods of Chemical Analysis," Vol. 1, p. 1162. D. Van Nostrand, Princeton, New York, London, 1962.
2. Ismail, I. A., Ghonaim, A. Kh., and Khalifa, H., Rapid potentiometric methods for vanadium and for analysis of ferrovanadium and some vanadium steels. *Microchem. J.* **21**, 209–214 (1976).
3. Kolthoff, I. M., and Belcher, R., "Volumetric Analysis," Vol. 3, p. 623. Wiley (Interscience), New York, 1957.
4. Kolthoff, I. M., and Elving, P. J., "Treatise on Analytical Chemistry," part II, Vol. 7, p. 463. Wiley (Interscience), New York, 1961.
5. Martinez Lope, M. J., Maceria, V. A., and Burriel- Marti, F., Separation of vanadium from large quantities of tungsten with thioacetamide in ammoniacal medium and determination of the tungsten with $HClO_4$. *An. Quim.* **70**(3), 223–227 (1974).
6. Mellor, J. W., and Thompston, H. V., "A Treatise on Quantitative Inorganic Analysis with Special Reference to the Analysis of Clays, Silicates and Related Materials," p. 442. Griffen, London, 1938.
7. Polyakov, B. I., and Rumyantesev, V. K., Precipitation of tungstic acid in the presence of iron, aluminium and lead. *Zhur. Analit. Kim.* **27**(7), 1412–1413 (1972).
8. Shivahere, G. C., Potentiometric determination of tungsten as silver tungstate. *Naturwissenschaften* **52**(7), 157 (1965).
9. Varughese, K., and Rao, K. S., Determination of tungstate by precipitation of silver tungstate from a homogeneous solution. *Anal. Chim. Acta* **57**(1), 219–221 (1971).
10. Welcher, F. J., "Standard Methods of Chemical Analysis," Vol. 2A, pp. 713–790. Van Nostrand, Princeton, 1963.

MICROCHEMICAL JOURNAL 24, 23–32 (1979)

Determination of Iron by Catalytic Oxidation of *p*-Aminophenol by Hydrogen Peroxide

C. PAPADOPOULOS, V. VASILIADIS, AND G. S. VASILIKIOTIS[1]

Laboratory of Analytical Chemistry, University of Thessaloniki, Thessaloniki, Greece

Received June 15, 1978

INTRODUCTION

In a continuation of our studies on the catalytic determination of iron, we have examined in a previous communication (*6*) the oxidation reaction of 2,4-diaminophenol by hydrogen peroxide. *p*-Aminophenol is also oxidized to a colored product.

The oxidation reaction of *p*-aminophenol in aqueous solution has been studied by Eggers *et al.* (*2*). They have proposed that *p*-quinoneimine is the product of this oxidation. Later Snead and Remick (*5*) studied the redox mechanism of *p*-aminophenol. Guilbault (*3*) has studied the oxidation of *p*-aminophenol by cerium(IV) perchlorate in 3F perchloric acid and a selective spectrophotometric determination of *p*-aminophenol was proposed, based on the purple compound which was formed and believed to be a phenoxazine derivative. The oxidation reaction of *p*-aminophenol is generally catalyzed by several ions, such as Cu^{2+}, Fe^{3+}, VO_3^-, and WO_4^{2-}. The catalytic effect of Cu^{2+} was studied by Dolmanova *et al.* (*1*). They found that the catalytic activity of Cu^{2+} on the oxidation of the aminophenols by H_2O_2 decreases in the order of *p*→*m*→*o*-aminophenol.

According to the above facts it was found interesting to study the catalytic activity of Fe^{3+} on the oxidation reaction of *p*-aminophenol (PAP) by H_2O_2 and to apply this reaction for the catalytic microdetermination of ferric ions. The results of this study are reported in the present paper.

EXPERIMENTAL

Reagents. Analytical-grade reagents from Merck A.G. were employed and their solutions were prepared from twice distilled water. *p*-Aminophenol (PAP) was a reagent grade chemical also from Merck A.G. It was recrystallized twice from 1 *N* hydrochloric acid. Solutions of PAP were prepared always just before their use to avoid any oxidation by standing in the diffused light.

[1] To whom all inquiries should be addressed.

0026-265X/79/010023-10$01.00/0

Standard ferric ion solution was prepared by dissolving in 0.1 *N* HCl the appropriate amount of $FeCl_3 \cdot 6H_2O$. This solution was standardized gravimetrically.

Hydrogen peroxide standard solution was prepared by appropriately diluting with water the 30% v/v reagent. Buffer solutions were prepared by mixing glycine and hydrochloric acid solutions in the appropriate ratio, and their ionic strength was kept constant at 0.1 *M* by using 1 *M* sodium chloride solution.

Instruments. A Unicam SP-700 spectrophotometer, connected with a Sargent thermocirculating bath, was used for absorbance measurements. For pH measurements a Beckmann 1019 research pH meter was used with absolute accuracy limits of pH measurement defined by NBS buffers.

Procedure. In order to get reproducible results it was found necessary to employ the following procedure: In a 50-ml volumetric flask were added (a) solutions of hydrogen peroxide, (b) solutions of ferric ions, (c) buffer solutions, and (d) solutions of *p*-aminophenol (PAP) in this order always. Then the flask was filled with water up to the mark, with continuous mixing. The starting time of the reaction was taken the moment the half volume of the PAP solution was added.

An aliquot of the reaction mixture was transferred to the cell (in the spectrophotometer) and, exactly 2 min from the starting time, the recording system of the spectrophotometer was put on. The recorded values were optical density vs time. The pH measurements were performed with the rest of the reaction mixture.

To find the experimental constant rate K^* of the studied catalytic reaction, the intergration method was used according to relations (1), (2), and (3):

$$\log (a - x) \log a - K_1 C t \tag{1}$$

$$\text{or } \log(a - x) \log a - K^* t \tag{2}$$

$$\text{or } \log(D_0 - D_t) \log D_0 - K^* t \tag{3}$$

where a and $(a\text{-}x)$ are the initial and observed concentration of a starting material, C is the concentration being determined, K_1 is a function of all the remaining substances participating in this reaction (K^* is equal to the product $k_1 C$), D_0 is the optical density of a fully oxidized form of PAP, and D_t is the optical density of the partially oxidized PAP at a time t, calculated from the graphs. So, the method of tangents was used according to Yatsimirskii (*7*).

RESULTS AND DISCUSSION

In Fig. 1, the absorption spectrum of the oxidized form of PAP in water is given. The maximum absorption is at 520 nm. Guilbaut (*3*) reported that

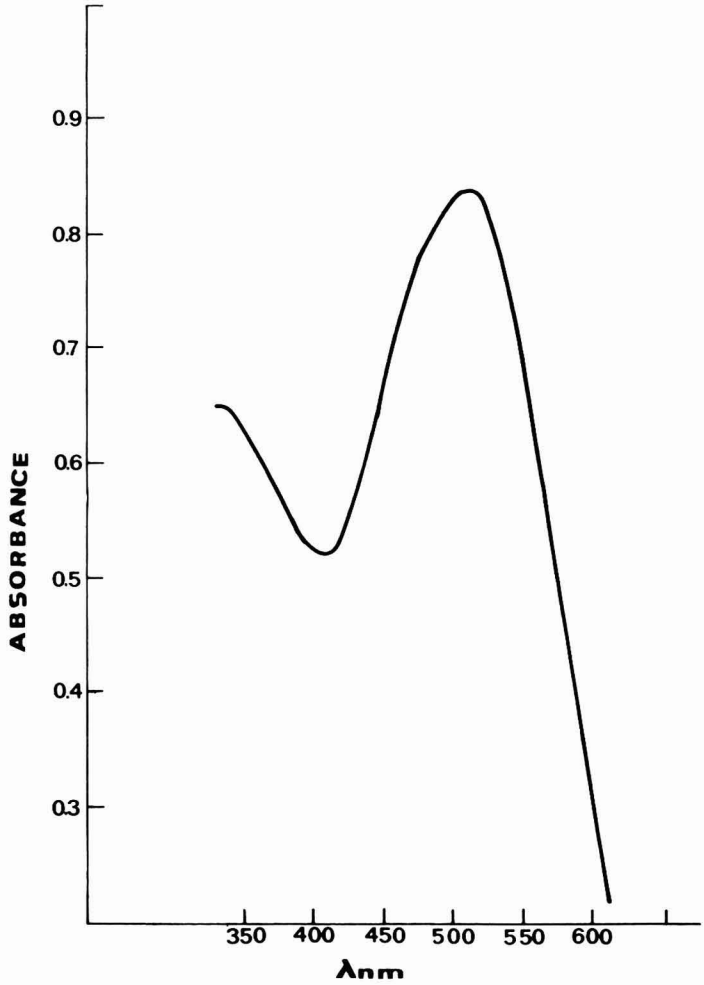


FIG. 1. Absorption spectrum of the oxidized form of PAP.

this product is stable for at least 15 min and Beer's law is obeyed. At 15 min the molar absorptivity was found to be 1.5×10^2 $1 \cdot mol^{-1} \cdot cm^{-1}$.

To select the optimal conditions for carrying out this oxidation reaction, we studied the dependence of the catalytic reaction rate on several factors and results are given as follows.

Effect of pH

In Fig. 2, the increase of the reaction rate with the increase of the pH values, in the range of 1.7 to 2.8, is shown. At higher pH values, the reaction rate decreases, probably because of the ferric hydroxide formation. The pH values from 2.5 to 2.8 were accepted as the optimum range for this work.

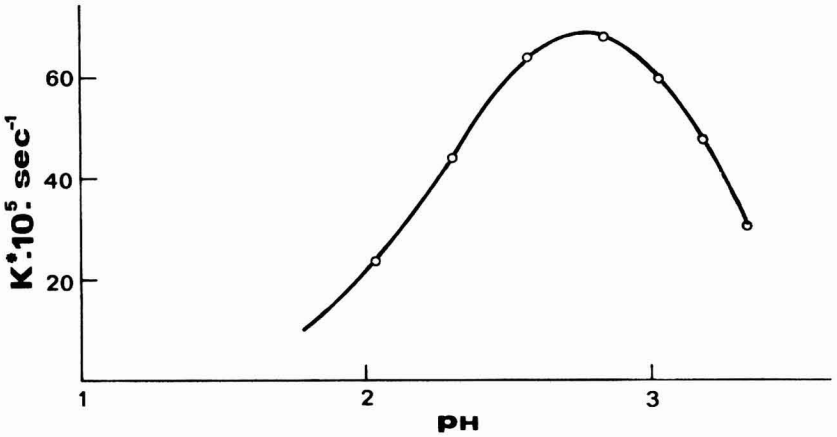


FIG. 2. Dependence of the K^* on the pH values of the solution (PAP = $2 \times 10^{-3} M$; H_2O_2 = $1 \times 10^{-1} M$; Fe = $1.918 \times 10^{-5} M$; ionic strength, $I = 0.1$ with NaCl; buffer, glycine + HCl; temperature, 25 ± 0.1°C).

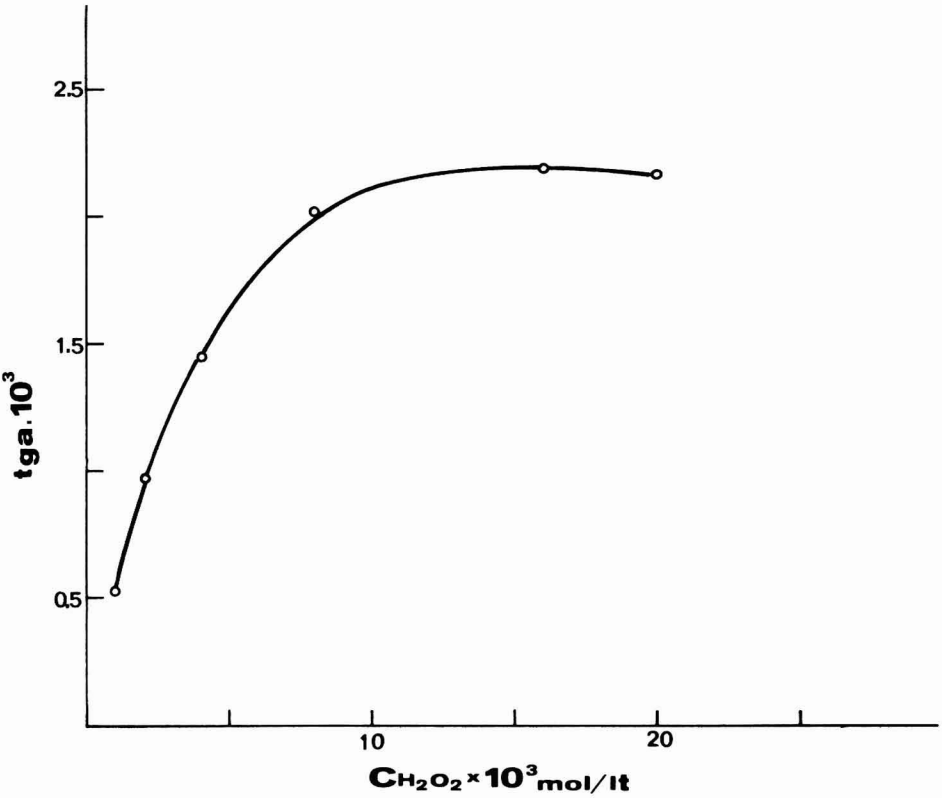


FIG. 3. Dependence of the reaction rate on the concentration of H_2O_2 (PAP = $2 \times 10^{-3} M$; Fe = $2.87 \times 10^{-5} M$; pH = 2.56; buffer, glycine + HCl; temperature, 25 + 0.1°C; ionic strength, $I = 0.1$).

Effect of H_2O_2 and PAP Concentration

The effect of H_2O_2 concentration was examined over the range of 0.1×10^{-2} to 2.0×10^{-2} *M*. As Fig. 3 shows, there is initially an increase of the reaction rate as the H_2O_2 concentration increases and then, after a definite value, remains constant. This fact is in agreement with the views of Lewis *et al.* (*4*) that a 1:1 catalytically active complex is formed between ferric ions and hydrogen peroxide.

Considering that a simple reaction takes place, without any stepwise complex formation,

$$nA + K \rightleftarrows KAn$$

where *A* is the initial compound (H_2O_2), *K* is the catalyst (Fe^{3+}), *KAn* is the formed intermediate complex, and b_n is the equilibrium constant, we can apply the following relation given by Yatsimirskii (*8*)

$$\frac{1}{v} = \frac{1}{K_1 C_K^{\circ}} \left(1 + \frac{1}{b_n} \cdot \frac{1}{|A|^n}\right)$$

where v is the reaction rate, C_k° is the overall concentration of catalyst, and K_1 is a function of the concentration of the other reactants.

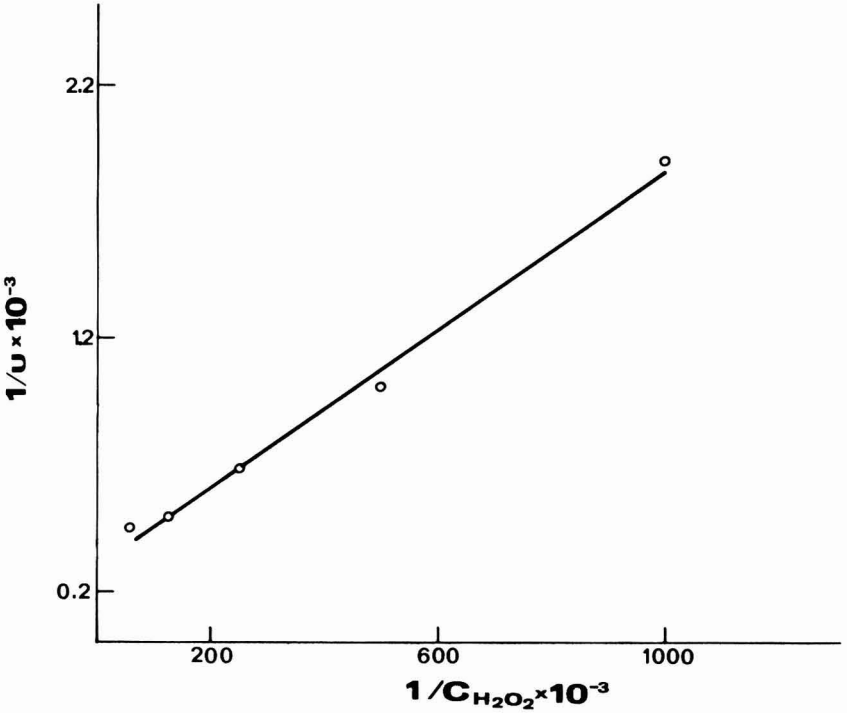


FIG. 4. Plot of the reciprocal of the reaction rate against the reciprocal H_2O_2 concentration (PAP = 2×10^{-3} *M*; Fe = 2.87×10^{-5} *M*; pH = 2.56; buffer, glycine + HCl; temperature, 25 ± 0.1°C; ionic strength, I = 0.1).

If we put $1/v$ on the ordinate and $1/[A]^n$ on the abscissa then all the experimental points must lie on a straight line. In Fig. 4, this line is actually shown and this indicates that the reaction is of a complex nature and presumably the result of the formation of a catalytically active complex Fe(III)$-H_2O_2$.

In Fig. 5 we have a plot of the reaction rate vs the concentration of PAP. There is an increase of the reaction rate as the PAP concentration increases and a linear relation exists.

Effect of Temperature. Activation Energy

The dependance of the reaction rate on the temperature is given in Fig. 6. From Fig. 7 (a graph of log K^* vs the reciprocal of the absolute temperature) and the given values of Table 1, the order of the activation energy of this catalytic reaction was calculated (*9*) and it was found to be 14.6 kcal·mol^{-1}.

On the basis of the investigations described above, we summarize the optimal conditions which we suggest for the catalytic ultramicrodetermination of iron:

(a) H_2O_2 concentration: 1.6×10^{-2} *M*, i.e., 8.0 ml of a 1.6×10^{-1} *M* solution of H_2O_2 in a 50-ml volumetric flask.

(b) FE(III) concentration for the reference curve: from 0.5 to 4.0 ml of a

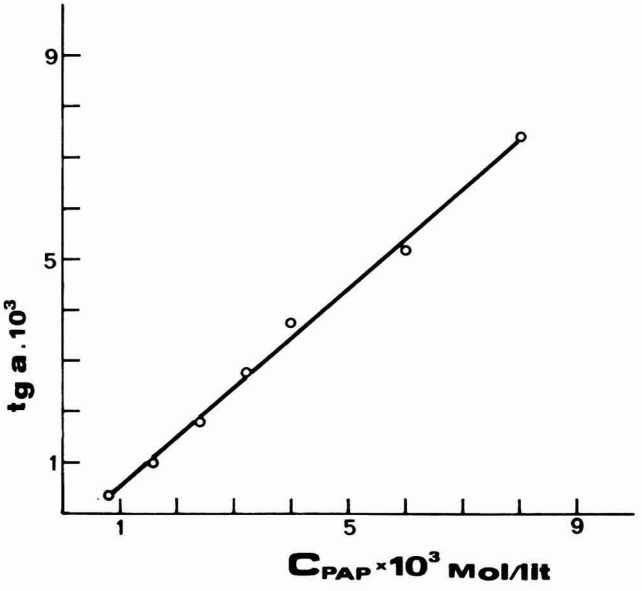


FIG. 5. Dependence of the reaction rate on the concentration of PAP: (H_2O_2 = 1.6×10^{-2} M; Fe = 1.918×10^{-5} *M*; pH = 2.83; buffer, glycine + HCl; temperature, 26.5 ± 0.1°C; ionic strength, I = 0.1).

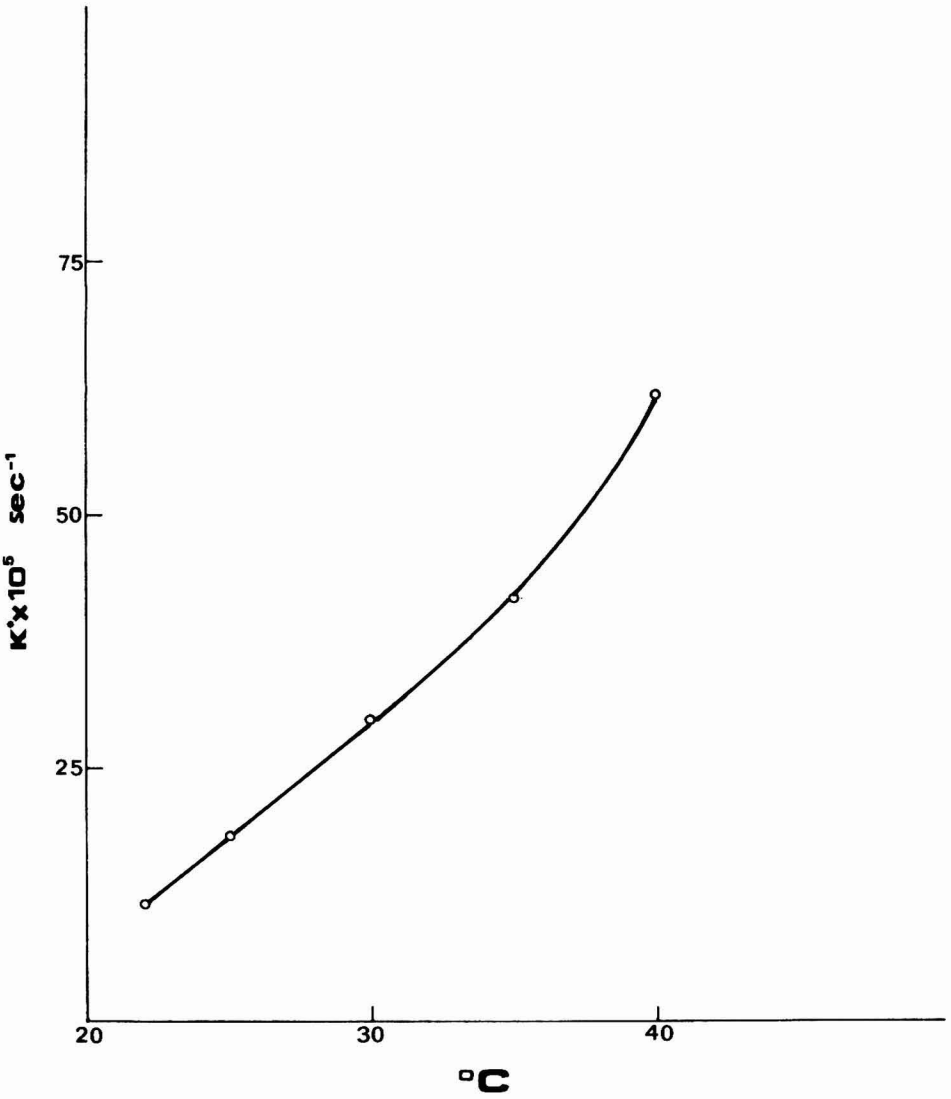


FIG. 6. Plot of the reaction rate against the temperature ($H_2O_2 = 1.6 \times 10^{-2} M$; PAP = $4 \times 10^{-3} M$; Fe = $3.83 \times 10^{-6} M$; pH = 2.79; buffer, glycine + HCl; ionic strength, $I = 0.1$).

$10 \times 10^{-5} M$ solution of Fe(III) (we used 0.5, 1.0, 2.0, 3.0, and 4.0 ml of a $9.59 \times 10^{-5} M$ solution).

(c) Buffer solution to keep the pH range from 2.5 to 2.8.

(d) PAP concentration: $4.0 \times 10^{-3} M$, i.e., 10 ml of a $2 \times 10^{-2} M$ solution of PAP in the above flask and then fill it with water up to the mark. The solutions were thermostated at 25 ± 0.1°C before the beginning of the

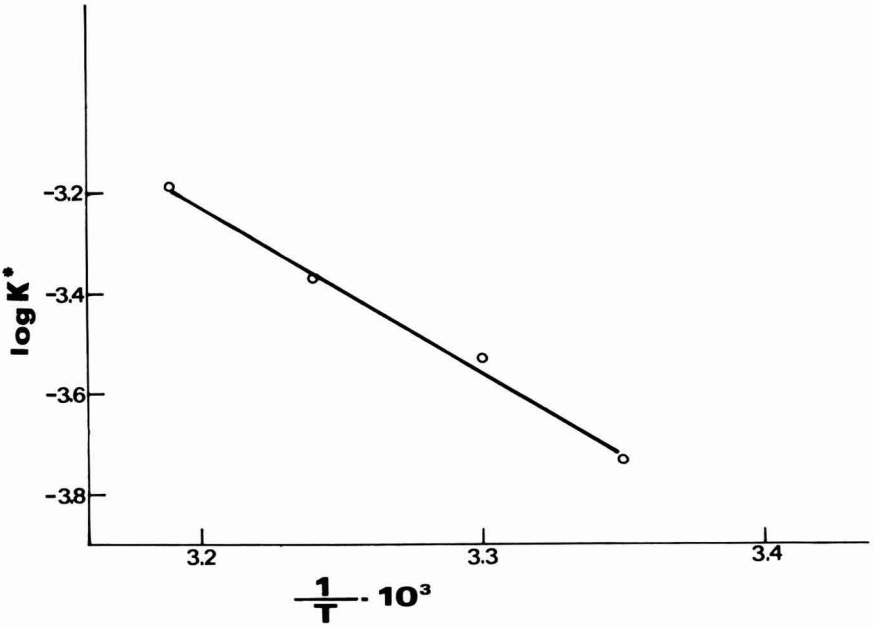


FIG. 7. Plot for calculation of the activation energy, log K^* vs reciprocal of the absolute temperature ($H_2O_2 = 1.6 \times 10^{-2}\ M$; PAP = $4 \times 10^{-3}\ M$; Fe = $3.83 \times 10^{-6}\ M$; pH = 2.79; buffer, glycine + HCl; ionic strength, $I = 0.1$).

reaction. During the oxidation of PAP by hydrogen peroxide the solution gradually develops its color and the progress of the reaction is observed spectrophotometrically.

The application of the oxidation reaction of PAP by H_2O_2 allows the determination of ferric ions in concentrations from 0.5×10^{-1} to 4.5×10^{-1} μg/ml as is shown in Fig. 8, where a reference curve is represented.

In Table 2 the results of several determinations are given. The influence of several ions on the accuracy of the determination of ferric ions was tested and the results are given in Table 3.

TABLE 1
VALUES FOR ACTIVATION ENERGY CALCULATIONS

T	$1/T$	log K^*	K
298	3.35	−3.7361	18.36
303	3.30	−3.5297	29.53
308	3.24	−3.3771	41.97
313	3.19	−3.2100	61.62

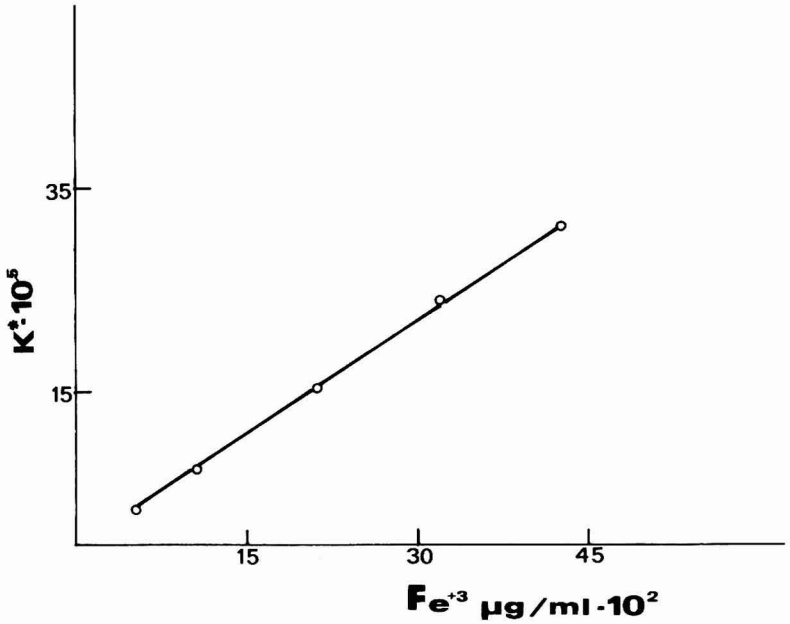


FIG. 8. Working curve for iron determination (H_2O_2 = 1.6 × 10^{-2} M; PAP = 4 × 10^{-3} M; pH = 2.62; buffer, glycine + HCl; temperature, 25 ± 0.1°C; ionic strength, I = 0.1).

SUMMARY

The oxidation reaction of *p*-aminophenol by hydrogen peroxide was studied in the presence of ferric ions, which catalyzes it. The corresponding rate constants were calculated and the activation energy was determined. A method for ultramicrodetermination of ferric ion was developed using the method of tangents. Determination of ferric ions from 0.5 × 10^{-1} up to 4.5 × 10^{-2} µg/ml is possible with a relative mean error of ±1.55%. The influence of several ions was also investigated.

TABLE 2
DETERMINATION OF Fe(III) (CATALYST)

µg/ml taken (×10^{-2})	µg/ml found[a] (×10^{-2})	Mean error (%)
7.71	7.76	+0.71
16.07	15.68	−2.45
26.78	26.59	+0.72
37.50	37.05	−1.20
40.70	41.79	+2.70
		Relative mean error, ±1.55%

[a] Each value is the mean value of three determinations.

TABLE 3
INFLUENCE OF FOREIGN IONS ON THE DETERMINATION OF Fe(III)

Ion	Maximum concentration which does not interfere (M)
NO_3^-	2.8×10^{-5}
Cu^{2+}	2.0×10^{-5}
Sn^{2+}	2.0×10^{-5}
CrO_4^{2-}	1.0×10^{-5}
Ce^{4+}	4.0×10^{-6}
VO_3^-	2.5×10^{-6}
Ce^{3+}	1.0×10^{-6}
WO_4^{2-}	1.0×10^{-7}

REFERENCES

1. Dolmanova, I. F., Poddubienco, V. P., and Peshkova, V. M., Mechanism of the catalytic action of copper in the oxidation of a series of organic compounds by hydrogen peroxide. *C.A.* **74**, 41660m (1971).
2. Eggers, J., Posse, R., and Scheibe, G., Oxidation of aminohydroxybenzenes. *Z. Electrochem.* **58**, 731–743 (1954).
3. Guilbault, G., Selective spectrophotometric method for the determination of *p*-aminophenol. *Anal. Chem.* **35**, 828–830 (1963).
4. Lewis, T. J., Richards, D. H., and Salter, D. A., *J. Chem. Soc.* 2434 (1963).
5. Snead, W. K., and Remick K. A., Studies on oxidation–reduction mechanism. II. The anodic oxidation of *p*-aminophenol. *J. Amer. Chem. Soc.* **79**, 6121–6127 (1957).
6. Vasilikiotis, G. S., Papadopoulos, C., Sofoniou, M., and Themelis, D., Catalytic microdetermination of iron. *Microchem. J.* **22**, 541–547 (1977).
7. Yatsimirskii, K. B., "Kinetic Methods of Analysis," p. 39. Pergamon, New York, 1966.
8. Yatsimirskii, K. B., "Kinetic Methods of Analysis," p. 70. Pergamon, New York, 1966.
9. Yatsimirskii, K. B., "Kinetic Methods of Analysis," p. 33. Pergamon, New York, 1966.

MICROCHEMICAL JOURNAL 24, 33–39 (1979)

Intermolecular Interactions between the Mobile Phase Components and Isomeric Naphthols in a Thin-Layer Chromatography

JÓZEF ŚLIWIOK, ANTONINA MACIOSZCZYK, AND BOŻENA KÓCJAN

Institute of Chemistry, Silesian University, 40-006 Katowice, Poland

Received June 20, 1978

INTRODUCTION

An important role in the process of adsorption is played by intermolecular interactions between the layer surface, the examined substance molecules, and those of the mobile phase *(1)*. The following are consecutive stages of developing a chromatogram: (a) placing of a sample on an adsorbent—adsorption of a substance; (b) developing of a chromatogram—solvatation of a substance by means of solvent molecules; adsorption and desorption of the obtained system; and (c) stopping of the developing process, removal of the solvent molecules from the adsorbent surface and final adsorption of the examined substance. One of the factors particularly responsible for the R_f coefficient value with a given substance is quality and the scope of intermolecular interactions between the mobile phase components and this substance.

The purpose of our work was to test intermolecular interactions between the components of a mobile phase acetone–benzene and both isomeric naphthols in adsorption thin-layer chromatography.

EXPERIMENTAL

The ready-made glassplates (E. Merck, Darmstadt, West Germany) covered with silica gel G-60 (thickness of a layer, 0.25 mm) were activated at 110 ± 5°C for 0.5 hr. The 0.5% solutions of isomeric α- and β-naphthol in toluene were placed in amounts of 10 μl at the starting points, approximately 2 cm above the lower edge of a plate. The mobile phase was composed of benzene and acetone in different volume ratios. Each plate was developed 14 cm high. After drying chromatograms were visualized in the iodine vapors *(2)*. The obtained R_f and R_m coefficient values, depending upon the volume ratio of the mobile phase, are given for α- and β-naphthol in Table 1. The presented R_f values were mean values taken from 12 measurements, with the deviation equal ± 0.02 R_f units. In Fig. 1 dependence between the R_f coefficient values of the examined naphthols and the volume ratio of the mobile phase is shown. Simultaneously the

0026-265X/79/010033-07$01.00/0

TABLE 1
DEPENDENCE BETWEEN THE R_f AND R_m VALUES AND THE COMPOSITION OF THE MOBILE PHASE

Number	The benzene–acetone volume ratio	Dielectric constant of a mobile phase	α-Naphthol		β-Naphthol		
			R_f	R_m	R_f	R_m	ΔR_m
1	10:0	2.28	0.26	0.454	0.17	0.689	−0.235
2	9:1	3.80	0.54	−0.070	0.51	−0.017	−0.053
3	8:2	5.40	0.78	−0.550	0.73	−0.432	−0.118
4	6:4	9.00	0.83	−0.689	0.81	−0.630	−0.059
5	4:6	11.40	0.88	−0.865	0.87	−0.826	−0.039
6	2:8	15.30	0.92	−1.061	0.90	−0.954	−0.107
7	1:9	18.80	0.94	−1.195	0.92	−1.061	−0.134
8	0:10	21.20	0.96	−1.380	0.95	−1.279	−0.101

areas of the obtained chromatographic spots were calculated depending upon the changing volume ratio of benzene and acetone in the applied mobile phases. This dependence is given in Fig. 2. The chromatographic investigations proved that the chromatographic spot areas and the R_f coefficient values of the examined naphthols depend mainly upon the composition and the polarity of the mobile phase. To describe the polarity of the applied mobile phases their dielectric constants were measured. They were measured with the help of decameter, type OH-302 produced

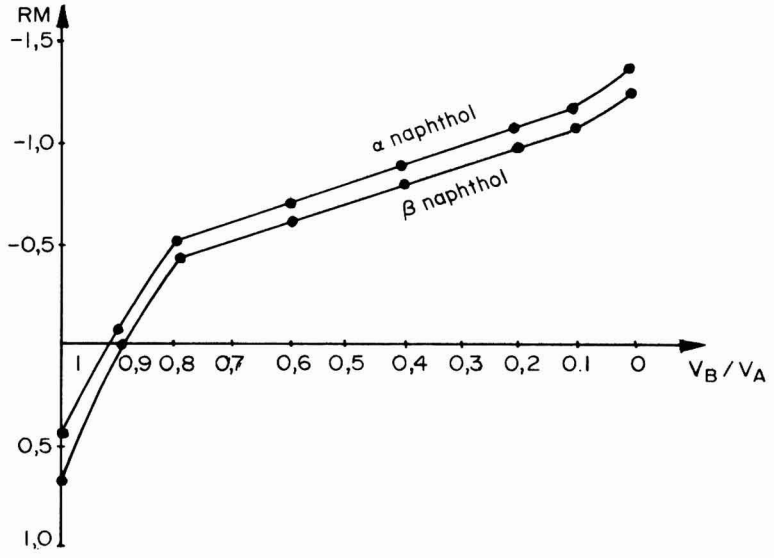


FIG. 1. The R_m coefficient values for α- and β-naphthol vs the volume composition of the benzene–acetone mobile phase (V_B/V_A).

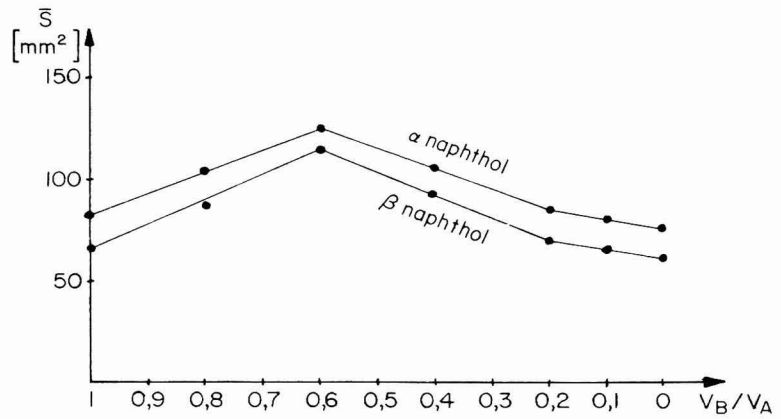


FIG. 2. The chromatographic spot surfaces of α- and β-naphthol vs the volume composition of the benzene–acetone mobile phase (V_B/V_A).

by Radelkis, Hungary. The measurements were performed at 22°C. From the equation $\epsilon_A = \epsilon_1 \cdot x_1 + \epsilon_2 \cdot x_2$ the additive dielectric constant values were calculated (where x_1 and x_2 = the molar fractions of both components of a system, and ϵ_1 and ϵ_2 = their dielectric constant values), and on this basis the deviations from additivity $\Delta\epsilon_A = \epsilon_{EXP} - \epsilon_A$. The obtained results are shown in Fig. 3 as dependences $\epsilon_{EXP} = f(V_B/V_A)$; $\epsilon_A = f(V_B/V_A)$; $\Delta\epsilon_A = f(V_B/V_A)$ for the benzene-acetone systems. As shown from the above presented data, the greatest deviations from additivity for the discussed benzene–acetone systems can be observed in the following ranges of volume ratios: 0.5 to 0.2 (V_B/V_A).

Then the influence of the isomeric α- and β-naphthol on the dielectric constant values has been established. For this purpose the 0.5% solutions of α- and β-naphthol in each mobile phase was prepared. The obtained results are given in Fig. 4. From the given data it can be seen that intermolecular interactions between the investigated naphthols on one hand and the components of the mobile phase on the other are to be traced starting from the volume ratio 0.4 of benzene.

Acetone owing to its carbonyl group has satisfactory conditions to interact with the -OH naphthol group through hydrogen bonds. This enabled us to run spectra of the examined naphthols in the mobile phases using the absorption IR spectroscopy technique. The series of the α- and β-naphthol solutions in benzene and acetone was prepared, preserving the same concentration of 0.5%. All the spectra were run using the UR-20 spectrophotometer (Carl Zeiss, Jena, East Germany). The following measuring conditions were applied: optical pathway, 0.58 mm; registration speed, 64 cm^{-1}/min; registration width, 20 mm/100 cm^{-1}.

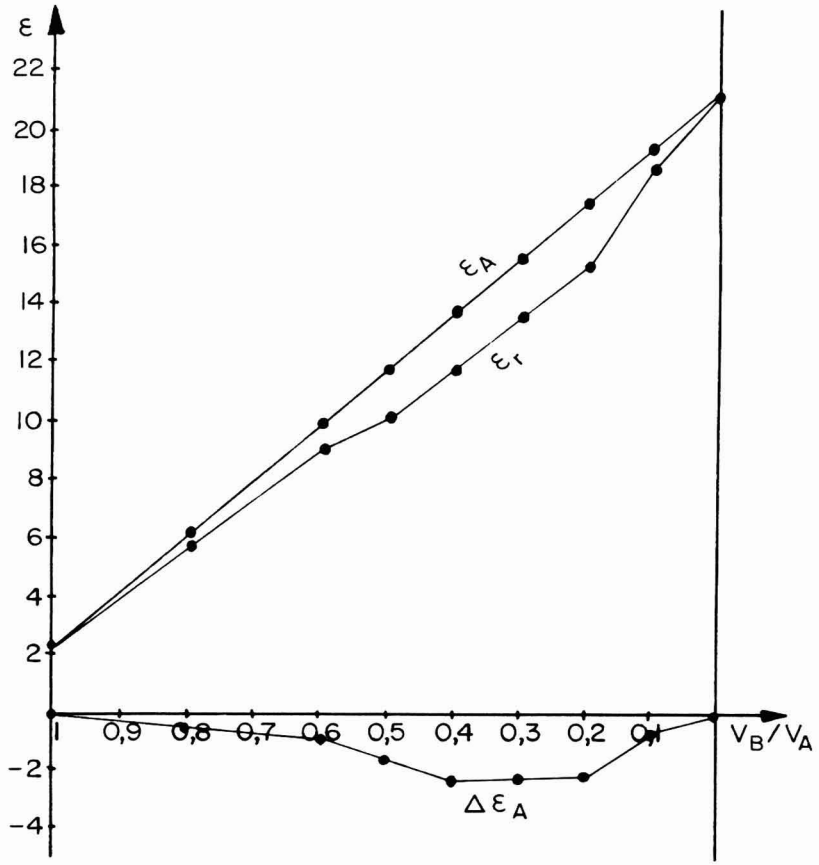


FIG. 3. The $\epsilon_{EXP} = f(V_B/V_A)$; $\epsilon_A = f(V_B/V_A)$; $\Delta\epsilon_A = f(V_B/V_A)$ dependences for the benzene–acetone systems.

For the benzene solutions of α- and β-naphthol the narrow valency absorption band was observed of the monomeric hydroxyl group. Its maximum was with α-naphthol, $\bar{\nu} = 3541$ cm^{-1}, and with β-naphthol, $\bar{\nu} = 3544$ cm^{-1}.

In the case of the acetone solutions of α- and β-naphthol the broad absorption bands were observed in a wide range from 3670 to 3100 cm^{-1}. The obtained spectra from both the benzene and the acetone solutions are given in Figs. 5 and 6. The broad absorption band with the acetone solutions of α- and β-naphthol is connected with the valency vibrations of the bonded hydroxyl groups. It can be explained with interactions between the "acidic" hydrogen atoms from the functional group of naphthols and the "basic" oxygen atoms from the functional group of acetone. At the

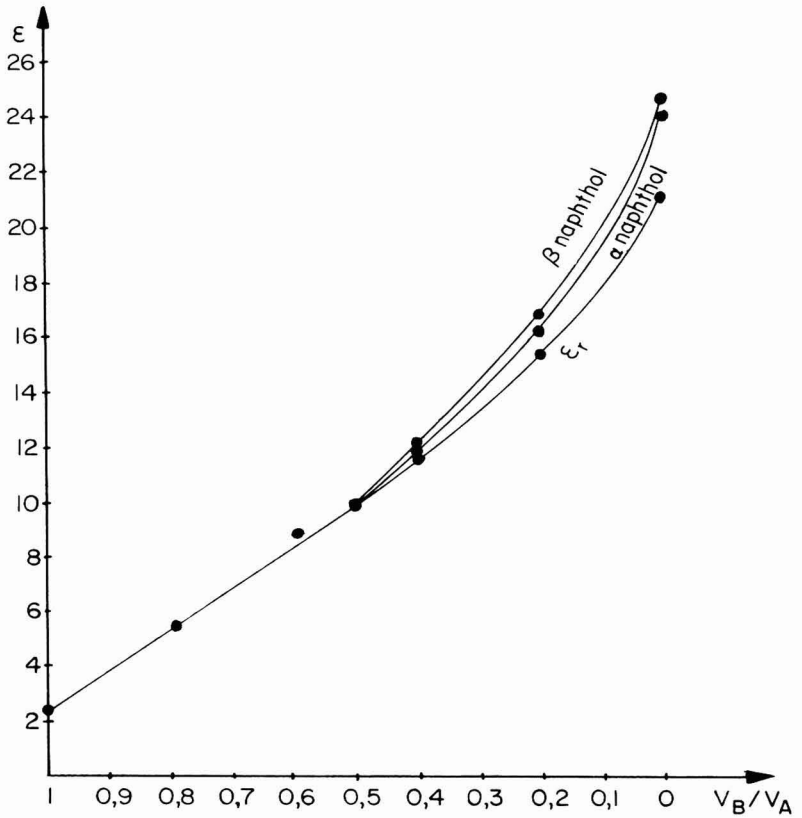


FIG. 4. The dielectric constant values for the α- and β-naphthol solutions in the applied benzene–acetone mobile phases.

broad absorption band due to the bonded naphthol hydroxyls a distinct absorption shoulder can be noticed, caused by the stretching vibrations of the nonbonded hydroxyls.

As seen from the comparison of both types of spectra, with the strongly diluted benzene solutions one observes the discussed naphthols almost exclusively in the monomeric form, while with the acetone solutions they seem to be bonded with acetone and only some rather insignificant proportion of the molecules appears as monomers.

The chromatographic investigations made it clear, that the R_m coefficient value of naphthols is significantly dependent on the composition of the mobile phase. In some defined range of the volume ratios of benzene and acetone the $R_m = f(V_B/V_A)$ dependence with both α- and β-naphthol tending toward linearity. Deviations from linearity are to be observed

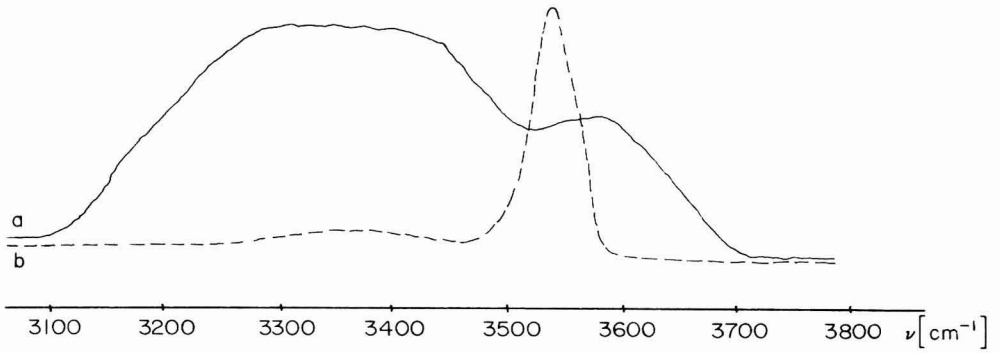


FIG. 5. The IR spectra of α-naphthol in the range 3800 to 3100 cm^{-1}: (a) in acetone (———, 0.2 *M*/liter); (b) in benzene (- - -, 0.2 *M*/liter). Optical pathway, 0.58 mm.

with the volume fractions of benzene (V_B) equal to 0.8 and 1, and with the volume fraction of acetone (V_A) equal to 1. The increasing concentration of acetone in a solution evokes association between the naphthol molecules and those of acetone. Change of the V_B/V_A ratio values means simultaneously the increasing polarity of a mobile phase compared with the obtained associative multimers. This situation is responsible for the occurrence of the new equilibria between the components of the mobile phase, the examined naphthols, and the adsorbent surface.

The chromatographic spot areas of α- and β-naphthol increase up to the benzene volume fraction equal to 0.6 and then succesively decrease. This fact is probably connected with the different behavior of associative multimers toward the quantitative changes of the mobile phase.

The R_m coefficient values show a rather slow but systematic decrease of adsorption with α- and β-naphthol. In the further course of investigations

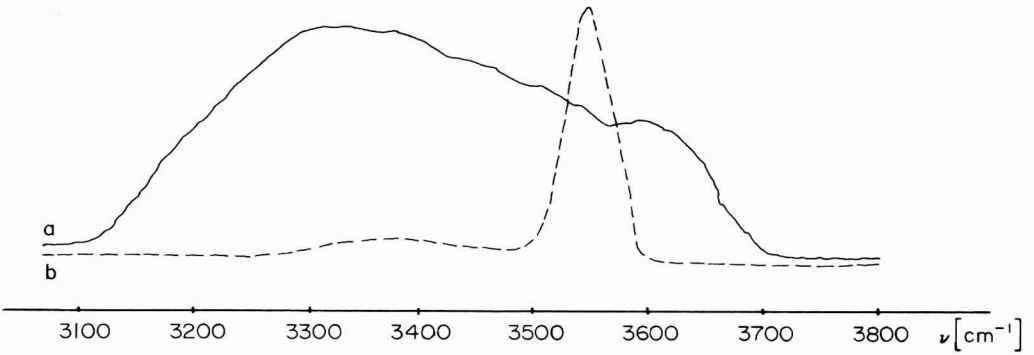
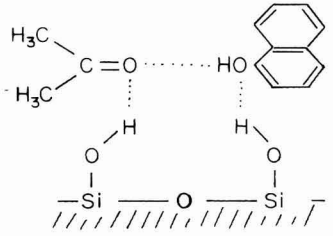


FIG. 6. The IR spectra of β-naphthol in the range 3800 to 3100 cm^{-1}: (a) in acetone (———, 0.2 *M*/liter); (b) in benzene (- - -, 0.2 *M*/liter). Optical pathway, 0.58 mm.

it has been established that the $R_m = f(V_A/V_B)$ functions for α- and β-naphthol in the entire range of concentrations have the parallel course. The α-naphthol R_m values are higher than those of the β-isomer. It is caused by the better adsorption of β-naphthol on silica gel.

On the basis of the performed chromatographic, dielectric, and IR measurements it can be stated that in the case of a mobile phase composed exclusively of benzene the investigated naphthols occur mainly as monomers. The α- and β-naphthol monomers should in such a case be adsorbed directly on silica gel, due to the high polarity of SiO_2. The mobile phase including acetone is responsible for forming the associative system acetone–naphthol, which most probably is going to be adsorbed on a layer according to the following scheme:



With the systematic increase of acetone concentration in the applied mobile phases the effectiveness of interactions between the molecules of naphthol and acetone also increased, which was demonstrated by measuring the dielectric constant values with systems composed of a given naphthol, acetone, and benzene.

The described method can help to qualitatively evaluate intermolecular interactions in chromatographic systems, and, more precisely, the interactions between mobile phase and the separated compounds.

SUMMARY

The purpose of our work was to investigate intermolecular interactions between the components of a mobile phase (acetone–benzene) and the isomeric naphthols. On the basis of chromatographic, dielectric, and IR measurements it was established that with the mobile phase composed exclusively of benzene the α- and β-naphthol occurred mainly as monomers and were directly adsorbed on silica gel throughout the whole chromatographic process. With the increase of acetone concentration in the applied mobile phases the effectivity of intermolecular interactions between the α- and β-naphthol molecules and those of acetone also increased. The described method can help give approximate evaluations of intermolecular interactions in the chromatographic systems composed of the mobile phase and separated substances.

REFERENCES

1. Ościk, J., "Adsorpcja" (= "Adsorption"). PWN, Warszawa, 1973.
2. Randerath, K., "Dünnschicht-Chromatographie." Verlag Chemie, Berlin, 1962.

MICROCHEMICAL JOURNAL 24, 40–45 (1979)

Spectrophotometric Determination of Vanadium(V) and Its Application to Vanadium Steel Containing Chromium, Molybdenum, Manganese, and Nickel

H. SANKE GOWDA AND B. NARAYANA ACHAR

Department of Post-graduate Studies and Research in Chemistry, University of Mysore, Manasa Gangotri, Mysore 570 006, India

Received March 18, 1977

INTRODUCTION

Most of the reagents proposed for the spectrophotometric determination of vanadium(V) are extractive–photometric reagents. The deficiencies of many of these reagents are narrow ranges of determination, lengthy procedures, and interference by common ions (*5*, *9*, *14*). For example, 4-(2-pyridylazo)resorcinol(*5*) was proposed as the most sensitive reagent in the narrow range of 0.04 to 1.0 ppm. The reagent absorbs strongly at the wavelength of the maximum absorption of the complex. In addition, it does not tolerate the presence of uranium(VI), niobium(V), titanium(IV), and zinc(II). 2-(3,5-Dibromo-2-pyridylazo)5-dimethylamino phenol(*9*) cannot be used in the presence of iron(III), chromium(III), copper(II), and titanium(IV). 4-Methoxy benzothiohydroxamic acid(*14*) is not satisfactory in the presence of niobium(V), molybdenum(VI), iron(III), and titanium(IV). In the present investigation the authors studied the reaction of promethazine hydrochloride (PH), 10-(2-dimethylaminopropyl)phenothiazine hydrochloride, with vanadium(V), and proposed PH as a sensitive reagent for the spectrophotometric determination of vanadium(V). The proposed method offers the advantages of simplicity, selectivity, high sensitivity, wider range of determination, and reasonable stability without the need for extraction or heating.

EXPERIMENTAL

Reagents

Vanadium(V) solution. A stock solution of vanadium(V) was prepared from sodium metavanadate in doubly distilled water and standardized against a standardized iron(II) solution. The stock solution was further diluted to give a standard solution of 25 μg/ml.

PH solution. A 0.2% aqueous solution of PH was prepared and stored in an amber bottle in a refrigerator.

0026-265X/79/010040-06$01.00/0

Diverse ions. Solutions of the diverse ions of suitable concentrations were prepared using analytical grade reagents.

Apparatus

Beckman Model DB spectrophotometer with 1-cm silica cells, from Beckman Instruments, Inc., Fullerton, Calif., was used for absorption measurements.

Standard Procedure

An aliquot of the stock solution containing 2.5 to 175 μg of vanadium(V), 12 ml of 13 *M* phosphoric acid, and 3 ml of 0.2% PH solution was taken in a 25-ml volumetric flask, and the solution was diluted to the mark with doubly distilled water. The solution was mixed well, and the absorbance was measured at 517 nm against a reagent blank prepared in the same manner. The amount of vanadium in the sample solution was then deduced from the standard calibration curve.

RESULTS AND DISCUSSION

PH is oxidized by vanadium(V) in sulfuric, hydrochloric, or phosphoric acid medium to a red-colored species believed to be a radical cation (*7, 8, 10*). The red color is not stable either in sulfuric or hydrochloric acid medium. The reagent gives full development of red color instantaneously at room temperature (27 ± 1°C) in 6.0–7.5 *M* phosphoric acid solutions as shown by the constancy of λ_{max}. The absorbance of the red-colored species remains constant for 35 min. The maximum color intensity is not observed below 6.0 *M* phosphoric acid. The reagent is slowly oxidized in a phosphoric acid concentration higher than 7.5 *M*. An acid strength of 6.5 *M* phosphoric acid has been chosen for all further work.

Spectral Characteristics

The red-colored species which appears to be an intermediate oxidation product of PH exhibits an absorption maximum at 516–518 nm (Fig. 1). The absorption spectra of the reagent and vanadium(V) under similar conditions show that they do not absorb around this wavelength, thus promoting an excellent analytical condition.

Effect of Reagent Concentration

The effect of reagent concentration has been examined by measuring the absorbance at 517 nm of solutions containing 2 μg of vanadium(V) and various amounts of PH. A 16-fold excess of the reagent is necessary for the full development of red color. Addition of more reagent has no effect on the absorbance reading.

Sequence of Addition of Reagents

There is no appreciable change in the absorbance if the order of addition of reactants is varied.

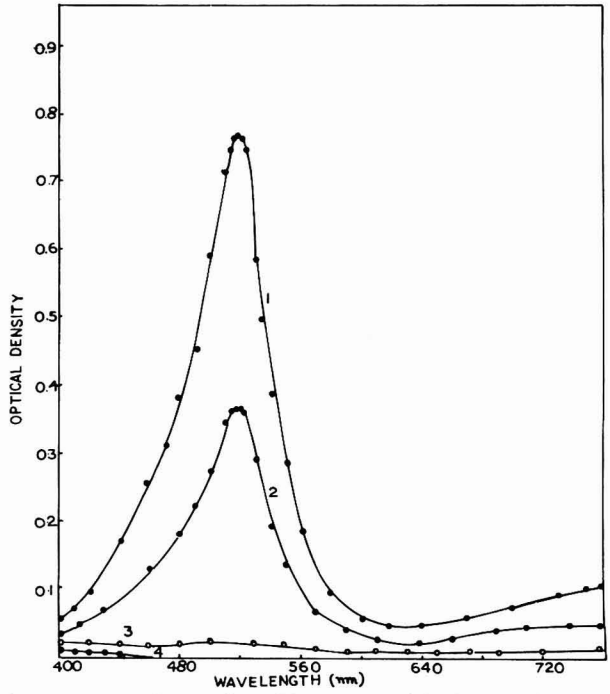


FIG. 1. Absorption spectra of vanadium(V), reagent blank, and the red-colored species in 6.5 *M* phosphoric acid. (1) Red-colored species vs reagent blank [concentration of V(V) = 4 μg/ml]. (2) Red-colored species vs reagent blank [concentration of V(V) = 2 μg/ml]. (3) Reagent blank vs water. (4) Vanadium vs water [concentration of V(V) = 4 μg/ml].

Effect of Temperature

The absorbance values are insensitive to temperature in the range of 10 to 65°C.

Adherence to Beer's Law and Optimum Concentration

Beer's law is obeyed in the range of 0.1 to 7.0 ppm of vanadium. The optimum concentration range for the effective spectrophotometric determination of vanadium by Ringbom's method (*2*, *12*) is 0.5 to 7.0 ppm.

Precision and Accuracy

The precision and accuracy of the method have been studied by analyzing solutions containing known amounts of vanadium. The results are presented in Table 1.

Sensitivity and Molar Absorptivity

For $\log (I_0/I) = 0.001$, the sensitivity of the reaction as calculated from Beer's law data is 0.005 μg cm^{-2}, and the molar absorptivity is 9.60×10^3 liter mol^{-1} cm^{-1} at 517 nm.

TABLE 1
PRECISION AND ACCURACY

Experiment	No. of determinations	Vanadium (V) (ppm)		Relative error (%)	SD (ppm)	Range (ppm)
		Taken	Found			
1	10	0.5	0.498	−0.4	0.003	0.010
2	10	1.2	1.201	+0.08	0.001	0.025
3	10	2.0	2.00	0.00	0.001	0.009
4	10	3.8	3.77	−0.8	0.002	0.011
5	10	5.0	5.05	+1.0	0.008	0.015
6	10	6.5	6.54	+0.63	0.007	0.008

PH is superior to the most sensitive reagents (*5*, *9*, *14*) in that it selectively reacts with vanadium(V) in the presence of large amounts of uranium(VI), titanium(IV), zinc(II), copper(II), iron(III), chromium(III), and molybdenum(VI) and enables very rapid determination of vanadium(V). It is more sensitive than *N*-phenyl-3-styryl-acrylohydroxamic acid (*4*), 2-naphthohydroxic acid (*3*), *m*-nitro-*N*-phenylbenzohydroxamic acid(*11*), *N*-*p*-tolyl-2-flurohydroxamic acid(*1*), Tropolene [2-hydroxy cyclohepta-2,4,6-trien-1-one](*13*), and 3-hydroxy-1,3-diphenyltriazin(*6*) which have been proposed as sensitive reagents.

Effect of Diverse Ions

To assess the possible analytical applications of this colored reaction, the effects of some ions which often accompany vanadium have been studied by adding different amounts to 2 μg/ml of vanadium in solution. The color is developed as outlined in the procedure. An error of 2% in the absorbance reading is considered tolerable. The following amounts (μg/ml) of foreign ions are found to give less than 2% error in the determination of 2 μg/ml of vanadium: Ni(II), 520; Cu(II), 260; Co(II), 100; Fe(III), 600; Fe(III), 3000 (in presence of 150 mg of citrate); Cd(II), 1600; Hg(II), 1000; Cu(II), 6800; Mg(II), 500; Be(II), 3200; Mn(II), 800; Al(III), 950; Mo(VI), 1500; W(VI), 80; U(VI), 920; Zr(IV), 820; Zn(II), 800; Sn(IV), 5000; V(IV), 1000; Cr(III), 150; fluoride, 7200; chloride, 2800; bromide, 3800; iodide, 0.2; nitrate, 3500; sulfate, 15000; thiosulfate, 0.5; oxalate, 5000; tartrate, 6000; citrate, 6000; and EDTA, 450. Cations such as Cr(VI), Ce(IV), and Fe(II) interfere in all concentrations. The major advantage is that iron(III) which is associated with vanadium steel is tolerated up to 3000 ppm in the presence of phosphoric acid and citrate ions.

Analysis of Vanadium Steel

The composition of vanadium steel supplied by Guest Keen Williams Ltd., Howrah, is 0.54% C, 0.89% Mn, 0.018% S, 0.034% P, 0.33% Si,

1.02% Cr, and 0.13% V. The vanadium steel, heat No. 6-4828, 58 CrV_4, supplied by Hindustan Steel Ltd., Durgapur, has the composition of 0.62% C, 0.86% Mn, 0.033% P, 0.011% S, 0.29% Si, 1.12% Cr, 0.12% Mo, 0.14% Ni, 0.017% Al, and 0.09% V. The two samples were analyzed for vanadium by the method described below.

Procedure. About 0.5 g of vanadium steel was accurately weighed into a covered 250-ml beaker and treated with 15 ml of 10 *N* sulfuric acid, 2 ml of syrupy phosphoric acid (sp.gr., 1.75), and 1 ml of concentrated nitric acid. When the initial brisk reaction had subsided, the solution was boiled for 2 to 4 min to expel the oxides of nitrogen. The solution was cooled and diluted to about 50 ml with doubly distilled water. A 0.1 *N* potassium permanganate solution was added dropwise until the solution just appeared pale pink. The solution was left aside for about 5 min, and 0.01 *N* oxalic acid solution was added slowly with stirring until the pink color of the solution disappeared. The solution was transferred into a 100-ml volumetric flask and diluted to the mark with doubly distilled water. A suitable aliquot portion of the solution and 150 mg of sodium citrate were taken in a 25-ml volumetric flask. The solution was then treated, and absorbance was measured as in the standard procedure.

The precision and accuracy of the method have been studied by analyzing (six times) the two sample steels. The results are presented in Table 2.

ACKNOWLEDGMENTS

Authors are grateful to May and Baker (India) Private Ltd., Bombay, for supply of pure PH as free sample. B.N.A. thanks the University of Mysore for the research grant.

SUMMARY

Promethazine hydrochloride forms a red colored species with vanadium(V) in 6.0–7.5 *M* phosphoric acid. A 16-fold molar excess of the reagent is necessary for full development of color intensity. Beer's law is valid over the concentration range of 0.1–7.0 ppm. The optimum concentration range as evaluated by Ringbom's method is 0.5–7.0 ppm. The

TABLE 2
DETERMINATION OF VANADIUM IN VANADIUM STEEL

Steel sample	No. of determinations	Certified value of vanadium (%)	Vanadium found (%)	Relative error (%)	SD (%)
Heat No. 6-4828, 58 CrV_4	6	0.090	0.091	+1.1	0.002
Vanadium steel, Guest Keen Williams Ltd.	6	0.130	0.132	+1.5	0.0018

sensitivity of the reaction is 0.005 μg cm^{-2} and the molar absorptivity is 9.60×10^3 liter mol^{-1} cm^{-1} at 517 nm. The effects of acidity, time, temperature, order of addition of reagents, reagent concentration, and the interferences from various ions were reported. Vanadium in vanadium steel containing chromium, molybdenum, manganese, and nickel was determined.

REFERENCES

1. Agarwal, Y. K., *N*-*p*-Tolyl-2-flurohydroxamic acid as extracting and spectrophotometric reagent for vanadium(V). *Anal. Lett.* **7**, 729–741 (1974).
2. Ayres, G. H., Evaluation of accuracy in photometric analysis. *Anal. Chem.* **21**, 652–657 (1949).
3. Bass, V. C., and Yoe, J. H., Spectrophotometric determination of vanadium(V) with 2-naphthohydroxamic acid. *Anal. Chim. Acta* **35**, 337–344 (1966).
4. Bhura, D. C., and Tandon, S. C., Unsaturated *N*-arylhydroxamic acids as colorimetric reagents for vanadium(V). Spectrophotometric determination with *N*-phenyl 3-styryl-acrylohydroxamic acid. *Anal. Chim. Acta* **53**, 379–386 (1971).
5. Budevsky, O., and Johnova, L., Colorimetric determination of vanadium(V) with 4-(2-pyridylazo)resorcinol. *Talanta* **12**, 291–295 (1965).
6. Chakraborti, D., 3-Hydroxy-1,3-diphenyltriazen and its substituted derivatives as spectrophotometric reagents for vanadium(V). *Anal. Chim. Acta* **71**, 196–201 (1974).
7. Dwivedi, P. C., Gurudath Rao, S. N., Bhat, S. N., and Rao, C. N. R., Spectrophotometric studies of electron donor properties and radical cations of phenothiazine derivatives. *Spectrochim. Acta* **31A**, 129–135 (1975).
8. Forrest, I. S., Forrest, F. M., and Berger, M., Free radicals as metabolites of drugs derived from phenothiazine. *Biochim. Biophys. Acta* **29**, 441–442 (1958).
9. Gusev, S. I., Poplevina, L. V., and Shalamova, G. G., 2-(3,5-Dibromo-2-pyridylazo)-5-diethyl-aminophenol as a reagent for antimony(III) and vanadium(V). *Uch. Zap. Perm. Gos. Univ.* **178**, 214–218 (1968).
10. Katritzky, A. R., and Boulton, A. J. (Eds.), "Recent Advances in the Chemistry of Phenothiazines—Advances in Heterocyclic Chemistry," Vol. 9, p. 364. Academic Press, New York, 1968.
11. Nripendra Nath Ghosh and Gautam Siddhanta, Extraction–photometric determination of vanadium(V) with *M*-nitro-*N*-phenylbenzohydroxamic acid. *Z. Anal. Chem.* **253**, 207–208 (1971).
12. Ringbom, A., Accuracy of colorimetric determinations. *Z. Anal. Chem.* **115**, 332–343 (1939).
13. Rizvi, G. H., and Singh, R. P., Tropolone [2-hydroxycyclohepta-2,4,6-trien-1-one] as a specific reagent for the determination of vanadium(V). *Talanta* **19**, 1198–1201 (1972).
14. Skorko-Trylula, Z., Spectrophotometric determination of vanadium by means of 4-methoxybenzo thiohydroxamic acid. *Chemia Anal.* **10**, 831–836 (1965).

MICROCHEMICAL JOURNAL 24, 46–49 (1979)

Correlation of NMR and UV Results to Evaluate the Stability Constants of Carbon Tetrachloride Complexes

H. MODARESS AND M. MOHAMMADI

Department of Chemistry, Tehran Polytechnic, Tehran, Iran

Received July 3, 1978

INTRODUCTION

Inconsistent values of stability constants K^{AD} have been reported for the complexes of CCl_4 and hexamethylbenzene by different workers. Weimer and Prausnitz (*17*) obtained the value of 0.64 liter mol^{-1}. While for the same system, a smaller value (0.02 liter mol^{-1}) has been reported by Doerr and Buttgereit (*5*). Rosseinsky and Kellawi (*15*) have obtained a value (0.55 liter mol^{-1}) close to that of Weimer and Prausnitz. The reported K^{AD} values for this system (0.64 and 0.55 liter mol^{-1}) are large compared to the systems where CCl_4 has formed complexes with other aromatic compounds (*3*). Deranleau (*4*) and Person (*13*) have emphasized a concentration region which should be regarded to obtain accurate values of stability constants from spectroscopic measurements. In CCl_4, hexamethylbenzene system the concentrations used were not sufficiently high and thus their accuracy are open to question. Shortcomings have also been noted in the equations used to evaluate the K^{AD} from the spectroscopic measurements (*7*). Thus the disagreement in the reported results could partly be due to erroneous evaluation of K^{AD} from these equations.

Choosing the concentrations in the proper region, we studied the system of hexamethylbenzene, CCl_4 by UV and NMR spectroscopy. An equation was derived to correlate the NMR and UV results and to calculate the K^{AD}.

EXPERIMENTAL AND RESULTS

The solution preparation and experimental procedure in NMR measurements have been described in an earlier work (*12*). In this work the proton chemical shift (δ) of hexamethylbenzene was measured against TMS and *n*-hexane as the internal references for solutions of different concentrations of CCl_4 in *n*-hexane. The results are reported in Table 1.

UV measurements were done with a Pye Unicam sp 1700 spectrophotometer. The spectra of the solutions showed evidences of complex

0026-265X/79/010046-04$01.00/0

TABLE 1
NMR AND UV RESULTS FOR HEXAMETHYLBENZENE (A), CCl_4, n-HEXANE SYSTEM

Concn (mol liter^{-1})		Absorbance	Chemical shift (Hz)	
A	D	(λ = 243 nm)	TMS (INT)	n-hexane (INT)
0.025	0.0	0.0	216.321	640.631
	0.025	0.105	216.451	642.539
	0.051	0.153	216.568	644.331
	0.093	0.235	216.678	646.592
	0.116	0.284	216.787	647.791
	0.154	0.325	216.929	646.532
	0.181	0.380	217.018	650.905
	0.220	0.405	217.132	652.333
	0.281	0.475	217.313	654.681
	0.345	0.492	217.416	657.146
	0.395	0.551	217.559	658.831
	$\epsilon^{A}_{243} = 4.440$		$\epsilon^{D}_{243} = 2.825$	

formation around the wavelength 243 nm. The absorbance measurements were done at this wavelength. The results are reported in Table 1.

DISCUSSION

For the complexing reaction of the form

$$A + D \rightleftharpoons AD$$

the measured chemical shift (δ) is the population average of the shift due to pure A (δ_A) and the shift of pure complex AD (δ_{AD}) in all solutions according to the following equation (*8*).

$$\delta = \frac{[A]_0 - [AD]}{[A]_0}\delta_A + \frac{[AD]}{[A]_0}\delta_{AD}, \tag{1}$$

where the symbols A, D, and AD were used to represent hexamethylbenzene, CCl_4, and complex, respectively. The symbols in the square brackets are the concentrations which are related to the stability constant K^{AD} by the equilibrium expression:

$$K^{AD} = \frac{[AD]}{([D]_0 - [AD])([A]_0 - [AD])}. \tag{2}$$

The measured absorbance (A) of the solutions is also related to the concentrations of A, D, and AD in the following manner (*14*):

$$A = \epsilon_\lambda^A l([A]_0 - [AD]) + \epsilon_\lambda^D l([D]_0 - [AD]) + \epsilon_\lambda^{AD} l[AD] \quad (3)$$

where the ϵs are the molar absorbancies and l is the cell length.

For the cases where $[D]_0 >> [A]_0$ from a combination of Eqs. (1) and (2), for treatment of NMR results, or Eq. (2) and more simplified form of Eq. (3) for treatment of UV results, various equations have been derived (*6*). These equations, which are based on the simple linear relationships, have been used to evaluate K^{AD}. The results have shown that K^{AD}'s obtained from NMR and UV did not agree with each other. It seems in most cases the disagreement is centered around the problem of separating the products of K^{AD} ϵ_λ^{AD} in UV measurements and $K^{AD}(\delta_A - \delta_{AD})$ in the NMR measurements (*7*).

To avoid this problem in the system we studied, an equation was derived by substituting for $[AD]_0$ from Eqs. (1) and (3) in Eq. (2) to obtain:

$$[A]_0 + [D]_0 - \frac{A - \epsilon^D l[D] \cdot - \epsilon^A l[A] \cdot}{\epsilon_\lambda^{AD} l\ \epsilon_\lambda^D l\ \epsilon_\lambda^A l} = \Delta_{AD}\left(\frac{[D]_0 \cdot}{\Delta}\right) - \frac{1}{\bar{K}^{AD}}, \quad (4)$$

where $\Delta = \delta_A - \delta$ and Δ_{AD} $\delta_A - \delta_{AD}$. According to Eq. (4), a plot of the left-hand side of this equation against the values of $[D]_0/\Delta$ should be linear and K^{AD} and Δ_{AD} are evaluated from intercept and slope, respectively. Use of Eq. (4) has the following advantages: (i) it is not necessary that $[D]_0 >> [A]_0$ and can be used under any condition, and (ii) K^{AD} can be calculated from it, directly without recourse to extrapolation to infinite concentration.

To treat the results, a computer program was used which calculated the left-hand side of Eq. (4) for insert values of ϵ_λ^{AD} and then they were examined in Eq. (4) to find the best straight line. Table 2 shows excellent agreement between the K^{AD}s for the two reference.

In our calculations of K^{AD} the molarity concentration unit was used. A great deal of work has been done to examine which concentration unit is correct for the spectroscopic studies of the stability constants (*9–11, 16*). Theoretically it can be suggested that molarity is a correct concentration unit. Since from the theory of electronic and vibrational absorption (*1*), and kinetic considerations of chemical equilibrial (*18*), it seems that the

TABLE 2
STABILITY CONSTANTS (K^{AD}), EVALUATED FOR THE HEXAMETHYLBENZENE, CCl_4, *n*-HEXANE

	TMS (INT)	*n*-hexane (INT)
K^{AD} (liters mol^{-1})	0.412	0.424

most appropriate unit for expressing the concentrations are molarities. In NMR studies also, the way in which Eq. (1) originates suggests that molarities are the correct units (*2*).

SUMMARY

An equation has been suggested which correlate the NMR and UV results to evaluate the stability constant of electron donor acceptor complexes. Using this equation the stability constant of complexation between carbon tetrachloride and hexamethylbenzene has been studied by UV and NMR spectroscopy.

REFERENCES

1. Barrow, G. M., "Introduction to Molecular Spectroscopy," p. 71. McGraw–Hill, New York, 1962.
2. Bovey, F. A., "Nuclear Magnetic Resonance Spectroscopy," p. 184. Academic Press, New York, 1969.
3. Davis, K. M. C., and Farmer, M. F., Charge-transfer complexes. Part II. Complex formation between halogenomethanes and aromatic amines. *J. Chem. Soc. (B)*, 28–32 (1967).
4. Deraneau, D. A., Theory of the measurements of week molecular complexes. I. General considerations. *J. Amer. Chem. Soc.* **91**, 4044–4049 (1969).
5. Doerr, F., and Buttgereit, G., Loose electron transfer compounds of hexamethylbenzene with halogen containing solvents. *Ber. Bunsenges. Physik. Chem.* **67** (9), 867–887 (1963).
6. Foster, R., "Organic Charge-Transfer Complexes," pp. 128–147. Academic Press, New York, 1970.
7. Foster, R., and Fyfe, C., *In* "Progress in NMR Spectroscopy" (J. M. Emsley, J. Feeney, and L. M. Sutcliffe, eds.), p. 78. Pergamon Press, New York. 1969.
8. Gutowsky, H. S., and McCall, D. W., Nuclear magnetic resonance multiplets in liquids. *J. Chem. Phys.* **21**(2), 279–292 (1953).
9. Hanna, M. W., and Rose, D. G., Effect of donor nonidelity on molecular complex equilibrium. *J. Amer. Chem. Soc.* **94**, 2601–2605 (1972).
10. Homer, J., Everdell, M. H., Jackson, C. J., and Whitney, P. M., Some comments on the processing data obtained from nuclear magnetic resonance studies. *Trans. Faraday Soc.*, 874–884 (1972).
11. Kuntz, I. D., Jr., Gasparro, F. D., Johnston, M. D., Jr., and Taylor, R. P., Molecular interactions and the Benesi–Hidebrand equation. *J. Amer. Chem. Soc.* **90**, 4778–4781 (1968).
12. Modaress, H., and Edrissi, M., NMR measurements of stability constants for complexes of carbon tetrachloride and some aromatic compounds. *Microchem. J.* **22**(4), 451–456 (1977).
13. Person, W. B., A criterion of reliability of formation constants of weak complexes. *J. Amer. Chem. Soc.* **87**, 167–170 (1965).
14. Rose, N. J., and Drago, R. S., Molecular addition of compounds of iodine. I. An absolute method for the spectroscopic determination of quilibrium constants. *J. Amer. Chem. Soc.* **81**, 6138–6141 (1959).
15. Rosseinsky, D. R., and Kellawi, H., A statistical assessment of association constants. *J. Chem. Soc. (A)*, 1207–1211 (1969).
16. Slejko, F. L., and Drago, R. S., On making corrections for donor nonideality on molecular complex equilibria. *J. Amer. Chem. Soc.* **94**, 6546–6548 (1969).
17. Weimer, R. F., and Prausnitz, J. M., Complex formation between carbon tetrachloride and aromatic hydrocarbons. *J. Chem. Phys.* **42**, 3643–3644 (1965).
18. Westion, R. E., Jr., and Schwartz, H. A., "Chemical Kinetics," p. 71. McGraw–Hill, New York, 1962.

MICROCHEMICAL JOURNAL 24, 50–55 (1979)

Determination of α-Amylase Activity from Cotton Leaves with Amylopectin-Azure

CHONG W. CHANG

Federal Research, Science and Education Administration, USDA, Western Cotton Research Laboratory, Phoenix, Arizona 85040

Received August 8, 1978

INTRODUCTION

In our laboratory, determinations of α-amylase activity in crude enzyme preparations from numerous small leaf samples were needed. The method of Abbott and Matheson (*1*) for determining α-amylase activity from tobacco leaves was tested with cotton leaves and rejected. The amount of residual starch unaffected by the enzyme was determined by the procedure of Shuster and Gifford (*8*). Components in the crude enzyme preparation interfered with the formation of a complex between iodine and the substrate polymer. As a second trial, the phenolics were separated from the cotton leaf homogenate by use of a column of Bio-gel P-60[1] eluted with borate buffer (0.2 *M*, pH 7.6). The activity of enzyme without phenolics was assayed in the presence of calcium acetate (0.2 *M*). Calcium is known to activate α-anylase activity (*3*). The amount of polymer hydrolyzed by the enzyme was determined in a way similar to the previous experiments. Under these conditions, α-amylase activity could not be detected from 5 g of fresh cotton leaves (the top leaves of a plant 60 days old). A substrate which was specific for the enzyme and also sensitive enough to detect the low activity of α-amylase was needed to overcome these difficulties.

Amylopectin-azure (a product of Calbiochem[1]) is a bona fide substrate for amylase. The blue dye, azure, is covalently labeled with the primary hydroxyl group of the polymer. This dye is not released by β-amylase or phosphorylase, but is specifically freed by the enzymatic action of α-amylase (*5*). The use of amylopectin-azure for determining the low activity of enzyme from phenolic-rich plant tissues, e.g., cotton leaves (*6*), has not been reported.

The purposes of this paper are: (a) to present the procedures for determining α-amylase activity with amylopectin-azure; (b) to demonstrate a successful measurement of the activity with a minute amount of protein (about 25 μg); and (c) to present evidence that interfering substances in

[1] Mention of a specific trade name is made for identification only and does not imply endorsement by the United States Department of Agriculture.

0026-265X/79/010050-06$01.00/0

the crude preparation did not influence enzyme activity due to substrate specificity.

MATERIALS AND METHODS

Glandless cotton plants (*Gossypium hirsutum* L., cv. Coker 100) were raised as previously described (*4*).

Reagents

Sodium chloride, 0.02 *M*.
Calcium chloride, 0.1 *M*.
Ammonium sulfate, powder.
Calcium acetate buffer, 0.05 *M*, pH 5.2.
Mercaptoethanol.
Amylopectin-azure.
Sodium phosphate buffer, 0.02 *M*, pH 7.0.

Procedure

(a) Preparation of crude enzyme solution. Weigh 5 to 7 g of fresh cotton leaves and cut into small pieces of about 1 cm^2. Homogenize the material in 0.02 *M* NaCl solution (3 ml/g fresh weight) for 3 min at 35% of full speed by use of a Sorvall mixer.[1] Strain the homogenate through two layers of cheesecloth. Centrifuge the filtrate at 37,000*g* for 10 min. Add 0.1 *M* $CaCl_2$ to the decanted supernatant (1 ml of $CaCl_2$/9 ml). Adjust the pH of the

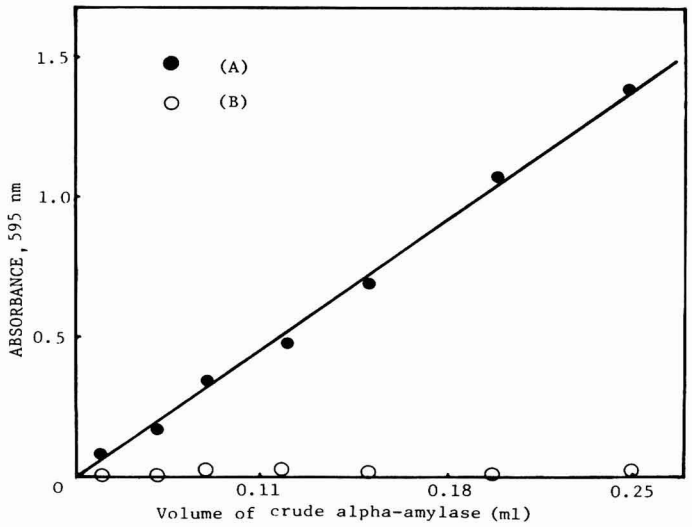


FIG. 1. (A) Effect of partially purified enzyme concentration on the linearity of α-amylase activity in the presence of amylopectin-azure. Enzyme assay conditions were the same as those described in the text. (B) Effect of partially purified enzyme concentration on the activity determined in the presence of soluble starch by the procedure of Shuster and Gifford (*8*).

mixture to 7.0 with 0.5 *M* NaOH. Heat the samples at 70°C for 20 min in a water bath, then cool in ice for 10 min (*1*). Centrifuge the mixture at 12,000*g* for 20 min. Lyophilize the decanted supernatant. Dissolve the powdered sample in about 5 ml of water. Precipitate protein by the addition of one volume of saturated ammonium sulfate. After centrifugation, dissolve the residues in 2 to 3 ml of 0.1 *M* $CaCl_2$. Dialyze the partially purified enzyme sample overnight against a solution composed of 0.05 *M* calcium acetate at pH 5.2 containing mercaptoethanol (10 drops/liter). Centrifuge the dialysate (27,000*g* for 7 min), and use the supernatant for the enzyme assays.

(b) Assay for α-amylase activity. Weigh 30 mg of amylopectin-azure into a container. Add 1 ml of 0.02 *M* sodium phosphate buffer, pH7, containing 0.015 *M* NaC1(7). Add also 1 ml of crude enzyme preparation. Stir the mixture 10 times only (5 sec) with a glass rod for a uniform suspension. Place the container of assay suspension in a shaker at 37°C. Shake for 10 min. Add 0.5 ml of concentrated glacial acetic acid to stop enzyme action. Centrifuge the mixture (12,000*g* for 10 min). Pipet out the small volume of supernatant. Read the blue color intensity of the sample at 595 nm after 5 min. Blank is prepared similarly but with dialysis solution instead of enzyme. Enzyme activity is expressed by units of optical density for absorbance at 595 nm per 10 min per mg protein.

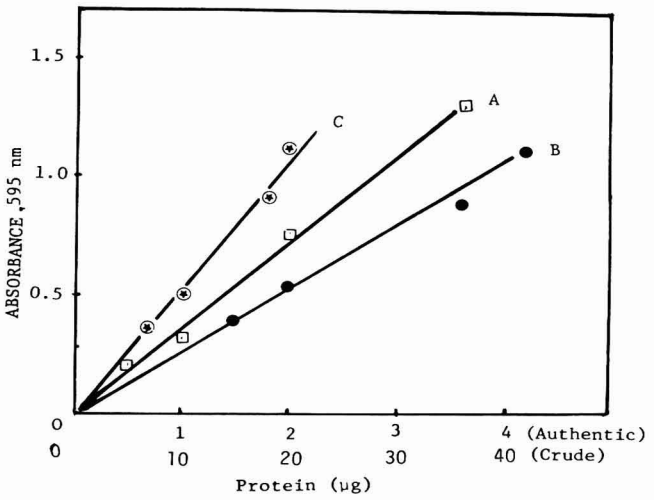


FIG. 2. Effects of crude α-amylase activity on purified authentic α-amylase (Calbiochem[1]). (A) Activity of purified authentic α-amylase; (B) activity of crude enzyme from cotton leaves; (C) activity of the mixture prepared with the equal parts of (A) and (B). Assay conditions were the same as those described in the text.

RESULTS AND DISCUSSION

As shown in Fig. 1, the activities of various crude enzyme dilutions were found to be linear up to the level of about 1.3 optical density units for absorbance at 595 nm (A). In these experiments, each volume of sample contained not only increasing amounts of enzyme, but also increasing amounts of interfering substances. The latter substances did not influence the linearity by various enzyme concentrations. Such a linearity was achieved by the substrate specificity in the presence of interfering substances. This was evidenced by the fact that the assay with similar crude enzyme preparations in the presence of soluble starch by the procedure of Shuster and Gifford (*8*) failed to detect the activity (B). This indicates that the enzyme preparations contained some interfering substances which were firmly associated with the final samples and inhibited the formation of a complex between the residual substrate and iodine, although the enzyme preparations were partially purified.

As shown in Fig. 2, addition of the crude enzyme preparation to the purified authentic α-amylase (Calbiochem) resulted in a curve of enzyme activity that was almost additive and linear. The data demonstrate that the crude preparation did not seriously influence the enzyme activity of pure enzyme.

As shown in Fig. 3, the proposed procedures successfully determined activities of the crude enzyme protein (a minimum of about 25μg) from 5 g at each of the various leaf levels of plants 60 days old. However, the assay procedures by Shuster and Gifford (*8*) detected only one-half the magnitude of activity by this proposed method and therefore failed to determine the activities for the top five leaf levels.

The level of calcium required for maximum enzyme stability was ap-

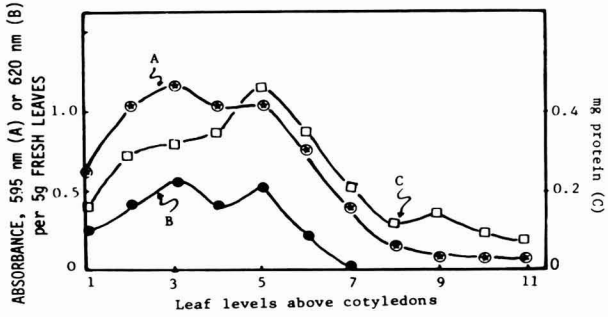


FIG. 3. Distributions of α-amylase activity and protein in various leaf levels of cotton plants 60 days old. (A) Activities by the proposed procedures; (B) activities assayed by the procedures of Shuster and Gifford (*8*) after phenolics were removed; (C) protein levels determined from each assay volume by the procedure of Bio-Rad[1] Technical Bulletin 1051 (*2*). Assays for (A) or (B) were conducted with similar volumes of 2 ml.

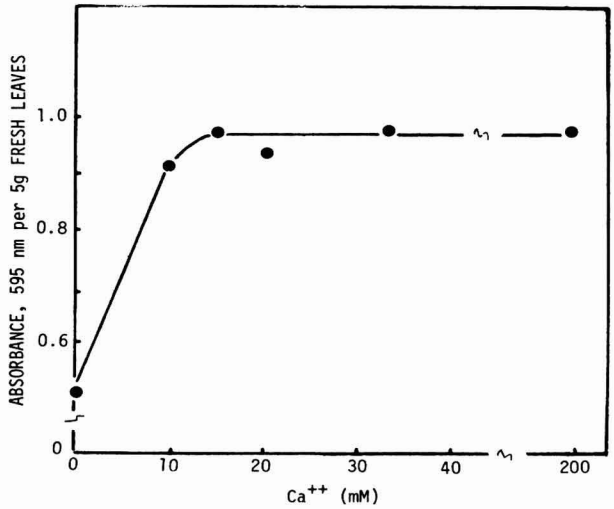


FIG. 4. Effects of various calcium concentrations on α-amylase activity. Activities were determined from 5 g of fresh cotton leaves according to the procedure described in the text. Calcium chloride was added to the supernatant after centrifuging the leaf homogenate in 0.02 *M* sodium chloride solution.

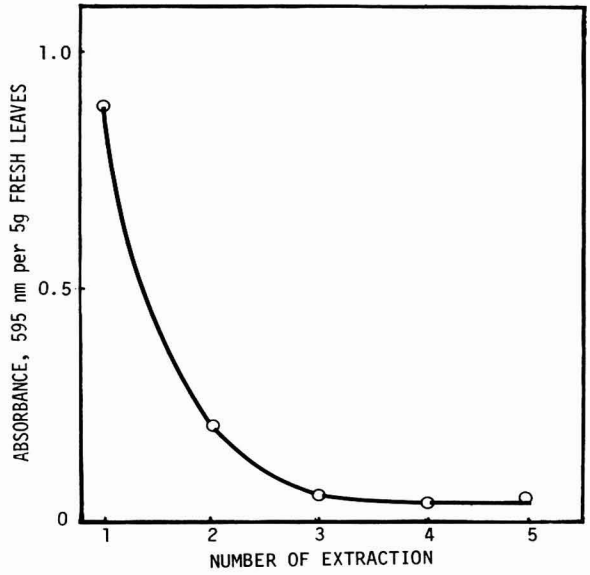


FIG. 5. Extractibility of enzyme protein from cotton leaves. Total protein was extracted from 5 g of fresh cotton leaves in 0.02 *M* sodium chloride solution. Extractions were performed until the activity was constant.

proximately 15 m*M* (Fig. 4). At least three extractions with sodium chloride (0.05 *M*) solution were required to remove the maximum amount of enzyme protein (Fig. 5).

The present procedure overcomes the interferences which are firmly associated with the partially purified enzyme preparations, due to the substrate specificity for α-amylase. This procedure can also determine activities from many small leaf samples with a high sensitivity. Application of this method to other leaf materials would not be difficult since cotton leaves are known to contain higher polyphenolics than most of the other plant sources (*6*).

SUMMARY

α-Amylase activity from phenolic-rich plant tissues is successfully determined with an amylopectin-azure substrate. This procedure, due to the substrate's specificity for α-amylase, overcomes the effects of the interfering substances firmly associated with enzyme preparations. The procedure is more sensitive than the assay for activity with iodine solution.

REFERENCES

1. Abbot, I. R., and Matheson, N. K., Starch depletion in germinating wheat, wrinkled-seeded peas and senescing tobacco leaves. *Phytochemistry* **11**, 1261–1272 (1972).
2. Bio-Rad Protein Assay, Bio-Rad Laboratories, Technical Bulletin 1051 (1977).
3. Briggs, M. A., Alpha-amylase in germinating, decorticated barley—I. Alpha-amylase, conditions of growth, and grain constituents. *Phytochemistry* **7**, 513–529 (1968).
4. Chang, C. W., Activation energy for thermal inactivation and Km of carbonic anhydrase from the cotton plant. *Plant Sci. Lett.* **4**, 109–113 (1975).
5. Juliano, B. O., and Varner, J. E., Enzymic degradation of starch granules in the cotyledons of germinating peas. *Plant Physiol.* **41**, 886–892 (1969).
6. King, E. E., Extraction of cotton leaf enzymes with borate. *Phytochemistry* **10**, 2337–2341 (1971).
7. Rinderknecht, H., and Haverback, B. J., A new method for the determination of alpha-amylase. *Experimentia* **23**, 805 (1967).
8. Shuster, L., and Gifford, R. H., Changes in 3′-nucleotidase during the germination of wheat embryos. *Arch. Biochem. Biophys.* **96**, 534–540 (1962).

MICROCHEMICAL JOURNAL 24, 56–61 (1979)

Measurement of Total Protein in Urine: Comparison of Two Dye-Binding Procedures with a Gel Filtration/Modified Biuret Method

RICHARD R. SWAIN AND STEPHEN L. BRIGGS

Lilly Laboratory for Clinical Research, Wishard Memorial Hospital, Indianapolis, Indiana 46202

Received August 7, 1978

INTRODUCTION

Doetsch and Gadsden have described a method for the determination of urinary total protein which is claimed to be both specific and sensitive (*3*, *4*). This procedure requires two gel filtration steps: a preliminary one performed on the urine sample itself and a second one at a later stage to remove excess copper ions. Eventually, copper ions which have been complexed by peptide bonds are measured with the use of diethyldithiocarbamate. Subsequent to the publication of this method, two other urinary total protein procedures have been described which are based on the dye-binding properties of proteins (*7*, *8*). The method of Meola *et al.* (*8*) involves the adsorption of urinary protein onto cellulose powder followed by measurement of the amount of Ponceau S dye which is bound by the adsorbed protein. The method described by McIntosh (*7*) is based on that originally described by Bradford (*1*) and depends on the shift of the wavelength of adsorption which occurs when Coomassie Brilliant Blue G250 binds to protein. Both the cellulose-dye (CD) method and the Coomassie blue (CB) method are more amenable to routine use than the gel filtration/modified biuret (GFB) procedure. In theory, the GFB determination should be more accurate than the dye-binding methods since peptide linkages are being measured rather than the variable content of basic amino acid residues; and, indeed, this procedure has been published as a "Selected Method" (*3*). We were interested in assessing the accuracy of the two dye-binding methods by comparing the values generated by them with those given by the GFB procedure. We also wished to determine the relative freedom of all three procedures from some commonly encountered interferences.

MATERIALS AND METHODS

All three urinary protein procedures were standardized similarly with the use of a protein standard solution containing 5.0 g human albumin and 3.0 g human globulin per 100 ml (Protein Standard Solution, #540-10, Sigma Chemical Co., St. Louis, Mo.). A stock standard was made by

0026-265X/79/010056-06$01.00/0

diluting this solution to a final concentration of 800 mg/liter with NaCl solution (9 g/liter). Working standards were prepared fresh by dilution of the stock solution to 100, 200, or 400 mg/liter with NaCl solution.

The GFB and CB procedures were performed exactly as described (*1, 3, 4, 7*). The CD method (*8*) was modified to increase sensitivity with normal clinical specimens by use of 2.0 ml of sample and 0.25 ml of Ponceau S Fixative Dye solution. In the final step, dye bound to protein was eluted from the cellulose with 4.0 ml of 0.2 *N* NaOH.

Bovine serum albumin (BSA), ovalbumin, carbonic anhydrase, β-lactoglobulin, and lysozyme were obtained from Isolab, Inc., Akron, Ohio (Protein Calibration Kit, GE-66). Coomassie blue (Brilliant Blue G) and ovomucoid were Sigma products. Disodium carbenicillin and disodium ticarcillin were laboratory assay standards from Pfizer, Inc. (J. B. Roerig Div., New York, N.Y.) and Beecham Labs. (Bristol, Tenn.), respectively. Sodium cephalothin, cefazolin sodium, and sodium cephalexin are products of Eli Lilly & Co. (Indianapolis, Ind.). Bovine pancreatic polypeptide (BPP) and procine C-peptide were gifts of Dr. R. E. Chance of Lilly Research Laboratories. Ortho Control Urine II (Ortho Diagnostics, Inc., Raritan, N.J.) was used as a control throughout this study.

RESULTS

Table 1 shows the analytical results obtained when 20 fresh urine samples from apparently healthy individuals were analyzed by all three methods. These values indicate that the two dye-binding methods provide roughly equivalent results which are appreciably lower than those given by the GFB procedure. In a separate experiment, another 23 urines were analyzed by the CB and GFB methods only. The results further confirmed the relationship between the two procedures with the CB method always producing markedly lower values than the GFB determination. Since we regard the GFB method as our standard for assessing accuracy (and, therefore, as providing the "true" values in Table 1), the dye-binding procedures underestimate the protein concentration in urine specimens obtained from normal individuals.

Table 2 lists the responses given by solutions of specific pure proteins when assayed by the three systems. The purpose of this investigation was to evaluate the accuracy with which proteins of varying amino acid composition, carbohydrate content, and molecular weight would be measured by the dye-binding procedures as compared to a method (GFB) which is specific for peptide bonds. For these observations, the various proteins were dissolved in NaCl solution (9 g/liter) to provide a final concentration of 200 mg/liter (with the exception of the porcine C-peptide which was 100 mg/liter). These results show that considerable differences in response exist among the three procedures for the various protein species. There are even appreciable differences between the two dye-binding methods

TABLE 1
VALUES OBTAINED BY CD AND CB METHODS COMPARED TO THE GFB PROCEDURE

	Urinary total protein (mg/liter)		
	CD	CB	GFB
	45	53	103
	54	62	129
	171	170	258
	120	140	266
	19	25	66
	50	58	148
	22	29	61
	72	80	168
	81	88	220
	38	39	70
	84	137	290
	211	240	670
	37	51	80
	31	40	50
	62	84	120
	59	70	97
	31	45	68
	47	70	116
	30	34	71
	40	38	63
N	20	20	20
$\bar{X}$	65.2	77.6	155.7
Slope	2.61	2.45	
Y intercept	−14.65	−34.32	
Correlation coefficient	0.914	0.944	

with the CD procedure giving the higher results. The values given by BSA indicate that recovery of this protein by the GFB method under these conditions is not 100%. This has been noted before for this method (*4*) with recoveries for various proteins reported to range from 82−95%. Our experience has been (as the results in Table 2 show) that we frequently see somewhat lower recoveries with this particular assay.

Table 3 gives the results obtained by the three urinary protein procedures when selected chemical compounds are individually added to a urine sample prepared by pooling urine from normal individuals. The drugs chosen for testing were selected because they are frequently administered and then subsequently excreted in high amounts. These observations were made in an attempt to assess the relative freedom from interference of the methods. The results obtained indicate that there appears to be no considerable difference between the two dye-binding procedures and that both are free from marked interference by the substances tested. On the other hand, carbenicillin, ticarcillin, cephalexin, and, to a lesser

TABLE 2
VALUES GIVEN BY THE THREE METHODS WITH SPECIFIC PROTEINS

	MW	CB	CD	GFB
		Urinary total protein (mg/liter)		
BSA	68,000	200 (5.4)[a]	205[b]	152 (11.6)[c]
Lysozyme	14,500	96 (10.9)	154	119 (13.6)
Carbonic anhydrase	32,000	170 (3.8)	175	138 (3.2)
Ovalbumin	45,000	82 (12.8)	120	167 (11.5)
β-Lactoglobulin	18,000	45 (4.9)	148	134 (9.5)
Ovomucoid	27,300	49 (8.0)	70	130 (10.7)
BPP	4,226	—	150	91 (7.8)
C-peptide	2,744	—	0	62 (5.0)
Control[d]	—	216 (7.5)	225	176 (4.9)

[a] Mean (SD), six determinations.
[b] Mean, two determinations.
[c] Mean (SD), three determinations.
[d] Ortho urine control, diluted 3× with NaCl (9 g/liter).

extent, cefazolin and cephalothin interfere markedly with the GFB method at the concentrations listed.

DISCUSSION

The results of Table 1 show that for urines having relatively low protein concentrations, both dye-binding methods give values for total protein which are appreciably lower than those given by the GFB procedure even though all three assays are standardized identically. This observation prompted us to perform a simple recovery study in which known amounts of Sigma Human Protein Standard were added to a pool of urine collected from normal individuals. In this instance, the protein concentration of the pool was 130 mg/liter when measured by the CD method and 200 mg/liter

TABLE 3
INTERFERENCE BY SELECTED COMPOUNDS WITH THE THREE URINE PROTEIN PROCEDURES

Compound (mg/ml)		CB	CD	GFB
		Urinary total protein (mg/liter)		
Urine pool		220	200	270
Carbenicillin	(30)	275	170	—[a]
Cefazolin	(10)	230	180	360
Cephalothin	(10)	235	180	350
Ticarcillin	(30)	250	180	—[a]
Cephalexin	(10)	220	200	—[a]
Salicylate	(4.0)	240	190	245
Caffeine	(6.5)	210	200	210

[a] Absorbance too intense to measure.

by the GFB procedure. Sufficient protein was added to produce increments of 40, 80, 120, 160, and 320 mg/liter. Recoveries ranged from 95 to 105% for both the CD and GFB assays. Thus, in this experiment added albumins and globulins were detected similarly and completely by both methods. When we analyzed urine samples which had very high protein concentrations and which required dilution five- or ten-fold to achieve concentrations such as in Table 1, we found nearly identical results for all three assays. In other words, dilution of these samples obliterated the discrepancy between the dye-binding and GFB methods. This indicated to us that albumins and globulins react similarly in all three methods but that there exist constituents which form a large percentage of the total protein composition of normal urine which are not detected equally by all three assays. In urines with abnormally high protein concentrations, albumins and globulins would most likely predominate and overshadow these components, and we might expect to see equivalent results with all three procedures. Such a component which forms a large fraction (> 25%) of the protein of normal urine is the mucoprotein of Tamm and Horsfall (uromucoid) (*9*) which has a carbohydrate content of 28%. The results of Table 2 indicate that various proteins give different responses with the three procedures and that ovomucoid [which is similar to uromucoid in that it has a carbohydrate content of 20% (*6*)] is measured especially poorly by both dye-binding methods. With urines of low protein concentrations (i.e., normal urines), it would seem logical that the GFB method would be more accurate since the type of measurement it affords (peptide bonds) should be less subject to error than methods which are influenced by relative percentages of basic amino acid residues or by carbohydrate content. For this reason, the normal ranges for the dye-binding methods would be expected to be somewhat less than for the GFB method although similar values have been reported tentatively (*3*, *7*).

It is worthy of note that BPP (*5*) and porcine C-peptide (*2*) (4,226 and 2,744 MW, respectively) are both detected in the GFB procedure even though a preliminary gel filtration step with Sephadex G 50-80 is performed. The detection of low-molecular weight material by this method has been noted (*4*). Procine C-peptide [which contains no residues of basic amino acids (*2*)] gives no response with the CD method.

The results given in Table 3 indicate that the problem of interferences is less with the dye-binding methods than with the GFB procedure. This observation has been repeated several times with different lots of Sephadex G 50-80 having been used. Under these conditions, it would appear that the gel columns may be overloaded. Perhaps this problem can be circumvented by using sample volumes less than the 1.0 ml recommended (*3*).

We feel that both the CD and CB methods are suitable for routine clinical use. The day-to-day C.V.s for these two methods are similar at a

protein concentration of about 1000 mg/liter (*7*, *8*). Although neither method was affected by the interferants we studied, the CD procedure would probably be generally safer in this regard since adsorption and washing steps are performed rather than a direct urine assay. The results of Table 2 also show that the CD procedure gives greater responses with the various proteins than the CB method. Although the dye-binding assays will give lower results with urine samples having normal or somewhat above normal protein concentrations than the GFB method, it is unlikely that significant proteinuria would be missed by these procedures. Both dye methods seem remarkably free from interferences relative to the GFB procedure, a fact which is surprising because the latter involves a preliminary gel filtration step. Since peptides are also detected by the GFB procedure, there would seem to be no basis to prefer this assay over a dye-binding procedure to avoid detecting these substances.

SUMMARY

Two dye-binding methods for the determination of urinary total protein concentration have been compared to a biuret-based procedure requiring a preliminary gel filtration step. The biuret-based procedure is claimed to be both specific and sensitive for the measurement of protein in urine. With those urines having relatively low protein concentrations, the dye-binding methods provide values about one-half of those given by the modified biuret procedure. This difference disappears when urines containing high levels of protein are measured. The lower results obtained at normal protein levels are most likely caused by the inability of dye-binding methods to measure proteins of high carbohydrate content (such as uromucoid) accurately. In spite of this limitation, both dye-binding methods appear suitable for routine use. In contrast to the biuret-based procedure, we found the dye-binding methods to be free from interferences by compounds known to appear in urine in high concentrations.

REFERENCES

1. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254 (1976).
2. Chance, R. E., Ellis, R. M., and Bromer, W. W., Porcine proinsulin: Characterization and amino acid sequence. *Science* **161**, 165–167 (1968).
3. Doetsch, K., and Gadsden, R. H., Determination of urinary total protein by use of gel-filtration and a modified biuret method. *Clin. Chem.* **21**, 778–781 (1975).
4. Doetsch, K., and Gadsden, R. H., More on urinary protein determination technique. *Clin. Chem.* **23**, 1186–1187 (1977).
5. Lin, T. M., Evans, D. C., Chance, R. E., and Spray, G. F., Bovine pancreatic peptide: action on gastric and pancreatic secretion in dogs. *Amer. J. Physiol.* **232**, E311–E315 (1977).
6. Lineweaver, H., and Murray, C. W., Identification of the trypsin inhibitor of egg white with ovomucoid. *J. Biol. Chem.* **171**, 565–581 (1947).
7. McIntosh, J. C., Application of a dye-binding method to the determination of protein in urine and cerebrospinal fluid. *Clin. Chem.* **23**, 1939–1940 (1977).
8. Meola, J. M., Vargas, M. A., and Brown, H. H., Simple procedure for measuring total protein in urine. *Clin. Chem.* **23**, 975–977 (1977).
9. Tamm, I., and Horsfall, F. L., Jr., A mucoprotein derived from human urine which reacts with influenza, mumps, and newcastle disease viruses. *J. Exp. Med.* **95**, 71–97 (1952).

MICROCHEMICAL JOURNAL 24, 62–63 (1979)

Suggestions for Improved Performance of the Thermal Energy Analyzer

WILLIAM F. TULLY AND WILLIAM C. KURYLA

Union Carbide Corporation, Research and Development Department, South Charleston, West Virginia 25303

Received August 11, 1978

INTRODUCTION

Since the introduction of the Thermal Energy Analyzer (TEA) by Thermal Electron Corporation, numerous authors have published material (*1–7*) on the quantitation of nitrosoamines (NAs) in various matrices, i.e., air, water, soil, cosmetics, cutting fluids, etc. The published material generally addresses the liquid chromatographic or gas chromatographic parameters which were established for the particular determination being reported without alluding to operation parameters of the TEA.

To date nothing has been reported on the analysis of nitrosoamine precursors. Attempts to determine nitrosoamines in neat alkanolamines resulted in operational problems with the Thermal Energy Analyzer when coupled to a liquid chromatograph and using a solvent system compatible with the analyzer. These difficulties were greatly reduced by staging the temperatures of the traps and the installation of a diverting valve after the liquid chromatograph analytical column to reduce the quantity of amines and eluent which reach the analyzer.

DISCUSSION

The development and introduction of the highly sensitive Thermal Energy Analyzer by Thermal Electron Corporation has created the means to determine ppb levels of NAs in a wide variety of materials. The ability to determine NAs with a high degree of specificity has revealed the ubiquitous nature of these known carcinogens in the ecosystem. The result is that any precursor of NAs which has industrial and/or domestic application becomes suspect of containing NAs.

Alkanolamines have wide application in both industrial and consumer products. Gas chromatographic separations of the mono-, di-, and triethanolamines are extremely difficult. While they can be achieved, drastic thermal conditions are necessary which could decompose any NAs present. High-pressure liquid chromatography (HPLC) presented another approach to achieving a separation of the amines and NAs. Difficulties experienced with separation and concentration of NAs from prepared samples left the direct analysis of alkanolamines for NAs by

0026-265X/79/010062-02$01.00/0

HPLC–TEA the most probable route to success. However, solvent systems normally used with HPLCs having conventional detectors gave excessive background levels which reduced sensitivity of the TEA. Hexane–acetone mixtures improved the background level but still presented a problem. Since the trap temperature of the TEA influences the background signal, reduction below −78°C (dry ice–acetone) freezes the hexane–acetone solvent and results in a malfunction of the TEA which could possibly damage the pyrolysis oven. It was found that staging the temperature of the double glass traps overcame the problem of trap freezing. The first trap was immersed in a dry ice–acetone mixture and the second in liquid nitrogen. The traps were then removed from the TEA for emptying rather than following the dumping routine.

The volume of solvent which passed through the TEA was reduced by the installation of a diverting valve after the HPLC column. Alkanolamine samples which had been spiked with the corresponding NA were injected into the HPLC via a high-pressure sampling valve and allowed to pass into the column. The contained NA elute ahead of a broad alkanolamine peak. Once the NA had eluted from the column and entered the TEA, the eluent was diverted to a waste container. This approach greatly shortened the turnaround time between sample injections and resulted in substantially lower background levels as well as enhancing the sensitivity of the TEA.

REFERENCES

1. Fine, D. H., Lieb, D., and Refeh, F., Principle of operation of the thermal energy analyzer for the trace analysis of volatile and non-volatile nitroso compounds. *J. Chromatogr.* **107**, 351–357 (1975).
2. Fine, D. H., Refeh, F., and Lieb, D., Roundbehler, D. P., Description of the thermal energy analyzer (TEA) for trace determination of volatile and non-volatile *N*-nitroso compounds. *Anal. Chem.* **47**, 1188–1191 (1975).
3. Fine, D. H., Ross, R., Roundbehler, D. P., Silvergleid, A., and Song, L., Analysis of nonionic non-volatile *N*-nitroso compounds in foodstuff. *J. Agric. Food Chem.* **24**, 1069–1971 (1976).
4. Fine, D. H., and Roundbehler, D. P., Trace analysis of volatile *N*-nitroso compounds by combined gas chromatography and thermal energy analysis. *J. Chromatogr.* **109**, 271–279 (1975).
5. Fine, D. H., Roundbehler, D. P., Belcher, N. M., and Epsteine, S. S., *N*-Nitroso compounds: Detection in air, *Science* **192**, 1328–1330 (1976).
6. Fine, D. H., Roundbehler, D. P., Roundbehler, A., Silvergleid, A., Sawicki, E., Krost, K., and DeMarrais, A., Determination of dimethylnitrosamine in air, water, and soil by thermal energy analysis: Measurements in Baltimore, Md. *Environ. Sci. Technol.* **11**, 581–584 (1977).
7. Fine, D. H., Roundbehler, D. P., Sawicki, E., and Krost, K., Determination of dimethylnitrosamine in air and water by thermal energy analysis: Validation of analytical procedures. *Environ. Sci. Technol.* **11**, 577–580 (1977).

MICROCHEMICAL JOURNAL 24, 64–72 (1979)

Computerized Technique in Organic Microelemental Analysis

Part II. Automatic Determination of Sulfur in Organic Compounds

G. M. MACIAK,* P. W. LANDIS,* AND E. KOZLOWSKI†

*The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, †Department of Chemical Engineering and Measuring Techniques, Polytechnic Gdańsk, Gdańsk, Poland

Received September 11, 1978

INTRODUCTION

In the course of automation and computerization of the Lilly Research Laboratories Microanalytical Laboratory (*6*), it was necessary to develop instrumentation and methodology for the automatic determination of sulfur and other frequently occurring elements. At the present time, no such method is sufficiently developed, and instruments are not commercially available.

The only instrument capable of determining sulfur in organic compounds in a truly automatic mode was described by Culmo (*2*) utilizing the well-established Perkin—Elmer 240 analyzer. This method is limited to sulfur determination only, whereas our new method has the further capability of analyzing for halogens, including fluorine.

The automatic mode of analysis has two important advantages: (i) rapid analysis and (ii) simplicity of operation by less qualified personnel. We therefore elaborated a totally automatic system for sulfur determination in organic compounds consisting of rapid combustion with a transfer of combustion products to a titration system interfaced to a computer.

TECHNIQUE

Combustion

The success of any microanalysis depends greatly on a properly conducted combustion. Of the many methods of combustion, we selected the dynamic oxidative type. This mode of combustion is ideally suited for an automated procedure, producing a dynamic flow of combustion products to the end of the tube. Combustion products are pure, usually free from most of the interfering elements, and, con-

0026-265X/79/010064-09$01.00/0

sequently, simple for the final determination. This type of mineralization in a combustion tube was reported in many versions and modifications since the time of the introduction of sulfur determination.

Techniques of combustion, as well as instrumentation and methods, vary greatly with regard to oxygen flow, tube diameter, length of the heating zone, temperature, presence of a catalyst, time of combustion, and the form of sample decomposition. We chose the following conditions for the rapid determination of sulfur: empty tube combustion method in a quartz tube of 15-mm inside diameter, oxygen flow at 35–40 ml/min, and temperature of about 950°C in a 200-mm-long furnace.

The substance, in a platinum boat, is decomposed with the movable heater (800°C); the movement is set at 1 cm/min. The decomposition and mineralization of a micro sample is completed in about 3 min by placing the boat close (2–3 cm) to the stationary furnace. The availability of large amounts of oxygen and a relatively slow flow through the large-diameter tube at 950°C provide sufficient time for complete combustion of any organic compound and for the conversion of sulfur to sulfur oxides. The presence of a catalyst in the combustion tube, particularly Pt, interferes with the proposed colorimetric determination. It was shown that Pt in the presence of halogens will volatilize (*5*) and can reach the titration vessel, reacting with the indicator.

Transfer of Combustion Products from the Combustion Site to the Determination Site

Combustion products of sulfur, as well as fluorine and other halogens, are condensed with moisture at the end of the combustion tube in the form of the corresponding acids. In the case of sulfur determination, the combustion products are a mixture of SO_2 and SO_3 and require additional chemical reactions to convert them into a well-characterized substance. The traditionally applied oxidation with H_2O_2 converts sulfurous acid into sulfuric acid, which is readily determinable. In our analysis, this chemical process, as well as the transfer of the product to the determination vessel, is achieved automatically.

Utilizing oxygen flow from one direction, and pumping hydrogen peroxide from the other direction, the mixture is forced to a side exit (Fig. 1). This type of washing was first utilized by Walisch and Jeanecke (*9*) and others (*4*, *7*). The transfer technique was modified and simplified by constructing a one-piece arrangement as an internal part of the tube, omitting an additional heater (*7*). Utilizing the radiant heat from the combustion furnace, the wash area can be kept warm, preventing condensation of sulfuric acid, provided it is within proper

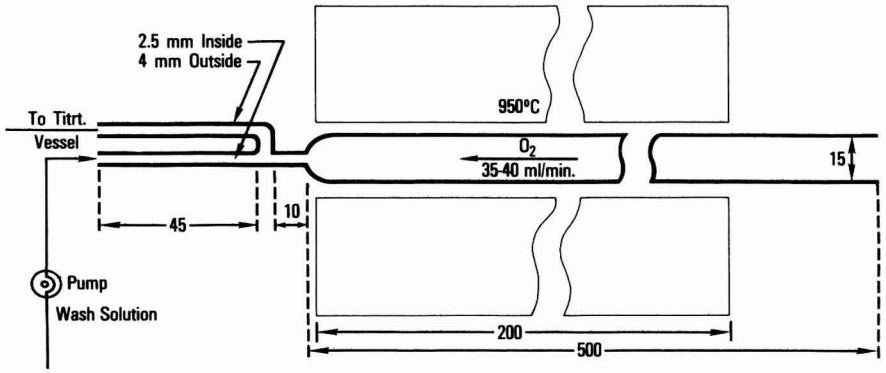


FIG. 1. Combustion tube with wash arrangement.

distance of the furnace. The wash solution is pumped (1 ml/min) in a countercurrent direction to the oxygen flow. In the contact area is a side arm in the form of a T, enabling the oxidized combustion product to exit by means of oxygen pressure to the vessel for determination. Dimensions of the combustion tube are given in Fig. 1.

Determination

Among the many methods of determining sulfate ions, titrimetric procedures are the quickest and easiest to be adapted for automation and computerization. Detection of the endpoint can be accomplished by colorimetry. For the colorimetric titration, there are two well-established indicators: thorin (*3, 8*) and dimethylsulfonazo III (*1*). We first examined the two indicators by obtaining their potentiographic curves. We then titrated sulfuric acid in 90% isopropanol (in the presence of a buffer) with $Ba(ClO_4)_2$ against the indicators, and recorded the potential change obtained from the colorimeter caused by the color change of the indicator at the equivalent point (Fig. 2). Obviously, a larger (40 mV) and sharper potential jump at the equivalent point, using dimethylsulfonazo III, caused by the color change of the indicator will be more precise for the determination. We elected to use a colorostatic $Ba(ClO_4)_2$ titration for the sulfate ion determination using a Fiberglas optic colorimeter as the endpoint detection. For this procedure, we suggest the name chromostatic or colorostatic titration. Titration assembly for such an automatic titration consists of a potentiostat, an electric burette, and a Fiberglas optic colorimeter.

MATERIALS AND METHODS

Reagents

Purified-grade oxygen gas used without additional treatment.

Wash solution: 3% hydrogen peroxide.

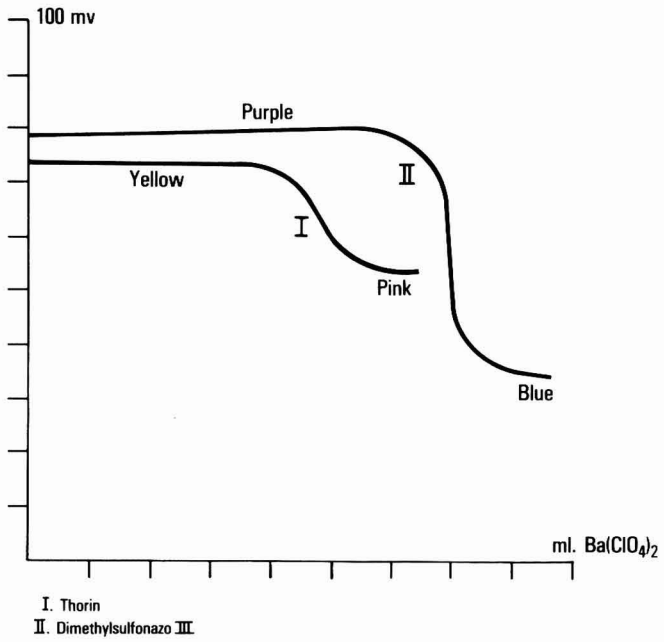


FIG. 2. Potentiometric titration of sulfate (color changes of the indicator).

Absorption solution: 10 ml buffer solution* and 5 ml indicator solution** per 1 liter of 90% aqueous isopropanol.

Buffer solution: *50 ml collidine dissolved in 500 ml 50% isopropanol adjusted to pH2 with 70½ perchloric acid, ca. 25 ml (R. Kübler, private communication).

Indicator solution: **0.1% solution of dimethylsulfonazo III–Na salt in water (J. T. Baker Chemical Co.).

Titrant: 0.005 *N* $Ba(ClO_4)_2$ in 90% aqueous isopropanol (G. Frederick Smith Chemical Co.).

Equipment

Gas flow meter, tube size 1–15–16 (Brooks Instrument Co., Hatfield, Pa.).

Combustion oven, 200-mm stationary furnace, orifice 24 mm, and a movable heater with 1 cm/min travel speed (Hösli, Bischofszell, Switzerland).

Quartz combustion tube, 550-mm over-all length, 16-mm outside diameter, with the attached washing arrangement as shown in Fig. 1.

Peristaltic pump, Ismatec Model 2-4, adjusted for 1 ml/min delivery of liquid (Brinkmann Instrument Co.).

Automatic titration assembly consisting of: one 10-ml electric

burette, "Dosimat" E-415 Metrohm, equipped with an electric pulse generator (for computer interfacing).

Potentiostat, "Impulsomat" E-473 Metrohm.

Metrohm titration vessel assembly with magnetic stirrer.

Fiberglas colorimeter P/C-1000, with a 1-cm light path, 670-nm optical filter, and 1-V electric output (Brinkmann Instrument Co.).

Cam switch timer, Model Micromat NA 4112 B12, 6-min cycle (Micro-Electric, Zurich, Switzerland).

Three-way Teflon solenoid valve, Model 72P16T3 (Valcor Co.).

Two two-way Teflon solenoid valves, Model P/N 2-15-900, S/N 621 (General Valve Co.).

Polyethylene tubing, Intermedic #PE-240.

Teflon tubing, ⅛-in. outside diameter, $^1/_{16}$-in. inside diameter.

Arrangement for vacuum.

Experimental

An automatic analyzer was set up as shown by the diagrams in Figs. 3, 4, and 5, and according to the manufacturers' manuals. The burette's three-way stopcock was removed and replaced with a three-way solenoid valve. The absorption solution reservoir was placed about 1 m above the instrument. Since filling the titration vessel with absorption solution is dependent upon both gravity and time, and in order to obtain equal volumes independent of the solution level, a Mariott-type arrangement was utilized. The equivalence point for the titration was set in the Impulsomat to the color of the indicator used in the absorption solution. This setting was previously established by titration (Fig. 2) and corresponds to approximately 60 mV or 60% transmittance. The combustion tube was washed with dilute

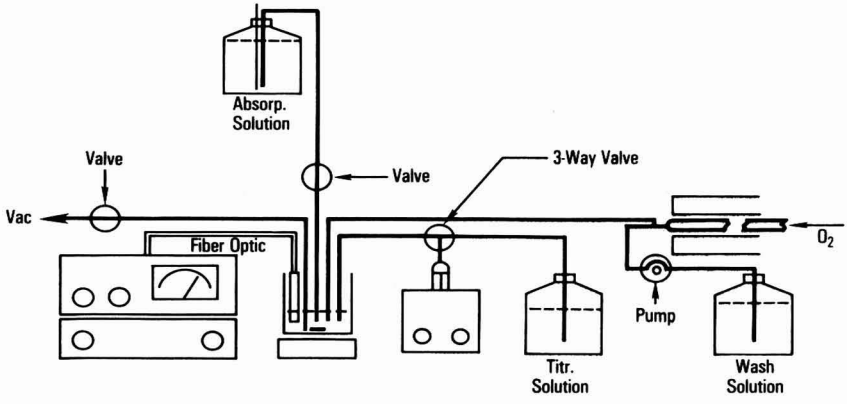


FIG. 3. Set-up for automatic sulfur determination.

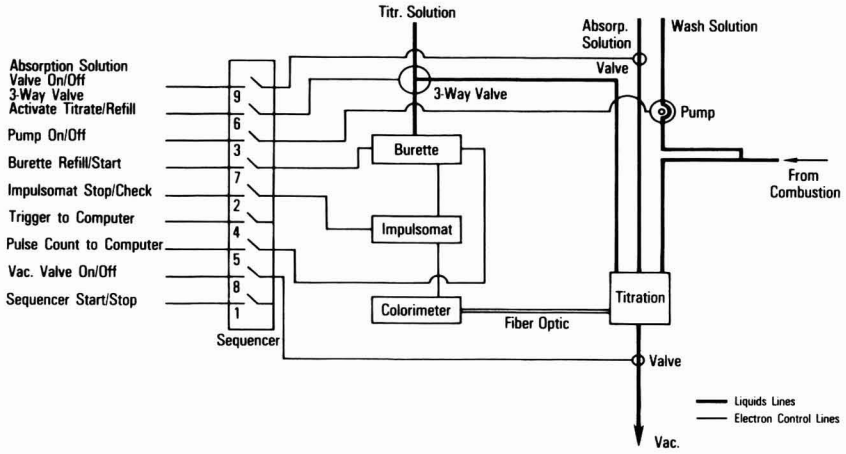


FIG. 4. Diagram of the electronic interface.

HF to remove any alkali salts from the surface, and then it was placed into the combustion furnace. The stationary furnace was set at 950°C and the movable heater at 800°C, while the oxygen flow was established at 35–40 ml/min. The delivery of fresh absorption solution to the titration vessel was adjusted for a 30-ml delivery, and the pump was set for wash-solution delivery of of 1 ml/min.

Procedure

A sulfur-containing sample was weighed into a platinum boat on a computer-interfaced electronic microbalance (Mettler ME22 or Sar-

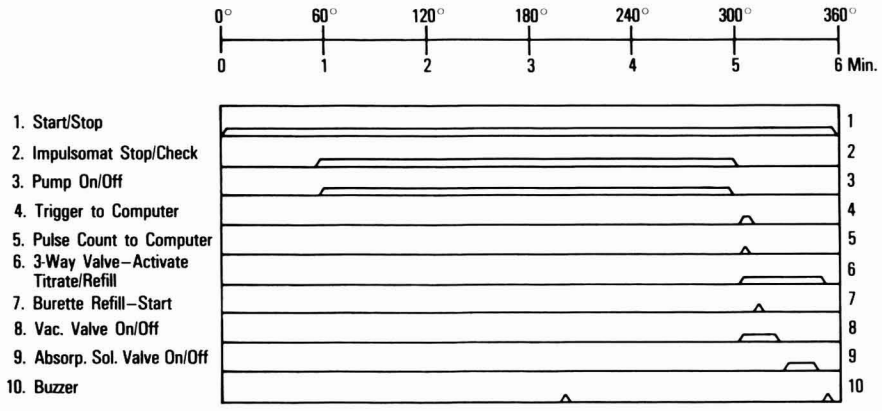


FIG. 5. Sequencer time schedule.

torius 4431) and placed in the combustion tube about 3 cm from the stationary furnace. The sequencer and combustion were initiated, and, after about 1 min, the pump was started by the sequencer. The combustion products were washed to the titration vessel. The presence of sulfate ions caused a change in the indicator color (reddish purple), and the colorimeter responded and activated automatic titration until the indicator color returned to a neutral blue color. After 3 min, combustion was completed and the movable heater was removed in order to cool the combustion tube in preparation for the next analysis. Washing and titration continued for another 2 min. In the fifth minute of the programmer cycle, the titration data, in the form of number of pulses (100 pulses = 1.00 ml), were received by the computer. Shortly afterwards, the burette was refilled and set at zero, as was the pulse counter. The titration vessel was emptied via vacuum and refilled with 30 ml fresh absorption solution. The system is now ready for the next determination.

Computer Interfacing

The computer system as previously described (*6*) has been expanded, updated, and converted to a departmental time-sharing operation, which makes possible the storing of the last 200 results on magnetic discs. Also, the teletypes were replaced by cathode ray terminals.

The sulfur analyzer was interfaced to the computer via a pulse counter. The accumulated electronic pulses produced by the function of the burette were transmitted to the computer and identified as to the unit number. Data from the balance and analyzer were calculated and reported to the operator on the CR terminal. The format of the report is analogous to that described for CHN determination (*6*). A particular report is then stored on the magnetic disc for further processing.

RESULTS AND DISCUSSION

During the last 4 years, we performed many thousands of sulfur analyses on various organic and organometallic compounds using the described automatic procedure.

Organometallic compounds were combusted in the presence of vanadium pentoxide (V_2O_5) as an additive to the analyzed substance. Volatile compounds were analyzed in sealed capsules and liquids in capillaries. Sample size ranged from 1 to 7 mg or more, depending on the expected sulfur content. The method functions blank free.

Interference of common heteroelements such as phosphorus and iodine was noticed. The presence of elemental iodine in the titration solution caused a change of the indicator color from green to gray. The colorimeter, however, responded correctly in this color system.

In most cases, phosphoroorganic compounds analyzed accurately or with only slightly higher results (0.2−0.5%) due to the presence of trace amounts of H_3PO_4 in the titration solution. The combustion tube was frequently removed and washed with HF, particularly after a series of analyses of metallo- or phosphoroorganic compounds. Formation of $BaSO_4$ during the titration, in the form of a dense precipitate, did not interfere with the colorimetric measurement of the solution.

Among these sulfur determinations, we also performed 16 sulfur determinations on cystine (E. M. Laboratories, microanalytical standard, theoretical value: 26.69% S). These were performed over a period of 3 weeks, as a daily instrument-control analysis, with the following results:

Sample size, 1.8−4.9 mg.

Titrant consumption, 3.0−8.2 ml.

The results were in the range of −0.16 to +0.25% relative to the theory, of which eight determinations were below and the remaining above the theoretical value.

Based on these results:

$$\text{Mean error: } \Delta\bar{x} = \frac{\sum \Delta x}{n} = 0.01$$

$$\text{Variance: } V = \frac{\sum (x - \bar{x})^2}{n} = 0.015$$

$$\text{Std. deviation: } S = \frac{\sum (x - \bar{x})^2}{n - 1} = 0.128$$

Least-square regression: $A_0 = 0.00$

$A_1 = 1.69$

$y = A_0 + A_1 x$

where y = titrant consumption

x = sample size

The above calculations were computed on a Hewlett−Packard 9815-A computer using HP statistical computer programs.

SUMMARY

Described is the automatic and computerized rapid microanalytical determination of sulfur in organic and organometallic compounds. The procedure consists of the combustion of the compound in an empty tube, transfer of combustion products to the titration vessel by automatic wash, and, finally, colorostatic titration of sulfuric acid with $Ba(ClO_4)_2$ against dimethylsulfonazo III.

The entire analysis and preparation of the equipment for the next determination are programmed in time of the 6-min cycle.

The analyzer is interfaced to the departmental real-time time-sharing computer, along

with electronic microbalances and other analyzers, as a part of the microanalytical laboratory computer service.

Results of the analyses are reported on CR terminals and are also stored on the magnetic disc for further processing.

ACKNOWLEDGMENTS

We wish to express our thanks to: Dr. G. L. Kirschner, Mr. J. B. Deeter, and Mr. D. O. Fegenbush for computer interfacing and programming; Mr. R. W. Sanders for providing electronic controls; and Mrs. J. L. Dyer, Mr. D. L. Cline, and Mr. J. A. Swallow for technical assistance.

REFERENCES

1. Budesinsky, B., and Varzalova, D., Determination of organic sulfur and sulfates with dibromosulfonazo III and dimethylsulfonazo III. *Chem.-Anal.* **55**, 110 (1966).
2. Culmo, R. F., The microdetermination of sulfur with an automatic elemental analyzer. *Microchem. J.* **17**, 499–507 (1972).
3. Fritz, J. S., and Yamamura, S. S., Rapid microtitration of sulfate. *Anal. Chem.* **27**, 1461–1464 (1955).
4. Kainz, G., and Müller, H. A., Automatisierung der Mikrohalogen-bestimmung in organischen Verbindungen. *Fresenius' Z. Anal. Chem.* **253**, 180–184 (1971).
5. Kozlowski, E., Jodogravimetrische Methoden in der organischen Elementaranalyse. VII. Uber die Verbrennung halogen-haltiger. Substanzen on feinverteiltem Platinkontakt. *Bull. Acad. Polon. Sci. Ser. Sci. Chim.* **13**, 361–364 (1965).
6. Maciak, G. M., Byers, R. A., and Landis, P. W., Computerized technique in organic microelemental analysis: Determination of C, H, N, and O. *Microchem. J.* **18**, 8–17 (1973).
7. Scheidl, F., and Toome, V., Automated potentiostatic microdetermination of Cl, Br, and J in organic compounds. *Microchem. J.* **18**, 42–50 (1973).
8. Wagner, H., Beitrag zur Mikrobestimmung des Schwefels in organischen Substanzen. *Mikrochim. Acta* 1957, 19.
9. Walisch, W., and Jaenicke, O., Messanordnung zur automatischen Ultramikrobestimmung von Halogenen in organischen Verbindungen. *Mikrochim. Acta* 1967, 1147–1163.

MICROCHEMICAL JOURNAL 24, 73–79 (1979)

Potentiometric Microdetermination of 1,3,5-Trinitro-1,3,5-triazacyclohexane and 1,3,5,7-Tetranitro-1,3,5,7-tetrazacyclooctane in Several Solvents[1,2,3,4]

WALTER SELIG

Lawrence Livermore Laboratory, University of California, Livermore, California 94550

Received October 13, 1978

INTRODUCTION

Our interest in nonaqueous titrimetry prompted us to investigate solvents not commonly used in such work: 1,1,3,3-tetramethylguanidine (TMG) and hexamethylphosphoramide (HMPT). These solvents, as well as pyridine, were used for the microdetermination of two weakly acidic compounds of ordnance interest, 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (HMX). Although both nitramines have previously been determined by nonaqueous titration (*1,3,6,14*) TMG and HMPT had not been used as solvents. We have also used constant current potentiometry (*13*) to show that a significant improvement in precision is possible even in solvents yielding poor endpoint breaks by direct potentiometric titration.

An integrated automated titration system controlled by a Tektronix 4051 graphics system (*11*) was used to generate titration curves and to evaluate the experimental results.

EXPERIMENTAL

The titrant was approximately 0.05 *N* tetrabutylammonium hydroxide (Bu_4NOH) prepared from a 25% solution in methanol (Eastman Organic

[1] This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately-owned rights.

[2] Reference to a company or product names does not imply approval or recommendation of the product by the University of California or the U.S. Department of Energy to the exclusion of others that may be suitable.

[3] Work performed under the auspices of the U.S. Department of Energy under contract No. W-7405-Eng-48.

[4] Parts of this article were presented at the Tenth Symposium on Explosives and Pyrotechnics, on February 14–16, 1979, in San Francisco, California.

0026-265X/79/010073-07$01.00/0

Chemicals) by dilution with reagent grade toluene (*12*). Benzoic acid was ACS reagent grade. Pyridine was Mallinckrodt analytical reagent, purified by stirring 24 hr over Dowex 1-X10 (*4*). 1,1,3,3-Tetramethylguanidine (TMG) was from Eastman Organic Chemicals, purified by stirring a minimum of 2 days over molecular sieves (Fisher Scientific Co., Type 4A, 14-30 mesh). Hexamethylphosphoramide was from Aldrich Chemical Co., purified by stirring for 2 days over a mixed resin bed (Fisher Scientific Co., Rexyn 300) (*2*). The solvent blanks were $<3 \times 10^{-3}$ mmol of Bu_4NOH per 50 ml for pyridine and HMPT and <0.01 mmol for TMG.

The titration system was controlled by a Tektronix 4051 graphics system as previously described (*11*). Emf's were monitored by a glass indicator electrode and a ceramic fiber-junction calomel reference electrode (Beckman 39402) in which the salt bridge was a saturated solution of tetramethylammonium chloride in methanol.

Stirring was provided by a magnetic stirrer. The stirring motor was separated from the titration vessel by a water-cooling plate and an aluminum plate connected to ground.

For the glass/modified calomel electrode system, titration endpoints were calculated according to Savitsky and Golay (*7*). A convolute was used for a third-order second derivative using 25 points. The zero crossing was found by linear interpolation near the sign change.

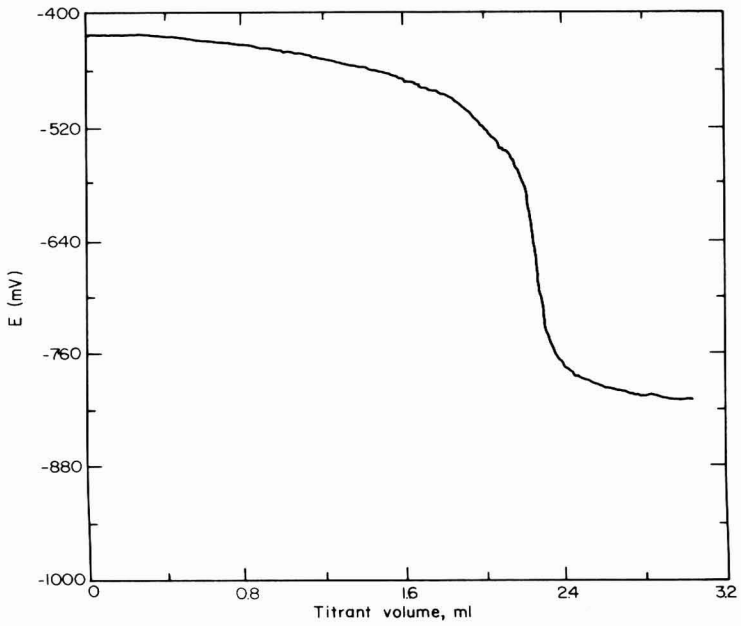


FIG. 1. Titration of 7.44 mg of HMX vs Bu_4NOH in tetramethylguanidine.

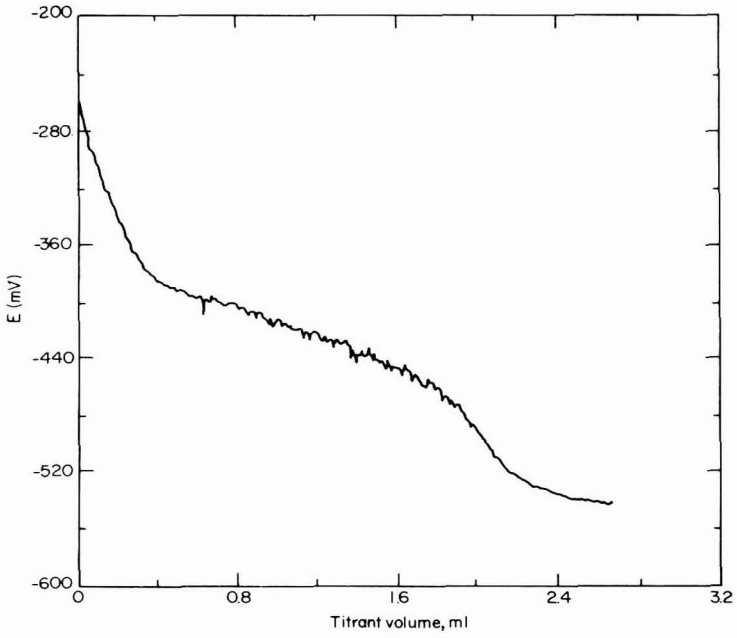


FIG. 2. Titration of 7.60 mg of HMX in pyridine vs Bu_4NOH; electrodes: glass/modified calomel.

Some titrations were monitored by two platinum electrodes, polarized by a constant current of 5 μA according to Shain and Svoboda (*13*). The endpoint was located as the emf before the abrupt dropoff (see Fig. 4).

Titrations were performed at ambient temperature (23 ± 1°C) under a nitrogen blanket.

RESULTS AND DISCUSSION

RDX has been determined by nonaqueous titration in *N,N*-dimethylformamide (DMF) as early as 1955 (*3*). The titrant was sodium methoxide; endpoints were determined visually using azo violet. Sarson (*6*) determined RDX in methylisobutyl ketone (MIBK) and DMF by titration with Bu_4NOH; endpoints were determined electrometrically. Sinha *et al.* (*14*) determined RDX and HMX in MIBK:isopropanol (4:1) by titration with sodium methoxide. Fauth and co-workers (*1*) investigated a wide variety of solvents for the potentiometric titration of RDX and HMX with Bu_4NOH. Recoveries for RDX were of the order of 98 to 100% for RDX, with a somewhat greater spread for HMX. All these methods were on the macroscale, using more than 60 mg of the explosive for each titration.

Our interest in nonaqueous titrimetry as well as in the determination of compounds of ordnance interest prompted us to investigate the micro-

TABLE 1
COMPARISON OF VARIOUS SOLVENTS FOR POTENTIOMETRIC TITRATION OF RDX AND HMX

Solvent	Compound	Amount taken (mg) (aliquots)	Number of replicates	Standard deviation (%)	Mean endpoint break (mV)
Pyridine	RDX	7.30	3	0.37	110
Pyridine	HMX	7.60	4	1.20	55
HMPT	RDX	7.31	4	0.28	260
HMPT	HMX	7.83	4	0.23	190
TMG	RDX	14.64	4	0.29	350
TMG	HMX	14.72	3	0.62	300
TMG	RDX	6.86	3	0.02	
TMG	HMX	7.44	3	0.71	

determination of RDX and HMX in solvents not heretofore used for this analysis. We have previously reported a nonaqueous titrimetric method for pentaerythritol tetranitrate (PETN) which is a considerably weaker acid than RDX and HMX (*10*). The solvent was TMG, a strong base which has previously been suggested for nonaqueous titration by Williams *et al.*

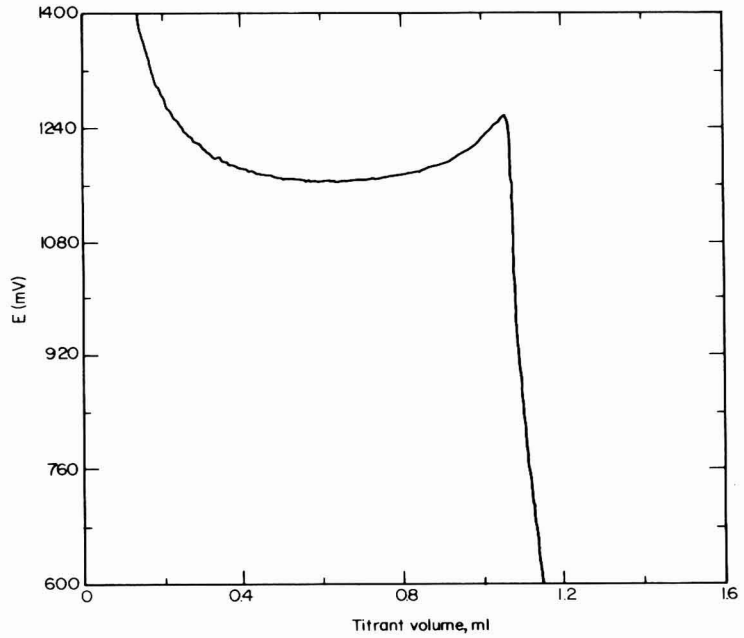


FIG. 3. Titration of 6.06 mg of benzoic acid in pyridine vs Bu_4NOH; electrodes: Pt/Pt, polarized at +5 μA.

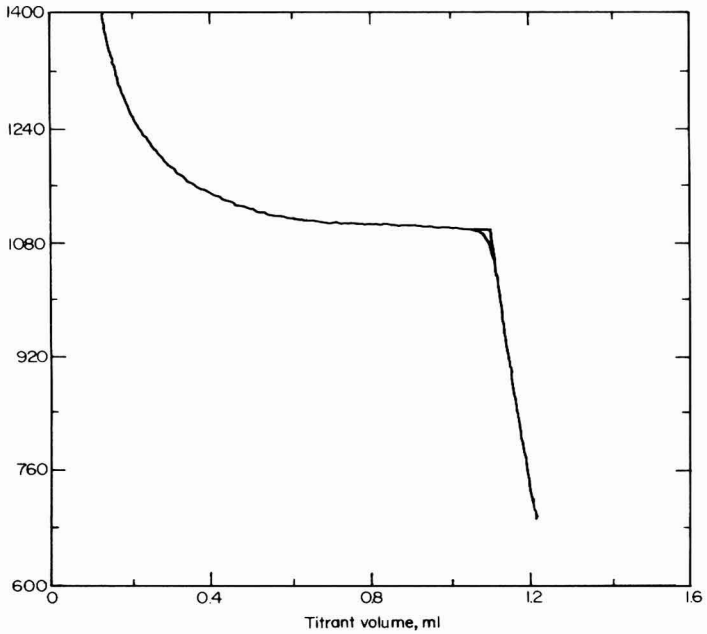


FIG. 4. Titration of 3.71 mg of HMX in pyridine vs Bu_4NOH; electrodes: Pt/Pt, polarized at +5 μA.

(*16, 17*). A fairly strong base is advantageous in the titration of very weak acids because it enhances their proton-donor ability.

We have found that TMG indeed yields a considerable enhancement in the endpoint breaks for RDX and HMX in their potentiometric titration with Bu_4NOH. A titration curve for 7.44 mg of HMX in TMG vs Bu_4NOH is shown in Fig. 1, and for similar amounts of HMX in pyridine in Fig. 2. It is evident that the endpoint break in pyridine is small and not sharp, while a sharp break of much greater magnitude is obtained in TMG. RDX is a slightly stronger acid than HMX and yields somewhat larger endpoint breaks.

HMPT is a solvent which to our knowledge has not previously been used in nonaqueous titrimetry. The properties of this solvent have been discussed by Normant (*5*). This compound has a fairly high dielectric constant (30 at 20°C) and has remarkable solvent as well as complexing properties. Indeed, we have previously described adducts of both RDX and HMX with HMPT (*8,9*). We found that this solvent is easily purified by stirring over a mixed resin bed (*2*) reducing the solvent blank to $<3 \times 10^{-3}$ mmol of Bu_4NOH per 50 ml. The titration curves for RDX and HMX in HMPT were of similar shape to those in pyridine (Fig. 2) although the magnitude of the endpoint breaks was larger. Although these compounds

form adducts with HMPT, titrations nevertheless proved feasible. A comparison of the solvents pyridine, HMPT, and TMG for the microtitration of RDX and HMX is given in Table 1. The half-neutralization potentials of RDX and HMX are so similar that differentiation of these nitramines is not possible by nonaqueous titration.

3-Methyl-2-oxazolidone is a solvent of very high dielectric constant (77.5 at 25°C) which has been suggested as solvent for acid–base titrations (*15*). We found, however, that the two nitramines did not yield titration curves in this solvent vs Bu_4NOH.

We previously used constant-current potentiometry (*13*) to enhance the precision of the titration of PETN in TMG (*10*). This method was applied to the weaker of the two acids, HMX, in the solvent yielding the poorest endpoint breaks in this study, pyridine. Two platinum electrodes were polarized by a constant current of 5 μA. A typical titration curve for a fairly strong acid, benzoic acid, which is commonly used to standardize Bu_4NOH, is shown in Fig. 3. The difference in the potential of the platinum electrodes reaches a very pronounced maximum at the equivalence point. For HMX the curve was of different shape as shown in Fig. 4 for a sample of 3.71 mg in pyridine. Although no maximum in potential was obtained at the equivalence point, the endpoint can easily be located at the intersection of the two straight lines. The improvement in precision when using this method over the direct potentiometric titration is shown in Table 2. This method may also prove useful for the nonaqueous titration of other very weak acids where direct titration yields poor endpoint breaks and poor precision.

In all our experiments we have standardized the titrant vs the sample of nitramine to be analyzed. When the titrant was standardized vs benzoic acid the recoveries of RDX and HMX were from 102 to 104%. High recoveries were already found by Fauth *et al.* (*1*). This may be caused by small amounts of acidic impurities present in the samples. The method should prove useful for the analysis of small amounts of composites containing nonacidic binders.

TABLE 2
COMPARISON OF ENDPOINT DETECTION METHODS FOR RDX AND HMX IN PYRIDINE

Compound	Amount taken, (mg) (aliquots)	Number of replicates	Standard deviation (%)	Electrode system
RDX	7.30	3	0.37	Glass/modified calomel
HMX	7.60	4	1.20	Glass/modified calomel
RDX	7.38	4	0.11	Pt/Pt polarized
HMX	7.94	4	0.35	Pt/Pt polarized
HMX	3.71	4	0.49	Pt/Pt polarized

SUMMARY

Pyridine, tetramethylguanidine, and hexamethylphosphoramide were evaluated as solvents for the microdetermination of two weakly acidic compounds of ordnance interest, 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX) and 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclooctane (HMX). The titrant was tetrabutylammonium hydroxide in methanol/toluene. Endpoints were determined potentiometrically by a glass/modified calomel electrode system or by two polarized platinum electrodes.

The largest potentiometric break was found in the very basic solvent tetramethylguanidine. Endpoint detection using two polarized platinum electrodes significantly enhanced the precision of the analysis.

REFERENCES

1. Fauth, M. I., Frandsen, M., and Havlik, B. R., Titration characteristics of hexahydro-1,3,5-trinitro-s-triazine and octahydro-1,3,5,7-tetranitro-*s*-tetrazine in nonaqueous solvents. *Anal. Chem.* **36,** 380–382 (1964).
2. Ferrari, H. J., and Heider, J. G., Purification of dimethylformamide for use in aqueous and nonaqueous microtitrations. *Microchem. J.* **7,** 194–202 (1963).
3. Kaye, S. M., Nonaqueous titration method for determination of the purity hexahydro-1,3,5-trinitro-*s*-triazine. *Anal. Chem.* **27,** 292–294 (1955).
4. Moskalyk, R. E., Chatten, L. G., and Pernarowski, M., Purification of dimethylformamide for use in nonaqueous titrations. *J. Pharm. Sci.* **50,** (1961).
5. Norman, H., Hexamethylphosphoramide. *Angew. Chem. Int. Ed.* **6,** 1046–1050 (1967).
6. Sarson, R. D., Analysis of explosives by nonaqueous titration. *Anal. Chem.* **30,** 932–937 (1958).
7. Savitsky, A., and Golay, M. J. E., Smoothing and differentiation of data by simplified least-square procedures. *Anal. Chem.* **36,** 1627–1639 (1964).
8. Selig, W., A cyclomethylenetrinitramine:hexamethylphosphoramide complex. *Explosivstoffe* **14,** 174–177 (1966).
9. Selig, W., Some complexes of cyclomethylenetetranitramine (HMX). *Explosivstoffe* **15,** 76–87 (1967).
10. Selig, W., The nonaqueous microtitration of pentaerythritol tetranitrate (PETN). *Mikrochim. Acta* **1978 II,** 169–175 (1978).
11. Selig, W., Potentiometric semimicro determination of some anions with 1,2,4,6-tetraphenylpyridinium acetate. I. Inorganic anions. *Mikrochim. Acta* **1978 II,** 75–81 (1978).
12. Selig, W., Evaluation of toluene as a replacement for benzene in tetrabutylammonium hydroxide titrant. *Microchem. J.* **23,** 466–468 (1978).
13. Shain, I., and Svoboda, G. R., Application of constant current potentiometry to nonaqueous titration of weak acids. *Anal. Chem.* **31,** 1857–1860 (1959).
14. Sinha, S. K., Kulkarni, R. M., and Rao, K. R. K., Determination of hexahydro-1,3,5-trinitro-*s*-triazine in a mixture with octahydro-1,3,5,7-tetranitro-*s*-tetrazine by nonaqueous titration in mixtures. *Anal. Chem.* **36,** 894–895 (1964).
15. Taphorn, J. E. III, Davies, G. M., and Caruso, J. A., 3-Methyl-2-oxazolidone as solvent for acid–base titrations. *Talanta* **22,** 611–614 (1975).
16. Williams, T. R., and Custer, J., Tetramethylguanidine: A new solvent for titration of weak acids. *Talanta* **9,** 175–180 (1962).
17. Williams, T. R., and Lautenschleger, M., Titration of weak acids with tetramethylguanidine as solvent. *Talanta* **10,** 804–808 (1963).

MICROCHEMICAL JOURNAL 24, 80–87 (1979)

Arsenic Colorimetry with Silver Diethyldithiocarbamate

B. W. BUDESINSKY

Phelps Dodge Corporation, Morenci, Arizona 85540

Received August 31, 1978

INTRODUCTION

Besides flameless atomic absorption (*14*) and plasma emission spectrometry (*8*), the colorimetric method employing silver diethyldithiocarbamate (*16*) is the most important routine method for determination of trace amounts (1–30 μg) of arsenic. The latter method uses arsine generation in the medium of metallic zinc (2–10 g) and aqueous solution of potassium iodide (2 ml of 15–20% solution), stannous chloride (0.2–1 ml of 15–40% solution in hydrochloric acid), and hydrochloric acid (5–10 ml of concentrated acid per total volume of 20–45 ml) introduced in the framework of Gutzeit method (*10*) more than 50 years ago (*19*).

Metallic aluminum, iron, and tin have been tested in the place of zinc (*11*), however, practical availability of arsenic-free zinc is the main factor of its preference. The amount of zinc varies due to its different surface (granules, shots, or sticks) and present interfering metal ions. Stannous chloride and potassium iodide have been used to reduce arsenic(V) to arsenic(III) and to remove some other oxidants. Water mist and traces of hydrogen sulfide as byproducts of hydrogen and arsine evolution are caught by a cotton or glass-wool plug impregnated with lead acetate.

The absorption of arsine in pyridine solution of silver diethyldithiocarbamate (the ligand L) was investigated in detail (*2, 3*). It is based on the following reactions

$$AsH_3 + 6AgL + 3B = AsAg_3 \cdot 3AgL + 3L^- + 3HB^+$$

$$AsAg_3 \cdot 3AgL + 3B = 6Ag + AsL_3 + 3L^- + 3HB^+$$

where B is a suitable base (= pyridine). Colloidal form of metallic silver is responsible for the final red–brown color of the absorption solution (*2, 3, 16*). The absorption peak is relatively flat with a maximum at 535–540 nm. It is affected by the purity of silver diethyldithiocarbamate; the contamination with silver nitrate shifts the peak to higher wavelengths (*6*). The volume of hydrogen necessary to sweep arsine into the pyridine solution was estimated as 750–1000 ml (for 3 g of zinc and 42 ml 1.6–2.2 M hydrochloric acid) (*7*). The reduction time varies due to the different conditions and interferences between 30 and 180 min. Quinoline, di-

0026-265X/79/010080-08$01.00/0

methylformamide, and several other amines (*2*, *5*) have been tested in the place of pyridine, however, the price and volatility of some of those solvents and/or decrease in sensitivity of the method result in the fact that pyridine is far the most frequently used solvent in spite of its disagreeable odor. Even the attempts to substitute silver diethyldithiocarbamate by some other compound have been not successful (*5*, *17*). Most of the other thio- and mercapto-compounds are decomposed by a base within the formation of silver sulfide.

The interference of other metallic ions is the most controversial aspect of the silver diethyldithiocarbamate method (*1*, *6*, *9*, *12*, *15*, *18*). For that reason we have studied that problem in detail and presented our results in this paper.

MATERIALS AND METHODS

Reagents. Silver diethyldithiocarbamate was prepared by dissolution of 22.5 g sodium diethyldithiocarbamate (trihydrate) in 250 ml of water and addition of 17.0 g silver nitrate in 250 ml water within a mechanical stirring. After 2 hr standing the reaction mixture was filtered with suction by means of a medium sintered-glass filter. The precipitate was washed with 500 ml of water (negative brucine–nitrate reaction) and dried at room temperature in a vacuum desiccator for 24 hr. The 0.5% (w/v) solution of the reagent in pyridine (GC spectrophotometric quality) had been prepared every third day and kept in dark. Granular zinc −30 mesh contained less than 0.1 ppm of arsenic. The solution of stannous chloride had been prepared by dissolution of 45 g salt (dihydrate) in 75 ml concentrated hydrochloric acid and 150 ml water. The aqueous solution of potassium chloride was 20% (w/v). The aqueous stock solutions of metal salts had been prepared in the usual way and contained 1000 ppm (w/v) of the corresponding metal. The lower concentrations had been prepared by a subsequent dilution of those solutions. The final standard solution of arsenic(III) contained 1.00 ppm of that element. The Baker's Ultrex and Analyzed Reagents had been used for preparation of all solutions.

Instruments. A standard arsenic apparatus Model JA-9540 (SGA Scientific Co., Bloomfield, N.J.) has been used for determinations of arsenic. The glass-wool plug had been 20 mm high and contained 2–3% (w/w) of lead acetate. Photometric measurements had been made with a double-beam Cary 118C spectrophotometer (Varian Assoc., Palo Alto, Calif.) and 1-cm glass flow-cell. Extractions had been accomplished with a S-1070 shaker (Eberbach Corp., Ann Arbor, Mich.). X-Ray measurements had been performed with a Universal X-Ray Vacuum Spectrometer (Norelco Corp., Mount Vernon, N.Y.) containing a chromium target tube and data control and processor. For parameters of measurement of individual elements see (*4*).

Procedure. A sample containing 10–100 μg of arsenic is dissolved in 20 ml of concentrated nitric acid and 10 ml of 50% hydrofluoric acid in a 250-ml Teflon beaker. The solution is evaporated to dryness, 20 ml of concentrated hydrochloric acid is added, evaporated to dryness again, and this is repeated twice more. For samples containing more than 100 μg of antimony, phosphorus, selenium, and tellurium see under Note. Finally the residue is dissolved in 10 ml of hydrochloric acid and 20–30 ml of water, filtered if necessary, and diluted to mark in a 100-ml volumetric flask. A 10-ml aliquot is placed into the flask of the arsenic apparatus, 5 ml of concentrated hydrochloric acid, 10 ml of water, and 5 ml of potassium iodide are added, the solution is mixed, and free iodine is removed by addition of 0.5–2.0 ml of stannous chloride solution with manual shaking. Meanwhile, 3-g portions of granular zinc are prepared (according to the number of solutions) and exactly 5-ml portions of silver diethyldithiocarbamate solution are placed into the corresponding number of arsenic apparatus absorbers connected with the lead acetate plug interabsorption. Zinc is placed by means of a dry funnel (5-mm internal stem diameter) into each flask as quickly as possible and immediately the flask is connected with the prepared absorbers. The evolution of hydrogen-arsine and absorption are maintained for 30 min. Then the arsine absorber is disconnected and the absorbance of the solution is measured at 540 nm in a 1-cm flow-cell against a reagent blank. The absorbance corresponding to 1 μg of arsenic is about 0.031–0.032. Lower absorbances are caused by the poor quality of silver diethyldithiocarbamate and/or pyridine. The standard should be run if not every day then at least with every new solution of silver diethyldithiocarbamate.

Note. If those elements are present arsenic must be separated by extraction of arsenic(III) chloride. After the last evaporation the residue is dissolved in 20 ml concentrated hydrochloric acid containg 1.5 g cuprous chloride and 50% (w/w) hypophosphorous acid is dropwise added until the solution brightens. Then it is extracted with two 10-ml portions of dry *p*-xylene (5 min shaking for each portion) and from combined extracts arsenic is reextracted into water by shaking with three 20-ml portions of water (5 min shaking for each portion). Combined aqueous extracts are placed into 100-ml volumetric flask, the solution is diluted to mark with water and mixed, and the subsequent arsenic determination is the same as above.

RESULTS AND DISCUSSION

The spectrum of colloidal silver formed from silver diethyldithiocarbamate by arsine and stibine is shown in Fig. 1. The rate of evolved hydrogen and arsine varies with the quality and quantity of interferences [see

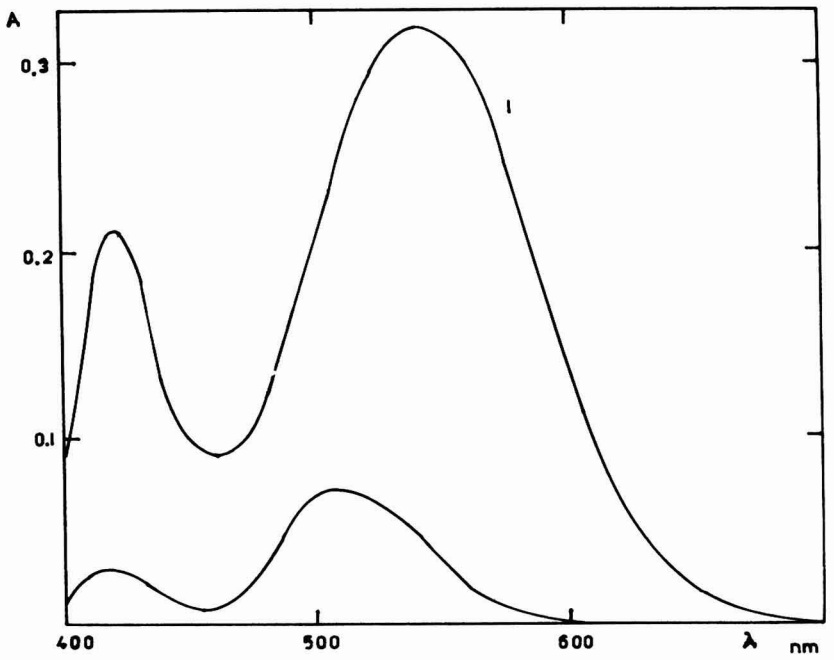


FIG. 1. Absorbance curves of the colloid silver corresponding to 10 μg of arsenic (1) and 10 μg of antimony (2). The reduction was performed in the absence of potassium iodide and stannous chloride.

below (d)]. Typical values expressed as absorbance at 540 nm corresponding to 10 μg of arsenic (5 ml concentrated hydrochloric acid, 5 ml potassium iodide, 0.5 ml stannous chloride, 10 ml water, and 3 g zinc) with time of reduction in minutes given in parentheses are as follows: 0.220 (5), 0.290 (10), 0.310 (15), 0.315 (20), 0.318 (25), 0.318 (30). The effect of various interferences is illustrated in Table 1.

Interference of other elements with the method occurs in four ways:

(a) Element forms volatile hydride that is also reducing silver diethyldithiocarbamate to colored colloidal silver. The interference of this type exists with antimony and phosphorus. Appreciable amounts of those elements (> 50 μg) must be removed by the extraction of arsenic.

(b) Element forms volatile hydride that forms an unidentified complex with silver diethyldithiocarbamate. The interference of that kind takes place with germanium, tellurium, and selenium. The amounts of those elements exceeding 30 μg (germanium) or 20 μg (tellurium and selenium) must be removed either by volatilization (germanium by repeated evaporation with hydrochloric acid) or by arsenic extraction (tellurium and selenium).

TABLE 1
EFFECT OF INTERFERENCES[a]

Element	Used as	Absorbance at 540 nm (mg of element)
None	—	0.096(3)[b], 0.159(5), 0.318(10), 0.474(15), 0.789(25)
Ag[c]	Ag_2SO_4	0.159(0.1), 0.157(1), 0.160(10)
Bi[c]	$BiCl_3$	0.155(0.1), 0.158(1), 0.125(10)
Co	$CoCl_2$	0.157(0.1), 0.155(1), 0.159(10)
Cu	$CuCl_2$	0.157(10), 0.159(25), 0.157(50), 0.154(100)
F	KF	0.155(0.1), 0.157(10), 0.155(100)
Fe	$FeCl_3$	0.160(10), 0.159(25), 0.156(50), 0.145(100)[d]
Ge	GeO_2	0.154(0.02), 0.160(0.05), 0.186(0.1)
Hg[c]	$HgCl_2$	0.152(0.005), 0.154(0.05), 0.151(0.5)
I	KI	0.153(150), 0.152(300), 0.151(1500), 0.152(3000)
Mo	$(NH_4)_6Mo_7O_{24}$	0.154(0.1), 0.151(1), 0.132(10)[e]
Ni	$NiCl_2$	0.152(0.01), 0.150(0.1), 0.155(1)
P	K_2HPO_4	0.153(0.01), 0.148(0.05), 0.122(0.1)
Pb	$PbCl_2$	0.156(0.01), 0.150(0.1), 0.152(1)
Pd	$PdCl_2$	0.155(0.01), 0.155(0.1), 0.150(1)
Pt	H_2PtCl_6	0.157(0.01), 0.150(0.1), 0.149(1)
S[f]	Na_2S	0.155(0.01), 0.150(0.05), 0.133(0.2), 0.090(0.5)
S	Na_2SO_4	0.152(200), 0.158(1000), 0.156(3000)
Sb[c]	$SbCl_3$	0.158(0.03), 0.162(0.05), 0.174(0.08), 0.187(0.1)[d]
Se	SeO_2	0.156(0.01), 0.138(0.05), 0.106(0.1)
Sn	$SnCl_2$	0.155(7), 0.158(40), 0.154(160)
Te	TeO_2	0.156(0.01), 0.138(0.05), 0.102(0.1)

[a] 5.0 μg As, 5 ml 20% KI, and 0.5 ml 15% $SnCl_2 \cdot 2H_2O$ has been used unless otherwise stated.
[b] In this line the numbers in parentheses indicate the micrograms of As.
[c] 10 ml 20% KI has been used.
[d] Acceleration of the reaction with increasing concentration of the element.
[e] Slow-down of the reaction with increasing concentration of the element.
[f] Without any oxidation.

(c) Element forms volatile hydride that gives a complex (usually insoluble) with silver. Sulfur(II) and selenium(II) represent this type of interference [besides that selenium(II) also reacts as indicated in (b)]. In practice a great deal of it is controlled by the cotton or glass-wool plug impregnated with lead acetate. Sulfur(II) is transformed to sulfate by oxidation with concentrated nitric acid.

(d) Ion of the corresponding element M^{n+} accelerates the dissolution of metallic zinc by means of reaction

$$\frac{n}{2}Zn + M^{n+} = \frac{n}{2}\,Zn^{2+} + M. \tag{3}$$

However, that acceleration is stopped as soon as appreciable amount of

TABLE 2
EXTRACTION OF SOME METAL CHLORIDES[a]

Compound	Percentage extraction (solvent)[b]	Compound	Percentage extraction (solvent)[b]
$AsCl_3$	91.0(B), 91.0(X)	$TeCl_4$	0.8(B), 0.7(X)
	83.0($CHCl_3$), 76.0(CCl_4)	CuCl	0.2(B), 0.1(X)
	54.3(iso-octane)	$CuCl_2$	0.1(B), 0.1(X)
$GeCl_4$	56.7(B), 56.3(X)	$FeCl_2$	0.1(B), 0.1(X)
$SbCl_3$	0.8(B), 0.3(X)	$FeCl_3$	0.1(B), 0.1(X)
$SeCl_4$	11.7(B), 13.9(X)	$SnCl_2$	0.1(B), 0.1(X)
		$SnCl_4$	0.1(B), 0.4(X)

[a] Concentration 0.05 *M* of the corresponding chloride in 12 *M* hydrochloric acid (aqueous phase), equal volumes of aqueous and organic phase, temperature of 20–21°C, and time of shaking 10 min had been used throughout. The yield extraction had been calculated from the X-ray fluorescence intensity measurement of aqueous phase before and after extraction.
[b] B, benzene; X, *p*-xylene.

deposited *M* is formed to cover the surface of zinc. For that reason the result of arsenic determination is usually low. The equilibrium constant β of reaction (3) is described by standard redox potentials E°_{M} and E°_{Zn}

$$\log \beta = nF(E^{\circ}_{M} - E^{\circ}_{Zn})/(2.303RT) =$$
$$\log ([Zn^{2+}]^{n/2}[M][Zn]^{-n/2}[M^{n+}]^{-1})$$

where *F* is the Faraday constant (96,487 C), *R* is the gas constant (8.3143 $J \cdot K^{-1}$ mol^{-1}), and *T* is the absolute temperature (in K°). This kind of interference occurs with all metal ions with the value of standard redox potential more positive than the value of zinc (−0.763 V for 25°C). That means it occurs with ions Ag^{+}, Bi^{3+}, Co^{2+}, Cr^{3+}, Cu^{+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mo^{3+}, Mo^{4+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Pd^{2+}, Pt^{4+}, Sb^{3+}, Sn^{2+}, Sn^{4+}, etc. Some of those ions (Ag^{+}, Bi^{3+}, Cu^{+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Pd^{2+}, Pt^{4+}, Sb^{3+}) form very stable complexes with iodide so that a great deal of their interference can be eliminated in that way. The concentration of potassium iodide can be varied in a wide range (see Table 1). For really large excess of many of those ions the arsenic extraction is a very efficient method of separation (see Table 2). The reduction of arsenic(V) with cuprous chloride and hypophosphorous acid was described by Nall (*13*).

It is suggested (*1*) that the effect of some interferences like iron, copper, nickel, and cobalt can be eliminated by running the standards in the presence of the same amounts of those elements. However, that alternative does not remove the basic drawback that is in nonquantitative evolution of arsine and very fast reaction on the beginning of reduction. The amounts of tin exceeding 1.5 ml of the stannous chloride solution acceler-

TABLE 3
DETERMINATION OF ARSENIC IN SOME COPPER INTERMEDIATES

Material	ppm (w/w) As		Material	ppm (w/w) As	
	a	*b*		*a*	*b*
Copper ore	3.1	2.9	Reverberatory matte	30.1	28.5
Copper concentrate	57	59	Converter flux	10.6	12.1
Reverberatory dust	1160	1150	Anode copper	3.9	4.7
Converter dust	2570	2590			

[a] Method of this paper.
[b] Flameless atomic absorption spectrometry.

ates the reduction beyond an acceptable limit. Volatile oxidants (nitric or perchloric acid) must be removed by evaporation as much as possible because of their detrimental effect on the reduction.

The developed procedure has been tested with some intermediates of copper production and compared with flameless atomic absorption spectrometry (*14*). The results are collected in Table 3. A reasonable agreement can be seen.

The distillation method of arsenic separation, for copper intermediates, with iodometric finish gives relatively 10–100% higher results than the described method. On the other hand, molybdenum blue finish results in a 10–20% increase. Those facts indicate that the distillation is not that efficient a method of separation as is sometimes supposed.

SUMMARY

The effect of 22 interferences had been studied. Sulfur(II) must be oxidized by nitric acid; germanium, nitric acid, and perchloric acid must be removed by repeated evaporation with hydrochloric acid. Antimony, phosphate, selenium, tellurium, iron, tin, copper, nickel, and many other elements are eliminated by extraction of arsenic(III) chloride in concentrated hydrochloric acid with *p*-xylene or benzene. Metal ions forming complex or insoluble iodide can be blocked with an excess of potassium iodide. The method enables the determination of 2–30 μg of arsenic with a relative error < 10%. It was tested with the materials of copper production.

REFERENCES

1. Bhargava, O., Donovan, J. F., and Hines, W. G., Rapid spectrophotometric determination of arsenic in iron and steel. *Anal. Chem.* **44**, 2402–2404 (1972).
2. Bode, H., and Hachmann, K., Photometric arsenic determination with silver diethyldithiocarbamate (in German). *Z. Anal. Chem.* **229**, 261–266 (1967).
3. Bode, H., and Hachmann, K., The reaction between arsine and silver diethyldithiocarbamate (in German). *Z. Anal. Chem.* **241**, 18–30 (1968).
4. Budesinsky, B. W., Dilution method in x-ray fluorescence spectrometry. *Microchem. J.* **23**, 469–472 (1978).
5. Budesinsky, B. W., Unpublished results, 1974–1978.

6. Dubois, L., Teichman, T., Baker, C. J., Zdrojewski, A., and Monkman, J. L., Interferences in the measurement of arsenic by the method of Vašák and Šedivec. *Mikrochim. Acta* **1969,** 185–192.
7. Dubois, L., Teichman, T., and Monkman, J. L., Determination of arsenic by the Vašák and Šedivec method (in French). *Mikrochim. Acta* **1966,** 415–425.
8. Fassel, V. A., Current and potential applications of inductively coupled plasma atomic emission spectroscopy. *Pure Appl. Chem.* **49,** 1533–1545 (1977).
9. Gastinger, E., Spectrophotometric determination of arsenic with silver diethyldithiocarbamate (in German). *Mikrochim. Acta* **1972,** 526–543.
10. Gutzeit, H. W., Determination of arsenic (in German). *Pharm. Z.* **24,** 236–238 (1879).
11. Kodama, K., "Methods of Quantitative Inorganic Analysis," p. 195. Wiley, New York, 1963.
12. Kopp, J. F., Ephedrine in chloroform as solvent for silver diethyldithiocarbamate in the determination of arsenic. *Anal. Chem.* **45,** 1786–1787 (1973).
13. Nall, W. R., An improved method for the determination of arsenic in steel. *Analyst* **96,** 398–402 (1971).
14. Ratcliffe, D. B., Byford, C. S., and Osman, P. B., The determination of arsenic, antimony and tin in steels by flameless atomic absorption spectrometry. *Anal. Chim. Acta* **75,** 457–459 (1975).
15. Skonieczny, R. F., and Hahn, R. B., "Arsenic in Treatise on Analytical Chemistry" I. M. Kolthoff and P. J. Elving, eds.), Part II, Vol. 10, p. 259. Interscience-Wiley, New York, 1978.
16. Vašák, V., and Šedivec, V., Colorimetric determination of arsenic (in Russian). *Collection Czech. Chem. Commun.* **18,** 64–72 (1953).
17. Vašák, V., New color reactions for the detection of phosphine (in Czech). *Chem. Listy* **50,** 1116–1119 (1956).
18. Warner, P. O., "Analysis of Air Pollutants," p. 157. Wiley, New York, 1976.
19. Yoe, J. H., "Photometric Chemical Analysis," p. 125. Wiley, New York, 1928.

MICROCHEMICAL JOURNAL 24, 88–91 (1979)

A Simple and Easily Fabricated Device for Desorbing Volatiles from Porous Polymers and Other Adsorbents

L. J. PRIESTLEY, JR., AND B. E. WILKES

Union Carbide Corporation, Chemicals and Plastics Division, Research and Development Department, Technical Center, P. O. Box 8361 South Charleston, West Virginia 25303

Received September 18, 1978

A device has been developed for the rapid desorption of organic compounds from adsorbent traps. Other advantages over previous devices (*1–3*) include its ease of fabrication from inexpensive and readily obtainable materials, and its high portability between instruments. No major modification of the GC inlet system is necessary and in a matter of seconds the desorber can be removed from the injection port for normal syringe injections. The adsorbent tubes can be transported into the field for sampling and returned to the laboratory for desorption and analysis by GC or GC-MS. Additionally, the device is useful for headspace analysis, volatile organics analysis in aqueous solutions, and impurities in gaseous feedstreams.

In other more complex desorption systems (*2, 3*) the sample tube is desorbed over a period of 5 to 10 min and the eluents are trapped in a sample loop at liquid nitrogen temperature. The sample loop is then heated to release the sample to the gas chromatographic column. With the system described here, the sample trap is positioned in the cool portion of the desorber to purge air from both the trap and the desorber for about 2 min. The trap is then manually moved into the heated zone to achieve the desorption within a few seconds. The sample is quickly desorbed in a "slug" to the entrance of the gas chromatographic column. The temperature used in this laboratory to desorb the traps is 275°C. Temperatures to 300°C, however, can be used if required.

EXPERIMENTAL

Traps. The use of Tenax-GC as an adsorbent for the concentration of volatile organic compounds is well known (*1–3*). The adsorptive capacity of Tenax-GC is rather low when compared to that of other porous polymers with greater surface areas [such as Chromosorb 105 (*3*)]; therefore, compounds having a medium volatility (such as benzene) are not wholly retained. Chromosorb 105 has a greater adsorptive capacity than Tenax-

0026-265X/79/010088-04$01.00/0

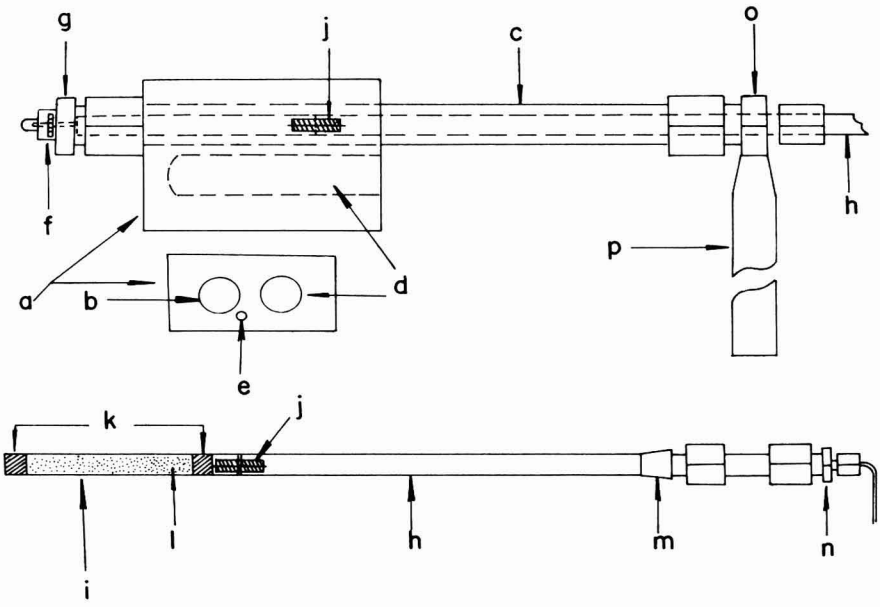


FIG. 1. Diagram of desorber. The parts shown in the diagram are not drawn to scale; however, the necessary dimensions are shown here. All parts are of stainless steel except where noted. (a) Aluminum heater block (1 × 1.5 × 3 in.); (b) hole for oven tube (3/8-in.); (c) oven tube (3/8-in.-o.d. × 8-in. tubing); (d) hole for 250-W cartridge heater (3/8 × 2.5 in. deep); (e) hole for thermocouple (1/16 × 1 in. deep); (f) luer hub fitting; (g) Swagelok tubing cap (3/8 in.); (h) probe (1/4-in.-o.d. × 7-in. tubing); (i) adsorbent trap (1/4-in.-o.d. × 3-in. tubing, tapped one end 10–32); (j) bolt (10–32 × 1/2-in. long with a 1/16-in. hole through the center); (k) glass wool plugs; (l) adsorbent; (m) Teflon front ferrule; (n) Swagelok reducing union (1/4 × 1/16 in.); (o) Swagelok reducing union (3/8 × 1/4 in); (p) tubing (3/8-in. o.d. × 8 in., flattened on one end and silver soldered to 3/8 × 1/4-in. reducing union). This tubing is clamped to suitable lab-stand.

GC, but does not have the thermal stability often required for desorption of compounds with lower volatility. In order to obtain both adsorptive capacity and temperature stability, this laboratory is using a mixture of 1:2 silica gel to Tenax-GC for adsorbent traps. The mixed-adsorbent traps are baked, between uses, at 300°C for a minimum of 1 hr with a N_2 or He purge.

Desorber design. The design of the desorbing device is presented in Fig. 1. The heater block is a 1 × 1.5 × 3-in. block of aluminum drilled for the 3/8-in. oven tube and drilled with blind holes for the cartridge heater and thermocouple. The temperature is controlled by a variable voltage auto-transformer and is monitored by a suitable pyrometer. After assembly of the device, the heater block is insulated with 1/2-in. thick glass wool pad, or with other suitable insulation.

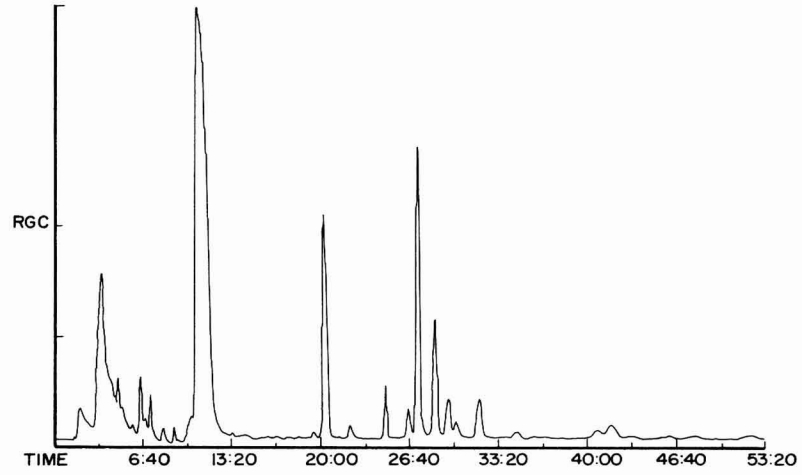


FIG. 2. Reconstructed chromatogram of volatile organics purged from a sample of untreated waste water and desorbed from the sample trap by the device described here. This reconstruced chromatogram was obtained on a Finnigan 4000 GC-MS, interfaced with an INCOS data system.

A luer hub fitting, removed from a 20-ml glass syringe, is used on the injection end of the desorber to allow attachment of a 20-gauge hypodermic needle. A needle smaller than 20 gauge offers too much restriction to carrier gas flow. The cup end of the luer hub is removed and the flange is filed flat. The luer hub is then centered on a 3/8-in. Swagelok tubing cap where it is attached by silver soldering. A 1/16-in. hole is then drilled through the cap.

The end of the probe unit is tapped with a 10–32 bolt tap, as is the adsorbent tube. A 1/16-in. hole is drilled through the bolt which is then screwed into the probe tip. The bolt is locked into the probe by dimpling the 1/4-in. tubing with a center punch or by drilling just through the tubing into the bolt and silver soldering.

Plumbing. A suitable two-way valve is plumbed into the carrier gas line downstream of the flow regulator, so that the carrier gas can be routed to the injection port or to the desorber. A suitable length of 1/16-in. tubing is used to connect the desorber to the routing valve. It is desirable to "spring-coil" the tubing in order to provide necessary flexibility.

Sample injection procedure. The trap is screwed onto the probe tip and then inserted into the cool end of the oven assembly. The 1/4-in. tubing nut is then screwed onto the oven fitting "finger-tight." The carrier gas routing valve is then turned to the desorber position and the system is purged for approximately 2 min to remove air from the system. The desorber needle is then inserted into the injection port. The septum retaining nut should be loosened slightly before the septum is pierced and then

retightened; otherwise the 20-gauge needle is likely to be plugged by a sliver of rubber cut from the compressed septum. The sample is now ready for injection. Injection is accomplished by merely pushing the sample probe all the way into the desorber oven. The carrier gas valve can be left in the desorber position throughout the GC or GC/MS run.

RESULTS AND DISCUSSION

An example of a routine application of this desorber is presented in Fig. 2. We have observed no marked differences in the resultant data between the use of the device described here and a commercially available device which we have used in this laboratory. The commercial device has a fixed adsorbent trap and can be used only for "in-laboratory" work such as head space analysis and the analysis of volatile organics from aqueous solutions. Additionally, its trap must be cooled and then heated each time a sample is collected and desorbed. The temperature of the desorber described here remains constant, allowing consecutive analyses to be performed with very little time lag.

SUMMARY

A device has been developed for the rapid desorption of organic compounds from adsorbent traps. Some advantages over previous devices include its ease of fabrication from inexpensive and readily obtainable materials, and its high portability between instruments. No major modification of the gas chromatograph (GC) inlet system is necessary and in a matter of seconds the desorber can be removed from the injection port for normal syringe injections. The adsorbent tubes can be transported into the field for sampling and returned to the laboratory for desorption and analysis by GC or GC-MS. Additionally, the device is useful for head space analysis, volatile organics analysis in aqueous solutions, and impurities in gaseous feed streams.

REFERENCES

1. Bursey, J. T., Smith, D., Bunch, J. E., Williams, R. N., Berkley, R. E., and Pellizzari, E. D., Application of capillary GC-MS computer techniques to identification of organic components in environmental samples. *Amer. Lab.* **9**, 35–41 (1977).
2. Lingg, R. D., Melton, R. G., Kopfler, F. C., Coleman, W. E., and Mitchell, D. E., Quantitative analysis of volatile organic compounds by GC-MS. J.AWWA, **69**, 605–612 (1977).
3. Murray, K. E., Concentration of headspace, airborne and aqueous volatiles on Chromosorb 105 for examination by gas chromatography and gas chromatography–mass spectrometry. *J. Chromatogr.* **135**, 49–60 (1977).

MICROCHEMICAL JOURNAL 24, 92 – 96 (1979)

Indirect Ultraviolet Determination of Cyanide with Mercury Complexes

RAY E. HUMPHREY, GEORGE S. INGRAM, AND DAVID A. WAAK

Department of Chemistry, Sam Houston State University, Huntsville, Texas 77340

Received October 12, 1978

INTRODUCTION

Mercury (II) compounds react with a large excess of bromide, chloride, iodide, or thiocyanate ion to form the corresponding mercurate ion, HgX_4^{2-}. These complexes absorb intensely in the ultraviolet region and have been used for the determination of chloride ion (*4*) and sulfite ion (*3*). The complex formed between mercury (II) and sulfite ion, $Hg(SO_3)_2^{2-}$ also absorbs strongly in the ultraviolet and can be employed for determination of sulfite (*3, 5*). The sulfite complex is very stable so that an excess of sulfite is not required.

In a study of the use of the iodide complex, HgI_4^{2-}, for the ultraviolet determination of mercury (II), it was found that cyanide ion interfered in a nonlinear manner (*6*). In an investigation of mixed ligand complexes involving mercury (II) with iodide and cyanide ions, addition of cyanide to solutions containing mercury (II) and a large excess of iodide decreased the absorbance of the HgI_4^{2-} complex in a somewhat linear manner (*2*). It has been found that cyanide can be determined spectrophotometrically by the decrease in ultraviolet absorption of the HgI_4^{2-} complex on adding cyanide ion (*1*).

We have found that cyanide ion reacts with all of the mercury (II) complexes mentioned above to presumably form mercury (II) cyanide or a mixed complex of some type which does not absorb at the absorption peak of the original species. The decrease in ultraviolet absorption is linear with cyanide concentration in the low parts per million range. The reaction is fast and the complexes involved are reasonably stable.

MATERIALS AND METHODS

Equipment. Ultraviolet absorption was measured with a Beckman ACTA III spectrophotometer.

Chemicals and solutions. The compounds $HgCl_2$, KCN, NaCl, KBr, KSCN, KI, and Na_2SO_3 were the best available reagent chemicals. The pH 6.86 buffer was a solution of 0.025 *M* in both KH_2PO_4 and Na_2HPO_4. The pH 9.01 buffer was a 0.010 *M* $Na_2B_4O_7$ solution. All mercury (II)

0026-265X/79/010092-05$01.00/0

solutions were approximately 0.001 *M* in $HgCl_2$. The complexing anion, with the exception of sulfite, added as the sodium or potassium salt was present in considerably higher concentration than the $HgCl_2$. The mercury (II) solution was 2 *M* in Cl^- for the $HgCl_4^{2-}$ species, 1 *M* in Br^- for $HgBr_4^{2-}$, 0.03 *M* in I^- for HgI_4^{2-}, and 0.25 *M* in SCN^- to yield $Hg(SCN)_4^{2-}$. One $Hg(SO_3)_2^{2-}$ solution had essentially stoichiometric amounts of $HgCl_2$ and Na_2SO_3 while a second solution had a twofold excess of sulfite ion. The solutions of the sulfite complex also contained low concentrations of EDTA, being about 0.1 m*M* in the disodium salt. The solutions of KCN were prepared in distilled water or in a buffer and were usually close to 0.2 or 0.5 m*M*.

Procedure. An appropriate volume of distilled water or buffer solution was placed in a flask. After adding 1.0 to 1.5 ml of the solution of the mercury complex, different volumes of the stock KCN solution were introduced. The final volume in each case was 10 ml. Absorbance values were then measured at the wavelength maximum for the complex being used.

RESULTS AND DISCUSSION

The decrease in absorbance for a solution of each of these mercury complexes was found to be linear with increasing cyanide concentration. The concentration of the mercury complex was chosen so that the absorbance of the stock solution would be 2.0 to 2.5. The decrease in absorbance was divided by the cyanide concentration in the reaction solution to obtain an effective molar absorptivity. This data for the $Hg(SO_3)_2^{2-}$ complex, which was studied most extensively, is shown in Table 1. The sensitivity for cyanide and reproducibility for this species is possibly somewhat better than for the other solutions. Reproducibility, as indicated by recovery data for cyanide from known solutions employing the sulfite

TABLE 1
DATA FOR REACTION OF CN^- WITH $Hg(SO_3)_2^{2-}$

CN^- ($M \times 10^5$)	CN^- (ppm)	*A* (230 nm)	ΔA	ϵ, Effective[a]
—	—	1.94	—	(24,000)[b]
2.0	0.52	1.70	0.24	12,000
4.0	1.0	1.37	0.57	14,000
6.0	1.6	1.09	0.85	14,000
8.0	2.1	0.85	1.09	14,000
10	2.6	0.58	1.36	14,000
12	3.1	0.37	1.57	13,000

[a] ϵ, Effective $= -\Delta A/[CN^-]$.

[b] This value is based on the assumed concentration of $Hg(SO_3)_2^{2-}$ of 8.1×10^{-5} *M*, the concentration of $HgCl_2$ used.

TABLE 2
RECOVERY DATA FOR CN^- WITH $Hg(SO_3)_2^{2-}$

CN^- present (ppm)	CN^- found (ppm)[a]
0.78	0.75
1.3	1.3
1.8	2.0
2.3	2.3
2.9	2.8
3.3	3.1

[a] Wavelength 230 nm.

complex, is presented in Table 2. Concentration ranges for cyanide and effective molar absorptivity values for the HgX_4^{2-} complexes studied are summarized in Table 3.

It is apparent that cyanide forms by far the most stable complex or compound with mercury (II). The sulfite complex would appear to be next in stability. The HgX_4^{2-} species with the halide ions and thiocyanate all require a very large excess of the anion to repress dissociation. Values for the stability constants are shown in Table 4 (*7*). Solutions of $Hg(SO_3)_2^{2-}$ follow Beer's law while solutions of the HgX_4^{2-} show considerable deviation due to dissociation.

The reaction of cyanide with $HgBr_4^{2-}$ and $HgCl_4^{2-}$ complexes showed a greater decrease in absorbance when the bromide or chloride concentration was relatively higher. The dissociation of $HgBr_4^{2-}$ was almost completely repressed, as indicated by the molar absorptivity value, when the ratio of Br^- to Hg(II) was about 1000. The effective molar absorptivity for cyanide using $HgBr_4^{2-}$ was about 5000 when the ratio of Br^- to Hg(II) was 25 as compared to 15,000 in 1 *M* KBr where the ratio was 1000. In the case of the $HgCl_4^{2-}$ species, the calculated molar absorptivity for the complex was 19,000 when the ratio of Cl^- to Hg(II) was 2100 compared to 28,000 for the complex in very concentrated chloride solution (*4*). The "tet-

TABLE 3
SUMMARY OF DATA FOR MONOVALENT ANION COMPLEXES

Complex	Wavelength (nm)	ϵ, Effective[a]	CN^- range (ppm)
$HgBr_4^{2-}$	248	15,000	0.50–4.0
$HgCl_4^{2-}$	230	13,000	0.50–4.0
HgI_4^{2-}	322	5,000	1.0–10
$Hg(SCN)_4^{2-}$	280	7,000	1.0–10

[a] $-\Delta A/[CN^-]$.

TABLE 4
STABILITY CONSTANT VALUES FOR MERCURY (II) COMPLEXES[a]

Ligand	Log K_1	Log K_2	Log K_3	Log K_4
CN^-	18.00	16.70	3.83	2.98
SO_3^{2-}		22.9[b]		
I^-	12.87	10.95	3.67	2.37
SCN^-		17.26[b]	2.71	1.72
Br^-	8.94	7.94	2.27	1.75
Cl^-	6.74	6.48	0.95	1.05

[a] Ref. (6).
[b] Log $K_1 \times K_2$.

rachloromercurate'' solution used for trapping atmospheric sulfur dioxide which contains stoichiometric amounts of $HgCl_2$ and NaCl for the composition $HgCl_4^{2-}$ (*8*) probably contains very little, if any, of the complex.

Cyanide was found to react with $Hg(SCN)_4^{2-}$ in 1 *M* KSCN solution but the sensitivity was no higher than in a 0.25 *M* solution. The reaction between cyanide and HgI_4^{2-} was far less extensive in 1 *M* KI than in 0.03 *M* solution. There was very little reaction in pH 6.86 buffer while the reaction in water showed a lower effective molar absorptivity and poor reproducibility. The reaction of cyanide in the range of 0.3 to 1.4 m*M* with 0.05 m*M* HgI_4^{2-} in 5 *M* NaI solution was reported to decrease the absorbance of the complex in a somewhat linear manner (*2*). Cyanide, when present in a 10-fold molar excess to mercury (II), was found to cause a large negative error in the determination of mercury (II) as the HgI_4^{2-} complex in 1 *M* KI solution (*6*). The decrease in absorption was reported to be a nonlinear function of the cyanide ion concentration (*6*). The application of mercury (II) in the presence of excess iodide for the determination of cyanide was found to be useful from 5×10^{-5} to 5×10^{-4} *M* cyanide ion. The effective molar absorptivity for cyanide appears to be about 1000 based on the data presented (*1*).

A plot of absorbance versus concentration of cyanide for the $Hg(SO_3)_2^{2-}$ complex with a stoichiometric ratio of sulfite to mercury or with a ratio of SO_3^{2-} to Hg(II) of 4.0 extrapolated to zero absorbance at a ratio of CN^- to Hg(II) of 2.0. For the other complexes, where there was a large excess of the complexing anion, the absorbance extrapolated to zero at a ratio of cyanide to mercury of 3–4. The fact that cyanide bonds to mercury (II) in these solutions in competition with the much greater number of other complexing anions is an indication of the much higher stability of the mercury (II) cyanide species formed.

The cyanide–mercury complex reactions were studied in distilled water, a pH 6.86 buffer, and a pH 9.01 buffer. The sensitivity values for the

HgX_4^{2-} species were not appreciably different in the different solutions. Reproducibility and sensitivity for the reaction of cyanide with the sulfite complex were significantly better in the pH 6.86 buffer than in a pH 9.01 buffer or distilled water.

Solutions of the mercury (II) complexes were stable for at least 2–3 weeks. The presence of a small amount of EDTA seemed to improve the stability of the sulfite complex in solution. The absorbance values of the reaction solutions did not change over a period of 10–12 hr.

Sulfide ion was found to react with these complexes, even with the very high levels of the complexing anions, to form the extremely insoluble mercuric sulfide. Sulfite ion had little effect on the absorption of the HgX_4^{2-} species with the high concentration of the monovalent anion present. It does not seem likely that any of these monovalent anions would interact with any of these mercury complexes under the conditions employed in this work.

SUMMARY

Cyanide ion can be determined in the range of 0.50 to 10 parts per million by the decrease in ultraviolet absorption of the mercury complexes $HgBr_4^{2-}$, $HgCl_4^{2-}$, HgI_4^{2-}, $Hg(SCN)_4^{2-}$, and $Hg(SO_3)_2^{2-}$. The decrease in absorbance is linear with cyanide concentration. Reaction of cyanide with the complexes is rapid in pH 6.86 and 9.01 buffers and in water. Solutions of the complexes are reasonably stable.

ACKNOWLEDGMENT

This research was supported in part by the Faculty Research Fund of Sam Houston State University.

REFERENCES

1. Clyde, D. D., and Warner, M. A., Spectrophotometric determination of cyanide by use of aqueous potassium iodide and mercury (II) iodide. *Analyst* **103**, 648–651 (1978).
2. Coleman, J. S., Penneman, R. A., Jones, L. H., and Kressen, I. K., Mixed ligand complexes in mercury (II) cyanide-iodide solutions. A Raman and ultraviolet study. *Inorg. Chem.* **7**, 1174–1178 (1968).
3. Hinze, W. L., Kippenberger, D. J., and Humphrey, R. E., Determination of sulfite by reaction with mercury (I) chloride and spectrophotometric measurement of mercury (II) complexes. *Microchem. J.* **20**, 43–49 (1975).
4. Humphrey, R. E., Clark, R. R., Houston, L., and Kippenberger, D. J., Mercuric iodate as an analytical reagent. Measurement of chloride ion by ultraviolet absorption of mercury complexes. *Anal. Chem.* **44**, 1299–1301 (1972).
5. Okutani, T., Ito, S., and Utsumi, S., Ultraviolet spectrophotometric determination of micro amounts of sulfite. *Nippon Kagaku Zasshi* **88**, 1296–1299 (1967).
6. Pappas, A. J., and Powell, H. B., Spectrophotometric determination of mercury (II) in aqueous potassium iodide media. *Anal. Chem.* **39**, 579–581 (1967).
7. Sillen, L. G., and Martell, A. E., Stability constants of metal-ion complexes. *Chem. Soc. Special Publ., No. 17* (1964).
8. West, P. W., and Gaeke, G. C., Fixation of sulfur dioxide as disulfitomercurate (II) and subsequent colorimetric estimation. *Anal. Chem.* **28**, 1816–1819 (1956).

MICROCHEMICAL JOURNAL 24, 97–101 (1979)

A Cellulose Column Cleanup for Organophosphorus Pesticides

H. M. STAHR, M. GAUL, W. HYDE, AND R. MOORE

Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa 50011

Received July 17, 1978

INTRODUCTION

Since the inception of pesticide analysis, the need to remove extraneous materials has been felt. The most widely used system (Florisil) (*2*) of cleanup of pesticide extracts removes mercapto groups from organophosphorus pesticides (*3*).

A carbon column is described by Sherma (*3*), which is useful for cleanup but involves the use of obnoxious chemicals and finely divided carbon, a most unpleasant laboratory reagent. Reichling (*2*) has suggested a zinc carbonate cleanup, but this suffers from the same defects as the Florisil cleanup.

We tried cellulose because it was less reactive than the absorbent mentioned (*3*). Cellulose poses no problems in handling in the laboratory.

REAGENTS AND APPARATUS

Whatman cellulose CF-11 activated by heating in a vacuum oven overnight at 85°C (35 in. of vacuum) and nanograde benzene and hexane (Mallinckrodt) or equivalent, anhydrous granular sodium sulfate (Mallinckrodt) were used. The apparatus used consisted of the following: chromatographic columns (10 × 74 cm) with glass disc and Teflon stopcocks, Kontes pesticide concentrating glassware, Kuderna Danish Snyder columns, and concentration tubes from Kontes, and a Perkin–Elmer 3920 gas chromatograph with glass columns 6 feet long packed with OV-101 (10% on Gas ChromQ) and equipped with phosphorus and sulfur flame photometric detectors.

PROCEDURE

(1) Twenty grams of activated cellulose is placed in 50 ml of hexane, suspended, and decanted into the column. The fluid is withdrawn until the level of solvent is approximately at the top of the cellulose. One inch of anhydrous sodium sulfate is added to the top of the column (see Fig. 1).

(2) The sample in question (e.g., mixed feed) is extracted with 100 ml acetonitrile per 20g of sample. It is blended in an explosion-proof blender for 1 min or shaken for 10 min if already finely divided.

0026-265X/79/010097-05$01.00/0

(3) The sample is filtered into a separatory funnel (an FG-4 Whatman glass fiber filter paper works well). The exact volume which is recovered is recorded.

(4) To the separatory funnel is added 250 ml of 2% NaCl and 100 ml of hexane.

(5) The contents of the funnel are shaken together for 5 min and allowed to separate. The hexane layer is removed.

(6) The hexane layer is concentrated to 0.5 ml by means of Kuderna Danish Snyder column (3 ball) and concentrator apparatus.

(7) The concentrated extract is added to the cellulose column, and 50 ml hexane are added. This eluate is retained.

(8) One hundred milliliters of 8% benzene and 92% hexane are added to the column, allowed to elute from the column, and are saved.

(9) The combined hexane and hexane–benzene solvent samples are concentrated to 1 ml and analyzed by gas chromatography, high performance liquid chromatography, thin-layer chromatography, or GC/mass spectrometry, as appropriate.

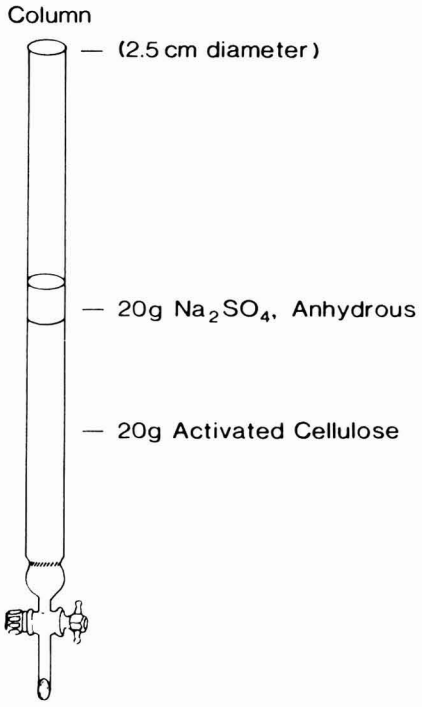


FIG. 1. Diagram of cellulose column.

TABLE 1

PROCEDURE OUTLINE FOR COLUMN PREPARATION

Cellulose CF-11 activated at 85°C, 35 inches of vacuum overnight
Slurry pack column in pet. ether
Add upper layer of anhydrous sodium sulfate

TABLE 2

PROCEDURE OUTLINE FOR SAMPLE PREPARATION

1. Extract sample in 100 ml acetonitrile.
2. Add 250 ml of 2% NaCl in H_20, 100 ml pet. ether; shake.
3. Remove pet. ether layer; repeat with another 100 ml pet. ether; combine pet. ether extracts.
4. Concentrate to 1 ml.
5. Place 0.5 ml on cellulose column.
6. Elute with 100 ml of 8% benzene, 92% pet. ether (volume).
7. Concentrate to 0.5 ml, analyze.

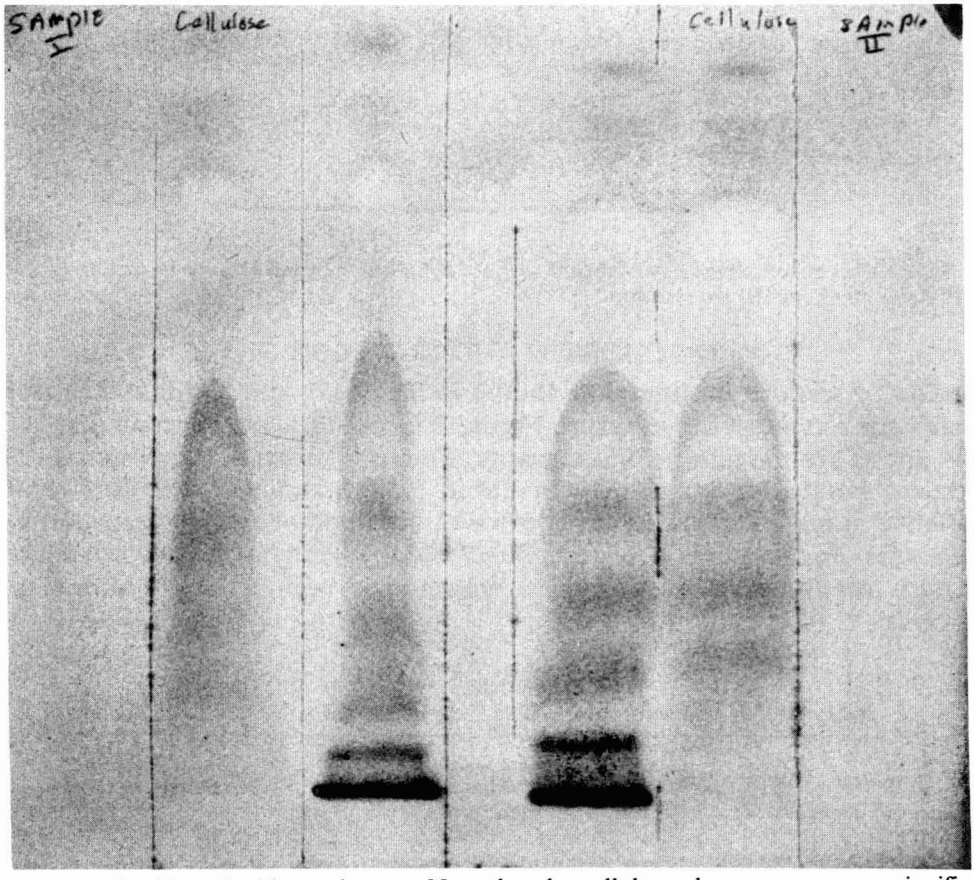


FIG. 2. TLC with and without cleanup. Note that the cellulose cleanup removes a significant amount of extraneous material from the extract (especially low R_f). The two inner bands were not cellulosed.

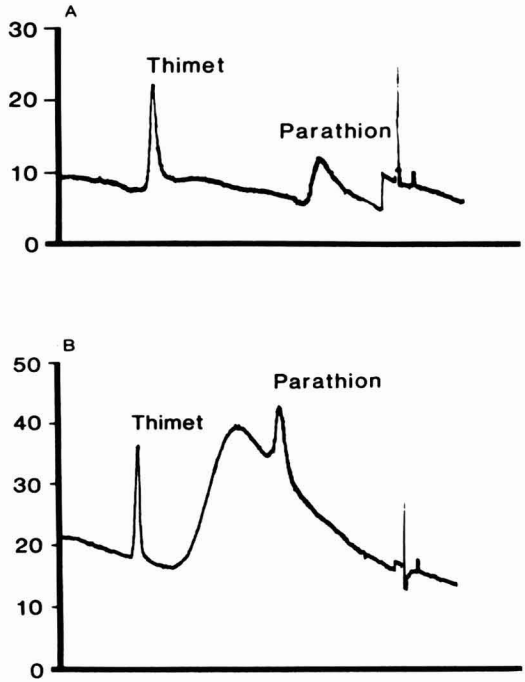


FIG. 3. Gas chromatography with and without cellulose cleanup prior to injection. (A) with cellulose cleanup; (B) no cleanup.

DISCUSSION AND CONCLUSION

The procedure outlines are shown in Tables 1 and 2. Figures 2 and 3 show the extent of the cleanup. Figure 2 is the TLC photograph and Figs. 3A and B are the gas chromatograms. The recoveries of four organophosphorus pesticides are shown in Table 3. The range is 80–90%, and a relative standard deviation of ± 5% was obtained. The recoveries for carbamates are somewhat lower, 50–75%, but the increased cleanliness which results is a significant advantage in mixed feeds and forage samples.

TABLE 3
RECOVERY OF ORGANOPHOSPHORUS PESTICIDES FROM CELLULOSE COLUMN

Pesticide	Cellulose column	Without cellulose column
Thimet	80%	100%
Methyl Parathion	82%	86%
Malathion	87%	95%
Parathion	90%	96%

SUMMARY

A simple, rapid method to clean up toxicological samples has been developed. The method greatly improves the matrix contribution to TLC or GLC analysis.

ACKNOWLEDGMENT

We thank the Biomedical Communications staff of the College of Veterinary Medicine for their help in the preparation of Figures 3A and B.

REFERENCES

1. "Pesticide Analytical Manual," 1978 revision. U.S. Department of Health, Education and Welfare, FDA, 5600 Fishers Lane, Rockville, Md., 1978.
2. Reichling, J., Solubility of basic zinc carbonate for the analysis of pesticide residues. *Fresnius Z. Anal. Chem.* **261**(2), 139–40 (1976).
3. Sherma, J., "Manual of Analytical Quality Control for Pesticides and Related Compounds," EPA 600/1-76-017. EPA, 1976.

MICROCHEMICAL JOURNAL 24, 102–106 (1979)

Potentiometric Determination of EDTA and NTA in Detergents

M. TADDIA, M. T. LIPPOLIS, AND L. PASTORELLI

"G. Ciamician" Chemical Institute of the University, Bologna 40126, Italy

Received September 8, 1978

INTRODUCTION

The sequestering agents EDTA (ethylenediaminetetraacetic acid) and NTA (nitrilotriacetic acid) as sodium salts may both be contained in laundry products, bathroom cleaners, and other detergents. They react with calcium and magnesium in hard water, forming stable and soluble chelates. As water softeners they have partly replaced polyphosphates to reduce eutrophication phenomena. Anyhow they contribute to the pollution problem owing to their toxicity. Methods for evaluation of EDTA and NTA content of detergents are based on complexometric titrations with different ions, in the presence of visual indicators to detect the end-point (*1*, *4*, *5*). Recently an instrumental method based on a spectrophotometric titration has been reported (*2*). The present paper describes a new procedure based on a consecutive potentiometric titration with iron(III), in the presence of ferroin, a platinum electrode being used as an end-point detector. The theory of complexometric titration of EDTA and NTA and results obtained by analyzing synthetic mixtures has been reported (*3*). The difficulties encountered by analyzing EDTA and NTA in detergents are rather enhanced because of the complexity of manufactured products and phosphates interference. The proposed procedure adopts magnesium(II) to remove phosphates as magnesium ammonium phosphate, after conversion of pyro- and triphosphate into orthophosphate by hydrolysis.

EXPERIMENTAL

Reagents. Redistilled water and analytical grade reagents were used. Iron(III) chloride 0.1 *M* in 0.1 *N* sulfuric acid was standardized complexometrically. Ferroin solution was prepared as described (*3*). Nitrilotriacetic acid and disodium ethylenediaminetetraacetate were standardized complexometrically.

Apparatus. Potentials were measured to 0.5 mV with a Knick pH 35 mV/pH meter. A platinum foil of approximately 0.15 cm^2 geometric area was used as indicator referred to a SCE. The reproducibility and stability of the potential measurements were significantly improved by

0026-265X/79/010102-05$01.00/0

storing the platinum electrode in thiosemicarbazide 0.3 *M* in 1 *M* hydrochloric acid. Before use the electrode was rinsed with redistilled water.

Procedure. A 3- to 4-g sample of detergent powder containing not less than 1% EDTA or 0.5% NTA to which 2.5 g magnesium sulfate eptahydrate (for detergents containing not more than 40% sodium triphosphate) was added was stirred for 5 min with 60 ml of water at 50–60°C. The pH was adjusted to 9 ± 0.1 with 0.1 *M* sodium hydroxide. The mixture was transferred to an appropriate test tube and centrifuged at 5000*g* for 10 min. The extract supernate was transferred to a 250-ml conical flask, the residue was twice rinsed with 15 ml of water, and the rinsings were added to the first supernate. To the combined extracts 18 ml of 36% w/w hydrochloric acid was added, and the solution was gently boiled for 30 min and filtered through fast filter paper to remove coagulated fatty material. The filter was rinsed twice with 10 ml warm 25% hydrochloric acid. The solution was cooled to room temperature and the acidity was first neutralized to pH 2–3 with 40% w/v sodium hydroxide then to phenolphthalein with 32% w/w ammonia. An 1-ml ammonia excess was still added, and the precipitation mixture containing magnesium ammonium phosphate was maintained at least 1 hr at room temperature and then filtered or (when necessary) centrifuged. The precipitate was rinsed three times with 15 ml ammonia (1:20) and the combined aqueous phases were transferred in a 250-ml beaker. The pH was first adjusted to 2.5–3.0 with 36% hydrochloric acid then to 4.7 ± 0.1 with 2 ml 1 *M* sodium acetate to obtain a buffered appropriate system. Finally a potentiometric titration was carried out under constant stirring, in the presence of 2 ml ferroin solution, by using platinum–calomel electrodes and 0.1 *M* iron(III) as titrant. The EDTA content was given from the iron consumed to the first inflection point, the NTA from that consumed to the second (see Fig. 1).

RESULTS AND DISCUSSION

Accuracy of the method was checked by adding known amounts of EDTA and NTA to detergent powders free from these sequestering agents. Results are shown in Table 1. For a concentration range from ca. 1.7 to 4.7% EDTA or NTA, the mean recovery was 93% for EDTA and 89% for NTA. Losses during EDTA and NTA determination in detergents are also quite common by employing different methods. Therefore Longman (*5*) suggested to multiplying all results by 1.1, in order to obtain the correct figure. Other authors (*2*) found 8.75% NTA in a commercial sample whose content according to the

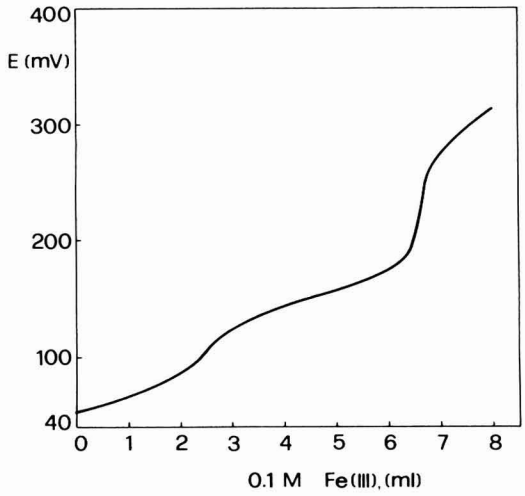


FIG. 1. Experimental titration curve using iron(III) as titrant, for the EDTA and NTA determination in detergents. Shown is the titration of a detergent sample (3.141 g) containing 3.1% EDTA and 2.6% NTA.

manufacturer was 10%. In the present paper the use of magnesium is proposed to remove phosphates as well to reduce EDTA losses. Magnesium reacts at pH 9 with EDTA to form the well-known chelate, so occlusion and coprecipitation in the residue during the extraction step may be limited. When the use of magnesium was omitted the recovery was checked to fall to 73%. The effect over the NTA recovery was negligible according to log $\alpha_{m(L)}$ values at pH 9: 2.6 for MgNTA and 5.8 for MgEDTA (*6*). The presence of magnesium does not interfere in the course of the final titration with iron(III) at pH 5 results: log $\alpha_{MgEDTA} = 0.7$ and log $\alpha_{MgNTA} = 0.1$. An important advantage inherent in the use of magnesium is the possibility of removing phosphate as magnesium ammonium salt. It is known that polyphosphates must be hydrolyzed to orthophosphate to prevent their sequestering effect toward the titrant (*5*).

This procedure needs orthophosphate remotion too, in order to prevent formation of iron phosphates that result in a large positive error for NTA. Remotion of orthophosphate is also advisable when the complexometry with copper(II) and indicators has to be performed, as it makes the color change sharp and stable. In this case lithium was found more convenient than magnesium. It does not form in practice complexes with EDTA and NTA so the conditional constant copper−EDTA or NTA is unaltered. Results obtained by titrating some samples listed in Table 1 with indicators (*5*), after phosphates remo-

TABLE 1

PERCENTAGE RECOVERY OF EDTA AND NTA FROM DETERGENT SAMPLES

Detergent (g)	Percentage sodium triphosphate[a]	EDTA			NTA		
		Added (g)	Found (g)	Percentage recovery	Added (g)	Found (g)	Percentage recovery
3.082	19.0	0.0573	0.055	96.0	0.0535	0.048	89.7
3.036	19.0	0.0693	0.064	92.3	0.0700	0.059	84.3
3.000	19.0	0.0785	0.075	95.5	0.0745	0.065	87.2
3.000	31.3	0.0791	0.074	93.5	0.0621	0.055	88.6
3.002	19.0	0.0905	0.083	91.7	0.0602	0.054	89.7
3.055	31.3	0.0961	0.087	90.5	—	—	—
3.041	31.3	—	—	—	0.0984	0.088	89.4
3.041	31.3	—	—	—	0.0984	0.089	90.4
3.120	19.0	0.0980	0.092	93.9	0.1191	0.109	91.5
3.141	19.0	0.1022	0.092	90.0	0.0872	0.078	89.4
2.989	19.0	0.1046	0.101	96.5	0.1421	0.128	90.1
3.043	19.0	—	—	—	0.1168	0.104	89.0
3.063	19.0	0.1122	0.101	90.0	0.0808	0.071	87.9
3.068	19.0	0.1150	0.107	93.0	0.0950	0.087	91.6
3.036	19.0	0.1388	0.130	93.6	0.0760	0.067	88.2
3.317	19.0	—	—	—	0.1210	0.111	91.7
2.969	31.2	0.1415	0.129	91.2	0.1273	0.110	86.4
2.994	19.0	—	—	—	0.1080	0.096	88.9
				Mean recovery: 93%			Mean recovery: 89%

[a] Determined by alkalimetric titration (*5*).

tion with lithium, confirm those obtained potentiometrically. A commercially available sample containing NTA was examined in order to check the precision of the method proposed in this paper. Results were corrected for an 89% mean recovery. A series of five determinations gave an NTA content of 0.8 ± 0.06%.

SUMMARY

A method is described for the potentiometric titration with iron(III) of EDTA and NTA in detergents, a platinum electrode being used as indicator. EDTA and NTA were extracted at pH 9 and 50–60°C in the presence of magnesium(II). Interference from polyphosphates was minimized by hydrolysis to orthophosphate followed by remotion as magnesium ammonium phosphate. Complexometric titration was carried out at pH 4.7 in acetate medium, in the presence of ferroin. A preliminary cleaning of the platinum electrode with thiosemicarbazide in hydrochloric acid was found to improve significantly the potential measurements. A mean recovery of 93% for EDTA and 89% for NTA was observed.

REFERENCES

1. Clinckemaille, G. G., Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in granular detergent formulation. *Anal. Chim. Acta* **43**, 520–522 (1968). *Acta* **43**, 520–522 (1968).
2. Harju, L., and Sara, R., Determination of nitrilotriacetic acid in detergent formulations. *Anal. Chim. Acta* **91**, 393–396 (1977).
3. Horáček, J., and Přibil, R., Potentiometric determination of NTA, EDTA and DTPA in mixtures. *Talanta* **16**, 1495–1499 (1969).
4. Kump, K. I., Palocsay, F. A., and Gallaher, T. N., Determination of the percent Na_4EDTA in bathroom cleaners. *J. Chem. Ed.* **55**, 265–266 (1978).
5. Longman, G. F., "The Analysis of Detergents and Detergent Products," pp. 403–408, 480–482, 487–488. John Wiley, London, 1975.
6. Ringbom, A., "Les complexes en chimie analytique," pp. 334. Dunod, Paris.

MICROCHEMICAL JOURNAL 24, 107–116 (1979)

The Application of Metal-Coated Graphite Tubes to the Determination of Trace Metals in Biological Materials

I. The Determination of Lead in Blood Using a Tungsten-Coated Graphite Tube

PAUL BAILY,[1] ELSA NORVAL,[2] TERENCE A. KILROE-SMITH,[1] MAURICE I. SKIKNE,[1] AND HELEN B. RÖLLIN[1]

[1] *National Research Institute for Occupational Diseases of the South African Medical Research Council, P.O. Box 4788, Johannesburg, 2000, and* [2] *National Physical Research Laboratory of the Council for Scientific and Industrial Research, P.O. Box 395, Pretoria, 0001, South Africa.*

Received September 11, 1978

INTRODUCTION

Matrix effects have been responsible for many of the difficulties encountered in the determination of trace metals in biological specimens, particularly of lead. Some interferences have been listed, for example (a) the presence of variable amounts of both cations and anions, and (b) light scattering by molecular vapors of salts present (*4, 8*). Fuller (*7*) has commented on the "condition" of the graphite tube and suggested that the vapor phase effect of interfering salts could be overcome by a solid phase reaction or by a soft powdery carbon coating which is present on old graphite tubes. The use of pyrolytically coated furnaces has been advocated since the inception of the technique (*1, 10*) and various evaluation studies of such tubes have been undertaken (*11, 17*). A major disadvantage of this type of coating is that it is progressively removed from the surface during use. Recoating of tubes may require recalibration. Hodges (*8*) altered the characteristics of graphite tubes by pretreatment with ammonium molybdate. On the addition of orthophosphoric acid to the specimen of urine he found that both chlorides and sulfates were removed as free acids during the drying and ashing stages. The pretreatment of the carbon tube with molybdenum resulted in a low nonspecific absorption signal. Other reports of pretreatment of tubes have been published (*3, 14, 16, 18*) but none of these gives details regarding the reproducibility of the treatment process. To counter the interelement effects of alkaline earths, Regan and Warren (*15*) altered the matrix of their specimens by the addition of ascorbic acid at the 1% level. They found enhanced peak height signals ranging from a factor of about 1.4 for lead to 1.95 for copper and 20

0026-265X/79/010107-10$01.00/0

for gallium. However, McLaren and Wheeler (*12*) found that although additives such as ascorbic acid and hydrofluoric acid resulted in considerable enhancement of the absorption signal, the peaks in all instances were double peaks.

In the studies presented here we show that the surface characteristics of untreated graphite tubes are so altered in the presence of a complex matrix such as blood that it is impossible to obtain accurate and reproducible results for lead in blood in an argon atmosphere nor is it possible to use pure acid lead standards for the determination of lead in blood as suggested by Fernandez (*6*). When the surface characteristics of the graphite tube were altered by applying a tungsten coating process (*13*) which is highly reproducible and which results in a tube lifetime of more than 2000 firings (E. Norval and H. G. C. Human, to be published), both accurate and precise results for lead in blood were obtained.

MATERIALS AND METHODS

Instrumentation

Atomic absorption spectroscopy. A Perkin–Elmer Model 603 double-beam atomic absorption spectrophotometer was equipped with an Intensitron hollow cathode lamp as light source operated at 8 mA. Simultaneous background correction was provided by a deuterium arc background corrector which compensated for light scattering and molecular absorption of any undissociated species resulting from the complex blood matrix. The HGA-2200 graphite furnace had an optical temperature sensor for continuous monitoring of the furnace temperature and reproducible temperature control. The power unit (210–240 V, 50/60 Hz, 15 A) was fitted with variable timers for the sequential operation of the three power cycles, drying, charring, and atomization. A variable internal gas flow controller allowed the argon flow inside the graphite tube to be stopped automatically for a set period at the start of atomization. In addition the HGA ramp accessory permitted the gradual increase and holding of temperatures for all three cycles of the furnace operation. A fourth cycle, the auto high temperature stage, could not be used in this operation as it was found that in the automatic operation the graphite tube had not cooled sufficiently to accept the next sample.

An automatic sampler, Perkin–Elmer Model AS-1, was used to dispense 10-μl samples into the graphite furnace. The precision syringe pump was found to take up and accurately dispense the prepared blood specimens with a precision of volume delivery within 1% while the washing system of the AS-1 was found to be adequate to ensure no carryover. Approximately 750 μl prepared blood specimens were contained in small disposable plastic cups in a sample tray capable of holding 30 cups. A Model 56 recorder, which permits full-scale pen travel in less than 0.5 sec,

TABLE 1
HGA-2200 OPERATING PROGRAM

	Drying	Ramp	Charring	Ramp	Atomization	Flow time stop	Integration
Time (sec)	60	50	60	50	7	7	7
Temperature (°C)	150		600		2400	—	—

was used to record all traces and data were logged on a PRS-10 printer sequencer.

HGA-2200 graphite furnace operating program. The operating program of the HGA-2200 graphite furnace is shown in Table 1. Times and temperatures shown were arrived at after several trial runs. For example, the optimum charring temperature was found by measuring the maximum peak height signals for successive temperatures at 100°C intervals given by a 1 ppm standard lead solution in blood while keeping the atomization temperature constant. Similarly, the optimum atomization temperature was found by keeping the previously found charring temperature constant. An atomization time of 7 sec was sufficient for maximum signal and this was combined with a flow time stop of 7 sec during which the internal argon flow was reduced to approximately 48 ml/min. The "high temperature" (2650 ± 100°C) was used only after each set of runs had been completed, that is after 40 firings, to remove any sample residues.

Electron microscopy. Electron microscopy of the residues found in the graphite tubes was carried out with an A.E.I. electron microscope–microprobe analyzer EMMA-4, fitted with an Ortec Model 7000 energy dispersive Si(Li) X-ray fluorescence analyzer. A minilens to focus the electron beam in the microprobe mode enabled a probe beam of 200-nm diameter to be obtained. Energy data were collected and displayed in a multichannel analyzer. It is possible to identify elements of atomic weight greater than or equal to 23 with this instrument. An accelerating voltage of 60 kV was used and the microprobe current was set at 0.2 μA. The probe diameter was 100 nm at 20,000 magnification and counting time for X rays was 100 sec.

Glassware and reagents. All glassware was acid washed overnight in chromic acid and then repeatedly rinsed with water, double distilled in glass. All reagents used, including those for precipitating the proteins in blood, were of analytical-reagent grade with the exception of Triton X-100 (iso-octylphenoxypolyethoxyethanol), which is a British Drug House laboratory reagent.

Standard solutions of lead were prepared by diluting a stock solution, 1000 ppm (m/V) in 0.1 *M* perchloric acid (Hopkins and Williams Ltd., England), with 0.01 *M* hydrochloric acid. The working solutions (1 μg

ml^{-1}, 2 $\mu g\ ml^{-1}$, etc.) were prepared fresh daily and stored in lead-free polyethylene bottles.

Preparation of blood specimens. Two techniques were used for preparing the blood specimens. For the determination by graphite tube, aliquots of lead standard were added to 1.0 ml heparinized blood and thoroughly mixed on a vibration mixer for 2 min. The blood was then made up to 10.0 ml with 0.1% Triton X-100 and again thoroughly mixed as before. Aliquots of the prepared blood, for which the final dilution was to 10 volumes, were placed in disposable plastic cups for total lead content determination.

In order to test the accuracy of the results obtained by the graphite tube method, blood was prepared by the modified Einarsson and Lindstedt method (*5*) previously outlined (*2*). These determinations were then carried out by the flame method and compared with results obtained by graphite tube. All determinations (both flame and graphite tube) were carried out using a wavelength of 283.3 nm.

Preparation of specimens for electron microscopy. Scrapings were made of the residues found in the untreated graphite tubes, care being taken to avoid removal of any pure carbon. The scrapings were finely ground in an agate mortar and the particles were suspended in glass double-distilled water using an ultrasonic vibrator. Droplets of the particle suspensions were then pipetted onto 3.0-mm electron microscope grids, on which carbon-coated collodion support films had been previously mounted. The droplets were allowed to evaporate in a dust-free atmosphere before being placed in the electron microscope microprobe analyzer.

Tungsten-coated graphite tubes. A novel process for coating commercial graphite tubes with tungsten (*13*) has been employed for this study and a description of this process will be given in a subsequent paper. The coating method ensures a more durable surface which eliminates surface interference effects. The functional lifetime of the tube was prolonged by this technique (over 2000 firings at 2700°C under severe corrosive conditions) and significant variations in tube performance from batch to batch were eliminated.

RESULTS

Figure 1 shows the graphs obtained for lead-in-blood results using uncoated graphite tubes when the method outlined by Fernandez (*6*) was followed. Although linear graphs were obtained as shown by graphs A and B, when lead standards in 0.01 *M* HCl were interspersed between the two sets of readings (A and B), the effect of this interspersion was to increase the slope of the graph from B to A. In a subsequent experiment a standard curve was drawn by conditioning the graphite tube with a blood specimen to give a constant value, then reading an acid standard twice. Blood was

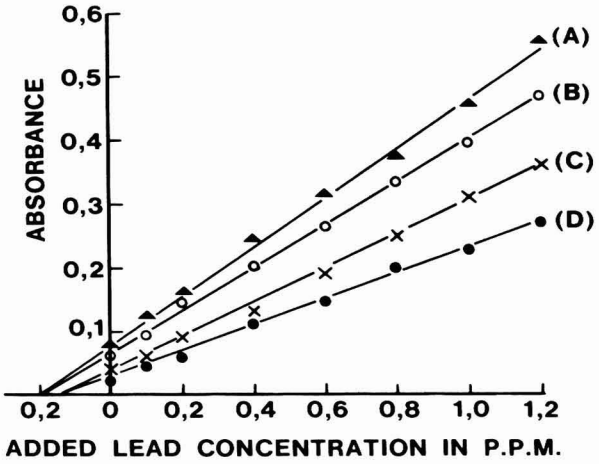


FIG. 1. Lead in blood graphs using untreated graphite tubes. Explanation of graphs A, B, C, and D is given in text.

again introduced until a constant value was obtained and the next acid standard was read twice as before. This was repeated for all subsequent standards. Separate graphs were drawn for the first and second readings of the standards. The two lines were divergent as shown by graphs C and D with the first reading (D) lower than the second (C). Figure 2 illustrates the divergence of the graphs when lead in 0.01 *M* HCl is compared with lead in blood. It is evident that the method suggested by Fernandez (*6*) is not valid particularly from 0 to 80 μg Pb/100 ml blood.

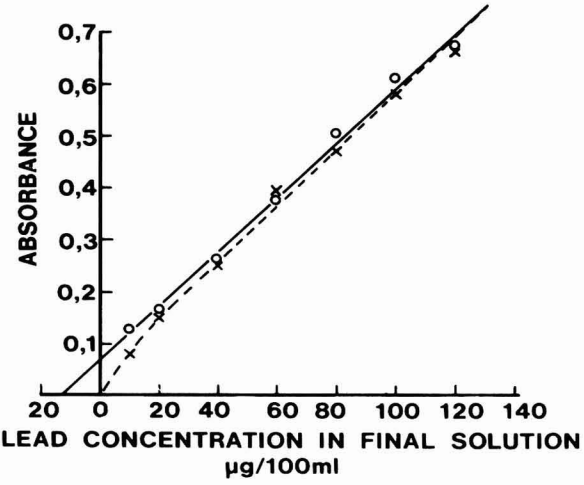


FIG. 2. A comparison of the graphs of lead standards in blood with aqueous (0.01 *M* HCl) lead standards. ×- - - -, Aqueous (0.01 *M* HCl) lead standard; ○ ———, blood lead standard.

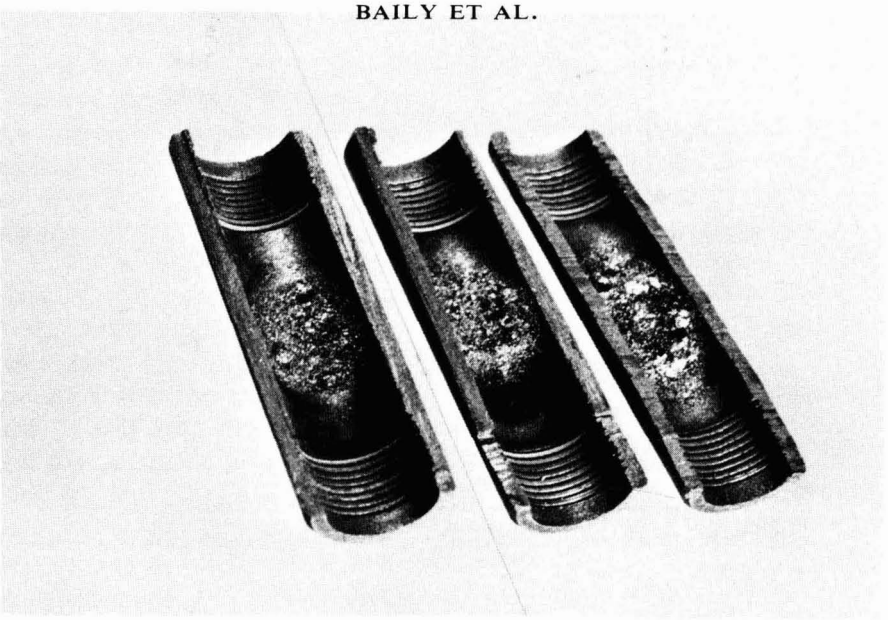


FIG. 3. Longitudinal section of untreated graphite tubes after approximately 200 firings showing residues from blood lead determinations.

Figure 3 shows a longitudinal section of uncoated graphite tubes after approximately 200 firings from determinations of lead in blood and in dilute acid. The residues in the tubes are clearly visible. Electron microscopy of the scrapings taken from the tubes (Fig. 3) shows that two types of particles were qualitatively distinguishable based on their elemental

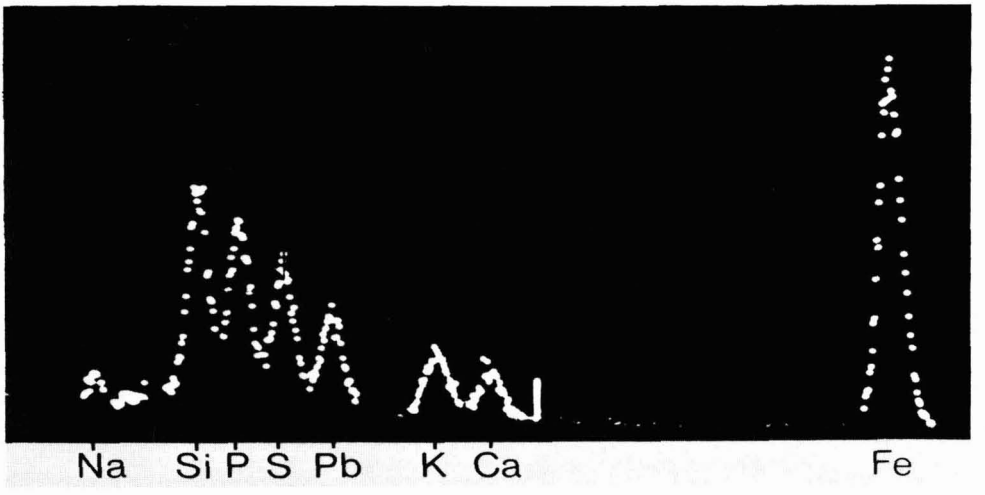


FIG. 4. Energy dispersive Si(Li) X-ray fluorescence analyzer spectrum obtained from scrapings of residues in untreated graphite tubes. Major peaks for Si (1.8 eV), P (2.1 eV), S (2.4 eV), Pb (2.6 eV), and Fe (6.4 eV). Minor peaks for K (3.4 eV) and Ca (3.7 eV).

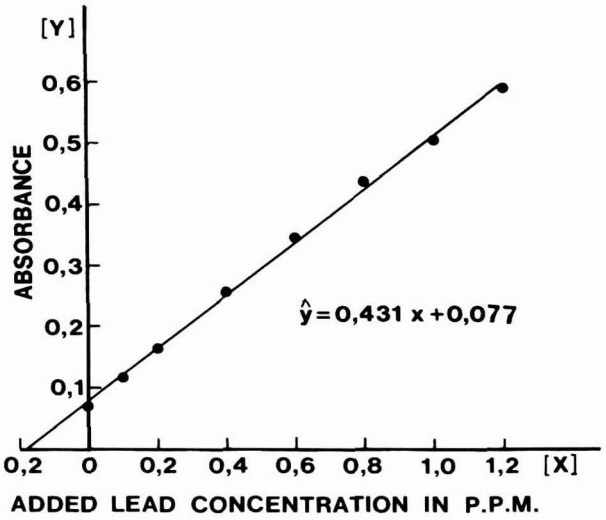


FIG. 5. Calibration graph for lead in blood using tungsten-coated graphite tube. Each point on the graph represents the mean of 50 determinations over a period of 5 days.

spectra. Twenty of each type were assessed. However, both types of particles observed contained the following elements in common: Si (1.8 eV, $K\alpha$), S (2.4 eV, $K\alpha$), K (3.4 eV, $K\alpha$), and Ca (3.7 eV, $K\alpha$). In the one type of particle, extra peaks were present for P (2.11 eV, $K\alpha$), Pb (2.6 eV, $M\beta$), and Fe (6.4 eV, $K\alpha$). In this particle (Fig. 4), the high peak for Fe is indicative of a high concentration of Fe in association with Pb and P. It is possible that the silica found is a contaminant from the agate pestle and mortar. The other elements found, with the exception of lead, are normal physiological constituents of blood.

The tungsten-coated graphite tube used was fired for 652 determinations for lead in blood. Lead in dilute acid was not interspersed with the lead in blood determinations and each point on the linear calibration graph (Fig. 5) is the mean of 50 determinations. The determinations were carried out twice daily for 5 consecutive days and blood specimens were freshly prepared each day by adding the requisite amount of lead standard. It was found that (Fig. 5) $r(6) = 0.999$ and $100r^2 = 99.9\%$ which is the percentage of the variation explained by the regression y on x. The standard error of the straight line y on x ($Sy \cdot x$) is 0.0073. The fact that $100r^2$ is almost 100% shows that the analytical conditions for generating a straight line calibration are excellent. This is further borne out by the very small $Sy \cdot x$ value. Using the above graph, lead was determined on two representative samples of blood, giving 13.3 and 53.1 μg/100 ml, respectively. The flame method gave 11.3 and 50.5 μg/100 ml, respectively.

The confidence intervals for the estimate of Pb in blood using our calibration graph and taking into account all the variances involved within

days and from day to day, as well as operator errors, machine errors, and other undetermined errors, are lowest at the center of the graph and highest at the extremes (Table 2). It also depends on the number of observations taken in determining a value (K) and on the value of α required (where α is the probability of being wrong). Taking the least favorable case where $K = 3$, the interval at the ends of the calibration line is $\pm$ 2.4 μg/100 ml at 5% confidence limit and $\pm$ 3.8 μg/100 ml at 1% limit. At the center of the line the confidence interval is lower. If $K = 6$, the confidence limit drops to 2.1 μg/100 ml at 5% level and 2.9 μg/100 ml at the 1% level at the ends of the calibration line.

With the flame method the confidence interval is about 4 μg/100 ml above or below the mean which represents ca. 1% of full scale deflection on the recorder. Hence the results on the flame compare favorably with the coated graphite tube, the latter showing a higher degree of precision.

DISCUSSION

Fernandez (*6*) described a very convenient method for lead in blood determinations which requires very little preparation of the blood specimens. However, the method recommends the use of a set of aqueous (dilute acid) lead standards in a calibration graph to be used for reading the absorbance values obtained for blood specimens. It was found that this method could not be used with any degree of assurance since the slope of the aqueous standard graph appeared to depend on the history of the graphite tube. Thus if a standard graph was made with aqueous lead standards and the tube was then used for determining lead in blood, the characteristics of the tube appeared to be changed so that a subsequent graph drawn with aqueous standards was different from the previous aqueous standard graph. It was also found that a new untreated tube took about 100 firings before it was stabilized, using a blood matrix. Standard graphs then drawn from the same blood with the stabilized tube gave slopes varying by not more than $\pm$ 10%. However, no other matrix (e.g., lead in dilute acid) could be introduced without altering the tube charac-

TABLE 2
CALCULATED CONFIDENCE LIMITS OF ESTIMATED LEAD IN BLOOD

Average absorbance based on five readings	μg Pb/100 ml blood	Confidence limits	
		At 5% level	At 1% level
0.1	23.2	$\pm$ 2.2	$\pm$ 3.1
0.2	46.4	$\pm$ 1.9	$\pm$ 2.7
0.3	69.6	$\pm$ 1.8	$\pm$ 2.5
0.4	92.8	$\pm$ 1.9	$\pm$ 2.7
0.5	116.0	$\pm$ 2.1	$\pm$ 3.1

teristics since the same blood would then give completely different readings.

The cause of the problem appears to be the residue which accumulates in the tube when blood is analyzed. Apart from the elements found as shown by the electron micrograph (Fig. 4), the residue was found to consist of about 50% carbonaceous material which was oxidizable in air at 500°C. The presence of lead in the residue indicated that the atomization of lead was not completed once it became trapped in this matrix. Koirtyohann and Hopkins (*9*) found losses of trace metals by retention in an acid-insoluble form in the ashing vessel. They concluded that the container material, condition of the surface, type of tissue, and ashing temperature were important variables which affected this loss.

In the study presented here the alteration of the surface characteristics of the graphite tube, by applying a highly reproducible coating which results in a greatly lengthened tube lifetime, appeared to be largely responsible for preventing the formation of the residue found in the untreated tube.

SUMMARY

A new technique is introduced in trace metal determinations in biological materials by coating the graphite tube with tungsten by a highly reproducible process. This coating appeared to be responsible for the prevention of residue formation that occurred in untreated graphite tubes when lead in blood was analyzed. The life of the graphite tube was thus increased by about 300% for this matrix.

ACKNOWLEDGMENT

The authors wish to thank Michael J. A. Andrew of the Institute of Biostatistics of the South African Medical Research Council for assistance with the statistical analyses.

REFERENCES

1. Aspila, K. I., Chakrabarti, C. L., and Bratzel, M. P., Pyrolytic graphite-tube microfurnace for trace analysis by atomic absorption spectrometry. *Anal. Chem.* **44**, 1718–1720 (1972).
2. Baily, P., and Kilroe-Smith, T. A., Effect of sample preparation on blood lead values. *Anal. Chim. Acta* **77**, 29–36 (1975).
3. Cioni, R., Mazzucotelli, A., and Ottonello, G., Matrix effects in the flameless atomic absorption determination of trace amounts of barium in silicates. *Anal. Chim. Acta* **82**, 415–420 (1976).
4. Delves, H. T., A simple matrix modification procedure to allow the direct determination of cadmium in blood by flame micro-sampling atomic-absorption spectrophotometry. *Analyst* **102**, 403–405 (1977).
5. Einarsson, Ö., and Lindstedt, G., A non-extraction atomic absorption method for the determination of lead in blood. *Scand. J. Clin. Lab. Invest.* **23**, 367–371 (1969).
6. Fernandez, F. J., Micromethod for lead determination in whole blood by atomic absorption, with use of the graphite furnace. *Clin. Chem.* **21**, 558–561 (1975).
7. Fuller, C. W., The effect of graphite tube condition on the determination of lead in the presence of magnesium chloride by electrothermal atomic absorption spectrometry. *Atom. Absorpt. Newslett.* **16**, 106–107 (1977).

8. Hodges, D. J., Observations on the direct determination of lead in complex matrices by carbon furnace atomic-absorption spectrophotometry. *Analyst* **102**, 66–69 (1977).
9. Koirtyohann, S. R., and Hopkings, C. A., Losses of trace metals during the ashing of biological materials. *Analyst* **101**, 870–875 (1976).
10. L'vov, B. V., "Atomic Absorption Spectrochemical Analysis," p. 206. Adam Hilger, London, 1970.
11. Manning, D. C., Fernandez, F. J., and Peterson, G. E., Advantages and limitations of pyrolytic graphite coated sampling tubes in atomic absorption spectroscopy. Perkin-Elmer Atomic Absorption Application Study No. 631 (1976). Presented at 3rd Annual FACSS Meeting and XIX CSI and VI ICAS November 15–19, 1976, Civic Centre, Philadelphia, Pa.
12. McLaren, J. W., and Wheeler, R. C., Double peaks in the atomic-absorption determination of lead using electrothermal atomisation. *Analyst* **102**, 542–546 (1977).
13. Norval, E., South African patent application filed (1978).
14. Ortner, H. M., and Kantuscher, E., Metallsalzimprägnierung von graphitrohren zur verbesserten AAS-siliziumbestimmung. *Talanta* **22**, 581–586 (1975).
15. Regan, J. G. T., and Warren, J., A novel approach to the elimination of matrix interferences in flameless atomic-absorption spectroscopy using a graphite furnace. *Analyst* **101**, 220–221 (1976).
16. Stiefel, Th., Schulze, K., Tölg, G., and Zorn, H., Ein verbundverfahren zur bestimmung von beryllium in biologischen matrices durch flammenlose atomabsorptionsspektrometrie. *Anal. Chim. Acta* **87**, 67–78 (1976).
17. Sturgeon, R. E., and Chakrabarti, C. L., Evaluation of pyrolytic-graphite-coated tubes for graphite furnace atomic absorption spectrometry. *Anal. Chem.* **49**, 90–97 (1977).
18. Zatka, V. J., Tantalum treated graphite atomizer tubes for atomic absorption spectrometry. *Anal. Chem.* **50**, 538–541 (1978).

MICROCHEMICAL JOURNAL 24, 117–123 (1979)

Extraction–Spectrophotometric Determination of Palladium with Triphenylphosphine (TPP)

M. MOJSKI AND M. PLESIŃSKA[1]

Department of Analytical Chemistry, Warsaw Technical University, Noakowskiego 3, 00–664 Warsaw, Poland

Received September 18, 1978

INTRODUCTION

The formation of complexes of triphenylphosphine (TPP) and other phosphines with noble metals follows from Ahrland's (*1*) and Pearson's (*5*) general rule of affinity of "soft" bases to metal ions that are "soft" acids. Some of these compounds have been used for extraction of silver (*2*) and gold (*8*).

Extraction of palladium (II) complexes from halide medium with triphenylphosphine and its arsenic and antimonic analogs in various organic solvents has been observed by Senise and Levi (*6*). Extraction of iodide complex of palladium with triphenylarsine has been used for a spectrophotometric determination of this metal (*7*). The interference of small amounts of platinum with the determination of palladium can be eliminated by sulfite addition.

The extraction efficiency of halide complexes of palladium and platinum with neutral solvents, e.g., dialkyl sulfides (*4*), increases in the order: chlorides, bromides, iodides. From the viewpoint of selectivity it is then of advantage to carry out the extraction from chloride medium, provided that the extraction efficiency is high enough.

The studies carried out in this work on the extraction of palladium with TPP solution in benzene from chloride medium were aimed at elaboration of a simple spectrophotometric method of the determination of palladium under conditions of limited interference of platinum.

MATERIALS AND METHODS

Reagents. Triphenylphosphine (TPP) (Chemapol, Chechoslovakia) was reagent grade and was recrystallized twice from warm ethanol. The melting point found for TPP (81–82°C) was in agreement with the published data.

Standard palladium (II) solution of a concentration of 1 mg/ml was prepared by dissolving palladium chloride in 4 *M* hydrochloric acid. The solution was standarized gravimetrically with dimethylglyoxime.

[1] Present address: Department of Analytical Chemistry, Jagiellonian University, Krupnicza 41, 30–060 Cracow, Poland.

0026-265X/79/010117-07$01.00/0

Platinum (IV) solution in 4 *M* hydrochloric acid of a concentration of 19.6 mg/ml was obtained by dissolving the pure metal in aqua regia. The solution was evaporated several times with concentrated hydrochloric acid and the residue was dissolved in 4 *M* hydrochloric acid.

Other reagents used throughout this work were of analytical purity grade.

Instruments. Photometric measurements were carried out with a VSU−2P spectrophotometer and a SPECORD UV VIS recording spectrophotometer (Carl Zeiss Jena) using 1- and 5-cm quartz cells.

RESULTS AND DISCUSSION

Optimum Conditions of Palladium Extraction

TPP solution in benzene extracts palladium from hydrochloric acid solutions, with the formation of a complex, which shows an absorption maximum at 346 nm. The absorption curves of a TPP solution in benzene (curve 1), of the palladium complex (curve 3), and of the palladium complex with an excess of TPP (3) are shown in Fig. 1.

The absorbance of TPP at the absorption maximum of the complex is low, and above 350 nm it is practically negligible. The molar absorptivity of the palladium complex with triphenylphosphine at 346 nm amounts to $2.26 \times 10^4\ M^{-1}$ liter cm $^{-1}$.

The effect of time on the extraction of palladium is shown in Fig. 2. A period of at least 15 min is necessary for effective extraction.

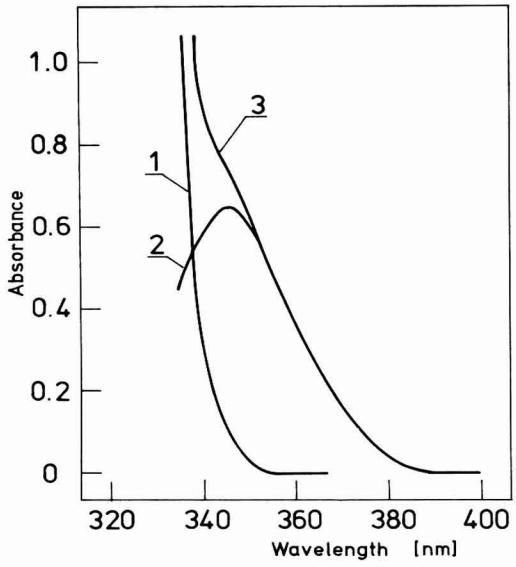


FIG. 1. Absorption curves of: (1) TPP solution in benzene, (2) Pd−TPP complex, (3) Pd−TPP complex with an excess of TPP; $c_{Pd} = 3\ \mu g/ml$, $c_{TPP} = 1.5\%$, $c_{HCl} = 2\ M$.

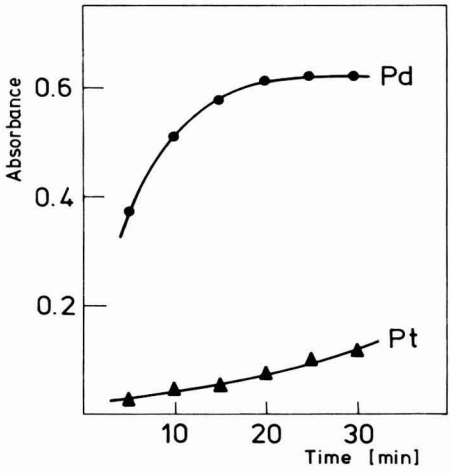


FIG. 2. The dependence of the extraction of palladium and platinum with TPP solution in benzene on the shaking time; c_{Pd} = 3 μg/ml; c_{Pt} = 2 mg/ml; c_{TPP} = 1.5%; c_{HCl} = 2 *M*.

The concentration of hydrochloric acid in the aqueous phase, in the range 0.5–6 *M* (Fig. 3), does not practically affect the extraction of palladium with TPP solution in benzene. A considerable excess of the reagent is necessary for extraction of palladium. First at TPP concentrations above 1% good extraction efficiency is attained (Fig. 4).

The Composition of the Extracted Species

The dependence of the distribution ratio of palladium on the TPP concentration in the organic phase was examined. The dependence is shown

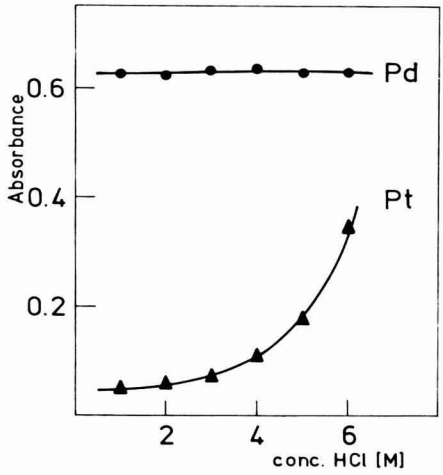


FIG. 3. The effect of hydrochloric acid concentration on the extraction of palladium and platinum with TPP solution in benzene; c_{Pd} = 3 μg/ml; c_{Pt} = 2 mg/ml; c_{TPP} = 1.5%.

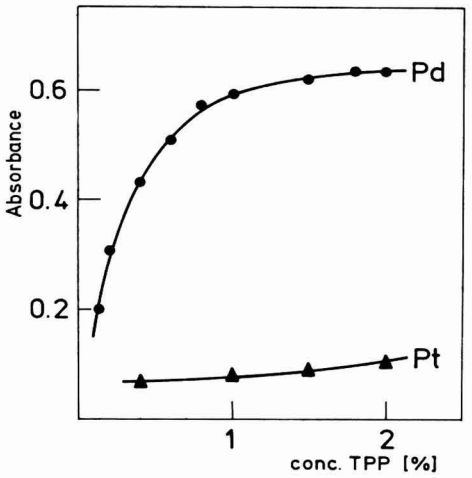


FIG. 4. The effect of TPP concentration on the extraction of palladium and platinum; c_{Pd} = 3 μg/ml; c_{Pt} = 2 mg/ml; c_{HCl} = 2 *M*.

in Fig. 5, in the logarithmic coordinate system. The slope of the curve, approximately equal to 2, indicates that the extracted species contains two molecules of triphenylphosphine.

The palladium complex obtained by shaking 0.01 *M* triphenylphosphine solution in benzene with 0.1 *M* palladium (II) solution in 2 *M* hydrochloric acid was isolated by evaporating the solvent. The obtained product was

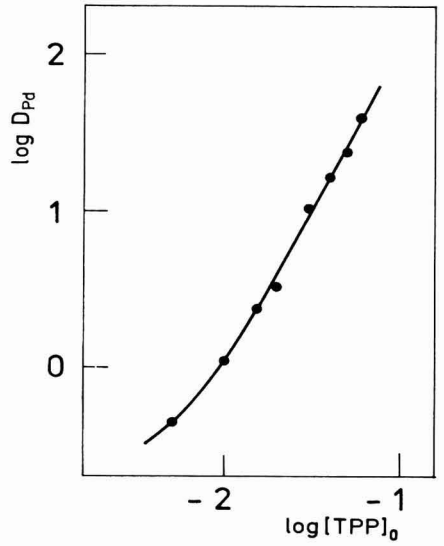


FIG. 5. The dependence of the distribution ratio of palladium on the equilibrium concentration of TPP in the organic phase; $c_{Pd} = 1 \times 10^{-4}$ *M*; c_{HCl} = 2 *M*.

recrystallized (twice) from a mixture of benzene and alcohol and the content of palladium, chlorine, and phosphorus in the complex was determined. It has been found that the composition of the complex corresponds to the formula—$PdCl_2 \cdot 2$ TPP.

The extraction of palladium (II) with triphenylphosphine from hydrochloric acid solutions can be presented as follows:

$$PdCl_4^{2-} + 2\ TPP_{/0/} \rightleftharpoons PdCl_2 \cdot 2TPP_{/0/} + 2\ Cl^-$$

The Effect of Foreign Ions

A 20-fold excess of platinum metals, gold, silver, mercury, copper, nickel, cobalt, and other metals has no effect on the spectrophotometric determination of palladium with triphenylphosphine.

Complexes of the type $MeCl_n \cdot mPR_3$ were prepared for all platinum metals (*3*). From 2 *M* hydrochloric acid all platinum metals except palladium are not extracted with TPP and platinum is only slightly extracted. This is presumably due to the fact that electronegative chloride complexes of platinum metals are highly inert. A similar behavior was observed in the extraction of platinum metals with organic sulfides (*9*).

Extraction of Platinum with TPP

At higher platinum(IV) concentrations this metal is slightly extracted with TPP solution and shows absorption at absorption maximum of the palladium complex. Studies were carried out in order to find conditions under which the effect of platinum on the determination of palladium is as small as possible and to determine the lowest admissible amount of platinum in the aqueous phase.

The extraction of platinum increases with the shaking time (Fig. 2) and with increasing hydrochloric acid concentration (Fig. 3). It follows then that the determination of palladium should be carried out at low hydrochloric acid concentration and the time of shaking should be as short as possible. These conditions are fulfilled at 1−2 *M* hydrochloric acid solution as extraction medium and a shaking time of 15 min.

At platinum concentrations in the range 1−5 mg Pt/ml the absorbance of the extract is so low, that spectrophotometric determination of palladium in the presence of platinum is possible provided that the result is corrected for the blank. However the extraction of palladium decreases with increasing platinum concentrations and at platinum concentrations higher than 5 mg/ml palladium is only partly extracted.

Determination of Pd in Platinum Samples

The elaborated method was applied to the analysis of platinum samples for palladium. The results of the determination, shown in Table 1, indicate

TABLE 1
DETERMINATION OF PALLADIUM IN PLATINUM SAMPLES WITH TRIPHENYLPHOSPHINE

Sample	Pd found[a] (%)	Standard deviation s (%)	Pd added (%)	Total Pd found (%)
1	1.1×10^{-2}	3.1×10^{-3}	1.0×10^{-1}	2.0×10^{-2} 1.9×10^{-1}
2	6.6×10^{-2}	1.8×10^{-3}	5.0×10^{-2}	1.1×10^{-1} 1.1×10^{-1}
3	8.1×10^{-3}	0.24×10^{-3}	5.0×10^{-3}	1.2×10^{-2} 1.4×10^{-2}

[a] The average of five determinations.

good precision and accuracy of the method. Bearing in mind the admissible concentration of platinum in the solution the method can be used for the determination of palladium concentrations not lower than 10^{-3}%.

SUMMARY

Palladium is extracted with triphenylphosphine (TPP) solution in benzene from hydrochloric acid medium as the $PdCl_2 \cdot 2TPP$ complex showing maximum absorption at 346 nm and a molar absorptivity of $\epsilon = 2.26 \times 10^4$.

The conditions of palladium extraction have been examined and the composition of the extracted species has been found to be $PdCl_2 \cdot 2TPP$.

A 20-fold excess of other platinum and transition metals has no effect on the palladium extraction. Palladium can be determined at platinum concentrations up to 5 mg/ml provided that the result is corrected for the blank. The elaborated method has been applied to the analysis of platinum samples containing not less than 10^{-3}% of palladium.

REFERENCES

1. Ahrland, S., Chatt, J., and Davies, N. R., The relative affinities of ligand atoms for acceptor molecules and ions. *Quart. Rev.* **12**, 265–276 (1958).
2. Fishkova, N. L., and Petrukhin, O. M., Atomic-absorption determination of silver after its solvent extraction with triphenylphosphine (in Russian), *Zh. Anal. Khim.* **28**, 645–648 (1973).
3. Livingstone, S. E., "The Chemistry of Ruthenium, Rhodium, Palladium, Osmium, Iridium and Platinum." Pergamon Press, Oxford, 1975.
4. Mojski, M., Extraction of gold, palladium and platinum from chloride, bromide, and iodide solution with di-*n*-octyl sulphide (DOS) in cyclohexane. *Talanta* **25**, 163–165 (1978).
5. Pearson, R. G., Hard and soft acids and bases. *J. Amer. Chem. Soc.* **85**, 3533–3539 (1963).
6. Senise, P., and Levi, F., Solvent extraction studies of addition compounds of metal halides and triphenylphosphine, -arsine and -stibine. Part I. Detection of palladium. *Anal. Chim. Acta* **30**, 422–426 (1964).
7. Senise, P., and Levi, F., Solvent extraction studies of addition compounds of metal halides and triphenylphosphine, -arsine and -stibine. Part II. Spectrophotometric determination of palladium. *Anal. Chim. Acta* **30**, 509–514 (1964).

8. Serebryany, B. L., Fishkova, N. L., Petrukhin, O. M., and Rakovsky, E. E., Atomic-absorption determination of gold after its extraction by triphenylphosphine from hydrochloric acid and cyanide solutions (in Russian). *Zh. Anal. Khim.* **28**, 2333–2336 (1973).
9. Shelkovnikova, O. S., Nikolaev, A. V., and Novoselov, R. I., Increase in the extraction rate of the chloride complexes of the platinum metals with dibutylsulphide in the presence of silver(I). *Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* **1975** (3), 88.

MICROCHEMICAL JOURNAL 24, 124–126 (1979)

Spectrophotometric Determination of Ascorbic Acid

V. K. S. SHUKLA,[1] C. K. KOKATE,[1] AND K. C. SRIVASTAVA[2,3]

[1] *Federal Center of Lipid Research, Piusalle 68, D-4400 Münster, West Germany, and* [2] *Department of Community Health and Environmental Medicine, Odense University, J. B. Winsløwsvej 19, DK-5000 Odense C, Denmark*

Received July 3, 1978

INTRODUCTION

Numerous methods are available for the quantitative determination of ascorbic acid, depending upon various analytical principles. Thus Bessey (*1*) reported a visual titrimetric method employing 2,6-dichlorophenolindophenol. Deutsch and Weeks (*3*) described a microfluorometric method for the determination of ascorbic acid. Roe and co-workers (*10*) evolved a colorimetric method using 2,4-dinitrophenylhydrazine. Various other methods, for example, polarographic, (*5*) chromatographic, (*11*) and turbidimetric (*9*) have also been recently investigated for the assay of ascorbic acid. Recently Shukla and Clausen (*12*) determined ascorbic acid using bromine monochloride in water–acetic acid medium.

The Folin–Ciolcateu (*4*) reagent yields a blue color with phenolic compounds, cysteine, (*2*) thioureas, (*8*) and some buffers and monosaccharides when present in high concentration (*6, 7*). The observation that ascorbic acid readily forms a blue-colored complex with Folin–Ciocalteu reagent led to the development of a simple and accurate procedure for the microgram determination of ascorbic acid.

EXPERIMENTAL

Apparatus. Absorbance (1-cm light path) was read with a CARL ZEISS PMQ II spectrophotometer.

Reagents and solutions. All the reagents used were of analytical grade. Stock solutions of ascorbic acid were prepared by dissolving 5 to 35 mg in distilled water and making up to the mark in a 100-ml standard flask.

Procedure. A 1-ml aliquot of the stock solution was transferred to a 25-ml standard flask and made up to the mark with 1% of freshly diluted Folin–Ciocalteu reagent. The reaction mixture was allowed to stand at

[1] *Federal Center of Lipid Research, Piusalle 68, D-4400 Münster, West Germany,* and [2] Department of Community Health and Environmental Medicine, Odense University, J. B. Winsløwsvej 19, DK-5000 Odense C, Denmark

[3] To whom correspondence should be addressed.

0026-265X/79/010124-03$01.00/0

TABLE 1
MICROGRAM DETERMINATION OF ASCORBIC ACID

Sample number	Sample weight[a] (μg)		Deviation (%)
	Taken	Recovered	
1	66	65	−1.5
2	132	133.7	+1.3
3	198	195	−1.5
4	264	260	−1.5
5	330	336.2	+1.8

[a] All determinations were done in triplicate.

room temperature for 40−50 min. The absorbance of the blue-colored complex was measured against a reagent blank at 730 nm. A calibration curve was prepared by treating various aliquots of the standard solution as described.

RESULTS AND DISCUSSION

The above procedure was employed to determine microgram quantities of ascorbic acid and the results obtained are presented in Table 1. The results are moderately precise and the maximum deviation is about ± %1.8. Beer's law is obeyed in the range of 50−350 μg.

The development of color depended on time and the concentration of the Folin−Ciocalteu reagent. The absorbance increased with time and, after 40 min, it became constant. Therefore the reaction mixture was kept for 40 min for optimal color development. The principal advantage of this method is its reproducibility and simplicity.

REFERENCES

1. Bessey, O. A., A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. *J. Biol. Chem.* **126**, 771−784 (1938).
2. Chou, S., and Goldstein, A., Chromogenic groupings in the Lowry protein determinations. *Biochem. J.* **75**, 109−115 (1960).
3. Deutsch, M. J., and Weeks, C. E., Microfluorimetric assay for vitamin C. *J. Assoc. Offic. Agric. Chem.* **48**, 1248−1256 (1965).
4. Folin, O., and Ciocalteu, V., Tyrosine and tryptophan determinations in proteins. *J. Biol. Chem.* **73**, 627−650 (1927).
5. Lindquist, J., and Farroha, S. M., Application of differential pulse polarography to the assay of vitamins. *Analyst* **100**, 377−385 (1975).
6. O'Sullivan, J., and Mathison, G. E., Interference by monosaccharides with the estimation of tyrosine and proteins using the Folin−Ciocalteu phenol reagent. *Anal. Biochem.* **35**, 540−542 (1970).
7. Peters, M. A., and Fouts, J. R., Interference by buffers and other chemicals with the Lowry protein determination. *Anal. Biochem.* **30**, 299−301 (1969).
8. Rakitzis, E. T., Reaction of thioureas with the Folin−Ciocalteu reagent. *Anal. Chim. Acta* **78**, 495−497 (1975).

9. Ralls, J. W., Turbimetric determination of ascorbic acid in foods. *J. Agric. Food Chem.* **23**, 609–610 (1975).
10. Roe, J. H., Mills, M. B., Oesterling, M. J., and Damron, C. M., Determination of diketo-L-gluconic acid, dehydro-L-ascorbic acid and L-ascorbic acid in the same tissue extract by the 2,4-dinitrophenylhydrazine method. *J. Biol. Chem.* **174**, 201–208 (1948).
11. Schlack, J. E., Quantitative determination of L-ascorbic acid by gas– liquid chromatography. *J. Assoc. Offic. Anal. Chem.* **57**, 1348–1356 (1974).
12. Shukla, V. K. S., and Clausen, J., Microdetermination of ascorbic acid using bromine monochloride in water–acetic acid medium. *Michrochem. J.* **22**, 475–478 (1977).

MICROCHEMICAL JOURNAL 24, 127–134 (1979)

BOOK REVIEWS

Aldehydes in Biological Systems. By E. SCHAUENSTEIN, H. ESTERBAUER, AND H. ZOLLNER. Academic Press, London, 1977. 205 pp., £.9.00.

Aldehydes are found in a wide range of living systems where they have a number of significant biological and biochemical functions as metabolic intermediates and also have highly specific effects on biological processes such as energy metabolism and biosynthesis. The objective of this monograph is to present information on aldehydes in biological systems gathered from widely diverse sources, for students and research scientists interested in this class of compounds.

The book includes eight chapters, grouped into an introduction, parts 1 and 2, and a subject index. The introduction presents some basic organic chemistry aspects of aldehydes. Part 1 includes chapters on: saturated aldehydes; alpha-, beta-unsaturated alkanes; alpha-hydroxyaldehydes; dicarbonyl compounds; and stable derivatives of aldehydes. Part II includes chapters on naturally occurring aldehydes and their specific biological functions. Each chapter is followed by an extensive list of references to the world literature on aldehydes in biological systems. The many illustrations and tables add greatly to the discussions presented.

There is material in this book of interest to both the specialist in this area and for the inexperienced student. Both will benefit by exposure to concepts presented. This is a worthwhile addition to the Pion advanced biochemistry series, edited by J. R. Lagnado.

DONALD F. LOGSDON, JR.,
USAF Occupational and Environmental Health Laboratory,
McClellan AFB, California 95652

Electrochemical Studies of Biological Systems. Edited by DONALD T. SAWYER. American Chemical Society, Washington, D.C., 1977. viii + 216 pp., $15.50.

This volume, the ACS Symposium Series 38, presents the proceedings of a symposium held at the 172nd National Meeting of the American Chemical Society at San Francisco, California on August 30, 1976. (The Symposium was sponsored by the Division of Analytical Chemistry.) It consists of 12 distinguished papers discussing the recent development of the use of electroanalytical methods in characterizing biological systems. In particular, these papers are concerned with the versatile and uniquely effective applications of electroanalytical techniques to the determination of the stoichiometries, thermodynamics, and kinetics of electron-transfer reactions, atom-transfer reactions, and oxidation–reduction processes. Each paper constitutes a separate chapter and provides the available and related literatures. An index is also included at the end of the book.

The first paper by H. B. Mark, Jr., and T. M. Kenyhercz discusses three aspects of the spectroelectrochemical study of Vitamin B_{12} and related cobalamins. These are: (1) the time-resolved spectral study of the autooxidation of cob(I)alamins under various experimental conditions; (2) the electrochemical behavior of the cob(III)alamins, and (3) the electrochemical behavior of 5′-deoxyadenosylcobalamin. It is an interesting paper dealing with the combined use of optical and electrochemical techniques to the interpretation of the complicated mechanisms of electrokinetic processes.

0026-265X/79/010127-08$01.00/0

The second paper by C. C. Y. Ting, J. Jordan, and M. Gross reports the preliminary findings on the electrochemical behavior of porphyrin C and heme C (the prosthetic group of cytochrome *c*). D.C. Polarography, cyclic voltammetry, and coulometry were used in this investigation.

In Chapter 3, a study of the control of the potentials of metal ion couples in complexes of macrocyclic ligands by ligand structural modifications conducted by D. H. Busch and eight other co-workers is presented. Special attention is focused here on the oxidation–reduction processes of the central metal ion. The results are critically interpreted.

Chapter 4 by K. M. Kadish, L. K. Thompson, D. Beroiz, and L. A. Bottomley deals with electrochemical studies on the thermodynamics of electron-transfer and ligand-binding of several metalloporphyrins in aprotic solvents. The systems measured include H_2TPP, Zn(II)TPP, Mg(II)TPP, Mn(II)TPP, Fe(II)TPP, and Co(II)TPP (where TPP is tetraphenylporphrin) in various solvents of DMSO, DMF, and butyronitrile. In the succeeding chapter, Kadish *et al.* also report the electron-transfer properties of an *N*-bridged dimer, μ-nitridobis[α, β, γ, δ-tetraphenylporphinatoiron] in methylene chloride. Cyclic voltammetry and differential pulse polarography were extensively and comparatively utilized.

A general framework for understanding the mode of production of active catalysts from binuclear Mo(V) (i.e., $Mo_2O_4^{2+}$) complexes and the mechanism by which these species catalyze the reduction of nitrogenase substrates was described by Schultz, Ledwith, and Leazenbee in Chapter 6.

The next paper by Howie, Morrison, and Sawyer summarizes the results of the studies of manganese(II) and -(III) 8-quinolinol complexes acting as model compounds for mitochondrial superoxide dismutase. A sequence of possible redox reaction mechanisms for this particular system is proposed and reviewed.

The interfacial behavior of biologically important purines and pyrimidines and various adenine derivatives at pH 9 at the mercury solution interface is demonstrated in Chapter 8 by Kinoshita, Christian, Kim, Baker, and Dryhurst. Two major surface electrochemical techniques, the capillary electrometer and differential capacitance measurements, were described in detail in this paper.

In Chapter 9, Szentrimay, Yeh, and Kuwana describe an indirect coulometric titration (ICT) method using mediator-titrants (M-T's) for the accurate assessment of stoichiometry (*n* value) and energetics ($E^{\circ\prime}$ value) of bioredox components such as cytochrome *c* oxidase and "blue" copper laccases. Some 60 possible M-T's are being studied.

A rotating ring disk enzyme electrode for biocatalysis studies was discussed in Chapter 10. Using this electrode, the effects of substrate mass transport and kinetic control of surface catalyzed reactions, particularly immobilized biosurfaces, can be investigated.

The eleventh paper by Findl and Kurtz deals with the examination of electrokinetic potentials in a left ventricle/aorta simulator. The simulators produced pulsed turbulent flows, simulating mammalian heart pumping conditions. It has been shown that EKG (an electrocardiogram) like signals were generated by the motion of the electrolyte through the simulators. It is an interesting paper to go through carefully.

The closing paper contributed by R. J. Stolzberg is concerned with the analysis of synthetic seawater and phytoplankton media for ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA) by differential pulse polarography. Several cations such as Ca, Ni, Co, Zn, and Cu with the addition of Cd were examined for the electrode response.

In summary, these 12 papers are well written. They provide a representative cross-section of the kinds of electrochemical techniques and instrumentation that are used to study biological systems. This volume should be considered as an excellent reference book in that special field.

GEORGE W. C. HUNG,
Woodson–Tenent Laboratories,
Hill Top Testing Services,
American Biomedical Corporation,
Memphis, Tennessee 38101

Colorimetric and Fluorimetric Analysis of Steroids. By J. BARTOS AND M. PESEZ. Academic Press, New York, 1976. xii + 274 pp., $21.50.

This is number 11 in the series of monographs edited by Belcher and Anderson on the Analysis of Organic Materials.

This volume is aimed at the pharmacist, biochemist, and clinical chemist who is not specialized in steroids but who occasionally needs a method of estimation or who wishes to acquire a background of knowledge in this field. Thus a rigorous selection was made from the thousands of papers dealing with the characterization and determination of natural and synthetic steroids. The rules of the selection were that only methods based on the chemical reactions performed in solution and yielding a color or fluorescence or exceptionally strong absorption in the near-UV are included. Qualitative tests are quoted only when quantitative tests can be derived from them. Even within these parameters, although all of the colorimetric and fluorimetric methods which have aroused interest are believed by the authors to have been included, it is still not claimed to be exhaustive.

When procedures are detailed they have been tested subsequent to their original publication and found to be satisfactory.

A short chapter on nomenclature is included followed by chapters on functional group analysis, halochromism, and halofluorism. With these as introduction, the remainder of the book divides the steroids into ten sections: Sterols and Vitamin D, Bile Acids, Estrogens, Gestogens, Androgens, Corticosteroids, Contraceptive Progestogens, Cardiac Glycosides, Steroid Saponins and Sapogenins and Steroid Alkaloids.

Each chapter opens with an introduction defining its subject, a section on general reactions of the group and then specific reactions of selected representatives of the group, and closes with a substantial bibliography.

The book contains an author and subject index.

Within the limits set by the authors, this volume contains a wealth of information and this reviewer feels they have met the objectives set.

BILL ELPERN,
9 Surrey Way,
White Plains, New York 10607

Principles and Techniques of Electron Microscopy, Vol. 7. Biological Applications. Edited by M. A. HAYAT. Van Nostrand Reinhold, New York, 1977. xix + 383 pp. $27.50.

This is the seventh volume of Hayat's multivolume series on the principles and techniques employed for studying biological specimens with the aid of an electron microscope (EM). It has been developed over the years through the joint effort of 10 distinguished author–scientists. It consists of 9 chapters, including 153 well-reproduced electron micrographs and illustrations, 7 tables, and some 665 up-to-date literature references. The basic approach and format in this volume are similar to those in the previous six volumes. Complete author and subject indexes are included at the end of the book.

The first chapter by Isaacson is concerned with the radiation damage of specimens in the EM. It discusses briefly the electron scattering process related to potential damage, the experimental measurements of radiation damage relating to EM, the damage mechanisms, and the proposed techniques to reduce radiation damage. It thus shows electron microscopists the need to quantitate carefully their experimental data and methods with regard to radiation damage.

The second chapter by Nermut describes one of the most successful techniques—freeze-drying for preserving the native state of biological structures for EM. Both theoretical and

practical aspects of the technique and its biological applications in the study of macromolecules, viruses, and subcellular fractions are reviewed and discussed.

In Chapter 3, Peters introduces and describes an interesting technique—equidensite integration analysis for image reconstruction of electron micrographs. By this technique, defined contrast filtration is possible by employing a photographic copying process using equidensite negatives to control the integration of periodic structures as well as producing an average image (equidensite rotation method). By means of such copying, a structural correlation of the contrast filtered image to the original is carried out, thus making possible the reconstruction of the image analogous to optical filtration. This chapter is therefore useful for a comprehensive study for applications.

Chapter 4 by Ruzicka considers G-banding of chromosomes. G-banding involves procedures carried out on chromosome preparations and subsequent staining with Giemsa (G-banding). It enables chromosomes (including those of the human C-, D-, F-, and G-group chromosomes) to be differentiated. Methods developed by the author and by others are critically described and compared. Observations on the ultrastructure of chromosomes after various specific treatments are comprehensively discussed and illustrated.

In Chapter 5, Geuskens discusses two methods of autoradiographic localization of DNA in nonmetabolic conditions. The first method deals with the localization of double-stranded DNA by [^{3}H]actinomycin D binding, while the second method is concerned with the detection of specific repetitive DNA sequences by *in situ* molecular hybridization. The former by combining with autoradiography is very useful for detecting small amounts of double-stranded DNA at the ultrastructural level, while the latter performed at the EM level is still in its infancy and needs to be further developed for future applications.

The optical analysis of the micrograph for structural detail and its optical reconstruction with that detail enhanced are demonstrated by Gibbs and Rowe in Chapter 6. While there have been achievements in the use of complex filters to modify phase as well as amplitude to enable all types of spatial filtering to be performed on an analog basis, much further research is required in this aspect before it becomes an economic and practical possibility in the ordinary laboratory. It is anticipated that high-quality, high-resolution reconstructed images can be produced in a modern optical diffractometer in the near future.

Chapter 7 by Gvosdover and Zel'dovich deals with the physical principles of the mirror electron microscope (MEM). The MEM is an instrument for investigating topographical inhomogeneities, potential distributions, and magnetic structures on the surface of bulk specimens. The materials covered in this chapter include a brief description of the MEM design and its modes of operation; a detailed treatment of the classical theory of image contrast formation; a display of the experimental results obtained in the shadow projection imaging mode of the MEM; a discussion of the theoretical and experimental results on the measurements of microfields by the MEM; the quantum effects in the MEM; and the applications of the MEM.

In Chapter 8, Burkholder comments on the methods of collection and preparation of metaphase cells, preparation of chromosomes, G-banding and C-banding of chromosomes, and chromosome staining for EM. A particular interest has been placed on the study of human and other mammalian chromosomes.

The last chapter by Ellison and Jones reviews some neurobiological applications of equidensitometry. Equidensitometry is the study of lines and areas of equal tonal density within an image. This method has been successfully used in the study of interferograms and spectrograms, and in the fields of astrophysics, illumination engineering, hydrology, photography, and radiation physics. However, its biological applications are still under development and require further work.

GEORGE W. C. HUNG, *Woodson–Tenent Laboratories, Hill Top Testing Services, American Biomedical Corporation, Memphis, Tennessee 38101*

Electron Microscopy in the Study of Materials. By P. J. GRUNDY AND G. A. JONES. Edward Arnold (Publishers) Limited, London, 1976. 174 pp., $19.50 (cloth), $9.50 (paper).

The authors of volume seven in the series "The Structures and Properties of Solids" describe the basic concepts behind the design of electron microscopes used in the study of solid materials. The uses of various electron optical devices are surveyed and the limitations and strengths of each are discussed which gives the reader a good understanding of the possible applications of each instrument.

A short historical presentation which includes the major concepts in determining resolution of an optical system is presented in Chapter 1. This is followed by a well-prepared chapter on the interactions of electrons with matter. The authors understand that these interactions must be appreciated before contrast effects can be introduced. Chapter 3 discusses the practical aspects of electron microscope design. As the chapter develops, the resolution of the microscopes based on design factors is introduced. The merits and disadvantages of electron microscopes are discussed at the end of this chapter.

Chapters 4 and 5 cover, in a concise manner, conventional transmission electron microscopy and scanning electron microscopy. These two chapters are very well written and would provide good background for a person entering the field. The content of these chapters comprise the core of the book. There is a final chapter on recent developments in electron microscopy which gives the essential information on the newer electron optical devices. There are eight appendices which contain basic crystallographic ideas, relationships, and methods of solution of single crystal, polycrystal, and kikuchi patterns.

The authors have condensed into a rather short book the information required to understand electron microscopes. They haven't included the long mathematical developments but give the basic equations needed at the proper time. The more advanced reader can go to the complete reference list to develop the topic while the novice gets the concept without being lost in extended mathematical presentations. This book is highly recommended, especially for those whose interest in electron microscopy is just developing.

ROBERT J. DEANGELIS,
Institue for Mining & Minerals Research,
University of Kentucky,
Lexington, Kentucky 40506

Analytical and Quantitative Methods in Microscopy. Edited by G. A. MEEK AND H. Y. ELDER. Cambridge University Press, New York, 1977. vii + 276 pp. $24.95 (Paperback: $8.50).

This volume, Seminar Series 3, containing the proceedings of a symposium held by the Society for Experimental Biology in England, describes some of the latest numerical and quantitative methods on biological problems employing microscopical techniques. It is designed purposely to provide the biologists with the mathematical background and numerical techniques for microscopy so that they can effectively utilize analytical methods to obtain accurate quantitative information from their biological specimens and to interpret certain features of the experimental results with greater certainty and precision.

The book consists of twelve papers. All papers were contributed by active scientists and leading authorities in their specific fields. The analytical techniques presented range from the simple methodology of manual stereology to the very complex and sophisticated computerized electronic equipment used in automatic image analysis. Some 109 technical figures

and electron micrographs, 15 informative tables, a total of 335 up-to-date references, and a complete index are provided. The materials thus presented involve principles and techniques applicable to both light and electron microscopy.

In the Introduction, Professor Meek briefly reviews the idea and content of each paper and introduces and comments on the organization of the symposium, the basic aim of all forms of microscopy, the current status of experimental biology, and the future approach and advancement in analytical and quantitative methods in microscopy. This section provides the readers with a very useful guide for reading and understanding the basic idea and principles of each article presented.

Stereology, which is defined as the science whereby three-dimensional information on structures can be derived from only two-dimensional sections of those structures, is discussed comprehensively by Dr. James in the first paper of the book. It can be used in the study of structures independently of their size or their composition. It is a very simple, accurate, and powerful technique for analyzing the biological micrographs. Its principles and applications are described in detail in this paper and are worth careful study.

The second paper by Professor Horne illustrates an optical diffraction analysis of periodically repeating biological structures. Optical diffractometry is also a simple and powerful method for the analysis of conventional images. The method is capable of resolving the structure of macromolecules down to the molecular level.

The techniques of quantitative fluorescence microscopy and its applications are reviewed by Dr. Ploem in the third paper. Microfluorometry is especially sensitive for low concentrations of reaction products which are present in a microscopical specimen. In this method, the direct image can be analyzed before recording. In the following paper, Dr. Bradbury of Oxford explains the principles and apparatus (The Quantimet 720 System) in automated direct quantitative image analysis. It is an excellent demonstration of typical and practical optical image analyzers. All the basic methods of stereology can be applied to automatic image analysis, producing stereological data far more radpily and accurately than can point-counting methods.

The next two papers, "Integrating Microdensitometry" and "Scanning Microinterferometry," are contributed by Dr. Goldstein. In the former, the techniques for the use of the flying-spot light microscope, which measures the optical density, integrates the total absorbance with a simple computer, and shows it as a direct readout, are discussed; while in the latter, the principles and applications of scanning microinterferometry, which measures the refractive index of the dry mass of an unstained cell component with a flying-spot microscope, calculated and displayed as an integrated digital readout, are illustrated in detail.

In article seven, Dr. Elder comments on the recent development of the scanning transmission electron microscope (STEM) and emphasizes its great potential use in the biological sciences at the molecular level. The eighth paper, by Professor Burge of London, also concentrates on the demonstration of high-resolution imaging of the STEM. The theory, instrumentation, and operation of the STEM and its competitive applications with other microscopical systems are critically described here.

Electron energy loss spectrometry, a very new and promising technique for elemental and chemical microanalysis of the biological specimen, is mentioned by Professor Ferrier of Glasgow in the ninth paper. Theoretically, energy loss spectrometry can be made several orders of magnitude more sensitive than X-ray microanalysis, particularly for the elements of low to medium atomic number. It is recommended that the techniques be further developed to become a powerful microanalytical tool for the study of biological and biochemical systems.

The last three chapters of this volume are devoted to X-ray microanalysis. Two main types of X-ray detector are currently in use; the energy-dispersive (ED) and the wavelength dispersive (WD) systems. Each has its own advantages and disadvantages. The paper by

Professor Hutchinson of Minnesota discusses the application of the ED system to the elemental microanalysis of frozen hydrated biological tissues. While the article by Dr. Chandler of Cardiff is concerned chiefly with the chemical microanalysis of biological material with the WD techniques. The techniques for preparing ultrathin sections of frozen material—"cyro-ultramicrotomy" for X-ray microanalysis of diffusible elements in biological systems are described by Dr. Appleton of Cambridge in the concluding chapter. This technique enables biologists to study the chemical nature of cells and their constituents under varying experimental conditions and at the ultrastructural level.

In summary, all materials and information, principles, techniques, and analytical methods presented and described in this volume are very useful, practical, and up-to-date. It is not only a valuable reference book for microscopists and biologists but also an excellent guidebook of microtechniques for biochemists, chemists, and other research scientists concerning their research or analytical work at the microquantitative or molecular level.

GEORGE W. C. HUNG,
Woodson–Tenent Laboratories, American Biomedical Corporation, Memphis, Tennessee 38101

Protein Crosslinking, Nutritional and Medical Consequences, Edited by MENDEL FRIEDMAN. Advances in Experimental Medicine and Biology, Volume 86B. Plenum Press, New York, 1977. xx + 740 pp. $59.50.

This is the second volume of the proceedings of the Symposium on Protein Crosslinking held at the A.C.S. meeting in San Francisco, California in September 1976. This second volume consists of thirty-nine contributions. While the first volume is concerned with the chemistry and biochemistry of protein polyfunctional affinity agents and crosslinking agents, this second volume is more heavily oriented toward medical and nutritional consequences of crosslinking.

Those nutritional aspects of crosslinking discussed include: lysinoalanine formation in protein food ingredients and in wool as well as the chemistry of lysinoalanine formation and cleavage under alkaline conditions; reactions of proteins with dehydroalanines; heat-induced crosslinks in milk proteins; effects of Maillard browning reaction on nutritional quality of protein; the biological significance of carbohydrate–lysine crosslinking during heat treatment of food proteins; formation of a Schiff's base between lysine and lactose in milk; and protected proteins in ruminant nutrition.

The biomedical aspects of crosslinking that are discussed include: metabolic transit of lysinoalanine; therapeutic implications of the crosslinkage theory of aging (by J. Bjorksten); relationship of collagen crosslinking to aging and nutrition; pharmacological and therapeutic actions of penicillamine; and effects of bone collagen and arterial elastin crosslinking on nutritional copper deficiency and penicillamine administration.

Some additional topics include: the stereochemistry and nomenclature of crosslinked amino acids; enzyme hydrolysis of crosslinked proteins; new amino acid derivatives formed by alkaline treatment of proteins; a very useful mathematical analysis of the kinetics of consecutive, competitive reactions of protein amino groups; and details of the crosslinking of collagen and of elastin.

The editor himself contributed a significant fraction of the manuscript. He deserves credit for selecting an exciting array of contributions ranging from fundamental chemical to biomedical research. The articles are well presented, the volumes are direct offprints from the typed manuscripts. The contributions are up-to-date; referencing into 1977 is in evidence

throughout. As perhaps expected for edited volumes of this type, there is no author index and only a rather cursory subject index. It is unfortunate that these very valuable collections can only be recommended for library acquisition on account of the rather prohibitive prices.

FRANK JORDAN, *Department of Chemistry,*
Newark College of Arts and Sciences,
Rutgers University,
Newark, New Jersey 07102

Homoatomic Rings, Chains and Macromolecules of Main-Group Elements. Edited by ARNOLD L. RHEINGOLD. Elsevier, New York/Amsterdam, 1977. xv + 615 pp. $79.95.

In April, 1976 the IXth Hudson Symposium was held to discuss the subject of this volume. One of the principle aims of the Symposium and of this book is to provide a broad appraisal of the chemistry of the main-group homoatomic structures. For obvious reasons discussions of carbon have been omitted as have several topics which have been the subject of extensive reviews since 1973. For the rest, the literature up to December 1976 has been covered.

The first four chapters deal with thermodynamic data, spectroscopic and molecular orbital investigations, and a discussion of the electrostatic forces operating within the molecules; information which applies, in general, to all of the subject structures. The remaining twenty chapters deal with specific elements. As might be expected, phosphorus and silicon receive major attention.

The availability of a wide assortment of carbon compounds in nature is a luxury that is not afforded the chemists who work in this area. Accordingly, much of the work reported here is of physical measurements and theoretical discussions. This is a guide book to future work potential and not a lab manual of the past.

There is an ample bibliography following each chapter and a computer-compiled subject index at the end of the volume. The book is well done as a book but the price is rather steep for a volume of this nature.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

MICROCHEMICAL JOURNAL 24, 135 (1979)

ANNOUNCEMENTS

1979 Eastern Analytical Symposium

The 1979 Eastern Analytical Symposium will be held at the Hotel Americana, New York City, on October 31 and November 1 and 2, 1979. For further information contact:

Ivor Simmons
EAS Publicity
M&T Chemicals, Inc.
P.O. Box 1104
Rahway, New Jersey 07065

The American Microchemical Society is soliciting nominations for its 15th annual Benedetti–Pichler Award. This award is given in recognition of service to microchemistry in its broadest sense, including research, application, administration, teaching, or other means of promoting the advancement of microchemistry. The nominee need not be a member of the society. Nominations should be made in writing, stating the reason for nomination and citing the work of the nominee. All nominations must be received by June 1, 1979. Nominations and requests for further information should be addressed to:

Lisa Hallquist
J. T. Baker Chemical Company
222 Red School Lane
Phillipsburg, NJ 08865