Vol. 111 December 1, 1979
Complete in one issue

ISSN 0003-2670

ANALYTICA CHIMICA ACTA

International journal devoted to all branches of analytical chemistry

ELSEVIER SCIENTIFIC PUBLISHING COMPANY

ANALYTICA CHIMICA ACTA

International journal devoted to all branches of analytical chemistry
Revue internationale consacrée à tous les domaines de la chimie analytique
Internationale Zeitschrift für alle Gebiete der analytischen Chemie

PUBLICATION SCHEDULE FOR 1979 (incorporating the section on Computer Techniques and Optimization).

	J	F	M	A	M	J	J	A	S	O	N	D
Analytica Chimica Acta	104/1	104/2	105	106/1	106/2	107	108	109/1	109/2	110/1	110/2	111
Section on Computer Techniques and Optimization			112/1			112/2			112/3			112/4

Scope. *Analytica Chimica Acta* publishes original papers, short communications, and reviews dealing with every aspect of modern chemical analysis, both fundamental and applied. The section on *Computer Techniques and Optimization* is devoted to new developments in chemical analysis by the application of computer techniques and by interdisciplinary approaches, including statistics, systems theory and operation research. The section deals with the following topics: Computerized acquisition, processing and evaluation of data. Computerized methods for the interpretation of analytical data including chemometrics, cluster analysis, and pattern recognition. Storage and retrieval systems. Optimization procedures and their application. Automated analysis for industrial processes and quality control. Organizational problems.

Submission of Papers. Manuscripts (three copies) should be submitted to:
for *Analytica Chimica Acta:* Dr. A. M. G. Macdonald, Department of Chemistry, The University, P.O. Box 363, Birmingham B15 2TT, England;
for the section on *Computer Techniques and Optimization:* Dr. J. T. Clerc, Universität Bern, Pharmazeutisches Institut, Sahlistrasse 10, CH-3012 Bern, Switzerland.
American authors are recommended to send manuscripts and proofs by **INTERNATIONAL** AIRMAIL.

Information for Authors. Papers in English, French and German are published. There are no page charges. Manuscripts should conform in layout and style to the papers published in this Volume. Authors should consult Vol. 111, p. 343 for detailed information. Reprints of this information are available from the Editors or from: Elsevier Editorial Services Ltd., Mayfield House, 256 Banbury Road, Oxford OX2 7DE (Great Britain).

Reprints. Fifty reprints will be supplied free of charge. Additional reprints (minimum 100) can be ordered. An order form containing price quotations will be sent to the authors together with the proofs of their article.

Advertisements. Advertisement rates are available from the publisher.

Subscriptions. Subscriptions should be sent to: Elsevier Scientific Publishing Company, P.O. Box 211, 1000 AE Amsterdam, The Netherlands. The section on *Computer Techniques and Optimization* can be subscribed to separately.

Publication. *Analytica Chimica Acta* (including the section on *Computer Techniques and Optimization*) appears in 9 volumes in 1979. The subscription for 1979 (Vols. 104–112) is Dfl. 1179.00 plus Dfl. 135.00 (postage) (total approx. U.S. $641.00). The subscription for the *Computer Techniques and Optimization* section only (Vol. 112) is Dfl. 131.00 plus Dfl. 15.00 (postage) (total approx. U.S. $71.00). Journals are sent automatically by airmail to the U.S.A. and Canada at no extra cost and to Japan, Australia and New Zealand for a small additional postal charge. All earlier volumes (Vols. 1–103) except Vols. 23 and 28 are available at Dfl. 150.00 (U.S. $73.00), plus Dfl. 10.00 (U.S. $5.00) postage and handling, per volume.

Claims for issues not received should be made within three months of publication of the issue, otherwise they cannot be honoured free of charge.

Customers in the U.S.A. and Canada who wish to obtain additional bibliographic information on this and other Elsevier journals should contact Elsevier/North Holland Inc., Journal Information Center, 52 Vanderbilt Avenue, New York, NY 10017. Tel: (212) 867-9040.

Biological Magnetic Resonance
Volume 2
edited by **Lawrence J. Berliner**
Ohio State University
and **Jacques Reuben**
University of Houston

The study of biological magnetic resonance has expanded very rapidly in recent years with the introduction of sophisticated and continuously improving instrumentation. The second volume in this series focuses on the use of phosphorus-31 NMR for the study of biological systems and the synthesis and applications of a new class of nitroxides for spin-label use. Other topics covered include the ESR of molybdenum and iron, and high resolution multinuclear NMR spectra of peptides and peptide hormones. approx. 390 pp., illus., 1979, $39.50 ($47.40/£24.89 outside US)

Multiple Electron Resonance Spectroscopy
edited by **Martin M. Dorio**
General Electric
and **Jack H. Freed**
Cornell University

Multiple Electron Resonance Spectroscopy presents an account of the techniques and applications of multiple resonance that use electron paramagnetic resonance (EPR) for detection. The book details theoretical and experimental material on inorganic, organic, biochemical, and polymer systems, and critically points out areas of controversy and future research. 524 pp., illus., 1979, $49.50 ($59.40/£31.19 outside US)

Introduction to Analytical Electron Microscopy
edited by **John J. Hren**
University of Florida
Joseph I. Goldstein
Lehigh University
and **David C. Joy**
Bell Laboratories, New Jersey

This is a tutorial guide for the practicing electron microscopist wishing to learn newly available methods of analysis; the approach is practical, with heavy reliance on examples. 618 pp., 1979, $35.00 ($42.00/£22.05 outside US)

Advances in X-Ray Analysis
Volume 22
edited by **Gregory J. McCarthy**
The Pennsylvania State University
Charles S. Barrett, Donald E. Leyden, John B. Newkirk, and **Clayton O. Ruud**
University of Denver

Presenting up-to-date information on the technique and its theoretical and practical applications, this new volume in an ongoing series reports on the latest X-ray methods for microstructural and analytical characterization of materials, including X-ray diffraction and fluorescence analyses. Among the examples cited are applications in energy production, materials optimization, and mineral characterization. 510 pp., 1979, $42.50 ($51.00/£26.78 outside US)

Practical Mass Spectrometry
A Contemporary Introduction
edited by **Brian S. Middleditch**
University of Houston

Practical Mass Spectrometry is a unique text directed to the novice who wishes to acquire a working knowledge of the techniques and capacities of modern mass spectrometers. The text provides a detailed examination of three currently available mass spectrometers, an introduction to gas chromatography–mass spectrometry, available methods for comparing spectra, and applications of mass spectrometry in a broad range of disciplines. approx. 370 pp., illus., 1979, $29.50 ($35.40/£18.59 outside US)

Ion-Selective Electrodes in Analytical Chemistry
Volume 1
edited by **Henry Freiser**
University of Arizona

"[This volume] is a comprehensive monograph treating both conceptual and practical aspects, and serves as an introduction to ion-selective electrodes while stimulating interest in the development of applications. . . . A recommended acquisition for scientists and engineers."
—Electrochemical Progress

A volume in the Modern Analytical Chemistry series. 454 pp., 1978, $42.50 ($51.00/£26.78 outside US)

THE LANGUAGE OF SCIENCE
Plenum
PUBLISHING CORPORATION

227 West 17th Street, New York, N.Y. 10011
In United Kingdom: Black Arrow House,
2 Chandos Road, London NW10 6NR, England

ANALYTICA CHIMICA ACTA
VOL. 111 (1979)

ANALYTICA CHIMICA ACTA

International journal devoted to all branches of analytical chemistry

ELSEVIER SCIENTIFIC PUBLISHING COMPANY

Anal. Chim. Acta, Vol. 111 (1979)

Printed in The Netherlands.

Analytica Chimica Acta, 111 (1979)

PUBLISHER'S NOTE

It is with regret that we announce that Dr. D. M. W. Anderson is ending his period of service as Joint Editor of Analytica Chimica Acta.

We are grateful to him for all his work on the journal's behalf over the last five years, and are sorry that he has now expressed the wish to withdraw from the joint editorship.

Analytica Chimica Acta, 111 (1979) 1—56

Review

THEORY AND APPLICATIONS OF ION-SELECTIVE ELECTRODES PART III*

JIŘÍ KORYTA

J. Heyrovský Institute of Physical Chemistry and Electrochemistry, Czechoslovak Academy of Sciences, Opletalova 25, 110 00 Prague 1 (Czechoslovakia)

(Received 14th June 1979)

SUMMARY

This review of ion-selective electrodes is arranged in the same way as Parts I and II. The continuous growth of the whole subject should be noted. Theory has particularly progressed through mechanistic studies. Among new types of electrodes, ISFET systems have become important. Among new applications of both solid and liquid membrane electrodes, the fluoride electrode still predominates, closely followed by potassium electrodes. In the field of biological and medical applications, the steady growth is significant. More than 800 papers published between mid-1976 and the end of 1978 are mentioned in the review.

CONTENTS

*Part II: Anal. Chim. Acta, 91 (1977) 1.

The steady growth of publications on ion-selective electrodes is still quite marked. This progress has involved not only the production of electrodes and automated equipment based on these electrodes, but especially the application field. Part I of Theory and Applications of Ion-Selective Electrodes [1] covered the literature to the beginning of 1972. In addition to the general theory of membrane potentials of various types, the theoretical basis of the potential of ion-selective electrodes and the applications of non-glass electrodes, Part I included the theory of the glass electrode with the basic facts about glass electrodes sensitive to cations other than hydronium. It also dealt in some detail with the general properties of neutral ion-carriers (ionophores). Part II [2] covered papers published before about mid-1976. Glass electrodes were not discussed except for some chalcogenide glasses, and neutral ion-carriers were described only in connection with their function in ion-selective electrodes. The present review deals with papers published before the end of 1978. During these two and a half years, more than 800 papers were published, which compares well with about 1200 references included in the preceding review. The subject is treated in an order similar to that used previously. First, progress in theory is dealt with, then some points common to all ion-selective electrodes are discussed, and finally new information on the basic properties of various types of electrodes, together with their analytical applications, is reviewed.

Between 1976 and 1978, several symposia on ion-selective electrodes were organized and the proceedings published [3–6]. The theory and applications of ion-selective electrodes have been dealt with in several books [7—14], all of which are oriented towards analysis. Four of them give general surveys of analytical applications [7, 9, 10, 14]; Cammann's book has appeared in a 2nd edition [9] and in a Polish translation [10]. Three deal with special fields of analysis: medicine [11], water [13] and organic species [8]. The first volume of a comprehensive handbook on ion-selective electrodes has been edited by Freiser [12]; it deals with theory (R. P. Buck), precipitate-based ion-selective electrodes (E. Pungor and K. Tóth), ion-selective electrodes based on neutral carriers (W. E. Morf and W. Simon), poly(vinyl chloride) matrix membrane ion-selective electrodes (G. J. Moody and J. D. R. Thomas), sources of error in ion-selective electrode potentiometry (R. A. Durst) and applications of ion-selective electrodes (G. J. Moody and J. D. R. Thomas). Several biblio-

graphies on ion-selective electrodes have been published [15—18]. Numerous general reviews have appeared in international and national journals [1, 2, 19—33]. The specialized reviews [34—82] have been listed in Table 1.

There have been no important changes in the list of firms producing ion-selective electrodes since 1977. Automatic instruments based on ion-selective electrodes will be dealt with in section B.

A. THEORY OF MEMBRANE POTENTIALS [83—110]

(i) General studies

In his review [84] Buck has successfully included various types of ion-selective sensors into the framework of electrode systems in general electrochemistry. The potential-generating processes in the systems electrolyte (redox)/metal, electrolyte (ion exchange)/metal, electrolyte (ion exchange)/membrane and electrolyte (redox)/semiconductor resemble each other and, on the whole, are well known (cf., e.g. ref. 110a). In contrast, the systems

electrolyte | membrane | insulator (1)
(reversible) (blocked)

electrolyte | membrane | insulator (2)
(blocked) (blocked)

electrolyte | membrane | electronic conductor (3)
(reversible) (blocked)

TABLE 1

Specialized reviews on ion-selective electrodes

Subject	Reference	Subject	Reference
Analysis	35, 47, 60, 68, 73, 74, 80	Soil analysis	49, 63, 82
		Agrochemical analysis	51, 63
		Environment	52, 55, 61, 62
Ion passive transport study	34		
Nutrient uptake by plants	36, 61a, 64	Tobacco and smoke	57
Gas-sensitive ion-selective electrodes	37	Industrial waste	58
		Non-aqueous solvents	59
Pollution control	38	Blast furnace effluents	65
Pharmaceutical analysis	39, 54	Electrodes CRYTUR	66
Paper industry	40	Industrial analysis	71
Water analysis	41, 44, 69, 70, 77	Anion-forming elements determination	75
Student laboratory use	42, 50	Heavy metal traces	76
Clinical applications	43, 53, 54, 56, 67	Dairy products	78
		Liquid-exchanger microelectrodes	79
Serum electrolyte determination	45		
Urine electrolyte determination	46	Metal finishing	81
Electrophysiological applications	48, 72		

are not generally understood (the term "blocked" means that neither electron nor ion exchange is possible across the interface). Systems (1) and (2) are further complicated because they require a detector of the interfacial potential (to the right of the insulator on the scheme) which is a metal or semiconductor with bent bands in response to the generated interfacial potential difference. The author has given a clear account of the processes at "reversible" (cf. Part I [1]) and blocked interfaces. The results obtained for the blocked interface may apply to the cases of coated-wire electrodes (Part I, p. 380; and Part II, p. 16) and of the immunoelectrode (ref. 1112 of Part II). For example, the coated-wire electrode comprises two interfaces, one between the coating and the analyte, the other between platinum and the coating. The outer interface shows a reversible Nernstian behaviour towards the appropriate ionic species. The coating is an ionic conductor with a zero inner field. The blocked platinum—coating interface behaves like an ideally polarized electrode, showing a constant potential difference independent of the composition of the analyte (capacitive coupling). In contrast to the usual type of ideally polarized electrode, the charge on the metal depends entirely on the charge in the coating (which depends on the analyte composition only) and cannot be arbitrarily varied by the external source. Thus, the total e.m.f. of the measuring cell consisting of all potential differences in the system changes only with variations in the potential difference at the coating—analyte interface. This situation can, of course, be influenced by spurious effects such as electrode processes of oxygen (p. 57 [110b]). Similarly, in the case of an ISFET (see [2]), the electrical field generated at the analyte—membrane interface is transmitted through the insulating gate to the insulator—semiconductor interface (for more details, see section B).

Usually, a metallic electrode in the presence of an oxidation—reduction system simultaneously possesses the properties of a charge-exchanger and of a capacitor (of an ideally polarized electrode). Both those properties are shown, in an analogous manner, by the membranes of ion-selective electrodes [87, 88]; this has been discussed in detail for liquid—liquid interfaces [97].

The rate of an oxidation—reduction process taking place at a metallic electrode, $Ox + e^- \rightleftharpoons Red$, is usually described by the basic equation of electrochemical kinetics

$$j = Fk \{\exp [(1-\alpha)F(E-E^{\circ})/RT]\, c_{Red} - \exp [-\alpha F(E-E^{\circ})/RT]\, c_{Ox}\} \tag{4}$$

where k is the standard rate constant of an electrode reaction, α is the charge-transfer coefficient, E is the electrode potential, E° the conditional standard (formal) electrode potential of the reaction, c_{Red} and c_{Ox} the concentrations of the species Red and Ox, and F, R and T have their usual significance. The exchange current density

$$j_0 = 10^3\, Fk c_{Ox}^{1-\alpha} c_{red}^{\alpha} \tag{5}$$

is normally used to characterize the rate of establishment of the Nernstian equilibrium at the electrode (which also means the rate of counter-action of the electrode if this equilibrium is disturbed by flow of current). The same equation applies to the membrane surface—electrolyte system [88, 100] if the E and E° terms are replaced by the electrical potential differences between the membrane phase and the aqueous electrolyte phase $\Delta\varphi$ and $\Delta\varphi^{\circ}$, and c_{Red} and c_{Ox} are replaced by the concentrations of the transferrable ionic species c_{J+} in the aqueous electrolyte and C_{J+} in the membrane. $\Delta\varphi^{\circ}$ is the equilibrium value of $\Delta\varphi$ for $c_{J+} = C_{J+}$. The values of exchange current densities obtained for various ion-selective electrodes by Cammann are listed in Table 2.

An ion-selective membrane resembles, in several aspects, a multiple (e.g., corroding) electrode. The concepts of the mixed-potential theory [110a] can be easily transferred to the field of ion-selective electrodes in order to explain non-Nernstian responses, interfering effects, etc. [88].

TABLE 2

Estimates of exchange current density [88, 89][a]

Electrode	Solution	Exchange current density (A cm^{-2})
Ag metal	1 M $AgNO_3$	0.1
Ag_2S	1 M $AgNO_3$	1
Fluoride (LaF_3)	1 M KF; 0.7 M K_2SO_4	5×10^{-5}
	1 M KOH; 0.7 M K_2SO_4	5×10^{-6}
	0.01 M KF; 0.7 M K_2SO_4	10^{-5}
	0.025 M $La(NO_3)_3$	7×10^{-7}
6×10^{-7} M valinomycin/ n-decanol	1 M KCl	3×10^{-7}
6×10^{-4} M valinomycin/ n-decanol[b]	1 M KCl	10^{-7}
6×10^{-4} M valinomycin/ n-decanol	1 M KCl	10^{-5}
6×10^{-3} M valinomycin/ n-decanol	1 M KCl	2×10^{-4}
2.7×10^{-3} M valinomycin/ diphenylether	1 M KCl	6×10^{-4}
	1 M K picrate	9×10^{-3}
	1 M RbCl	2.7×10^{-3}
	1 M CsCl	2×10^{-4}
	1 M NH_4Cl	3.4×10^{-6}
	1 M NaCl	10^{-6}
	1 M LiCl	8×10^{-7}
	0.1 M $MgCl_2$	2×10^{-7}

[a]Note that the exchange current densities were measured for the whole ion-selective electrode (not for a single interface) and are influenced by the ohmic drop in the system.
[b]Not conditioned.

(ii) Solid-state ion-selective electrodes

The approach of basic electrochemical kinetics is perhaps too simplified for solid-membrane ion-selective electrodes. Thus, the theory of response time based on a similar concept could not be verified for single crystal electrodes [85]. The possible influence of the dissolution rate has been accounted for in the theory put forward by Buffle and Parthasarathy [87]. The properties of the materials for solid-state membranes have been discussed from the standpoint of the theory of solid electrolytes [91]. An attempt has been made to account for the influence of diffusion initiated by the exchange reaction at the surface or inside the porous surface of a solid membrane [92, 93].

In an experimental study, Gratzl et al. [90] have stated the limits of application of the theory of pH-dependence of the cyanide-selective electrode put forward earlier (pp. 336—337 and 365 [1]).

(iii) Liquid membranes with dissolved ion-exchangers

In the earlier reviews, the important paper by Bonhoeffer et al. [83] on liquid membrane systems was omitted; in this paper, the ion-exchange properties of organic solvents containing hydrophobic ions were described. The basis of the selectivity of liquid-membrane electrodes [101—103] and the effect of ion-pairing in the membrane phase [95, 108] have been discussed in several papers. The influence of ion-pairing on the response time has been dealt with by Stover and Buck [109]. The results obtained by digital simulation [86] for the selectivity coefficient K_{21}^{Pot} of a liquid membrane with a monovalent exchange site as a function of the activities of monovalent and divalent ions present simultaneously in the bathing solution are shown in Fig. 1.

Jyo et al. [94] have investigated the influence of the presence of a co-ion

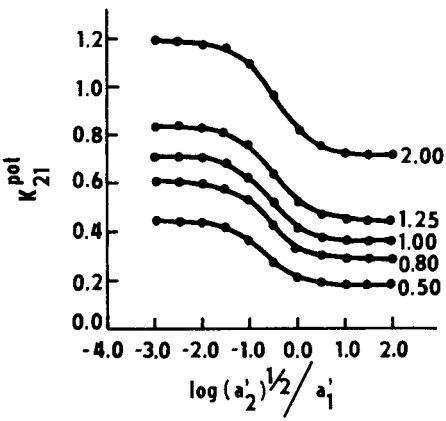


Fig. 1. Selectivity coefficient K_{21}^{Pot} calculated by Buck and Stover [86] vs. activity ratio for various values of the mobility ratio U_1/U_2. Suffixes 1 and 2 refer to the monovalent and divalent ion, respectively.

(the ion with the same charge sign as the ion-exchanging ion in the membrane) which can penetrate into the membrane. Their treatment of this problem (analogous to the Teorell—Meyer—Sievers approach [1]) based on solution of the appropriate Nernst—Planck equations resulted in an expression for the membrane potential in the case of monovalent ions:

$$\begin{aligned}\Delta\varphi_M &= \varphi(1) - \varphi(2) \\ &= \pm(RT/F) \ln a_A(1)/a_A(2) \mp (RT/F) \ln \{ [C_S + (C_S \\ &+ 4k_A k_B a_A(1)^2)^{\frac{1}{2}}]/2C_S\} \pm (RT/F)\,(U_A - U_B)/(U_A + U_B) \cdot \ln (\{(U_A \\ &+ U_B)\,[C_S + (C_S^2 + 4k_A k_B a_A(1)^2)^{\frac{1}{2}}]/2 - U_B C_S\}/U_A C_S) \qquad (6)\end{aligned}$$

In this equation, 1 denotes quantities relating to the bathing solution and 2 those relating to the filling solution of the ion-selective electrode; φ is the electrical potential of the appropriate phase, A the ion to be determined (analyte), B the co-ion, C_S the concentration of the ion-exchanging ion (counter-ion) in the membrane; U_A and U_B are the mobilities of A and B in the membrane, and k_A and k_B the individual partition coefficients of A and B between the membrane and the aqueous phase. These quantities depend, precisely speaking, on the potential difference between these two phases, but in the product the potential-dependent terms disappear with the result $k_A k_B = (k_{AB})^{\frac{1}{2}}$, where k_{AB} is the partition coefficient of the salt AB. The upper signs in eqn. (6) apply when A is a cation and the lower signs apply to anions. Jyo et al. [94] showed that for high values of the term $k_A k_B a_A(1)^2$ with respect to C_S, there will be a deviation from Nernstian behaviour. This was proved for the crystal violet nitrate electrode with nitrobenzene as solvent, where for low concentrations of crystal violet nitrate in the membrane the deviation increased in the sequence $(CH_3)_4N^+ > Cs^+ > Rb^+ > Na^+$, which accords with the relative increase of k_B. Analogous results were obtained with the methylephedrine electrode with tetraphenylborate as counter-ion.

(iv) Liquid membranes with dissolved neutral carriers [85, 96, 105, 106, 110]

The exchange kinetics at a valinomycin electrode was studied by Cammann [89] who used galvanostatic and potentiostatic methods. The exchange current densities decrease in the sequence $Rb^+ > K^+ > Cs^+ > NH_4^+ > Na^+ > Li^+ > N(CH_3)_4^+ > N(C_2H_5)_4^+$ for ions both in the membrane and in the bathing solution. The effect of interferences can be explained by the mixed potential theory. This theory also accounts for super-Nernstian phenomena. The effect of anions was also investigated; picrate caused instability of the membrane potential, while iodide and phthalate increased the current densities somewhat in comparison to Cl^-, Br^-, F^- and NO_3^-.

Several theories have been suggested to explain the conspicuous permselectivity of neutral carrier membranes for cations [1, 2]. Thus (a) electroneutrality is not preserved within the membrane (Ciani—Eisenman—Szabo theory of bilayer lipid membranes containing neutral ionophores, extended to thick membranes by Buck and Boles); (b) slow surface kinetics of anions

(Buck et al.); (c) there are anionic species fixed to the matrix of the membrane (proposed again by Kedem et al. [96] and Perry et al. [105a]); (d) the anions permanently present in the membrane are immobilized in the membrane owing to their poor water-solubility; and (e) anions with low mobility in the membrane have been extracted from water.

However, Thoma et al. [110] (cf. [105]) showed by electrodialysis experiments that the permselectivity of the membrane cannot be explained by these hypotheses. The membrane is electroneutral and, besides low concentrations of chlorides coming from the bathing solution, the counter-ions to the cationic carrier complexes are hydroxide ions formed by exchange of K^+ from the bathing solution for protons from water dissolved in the membrane solvent. The transfer of protons was also proven by the change in pH of the bathing solution when it comes in contact with the membrane containing the carrier alone.

The acyclic ionophores introduced by Simon and co-workers (see [2]) form complexes containing several ligand molecules per metal ion. For this case, the equation for the selectivity coefficient reads [106]

$$K_{ij}^{\mathrm{Pot}} = \{k_j(1 + K_{js}[\mathrm{S}]^{n_{js}})/\{k_i(1 + K_{is}[\mathrm{S}]^{n_{is}})\} \quad (7)$$

where k_i and k_j are the partition coefficients of the individual uncomplexed ions i and j between the aqueous and the organic phase, K_{is} and K_{js} are the stability constants in the organic phase, [S] is the ligand concentration in the organic phase, and n_{is} and n_{js} are the number of ligand molecules per metal ion in the complex.

(v) Electrolysis at the interface of two immiscible electrolyte solutions [90a, 96a, 97—100, 107, 107a, 107b]

This approach to membrane phenomena was mentioned in Part II [2], where the pioneering work of Gavach and co-workers was referred to together with our own contribution. In this method, two solvents of very poor miscibility (for example, water and an organic solvent like nitrobenzene or dichloroethane) are used. Each solvent contains an electrolyte which is practically confined to that solvent when the two electrolyte solutions are in contact (e.g., lithium chloride for the aqueous phase and tetrabutylammonium tetraphenylborate for the organic phase). In the presence of the two base electrolytes only, the interface behaves like an ideally polarized electrode [97, 98a]. When a "semi-hydrophobic" ion is present, e.g. in the aqueous phase, it can be transferred to the organic phase by imposing a suitable potential difference between the two phases. This situation is quite analogous to that occurring at a metallic electrode when an oxidation—reduction system is present in the adjacent electrolyte solution (Fig. 2). The polarization of the interface cannot be achieved simply by means of two electrodes immersed in the electrolyte solutions as the electrode processes taking place at these electrodes and the ohmic potential drop between the electrode and the interface would totally distort the measurement. In order

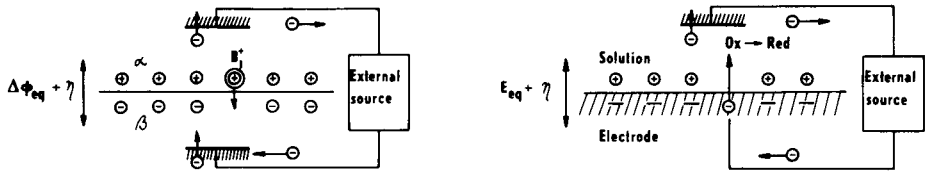


Fig. 2. Comparison of electrolysis at an interface between a metallic electrode and an electrolyte (right) and between two immiscible electrolyte solutions (left). The injection of a negative charge from the electrode into the electrolyte starts the electrolysis which proceeds with an overpotential η added to the equilibrium oxidation—reduction potential E_{eq}. The injection of a negative charge from an auxiliary electrode into one of the immiscible phases causes a semi-hydrophobic ion B_1^+ to cross the interface under the influence of an overpotential η added to the equilibrium potential difference between the two immiscible phases $\Delta\varphi_{eq}$.

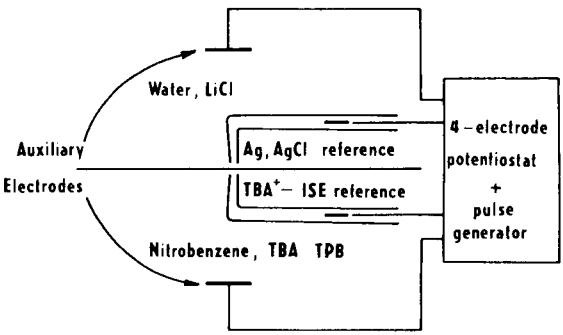


Fig. 3. A 4-electrode potentiostatic system applied to two immiscible electrolyte solutions, LiCl in water and tetrabutylammonium tetraphenylborate in nitrobenzene. The electric current is introduced by means of two auxiliary electrodes. The potential difference between the phases is controlled by means of two reference electrodes positioned close to the interface by Luggin capillaries. For the aqueous phase a silver—silver chloride reference electrode is used; for the nitrobenzene phase, a tetrabutylammonium-selective electrode (TBA in water with an internal Ag/AgCl electrode) is needed.

to avoid that, a four-electrode potentiostatic system must be used (Fig. 3); under these conditions the various modes of polarization of the interface can be applied, at present mainly cyclic voltammetry [107]. The example of transfer of tetramethylammonium ion is shown in Fig. 4. As in polarography and related methods, half-wave potentials are significant for current—voltage dependences. As a complete analogy of the standard electrode potential, which is directly related to the standard Gibbs energy of the half-cell reaction, the concept of the standard electrical potential difference $\Delta_0^w\varphi_i^0$ between the aqueous and the organic phase for ion i was introduced. This is related to the standard Gibbs energy of transfer of the ion from the organic to the aqueous phase, $\Delta G_{tr,i}^{0,o\rightarrow w}$, according to the equation

$$\Delta_0^w\varphi_i^0 = -z_iF\Delta G_{tr,i}^{0,o\rightarrow w} \qquad (8)$$

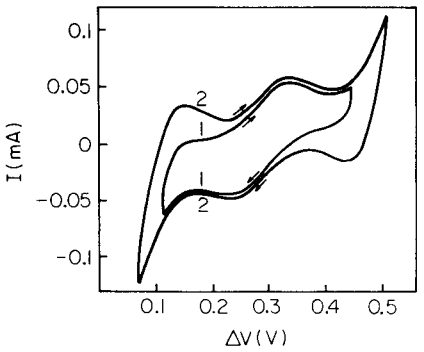


Fig. 4. Cyclic voltammograms for the transfer of tetramethylammonium cation, originally present in the aqueous phase, to nitrobenzene (upper curves) and back (lower curves). Polarization rate 5 mV s^{-1}. In curve 1 the original negative current corresponds to the transfer of the tetrabutylammonium ion from the organic to the aqueous phase after which transfer of tetramethylammonium ion ensues (the curve with the peak). The final current increase corresponds to the transfer of tetraphenylborate anion from the organic to the aqueous phase. The peaks at the start and end of curves 2 correspond to the transfer of tetrabutylammonium cation and of tetraphenylborate anion back to the nitrobenzene phase (both ions were transferred to water during prior polarization).

where z_i is the charge of ion i. The standard Gibbs transfer energy is connected with the partition coefficient of individual ion i (see eqns. 6 and 7)

$$k_i = \exp\left(-\Delta G^0_{\mathrm{tr},i}{}^{o\rightarrow w}/RT\right) \qquad (9)$$

but the value of k_i is not thermodynamically defined. The most frequently used extrathermodynamic assumption (TATB assumption) for the determination of standard Gibbs transfer energies is [110c] that these energies for tetraphenylarsonium and tetraphenylborate ions between an arbitrary pair of solvents have equal (but not constant) values. On the basis of this assumption, scales of $\Delta^w_0\varphi^0_i$ can be designed. On such a scale (if one of the solvents is water), the more hydrophobic the cation, the more negative its standard potential difference of transfer, and vice versa for the anions.

For reversible processes, the half-wave potential is linked to $\Delta^w_0\varphi^0_i$ in the same way as a polarographic half-wave potential at a metallic electrode is related to the standard electrode potential. The ion-transfer rates are, in fact, quite rapid [107a] so that for the current—potential curves the reversible course can be assumed. This is particularly true for polarographic curves obtained with an electrolyte dropping electrode [98, 98a] (for an advanced form equipped with a four-electrode system, see [107b]).

The selectivity of liquid-membrane ion-selective electrodes can be analysed by means of these current—voltage curves that have been either measured directly or constructed on the basis of data obtained by other methods (cyclic voltammetry, chronopotentiometry, partition equilibria, etc.).

In this analysis, the basic condition for the response of a membrane towards a given ionic species is the formation of an "anodic—cathodic" wave

on a polarogram of this ion present in both phases. The organic phase should be similar to the membrane solvent, and the counter-ion in the base electrolyte of the organic phase should be similar to the exchanging ion. The zero-current potential $\Delta\varphi(I = 0)$ of such undisturbed waves is connected with the potential of the ion-selective electrode by the relationship $\Delta\varphi(I = 0) = -E_{ISE}$ + const. The minus sign arises here because of the definition of $\Delta\varphi = \varphi(w) - \varphi(o)$. The value of $\Delta\varphi(I = 0)$ is related by the Nernst—Donnan equation to the ratio of the activities of the sensed ion in the aqueous and organic phases.

Figure 5 shows the polarogram of picrate dissolved simultaneously in nitrobenzene and in water with tetrabutylammonium as the counter-ion in the organic phase [90a]. In fact, the organic phase would contain the same concentrations of the sensed ion and the exchanging ion, from which a somewhat less advantageous situation would result, as shown in Fig. 6. Nernstian behaviour for a picrate electrode predicted from these results has been actually found with an electrode containing nonyl-*p*-nitroethyl ether (similar to but less polar than nitrobenzene) as the membrane solvent and dodecyltrimethylammonium ion (similar to but more hydrophobic than the tetrabutylammonium ion) as the exchanging ion [90b]. However, the same membrane system worked poorly as a perchlorate-selective electrode. This is obvious from the behaviour of perchlorate in cyclic voltammetry [96a, 97] where no distinct peak corresponding to perchlorate transfer from the aqueous to the nitrobenzene phase could be observed. Figure 7 shows the effect of superposition of the current of the ion-exchanging cation over the perchlorate wave. Thus, a perchlorate and even a nitrate response can be observed with very hydrophobic ion-exchanging cations such as crystal violet, the Ni(II)—bathophenanthroline complex or tetraphenylarsonium (Fig. 8).

The deteriorating effect of hydrophobic cations on the performance of

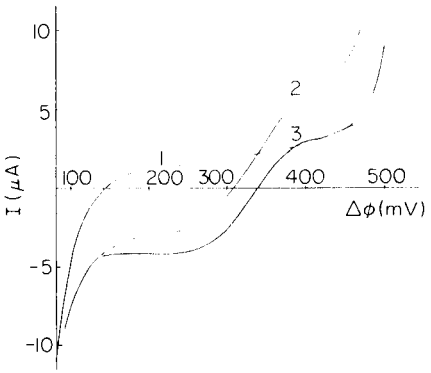


Fig. 5. Polarograms with the electrolyte dropping electrode: (1) base electrolyte with 0.05 M LiCl—1 M $MgSO_4$ as the aqueous phase and 0.05 M TBA—TPB in the nitrobenzene phase; (2) 0.2 mM picrate in both base electrolytes; (3) the resulting curve after subtraction of curve 1 from curve 2.

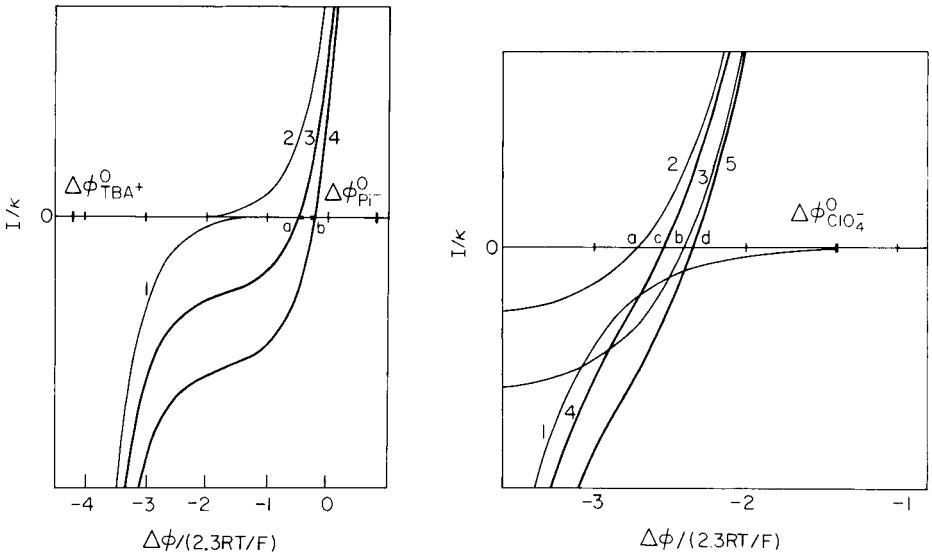


Fig. 6. Polarograms calculated for: (1) 10 mM TBA^+ in the organic phase: (2) 10 mM picrate in the organic phase; (3) 0.5 mM picrate in the aqueous phase and 10 mM TBA picrate in the organic phase; (4) 1 mM picrate in the aqueous phase and 10 mM TBA picrate in the organic phase. The shift of the zero-current potentials ab is 2.3 RT/F. log 2 = 18 mV (Nernstian slope). Equal diffusion coefficients of picrate in the aqueous and organic phase are assumed.

Fig. 7. Polarograms calculated for: (1) 10 mM TBA^+ in the organic phase; (2) 0.5 mM ClO_4^- in the aqueous phase and 10 mM ClO_4^- in the organic phase; (3) 1 mM ClO_4^- in the aqueous phase and 10 mM ClO_4^- in the organic phase. By addition of curves 1 and 2 and curves 1 and 4, composite polarograms 4 and 5 are obtained which correspond to the voltammetric behaviour of the picrate electrode with 0.5 mM picrate and 1 mM picrate, respectively, in the aqueous phase. Without the influence of the exchanging cation (TBA^+), the shift of the zero-current potentials would be Nernstian (ab = 2.3 RT/F = 18 mV) whereas under the influence of TBA^+ a sub-Nernstian slope (cd = 12 mV) is observed.

the perchlorate-selective electrode is easily explained by means of the diagram shown in Fig. 9. This effect is more pronounced with electrodes selective for more hydrophobic anions such as picrate.

Reversible cyclic voltammograms have been observed with macrocyclic ion carriers like dibenzo-18-crown-6 or valinomycin in the presence of alkali metal ions in the aqueous phase [96a, 97], in accordance with the reversible behaviour of the appropriate ion-selective electrodes. However, the wave of the potassium—valinomycin complex interferes with the current of tetrabutylammonium ion from the base electrolyte of the organic phase. Thus, cationic surfactants (or strong anionic surfactants) dissolved in the membrane of a valinomycin potassium-selective electrode will probably affect its functioning.

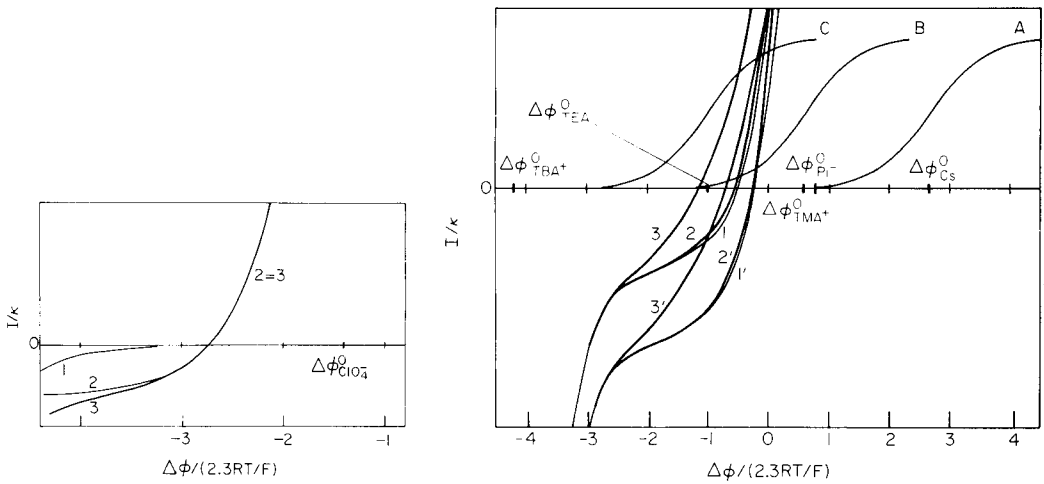


Fig. 8. Polarograms calculated for: (1) 10 mM tetraphenylarsonium in the organic phase; (2) 0.5 mM ClO_4^- in the aqueous phase and 10 mM ClO_4^- in the organic phase. The resulting curve 3 was calculated for 0.5 mM ClO_4^- in the aqueous phase and 10 mM TPA ClO_4^- in the organic phase. This composite curve and, particularly, the zero-current potential are not affected by the exchanging ion so that a Nernstian slope is expected for this perchlorate electrode.

Fig. 9. Polarograms demonstrating the influence of hydrophobic cations on the behaviour of the picrate electrode. (1) 0.5 mM picrate in the aqueous phase and 10 mM TBA picrate in the organic phase. (1') 1 mM picrate in the aqueous phase. The wave of 1 mM Cs^+ (curve A) in the aqueous phase has no influence on the composite waves 1 and 1'. Tetramethylammonium ion (1 mM) in the aqueous phase (curve B) causes a small shift of the zero-current potentials a and b to more negative values, c and d. Thus the zero-current potentials correspond to apparently smaller concentrations of the picrate in the aqueous phase while the slope remains practically Nernstian. In the presence of a more hydrophobic cation, 1 mM tetraethylammonium (curve C), there is a considerable shift of zero-current potentials to more negative potentials e and f. The resulting potential difference ef is considerably larger than the value 2.3 RT/F log 2. Thus a super-Nernstian slope is observed.

(vi) Liquid junction potential [104]

The theory of the liquid junction potential has already been discussed in detail [1]. Because of its somewhat involved mathematics, the Planck theory has not been dealt with (cf. [110b]). Recently, Morf [104] has succeeded in a simplified presentation of Planck's approach.

The Nernst—Planck equation for the flux of the ith ionic species in a liquid junction of thickness d

$$J_i = -U_i RT\, dc_i/dx - z_i F U_i c_i d\varphi/dx \tag{10}$$

can be written in the integral form

$$J_i = U_i RT\{c_i(d) \exp[z_i F\varphi(d)/RT] - c_i(0) \exp[z_i F\varphi(0)/RT]\}/ \int_0^d \exp[z_i F\varphi(x)/RT]\, dx \tag{11}$$

where U_i is the mobility of the ith species, c_i its concentration and z_i its charge number. The electrical potential has the values $\varphi(0)$ for $x = 0$ and $\varphi(d)$ for $x = d$. The cations are denoted below by the symbol M and the anions by X; their charge numbers are z_m and z_x and their fluxes J_M and J_X. Since no electric current flows across the liquid junction, we have

$$j/F = \sum_m z_m J_M + \sum_x z_x J_X = 0 \tag{12}$$

where j is the total current density.

For the electrical potential gradient, we have from eqns. (10) and (12)

$$\frac{d\varphi(x)}{dx} = -\frac{RT}{F} \frac{\sum_m z_m U_m [dc_m(x)/dx] + \sum_x z_x U_x [dc_x(x)/dx]}{\sum_m z_m^2 U_m c_m(x) + \sum_x z_x^2 U_x c_x(x)} \tag{13}$$

Planck's basic assumption requires the flux to be independent of x, i.e. the system is in a steady state. Electroneutrality is preserved in the whole system. All cations have the same charge number z_m and all anions z_x.

A mean electrolytic mobility is defined for the cations and the anions by the equations

$$\bar{U}_m = \sum_m J_m / \sum_m (J_m/U_m) \text{ and } \bar{U}_x = \sum_x J_x / \sum_x (J_x/U_x) \tag{14}$$

Insertion of these equations into eqn. (12) gives

$$z_m \bar{U}_m \sum_m (J_m/U_m) + z_x \bar{U}_x \sum_x (J_x/U_x) = 0 \tag{15}$$

From eqns. (10) and (15) a new expression is obtained for $d\varphi/dx$:

$$\frac{d\varphi}{dx} = -\frac{RT}{F} \frac{\bar{U}_m \sum_m z_m [dc_m(x)/dx] + \bar{U}_x \sum_x z_x [dc_x(x)/dx]}{\bar{U}_m \sum_m z_m^2 c_m(x) + \bar{U}_x \sum_x z_x^2 c_x(x)} \tag{16}$$

Integration of eqn. (16) is accomplished using the electroneutrality condition. The resulting equation for the liquid junction potential has the form

$$\Delta\varphi_L = \frac{\bar{U}_m - \bar{U}_x}{z_m U_m - z_x \bar{U}_x} \frac{RT}{F} \ln \frac{\sum_i c_i(0)}{\sum_i c_i(d)} \tag{17}$$

where i denotes either m or x.

From eqns. (11) and (14) the mean mobilities are given by

$$\bar{U}_i = \frac{\sum_i U_i c_i(d) \exp(z_i F \Delta\varphi_L/RT) - \sum_i U_i c_i(0)}{\sum_i c_i(d) \exp(z_i F \Delta\varphi_L/RT) - \sum_i c_i(0)} \tag{18}$$

$\Delta\varphi_L$ is easily computed by successive approximations. For $\Sigma_i c_i(0) = \Sigma_i c_i(d)$ the relationship is simple:

$$\Delta\varphi_L = \frac{RT}{F} \ln \frac{\sum_m U_m c_m(0) + \sum_x U_x c_x(d)}{\sum_m U_m c_m(d) + \sum_x U_x c_x(0)} \quad (19)$$

Morf [104] also gave examples of the application of Planck's mode to diffusion potentials inside a membrane.

B. TECHNOLOGY OF ION-SELECTIVE ELECTRODES [87—224]

(i) Construction [126, 128, 135, 136, 141, 142, 167, 168, 186, 189, 193, 198, 202, 207—211, 217, 218]

All-solid-state ion-selective electrodes have been prepared from fused sensing materials [198]. Grain boundary effects, particularly those caused by adsorption, have been discussed [211]. Solid-state ion-selective electrodes consisting of a thin layer of a sensing material deposited on an ionic conductor have been described [208—210]; the supporting materials were Ag_2S, Ag_3SBr, Ag_3SI and $Ag_{19}I_{15}P_2O_7$. Another two papers on gold-containing membranes (cf. Part II, p. 14) have been published [217, 218].

The problem of high resistance at the tips of ion-selective microelectrodes with liquid membranes has been partly overcome in a coaxial ion-selective microelectrode [207]. Another micropipette filled with saturated KCl solution is plugged into the ion-selective microelectrode. The technique of filling the microelectrodes has also been discussed [202]. A detailed investigation into the construction and measurement techniques of mini- and microelectrodes has been reported [174a].

The properties of ion-selective electrodes based on PVC matrices have been reviewed [168]. Two reviews on coated-wire electrodes have been published [128, 167]. Miniature coated-wire sensors for Ca^{2+}, NH_4^+, K^+ and Pb^{2+} [126] as well as coated-disc electrodes [189, 193] have been described.

Further contributions deal with a suitable construction of the body of liquid-membrane electrodes [186], with electrodes based on an anion-exchange resin with a hydrophobic site [142] and on epoxy resins [141], and finally on application of the traditional Japanese material Urushi (used for lacquer products) as a membrane matrix (135, 136].

(ii) Ion-selective field-effect transistors (ISFET) [111, 117, 118, 124a, 125a, 138, 139, 145, 146, 162, 169a, 169b, 188, 203, 223, 224]

As already briefly outlined [2], an ISFET is a modified MOSFET where an ion-selective membrane placed on the insulating layer covers the npn junction of a silicon semiconductor (Fig. 10). The electrical potential of the membrane, which depends on the ionic activities in the test solution, generates a field in the insulating layer which influences the current flowing between the source and drain contacts. Measurement of the membrane potential is based on measurement of the changes in the drain current, which is a function of the membrane potential. In this case, the electrical potential of the

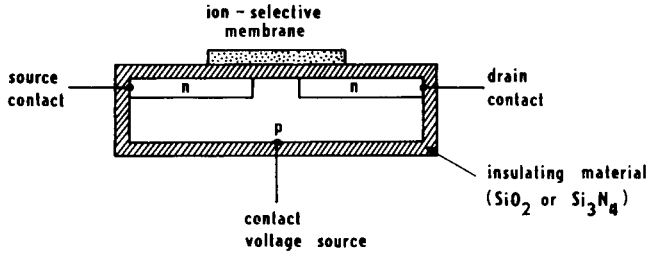


Fig. 10. An ion-selective field-effect transistor (ISFET). The potential difference between a reference electrode and the p-semiconductor wafer (including the potential differences on both surfaces of the membrane layer and an applied bias voltage) influences the drain current flowing between two diffused wells of the n-type. This current is measured as a function of the activity of the ions to which the membrane is selective.

membrane is a kind of Volta potential and the insulating layer plays the same role as vacuum between the phase under investigation and a metal phase in the usual Volta potential measurement [117].

ISFET theory has been reviewed in several papers [117, 139, 145, 146, 188, 224] (see also [125a, 138]). A conference on ISFETs has been held [203].

Since 1976, new ISFETs sensitive to hydronium [111, 124a, 162], sodium [124a], potassium [162, 169a] and calcium [162, 169a] ions have been developed. Direct casting of the PVC membranes on the insulating layer resulted in stable devices. The partially oxidized Si_3N_4 surface shows selectivity towards hydronium ions. The response times for ISFETs are similar to those for ion-selective electrodes. Their small size is advantageous for certain applications [162].

A sufficiently small reference electrode is an essential component of an ISFET assembly. Thus, a small Ag/AgCl reference electrode has been incorporated in the upper cavity of a catheter used as support for the ISFET [169b]. In another approach, two polymeric pH ISFETs have been placed on the same chip, one of which responds to the hydronium activity of the analyte while the other is in contact with an immobilized electrolyte bridge of constant pH [118]. Since ISFETs do not seem to be suitable for devices sensing several ions at the same time, an ion-selective gate-controlled diode has been constructed [223].

(iii) Calibration [115, 123, 131, 149, 158, 159a, 164—166, 185, 187]

Conventional activity scales are essential for any calibration of ion-selective electrodes. Developments in designing such scales have been reviewed [115] with particular accent on a new set of hydration numbers (Table 3) based on analysis of activity coefficient data. Recent work on the ion activities of mixtures of the type MX/NX_2 has been summarized (cf. [187]).

The biomedical applications require calibration methods for biological fluids [165]. On the basis of e.m.f. measurements at 37°C in synthetic

TABLE 3

Hydration numbers for 1:1 halides

Cation	Chloride	Bromide	Iodide
H^+	5.5	5.8	5.7
Li^+	5.3	5.4	5.3
Na^+	3.6	3.9	3.9
K^+	2.5	2.4	2.2
Rb^+	2.2	1.8	1.5
Cs^+	1.9	1.5	0.7

electrolyte mixtures simulating serum, a near-Nernstian response of ion-selective electrodes to ion concentrations has been proven; the deviations are caused by activity coefficient changes [166]. NBS standards for pH and ion-selectivity measurements in biological fluids have been worked out [123]. The activity coefficients of alkali chlorides in iso-ionic bovine serum albumin solutions have been evaluated [185].

The mean ionic activity coefficients of choline chloride have been determined at molalities up to 4 mol kg^{-1}. The choline-selective electrode [1] shows a near-Nernstian behaviour over the molality range 10^{-3}—4 mol kg^{-1} [159a].

Activity and interference effects in measurements of ionized calcium with three different types of ion-selective electrodes have been compared [131]. A calibration procedure to minimize errors in continuous analysis with ion-selective electrodes has been reported [149]. Non-ideal calibrations of electrodes caused by (a) the presence of the species to be determined in reagents added to sample solutions, (b) the presence of interfering species, and (c) the solubility of the material of the electrode, have been analysed [164]. A method of estimating the linear range of ion-selective electrodes has been proposed [158].

(iv) Selectivity [129, 151, 161, 175, 201]

Procedures for determination of selectivity coefficients have been described [129, 175, 201]. The measuring range and interferences for gas-sensing probes have been evaluated [161]. Anion interferences with neutral-carrier electrodes has been discussed [151].

(v) Response time [87, 150, 169]

A theoretical explanation of the sluggish response of ion-selective electrodes based on assumed inhomogeneity of the membrane phase has been presented [169]. The response time of neutral-carrier (valinomycin and acyclic synthetic compounds) electrodes has been studied [150]. An operational model for the response kinetics of solid-state electrodes has been worked out [87].

(vi) Detection limits, errors, etc. [124, 133, 143, 147a, 153—157, 177—180, 219]

The detection limit of the iodide-selective electrode has been confirmed as based on the solubility of the membrane material [147a]. The limits of detection of liquid-membrane electrodes have been studied [143]. The relationship between the detection limit and non-linearity of the electrode function has been discussed [154]. Upper limits of concentration determination by ion-selective electrodes have been determined [177—180]. The time-dependence of the electrode response has been discussed on the basis of a statistical approach [153, 155—157]. However, this method has been criticized [219]. Concentration errors in determinations by ion-selective electrodes have been discussed [124]. Methods of increasing the sensitivity of potentiometric analysis have been evaluated [133].

(vii) Measuring procedures [127, 137, 197, 200, 204—206, 214, 215]

Frazer et al. [127] have presented a new method for determination of the equivalence point in automatic potentiometric titrations. A computer helps to find the period during which the titration curve shows Nernstian response. Data obtained during this period are used to estimate the equivalence point.

The "zero current chronopotentiometry" [197] resembles in a certain sense the usual chronopotentiometric method; the method, which is suitable for gas-sensing probes, is based on an e.m.f.—time dependence which is characteristic for each concentration. Several papers deal with null-point potentiometry [204], electrochemical titrant generation [205, 206], effects of complexing agents on solid-state electrodes [214], experimental conditions for back-titrations [215], and computer evaluation of multiple standard additions [137]. A new application of an ion-selective electrode as reference electrode has been reported [200].

(viii) Automatic procedures [112—114, 116, 120—122, 125, 130, 134, 140, 143a, 148, 152, 159, 160, 170—174, 176, 181—183, 190—192, 194—196, 199, 212, 213, 220, 222]

Automatic analytical methods fall into two groups: the monitoring of concentration changes in order to obtain data for process control and the automatic analysis of serial samples (for a discussion with practical examples, see [171] and [183]).

On-line applications of ion-selective electrodes have been reviewed [112, 114] and critical comments have been made [122]. Continuous methods for cyanides [121] and for in vivo analysis [113, 191] have been described. Flow-through systems containing a copper-selective electrode [116] and several coated-disc ion-selective electrodes [192] have been dealt with. A computer-controlled interference correction for measurement in flowing systems has been suggested [130].

For automatic discontinuous analysis of clinical samples, a very stable flow-through system has been worked out [176]. In Table 4 the reproduci-

TABLE 4

Reproducibility of measurements [176][a]

Ion	Reproducibility of flow-through system based on ion-selective electrodes	Reproducibility of optical methods
Na^+	1.3	4
K^+	1.4	5.6
Ca^{2+}	2.6	6.8—10.8
Cl^-	1.3	4

[a]The reproducibility (%) corresponds to the 95% confidence interval (4 times the coefficient of variation).

bility of measurements with ion-selective electrodes is compared with those of atomic spectrometric techniques for Na^+, K^+ and Ca^{2+} and with the colorimetric method for chloride.

Table 5 lists recent commercial analysers based on ion-selective electrodes. In several papers the results obtained with some of these devices have been critically discussed [125, 148, 159, 160, 174, 181, 181a, 194—196, 199, 222].

Automated analysers for determinations of K^+ and Na^+ in whole blood [20] and whole blood and serum [134, 140] have been described. Methods for automatic water analysis have been proposed [152, 182]. Automatic methods for determination of several ions in biological samples and in synthetic mixtures [190, 220] have been reported. The use of combination electrodes in automated systems has been discussed [212, 213]. A microprocessor ion-analyser [173] and a voltage-frequency converter for high-precision on-line A/D conversion for ion-selective measurements have been described [143a]. The titration technique based on triangle-programmed reagent addition has definite advantages for automated systems [170, 172].

TABLE 5

Commercial analysers for blood and serum based on ion-selective electrodes

Analyser	Ions analysed	Producer
STAT/ION	Na^+, K^+, Cl^-, HCO_3^-	Technicon
Hitachi 702	Na^+, K^+, Cl^-	Hitachi
Electrion	Ca^{2+}	Applied Medical Technology
STAT/LYTE	K^+, Cl^-	Technicon
Orion 98, 99-Series	Na^+, K^+, Ca^{2+}, Cl^-	Orion Research
Orion Space-Stat	Na^+, K^+, Ca^{2+}, Cl^-	Orion Research
Orion 3300	Na^+, K^+, Ca^{2+}, Cl^-	Orion Research
Orion SS 20	Ca^{2+}	Orion Research
Orion SS 30	Na^+, K^+	Orion Research
Nova 1	Na^+, K^+	Nova Biochemical
OP-266	Na^+, K^+	Radelkis

(ix) In vivo measurements [119, 144, 163]

The measurement of local ion activities in blood has been discussed [163]. The application of solid membrane ion-selective electrodes in vivo measurement has been described [119]. Electrodes sensitive to membrane-permeable ions have been used to determine membrane potentials of cells [132]. Membrane potentials across energy-transducing membranes (submitochondrial particles and chromatophores) can be measured by means of thiocyanate or nitrate uptake determined with ion-selective electrodes [144].

(x) Miscellaneous [132, 147, 184, 216, 221]

The use of a Gran ruler has been discussed [216, 221]. Methods for evaluation of commercial ion-selective electrodes have been described together with several examples [147]. Another paper [184] deals with the use of ordinary pH meters with ion-selective electrodes.

C. FIXED-SITE ION-SELECTIVE ELECTRODES [87, 116, 147a, 181a, 199, 212, 213, 225–496]

Two mechanistic studies [87, 406] concern general properties of solid-state electrodes. A review on this subject has also been published [293].

(i) Silver halide electrodes

Several papers deal jointly with the properties of selective electrodes based on silver halides [230, 253, 278, 292, 304, 364, 405, 477, 480, 481, 489]. The simultaneous determination of halides [278], behaviour of halide-sensitive electrodes in mixed solvents [253] and analysis of halides in marine algae [489] have been described. The properties of membranes based on a mixture of silver halides and Ag_2S have been compared with those of a silver halide–Hg_2Cl_2 mixture [477, 480, 481]. The halide electrodes can be used for microdetermination of quinones [308].

Chloride-selective electrodes. Various applications of these electrodes are listed in Table 6. Various modifications of the electrode based on mercury(I) chloride (calomel) have been described [310, 446]. Inferferences in chloride determination have been discussed [362], particularly the situation when AgI is deposited on AgCl [355]. Another paper [358] deals with calibration of chloride-selective electrodes. A method for increasing the detection limit of the chloride electrode has been described [323]. An on-line computer has been used in a study of the dynamic response of the chloride electrode in the presence of iron(III) [245].

Applications of a chloride electrode based on Hg_2Cl_2 have been reported [233, 377]. Direct potentiometric measurement of sulfite by means of a Hg_2Cl_2/HgS electrode has been based on the reaction

$$Hg_2Cl_2 + 2\ SO_3^{2-} \rightarrow Hg + Hg(SO_3)_2^{2-} + 2\ Cl^-$$

Thus the chloride concentration sensed corresponds to the sulfite concentration in the analyte [459].

TABLE 6

Applications of chloride-selective electrodes

Use	Reference
Cl^- determination, in automated system	199, 212, 213
—, in intracellular conditions	232, 426
—, in sweat	248, 249, 250, 280, 354, 415, 486, 487
—, in waters	263, 325, 357, 366, 433, 456, 493
—, in serum	199, 289, 335
—, in soils	291, 441
—, in flux baths for soft soldering	303
—, in biological fluids	310a, 311, 472
—, in organic material	319a
—, in plant tissues	347
—, in catalysts	357
—, in the presence of $Fe(CN)_6^{4-}$	411
—, in caviar	490
—, general paper	452
Kinetic studies, in non-aqueous solvents	241
—, chloromethyldichloroacetate hydrolysis	359
Detector in gel chromatography	265
Orthophosphate determination as Ag salt	272

Bromide-selective electrodes. Applications of these electrodes to bromide determination in cereals [235], urine [228] and rocks [281] have been described. The electrode has been used as a detector in gas chromatography [356]. The calibration of the bromide electrode [298, 299] and its application to a further study of oscillating reactions (cf. [2]) have been described [297]. Another version of the bromide electrode is the Hg_2Br_2/HgS membrane [459].

Iodide-selective electrodes. Various applications of these electrodes are listed in Table 7. The properties of iodide-selective electrodes have been described [147a, 348, 447] including their behaviour in non-aqueous solvents [407] and under electrical current flow [410].

The Orion Research model 97—70 residual-chlorine electrode [422] is based on a cell consisting of a platinum electrode responding to the couple I_2/I^- and an iodide-selective electrode. The e.m.f. of this cell is given by

$$E = E_{I_2/I^-} - E_{ISE}$$
$$= E^0_{I_2/I^-} + (RT/F) \ln [I_2]^{\frac{1}{2}}/[I^-] - E_{0,ISE} + (RT/F) \ln [I^-]$$
$$= \text{constant} + (RT/2F) \ln [I_2]$$

In an acetate buffer (pH 4) containing iodide, active chlorine is converted to iodine stoichiometrically, thus the sensor indicates the concentration of active chlorine. This method is suitable for determining active chlorine in the range 3—100 ppb.

TABLE 7

Applications of iodide-selective electrodes

Use	Reference
I^- determination, in milk	261, 445
—, in waters	493
—, in alcohol	346
I_2 determination based on iodoform reaction	227
Thiocarbonyl and thiol group determination by titration with I_2	306
α-Amino acid determination by titration with Hg^{2+}	367
Arsenite, sulfite, ascorbic acid, hydrazine and hydroxylamine indirect determinations	259
Nitrate and nitramine determination	307
Oil iodine number determination	321
Hydrazine and hydroxylamine determination by potentiometric titration	330
Molybdenum catalytic determination on the basis of I^--perborate reaction	343
Sulfide indirect determination	398
Trace iodide determination in a refrigerant	414
Organic iodine determination	484
Iodate determination in table salt	409
Pd(II) and PdI_2 determination	464
Hg(II) determination in mixtures	462
Peroxodisulfate—iodide reaction study	360
Determination of L-amino acids and alcohols with the help of oxidase enzymes	360a

TABLE 8

Applications of the cyanide-selective electrodes

Use	Reference
CN^- determination, in waters	240, 300, 349, 471, 474
—, in steelwork effluents	264
—, continuous	319
—, in industrial waste	443
Indirect determination of Co^{2+} in excess of Zn^{2+}	271
Determination of cyanocobalamin by thermal dissociation of CN^-	290
Determination of *m*-dinitro groups after reaction with CN^-	305

Cyanide-selective electrodes. Applications of these electrodes are listed in Table 8. A review of cyanide-selective electrodes has been published [302]. The surface of the cyanide-selective electrode has been studied by means of energy-dispersive x-ray fluorescence spectroscopy [231].

For the continuous determination of cyanide, a HCN-sensing electrode based on an Ag^+-selective electrode in equilibrium with $Ag(CN)_2^-$ can be used [274, 320].

(ii) Silver sulfide ion-selective electrode

A construction of the Ag_2S electrode has been described [230]. The response of an Ag_2S electrode to silver ions has been compared with that of a silver metal electrode [266]; the metal electrode shows shorter response times. A sulfide antioxidant buffer has been described [269]. On prolonged use, the limit of Nernstian response of an Ag_2S electrode changes from 10^{-7} M to ca. 10^{-6} M [294]. This phenomenon originates from mixed potentials caused by accumulation of silver metal in the surface of the membrane. An analogous deterioration of the response of the electrode in the presence of iodine is due to surface oxidation [238]. The response of this electrode to sulfide, iodide and cyanide has been described. Applications of the Ag_2S electrode are listed in Table 9. A silver telluride ion-selective electrode has been evaluated [387].

(iii) Divalent metal chalcogenide electrodes

The construction of divalent metal chalcogenide electrodes has been discussed [229, 393]. The behaviour of electrodes based on silver or mercury(II) sulfide, selenide and telluride matrices has been described [431].

Copper-selective electrodes. The construction of conventional [315, 482, 494], porous flow-through [402], micro flow-through [468] and micro [475] copper-selective electrodes has been described. The formation of mixed copper sulfide/silver sulfide membranes for copper-selective electrodes has been studied by means of x-ray powder diffractometry, scanning electron

TABLE 9

Applications of Ag_2S ion-selective electrodes

Use	Reference
S^{2-} determination, in mineral waters	234
—, in heavy water plant effluents	295
—, during sulfidization of oxidized copper ores	337
—, during flotation of oxidized ores	338
Sulfur compounds determination	260
Free sulfhydryl content of protein determination	270
Ag^+ determination, in alloys	287
—, in ZnS, ZnSe, CdS and CdSe	333
—, in alkaline solution	420
2-Pyridylthioacetamide determination	296
Sulfate determination after reduction to S^{2-}	412, 434
Dithiooxamide determination	435
Automated immunoassay	440
General papers	350, 460

microscopy and solubility measurements [313]. A diffusion model for the copper-selective membrane has been worked out [327]. The effects of chloride and ionic strength [400] and of halides and acids [384] have been investigated. The effect of various complexing agents on the response of the copper-selective electrode has been studied [314, 432]. A buffer to prevent ligand interference has been based on a solution of triethylenetetramine, nitric acid and KNO_3 [339]; this buffer removes the interference of nitrilotriacetic and ethylenediaminetetraacetic acids. Applications of the copper-selective electrode are listed in Table 10.

Lead-selective electrodes. The construction of a lead-selective electrode with a mixed PbS/Ag_2S membrane has been described [317]. Several commerical lead-selective electrodes have been evaluated [352]. Mixed $PbSO_4/Ag_2S$ and PbS/Cu_2S membranes for sulfate determinations have been described [388]. Applications of lead-selective electrodes are listed in Table 11.

TABLE 10

Applications of copper-selective electrodes

Use	Reference
Cu^{2+} determination (in mM—μM range)	116, 385, 469
—, in waters	236, 328, 421
—, in waste streams	267
—, in palm oil	284
—, in silicon	449, 450
Sulfate determination with EDTA and Cu^{2+} indicator	239
Vanadyl determination with EDTA and Cu^{2+} indicator	390
Stability constant determination of Cu complexes of fulvic and humic acids	251, 252
Fe(III) determination	283
Determination of stability constants of Cu—D-penicillamine complexes	350a
Determination of extraction rate of Cu from aqueous solution	351
Determination of reducing substances	397, 404
Determination of drugs	418
Study of Cu complexes with poly(acrylic acid) and poly(itaconic acid)	491
Complexiметric titration of Mg and Ca with Cu^{2+} indicator	316

TABLE 11

Applications of lead-selective electrodes

Use	Reference
SO_4^{2-} determination, in water	326, 429
—, in μM range	341
—, general paper	470
Phosphate determination	453
Pb determination in soils	492
Stability constant determination	243, 244
Solubility product determination	257

Cadmium-selective electrodes. The construction [495], testing [345], standardization [428] and transient characteristics [396] of cadmium-selective electrodes have been described. A cadmium-selective electrode with a semiconductor function based on Ag or In doping has been studied [395]. Several commercial cadmium-selective electrodes have been evaluated [353]. Calibration of a cadmium-selective electrode by means of a diethylenetriamine metal buffer has been described [262]. EDTA titrations with a cadmium-selective indicator electrode have been studied [451, 463]. Cadmium complexes of poly(acrylic acid) and poly(itaconic acid) have been investigated [491].

(iv) The lanthanum trifluoride fluoride-selective electrode

Various aspects of the construction of this electrode have been studied [309, 368—371, 394]. The mechanism of the membrane response including kinetics [312, 313, 317] and double-layer effects [383] have been investigated. The use of BiF_3 [275] and CaF_2 [288] as membrane material was again attempted.

Sixteen methods of fluoride determination including potentiometry with a fluoride-selective electrode have been compared [437]. Various modifications of buffers for use with the fluoride-selective electrode have been suggested [232a, 263]. The behaviour of this electrode in organic and mixed solvents has been studied [286, 375, 408]. A particular increase of sensitivity was observed in a dioxane—water solvent [454]. Titration of fluoride with lanthanum chloride and biamperometric and bipotentiometric end-point detection has been described [285]. Applications of the fluoride-selective electrode are listed in Table 12.

(v) Other systems

Determination of nitrate with an Fe—chalcogenide glass electrode has been described [226]. The solubility and crystal structure of Hg, Pb, Cd, Ag, Zn and Cu nicotinates have been studied in attempts to achieve a nicotinate-selective electrode [255]. Papers on a strontium-selective electrode based on strontium arsenotungstate [336, 342], a Ni^{2+}-selective electrode based on nickel dimethylglyoximate [386] and a K^+-selective electrode based on potassium zinc hexacyanoferrate(II) [424] have been published. A phosphate-selective electrode based on silver phosphate, which shows a strong interference from chlorides, has been described [399]. Commerical electrodes for phosphate and chloride with unrevealed compositions have been announced [448].

D. LIQUID MEMBRANE ELECTRODES [120, 126, 131, 134, 148, 159, 160, 174, 194—196, 207, 497—811]

This section includes electrodes based both on ionized ion-exchangers and on neutral ion-carriers. Some of the systems with neutral ionophores like the

TABLE 12

Applications of the lanthanum trifluoride fluoride-selective electrode

Use	Reference
F^- determination, general papers	256, 268, 365, 389, 425
—, automated methods	181a
—, in fluorophosphate rocks	225, 416
—, at high ionic strengths	237
—, in titanium hydroxide during fluorination	242
—, in silicates	246
—, in waters	254, 276, 281a, 332, 342, 361, 392, 423, 483, 493
—, in baby foods	258
—, in tooth enamel	273, 413, 461, 488
—, in drinking water and in man	276
—, in silicate rocks	279, 340
—, in bone	281, 334
—, in blood plasma	282, 439
—, at low concentrations	301, 374
—, in air	318, 392
—, in rocks	322, 485
—, in soils	322, 380, 382
—, in emissions	324
—, after release from NaF and chlorhexidine difluoride mouth rinses	329
—, in rare metal industrial materials	331
—, in Nb and V oxides	332
—, in urine	334, 391, 403
—, in serum	334
—, in the presence of Al	344
—, in pharmaceuticals	372
—, in microemulsions	373
—, in automated fluoridation control	376
—, in automated atmospheric HF analyser	378
—, in concentrated solutions of phosphoric acid and ammonium phosphate	379
—, in potato	381
—, in vegetation	382, 438, 473
—, in aliphatic amine solutions	401
—, in pure tungsten	417
—, in beryllium	416
—, in large amounts	444
—, in fluorozirconite, fluorotitanite and fluoroborate	455
—, in phosphates	409, 457
—, in fluoride excretion study	467
—, in biological fluids	310a, 311
Orthophosphate determination as La-salt	272
MgF^+ stability constant determination	277
Determination of nanomolar amounts of Al	419
Study of mixed ligand complexes of Th(IV) with aminopolycarboxylic acids and fluoride	436
Determination of SiF_4 and HF in calcining gases	476
F_2 determination	492a

potassium or calcium electrodes have been used quite extensively and will, therefore, be considered together with the electrodes selective for the same ions but based on hydrophobic counter-ions. The other neutral-carrier systems will be discussed separately.

(i) Calcium-selective electrodes

The original versions of the calcium electrode based on long-chain alkyl esters of phosphoric acid as the ion-exchanger and on esters of phenylphosphoric acid as the solvent [1, 2] have been thoroughly investigated and modified [515, 555, 637, 638, 652—654, 711, 712, 757]. Electrodes based on calcium bis-di(*p*-1,1,3,3-tetrabutylphenyl)-phosphate or trialkylphosphates are subject to interference by transition and alkaline earth metal ions [711]. Calcium salts of nitro- and dinitro-phenyl esterified phosphoric acid in di-n-octyl-(3-nitrophenylphosphate) show no advantage over the phenyl esterified acids (which are superior to didecylphosphoric acids) [652]. Detection limits of the calcium electrodes have been determined [554]. These electrodes have also been studied by means of radiotracers [555]. An asymmetry potential at asymmetric calcium electrodes has been measured [757]. Solid contacts in a PVC-matrix calcium electrode have been carefully investigated [637, 638]. The divalent cation electrode [1] has been used for water hardness determination [749]. Application of the same electrode to magnesium determinations is complicated by interference from polyamines at the physiological pH level [653].

Another version of the calcium electrode is based on the synthetic neutral ionophore described earlier [2]; for reviews see [714, 775]. A microelectrode based on this system has been described [545, 732].

The most important application of calcium electrodes is the determination of ionized calcium in serum. Some basic work has been reported [194—196, 579, 816]. The automated method based on the Electrion system with AMT electrodes and the adjustment of pH of the samples with CO_2 [194—196] have been criticized [579].

Applications of calcium electrodes are listed in Table 13. Fig. 11 shows a typical time course of the calcium concentration in rat cerebellum measured with a neutral ligand microelectrode [725].

(ii) Nitrate-selective electrodes

In a thorough study of nitrate electrodes, various tetraalkylammonium cations have been compared as counter-ions [726]. The calibration curves are shown in Fig. 12. The tetradodecylammonium cation, which is similar to the tridodecylhexadecylammonium cation of the commercial Corning electrode, seems most suitable. A method for elimination of fluctuations with the nitrate electrode has been worked out [519]. Nitro-*p*-cymene and nitrophenyloctyl ether as membrane solvents have been compared [635].

The nitrate-selective electrode has shown up well in comparison with the nitration—distillation method based on nitration of 3,4-dimethylphenol and

TABLE 13

Applications of calcium-selective electrodes

Use	Reference
(a) Alkylphosphate ion-exchangers	
Ca determination, in serum	131, 148, 159, 160, 174, 194, 195, 196, 537, 549, 563, 579, 603, 605, 693
—, intracellular	502, 525, 539, 540, 541, 683, 738, 809, 816
—, general papers	507, 666, 758
—, interaction of Ca^{2+} with spectrin	522, 523
—, in heart	524
—, in blood	113, 562
—, with a miniature sensor	126
—, in waters	605, 636, 802
—, in flotation pulps	659
—, in the presence of chelating agents	660
—, Ca^{2+} interaction with serum albumin	686
—, Ca^{2+} interaction with Na silicates	747
—, in pancreas	787
—, in beer	808
Stability constant determination of organic acids with Ca^{2+}	655
(b) Neutral ligand	
Ca^{2+} determination, in serum	693, 759
—, in brain cortex	587, 724, 725, 783, 792
—, in cat cerebellum	722
—, with a microelectrode	732
—, in haemodialysis solution	745
—, general paper	775

with the reduction—distillation method in the determination of nitrate in plant samples [634]. Methods for the determination of nitrate in water based on brucine reaction, u.v. spectrophotometry, automatic hydrazine reduction and ion-selective electrodes have been compared [503].

Applications of nitrate electrodes are listed in Table 14. Applications of an analogous perchlorate electrode (see [1]) are listed in Table 15. The same ion-exchanger, iron(II)—bathophenanthroline has been used in electrodes for 2,4-dichlorophenoxyacetic acid [611] and tetrathionate [799] and in measurements of sulfa drugs [612].

(iii) Potassium-selective electrodes

The membrane-active materials of potassium-selective electrodes are either exchanger-ions (usually tetra(*p*-chlorophenyl)borate [2]) or valinomycin [1]. Applications of these electrodes are listed in Table 16. A general paper on microelectrode application has been published [803]. Potassium electrodes based on bis- and poly-crown ethers has been described [662]. The high

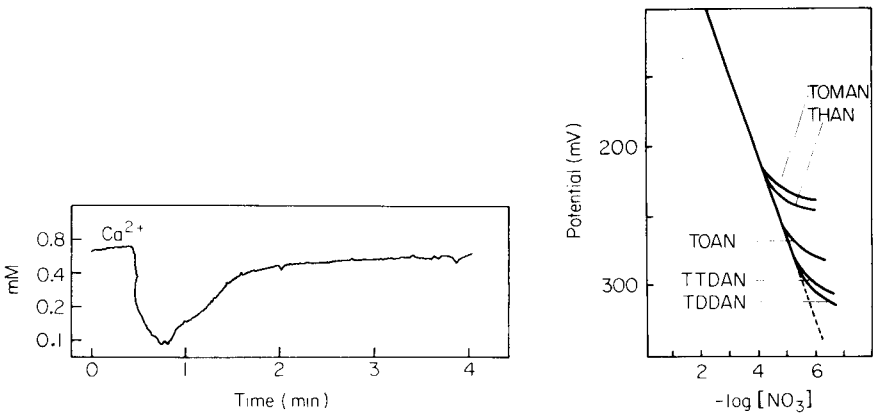


Fig. 11. Intercellular calcium concentration changes during spreading depression. Spreading was evoked by strong stimulation at 20 Hz. After Nicholson et al. [725].

Fig. 12. Calibration curves of nitrate-selective electrodes containing trioctylmethyl- (TOMAN), tetraheptyl- (THAN), tetraoctyl- (TOAN), tetratetradecyl- (TTDAN) and tetradodecyl-ammonium (TDDAN) ions dissolved in dibutylphthalate. All electrodes have been adjusted to the same E°_{ISE}. After Nielsen and Hansen [726].

TABLE 14

Application of nitrate-selective electrodes

Use	Reference
NO_3^- determination, in waters	503, 604, 636, 771
—, in soil	518, 521, 529a, 754
—, in the presence of NO_2^-	578
—, in nutrients	575, 707
—, in plants	596, 634, 752
—, in air	604
—, in agricultural samples	645
—, in cheese and cream	646
—, in strongly acidic solutions	697, 698
—, general paper	748, 790, 817
—, in serum	754
Determination of nitrogen oxide in air	571
—, in the presence of nitramines	767
—, in the presence of nitrites in smoked fish	772
—, at low concentrations	814

resistance which is prohibitive for application of the valinomycin system to microelectrodes can be reduced by using tetra(*p*-chlorophenyl)borate as counter-ion in the valinomycin electrode [733].

TABLE 15

Applications of perchlorate-selective electrodes

Use	Reference
Perchlorate determination, general papers	512, 530, 632, 648, 766
Study of periodate—aminoalcohol reaction catalysed by manganese	564
Carbohydrate determination by periodate	565
Study of periodate—arsenite reaction catalysed by Cr(III)	566
Study of periodate—acetylacetone reaction catalysed by manganese	567
Study of periodate—*vicinal* glycol reaction	568
Catalytic titrations with IO_4^- as indicator	602

TABLE 16

Applications of potassium-selective electrodes

Use	Reference
(a) Ionized ion-exchanger electrodes (used only in microelectrodes for K^+ determination)	
Review	545, 580
Coaxial construction	207
K^+ changes or state, in cat carotid body	497
—, in *Aplysia* neurones	501
—, in cardiac muscle	546, 658, 679, 709, 710, 777
—, in retina of drones	548
—, correlated with transmitter release at giant synapse of squid	569
—, in normal and glyotic cortex	574
—, in proximal tubule of frog	581
—, in mammalian cerebral cortex	586, 613, 614, 615, 616, 695, 743
—, —, during arousal reaction	706
—, in salivary glands of *Calliphora* during secretion	600
—, in snail neurones	617, 618, 741
—, during and after muscle activity	620
—, in mudpuppy retina	647
—, in rat skeletal muscles	657
—, in frog bladder cells	661
—, in mammalian spinal cord	511, 674, 675, 676, 677, 689, 778, 786, 787, 788, 804
—, in dorsal root	678
—, during repetitive interhemispheric stimulation	692
—, in cochlea	705
—, in frog retina	717

Table 16 (continued)

Use	Reference
—, in catfish cerebellum	721
—, in cat cerebellum	723, 724, 791
—, in avian hyperstriatum	731
—, in retina	735
—, in *Chironomus* salivary glands	739, 740
—, in salivary glands	744
—, during activity of medullar respiratory neurones	751
—, during spreading depression	800
—, in turgid cells	801
—, in cells of *Amphiuma* small intestine	810
—, in single cortical neurone in awake cat	813
(b) Valinomycin electrodes	
K^+ determination, in blood	120, 609, 662a, 815
—, in body fluids	134
—, in serum	181, 289, 335, 509, 537, 585, 690, 696, 797
—, with catheter electrode	510, 796
—, in nutrients	575
—, in study of K^+ binding to membrane proteins	607
—, in vivo	608
—, in muscle	619
—, with an electrode durable for several years	685
—, in human erythrocytes	694
—, general paper	714, 755, 760, 763, 764
—, in sea water	756
—, in infusion solutions	819
—, in amphibian gall-bladder	822
Titrations of organic cations	805, 806, 807

(iv) Other ion-exchanger systems

The tendencies noted earlier [2] have continued at a steady rate. However, several useful systems must be mentioned.

The chloride liquid ion-exchanger electrode [2] has been used in physiology, offering some advantages over the solid-state electrode (see Table 17). The choline [159a, 691] and acetylcholine electrodes (see [1]) have been studied further.

A basic study on trialkylbenzylammonium ions as ion-exchangers has been reported [784]. Solvent extraction of Cu, Ag, Au and Hg by trialkylmonothiophosphates and trialkylphosphine sulfides has been investigated [538].

Various quaternary ammonium salts (for general papers, see [727, 728]) have been used in electrodes which have moderate selectivity for sulfate

TABLE 17

Applications of chloride liquid-membrane electrode

Use	Reference
Cl^- determination, in *Balanus* photoreceptor	426
—, in frog sartorius	520
—, in blood serum	606, 703
—, in *Aplysia* neurones	753, 501
—, with coated disc electrode	761
—, in blood	785
—, in *Amphiuma* small intestine	811
—, in catfish cerebellum	721
—, in *Chironomus* salivary glands	739

[514], nicotinate [531—533, 594], phenobarbital [534], picrate [558, 559, 601, 601a], toluenesulfonate [582], chlorate [583], bile acids [588], perrhenate [592, 730], chloramine T [601a, 668—672], sulfanilate, cholate, barbiturate, nitrobenzoate, salicylate, sebacate, phenylacetate, chlorobenzoate, benzoate and hydrogenphthalate [631], perbromate [681, 682], anions of saturated monocarboxylic acids [701], anions of aromatic acids [702, 737], benzenesulfonate [729], chromate [624], bromide [595, 718, 780] and thiocyanate [770].

Various heavy metal cations can be determined as anionic complexes by electrodes containing quaternary ammonium ion-exchangers. This group includes Zn [535], Cd [536], Ag [550, 551], Hg(II) [552], Au(III) [570, 591, 769], Fe(III) [622, 626] and Bi(III) [734].

The hapten—antibody reaction has been studied by means of the trimethylphenylammonium electrode [708]. Various quaternary ammonium electrodes based on tetraphenylborate, particularly the dibenzyldimethylammonium electrode [560, 561, 719, 773], have been used for monitoring the corresponding cations for determinations of membrane potentials and membrane permeability.

A nitrate-selective electrode [630, 633] has been based on the tetraphenylphosphonium cation. The tetraphenylarsonium cation has been used in electrodes for dicyanoaurate [529, 818], perrhenate [818, 820, 821] and tetrafluoroborate [688].

Various cation-sensitive electrodes have been based on hydrophobic anionic substances such as tetraphenylborate for Cs^+ [513], alkyl phosphates [699] for Zn^{2+} [593] and UO_2^+ [708a], thiophosphates for Pb^{2+} [700], dipicrylaminate for K^+ [627], and tetra(*m*-trifluoromethylphenyl)borate for Tl^+ and Cs^+ [547]. Several surfactant-selective electrodes (cf. [2]) have been described [499, 500, 542—544, 556, 584, 720, 795]. Basic [498, 576, 577, 625, 628, 629, 704, 768, 798] and anionic [577, 650, 651] dyes have been used as ion-exchangers in various electrodes.

Hydrogen ion-selective liquid-membrane electrodes intended to replace the more fragile glass microelectrodes in physiology have been reported [667, 684]. The carbonate electrode [2] has been further studied [750, 774].

Other systems include electrodes sensitive to mercury(I) [505], mercury(II) [504, 506], vitamin B_6 [589], various drugs [590], novocain [623] and saccharin [610]. A system similar to that of Higuchi et al. (see ref. 264 of Part I [1]) was used again [779].

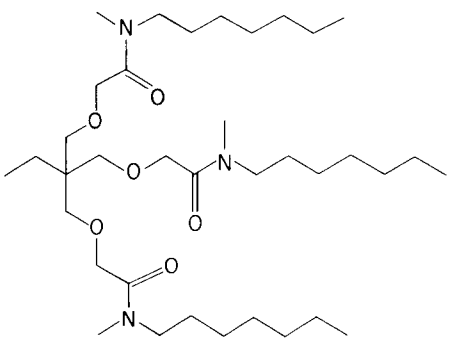
(v) Neutral-carrier electrodes

This field has been the subject of several reviews [680, 713, 715, 716, 776]. The response time of neutral-ligand electrodes [150] and the influence of the dielectric constant of the medium on the selectivity of these electrodes [573] have been discussed.

Electrodes based on nonactin and its homologues (see [1]) have been used for ammonium ion determination in waters [557, 644], urine [621], fruit juices and mineral waters [742] and beer [812]. The general properties of such electrodes have been discussed [762, 781, 789]. The monensin Na^+ electrode has been used for sodium determinations in tissue [656].

The synthetic acyclic ligands discussed previously [2] have been further investigated with respect to complex formation and ionophoric possibilities by n.m.r. spectroscopy and e.m.f. measurements [516, 517, 526—528, 746].

Various ligands for lithium electrodes have been investigated [598]. Lithium accumulation by snail neurones has been studied [794]. The sodium electrode has been optimized for the serum analysis [572] and has been used for measurements of large extracellular sodium transients [673]. Another ligand(I) has been reported [597] to have high selectivity for Na^+



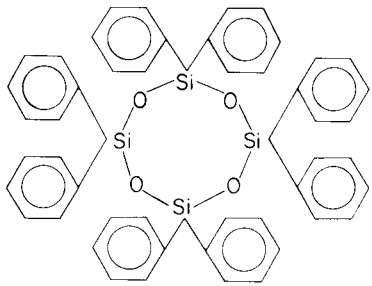
(I)

compared to K^+ and to have excellent stability. The properties of a barium electrode (see [2]) have been further investigated [599].

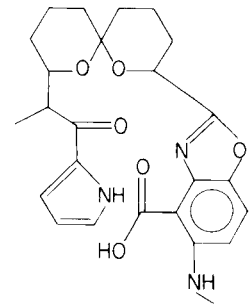
The application of polyglycol ether species [1] as ligands for barium and other alkaline-earth metal electrodes has been examined further [639—643, 736]. Thus, nonylphenoxypoly(ethyleneglycol)ethanol appears to be most

suitable for a barium-selective electrode [640, 642, 643, 736], while the poly(propyleneglycol) analogue can be used as the neutral ligand for the calcium electrode [639, 641].

Electrodes based on crown polyethers (see [1, 2]) have been reviewed [665]. A siloxane compound (II) appears to be suitable as the active com-



(II)



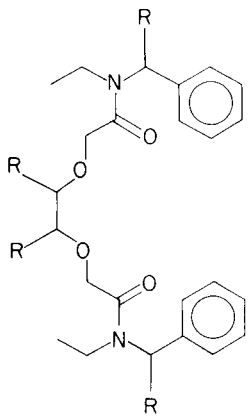
(III)

ponent for a lithium (or better a hydronium) electrode (765]. The naturally occurring antibiotic A 23187 (III) has been proposed for a calcium electrode [553].

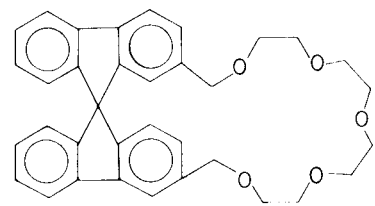
Not very high but distinct enantiomer-selectivity has been reported for electrodes based on the acyclic ligand (IV) and on 9,9′-spirobifluorene-20-crown-5 (V) towards optically active quaternary ammonium cations [749a, 793].

E. OTHER SYSTEMS [823–839]

Enzyme electrodes, including those based on ion-selective electrodes, have been reviewed [823, 825a, 826, 827, 832, 833, 837]. Yet another version of the urea electrode has been proposed [838]. Enzyme electrodes for acetyl-



(IV)



(V)

choline and acetyl-β-methylcholine based on acetylcholinesterase and choline-selective electrodes have been described [830, 831, 839]. A specific enzyme electrode for L-phenylalanine has been reported [828]. An enzyme electrode for O-acetyl-L-serin has been based on O-acetylserinsulfhydrylase and the Ag_2S electrode [834]. Fourier transform of the transient response of potentiometric enzyme electrodes has been described [824].

Finally, a new and interesting approach is the bacterial electrode; this is actually a version of an enzyme electrode in which living bacteria are used instead of an enzyme [825, 829, 835, 836].

The assistance of the Technical and Economic Research Institute of Chemical Industry, Prague, who supplied computerized Chemical Abstract condensates on ion-selective electrodes, is greatly appreciated as is the help from colleagues all over the world who send reprints and reports in the field of ion-selective electrodes. Their help in the future is essential for continuation of this work.

REFERENCES

1 J. Koryta, Anal. Chim. Acta, 61 (1972) 329.
2 J. Koryta, Anal. Chim. Acta, 91 (1977) 1.
3 M. Trojanowicz (Ed.), Elektrody jonoselektywne. Proc. Conf., Analytical Chemistry Commission, Polish Academy of Sciences, Warsaw, 1975.
4 E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977.
5 E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977 Akademia Kiadó, Budapest, 1978.
6 International Reference and Ion-selective Electrode Conference, University of Newcastle upon Tyne, 1978.
7 P. L. Bailey, Analysis with Ion-selective Electrodes, Heyden, London, 1976.
8 G. E. Baiulescu and V. V. Cosofret, Applications of Ion Selective Membrane Electrodes in Organic Analysis, Horwood—Wiley, Chichester, 1977.
9 K. Cammann, Das Arbeiten mit ionenselektiven Elektroden, 2nd edn., Springer-Verlag, Berlin, 1977.
10 K. Cammann, Zastosowanie elektrod jonoselektywnych (Use of ion-selective electrodes), Wydawnictvo naukovo-techniczne, Warsaw, 1977.
11 C. Fuchs, Ionenselective Elektroden in der Medizin, Thieme, Stuttgart, 1976.
12 H. Freiser (Ed.), Ion-selective Electrodes in Analytical Chemistry, Vol. 1, Plenum Press, New York, 1978.
13 D. Midgley and K. Torrance, Potentiometric Water Analysis, Wiley, Chichester, 1978.
14 J. Veselý, D. Weiss and K. Štulík, Analysis with Ion-Selective Electrodes, Horwood—Wiley, Chichester, 1978.
15 D. M. Cavagnaro, Natl. Tech. Inf. Serv., Springfield, Va., NT IS/PS-77/0875, 1977, p. 133.
16 D. M. Cavagnaro, Natl. Tech. Inf. Serv., Springfield, Va., NT IS/PS-77/0876, 1977, p. 116.
17 J. Pick, Hung. Sci. Instrum., 37 (1976) 37.
18 J. Pick, Hung. Sci. Instrum., 12 (1978) 37.
19 A. O. Brunfelt, Bull. Soc. Roy. Sci. Liege, 46 (1977) 133.
20 A. O. Brunfelt, Kjemi, 36 (1976) 34.
21 R. P. Buck, Anal. Chem., 50 (1978) 17R.

22 R. P. Buck, Proc. Anal. Div. Chem. Soc., 14 (1977) 332.
23 P. E. Childs, School Sci. Rev., 58 (1977) 677.
24 J. Comer, Prod. Finish. (London), 30 (1977) 17.
25 J. Koryta, Vesmír, 54 (1975) 304.
26 G. J. Moody and J. D. R. Thomas, Proc. Anal. Div. Chem. Soc., 14 (1977) 340.
27 Nanking Soil Laboratory, K'O Hsueh Shih Yen, 10 (1976) 36.
28 F. Oehme, Taschenbuch Abwasserbehandl. Metallverarb. Ind. 2, 50a, Hanser-Hartinger, Munich, 1977.
29 E. Pungor in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 161.
30 E. Pungor and K. Tóth, Ion selective electrodes, Euroanalysis II, Budapest, 1975, Masson, Paris, 1977.
31 E. Pungor, K. Tóth and G. Nagy, Mikrochim. Acta, (1978) 531.
32 P. A. Rock, in P. A. Rock (Ed.), Special Topics in Electrochemistry, Elsevier, Amsterdam, 1977.
32a T. Seiyama, Denki Kagaku, 46 (1978) 633.
32b J. C. Sternberg, S. J. Updike and D. P. Lehane, Mikrotech. Clin. Lab., 9 (1976) 129.
33 W. E. van der Linden, Chem. Weekbl., (1978) 379.
34 V. F. Antonov, A. S. Ivanov, E. A. Korepanova and V. V. Petrov, Ito Nauk. Tekhn. Biofiz., 5 (1975) 166.
35 J. Antson and T. Suntala, Tutkinues Tek., 9 (1975) 26.
36 A. Bekhar, A. Urumova and D. Chuldzhiyan, Dokl. Bolg. Akad. Nauk, 31 (1978) 245.
37 S. Braaten, Kjemi, 37 (1977) 8, 29, 31.
38 R. E. Burell, Ind. Pollut. Control Meas. Instrum., Proc. Spec. Conf., 1976, p. 263.
39 V. V. Cosofret and P. G. Gravescu, Rev. Chim. (Bucharest), 28 (1977) 785.
40 L. D'Anguiro, Ind. Carta, 14 (1976) 205.
41 D. Eicken, Vom Wasser, 49 (1978) 139.
42 G. H. Fricke and M. J. Kuntz, J. Chem. Educ., 54 (1977) 517.
43 C. Fuchs, D. Darn and C. J. Preusse, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 373.
44 A. V. Gordievskii and E. A. Zeinalova, Probl. Anal. Khim., 5 (1977) 136.
45 M. Iwata, Y. Takahashi and H. Kushiro, Eisei Kensa, 26 (1977) 883.
46 M. Iwata, Y. Takahashi and H. Kurshiro, Eisei Kensa, 26 (1977) 887.
47 T. Kambara and M. Kataoka, Kagaku, 33 (1978) 400.
48 J. Koryta, M. Březina, J. Pradáč and J. Pradáčová, in A. J. Bard (Ed.), Electroanalytical Chemistry, Vol. 11, Dekker, New York, 1979.
49 V. A. Kovda, E. A. Materova, G. K. Zykina, V. V. Snakin, T. L. Bystritskaya and A. N. Tyuryukanov, Dokl. Akad. Nauk SSSR, 235 (1977) 198.
50 R. E. Lamb, D. F. S. Natusch, J. E. O'Reilly and N. Watkins, J. Chem. Educ., 50 (1973) 432.
51 R. Lászlóné, Agrokém. Talajtan, 25 (1976) 1.
52 V. M. Leon'ev, L. P. Guk and G. K. Khokhlova, Biosfera Chel., Mater. 1. Vses. Simp., (1975) 286.
53 P. C. Meier, D. Ammann, H. F. Osswald and W. Simon, Med. Progr. Technol., 5 (1977) 1.
54 J. Mertens, H. Declercq, D. L. Massart, Y. Michotte, P. van den Winkel, L. Dryon and A. Henrion-Boeckstijns, Actual. Chim. Anal., Org. Pharm., 23 (1975) 33.
55 G. J. Moody and J. D. R. Thomas, in D. R. Williams (Ed.), Introduction to Bio-Inorganic Chemistry, Thomas, Springfield, Ill., 1976, p. 220.
56 G. J. Moody and J. D. R. Thomas, Prag. Med. Chem., 14 (1977) 51.
57 G. P. Morie, Beitr. Tabakforsch., 9 (1977) 19.
58 T. Murakami, Y. Ohshima and K. Matsumoto, Gesuido Jigyo Ghosahi Hokoku, 50 (1976) 37.
59 E. Pungor and K. Tóth, in J. J. Lagowski (Ed.), The Chemistry of Nonaqueous Solvents, Vol. 5A, Academic Press, New York, 1978.

60 E. Pungor, K. Tóth and G. Nagy, in E. Wänninen (Ed.), Essays in Analytical Chemistry, Pergamon, Oxford, 1977, p. 331.
61 H. Puxbaum and V. Simeonov, Hung. Sci. Instrum., 41 (1077) 17.
61a C. Radenović, V. Krsnik, M. Pencić, Z. Vucinić and M. Vesković, Zemljiste Biljka (Yugoslavia), 24 (1975) 199.
62 S. Ramamoorthy and D. J. Kustiner, NBS Spec. Publ. No. 464 (1977) 467.
63 L. Redly, Agrokem. Talajtan, 25 (1976) 191.
64 G. Schönhard and H. D. Schenke, Landwirtsch. Forsch., 29 (1978) 254.
65 P. Schweyer, Circ. Inf. Tech., Cent. Doc. Sider, 34 (1977) 1159.
66 M. Semler and B. Mánek, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 529.
67 W. Simon, D. Ammann, H. F. Osswald, P. C. Meier and R. E. Dohner, Adv. Autom. Anal., 7th Technicon Int. Congress, 1 (1977) 59.
68 R. Staroscik and K. Selinger, Farm. Pol., 30 (1974) 107.
69 K. Sykut, J. Dumkiewicz and R. Dumkiewicz, Metody Fizykochem.Oczyszania Wodsciekow, Ref. Konf. Nauk.-Tech., 2 (1976) 78.
70 D. Szyszlo, Tech. Poszukiwán geol., 15 (1976) 30.
71 E. Tassara, R. Ciurlo and G. B. Deferrari, Chim. Ind. (Milan), 59 (1977) 301.
72 G. Ten Bruggencate, R. Steinberg, H. Stöckle and C. Nicholson, in Ryall and Kelly (Eds.), Ionophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System, Elsevier—North-Holland, Amsterdam, 1978, p. 412.
73 J. Tenygl, Int. Rev. Sci., Phys. Chem., Ser. 2, 13 (1976) 1.
74 J. D. R. Thomas, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 175.
75 J. D. R. Thomas, Proc. Anal. Div. Chem. Soc., 14 (1977) 7.
76 I. Trachtenberg, Traces Heavy Met. Water Removal Processes Monit., Proc. Symp., (1973) 323.
77 M. Trojanowicz and A. Hulanicki, Chem. Anal. (Warsaw), 22 (1977) 615.
78 E. Tschager, Milchwirtsch., Ber. Bundesanst, Wolfpassing Rotholz, 56 (1978) 189.
79 J. L. Walker and H. M. Brown, Physiol. Rev., 57 (1977) 729.
80 D. Weiss, Sklář. Keram., 28 (1978) 21.
81 R. D. Wyvill, Prod. Finish. (London), 30 (1977) 21.
82 G. K. Zykina, T. L. Bystritskaya, E. A. Materova, A. L. Grekovich and V. V. Volkova, Pochv. Biogeotsenol. Issled. Priazov USSR, 1 (1975) 102.
83 K. F. Bonhoeffer, M. Kahlweit and H. Strehlow, Z. Elektrochem., 57 (1953) 614.
84 R. P. Buck, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 21.
85 R. P. Buck, D. E. Mathis and R. K. Rhodes, J. Electroanal. Chem., 80 (1977) 245.
86 R. P. Buck and F. S. Stover, Anal. Chim. Acta, 101 (1978) 231.
87 J. Buffle and N. Parthasarathy, Anal. Chim. Acta, 93 (1977) 111.
88 K. Cammann, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 297.
89 K. Cammann, Anal. Chem., 50 (1978) 936.
90 M. Gratzl, F. Rakiás, G. Horvai, K. Tóth and E. Pungor, Anal Chim. Acta, 102 (1978) 85.
90a D. Homolka, unpublished results.
90b D. Homolka, Collect. Czech. Chem. Commun., in press.
91 R. A. Huggins, in H. Gerischer and C. W. Tobias (Eds.), Advances in Electrochemistry and Electrochemical Engineering, Vol. 10, Wiley, New York, 1977, p. 323.
92 A. Hulanicki and A. Lewensztam, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 395.
93 A. Hulanicki and A. Lewensztam, Talanta, 24 (1977) 171.
94 A. Jyo, K. Fukamachi, W. Koga and N. Ishibashi, Bull. Chem. Soc. Jpn., 50 (1977) 670.

95 A. Jyo, H. Mihara and N. Ishibashi, Denki Kagaku, 44 (1976) 268.
96 O. Kedem, M. Perry and R. Bloch, Charged React. Polym., 4 (Charged Gels Membr. Part 2), (1976) 125.
96a W. Khalil, Thesis, J. Heyrovský Institute, Prague, 1978.
97 J. Koryta, Electrochim. Acta., 24 (1979) 293.
98 J. Koryta, P. Vanýsek and M. Březina, J. Electroanal. Chem., 67 (1976) 263.
98a J. Koryta, P. Vanýsek and M. Březina, J. Electroanal. Chem., 75 (1977) 211.
99 J. Koryta, P. Vanýsek, H. Jänchenová and M. Březina, Elektrokhimiya, 13 (1977) 706.
100 J. Koryta, P. Vanýsek, M. W. Khalil, V. Mareček and Z. Samec, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 441.
101 J. R. Luch, Thesis, University of Kansas, 1976.
102 E. A. Materova and V. V. Mukhovikov, Ionn. Obmen Ionometr. USSR, 1 (1976) 106.
103 C. McCallum and R. Paterson, J. Chem. Soc. Faraday Trans. 1, 72 (1976) 323.
104 W. E. Morf, Anal. Chem., 49 (1977) 810.
105 W. E. Morf and W. Simon, in ref. 5, p. 25.
105a M. Perry, E. Löbel and R. Bloch, J. Membr. Sci., 1 (1976) 223.
106 E. Pretsch, R. Büchi, D. Ammann and W. Simon, in E. Wänninen (Ed.), Analytical Chemistry — Essays in Memory of A. Ringbom, Pergamon, Oxford, 1977, p. 379.
107 Z. Samec, V. Mareček, J. Koryta and M. W. Khalil, J. Electroanal. Chem., 83 (1977) 393.
107a Z. Samec, V. Mareček and J. Weber, J. Electroanal. Chem., 100 (1979) 841.
107b Z. Samec, V. Mareček, J. Weber and D. Homolka, J. Electroanal. Chem., in press.
108 F. S. Stover, and R. P. Buck, Biophys. J., 16 (1976) 753.
109 F. S. Stover and R. P. Buck, J. Phys. Chem., 81 (1977) 2105.
110 A. P. Thoma, A. Viviani-Nauer, S. Arvanitis, W. E. Morf and W. Simon, Anal. Chem., 49 (1977) 1567.
110a See, e.g., J. Koryta, J. Dvořák and V. Boháčková, Electrochemistry, Methuen, London, 1970. Electrochemie, Springer-Verlag, Vienna, 1975.
110b See, e.g., J. Koryta, Ion-selective Electrodes, Cambridge University Press, Cambridge, 1975.
110c A. J. Parker, Electrochim. Acta, 21 (1976) 671.
111 M. A. Afromowitz and S. S. Yee, J. Bioenerg., 1 (1977) 55.
112 P. W. Alexander, Proc. Roy. Aust. Chem. Inst., 43 (1976) 358.
113 O. Aziz, J. G. Schindler and R. Dennhardt, Biomed. Technol., 23 (1978) 194.
114 P. L. Bailey, Anal. Chem., 50 (1978) A698.
115 R. Bates and R. A. Robinson, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 3.
116 W. J. Blaedel and D. E. Dinwiddie, Anal. Chem., 47 (1975) 1070.
117 R. P. Buck and D. E. Hackleman, Anal. Chem., 49 (1977) 2315.
118 P. A. Comte and J. Janata, Anal. Chim. Acta, 101 (1978) 247.
119 W. Crowe, A. Mayevsky and L. Mela, Am. J. Physiol., 233 (1977) C56.
120 R. A. Durst, Clin. Chim. Acta, 80 (1977) 225.
121 R. A. Durst, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 359.
122 R. A. Durst, NBS Spec. Publ., 464 (1977) 229.
123 R. A. Durst and R. G. Bates, NBS Spec. Publ. 450. Proceedings of a Workshop on pH and Blood Gases held at NBS, Gaithersburg, Maryland, July 7—8, 1975. Issued June 1977.
124 S. Ebel, E. Glaser and A. Seuring, Fresenius Z. Anal. Chem., 291 (1978) 108.
124a M. Esashi and T. Matsuo, IEEE Trans. Biomed. Eng. BME-25, (1978) 184.
125 W. A. Finnerty, H. Luttrell and S. Zudeck, Adv. Autom. Anal., 7th Technicon Int. Congr., 2 (1977) 210.

125a T. A. Fjeldly and K. Nagy, Kjemi, 7 (1978) 14.
126 B. Fleet, G. P. Bound and D. R. Sandbach, Bioelektrochem. Bioenerg., (1976) 158.
127 J. W. Frazer, W. Selig and L. P. Rigdon, Anal. Chem., 49 (1977) 1250.
128 H. Freiser, Res. Dev., 27 (1976) 28, 32.
129 S. Furuta, M. Okada and H. Matsushita, Chubu Kogyo Daigaku Kiyo, 13A (1977) 143.
130 P. D. Gaarenstrom, J. C. English, S. P. Perone and J. W. Bixler, Anal. Chem., 50 (1978) 811.
131 J. M. Grimat and M. J. D. Brand, Clin. Chem., 23 (1977) 2048.
132 L. L. Grinius, A. A. Jasaitis, Yu. P. Kadsiauskas, F. A. Liberman, V. P. Skulachev, V. P. Topali, L. M. Tsofina and M. A. Vladimirova, Biochim. Biophys. Acta, 216 (1970) 1.
133 B. M. Gulin and A. A. Reshetilo, Zh. Anal. Khim., 31 (1976) 2097.
134 J. Havas, L. Kecskés and R. Somodi, Orv. Tech., 15 (1977) 41.
135 K. Hiiro, Bunseki Kagaku, 2 (1978) 115.
136 K. Hiiro, T. Tanaka and A. Kawahara, Bunseki Kagaku, 26 (1976) 635.
137 G. Horvai, L. Domokos and E. Pungor, Z. Anal. Chem., 292 (1978) 132.
138 J. Janata, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
139 J. Janata and S. D. Moss, Biomed. Eng., 11 (1976) 241
140 T. Jancsó, F. Faragó, J. Havas and L. Kecskés, in ref. 5, p. 419.
141 K. M. Joshi and G. M. Ganu, Indian J. Technol., 16 (1978) 5305.
142 A. Jyo, T. Imato, K. Fukamachi and N. Ishibashi, Chem. Lett., 7 (1977) 815.
143 N. Kamo, N. Hazemoto and Y. Kobatake, Talanta, 24 (1977) 111.
143a K. Katsuhiko, Y. Imanishi, Y. Umezawa and S. Fujiwara, Bunseki Kagaku, 27 (1978) 180.
144 D. B. Kell, P. John, M. C. Sorgato and S. J. Ferguson, FEBS Lett., 86 (1978) 294.
145 R. G. Kelly, Electrochim. Acta, 22 (1977) 1.
146 R. G. Kelly, J. R. Jordan and A. E. Owen, Proc. Anal. Div. Chem. Soc., 14 (1977) 338.
147 P. Kivalo and R. Virtanen, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 151.
147a J. Kontoyannakos, G. J. Moody and J. D. R. Thomas, Anal. Chim. Acta, 85 (1976) 47.
148 L. Larsson and S. Ohman, Clin. Chem., 24 (1978) 731.
149 L. Liberti and A. Pinto, Anal. Chem., 49 (1977) 2377.
150 E. Lindner, K. Tóth, E. Pungor, W. E. Morf and W. Simon, Anal. Chem., 50 (1978) 1627.
151 E. Lindner, P. Wuhrmann, W. Simon and E. Pungor, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 159.
152 W. A. Lingerak, F. Bakker and J. Slanina, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 453.
153 C. Liteanu and E. Hopirtean, Fresenius Z. Anal. Chem., 288 (1977) 59.
154 C. Liteanu and E. Hopirtean and I. C. Popescu, Anal. Chem., 48 (1976) 2013.
155 C. Liteanu, E. Hopirtean and I. C. Popescu, Acta Chim. Acad. Sci. Hung., 97 (1978) 265.
156 C. Liteanu, E. Hopirtean and I. C. Popescu, Acta Chim. Acad. Sci. Hung., 97 (1978) 279.
157 C. Liteanu, E. Hopirtean, I. C. Popescu, I. Rica and E. Stefaniga, Anal. Chem., 50 (1978) 1202.
158 C. Liteanu, I. C. Popescu and E. Hopirtean, Anal. Chem., 48 (1976) 2010.
159 H. F. Loken, S. B. Arnaud and S. J. Rehfeld, Clin. Chem., 24 (1978) 2066.

159a J. B. Macaskill, M. S. Mohan and R. G. Bates, Anal. Chem., 49 (1977) 209.
160 S. Madsen and K. Olgaard, Clin. Chem., 23 (1977) 690.
161 M. Mascini and C. Cremisini, Anal. Chim. Acta, 97 (1978) 237.
162 P. T. McBride, J. Janata, P. A. Comte, S. D. Moss and C. C. Johnson, Anal. Chim. Acta, 101 (1978) 239.
163 P. C. Meier, M. Oehme and W. Simon, Bibl. Anal. 15, Recent Adv. Basic. Microcirc. Res., (1977) 120.
164 D. Midgley, Anal. Chem., 49 (1977) 1211.
165 M. S. Mohan and R. G. Bates, Clin. Chem., 21 (1975) 864.
166 M. S. Mohan and R. G. Bates, NBS Spec. Publ. (U.S.), 450 (1977) 293.
167 G. J. Moody and J. D. R. Thomas, Lab. Pract., 27 (1978) 285.
168 G. J. Moody and J. D. R. Thomas, Properties of Ion-Sensing Membranes Based on PVC Matrices, in ref. 4, p. 41.
169 W. E. Morf, Anal. Lett., 10 (1977) 87.
169a S. D. Moss, J. Janata and C. C. Johnson, IEEE Trans. Biomed. Eng., BME-25 (1978) 49.
169b S. D. Moss, J. B. Smith, P. A. Comte, C. C. Johnson and L. Astle, J. Bioenerget., 1 (1977) 11.
170 G. Nagy, Zs. Fehér, K. Tóth and E. Pungor, Anal. Chim. Acta, 91 (1977) 87.
171 G. Nagy, Zs. Fehér, K. Tóth and E. Pungor, Hung. Sci. Instrum., 41 (1977) 27.
172 G. Nagy, Zs. Fehér, K. Tóth and E. Pungor, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 477.
173 Nikkaki Co, Ltd., Japan, A & R, 15 (1977) 562.
174 S. Oleman, L. Larsson and L. Demetrion, Clin. Chem., 24 (1978) 2070.
174a M. Oehme, Beitrag zur Entwicklung ionenselektiver Mini- und Mikroelektroden und zu deren Messtechnik, Juris Verlag, Zürich, 1977.
175 S. Okazaki, Kagaku (Kyoto), 31 (1976) 839.
176 H. F. Osswald, R. E. Dohner, T. Meier, P. C. Meier and W. Simon, Chimia, 31 (1977).
177 L. Pataki, K. Harka, J. Havas and G. Keömley, Radiochem. Radioanal. Lett., 26 (1976) 223.
178 L. Pataki, K. Harka, J. Havas and G. Keömley, Radiochem. Radioanal. Lett., 27 (1976) 287.
179 L. Pataki, K. Harka, J. Havas and G. Keömley, Radiochem. Radioanal. Lett., 27 (1976) 385.
180 L. Pataki, K. Harka, J. Havas and G. Keömley, Radiochem. Radioanal. Lett., 30 (1977) 219.
181 S. Patal and P. O'Gorman, Clin. Chem., 24 (1978) 1856.
181a F. J. Philbert, M. N. Smith and O. El Kei, Adv. Automat. Anal., 7th Technicon Int. Congr., 2 (1977) 43.
182 J. R. Potts, Adv. Autom. Anal., 7th Technicon Int. Congr., 2 (1977) 38.
183 E. Pungor, K. Tóth, G. Nagy and Zs. Fehér, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 67.
184 D. G. Rands and C. F. Bayer, J. Am. Water Works Assoc., 67 (1975) 694, 704, 708.
185 M. D. Reboiras, H. Pfister and H. Pauly, Biophys. Chem., 9 (1978) 37.
186 K. Ren and W. Szczepaniak, Chem. Anal. (Warsaw), 21 (1976) 1365.
187 R. A. Robinson and R. G. Bates, Marine Chem., 6 (1978) 327.
188 J. F. Schenck, J. Coll. Interface Sci., 6 (1977) 569.
189 J. G. Schindler, Biomed. Technik, 20 (1975) 75.
190 J. G. Schindler, Biomed. Technik, 22 (1977) 235.
191 J. G. Schindler, R. Dennhardt and W. Simon, Chimia, 21 (1977) 404.
192 J. G. Schindler, A. Mönnich, W. Riemann and H. E. Braun, Biomed. Techn., 22 (1977) 209.

193 J. G. Schindler and W. Riemann, Biomed. Tech., 20 (1975) 75.
194 H. D. Schwartz, Clin. Chem., 22 (1976) 461.
195 H. D. Schwartz, Clin. Chem., 23 (1977) 610.
196 H. D. Schwartz, Clin. Chim. Acta, 64 (1975) 227.
197 I. Sekerka and J. F. Lechner, Anal. Chim. Acta, 93 (1977) 129.
198 J. Siemroth, I. Hennig and R. Claus, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 185.
199 D. Slaunwhite, J. C. Clements and G. Reynoso, Clin. Biochem., 10 (1977) 44.
200 Z. G. Szabó, L. Barcza, L. Ladányi, G. Pitter and I. Ruff, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest 1977, p. 207.
201 P. Szepesváry and L. Naszódi, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, 1977, p. 225.
202 P. J. Tetenes and P. A. Anninos, Brain Res. Bull., 2 (1977) 55.
203 P. W. Cheuny, D. G. Fleming, M. R. Neuman and W. H. Ko (Eds.), Theory, Design and Biomedical Applications of Solid State Chemical Sensors, CRC Press, Cleveland, 1978.
204 L. Tomcsányi, in ref. 4, p. 239.
205 K. Tóth, G. Nagy, Z. Fehér and E. Pungor, Fresenius Z. Anal. Chem., 282 (1976) 379.
206 K. Tóth and E. Pungor, Am. Lab., 8 (1976) 9.
207 E. Ujec, O. Keller, J. Machek and V. Pavlík, Physiol. Bohemoslov., 27 (1978) 570.
208 R. E. Van de Leest, Analyst, 101 (1976) 433.
209 R. E. Van de Leest, Analyst, 102 (1977) 509.
210 R. E. Van de Leest, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
211 R. E. Van de Leest and A. Geven, J. Electroanal. Chem., 90 (1978) 97.
212 M. Vandeputte, L. Dryon and D. L. Massart, Anal. Chim. Acta, 91 (1977) 113.
213 M. Vandeputte, L. Dryon and D. L. Massart, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 583.
214 W. E. van der Linden and G. J. M. Heijne, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 445.
215 J. M. van der Meer, G. den Boef and W. E. van der Linden, Anal. Chim. Acta, 85 (1976) 309.
216 J. M. van der Meer and J. C. Smit, Anal. Chim. Acta, 83 (1976) 367.
217 G. W. S. van Osch and B. Griepink, Fresenius Z. Anal. Chem., 283 (1977) 29.
218 G. W. S. van Osch and B. Griepink, Fresenius Z. Anal. Chem., 284 (1977) 267.
219 G. E. Veress, Acta Chim. Acad. Sci. Hung., 97 (1978) 278.
220 R. Virtanen, in ref. 5, p. 589.
221 C. C. Westcott, Anal. Chim. Acta, 86 (1976) 269.
222 J. O. Westgard, R. N. Carey, D. H. Feldbruegge and L. M. Jenkins, Clin. Chem., 22 (1976) 485.
223 J. N. Zemel, Ger. Offen. No. 2813170 (1978).
224 J. N. Zemel, Res. Dev., 28 (1977) 38.
225 T. Adachi, N. Shiraishi, G. Nakagawa and K. Kodama, Bunseki Kagaku, 26 (1977) 658.
226 J. D. Allen, Analyst, 99 (1974) 765.
227 A. Altinata, B. Pekin and S. Ulgü, Analyst, 102 (1977) 876.
228 J. Angerer, J. Clin. Chem. Clin. Biochem., 15 (1977) 201.
229 T. Aomi, Denki Kagaku, 46 (1978) 259.
230 T. Aomi, Denki Kagaku, 46 (1978) 343.
231 T. Aomi, Denki Kagaku, 46 (1978) 567.

232 W. McD. Armstrong, W. Wojtkowski and W. R. Bixenman, Biochim. Biophys. Acta, 465 (1977) 165.
232a J. Bagg, Anal. Chem., 48 (1976) 1811.
233 P. L. Bailey, J. Wilson, S. Karpel and M. Riley, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 201.
234 H. Z. Balog, M. Varhelyi and M. Kellermayer, Rheumatol. Balneol. Allergol., 17 (1976) 244.
235 H. J. Banks, J. M. Desmarchelier and J. A. Elek, Pestic. Sci., 7 (1976) 595.
236 J. Barica, J. Fish. Res. Board Can., 35 (1978) 141.
237 J. M. Bather, G. J. Kakabadse and E. C. Weller, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne 1976.
238 F. G. K. Bauke, Fresenius Z. Anal. Chem., 282 (1976) 105.
239 E. W. Baumann, Report DP-1442 (1976) 12.
240 T. B. Beider and K. R. Soldmenskaya, Metody Anal. Kontrolya Proizvod. Khim. Prom., 7 (1977) 44.
241 C. G. Beguin and Ch. Coulombeau, Anal. Chim. Acta, 90 (1977) 237.
242 F. A. Belinskaya and E. D. Makarova, Ionn-Obmen Ionometri. USSR, 1 (1976) 40.
243 C. Birraux, J.-Cl. Landry and W. Haerdi, Anal. Chim. Acta, 90 (1977) 51.
244 C. Birraux, J.-Cl. Landry and W. Haerdi, Anal. Chim. Acta, 93 (1977) 281.
245 J. W. Bixter, R. Nee and S. P. Perone, Anal. Chim. Acta, 99 (1978) 217.
246 J. B. Bodkin, Analyst, 102 (1977) 409.
247 H. J. Boniface and R. H. Jenkins, Analyst, 102 (1977) 739.
248 P. T. Bray, G. C. F. Clark, G. J. Moody and J. D. R. Thomas, A Perspective of Sodium and Chloride Ion-selective Electrode Sweat Tests for Screening in Cystic Fibrosis, University of Wales, Cardiff, 1975.
249 P. T. Bray, G. C. F. Clark, G. J. Moody and J. D. R. Thomas, Clin. Chim. Acta, 77 (1977) 69.
250 P. T. Bray, G. C. F. Clark, G. J. Moody and J. D. R. Thomas, Clin. Chim. Acta, 88 (1977) 333.
251 W. T. Bresnahan, C. Grant and J. H. Weber, Anal. Chem., 50 (1978) 1675.
252 J. Buffle, F.-L. Greter and W. Haerdi, Anal. Chem., 49 (1977) 216.
253 L. N. Bykova, N. A. Kazaryan, E. Pungor and N. S. Chernova, in ref. 5, p. 281.
254 L. F. Bystrova, V. B. Stradomskii and A. A. Nazarova, Gidrokhim. Mater., 63 (1976) 145.
255 L. Campanella, D. Gozzi and T. Ferri, Ann. Chim. (Rome), 67 (1977) 295.
256 L. P. Cellini, P. E. Barbolani and C. Bartoli, Rass. Chim., 28 (1976) 183.
257 E. E. Chao and K. L. Cheng, Talanta, 24 (1977) 247.
258 R. Chavdarova, E. R. Schmid and R. R. Becker, Mikrochim. Acta, (1975) 445.
259 R. Christova, M. Ivanova and M. Novkirishka, Anal. Chim. Acta, 85 (1976) 301.
260 H. Clysters and F. Adams, Anal. Chim. Acta, 92 (1977) 251.
261 G. S. Craven and M. C. Griffith, Aust. J. Dairy Technol., 32 (1977) 75.
262 M. Cromer-Morin and J.-P. Scharff, C. R. Acad. Sci. Paris, Ser., C283 (1976) 621.
263 T. Cserfalvi, T. Meisel, B. Tarnay, K. Seybold, F. Galina and E. Pungor, Fresenius Z. Anal. Chem., 282 (1976) 351.
264 P. J. Cusbert, Anal. Chim. Acta, 87 (1976) 429.
265 T. Deguchi, T. Kuma and R. Nagai, J. Chromatogr., 152 (1978) 349.
266 A. Dencks and R. Neeb, Fresenius Z. Anal. Chem., 285 (1977) 233.
267 A. L. Dent and C. A. Hendrick, Prepr. Pap. Natl. Meet., Div. Environ. Chem, Am. Chem. Soc., 15 (1975) 209.
268 M. R. Dimeski, God. Zb., Prir.-Mat. Fak. Univ. Skopje, 25–26 (1976) 245.
269 E. L. Donaldson and D. C. Mullan, Anal. Lett., A11 (1978) 39.
270 P. D'Orazio and G. A. Rechnitz, Anal. Chem., 49 (1977) 41.
271 A. Duca and F. Matei, Rev. Chim. (Bucharest), 23 (1977) 1186.

272 E. J. Duff and J. A. Stuart, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
273 S. Duke and G. C. Forward, Caries Res., 12 (1978) 12.
274 R. A. Durst, Anal. Lett., 10 (1977) 961.
275 K. Ebock and C. Neiser, Z. Chem., 18 (1978) 343.
276 J. Ekstrand, Caries Res., 12 (1978) 123.
277 B. Elgquist and M. Wedborg, Mar. Chem., 6 (1978) 243.
278 Y. I. Elmehrik, S. A. Marei and S. S. M. Hassan, Libyan J. Sci., 6A (1976) 23.
279 A. Farzanek and G. Troll, Geochem. J., 11 (1977) 177.
280 P. R. Finley, J. A. Dye, D. A. Lichti, J. M. Byers and J. R. Williams, Am. J. Clin. Pathol., 69 (1978) 615.
281 B. Courvoisier, A. Donath and C. A. Band (Eds.), Fluoride and Bone, Proc. 2nd Symposium of Centre d'Etude des Maladies Osteo-Articulaires, Nyon, Switzerland, 1977, Hans Huber Publishers, Bern, 1978.
281a A. Franke, Vom Wasser, 42 (1974) 161.
282 Ch. Fuchs, D. Dorn, Ch. A. Fuchs, H.-V. Henning, Ch. McIntosh, F. Scheler and M. Stennert, Clin. Chim. Acta, 60 (1975) 157.
283 Y. S. Fung and K. W. Fung, Anal. Chem., 49 (1977) 497.
284 Y. S. Fung and K. W. Fung, Analyst, 103 (1978) 149.
285 F. F. Gaál, L. S. Jovanović and V. D. Canić, Fresenius Z. Anal. Chem., 282 (1976) 439.
286 S. A. Gava, N. S. Poluektov and G. N. Karoleva, Zh. Anal. Khim., 33 (1978) 506.
287 M. Geissler, Anal. Chim. Acta, 90 (1977) 249.
288 M. Ghosh, M. R. Dhaneshwar, R. G. Dhaneshwar and B. Ghosh, Analyst, 103 (1978) 768.
289 H. J. Gibitz and H. Fenninger, Wien. Med. Wochenschr., 128 (1978) 125.
290 S. Goldstein and A. Duca, J. Pharm. Sci., 65 (1976) 1831.
291 N. B. Greenhill and K. I. Peverill, Comm. Soil Sci. Plant. Anal., 8 (1977) 579.
292 A. L. Grekovich, Yu. G. Vlasov, E. A. Materova, I. V. Murin, S. B. Kocheregin and S. S. Mikhailova, Ionn. Obmen Ionometr. USSR, 1 (1975) 170.
293 U. Gruenke and P. Hartmann, Hermsdorfer Tech. Mittl., 18 (1978) 1619.
294 J. Gulens and B. Ikeda, Anal. Chem., 50 (1978) 782.
295 J. Gulens, K. Jessome and C. K. Macneil, Anal. Chim Acta, 96 (1978) 23.
296 R. Gyenge, Gyogyszereszet, 22 (1978) 137.
297 R. Gyenge, E. Körös and K. Tóth, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 115.
298 R. Gyenge, K. Tóth, E. Körös and E. Pungor, Magy. Kem. Foly., 83 (1977) 135.
299 R. Gyenge, K. Tóth, E. Pungor and E. Körös, Anal. Chem. Acta, 94 (1977) 111.
300 R. Halas, K. Secenji and M. Kucsera, Hem. Ind., 30 (1976) 443.
301 A. S. Hallsworth, J. A. Weatherell and D. Deutsch, Anal. Chem., 48 (1976) 1660.
302 H. Harada, Tokoshi Nyusu, Kagaku Kogyo Shiryo, 13 (1978) 40.
303 P. Hartmann, U. Gruenke and H. Berge, Z. Chem., 17 (1977) 421.
304 C. Harzdorf and G. Hennig, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 379.
305 S. S. M. Hassan, Anal. Chem., 49 (1977) 45.
306 S. S. M. Hassan, Mikrochim. Acta, (1977) 405.
307 S. S. M. Hassan, Talanta, 23 (1976) 738.
308 S. S. M. Hassan and M. B. Elsayes, Mikrochim. Acta, (1978) 333.
309 J. Havas and L. Kecskés, Magy. Kem. Foly., 83 (1977) 529.
310 J. Havas and L. Kecskés, Magy. Kem. Foly., 83 (1977) 535.
310a J. Havas, L. Kecskés, K. Nyiro, M. Palko and I. Szoke, Hung. Sci. Instrum., 43 (1978) 7.
311 J. Havas, L. Kecskés, Kn. Nyiro, M. Palko and I. Szoke, Orv. Tech., 15 (1977) 1.
312 R. C. Hawkings, L. P. V. Carriveau, S. A. Kushnerink and P. Y. Wong, Anal. Chim. Acta, 102 (1978) 61.

313 G. J. M. Heijne and W. E. van der Linden, Anal. Chim. Acta, 93 (1977) 99.
314 G. J. M. Heijne and W. E. van der Linden, Anal. Chim. Acta, 96 (1978) 13.
315 G. J. M. Heijne, W. E. van der Linden and G. den Boef, Anal. Chim. Acta, 89 (1977) 287.
316 G. J. M. Heijne, W. E. van der Linden and G. den Boef, Anal. Chim. Acta, 98 (1978) 221.
317 G. J. Heijne, W. E. van der Linden and G. den Boef, Anal. Chim. Acta, 100 (1978) 193.
318 P. Hermann and W. Rode, Staub-Reinhalt. Luft, 35 (1975) 298.
319 G. Herrmann, Mess. Pruef., 6 (1976) 310, 312, 314.
319a G. W. Heunisch, Anal. Chim. Acta, 101 (1978) 221.
320 M. Hofton, Environ. Sci. Technol., 10 (1976) 277.
321 A. Honda, M. Kashimoto and S. Honda, Anal. Chim. Acta, 97 (1978) 391.
322 D. M. Hopkins, J. Res. U.S. Geol. Surv., 5 (1977) 589.
323 A. Hrabéczy-Páll, K. Tóth and E. Pungor, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 127.
324 A. Hrabéczy-Páll, F. Valló, K. Tóth and E. Pungor, Hung. Sci. Instrum., 41 (1977) 55.
325 A. Hulanicki, Z. Augustowska and M. Trojanowicz, Chem. Anal. (Warsaw), 22 (1977) 955.
326 A. Hulanicki, R. Lewandowski and A. Lewenstam, Analyst, 101 (1976) 939.
327 A. Hulanicki and A. Lewenstam, Talanta, 23 (1976) 661.
328 A. Hulanicki, M. Trojanowicz and T. Krawczynski, Water Res., 11 (1977) 627.
329 A. A. Hussain, J. Kraal and H. Wahner, J. Dental Res., 57 (1978) 872.
330 S. Ikeda and J. Motonaka, Anal. Chim. Acta, 90 (1977) 257.
331 S. B. Ilkova, E. A. Zazimko and K. K. Rivkina, Nauch. Tr. NIPIRP, 82 (1977) 79.
332 S. V. Ilkova, E. A. Zazimko and K. K. Rivkina, Zavod. Lab., 42 (1976) 658.
333 B. G. Iofis, N. I. Savvin, A. V. Vishnyakov and A. V. Gordievski, Zavod. Lab., 39 (1973) 267.
334 K. Irlweck and H. Soratin, Mikrochim. Acta, (1977) 25.
335 K. Itoh, H. Ueda, S. Matsumoto and M. Shibuya, Kotsu Igaku, 30 (1976) 351.
336 A. K. Jain, S. Srivastava, R. P. Singh and S. Agrawal, J. Appl. Chem. Biotechnol., 27 (1977) 630.
337 M. H. Jones and J. T. Woodcock, Proc. Aust. Inst. Min. Metall., 266 (1978) 11.
338 M. H. Jones and J. T. Woodcock, Transact. Sect. C Inst. Mining Metall. 87 (1978) C99.
339 A. Jyo, T. Hashizume and N. Ishibashi, Anal. Chem., 49 (1977) 1868.
340 S. Kanisawa, Ganseki Kobutsu Kosho Gakkaishi, 73 (1978) 26.
341 T. Kashima, M. Kawamura and M. Onuki, Kyonitsu Yakka Duigaku Kenkyu Nempo, 22 (1977) 1.
342 T. Karasawa and T. Tsujimoto, Ibaraki-Ken Kogai Giutsu Senta Nempo, 8 (1975) 188.
343 M. Kataoka and T. Kambara, Denki Kagaku, 45 (1977) 674.
344 P. Kauranen, Anal. Lett., 10 (1977) 451.
345 A. Kawahara, K. Hiiro and T. Tanaka, Osaka Kogyo Gijutsu Shikensho Kiho, 27 (1976) 85.
346 N. A. Kazaryan, L. N. Bykova and N. S. Chernova, Zh. Anal. Khim., 31 (1976) 334.
347 L. S. Kelday, D. J. F. Bowling and M. G. Penny, J. Exp. Bot., 28 (1977) 31.
348 G. O. Kim, A. G. Li and S. N. Kim, Punsak Hwahak, 3 (1975) 51.
349 G. O. Kim, A. G. Li and S. N. Kim, Punsak Hwahak, 15 (1977) 20.
350 G. O. Kim, A. K. Li and S. D. Yun, Punsak Hwahak, 15 (1977) 41.
350a M. A. Khalifa and M. Leszko, Chem. Anal. (Warsaw), 23 (1978) 599.

351 J. S. Kirchner and Q. Fernando, Anal. Chem., 49 (1977) 1636.
352 P. Kivalo, R. Virtanen, K. Wickstrom, M. Wilson, E. Pungor, G. Horvai and K. Tóth, Anal. Chim. Acta, 87 (1976) 401.
353 P. Kivalo, R. Virtanen, K. Wickstrom, M. Wilson, E. Pungor, K. Tóth and G. Sundholm, Anal. Chim. Acta, 87 (1976) 387.
354 B. Kjellman and B. Tengstrom, Laekartidningen, 73 (1976) 852.
355 H. A. Klasens and J. Goossen, Anal. Chim. Acta, 88 (1977) 41.
356 T. Kojima, M. Ichise and Y. Seo, Anal. Chim. Acta, 101 (1978) 273.
357 L. S. Kolesnik and E. A. Golovchenko, Metody Anal. Kontr. Kach. Prod. Khim. Prom., 4 (1978) 54.
358 N. P. Komar and L. P. Gudim, Zh. Fiz. Khim., 50 (1976) 2422.
359 P. O. Kosonen, M. J. Hotokka and J. A. Mannonen, Finn. Chem. Lett., (1978) 90.
360 P. O. Kosonen, J. A. Mannonen and M. J. Hottoka, Finn. Chem. Lett., (1977) 13.
360a M. Mascini and G. Palleschi, Anal. Chim. Acta, 100 (1978) 215.
361 A. E. Kosov, P. D. Novikov, O. T. Krylov and M. P. Nesterova, Okeanologiya (Moscow), 16 (1976) 815.
362 D. R. Krieg and D. Sung, Commun. Soil Sci. Plant. Anal., 8 (1977) 109.
363 P. Kuopiokauranan, Anal. Lett., 10 (1977) 451.
364 Y. S. Kwon, J. H. Kim and K. C. Park, Tachan Hwahak Hoechi, 20 (1976) 486.
365 P. C. Legittimo, E. B. Piccardi and C. Bartoli, Rass. Chim., (1976) 183.
366 M. Lendacká, Vodní Hospod., 26 (1976) 235.
367 C. Liteanu, E. Hopirtean and R. Vlad, Rev. Roum. Chim., 21 (1976) 933.
368 O. O. Lyalin, M. S. Turaeva and B. M. Mogilevskii, Elektrokhimiya, 13 (1977) 1716.
369 O. O. Lyalin and M. S. Turaeva, Elektrokhimiya, 13 (1977) 256.
370 O. O. Lyalin and M. S. Turaeva, Zh. Anal. Khim., 31 (1976) 1356.
371 O. O. Lyalin and M. S. Turaeva, Zh. Anal. Khim., 31 (1976) 1879.
372 W. Luethi and M. Sahli, Pharm. Acta Helv., 49 (1974) 270.
373 R. A. Mackay, C. Hermansky and R. Agarwal, Colloid Interface Sci. (Proc. Int. Conf.), 2 (1976) 289.
374 L. I. Manakova and N. V. Bausova, Zavod. Lab., 42 (1976) 635.
375 L. I. Manakova, N. V. Bausova, V. E. Moiseev, V. G. Bamburov and A. P. Sivoplyas, Zh. Anal. Khim., 35 (1978) 1517.
376 W. Manley, Proc. Ann. Conf. Am. Water Works Ass., Ottawa, Ont., (1975) 200.
377 G. B. Marshall and D. Midgley, Analyst, 103 (1978) 438.
378 M. Mascini, Anal. Chim. Acta, 85 (1976) 287.
379 M. Mauzac, F. Guérard, J. Mathieu and J. Laroche, Analusis, 4 (1976) 326.
380 J. R. McClenahen and E. R. Schulz, Soil Sci., 122 (1976) 267.
381 P. M. McElfresh, J. Agric. Food Chem., 26 (1978) 276.
382 N. R. McQuaker and M. Gurney, Anal. Chem., 49 (1977) 53.
383 J. Mertens, P. van den Winkel and J. Vereecken, J. Electroanal. Chem., 85 (1977) 277.
384 D. Midgley, Anal. Chim. Acta, 87 (1976) 19.
385 D. Midgley, Anal. Chim. Acta, 87 (1976) 7.
386 V. A. Mirkin, V. G. Goncharuk and V. V. Bakanina, Khim. Khim. Tekhnol., 20 (1976) 64.
387 V. A. Mirkin, M. A. Ilyushenko and V. V. Bakanina, Zh. Anal. Khim., 32 (1978) 2282.
388 W. Misniakiewicz and K. Raszka, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 467.
389 K. Nagy, T. A. Fjeldy and J. S. Johannessen, in ref. 5, p. 491.
390 A. Napoli and M. Mascini, Anal. Chim. Acta, 89 (1977) 209.
391 M. Naray, Munkavedelen, 21 (1975) 31.
392 M. Naumović, T. Bosković and O. Naumović, Mikrochim. Acta, 2 (1977) 537.
393 M. Neshkova and H. Sheytanov, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest, 1977, Akademia Kiadó, Budapest, 1978, p. 503.

394 G. F. Nichugovskii and V. S. Vasileva, Anal. Kontrol Khim. Veshchestv Okruzh. Sredy, (1977) 9.
395 E. Niki, Asahi Garasu Kogyo Gijutsu Shoreikai Kenkyu Hokoku, 29 (1976) 225.
396 E. Niki and H. Shirai, Elektrokhimiya, 14 (1978) 714.
397 D. P. Nikolelis, D. S. Papastathopoulos and T. P. Hadjiiouannou, Anal. Chim. Acta, 98 (1978) 227.
398 M. Novkirishka and R. Christova, Mikrochim. Acta, (1978) 483.
399 I. Novozamsky and W. H. van Riemsdijk, Anal. Chim. Acta, 85 (1976) 41.
400 G. B. Ogiesby, W. C. Duer and F. J. Millero, Anal. Chem., 49 (1977) 877.
401 A. N. Oparin, V. N. Filin and S. P. Kochetkov, Melody Anal. Kontr. Proizv. Khim. Prom., 7 (1977) 41.
402 E. A. Ostrovidov, Zavod. Lab., 42 (1976) 1056.
403 M. B. Pantůček, Česk. Hyg. (Prague), 23 (1978) 187.
404 D. S. Papastathopoulos, D. P. Nikolelis and T. P. Hadjiioannou, Analyst, 102 (1977) 852.
405 G. Papeschi, S. Bordi and M. Carla, J. Electrochem. Soc., 125 (1978) 1807.
406 N. Parthasarathy, J. Buffle and W. Haerdi, Anal. Chim. Acta, 93 (1977) 121.
407 L. Pataki, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 177.
408 H. Pokorná, Chem. Prum., 28 (1978) 238.
409 I. C. Popescu and M. Halalau, Rev. Chim. (Bucharest), 27 (1976) 161.
410 I. C. Popescu and M. Halalau, Rev. Roum. Chim., 22 (1977) 443.
411 I. C. Popescu and A. Lujerdean, Rev. Chim. (Bucharest), 27 (1976) 533.
412 S. R. Porter and A. P. Runnancles, Anal. Chim. Acta, 94 (1977) 449.
413 S. Poulsen, M. J. Larsen and R. H. Larson, Caries Res., 10 (1976) 227.
414 M. R. Powell, Oak Ridge Rep. K/TL-631, 1977.
415 C. P. Price and K. Spencer, Ann. Clin. Biochem., 14 (1977) 171.
416 M. M. Privalova, M. D. Tulina, E. M. Sheyanova, N. M. Borzova, B. D. Selivanova and L. F. Polyakova, Zh. Anal. Khim., 32 (1977) 1969.
417 M. M. Privalova, M. D. Tulina, E. M. Sheyanova and B. D. Selivanova, Zh. Anal. Khim., 31 (1976) 1185.
418 L. W. Przyborowski, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 519.
419 N. J. Radić, Analyst, 101 (1976) 657.
420 S. J. Redinha and M. L. P. Leitaõ, Rev. Port. Quim., 16 (1974) 14.
421 G. K. Rice and R. J. Jasinski, Natl. Bur. Stand. (U.S.), Spec. Publ., 422 (1976) 899.
422 L. P. Rigdon, G. J. Moody and J. W. Frazer, Anal. Chem., 50 (1978) 465.
423 C. J. Rix, A. M. Bond and J. D. Smith, Anal. Chem., 48 (1976) 1236.
424 P. A. Rock, T. L. Eyrich and S. Styer, J. Electrochem. Soc., 124 (1977) 530.
425 R. Ross and G. Kuester, Jahresbericht, Zentralinstitut. Kernforsch. DAW, ZFK-340, 150, 1977.
426 J. H. Saunders and H. M. Braun, J. Gen. Physiol., 70 (1977) 507.
427 V. S. Savenko, Okeanologiya (Moscow), 16 (1976) 825.
428 P. J. Scharff, Anal. Chim. Acta, 87 (1976) 499.
429 P. Scheide and R. A. Durst, Anal. Lett., 10 (1977) 55.
430 I. Sekerka and J. F. Lechner, Anal. Chim. Acta, 93 (1977) 139.
431 I. Sekerka and J. F. Lechner, Anal. Lett., 9 (1976) 1099.
432 I. Sekerka and J. F. Lechner, Anal. Lett., A11 (1978) 415.
433 I. Sekerka, J. F. Lechner and L. Harrison, J. Assoc. Off. Anal. Chem., 60 (1977) 625.
434 P. Y. Serre and H. Bozon, Analusis, 5 (1977) 304.
435 N. M. Sheina, V. P. Izvekov, M. K. Pápay, K. Tóth and E. Pungor, Anal. Chim. Acta, 92 (1977) 261.
436 S. Y. Shetty and R. M. Sathe, J. Inorg. Nucl. Chem., 39 (1977) 1838.
437 R. S. Sholtes, E. H. Meadows and S. B. Koogler, U.S. Nat. Tech. Inform. Serv., PB Rep. No 230954/OGA, 1973.

438 A. Siirde and B. Luiga, Eesti NSV Tead. Akad. Toim., Keem., 27 (1978) 1.
439 L. Singer and R. H. Ophaug, Anal. Chem., 49 (1977) 38.
440 R. L. Solsky and G. A. Rechnitz, Anal. Chim. Acta, 99 (1978) 241.
441 A. Sovová and N. Kasiková, Sb. Vys. Šk. Zeměd., Fak. Agron., (Prague), A 1 (1976) 13.
442 S. K. Srivastava, A. K. Jain, S. Agrawal and R. P. Singh, J. Electroanal. Chem., 90 (1978) 291.
443 J. P. Stahl, Circ. Inf. Tech. Cent. Doc. Sider., 34 (1977) 1151.
444 N. I. Stenina and N. S. Lapshanova, Tr. Ural. Nauchnoissled. Khim. Inst., 32 (1973) 50.
445 E. Sucman, M. Sucmanová and O. Synek, Z. Lebensmittel. Unters. Forsch., 167 (1978) 5.
446 K. Sykut and E. Nowakowska, Biul. Lubel. Tow. Nauk., Mat.-Fiz.-Chem., 19 (1977) 89.
447 K. Sykut and M. Przegalinski, Biul. Lubel. Tow. Nauk., Mat.-Fiz.-Chem., 16 (1974) 15.
448 J. Tacussel and J. J. Fombon, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 567.
449 M. Taddia, Microchem. J., 22 (1977) 369.
450 M. Taddia and P. Lanza, Ann. Chim. (Rome), 65 (1975) 719.
451 M. Taga, M. Mizuguchi, H. Yoshida and S. Hikime, Bunseki Kagaku, 25 (1976) 362.
452 K. Takamatsu, K. Takahashi, K. Saito and S. Kanno, Eisfi Kagaku, 22 (1976) 286.
453 T. Tanaka, K. Hiiro and A. Kawahara, Fresenius Z. Anal. Chem., 286 (1977) 212.
454 S. Tanikawa, T. Adachi, N. Shiraishi, G. Nakagawa and K. Kodama, Bunseki Kagaku, 25 (1976) 646.
455 S. Tanikawa, H. Kirihara, N. Shiraishi, G. Nakagawa and K. Kodama, Bunseki Kagaku, 24 (1975) 559.
456 K. Tomlinson and K. Torrance, Analyst, 102 (1977) 1.
457 K. T. Tran, Tap Chi Hoa Hoc, 14 (1976) 25.
458 P. K. C. Tseng and W. F. Gutknecht, Anal. Chem., 48 (1976) 1996.
459 P. K. C. Tseng and W. F. Gutknecht, Anal. Lett., 9 (1976) 795.
460 Ai-Min Tsou, Kuo-Hsiung Chang and Hou-Chi Wang, Hua Hsueh Tung Pao, 5 (1976) 299.
461 L. O. Turtola, Scand. J. Dent. Res., 85 (1977) 373.
462 M. Uchida, M. Akiba, S. Wada and T. Kashima, Kyoritsu Yakka Daigaku Kenkyu Nempo, 22 (1977) 6.
463 M. Uchida, K. Harada and T. Kashima, Kyoritsu Yakka Daigaku Kenkyu Nempo, 21 (1977) 11.
464 V. Vajgana and V. Kalajlijeva, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 577.
465 P. Van Den Winkel, J. Mertens, T. Boel and J. Vereecken, J. Electrochem. Soc., 124 (1977) 1338.
466 P. Van Den Winkel, J. Martens, J. Vereecken and D. L. Massart, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
467 M. Vandeputte, J. De Cock, L. Dryon, A. Vercruysse, F. Alexander and D. L. Massart, Clin. Chim. Acta, 75 (1977) 205.
468 W. E. van der Linden and R. Ostervink, Anal. Chim. Acta, 101 (1978) 419.
469 J. M. van der Meer, G. den Boef and W. E. van der Linden, Anal. Chim. Acta, 85 (1976) 317.
470 C. Vanroelen, R. Smits, P. van den Winkel and D. L. Massart, Fresenius Z. Anal. Chem., 208 (1976) 21.
471 A. Varduca, D. Virtosu, V. Murgulescu and C. Luca, Rev. Chim. (Bucharest), 27 (1976) 527.

472 N. Vick, E. G. Kleinschmidt, H. Berge and P. Hartmann, Coll. Inst. Nat. La Santé Rech. Med., 68, Inn. Eear Biol., (1977) 217.
473 B. Vickery and M. L. Vickery, Analyst, 101 (1976) 445.
474 D. Virtosu, A. Varduca, C. Luca, L. Popescu and O. Andrea, Stud. Prot. Calitatii Apelor, 17 (1976) 192.
475 H. V. Vishnyakov, A. F. Zhukov, T. A. Lyubchak, Yu. I. Urusov and A. V. Gordievsky, Zh. Anal. Khim., 32 (1977) 840.
476 T. N. Vladimirskaya and A. P. Gorskaya, Metody Anal. Kontr. Proizvod. Khim. Prom., 11 (1977) 53.
477 Y. G. Vlasov, A. L. Grekovich, E. A. Materova, I. V. Murin and S. B. Kocheregin, Ionn. Obmen Ionometr. USSR, 1 (1978) 170.
478 P. Venkateswarlu, Anal. Chem., 46 (1978) 46.
479 P. Venkateswarlu, Clin. Chim. Acta, 59 (1975) 277.
480 Yu. G. Vlasov and S. B. Kocheregin, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 597.
481 Y. G. Vlasov, S. B. Kocheregin and Y. E. Ermolenko, Elektrokhimiya, 13 (1977) 132.
482 Yu. G. Vlasov, S. B. Kocheregin and Yu. E. Ermolenko, Zh. Anal. Khim., 32 (1977) 1843.
483 T. V. Vornycheva, N. S. Filimonova and V. A. Kozhemyak, Zavod. Lab., 42 (1976) 522.
484 C. Y. Wang, Hua Hsueh Hsueh Pao, 34 (1978) 271.
485 Y.-X. Wang and Z.-X. Quian, Ti Ch'iu Hua Hsueh, 4 (1976) 285.
486 W. J. Warwick and L. G. Hansen, Clin. Chem., 24 (1978) 381.
487 W. J. Warwick and L. Hansen, Clin. Chem., 24 (1978) 2050.
488 J. A. Weatherell, G. Naylor and A. S. Hallsworth, Caries Res., 11 (1977) 231.
489 J. N. C. Whyte and J. R. Englar, Analyst, 101 (1976) 815.
490 J. N. C. Whyte and J. R. Englar, J. Fish. Res. Board Can., 35 (1978) 202.
491 F. Yaashita, T. Komatse and T. Nakagawa, Bull. Chem. Soc. Jpn., 4é (1976) 2073.
492 H. Y. Yung and K. K. Pu, Huan Ching K'o Hsueh, 2 (1978) 42.
492a M. Yoshida, Y. Makihara and T. Katsura, Nippon Kagaku Kaishi, 10 (1978) 1375.
493 E. A. Zeynalova and M. M. Senyavin, Zh. Anal. Khim., 30 (1975) 2207.
494 A. F. Thukov, A. V. Vishnyakov, Yu. I. Urusov and A. V. Gordievskii, Zh. Anal. Khim., 30 (1975) 1614.
495 A. F. Zhukov, A. V. Vishnyakov, Ja. L. Kharif, Yu. I. Urusov, F. K. Volynets, E. I. Ryzhikov and A. V. Gordievskii, Zh. Anal. Khim., 30 (1975) 1761.
496 R. L. Zimmerman and H. G. Bertrand, Anal. Lett., A11 (1978) 569.
497 H. Acker, Pfluegers Arch., 375 (1978) 229.
498 A. A. Al-Sibaai, Proc. Anal. Div. Chem. Soc., 12 (1975) 65.
499 D. F. Anghel and N. Ciocan, Anal. Lett., 10 (1977) 423.
500 D. F. Anghel and N. Ciocan, Colloid Polym. Sci., 254 (1976) 114.
501 P. Asher, D. Kunze and T. O. Neild, J. Physiol., 256 (1974) 441.
502 C. C. Ashley, T. J. Rink and R. T. Tsien, J. Physiol., 28 (1978) 27P.
503 U. Astrani, U.S. Nat. Tech. Inform. Serv., PB Rep., No. 238056/8GA, NTIS, 1973.
504 G. E. Baiulescu and N. Ciocan, Talanta, 24 (1977) 37.
505 G. E. Baiulescu and V. V. Cosofret, Talanta, 23 (1976) 677.
506 G. E. Baiulescu, V. V. Cosofret and M. Blasnic, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 207.
507 D. M. Band, C. H. Fry and T. Treasure, J. Physiol., 276 (1978) 1P.
508 D. M. Band, J. Kratochvíl and T. Treasure, J. Physiol., 265 (1977) 5P.
509 D. M. Band, J. Kratochvíl, P. A. P. Wilson and T. Treasure, Analyst, 103 (1978) 246.
510 D. M. Band and T. Treasure, J. Physiol., 266 (1977) 12P.

511 J. L. Barker, R. A. Nicoll and A. Padjen, J. Physiol., 245 (1975) 537.
512 T. Ya. Bart, E. A. Materova and O. G. Izmailova, Elektrokhimiya Ionitov, 2 (1977) 58.
513 E. W. Baumann, Anal. Chem., 48 (1976) 548.
514 E. W. Baumann, Anal. Chim. Acta, 99 (1978) 247.
515 B. J. Birch, A. Craggs, G. J. Moody and J. D. R. Thomas, J. Chem. Educ., 55 (1978) 740.
516 R. Bissig, U. Oesch, E. Pretsch, W. E. Morf and W. Simon, Helv. Chim. Acta, 61 (1978) 1531.
517 R. Bissig, E. Pretsch, W. E. Morf and W. Simon, Helv. Chim. Acta, 61 (1978) 1520.
518 A. S. Black and S. A. Waring, Plant Soil, 49 (1978) 207.
519 L. L. Blaine and F. R. Toman, Trans. Kentucky Acad. Sci., 39 (1978) 160.
520 T. B. Bolton and R. D. Vaughan-Jones, J. Physiol., 270 (1977) 801.
521 G. P. Bound, J. Sci. Food Agric., 28 (1977) 501.
522 E. Brauer, K. D. Kupka and G. Massig, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
523 E. Brauer, K.-D. Kupka and V. Rudloff, J. Electrochem. Soc., 123 (1976) 1313.
524 M. R. Bristow, H. D. Schwartz, G. Binetti, D. C. Harrison and J. R. Daniels, Circ. Res., 41 (1977) 565.
525 H. M. Brown, J. P. Pemberton and J. D. Owen, Anal. Chim. Acta, 85 (1976) 261.
526 R. Büchi and E. Pretsch, Helv. Chim. Acta, 60 (1977) 1141.
527 R. Büchi, E. Pretsch, W. E. Morf and W. Simon, Helv. Chim. Acta, 59 (1976) 2407.
528 R. Büchi, E. Pretsch and W. Simon, Helv. Chim. Acta, 59 (1976) 2327.
529 A. S. Bychkov, O. M. Petrukhin, L. V. Zarinskii, Yu. A. Zolotov, L. V. Bakhtinova and G. G. Shanina, Zh. Anal. Khim., 31 (1976) 2114.
529a L. F. Bystrova, V. B. Stradomskii and A. A. Nazarova, Gidrokhim. Mater., 70 (1977) 47.
530 M. Čakrt, J. Berčík and Z. Hladký, Fresenius Z. Anal. Chem., 281 (1976) 295.
531 L. Campanella, G. De Angelis, D. Gozzi and T. Ferri, Analyst, 102 (1977) 723.
532 L. Campanella, T. Ferri and D. Gozzi, Rev. Roum. Chim., 23 (1978) 281.
533 L. Campanella, T. Ferri, D. Gozzi and G. Scorcelletti, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, 1978, p. 307.
534 G. D. Carmack and H. Freiser, Anal. Chem., 49 (1977) 1577.
535 R. W. Cattrall and C.-P. Pui, Anal. Chim. Acta, 87 (1976) 419.
536 R. W. Cattrall and C.-P. Pui, Anal. Chim. Acta, 88 (1977) 185.
537 R. W. Cattrall and K. T. Fong, Talanta, 25 (1978) 541.
538 R. W. Cattrall, H. R. Martin and S. Tribuzio, J. Inorg. Nucl. Chem., 40 (1978) 687.
539 G. R. J. Christoffersen and E. S. Johansen, Anal. Chim. Acta, 81 (1976) 191.
540 G. R. J. Christoffersen and L. Simonsen, Acta Physiol. Scand., 101 (1977) 492.
541 G. R. J. Christoffersen and L. Simonsen, Acta Physiol. Scand., 103 (1978) 352.
542 N. Ciocan and D. F. Anghel, Anal. Lett., 9 (1976) 705.
543 N. Ciocan and D. F. Anghel, Fresenius Z. Anal. Chem., 290 (1978) 237.
544 N. Ciocan and D. Anghel, Tenside Deterg., 13 (1976) 188.
545 M. M. Civan, Am. J. Physiol., 234 (1978) F 261.
546 L. Cleemann and M. Morad, Science, 191 (1976) 90.
547 C. J. Coetzee and A. J. Basson, Anal. Chim. Acta, 92 (1977) 399.
548 J. A. Coles and M. Tsacopoulos, J. Physiol., 270 (1977) 12.
549 S. C. Conceicao, M. K. Ward, F. Alvarez-Ude, P. Aljima, P. Smith and D. N. S. Kerr, Clin. Chim. Acta, 86 (1978) 143.
550 V. V. Cosofret, Rev. Roum. Chim., 23 (1978) 1489.
551 V. V. Cosofret, C. Cristescu and P. G. Zugravescu, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 325.

552 V. V. Cosofret, P. G. Zugravescu and G. E. Baiulescu, Talanta, 24 (1977) 461.
553 A. K. Covington and N. Kumar, Anal. Chim. Acta, 85 (1976) 175.
554 A. Craggs, G. J. Moody, J. D. R. Thomas and B. J. Birch, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 335.
555 A. Craggs, G. J. Moody, J. D. R. Thomas and A. Willox, Talanta, 23 (1976) 799.
566 S. G. Cutler, P. Meares and D. G. Hall, J. Electroanal. Chem., 85 (1977) 145.
557 R. Dewolfs, G. Broddin, H. Clysters and H. Deelstra, Fresenius Z. Anal. Chem., 275 (1975) 337.
558 E. P. Diamandis and T. P. Hadjiioannou, Mikrochim. Acta, 2 (1977) 255.
559 E. P. Diamandis, M. A. Koupparis and T. P. Hadjiioannou, Microchem. J., 22 (1977) 498.
560 P. D'Orazio and G. A. Rechnitz, Anal. Chem., 49 (1977) 2083.
561 L. A. Drachen, A. A. Kondrashin, V. D. Samuilov and V. P. Skulachev, FEBS Lett., 50 (1975) 219.
562 L. J. Drop, C. Fuchs and P. M. Stulz, Clin. Chim. Acta, 89 (1978) 503.
563 J. W. Edmondson and T.-K. Li, J. Lab. Clin. Med., 87 (1976) 624.
564 C. E. Efstathiou and T. P. Hadjiioannou, Anal. Chem., 49 (1977) 414.
565 C. E. Efstathiou and T. P. Hadjiioannou, Anal. Chim. Acta, 89 (1977) 55.
566 C. E. Efstathiou and T. P. Hadjiioannou, Anal. Chim. Acta, 89 (1977) 391.
567 C. E. Efstathiou and T. P. Hadjiioannou, Talanta, 24 (1977) 270.
568 C. F. Efstathiou, T. P. Hadjiioannou and E. McNelis, Anal. Chem., 49 (1977) 410.
569 S. D. Erulkar and F. F. Weight, J. Physiol., 266 (1977) 209.
570 N. K. Evseeva, I. N. Kremenskaya, V. N. Golubev and S. K. Timofeeva, Zh. Anal. Khim., 31 (1976) 677.
571 B. I. Ferber, F. A. Sharp and R. W. Freedman, J. Am. Ind. Hyg. Assoc., 37 (1976) 1.
572 U. Fiedler, Anal. Chim. Acta, 89 (1977) 101.
573 U. Fiedler, Anal. Chim. Acta, 89 (1977) 111.
574 R. S. Fischer, T. A. Pedley and D. A. Prince, Brain Res., 101 (1976) 223.
575 R. Fleischer and F. Hoffman, Arch. Gartenbau, 24 (1976) 445.
576 A. G. Fogg, A. A. Al-Sibaai and K. S. Yoo, Anal. Lett., 10 (1977) 173.
577 A. G. Fogg and K. S. Yoo, in ref. 4, p. 369.
578 C. W. Francis and C. D. Malone, Proc. Soil. Sci. Soc. Am., 39 (1975) 150.
579 Ch. Fuchs and Ch. McIntosh, Clin. Chem., 23 (1977) 610.
580 M. Fujimoto and T. Kubota, Jpn. J. Physiol., 26 (1976) 631.
581 M. Fujimoto, T. Kubota and K. Kotera, Contrib. Nephrol., 6 (1977) 114.
582 T. Fujinaga, S. Okazaki and H. Hara, Chem. Lett., (1978) 1201.
583 K. Fukamachi and N. Ishibashi, Bunseki Kagaku, 26 (1977) 69.
584 K. Fukamachi and N. Ishibashi, Bunseki Kagaku, 27 (1978) 152.
585 I. Fukui, K. Soyama, M. Maeda, M. Imaki and J. Endo, Kyoto Furitsu Ika Daigaku Zasshi, 87 (1978) 126.
586 K. J. Futamachi and T. A. Pedley, Brain Res., 109 (1976) 311.
587 A. R. Gardner-Medwin and C. Nicholson, J. Physiol., 275 (1977) 66.
588 T. J. Gilligan, E. L. Cussler and D. F. Evans, Biochim. Biophys. Acta, 497 (1977) 627.
589 T. Goina and S. Hobai, Rev. Med. (Tirgu-Mures, Rom.), 23 (1977) 70.
590 T. Goina, S. Hobai and A. Rodeanu, Farmacia (Bucharest), 24 (1976) 89.
591 V. N. Golubev, N. K. Evseeva, I. N. Kremenskaya and S. K. Timofeeva, Elektrokhimiya, 2 (1976) 263.
592 V. N. Golubev and T. A. Filatova, Elektrokhimiya, 7 (1974) 1123.
593 L. Gorton and U. Fiedler, Anal. Chim. Acta, 90 (1977) 233.
594 D. Gozzi and G. Scorcelletti, J. Electroanal. Chem., 93 (1978) 109.
595 A. L. Grekovich, E. A. Materova, G. I. Shumilova and I. Yu. Ivanova, Ionn. Obmen Ionometr. USSR, 1 (1976) 127.
596 U. Gruenke, P. Hartmann and J. Siemroth, Hermsdorfer Tech. Mitt., 17 (1977) 1547. 1547.

597 M. Güggi, M. Oehme, E. Pretsch and W. Simon, Helv. Chim. Acta, 58 (1976) 2417.
598 M. Güggi, Dissertation, ETH Zürich (1977).
599 M. Güggi, E. Pretsch and W. Simon, Anal. Chim. Acta, 91 (1977) 107.
600 B. J. Gupta, M. J. Berridge, T. A. Hall and R. B. Morten, J. Exp. Biol., 72 (1978) 261.
601 T. P. Hadjiioannou and E. P. Diamandis, Anal. Chim. Acta, 94 (1977) 443.
601a T. P. Hadjiioannou, M. A. Koupparis and E. P. Diamandis, Proc. Anal. Div. Chem. Soc., 15 (1978) 78.
602 T. P. Hadjiioannou, M. A. Koupparis and C. E. Efstathiou, Anal. Chim. Acta, 88 (1977) 281.
603 A. C. Hansen, K. Engel, P. Kildeberg and S. Wamberg, Clin. Chim. Acta, 79 (1977) 507.
604 E. H. Hansen, A. K. Ghose and J. Růžička, Analyst, 102 (1977) 705.
605 E. H. Hansen, J. Růžička and A. K. Ghose, Anal. Chim. Acta, 100 (1978) 151.
606 K. Hartman, S. Luteratti, H. F. Osswald, M. Oehme, P. C. Meier, D. Amman and W. Simon, Mikrochim. Acta, 2 (1978) 235.
607 D. F. Hastings, Anal. Biochem., 83 (1978) 416.
608 J. Havas, L. Kecskés, J. Lantos and G. Halmagyi, Orv. Tech., 15 (1977) 97.
609 J. Havas, K. Kecskés and R. Somodi, Hung. Sci. Instrum., 41 (1977) 47.
610 N. Hazemoto, N. Kamo and Y. Kobatake, J. Assoc. Off. Anal. Chem., 57 (1974) 1205.
611 N. Hazemoto, N. Kamo and Y. Kobatake, J. Assoc. Off. Anal. Chem., 59 (1976) 1097.
612 N. Hazemoto, N. Kamo and Y. Kobatake, J. Pharm. Sci., 65 (1976) 435.
613 U. Heinemann and H. D. Lux, Brain Res., 93 (1975) 63.
614 U. Heinemann and H. D. Lux, Brain Res., 120 (1977) 231.
615 U. Heinemann, H. D. Lux and M. J. Gutnick, Exp. Brain Res., 27 (1977) 237.
616 U. Heinemann, H. D. Lux and K. J. Zander, in Ryall and Kelby (Eds.), Ionophoresis and Transmitter Mechanism in Mammalian CNS, Elsevier, Amsterdam, 1978, p. 419.
617 C. B. Heyer and H. D. Lux, J. Physiol., 262 (1976) 349.
618 C. B. Heyer and H. D. Lux, J. Physiol., 262 (1976) 319.
619 H. Hirche, IUPS Satellite Symposium on Theory and Application of Ion-selective Electrodes in Physiology and Medicine, Dortmund, 1977, p. 26.
620 P. Hník, M. Holas, I. Krekule, N. Kříž, J. Mejsnar, V. Smieško, E. Ujec and F. Vyskočil, Pfluegers Arch., 362 (1976) 85.
621 J. H. C. Hoge, H. J. A. Hazenberg and C. H. Gips, Clin. Chim. Acta, 55 (1974) 273.
622 E. Hopirtean, Rev. Roum. Chim., 22 (1977) 1385.
623 E. Hopirtean and F. Kormos, Stud. Univ. Babes-Bolyai, Ser. Chem., 22 (1977) 35.
624 E. Hopirtean, C. Liteanu and F. Kormos, Rev. Chim. (Bucharest), 28 (1977) 378.
625 E. Hopirtean, M. Preda and C. Liteanu, Chem. Anal. (Warsaw), 21 (1976) 861.
626 E. Hopirtean, M. Preda and C. Liteanu, Fresenius Z. Anal. Chem., 286 (1977) 65.
627 E. Hopirtean and E. Stefaniga, Chem. Anal. (Warsaw), 22 (1977) 845.
628 E. Hopirtean and E. Stefaniga, Rev. Roum. Chim., 23 (1978) 137.
629 E. Hopirtean, E. Stefaniga and C. Liteanu, Chem. Anal. (Warsaw), 21 (1976) 867.
630 E. Hopirtean, E. Stefaniga, C. Liteanu and I. Gusan, Rev. Chim. (Bucharest), 27 (1976) 346.
631 E. Hopirtean and E. Veress, Rev. Roum. Chim., 23 (1978) 273.
632 E. Hopirtean, E. Veress and V. Muresan, Rev. Roum. Chim., 22 (1977) 1243.
633 E. Hopirtean, P. Zugravescu and C. Achiri, Rev. Chim. (Bucharest), 27 (1976) 1085.
634 J. Hubáček and K. Bernatzik, Chem. Listy, 70 (1976) 513.
635 A. Hulanicki, M. Maj-Zurawska and R. Lewandowski, Anal. Chim. Acta, 98 (1978) 151.
636 A. Hulanicki, M. Maj-Zurawska and M. Trojanowicz, Zh. Anal. Khim., 32 (1977) 767.

637 A. Hulanicki and M. Trojanowicz, Anal. Chim. Acta, 87 (1976) 411.
638 A. Hulanicki and M. Trojanowicz, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 139.
639 A. M. Y. Jaber, G. J. Moody and J. D. R. Thomas, Analyst, 102 (1977) 943.
640 A. M. Y. Jaber, G. J. Moody and J. D. R. Thomas, J. Inorg, Nucl. Chem., 39 (1977) 1689.
641 A. M. Y. Jaber, G. J. Moody and J. D. R. Thomas, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 411.
642 A. M. Y. Jaber, G. J. Moody and J. D. R. Thomas, Proc. Anal. Div. Chem. Soc., 13 (1976) 328.
643 A. M. Y. Jaber, G. J. Moody and J. D. R. Thomas, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
644 G. Kahr and B. Kissling, Chem. Rundschau, 29 (1976) 1.
645 R. Kálmán, Agrokémia és Talajtan, 25 (1976) 415.
646 Y. Kaneda, T. Kanamori and M. Iwaida, Eisei Kagaku, 23 (1977) 301.
647 C. J. Karwoski and L. M. Prodenza, Brain Res., 142 (1978) 515.
648 M. Kataoka and T. Kambara, J. Electroanal. Chem., 73 (1976) 279.
649 M. Kataoka, M. Kudoh and T. Kambara, Denki Kagaku, 46 (1978) 548.
650 M. Kataoka, M. Shin and T. Kambara, Talanta, 24 (1977) 261.
651 M. Kataoka, M. Tsukamoto and T. Kambara, Denki Kagaku, 45 (1977) 100.
652 L. Keil, G. J. Moody and J. D. R. Thomas, Anal. Chim. Acta, 96 (1978) 171.
653 P. Kent, S. O. Bunce, R. A. Bailley and D. A. Aikens, Anal. Biochem., 62 (1975) 75.
654 L. Keil, G. J. Moody and J. D. R. Thomas, Analyst, 102 (1977) 274.
655 A. S. Kereichuk and N. V. Mokhnatova, Zh. Neorg. Khim., 21 (1976) 1195.
656 M. Kessler, J. Höper and B. A. Krumme, Anesthesiology, 45 (1976) 184.
657 R. P. Kernan and M. Macdermott, J. Physiol., 263 (1976) 158P.
658 R. Kline and M. Morad, Biophys. J., 16 (1976) 319.
659 V. V. Klimaro, L. D. Kovalchuk, A. G. Rodichev and L. A. Barskii, Izv. Vyssh. Ucheb. Zaved., Tsvetn. Met., 5 (1977) 26.
660 Y. S. Kim and G. M. Padilla, Anal. Biochem., 89 (1978) 521.
661 G. Kimura and M. Fujimoto, Jpn. J. Physiol., 27 (1977) 291.
662 K. Kimura, T. Maeda, H. Tamura and T. Shono, J. Electroanal. Chem., 95 (1979) 91.
662a J. Kiszel and J. Havas, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 425.
663 N. N. L. Kirsch, R. J. J. Funck, E. Pretsch and W. Simon, Helv. Chem. Acta 60 (1977) 2326.
664 R. K. Kobos and G. A. Rechnitz, Biochem. Biophys. Res. Commun., 71 (1976) 762.
665 Y. Kobuke, Kagaku, 74 (1978) 133.
666 N. P. Komar and S. I. Kharkvovk, Zh. Fiz. Khim., 51 (1977) 2037.
667 P. O. Kosonen, Finn. Chem. Lett., (1978) 86.
668 M. A. Koupparis and T. P. Hadjiioannou, Anal. Chim. Acta, 94 (1977) 367.
669 M. A. Koupparis and T. P. Hadjiioannou, Anal. Chim. Acta, 96 (1978) 31.
670 M. A. Koupparis and T. P. Hadjiioannou, Microchem. J., 23 (1978) 178.
671 M. A. Koupparis and T. P. Hadjiioannou, Mikrochim. Acta, (1978) 267.
672 M. A. Koupparis and T. P. Hadjiioannou, Talanta, 25 (1978) 477.
673 R. P. Kraig and C. Nicholson, Science, 194 (1976) 725.
674 N. Kříž and E. Syková, Physiol. Bohemoslov., 26 (1977) 263.
675 N. Kříž, E. Syková and L. Vyklický, Bioelectrochem. Bioenerget., 3 (1976) 582.
676 K. Krnjević and M. E. Morris, Can. J. Physiol. Pharmacol., 53 (1975) 912.
677 K. Krnjević and M. E. Morris, Can. J. Physiol. Pharmacol., 53 (1975) 923.
678 S. W. Kuffler, J. G. Nichols and R. K. Orkand, J. Neurophysiol., 29 (1976) 768.
679 D. L. Kunze, Circ. Res., 41 (1977) 122.
680 N. Lakshminarayanaiah and C. P. Bianchi, in T. Narahashi and C. P. Bianchi (Eds.)

Advances in General and Cellular Pharmacology, Vol. 2, Plenum, New York, 1977.
681 L. A. Lazarou and T. P. Hadjiioannou, Anal. Lett., 11 (1978) 779.
682 L. A. Lazarou and T. P. Hadjiioannou, Anal. Chim. Acta, 100 (1978) 207.
683 T. J. Lea, Nature, 269 (1977) 108.
684 O. H. Le Blanc, J. F. Brown, J. F. Klebe, L. W. Niedrach, G. M. J. Slusarczuk and W. H. Stoddard, J. Appl. Physiol., 40 (1976) 644.
685 O. H. Le Blanc and W. T. Grubb, Anal. Chem., 48 (1976) 1658.
686 C. E. Leme and H. B. Silva, Clin. Chim. Acta, 77 (1977) 287.
687 P. K. Leontevskaya, A. A. Pendin, M. A. Trofimov and I. M. Shults, Vzaimodeistviya Rastvorakh Okislitelno-Vosstanovit. Sistem, (1977) 174.
688 C. Liteanu, E. Hopirtean and E. Stefaniga, Rev. Roum. Chim., 22 (1977) 653.
689 E. H. Lothman and G. G. Somjen, J. Physiol., 252 (1975) 115.
690 J. A. Lustgarten, R. E. Wenk, C. Byrd and B. Hall, Clin. Chem., 20 (1974) 1217.
691 J. B. Macaskill, M. S. Mohan and R. G. Bates, Anal. Chem., 49 (1977) 209.
692 J. Machek, E. Ujec and V. Pavlík, Neurosci. Lett., 2 (1976) 147.
693 H. Maier, A. Röckel, A. Heidland, D. Schneider, Ch. Steffen, O. Aziz, R. Dennhardt, H.-O. Lindt and J. G. Schindler, Res. Exp. Med., 172 (1978) 75.
694 E. A. Mangubat, T. R. Hinds and F. F. Vincenzi, Clin. Chem., 24 (1978) 635.
695 P. Mareš, N. Kříž, G. Brožek and J. Bureš, Exp. Neurol., 53 (1976) 12.
696 H. J. Marsoner and K. Harnoncourt, Aerztl. Lab., 23 (1977) 327.
697 E. A. Materova, Z. C. Alagova and V. P. Zhesko, Elektrokhimiya, 10 (1974) 1568.
698 E. A. Materova and N. V. Garbuzova, Elektrokhimiya, 13 (1977) 1846.
699 E. A. Materova, A. L. Grekovich, Z. S. Alagova and V. V. Mukhovikov, Ionn. Obmen Ionometr. USSR, 1 (1976) 144.
700 E. A. Materova, A. L. Grekovich and N. N. Garbuzova, Ionn. Obmen Ionometr. USSR, 1 (1976) 137.
701 E. A. Materova and S. A. Ovchinnikova, Zh. Anal. Khim., 32 (1977) 331.
702 E. A. Materova, S. A. Ovchinnikova and S. A. Smekalova, Elektrokhimiya, 14 (1978) 71.
703 E. A. Materova, Z. Salagova, A. L. Grekovich, G. I. Shumilova and V. B. Keyer, Elektrokhimiya, 12 (1976) 1860.
704 A. Matsubara and K. Nomura, Mem. Fac. Sci. Kyushu Univ., Sec. C, 10 (1976) 37.
705 I. Melichar and J. Syka, Pflueger's Arch., 372 (1977) 207.
706 H. Melnikovová, Physiol. Bohemoslov., 27 (1978) 131.
707 C. Mergey and J. M. Bonnoit, Analusis, 6 (1978) 164.
708 M. Meyerhoff and G. A. Rechnitz, Science, 195 (1977) 494.
708a V. A. Mikhailov, V. V. Osipov and N. V. Serebrennikova, Zh. Anal. Khim., 33 (1978) 1154.
709 D. S. Miura, B. F. Hoffman and M. R. Rosen, J. Gen. Physiol., 69 (1977) 463.
710 D. S. Miura and M. R. Rosen, Circ. Res., 42 (1978) 333.
711 G. J. Moody, N. S. Nassory and J. D. R. Thomas, Analyst, 103 (1978) 68.
712 G. J. Moody, N. S. Nassory and J. D. R. Thomas, Hung. Sci. Instrum., 41 (1977) 23.
713 W. E. Morf, D. Ammann, R. Bissig, E. Pretsch and W. Simon, in J. J. Christensen and R. M. Izatt (Eds.), Multidentate Macrocyclic Molecules, Wiley—Interscience, New York, 1978.
714 W. E. Morf, M. Oehme and W. Simon, in E. Betz (Ed.), Ionic Actions Vasc. Smooth Muscle Spec. Regard Brain Vessels, 2nd Symp. ETH 1976, Springer, Berlin, 1976, p. 1.
715 W. E. Morf and W. Simon, Hung. Sci. Instrum., 41 (1977) 1.
716 W. E. Morf and W. Simon, in ref. 5, p. 149.
717 S. Mori, W. H. Miller and T. Tomita, Jpn. J. Physiol., 26 (1976) 219.
718 L. N. Moskvin and V. M. Krasnoperov, Zh. Anal. Khim., 32 (1978) 1559.
719 M. Muratsugu, N. Kamo, K. Kirihara and Y. Kobatake, Biochim. Biophys. Acta, 464 (1977) 613.

720 J. E. Newbery and V. Smith, Colloid Polymer Sci., 256 (1978) 494.
721 C. Nicholson and R. P. Kraig, Brain Res., 96 (1975) 384.
722 C. Nicholson, R. Steinberg, H. Stöckle and G. Ten Bruggencate, Neurosci. Lett., 3 (1976) 315.
723 C. Nicholson, G. Ten Bruggencate and R. Senekowitsch, Brain Res., 113 (1976) 606.
724 C. Nicholson, G. Ten Bruggencate, H. Stöckle and R. Steinberg, J. Neurophysiol., 41 (1978) 1026.
725 C. Nicholson, G. Ten Bruggencate, R. Steinberg and H. Stöckle, Proc. Nat. Acad. Sci., 74 (1977) 1287.
726 H. J. Nielsen and E. H. Hansen, Anal. Chim. Acta, 85 (1976) 1.
727 B. P. Nikolskii, E. A. Materova and A. L. Grekovich, Elektrokhimiya, 13 (1977) 740.
728 B. P. Nikolskii, E. A. Materova and A. L. Grekovich, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 171.
729 B. P. Nikolskii, E. A. Materova, C. V. Timofeev and L. K. Arkhangelskii, Elektrokhimiya, 14 (1978) 68.
730 Sh. K. Norov, V. V. Palchevskii and Ye. S. Gureev, Zh. Anal. Khim., 32 (1977) 2394.
731 T. Ookawa and J. Bureš, Brain Res., 97 (1975) 171.
732 M. Oehme, M. Kessler and W. Simon, Chimia, 30 (1976) 204.
733 M. Oehme and W. Simon, Anal. Chim. Acta, 86 (1976) 21.
734 K. Ohzeki and T. Kambara, J. Electroanal. Chem., 88 (1978) 85.
735 J. Olsen and R. F. Miller, J. Neurophysiol., 40 (1977) 752.
736 G. Ouzounian and G. Michard, Anal. Chim. Acta, 96 (1978) 405.
737 S. A. Ovchinnikova, E. A. Materova and V. S. Karavan, Vestn. Leningr. Univ., Fiz. Khim., 2 (1977) 117.
738 J. D. Owen, H. M. Brown and J. P. Pemberton, Anal. Chim. Acta, 90 (1977) 241.
739 L. G. Palmer and M. M. Civan, J. Membrane Biol., 33 (1977) 41.
740 L. G. Palmer and M. M. Civan, Science, 188 (1975) 1321.
741 L. D. Partridge and R. C. Thomas, J. Physiol., 254 (1976) 551.
742 A. Pepe, S. Constantini and C. Desena, Riv. Soc. Ital. Sci. Alim., 3 (1974) 59.
743 S. Prelević, W. M. Burnham and P. Gloar, Brain Res., 105 (1976) 437.
744 J. H. Poulsen, S. W. Bledsoe and L. Bonacquist, Am. J. Physiol., 234 (1978) E79.
745 Ž. Pranjić-Anušić, Z. Cimerman and Z. Štefanac, Acta Pharm. Jugosl., 27 (1977) 55.
746 E. Pretsch, W. E. Morf and W. Simon, Helv. Chim. Acta, 59 (1976) 2407.
747 J. Pysiak and W. Koźlak, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 523.
748 K. Raikai, Agrokem. Talajtan, 25 (1976) 415.
749 D. G. Rands and E. Stoica, J. Am. Water Works Assoc., 68 (1976) 309.
749a V. Prelog, Pure Appl. Chem., 50 (1978) 893.
750 G. A. Rechnitz, G. J. Nogle, M. R. Bellinger and H. Lees, Clin. Chim. Acta, 76 (1977) 295.
751 D. W. Richter, H. Camerer and U. Sonnhof, Pfluegers Arch., 376 (1978) 139.
752 R. Ruiz S. and U. Roldon, Agric. Tec. (Mexico), 38 (1978) 37.
753 J. M. Russel, Biophys. J., 22 (1978) 131.
754 J. Růžička, E. H. Hansen and E. A. Zagatto, Anal. Chim. Acta, 88 (1977) 1.
755 T. H. Ryan and B. Fleet, Abstracts, International Reference and Ion-selective Electrode Conference, Newcastle upon Tyne, 1976.
756 V. S. Savenko, Okeanologiya, 17 (1977) 1123.
757 O. F. Schäfer, Z. Naturforsch., 32A (1977) 789.
758 J. G. Schindler, Biomed. Techn., 20 (1975) 47.
759 J. G. Schindler, H. K. Dürr, W. Riemann, H. E. Braun and V. Keller, Biomed. Techn., 23 (1978) 45.
760 J. G. Schindler, W. Riemann and W. Schäl. Biomed. Techn., 21 (1976) 135.

761 J. G. Schindler and W. Schäl, Biomed. Techn., 21 (1976) 91.
762 J. G. Schindler, R. G. Schindler and O. Aziz, J. Clin. Chem. Clin. Biochem., 16 (1978) 441.
763 J. G. Schindler, G. Stork and H.-J. Strüh, Fresenius Z. Anal. Chem., 292 (1978) 391.
764 J. G. Schindler, G. Stork and H.-J. Strüh, Fresenius Z. Anal. Chem., 292 (1978) 396.
765 J. G. Schindler, G. Stork, H.-J. Strüh and W. Schäl, Fresenius Z. Anal. Chem., 290 (1978) 45.
766 W. Selig, Mikrochem. J., 22 (1977) 1.
767 W. Selig, Fresenius Z. Anal. Chem., 285 (1977) 251.
768 J. Šenkýř and J. Petr, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 559.
769 Yu. V. Shavnya, A. S. Bychkov, O. M. Petruzhin, V. A. Zarinskii, L. V. Bakhtinova and Yu. A. Zolotov, Zh. Anal. Khim., 33 (1978) 1531.
770 Yu. V. Shavnya and Yu. M. Chikin, Electrokhimiya, 14 (1978) 336.
771 H. Shechter and N. Gruener, J. Am. Water Works Assoc., 68 (1976) 543.
772 S. Sherken, J. Assoc. Off. Anal. Chem., 59 (1976) 971.
773 T. Shinto, N. Kamo, K. Kurihara and Y. Kobataka, Arch. Biochem. Biophys., 187 (1978) 414.
774 L. A. Shumilova, A. V. Gordievskii, Yu. A. Klyachko and N. G. Sarishvili, Zh. Anal. Khim., 32 (1977) 2368.
775 W. Simon, D. Ammann, M. Oehme and W. E. Morf, Ann. New York Acad. Sci., 307 (1978) 52.
776 W. Simon, W. E. Morf and A. P. Thomas, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 13.
777 R. B. Skinner and D. L. Kunze, Circ. Res., 39 (1976) 678.
778 G. G. Somjen, M. Rosenthal, G. Cordingley, J. LaManna and E. Lothman, Fed. Proc., 35 (1976) 1266.
779 S. Srianujata, W. R. White, T. Higuchi and L. A. Sternson, Anal. Chem., 50 (1978) 232.
780 G. L. Starobinets, E. M. Rakhmaniko and R. Del Toro Denis, Vest. Akad. Navuk B. SSR, Ser. Khim. Navuk, 4 (1978) 75.
781 O. K. Stefanova and I. V. Rusina, Elekrokhimiya, 14 (1978) 882.
782 C. Steffen, J. G. Schindler and O. Aziz, Biomed. Technik, 21 (1976) 176.
783 H. Stöckle and G. Ten Bruggencate, Exp. Neurol., 61 (1978) 226.
784 T. Stwarzewicz, B. Czapkiewicz-Tutaj and M. Leszko, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 199.
785 K. Sulko, S. Alagova, E. A. Materova and G. I. Shumilova, Zh. Anal. Khim., 32 (1977) 1596.
786 E. Syková, B. Shirayev, N. Kříž and L. Vyklický, Brain Res., 106 (1976) 413.
787 E. Syková and L. Vyklický, Neurosci., 3 (1978) 1061.
788 E. Syková and L. Vyklický, Neurosci. Lett., 4 (1977) 161.
789 É. Szepezváry, E. Pungor and P. Szepesváry, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 225.
790 O. G. Takaishvili, E. P. Motsonelidze, Zh. N. Fidler, Yu. M. Karachentseva and P. I. Davitaya, Zh. Anal. Khim., 32 (1977) 727.
791 G. Ten Bruggencate, C. Nicholson and H. Stöckle, Pfluegers Arch., 367 (1976) 107.
792 G. Ten Bruggencate, R. Steiberg, H. Stöckle and C. Nicholson, in Ryall and Kelly (Eds.), Ionophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System, Elsevier, Amsterdam, 1978, p. 412.
793 A. P. Thoma and W. Simon, in B. Pullman and N. Goldblum (Eds.), Metal-Ligand Interactions in Organic Chemistry and Biochemistry, Part 2, 2. 37, Reidel, Dordrecht, 1977.
794 R. C.Thomas, W. Simon and M. Oehme, Nature, 258 (1975) 754.
795 S. V. Timofeev, E. A. Materova, B. P. Nikolskii and L. K. Arkhangelskii, Vest. Leningrad, Univ., Fiz. Khim., (1978) 135.

796 T. Treasure and D. M. Band, J. Med. Eng. Technol., 1 (1977) 271.
797 T. Treasure and D. M. Band, Aroc. Anal. Div. Chem. Soc., 14 (1977) 334.
798 H. Y. Tu, C. Li and P. L. Chu, Hua Hsueh Tung Pao, 3 (1978) 150.
799 O. H. Tuovinen and D. J. D. Nicholas, Appl. Environ. Microbiol., 33 (1977) 477.
800 M. Ueda and J. Bureš, Electroencephal. Clin. Neurophysiol., 43 (1977) 666.
801 M. L. Verkhovskaya, E. S. Imasheva, G. A. Kurella and L. G. Yaglova, Biol. Nauki (Moscow), 19 (1976) 142.
802 D. M. Victor and D. F. Martin, J. Environ. Sci. Health, A12 (1977) 367.
803 L. N. Voroblev, Prib. Metody Mikroelektrodnogo Issled. Kletok, (1975) 171.
804 L. Vyklický, E. Syková and B. Mellerová, Brain Res., 117 (1976) 153.
805 K. Vytřas, Collect. Czech. Chem. Commun., 42 (1977) 3168.
806 K. Vytřas and V. Říha, Ceskoslov. Farm., 26 (1977) 9.
807 K. Vytřas, V. Říha and S. Katzký, Sb. Věd. Prací Vys. Šk. Chemickotechnol., Pardubice, 35 (1976) 41.
808 D. B. West, J. Assoc. Off. Anal. Chem., 59 (1976) 687.
809 J. O. Westgard, Clin. Chem., 22 (1976) 489.
810 J. F. White, Am. J. Physiol., 231 (1976) 1214.
811 J. F. White, Am. J. Physiol. 232 (1977) E553.
812 T. J. Wisk and K. J. Siebert, Am. Soc. Brew. Chem. Aroc., 1973 (1974) 63.
813 B. Wong and C. D. Woody, Exp. Neurol., 61 (1978) 219.
814 J. A. Wright and P. L. Bailey, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, Conference, Budapest 1977, Akademia Kiadó, Budapest, 1978, p. 603.
815 H. R. Wuhrmann, Biomed. Tech. (Stuttgart), 21 (1976) 191.
816 D. R. Wybenga, F. A. Ibbott and D. C. Cannon, Clin. Chem., 22 (1976) 1009.
817 K. Y. Yu. and P. M. Berthouex, J. Water Pollut. Control Fed., 49 (1977) 1896.
818 V. A. Zarinskii, O. M. Petrukhin, A. S. Bychkov, L. K. Shpigun and Yu. A. Zolotov, in E. Pungor and I. Buzás (Eds.), Ion-selective Electrodes, 2nd Symposium 1976, Akademia Kiadó, Budapest, 1977, p. 245.
819 S. Zadecky, D. Kuttel, J. Havas and L. Kecskés, Acta Pharm. Hung., 48 (1978) 131.
820 V. A. Zarinskii, L. K. Shpigun, V. M. Trepalina and I. V. Volobueva, Zavod. Lab., 43 (1977) 941.
821 V. A. Zarinskii, L. K. Shpigun, O. M. Petrukhin and V. M. Trepalina, Zh. Anal. Khim., 31 (1976) 1191.
822 T. Zeuthen, J. Membr. Biol., 39 (1978) 185.
823 K. Cammann, Fresenius Z. Anal. Chem., 287 (1977) 1.
824 P. W. Carr, Anal. Chem., 49 (1977) 799.
825 P. D'Orazio, M. E. Meyerhoff and G. A. Rechnitz, Anal. Chem., 50 (1978) 1531.
825a G. G. Guilbault, Biomed. Appl. Immobil. Enzym. Proteins, 2 (1977) 163.
826 G. G. Guilbault, Compr. Anal. Chem., 8 (1977) 1.
827 G. G. Guilbault, Methods Enzymol., 44 (1976) 579.
828 C. P. Hsiung, S. S. Kuan and G. G. Guilbault, Anal. Chim. Acta, 90 (1977) 45.
829 R. K. Kobos and G. A. Rechnitz, Anal. Lett., 10 (1977) 751.
830 R. Kobos and G. Rechnitz, Arch. Biochem. Biophys., 175 (1976) 11.
831 R. Kobos and G. Rechnitz, Biochem. Biophys. Res. Commun., 71 (1976) 762.
832 M. Nanjyo, Denki Kagaku Oyobi Kogyo Butsuri Kagaku, 44 (1976) 694.
833 M. Nanjyo, Tohoku Daigaku Senko Seiren Kenkyusho Iho, 32 (1976) 127.
834 T. Ngo and P. Shargool, Anal. Biochem., 54 (1973) 247.
835 G. A. Rechnitz, T. L. Riechel, R. K. Kobos and M. E. Meyerhoff, Science, 199 (1977) 367.
836 G. A. Rechnitz, T. L. Riechel, R. K. Kobos and M. E. Meyerhoff, Science, 199 (1978) 440.
837 F. Scheller and D. Pfeiffer, Z. Chem., 18 (1978) 50.
838 J. G. Schindler, R. G. Schindler and O. Aziz, J. Clin. Chem. Clin. Biochem., 16 (1978) 447.
839 M. C. Tran, R. Guyonet and J. Beaux, C. R. Acad. Sci. Paris, Ser. C, 286 (1978) 115.

Analytica Chimica Acta, 111 (1979) 57—70

DEVELOPMENT OF POLYMERIC MEMBRANES FOR ZINC ION-SELECTIVE ELECTRODES

ULLA FIEDLER-LINNERSUND* and KHAN M. BHATTI**

Department of Analytical Chemistry, University of Lund, P.O. Box 740, S 220 07 Lund (Sweden)

(Received 8th June 1979)

SUMMARY

Several polymeric membranes for zinc ion-selective electrodes have been investigated. By optimizing the choice of solvent mediator and ligand, selectivity for zinc ions can be obtained. The applicability of a theory proposing membrane selectivities as a result of both solvent and site properties is demonstrated. The concept of solubility parameters is used in discussing the detection limits obtained. The best electrode found is based on a PVC membrane containing the zinc salt of di(2-ethylhexyl)phosphoric acid dissolved in tri(2-ethylhexyl)phosphate. It is the first ion-selective electrode which responds primarily to zinc. The sensor, which has a lifetime of at least two months, is characterized by a rapid response, potential stability and good sensitivity caused by a super-Nernstian slope (43.8 mV/p Zn); the detection limit is 4.5 ± 0.1 pZn.

Potentiometric methods for determinations of zinc ions can be classified as direct and indirect methods, which may be further subdivided into zinc-sensitive and zinc-selective methods. Indirect selective methods have been reported, based on electrodes which measure zinc(II) as tetrathiocyanatozincate [1] or as tetrachlorozincate [2]. Recently, a direct zinc-sensitive method based on a polymeric membrane electrode was described [3]; with certain precautions, i.e. after mixing the sample with a calcium-precipitating buffer, the electrode could be made selective for zinc. However, all methods involving dilution of the original sample may change the equilibria between zinc ions and different zinc-containing complexes present. The aim of the work reported here was therefore to develop an electrode applicable for the direct measurement of ionized zinc in a sample.

In the search for a zinc ion-selective electrode, the choice of membrane constituents was guided by the observation that zinc(II) interferes strongly with calcium-selective electrodes based on organic phosphate complexes. This was first pointed out by Ross [4], when describing the new Orion calcium liquid-membrane electrode [5] based on calcium didecylphosphate dissolved in dioctylphenylphosphonate. A K_{CaZn} value of 3.2 was reported.

*Present address: AB DRACO, Fack, S 221 01 Lund, Sweden.
**Permanent address: Pakistan Council of Scientific and Industrial Research Laboratories, Off University Road, Karachi-39, Pakistan.

In later studies of PVC-matrix membranes containing this liquid ion exchanger [6], K_{CaZn} was found to be less than one over a wide activity range. When Cattrall and Freiser [7] reported a coated wire electrode based on the same exchanger, they found a K_{CaZn} value of 32.3 for their new construction compared to 1.44 for the Orion construction. Ansaldi and Epstein [8], however, who used yet another construction, in which the membrane contacted graphite directly, found selectivity coefficients less than one (0.27 and 0.29, respectively) for both their new construction and for the Orion electrode. Some of these discrepancies are partly due to the use of different methods for the evaluation of selectivity coefficients (mixed and separate solution method, different concentration ranges, etc). However, the zinc interference was clearly manifested in the response behaviour of the electrodes, which required prolonged contact with Ca^{2+} to recover from contact with Zn^{2+} [6]. Radiotracer studies [9] indicated that the ion-exchanging sites may have been blocked. This was in agreement with the results of Fleet et al. [10], who found an intermediate selectivity coefficient versus Zn^{2+} but a measurable increase in response time for Ca^{2+} when Zn^{2+} was present. They ascribed this to the kinetics of the interfering ion reaction, which they regarded as rate-limiting.

The modified calcium-selective ligand developed by Růžička et al. [11] was subject to smaller interference from zinc ions. A selectivity coefficient of 0.060 was reported. When the zinc salt of the ligand was used [3], an even higher preference for Ca^{2+} was obtained ($K_{ZnCa} = 1.6 \times 10^3$). This was also observed by Jagner and Østergaard-Jensen [12] who reported that membranes containing the zinc salt gave irreproducible results in the presence of calcium.

Cattrall et al. [13, 14] made an extensive investigation of some different alkylphosphoric acid esters dissolved in a phosphonate solvent for use in coated-wire calcium-selective electrodes. Many of the membranes prepared suffered from severe zinc interference, i.e. they might be useful for making zinc-selective electrodes.

The important role of the solvent mediator was demonstrated by Moody et al. [15] when investigating calcium-selective electrodes based on calcium bis[di(*p*-1,1,3,3-tetramethylbutylphenyl)phosphate] sensor and different trialkyl phosphates as mediators. It is noteworthy that for membranes containing tri(n-octyl)phosphate the selectivity coefficient versus zinc could not be determined, because the calibration graph in the presence of a fixed level of interferent (Zn^{2+}) had changed its original position. This can be ascribed to a very strong effect on the membrane composition, presumably caused by entrance of Zn^{2+} ions.

The purpose of this work was therefore to investigate the influence of different ligands as well as membrane solvents on the zinc selectivity of polymeric membrane electrodes. After an initial evaluation of solvent mediators like decanol, phosphonates and trialkyl phosphates, a more detailed study was made on a number of commercially available alkylphosphoric acid ligands.

THEORY

Polymeric membranes for use in ion-selective electrodes contain three components: ligand, solvent and polymer. There are certain rules for the choice of a successful combination of these constituents [16]. In the absence of ligand, metal ions are distributed between the aqueous sample solution and the organic membrane phase. The partition reaction involves two equilibria: ion pair formation in the aqueous phase and phase distribution of IX:

$$I^+(aq) + X^-(aq) \overset{K_F}{\rightleftharpoons} I^+X^-(aq) \; ; \; I^+X^-(aq) \overset{K_D}{\rightleftharpoons} I^+X^-(org)$$

I^+ denotes the primary metal ion and X^- an anion with preference for the aqueous phase. Combining the equilibrium expressions of these reactions gives the partition coefficient K_P:

$$K_P = K_F K_D = a_{I^+X^-(org)}/a_{I^+(aq)}a_{X^-(aq)} \tag{1}$$

The membrane selectivity between the primary ion I^+ and an interfering ion J^+ is described by the reaction

$$I^+X^-(org) + J^+(aq) \overset{K^x_{ij}}{\rightleftharpoons} J^+X^-(org) + I^+(aq)$$

The equilibrium constant determines the selectivity coefficient K^x_{ij}:

$$K^x_{ij} = K^j_p/K^i_p \tag{2}$$

where K^i_p and K^j_p are the partition coefficients for ions i and j, respectively.

With a charged ligand S^- in the membrane phase a competitive reaction takes place:

$$I^+X^-(org) + S^-(org) \overset{K^i_{ex}}{\rightleftharpoons} I^+S^-(org) + X^-(org)$$

where the equilibrium constant of the exchange reaction is described by

$$K^i_{ex} = a_{I^+S^-(org)}a_{X^-(org)}/a_{I^+X^-(org)}a_{S^-(org)} \tag{3}$$

When interfering ions J^+ are present in the sample solution, the selectivity coefficient K^s_{ij} is equivalent to the equilibrium constant of the reaction

$$I^+S^-(org) + J^+(ag) \overset{K^s_{ij}}{\rightleftharpoons} J^+S^-(org) + I^+(aq)$$

$$K^s_{ij} = K^j_p K^j_{ex}/K^i_p K^i_{ex} = K^x_{ij} \cdot K^j_{ex}/K^i_{ex} \tag{4}$$

K^s_{ij} is thus dependent both on the solvent and site properties of the membrane. A high selectivity for the primary ion is therefore obtained if one or both of the following requirements is fulfilled. First, the partition coefficient for the primary ion is much higher than for other interfering ions. This can be investigated by measuring selectivity coefficients for membranes without ligand (K^x_{ij}). Second, the exchange equilibrium constant (complex constant) is much higher for the primary ion than for other interfering ions. The relation between the complex constants can be calculated from selectivity data on membranes with (K^s_{ij}) and without (K^x_{ij}) ligand.

Furthermore, the membrane solvent should have low solubility in water and a low vapour pressure. It must also be able to dissolve the ligand and act as a plasticizer for the polymer, making the membrane phase a highly viscous solution. The polymer chosen is most often PVC, although other polymers like polyurethane could be used.

The mutual compatibility of the three membrane components is thus an obvious requirement in the development of polymeric membranes. Moreover, the electrode stability is influenced by the dissolution rate of the ligand in the aqueous sample solution. This solubility is closely related to the distribution ratio q of the ligand between membrane and aqueous phase, and the electrode stability will improve with an increase in q. Additionally, a high q value should lead to a decrease of the limit of detection and therefore to an extension of the dynamic measuring range.

EXPERIMENTAL

Electrode systems

The sensor systems investigated were based on polymeric membranes of the type described by Moody et al. [6]. The membrane composition (by weight) was 30% polymer, 64% solvent and 6% ligand. At a later stage, some measurements were also made on membranes with twice the content of ligand.

The chemicals listed in Table 1 were tested; all were of analytical-reagent grade. Ligands 9—12, denoted by product numbers, belong to a series of Emphos organic phosphate esters (Witco Chemical, New York) based on ethoxylated linear alcohols of the general formula $(RO(CH_2CH_2O)_n)_2PO_2H$.

After an initial evaluation of the acid ligands the most interesting ones (from the viewpoint of zinc(II) ion measurements) were converted to the corresponding zinc salts. This was done by equilibrating 100 ml of a methanolic solution containing 1.0 mmol of the acid ligand (7 or 8) with the stoichiometric amount (0.5 mmol) of an aqueous solution of zinc nitrate (50 ml 0.010 M $Zn(NO_3)_2$) for about 3 h. The white precipitate obtained was filtered, washed and dried.

TABLE 1

Chemicals tested for the preparation of zinc-selective electrodes

Polymer	1. Polyvinylchloride	Ligands	1. Dimethylphosphoric acid
Solvents	1. n-Decanol		2. Diethylphosphoric acid
	2. Di(n-butyl)butylphosphonate		3. Di(iso-amyl)phosphoric acid
	3. Di(n-amyl)amylphosphonate		4. Diphenylphosphoric acid
	4. Di(n-octyl)phenylphosphonate		5. Di(4-nitrophenyl)phosphoric acid
	5. Tri(n-octyl)phosphate		6. Dibenzylphosphoric acid
	6. Tri(2-ethylhexyl)phosphate		7. Di(2-ethylhexyl)phosphoric acid
			8. Di(octylphenyl)phosphoric acid
			9. PS 121
			10. PS 220
			11. PS 400
			12. PS 810

The master membranes prepared are denoted in the subsequent text by three-figure numbers, composed of the number of the polymer, the solvent and the ligand as given in Table 1. Electrode membranes were cut and incorporated into Philips IS-560 electrode bodies (N.V. Philips Gloeilampenfabrieken, Eindhoven, Holland).

Measuring system

The potential measurements were done with a multi-channel amplifier provided with a digital output and accurate to within ±0.1 mV. Saturated calomel electrodes were used as reference electrodes. The cell studied was

$Hg/Hg_2Cl_2/KCl$ (sat.)//sample solution/membrane/inner reference solution/

AgCl/Ag

In order to study the influence of the inner reference solution activity of Zn^{2+} and Cl^- separately, this solution was prepared to contain $Zn(NO_3)_2$ and KCl at various concentrations (usually 0.10 M of each).

The sample solutions were stirred and thermostated at 25.0 ± 0.1°C. The water used had been purified with a Millipore-Q system and all chemicals used for salt solutions were of the highest purity available.

Calibrations

Manual method. All the sample solutions were prepared at constant pH and constant ionic strength by adding an acetate buffer (0.01 M acetic acid—0.01 M potassium acetate; μ = 0.01; pH 4.8). Calibrations were done by repeatedly adding to 20 ml of buffer solution small volumes of zinc nitrate solutions of increasing concentrations. As activity standards the values proposed by Bates were used, i.e.

$$\log \gamma_{Zn^{2+}} = [-2.04\,\mu^{\frac{1}{2}}/(1 + 1.55\,\mu^{\frac{1}{2}})] + 0.2\,\mu \qquad (5)$$

All slopes were calculated by regression analysis on the linear part of the calibration curve. The detection limit (expressed as pZn) was defined as the intersection point between the calibration line and the horizontal response line obtained in the buffer.

Automatic method. The technique described by Horvai et al. [19] was adopted. The ion-selective electrode was immersed in a stirred solution in a flow-through cell of constant volume (20 ml). The initial solution contained zinc nitrate (0.01 M) in a buffer of constant ionic strength (0.01 M acetic acid—0.01 M potassium acetate). This buffer was then also used to wash out the metal ions by passing it through the cell at constant flow rate (ca. 7 ml min^{-1}) with a peristaltic pump (ISMATEC mp-ge).

The concentration, c, of Zn^{2+} ions in the solution is described by $c = c_0 \exp(-Wt/V)$, where c_0 is the initial concentration, W the flow rate, t the time elapsed from the beginning of the wash-out period and V the constant volume of the flow-through cell. The response of the ion-selective electrode can thus be expressed by

$$E = E_0 + S \log \gamma_{Zn^{2+}} c_{Zn^{2+}} = \text{const} - S\,Wt \log e/V \qquad (6)$$

i.e. E is a linear function of t. The electrode slope S can be evaluated from the recorder response line.

Selectivities

Selectivity coefficients were determined by the separate solutions technique on aqueous 0.10 M solutions of the metal ions, from the relationship

$$pK_{ZnM} = -\log K_{ZnM} = [(E_{Zn^{2+}} - E_{MZ^{+}})\, 2F/2.303\, RT] - \log (a_{Zn^{2+}}) + \log (a_{MZ^{+}})^{2/Z} \quad (7)$$

where $E_{Zn^{2+}}$ is the potential of the cell assembly for a 0.10 M Zn^{2+} solution, and $E_{MZ^{+}}$ is the potential with a 0.10 M solution of the interfering cation; all other symbols have their accepted meanings.

The best membrane composition was further investigated with respect to pH dependence by using the mixed solution technique with a fixed level of primary ion.

RESULTS AND DISCUSSION

Choice of membrane solvent

Polymeric membranes were prepared from PVC (polymer 1) and six different solvents (solvents 1—6) but without ligand (ligand 0). The membranes were investigated with respect to selectivities. The pK values obtained were plotted as a function of ionic radius for the alkali metal ions, alkaline earth metal ions and heavy metal ions (Fig. 1).

As discussed above, the selectivity coefficient for "empty" membranes, i.e. without a ligand, is a measure of the partition coefficient for the primary ions compared to that for the interfering ions in the presence of anions X^- which have a preference for the aqueous phase. It is evident that the use of decanol (membrane 1.1.0) produces a "water hardness" (calcium, magnesium or bivalent ion) electrode, while di(n-octyl)phenylphosphonate (membrane 1.4.0) is suitable for making a calcium-selective electrode [20]. The other phosphonate solvents tested (membranes 1.2.0 and 1.3.0) do not show any pronounced bivalent ion selectivity but Pb^{2+} ions are a serious interference. Both trialkyl phosphate solvents (membranes 1.5.0 and 1.6.0) would be suitable for producing zinc-selective electrodes, membrane 1.6.0 being superior. The only ions interfering with this "empty" membrane are lithium(I), which is in accordance with the known properties of this compound, since tri(2-ethylhexyl)phosphate is a suitable solvent mediator for neutral-carrier lithium-selective ligands [21].

Choice of ligand

Polymeric membranes were prepared from PVC, tri(2-ethylhexyl)phosphate (solvent 6) and twelve different ligands, all commercially available organophosphoric acids. First, the influence of the composition of the inner reference solution was studied. As the membrane needs to be conditioned on both

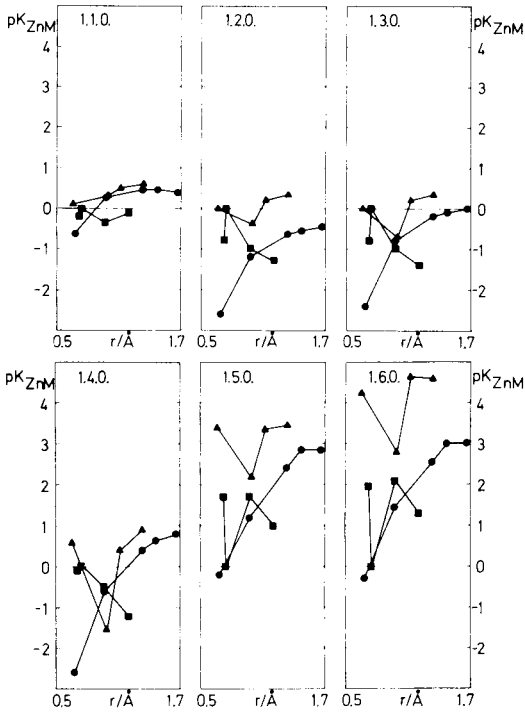


Fig. 1. Selectivities vs. ionic radii for polymeric membranes based on PVC (1), containing different solvents (1—6) but without a ligand (0). (•) Alkali metal ions: Li^+, Na^+, K^+, Rb^+ and Cs^+, respectively. (▲) Alkaline earth metal ions: Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}, respectively. (■) Heavy metal ions: Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}, respectively.

sides, a stable potential was rapidly reached when the Zn^{2+} content of the inner solution was high. Furthermore, the potential of the Ag/AgCl electrode is stabilized by a high content of chloride. Thus, all experiments were done with 0.10 M zinc nitrate and 0.10 M KCl as the inner reference solution, yielding a theoretical E_0 value of 92.6 mV vs. SCE.

After the membranes had been mounted in electrode bodies, the "fresh" electrodes were investigated by calibration and determination of the selectivity coefficients. The selectivity patterns, expressed as pK vs. ionic radius, are shown for all the membranes in Fig. 2. Clearly, the selectivities (K^x_{ij}) obtained for the "empty" membrane (1.6.0; Fig. 1) are changed (to K^s_{ij}) by the ligand tested. The most pronounced effect, although negative from the viewpoint of Zn^{2+} measurements, is obtained with ligands 8—12.

The pure effect of ligand complexation, expressed as log (K^i_{ex}/K^j_{ex}), is obtained by subtracting from the pK^s_{ij} values determined for the ligand-containing membrane, the corresponding pK^x_{ij} value determined for the "empty" membrane. Figure 3 shows the results obtained for ligands 7 and 8 dissolved in solvent 6 (cf. Fig. 2) and in solvent 4. Membrane 1.4.8 is, in fact,

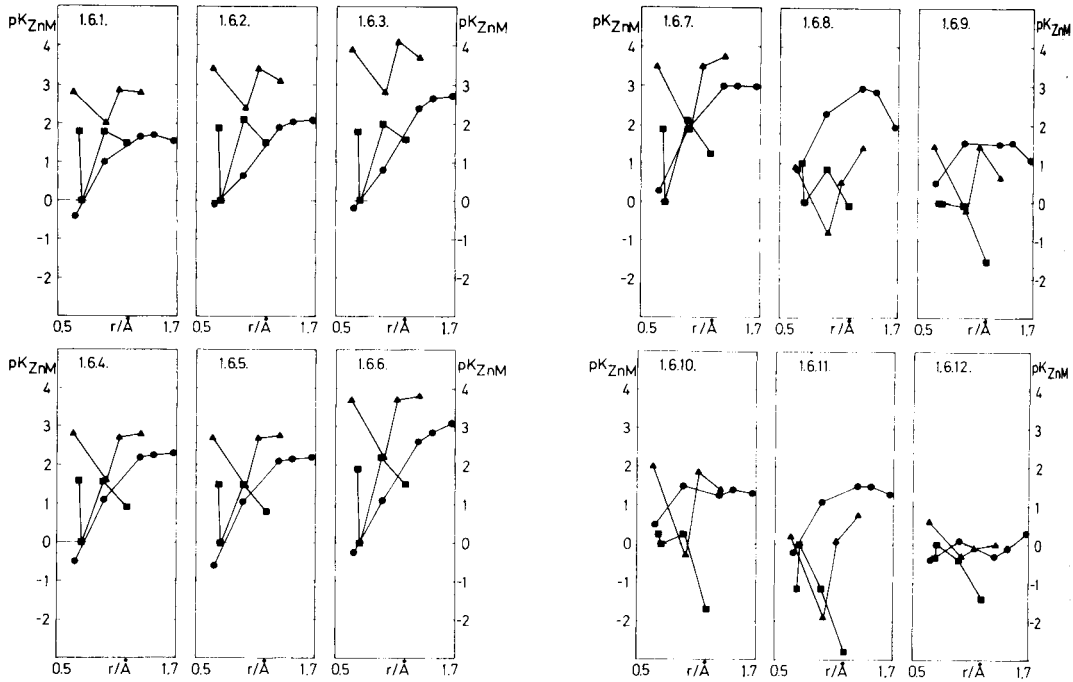


Fig. 2. Selectivities vs. ionic radii for polymeric membranes, based on PVC (1), tri(2-ethylhexyl)phosphate as a solvent mediator (6) and containing different ligands (1—12). For symbols, see legend to Fig. 1.

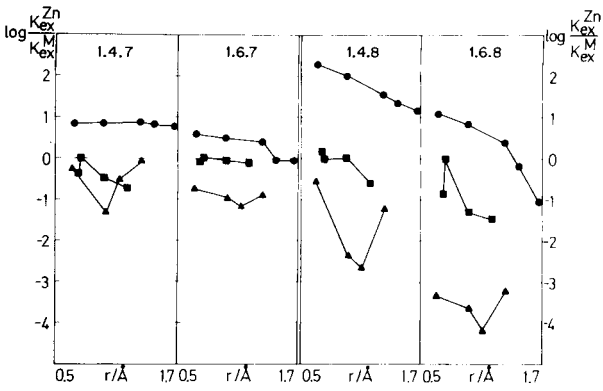


Fig. 3. Ligand complexation (expressed as log K_{ex}^{Zn}/K_{ex}^{M}) for di(2-ethylhexyl)phosphoric acid (ligand 7) and di(octylphenyl)phosphoric acid (ligand 8) in two different solvents, di(n-octyl)phenylphosphonate (4) and tri(2-ethylhexyl)phosphate (6). For symbols, see legend to Fig. 1.

the calcium-selective membrane reported by Růžička et al. [11]. Although a particular ligand would not be expected to have the same complexation constants in different media, the relation between them ($\log K^{i}_{ex}/K^{j}_{ex}$) seems to be practically unchanged in the solvents tested. This is clearly demonstrated by ligand 7. Ligand 8, however, shows greater differences although the selectivity sequence is the same in both solvents. It is further evident that both ligands prefer Ca^{2+} and/or Sr^{2+} ions. Thus, by choosing the appropriate solvent, the ligand effect can be either enhanced (as in membrane 1.4.8) or depressed (as in membrane 1.6.7).

Table 2 summarizes the results from calibration, i.e. the slope of the membranes and the E_0 value of the electrodes obtained by extrapolation from pZn 2 using the actual slope. It also includes some data on the ion-exchange selectivity, i.e. the ratio between the ligand complexation constants for zinc and sodium or calcium (obtained from Figs. 1 and 2).

In selecting the most appropriate ligand for a zinc-selective electrode, the following aspects should be considered: ligand complexation constants, membrane selectivities, detection limit, E_0 value, slope, and stability of the electrode potential.

Ligand complexation constants. The ligand should be selective for Zn^{2+} compared with Na^+ or Ca^{2+} if the electrode is to have bioanalytical applications. This implies that $\log(K^{Zn}_{ex}/K^{Na}_{ex})$ and $\log(K^{Zn}_{ex}/K^{Ca}_{ex})$ should be positive. The most advantageous ligands for suppressing interferences from Na^+ and Ca^{2+} are thus 3 or 7 (see Table 2), while ligands 8—12 all strongly favour Ca^{2+} ions.

Membrane selectivities. The combined effect of ligand and solvent selectivities determines the membrane selectivities. If the electrode is supposed to be "truly" zinc-selective, only membrane 1.6.7 (see Fig. 2) fulfils this requirement.

TABLE 2

Results for tri(2-ethylhexyl)phosphate-based PVC membranes

Membrane	$\log(K^{Zn}_{ex}/K^{Na}_{ex})$	$\log(K^{Zn}_{ex}/K^{Ca}_{ex})$	Slope (mV/pZn)		E_0 value (mV vs. SCE)
			Manual	Automatic	
1.6.0	—	—	49.0	52.0	—
1.6.1	−0.5	−0.8	43.0	38.0	+126.0
1.6.2	−0.8	−0.4	39.4	42.0	+118.5
1.6.3	−0.6	0	56.8	60.5	+113.4
1.6.4	−0.3	−1.2	58.8	59.0	+126.4
1.6.5	−0.4	−1.3	49.6	47.5	+117.6
1.6.6	−0.4	−0.6	52.1	55.5	+127.6
1.6.7	+0.5	−1.0	35.2	43.9	+105.4
1.6.8	+0.9	−3.6	31.4	29.8	+116.4
1.6.9	+0.1	−3.0	25.3	20.0	+88.1
1.6.10	0	−3.2	30.0	26.3	+100.2
1.6.11	−0.4	−4.7	30.7	29.2	+74.0
1.6.12	−1.3	−3.1	32.2	32.9	+95.2

Detection limit of electrode. Three main contributions must be considered: (a) the solubility and the dissolution rate of the zinc—ligand complex from the membrane into the aqueous phase; (b) interferences from ions in the buffer solution (i.e., K^+); and (c) traces of zinc ions in the water used. This third point was immediately eliminated when it was established that the total zinc content of the buffer solution used was less than 10^{-6} M.

As to the first point, it has been concluded [17, 18] that the detection limit of membrane electrodes is intimately connected with the solubility parameters of the membrane material and the ligand. It can be shown that for the distribution ratio q of the ligand

$$\ln q = [V_l V_m/RT][(d_l - d_{aq})^2 - (d_l - d_m)^2] \quad (8)$$

where V_l and V_m are volume fractions, and d_l and d_m are the solubility parameters of ligand and membrane material, respectively; d_{aq} is the solubility parameter of water. The solubility parameter of the "empty" membrane was calculated from $d_m = w_p d_p + w_s d_s$, where w refers to percentage by weight and d_p and d_s are the solubility parameters of the polymer and solvent, respectively. Solubility parameters for the ligands tested (except for 9—12, where the formulae were unknown) were calculated by the method of Small [22]. The results are summarized in Table 3, which also indicates the detection limits found experimentally. In order to optimize the distribution ratio q of the ligand, d_l should be as low as possible but still almost equal to d_m. Thus the choice of ligand 7 or 8 should favour low detection limits. The experimental detection limits obtained for ligands 9—12 indicate a close similarity between d_l and d_m for these ligands.

With regard to possible effects from ions in the buffer the use of constant

TABLE 3

Calculated solubility parameters and experimental detection limits for the ligands tested

Ligand	Solubility parameters (cal $cm^{-3})^{1/2}$		Detection limit (pZn)	
	$d_l(d_m)$	$d_m - d_l$	Manual	Automatic
0	9.05	0	3.8	3.5
1	8.00	1.05	3.4	3.6
2	8.27	0.78	3.7	3.8
3	8.50	0.55	3.7	3.4
4	8.20	0.85	3.6	3.5
5	8.16	0.89	3.5	3.6
6	7.78	1.27	3.7	3.3
7	8.76	0.29	4.3	4.0
8	8.83	0.22	4.4	4.8
9	—	—	4.6	5.3
10	—	—	4.0	4.6
11	—	—	4.8	4.7
12	—	—	3.9	4.1

ionic strength buffers in calibrating electrodes is essential for stable potential readings. However, it is important to minimize the interference by choosing moderate concentrations of weakly interfering ions. For membranes 1.6.1—1.6.8 the value of pK_{ZnK} is about 2, i.e. a 10^{-2} M potassium acetate solution yields the same potential as a 10^{-6} M Zn^{2+} solution. Table 3 shows that this interference does not determine the detection limit. In contrast, for membrane 1.6.12, with a pK_{ZnK} value of about zero, the buffer solution used corresponds to a 10^{-4} M Zn^{2+} solution and might therefore be the main contributor to the detection limit.

Thus, it seems as if the first contribution discussed predominates in determining the detection limit of the membranes investigated. The experimental results (Table 3) confirm this idea, showing low detection limits for membranes containing ligands 7, 8, 9 and 11.

E_0 *value of electrode.* Although the effect on q of the size of d_l and d_m is small (see eqn. 8), the E_0 value is more obviously influenced. It changes significantly with increasing numerical difference between the solubility parameters of the ligand and the membrane matrix. This is clear from Fig. 4, which shows the measured E_0 values ($E_{0,\,exp}$) for tri(2-ethylhexyl)phosphate-based membranes as a function of the d_l value of the ligand incorporated. The difference $E_{0,\,theor} - E_{0,\,exp}$ is negative for $d_l < d_m$, which agrees with the results obtained by Nielsen and Hansen (Fig. 4 [17]). From the $E_{0,\,exp}$ values of membranes containing ligands 9—12 (see Table 2), it can be concluded that the solubility parameters of the ligands are very close to d_m, in agreement with earlier results. The $E_{0,\,exp}$ value thus seems to be a measure of the compatibility of the ligand and the membrane material. Conversely, if the E_0 value differs from the theoretical value, the sign and magnitude of the difference will indicate in which direction the solubility parameter of the ligand should be changed in order to obtain a stable value of E_0.

Slope of calibration curve. The slopes of the calibration curves were determined manually, proceeding from low to high activity, and automatically in the reverse direction. The membranes containing ligands 8 and 10—12 have approximately Nernstian slopes while the others show a non-Nernstian behaviour (Table 2). One of the main assumptions made to obtain the simplified

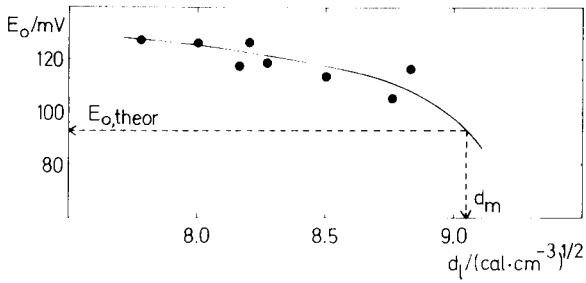


Fig. 4. The measured E_0 value of electrodes containing tri(2-ethylhexyl)phosphate-based PVC membranes as a function of the solubility parameter, d_l, of the ligand incorporated.

Nernst's equation, is that the ligand concentration is constant at the membrane/solution interface [23]. However, if the ligand site (S^-) transport within the membrane is not rapid enough or if the kinetics of the interfacial reaction are rate-limiting, the membrane composition at the sample solution interface may change during calibration, giving rise to a non-Nernstian slope. This was found experimentally for the "empty" membrane and for membranes containing ligands 1—7 and 9.

Stability of electrode potential. The most stable electrode potential with no drift of the E_0 value is obtained if d_l equals d_m. For ligand 7, d_l (8.76) is less than d_m (9.05) and the membrane material will dissolve more readily than the electroactive material. Thus, there will be an enrichment of ligand at the outer surface of the membrane. This time-dependent inhomogeneity will be registered as a drift of the E_0 value. For fresh membranes of type 1.6.7, the data (cf. Tables 2 and 3) obtained were: E_0 = 105.4 mV vs. SCE, slope = 35.2 mV/pZn, detection limit (pZn) = 4.3. A rapid drift of E_0 was then noted, so that after 24 h a value of 148.8 mV vs. SCE was obtained. This value was stable during the following 8 weeks, with a standard deviation of 3.9 mV. The values of the slope and detection limit also changed during conditioning, yielding results with excellent reproducibility. Within the 8 weeks mentioned, the slope was 43.8 mV/pZn (standard deviation 1.5 mV/pZn) and the detection limit was 4.5 ± 0.1 pZn. The change in E_0 can be explained by supposing that the membrane attains a higher concentration of ligand; when the membrane eventually becomes saturated with ligand, no further change is noted after the equilibrium period. Therefore, the detection limit remains the same even when a higher ligand concentration is used. This was confirmed experimentally by investigating a membrane of type 1.6.7 with twice the usual content of ligand; a slope of 41.2 mV/pZn and a detection limit (pZn) of 4.6 were obtained.

The composite membrane

The above results indicate that the optimum composition of a membrane for zinc ion-selective electrodes should be the membrane 1.6.7 which consists of 30% PVC, 64% tri(2-ethylhexyl)phosphate and 6% zinc bis[di(2-ethylhexyl)phosphate]. A typical response curve from a manual calibration and the corresponding calibration curve are shown in Fig. 5. The acidic nature of the ligand restricts the working pH range of the electrode to about pH 4.5—6.0. Figure 6 illustrates the pH dependence of the electrode when immersed in Zn^{2+} solutions of different concentrations. The characteristic dip, well-known in the literature for calcium-selective electrodes based on the same type of ligand [4, 6, 11], is situated at pH 3.3—4.3 (depending on the Zn^{2+} concentration). Above pH 6.0, solution side-reactions caused by hydrolysis of zinc occur. By running a pH curve (Fig. 6, curve d) in a solution containing excess of complexing agent (EDTA), the optimum pH for titration was found to be about 5.5.

It has been shown [11] that an increase in the extraction (or distribution)

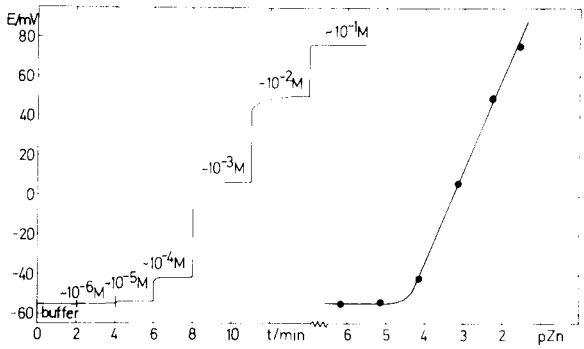


Fig. 5. Response curve and calibration curve for the zinc-selective electrode based on membrane 1.6.7 in acetate buffer.

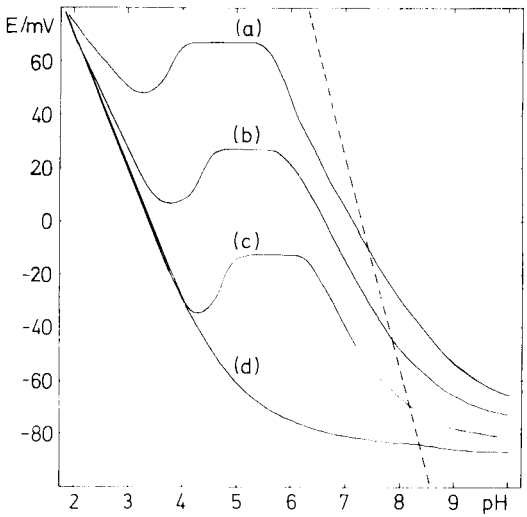


Fig. 6. Potential—pH diagrams for the zinc-selective electrode based on membrane 1.6.7 in (a) 10^{-2} M Zn^{2+} solution; (b) 10^{-3} M Zn^{2+} solution; (c) 10^{-4} M Zn^{2+} solution; (d) 10^{-3} M Zn—2 × 10^{-3} M EDTA solution. The dashed line represents the formation of $Zn(OH)_2$ (solubility product = 10^{-17} M^3).

value q is reflected in a decrease of the electrode potential. Thus, the results obtained here for the pH dependence of di(2-ethylhexyl)phosphoric acid-containing electrodes agree well with extraction studies. McDowell and Coleman [24] used the same ligand for the extraction of alkaline earth metal cations, and they found maxima in the pH region where dips occur for electrodes. By increasing the length of the alkyl chain of the substituted phosphoric acid, the proton will be less firmly bound, resulting in an extended pH working range. This was done by Ross, who used didecylphosphoric acid for the calcium-selective electrodes [4]. The electrode was further improved by Růžička

et al. [11], who introduced groups of electrophilic character (phenyl) into the alkyl chain. However, for the purpose of making zinc-selective electrodes, their ligand (8 in this work) was unfavourable with regard to selectivities. Thus, it remains to be found whether the use of didecylphosphoric acid or other long-chain analogues could further improve the behaviour of tri(2-ethylhexyl)phosphate-based PVC membranes for zinc-selective electrodes.

This work was supported by grants from the Swedish Natural Science Research Council. The authors thank Professor Gillis Johansson, University of Lund, for valuable discussions.

REFERENCES

1 A. G. Fogg, M. Duzinkewycz and A. S. Pathan, Anal. Lett., 6 (1973) 1101.
2 R. W. Cattrall and C.-P. Pui, Anal. Chim. Acta, 87 (1976) 419.
3 L. Gorton and U. Fiedler, Anal. Chim. Acta, 90 (1977) 233.
4 J. W. Ross, Science, 156 (1967) 1378.
5 Orion Research, Instruction Manual, Calcium Ion Electrode Model 92-20, 1968.
6 G. J. Moody, R. B. Oke and J. D. R. Thomas, Analyst, 95 (1970) 910.
7 R. W. Cattrall and H. Freiser, Anal. Chem., 43 (1971) 1905.
8 A. Ansaldi and S. I. Epstein, Anal. Chem., 45 (1973) 595.
9 A. Craggs, G. J. Moody, J. D. R. Thomas and A. Willcox, Talanta, 23 (1976) 799.
10 B. Fleet, T. H. Ryan and M. J. D. Brand, Anal. Chem., 46 (1974) 12.
11 J. Růžička, E. H. Hansen and J. Chr. Tjell, Anal. Chim. Acta, 67 (1973) 155.
12 D. Jagner and J. P. Østergaard-Jensen, Anal. Chim. Acta, 80 (1975) 9.
13 R. W. Cattrall, D. M. Drew and I. C. Hamilton, Anal. Chim. Acta, 76 (1975) 269.
14 R. W. Cattrall and D. M. Drew, Anal. Chim. Acta, 77 (1975) 9.
15 G. J. Moody, N. S. Nassory and J. D. R. Thomas, Analyst, 103 (1978) 68.
16 U. Fiedler and J. Růžička, Anal. Chim. Acta, 67 (1973) 179.
17 H. J. Nielsen and E. H. Hansen, Anal. Chim. Acta, 85 (1976) 1.
18 U. Fiedler, Anal. Chim. Acta, 89 (1977) 101.
19 G. Horvai, K. Toth and E. Pungor, Anal. Chim. Acta, 82 (1976) 45.
20 A. Craggs, L. Keil, G. J. Moody and J. D. R. Thomas, Talanta, 22 (1975) 907.
21 M. Güggi, U. Fiedler, E. Pretsch and W. Simon, Anal. Lett., 8 (1975) 857.
22 P. A. Small, J. Appl. Chem., 3 (1953) 71.
23 M. Sato, Electrochim. Acta, 11 (1966) 361.
24 W. J. McDowell and C. F. Coleman, J. Inorg. Nucl. Chem., 28 (1966) 1083.

Analytica Chimica Acta, 111 (1979) 71—77

POTENTIOMETRIC TITRATIONS OF SELENIUM WITH A FLUORIDE-SELECTIVE ELECTRODE

J L. BERNAL*, E. BARRADO and R. PARDO

Department of Analytical Chemistry, Faculty of Sciences, Prado de la Magdalena, Valladolid (Spain)

(Received 12th March 1979)

SUMMARY

Selenite can be determined with a fluoride-selective electrode by two methods: a direct procedure based on the joint titration of selenite and standard fluoride with lanthanum and an indirect method based on precipitation of selenite with excess of lanthanum followed by back-titration with fluoride. In the first case the end-point is located by the second derivative method, and in the second case by means of a Gran plot. Direct titrations are suitable for selenite concentrations above 5 mM; indirect titrations are useful for concentrations as low as 0.5 mM.

The fluoride-selective electrode [1] has been widely used in titrations in which fluoride is involved in precipitation or complex formation. The most widely studied procedures are those based on precipitation of lanthanum(III) and thorium(IV) fluorides [2—4]. Methods involving precipitation of fluorides have been described for determinations of lithium(I) [5] and aluminium(III) [6], and the formation of fluoride complexes of uranium(VI), thorium(IV) and zirconium(IV) has been utilized to determine these cations [7]. Some indirect methods of anion determination have also been described, e.g., for arsenate and phosphate [8].

In one of the methods proposed here for the determination of selenite, both selenite and fluoride are precipitated with a suitable cation, the fluoride precipitating after complete precipitation of the selenite. When the reactions are monitored with the fluoride-selective electrode, the e.m.f. jump occurs at the point corresponding to stoichiometric precipitation of fluoride.

The most appropriate precipitation reaction of fluoride is that with lanthanum(III) [2]. In order to see which anions might be determined by the general principle proposed, logarithmic graphs (log C_i vs. pLa) were plotted for the anions which precipitate with lanthanum(III) (Fig. 1). The pK_{so} value of LaF_3 considered was that proposed by Eriksson and Johansson [4]; the K_{so} value of $La_2(SeO_3)_3$ is unknown and was assumed to be similar to that of the analogous cerium(III) compound, an assumption that proved to be acceptable. The logarithmic graphs indicate that oxalate and selenite are precipitated by lanthanum(III) before the fluoride ion, so that the

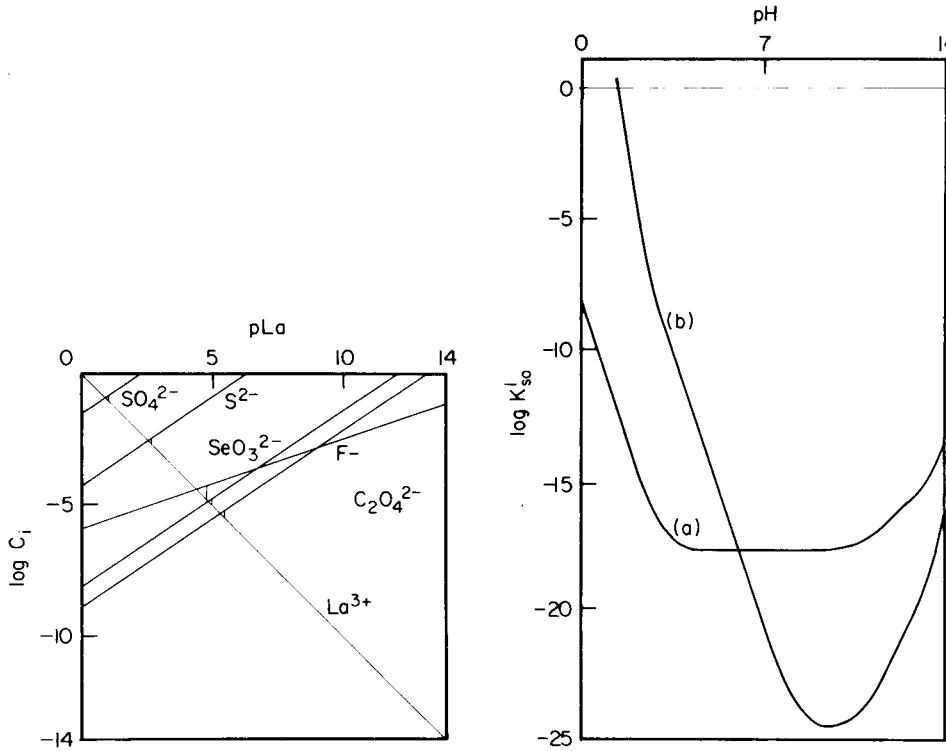


Fig. 1. Plots of log C_i vs. pLa for anions which precipitate with lanthanum(III), i.e. sulphate, sulphide, selenite, oxalate and fluoride.

Fig. 2. Conditional solubility products of (a) LaF_3 and (b) $La_2(SeO_3)_3$ as a function of pH.

method proposed can be applied only to these anions. Oxalate can affect the membrane of the fluoride selective electrode [3], however, and so this work was focussed on selenite.

In the precipitation of $La_2(SeO_3)_3$ and in that of LaF_3, pH plays a major role, as is clear from Fig. 2 which shows the K'_{so} values (conditional solubility products) as functions of the pH. It was therefore necessary to study the optimum pH for simultaneous precipitation, especially considering that fluoride is normally titrated with lanthanum(III) in neutral unbuffered medium.

EXPERIMENTAL

Equipment and reagents

A digital ion-activity meter (Philips PW 9414) was used with a fluoride-selective electrode (Orion 94—09) and a double-junction saturated calomel reference electrode (Philips R44/2-SD/1).

For the stock fluoride solution (0.1 M), 4.199 g of sodium fluoride was dissolved in 1 l of deionized water. Stock lanthanum(III) solution was prepared by dissolving 4.33 g of lanthanum(III) nitrate hexahydrate in 1 l of deionized water. For the stock selenite solution, 17.294 g of sodium selenite was dissolved in 1 l of deionized water.

All reagents used were of analytical grade.

Preliminary tests

In the direct method a mixture of selenite and fluoride is titrated with lanthanum(III) solution. For initial tests, a small indicating amount of fluoride was added to the selenite solution prior to titration with lanthanum(III) solution, but this method was unsuccessful because a significant amount of fluoride was needed to produce a detectable potential jump at the end-point. In further tests, a known significant amount of fluoride was added before the titration; the end-point was located from the second derivative of the titration curve.

In the indirect method, a known amount of lanthanum(III) was added to precipitate the selenite and the excess of lanthanum(III) was titrated with fluoride. The titration curves were difficult to evaluate by traditional methods because the e.m.f. values obtained when fluoride was added to lanthanum(III) solutions were unstable; end-points were therefore located by using Gran plots [9].

The pH has a strong influence on the precipitations (Fig. 2). To establish the optimum pH values experimentally, several solutions containing 0.5 mmol of selenite and 0.5 mmol of fluoride were titrated with 0.1 M lanthanum(III) solution. For unbuffered solutions, both the pH and the potential value of the fluoride-selective electrode change in an analogous manner during the titration (Fig. 3). In other titrations, the pH was adjusted to different values with appropriate buffer solutions under otherwise similar conditions; above pH 6 end-points were not detectable whereas below pH 6 results were low (Fig. 4). Accordingly, as in titration of fluoride alone [2], titrations must be done in neutral unbuffered media. Similarly, in the back-titration method, satisfactory results were obtained only in neutral unbuffered media.

Recommended procedures

Direct method. Add 5 ml of standard fluoride to the selenite solution, making up to 100 ml. Place the reference and selective electrodes into this solution and titrate with a lanthanum(III) solution having a concentration similar to that of the fluoride addition (0.1 M or 0.01 M). Continuous stirring is required, and near the end-point the titrant must be added in increments of 0.20 ml every 30 s. The end-point can be located from the second derivative of the titration curve. The lanthanum(III) solution must be previously standardized versus the standard fluoride under the same conditions. Figure 3 shows a typical titration curve.

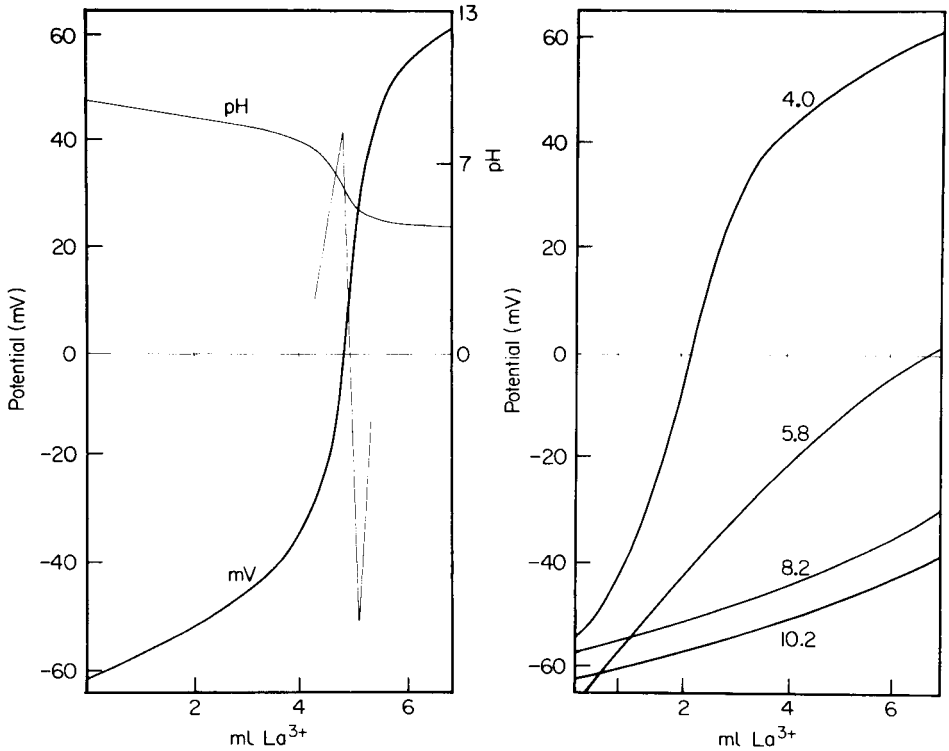


Fig. 3. Typical titration curves (e.m.f. and pH indication) for the direct method with the corresponding second-derivative plot.

Fig. 4. Effect of pH on the titration curves.

Indirect method. For determination of the blank, add 7 ml of standard fluoride solutions (0.1 M or 0.01 M) to 100 ml of deionized water, and then add up to 10 ml in 1-ml increments with continuous stirring, reading the e.m.f. values after each addition. To obtain a plot for zero selenite, dilute 2 ml of lanthanum(III) solution (0.1 M or 0.01 M, depending on the fluoride concentration used) to 100 ml. Place the electrodes in this solution and continue as for the blank, recording the e.m.f. values. For the analysis of samples, add 2 ml of lanthanum(III) solution (0.1 M or 0.01 M) to the sample solution, dilute to 100 ml and proceed as before.

Plot the data obtained on Gran plot paper (see Fig. 5) and draw straight lines through the points corresponding to each determination. The distance ($V_{zero} - V_{sample}$) obtained by extrapolation to zero ordinate is the volume of standard fluoride equivalent to the amount of selenite in the solution.

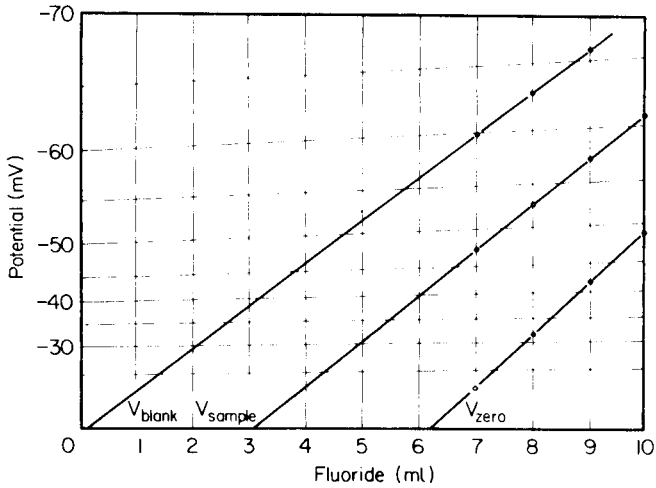


Fig. 5. Typical titration in a Gran-plot form.

RESULTS AND DISCUSSION

Direct method

When this procedure is used, the e.m.f. readings obtained are perfectly stable and the titration curves are easily drawn, although in the present case the monitored reaction between fluoride and lanthanum(III) gives rise to asymmetric titration curves, and therefore the end-point and the stoichiometric points do not match. This difficulty can, however, be avoided if the lanthanum(III) solution is standardized against standard fluoride solution under the same conditions.

Table 1 shows the results obtained when 0.1 M fluoride and lanthanum-(III) solutions were used. The results are low for selenite concentrations below 5 mM, but in the selenite concentration range 30—5 mM the mean recovery of selenium is 99.5% with a standard deviation of 0.87%.

Use of aqueous 0.01 M fluoride and lanthanum(III) solutions leads to large errors and therefore the threshold of the determination could not be lowered in this way. However, as the solubility of LaF_3 and $La_2(SeO_3)_3$ decreases very quickly in water—ethanol mixtures, an attempt was made to lower the determination threshold by performing the titration in a (1 + 1) water—ethanol mixture with 0.01 M solutions of fluoride and lanthanum-(III). These results are also given in Table 1. In this case, the mean recovery of selenium is 96.5% with a standard deviation of 2.3%. These results are low and therefore the titration in water—ethanol media is not recommended.

TABLE 1

Potentiometric titrations of selenite by the direct method

Selenium added (mg)	Selenite concentration (mol l^{-1})	Selenium recovered (mg)	Recovery (%)
With 0.1 M fluoride and lanthanum solutions			
236.9	3×10^{-2}	237.4[a]	100.2
157.9	2×10^{-2}	157.3[b]	99.6
79.0	1×10^{-2}	79.0[b]	100.1
39.5	5×10^{-3}	38.8[b]	98.5
15.8	2×10^{-3}	14.1[a]	89.5
With 0.01 M fluoride and lanthanum solutions in (1 + 1) water–ethanol			
15.8	2×10^{-3}	15.15[a]	95.9
11.8	1.5×10^{-3}	11.3[a]	95.25
7.9	1×10^{-3}	7.6[a]	96.7
3.95	5×10^{-4}	3.9[a]	98.8

[a]Mean of 2 results. [b]Mean of 3 results.

TABLE 2

Potentiometric titrations of selenite using the indirect method with 0.1 M fluoride and lanthanum(III) solutions
(Each value is the mean of 2 results)

Selenium added (mg)	Selenite concentration (mol l^{-1})	Selenium recovered (mg)	Recovery (%)
19.9	2.5×10^{-3}	19.8	99.4
15.9	2×10^{-3}	15.6	98.4
11.9	1.5×10^{-3}	12.6	105.8
7.95	1×10^{-3}	7.9	99.25
3.95	5×10^{-4}	3.78	94.8

Indirect method

The Gran plot paper used was designed for a slope of −59 mV and corrects automatically for the increase in volume produced by 10 increments of 1% of the initial volume. Table 2 shows the results obtained when the procedure was applied to selenite solutions with concentrations as low as 5 mM, by using 0.1 M fluoride and lanthanum(III) solutions. The mean recovery of selenium was 99.5% with a standard deviation of 5.6%, but for 0.01 M solutions of fluoride and lanthanum(III) the results were again very poor. Despite this, the indirect method, when compared with the direct one, allows the determination threshold to be significantly lowered.

In conclusion, the two methods proposed here cover a concentration

range of 30 mM to 0.5 mM for selenium as selenite. If the concentration is higher than 5 mM the direct method should be used, but for solutions with concentrations between 5 and 0.5 mM the indirect method is to be preferred.

REFERENCES

1 M. S. Frant and J. W. Ross, Science (N.Y.), 154 (1966) 3756.
2 J. J. Lingane, Anal. Chem., 39 (1967) 881; 40 (1968) 395.
3 T. Anfalt, D. Dyrssen and D. Jagner, Anal. Chim. Acta, 43 (1968) 487.
4 T. Eriksson and G. Johansson, Anal. Chim. Acta, 52 (1970) 465.
5 E. W. Baumann, Anal. Chem., 40 (1968) 1731.
6 E. W. Baumann, Anal. Chem., 42 (1970) 110.
7 F. C. Chang, H. T. Tsai and S. C. Wu, Anal. Chim. Acta, 71 (1974) 477.
8 W. Selig, Mikrochim. Acta, (1973) 349; (1974) 315.
9 G. Gran, Analyst, 77 (1952) 661.

Analytica Chimica Acta, 111 (1979) 79—87

ANODIC STRIPPING VOLTAMMETRY WITH A SYMMETRIC DOUBLE-STEP WAVEFORM

JOUKO J. KANKARE* and KEIJO E. HAAPAKKA

Department of Chemistry, University of Turku, SF-20500 Turku 50 (Finland)

(Received 29th May 1979)

SUMMARY

The high sensitivity of differential pulse anodic stripping voltammetry (d.p.a.s.v.) is based on discrimination against the charging current by using short current-sampling periods near the end of the applied pulses. In this paper, the capacitive current is eliminated by using a symmetric double-step waveform superimposed on the voltage ramp. The areas under the adjacent current peaks are subtracted from each other which effectively cancels the contribution of the double-layer charging current but preserves the changes in the faradaic current. By using a mercury thin-film electrode the base line is reasonably flat down to nanomolar levels of Cd, Cu and Pb with a deposition time of 3—5 min. Compared with d.p.a.s.v., the proposed method has the advantages of a higher scan rate and simple electronic circuitry.

Differential pulse anodic stripping voltammetry (d.p.a.s.v.) is reputedly the most sensitive of the voltammetric techniques. This superiority is based on the high level of discrimination against the charging current achieved by using short sampling periods near the end of the applied pulses where the charging current has decayed to an insignificant level but the faradaic current still has an appreciable contribution. A minor point is that by using a low duty cycle, an appreciable portion of the analyte is believed to be replated during the rest period between pulses as long as this period is cathodic of the reduction potential. Consequently, the same analyte may make repeated contributions to the current measured.

The price paid for this sensitivity is the slow pulse repetition and scan rate. The total stripping time may be 3—4 min, which is about the same as the duration of the plating step. Another point which is not usually mentioned in papers dealing with pulse voltammetric methods is that the discrimination method based on the slower decay of the faradaic current compared with the capacitive current is of low efficiency in using the available faradaic charge. Thus, for instance, in a paper dealing with the optimization of the parameters for d.p.a.s.v. [1], the optimum values of the current sampling duration and pulse duration are 1.5 ms and 7 ms, respectively. Consequently, the proportion of the charge utilized for the measurement is ca. 20% of the total charge.

This paper presents an alternative method for discriminating against the

charging current. In this method a pulse train (Fig. 1a) (called here a symmetric double-step waveform) superimposed on the voltage ramp is applied to the stripping electrode. Assuming that capacitance is reasonably constant within the step amplitudes, the capacitive current peaks are equal in magnitude. However, the changes in the faradaic current are not equal if the voltage is within the range of the stripping process. Integrating the total current during the steps (from A to B and B to C in Fig. 1a) and subtracting the integrals from each other effectively suppresses the charging current. The necessary electronic circuitry is described and the equipment is applied to the determination of cadmium, lead and copper.

EXPERIMENTAL

Apparatus

The cell was a rotating cell originally devised by Clem et al. [2] and manufactured by McKee-Pedersen Instruments Inc. (Pasadena, California). The working electrode was a mercury thin-film electrode on wax-impregnated graphite (WIGE), the effective area of which was ca. 8 mm^2. The counter electrode was a platinum disc and the reference electrode a calomel electrode with a glass-frit liquid junction filled with saturated aqueous sodium chloride (Suprapur, E. Merck).

The block diagram of the apparatus is shown in Fig. 2. The waveform generator (a detailed circuit diagram is shown in Fig. 3) generates the pulse train shown in Fig. 1(a). Pulses are summed with a ramp voltage in OA2 and

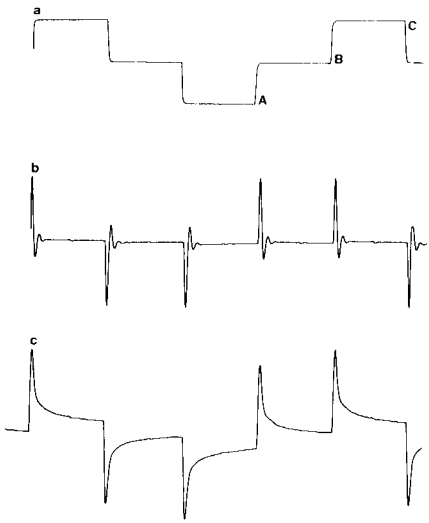


Fig. 1. Symmetric double-step waveform (superimposed on the voltage ramp) and cell current. (a) Potential—time waveform where A, B, and C refer to the gating points of the integrator (see text); (b) cell current with a fresh electrode; (c) cell current with a deteriorated electrode.

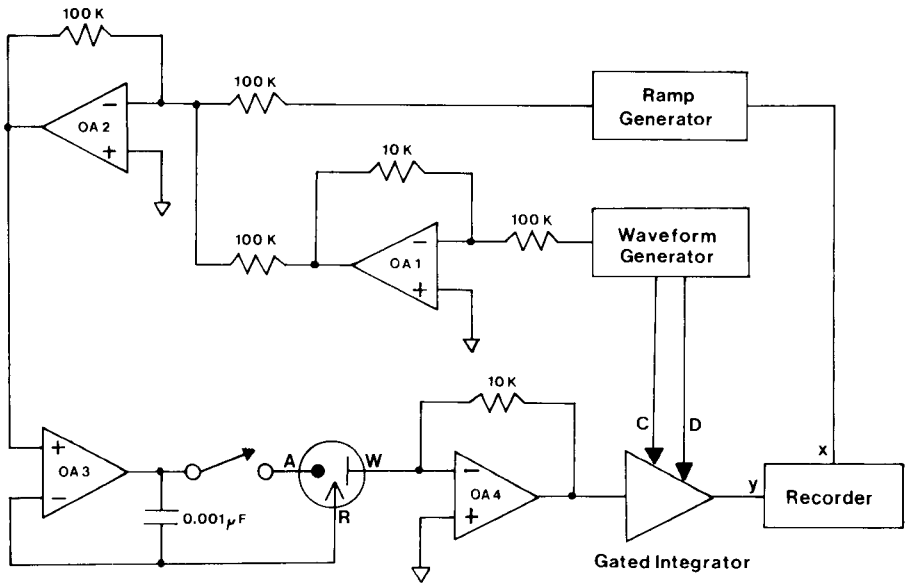


Fig. 2. Outline diagram of the voltammeter.

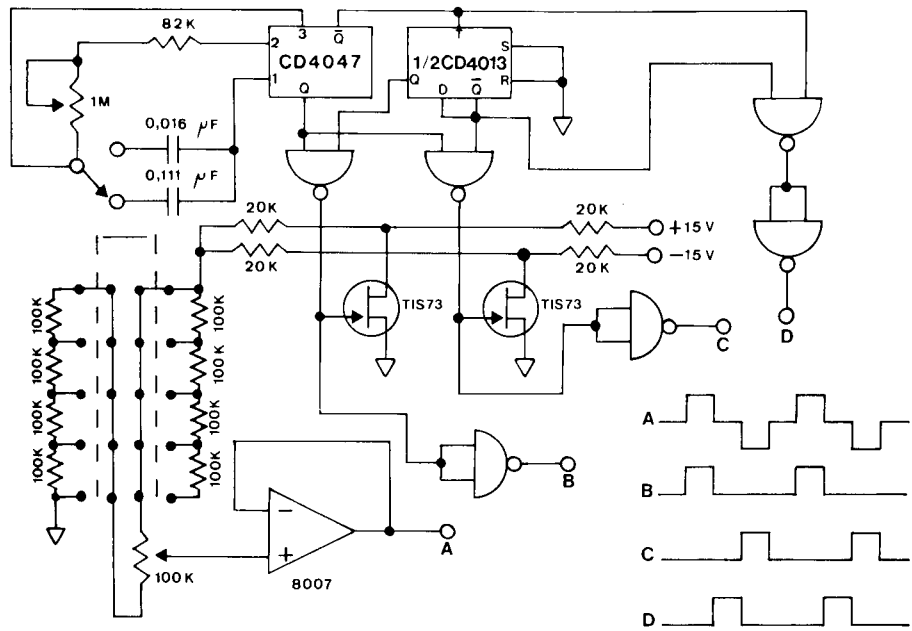


Fig. 3. Detailed circuit diagram of waveform generator.

taken to a potentiostat OA3. The cell current is converted to a proportional voltage in OA4 and demodulated in a simple gated integrator. Output from the gated detector was recorded on an X-Y recorder (Model 29350, Bryans Southern Instruments Ltd., England). The potentiostat was a single op amp (741) with a transistor booster.

The waveform generator is composed of a tunable astable multivibrator, D flip-flop and a few NAND gates (Fig. 3). The logic voltage levels are −15 V and 0 V in order to be able to control FETs without interface circuits. The FETs, a Kelvin—Warley bridge and an operational amplifier produce waveform A and the gate-control pulses B, C, and D shown in Fig. 3.

The gated integrator or "lock-in amplifier" (Fig. 4) is quite simple, consisting of a capacitor, two FET switches controlled by pulses from the waveform generator, and a differential amplifier.

Pulses in Fig. 1 were recorded on a transient recorder Datalab DL 901 (Data Laboratories Ltd., England).

Reagents

Supporting electrolyte was in most cases sodium perchlorate (analytical grade, E. Merck). It was found that at least this particular batch was extraordinarily pure containing negligible amounts of cadmium and lead and ca. 3×10^{-8} M of copper (cf. Fig. 11). The stock solutions of cadmium, lead, and copper were prepared from analytical-grade reagents and stored in polypropylene flasks. The solution of mercury nitrate used for the plating of WIGEs was prepared by dissolving triply distilled mercury in nitric acid.

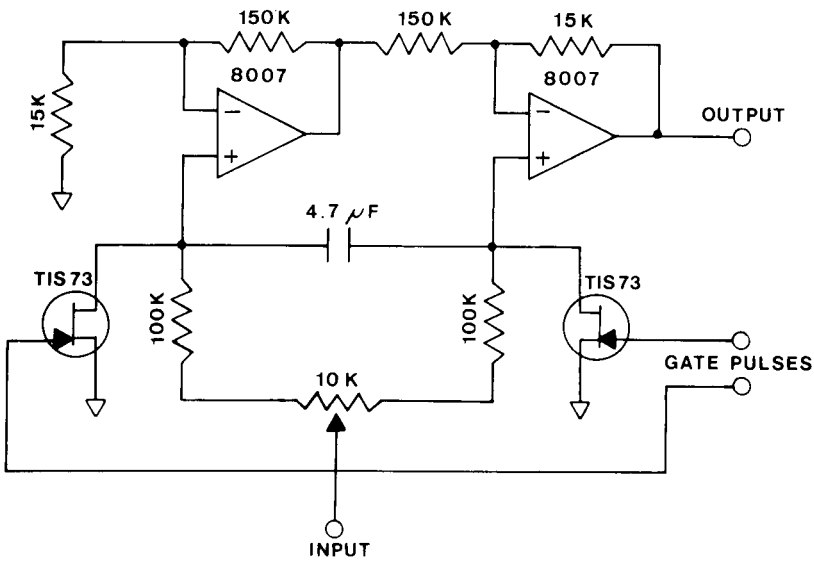


Fig. 4. Detailed circuit diagram of gated integrator.

All solutions were made in water distilled once in a conventional stainless-steel distilling system and then in a quartz double-distilling unit.

Procedure

A 15-ml aliquot of supporting electrolyte was pipetted into the cell, and 100 μl of 1.35×10^{-3} M mercury nitrate and various amounts of analyte solutions were added from Eppendorf microliter pipettes. Oxygen was sparged from the solution in 2 min by using a high flow rate of nitrogen and rotating the cell so that the solution rose as a thin layer against the walls of the cell. The flow rate of nitrogen was then decreased and the cell rotation switched to reciprocating for the deposition step. The deposition time was 3 or 5 min. After the deposition step the ramp voltage was started and the voltammogram recorded.

RESULTS AND DISCUSSION

Numerous criteria may be applied in assessing the quality of stripping voltammograms. The first criterion is the limit of detection, which, among other things, depends on the deposition time. The second criterion is the height of the background current, or, what is more important, the slope of the background. In most cases the sensitivity of the conventional linear ramp method is sufficient to detect the metals to be analyzed, but quantification is made difficult by the steep slope of the capacitive current. Thirdly, the resolution may have an influence on quantitative analysis although in most cases this is not a great problem in a.s.v. Of course, as in any analytical method, important points are also the reproducibility and linear concentration range of the determinations.

In the conventional linear ramp method, there is only one electric variable, the scan rate, which may be optimized in regard to the above criteria. Pulse voltammetric methods have additional parameters like pulse repetition rate, amplitude, etc., which each has its influence on the voltammogram.

Waveform

Figure 1(b) shows the cell current recorded on a transient recorder. The slight "ringing" after the capacitive current peaks may be due to the primitive potentiostat employed. Usually faradaic current could be seen only as a very slight current rise between the sharp charging current peaks, although it could be easily recorded by integrating the current between A and B and B to C (Fig. 1a) and subtracting the integrals from each other.

Figure 1(b) shows the cell current for a freshly prepared WIGE. During use, the background current increases until the gated integration can no longer distinguish between capacitive and faradaic currents. Figure 1(c) shows the current waveform obtained with a badly deteriorated electrode. Continuous monitoring of the cell current with an oscilloscope was found to be advantageous in assessing the condition of the electrode.

Effect of scan rate

The scan rate of the cell voltage has a pronounced influence on the voltammograms in most stripping methods. In the conventional linear ramp method, the increase in scan rate results in enhanced sensitivity which is however, partly compensated by higher capacitive current. In the differential pulse method, a slow scan rate is necessitated by the slow pulse repetition rate. In the proposed method, the scan rate has no dramatic influence on the sensitivity as can be seen in Fig. 5. A shallow maximum can be observed between 30 and 40 mV s^{-1}. What is more significant is the influence of scan

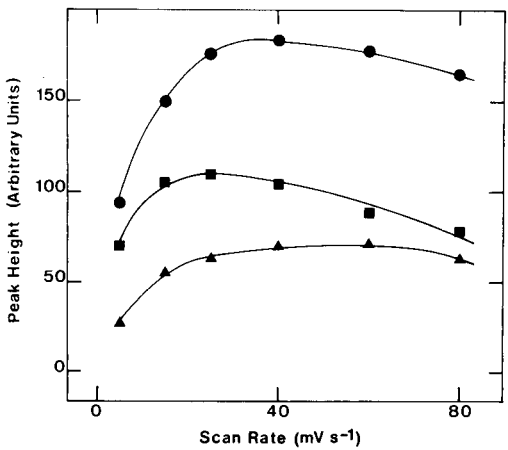


Fig. 5. Effect of scan rate on peak heights. Pulse height 15 mV; pulse width 8.8 ms; plating time 300 s at −1.3 V; supporting electrolyte 0.1 M $NaClO_4$. (▲) 4 × 10^{-7} M Cu; (■) 2 × 10^{-7} M Pb; (●) 2 × 10^{-7} M Cd.

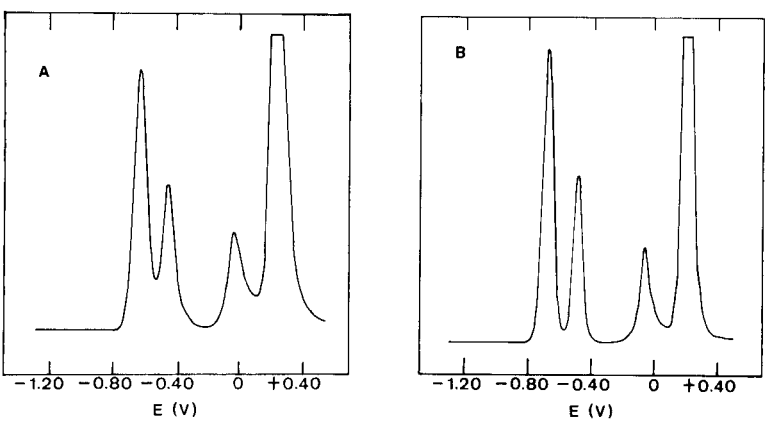


Fig. 6. Effect of scan rate on voltammogram. (A) Scan rate 80 mV s^{-1}; (B) scan rate 40 mV s^{-1}. Conditions and solution as for Fig. 5.

rate on the resolution. Figure 6 shows stripping voltammograms of a mixture of 2×10^{-7} M Cd (at −0.7 V), 2×10^{-7} M Pb (at −0.5 V) and 4×10^{-7} M Cu (at −0.1 V). Curve A was recorded at a scan rate twice that used for curve B. Impairment in resolution with increasing scan rate can be clearly observed.

Figures 5 and 6 indicate that the optimum scan rate should be near 40 mV s^{-1} although the acceptable range is quite large.

Effect of pulse amplitude and pulse width

The effect of pulse height on the sensitivity is rather peculiar in the present case. As Fig. 7 shows, cadmium and copper behave normally, i.e. the sensitivity increases with increasing pulse amplitude, but lead has a sensitivity maximum at around 70 mV. No reasonable explanation could be found for this phenomenon.

Although the sensitivity can be increased ca. tenfold by increasing the pulse height from 10 to 70 mV, the noise level is so low that this increase has no practical significance. High pulse amplitudes such as 70 mV have a deleterious effect on the peak form.

Osteryoung and Christie [3] have shown that in d.p.a.s.v. the peak current is inversely proportional to the pulse width. Shorter pulses can thus be used to enhance the signal. The same phenomenon is observed with the symmetric double-step waveform. Figure 8 shows the approximately hyperbolic relation between the peak height and pulse width.

Effect of stirring

The rate of solution stirring has a direct influence on the amount of metal plated onto the working electrode. However, according to Copeland et al. [1] stirring should not be continued during the stripping step because of the

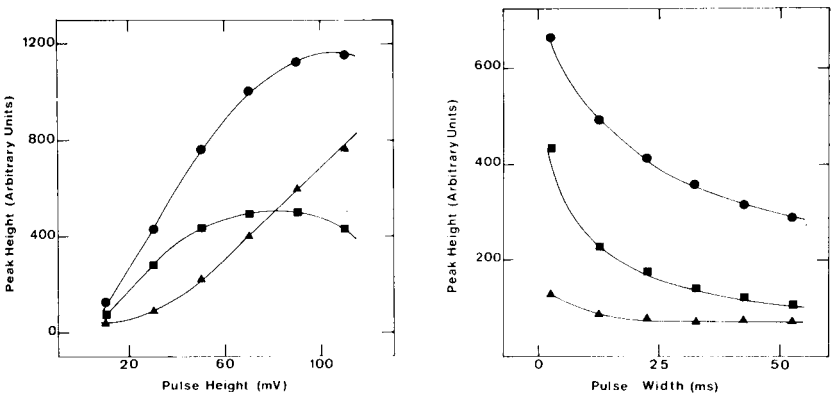


Fig. 7. Effect of pulse amplitude on peak heights. Scan rate 30 mV s^{-1}; pulse width 8.8 ms; solution and symbols as for Fig. 5.

Fig. 8. Effect of pulse width on peak heights. Pulse height 30 mV; scan rate 30 mV s^{-1}; solution and symbols as for Fig. 5.

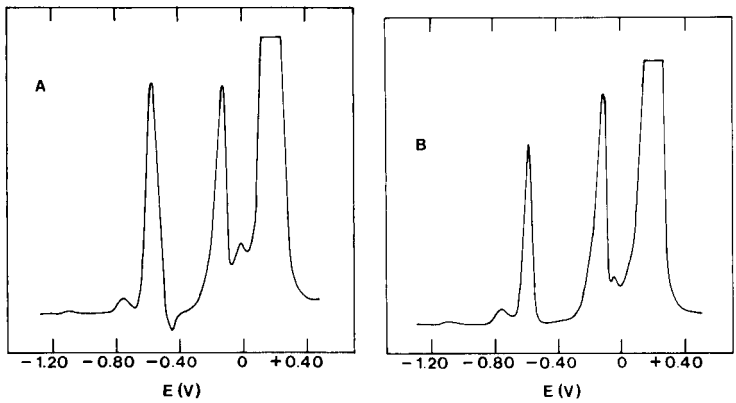


Fig. 9. Effect of stirring on voltammogram. (A) Stirring off; (B) stirring on. Pulse height 80 mV; pulse width 6.0 ms; scan rate 20 mV s^{-1}; plating time 180 s at −1.3 V; supporting electrolyte, 0.1 M acetate buffer (pH 4.65).

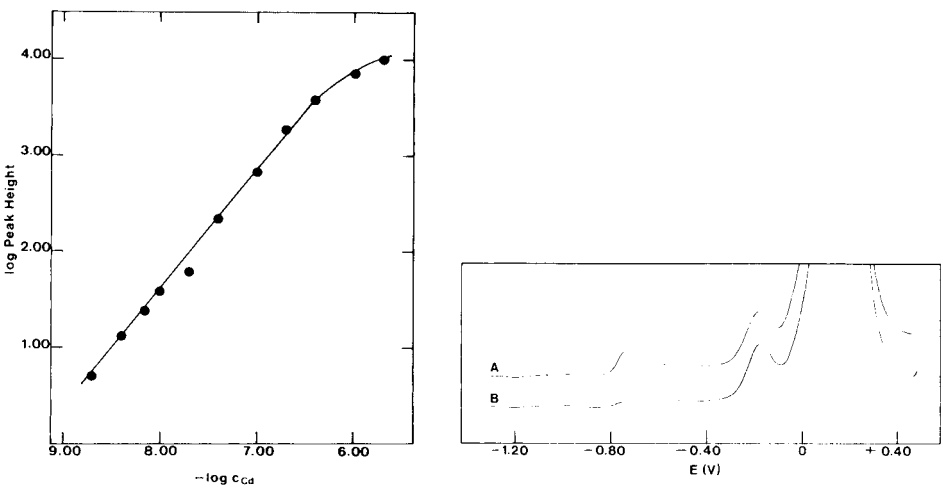


Fig. 10. Calibration curve for cadmium. Pulse height 80 mV; pulse width 6.0 ms; scan rate 40 mV s^{-1}; plating time 180 s at −1.3 V.

Fig. 11. Stripping voltammogram of the supporting electrolyte (0.1 M $NaClO_4$). (A) 6×10^{-9} M Cd added; (B) no additions. Conditions as for Fig. 10.

increased background noise and slight decrease in the peak current. In the present case, when working with the symmetric double-step waveform and reciprocating rotation of the cell, no increase in the background noise could be observed. In fact, the peaks are more symmetric as can be seen in Fig. 9. Sensitivity is slightly decreased by stirring, but the difference is insignificant. Consequently, almost all the determinations were performed with stirring on (at about 80 strokes per min) during the stripping step.

Linearity and sensitivity

The linear range of the symmetric double-step a.s.v. for cadmium can be seen in Fig. 10. The slight curvature can be explained by saturation of the mercury film on the WIGE by cadmium.

No systematic approach was made to determining the sensitivity of the method. Figure 11 shows that determinations at the 0.1 ppb level are fully possible without increasing the plating time from the 3 min used at present.

Conclusion

In view of the results presented herein, anodic stripping voltammetry with symmetric double-step waveform may be considered a rival technique to differential pulse a.s.v. The newcomer in the field, staircase stripping voltammetry [4, 5], shares the advantage of fast scan rate with the present method. It is difficult to compare the methods in terms of the reported data because of the large number of experimental variables involved. The more efficient use of the faradaic current in the present method may be advantageous in certain critical applications.

The use of symmetric double-step waveform in voltammetry evolved as a side result for a project dealing with photoelectrochemistry and as such did not create sufficient interest in theoretical calculations. It may well be that the theoretical treatment would reveal the way to optimize the main parameters, pulse width and amplitude in terms of background rejection and sensitivity, and at least would allow comparison with the other pulse methods.

Financial support for this work from the National Research Council for Sciences, Finland, and Emil Aaltonen Foundation is gratefully acknowledged. The paper was presented in part in Euroanalysis III, Dublin, August 1978.

REFERENCES

1 T. R. Copeland, J. H. Christie, R. A. Osteryoung and R. K. Skogerboe, Anal. Chem., 45 (1973) 2171.
2 R. G. Clem, G. Litton and L. D. Ornelas, Anal. Chem., 45 (1973) 1306.
3 R. A. Osteryoung and J. H. Christie, Anal. Chem., 46 (1974) 351.
4 J. H. Christie and R. A. Osteryoung, Anal. Chem., 48 (1976) 869.
5 U. Eisner, J. A. Turner and R. A. Osteryoung, Anal. Chem., 48 (1976) 1608.

Analytica Chimica Acta, 111 (1979) 89—102

FLUORIDE MICRODETERMINATION AND ITS APPLICATION TO THE ANALYSIS OF ROCKS, SOILS, PRECIPITATION, AND AIRBORNE DUST

JÜRGEN AUFFARTH and DIETER KLOCKOW*

Universität Dortmund, Abteilung Chemie, Postfach 50 05 00, D-4600 Dortmund 50 (Federal Republic of Germany)

(Received 16th July 1979)

SUMMARY

A method for the routine determination of nanogram amounts of fluoride is described. In a one-vessel technique, the fluoride is separated from the matrix by diffusion and then determined kinetically by its inhibiting effect on the zirconium-catalysed reaction between perborate and iodide. The method is applied to the analysis of geochemical materials, rain water, and aerosol filter samples.

The catalytic—kinetic technique described earlier [1] for the determination of nanomolar amounts of fluoride utilized the zirconium-catalysed reaction between perborate and iodide as the indicator reaction [10]. By addition of a small amount of ascorbic acid a Landolt-type reaction system was established, the induction period of which is a measure of the amount of fluoride present. The method was made specific for fluoride by combining it with a microdiffusion separation of fluoride from the matrix based on hexamethyldisiloxane (HMDSO) [2, 3]. Analysis of biological materials yielded results similar to those obtained by other methods for fluoride determination. The combination of the HMDSO diffusion with a potentiometric finish for fluoride has been applied to the investigation of toothpastes [4]. Yoshida et al. [5] have pointed out the potentialities of the HMDSO technique.

In order to make the earlier system applicable to routine work and to minute samples, it has now been modified to a micro-scale version. The entire analytical procedure (sample storage and preparation, separation, and determination of analyte) has been optimized to agree with some important advice for micro and trace analysis as given by Tölg [6]: "The best way of eliminating systematic errors would be to carry out all stages of a multi-stage procedure in a single closed system with a minimum surface area, and made of an inert material".

EXPERIMENTAL

Apparatus

Microdiffusion. The diffusion separation of fluoride from liquid samples by using HMDSO is done in disposable 1.5-ml polypropylene micro test

tubes (No. 3810, Eppendorf Gerätebau Netheler & Hinz, D-2000 Hamburg 63) if the sample volume does not exceed 200 µl. For samples in the milliliter range, high-density polyethylene vials (capacity ca. 20 ml) with polyethylene screw caps, as used in liquid scintillation counting, are employed. In both cases an alkali-coated lid of a 1.5-ml micro test tube serves as a receptor compartment for the fluorosilane and can be used afterwards as the reaction vessel for the catalytic—kinetic step (see below). Such lids are fitted to the polyethylene vials through a hole punched in the vial cap which holds the upper part of a micro test tube (see Fig. 1). To accelerate the diffusion process, a rotator device is used similar to that described earlier [1]. The micro test tubes or the polyethylene vials, respectively, are mounted on suitable rotator discs.

Catalytic—kinetic determination. After the fluoride separation, the receptor lid, now used as a reaction vessel, is mounted upside down with a spring clamp on top of the hollow spindle of an electric stirrer (see Fig. 2). Thermostated demineralized water (20.0 ± 0.1°C) is pumped through a concentrically arranged feed pipe against the bottom of the reaction vessel to maintain proper temperature conditions during a series of measurements. By this means the reaction vessel can be turned ("stirred") at ca. 300 rpm around two micro platinum electrodes connected to a biamperometric detector with timer [1]. The electrodes can be lifted for changing the reaction vessels. The dimensions of the electrodes must be such that the platinum tips are totally immersed in the small reaction volume (ca. 80 µl) during the measurements (see Fig. 2).

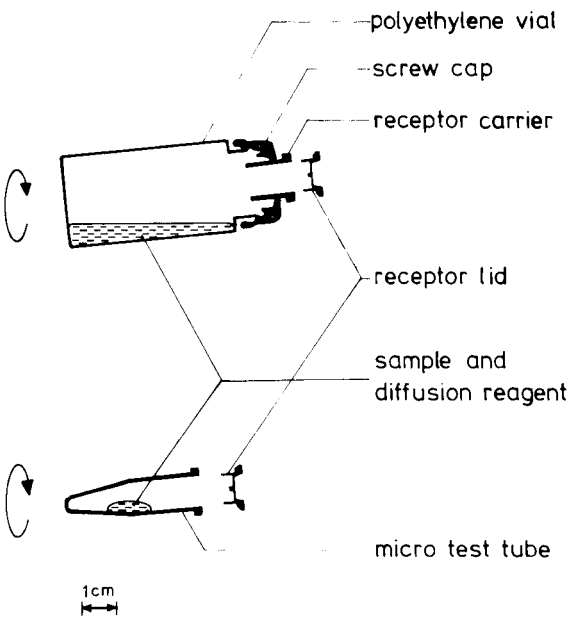


Fig. 1. Cells for microdiffusion of fluoride.

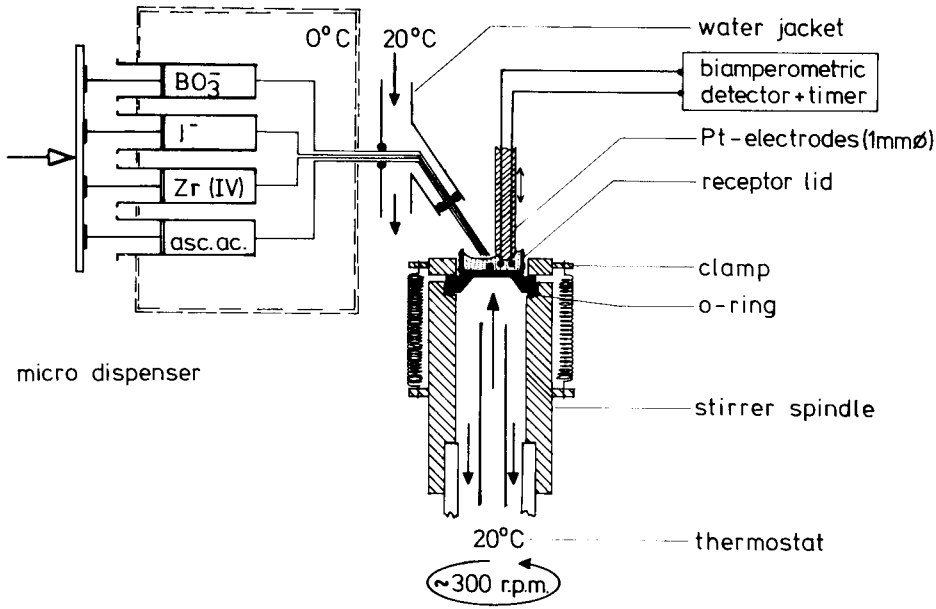


Fig. 2. Set-up for the catalytic—kinetic microdetermination of fluoride.

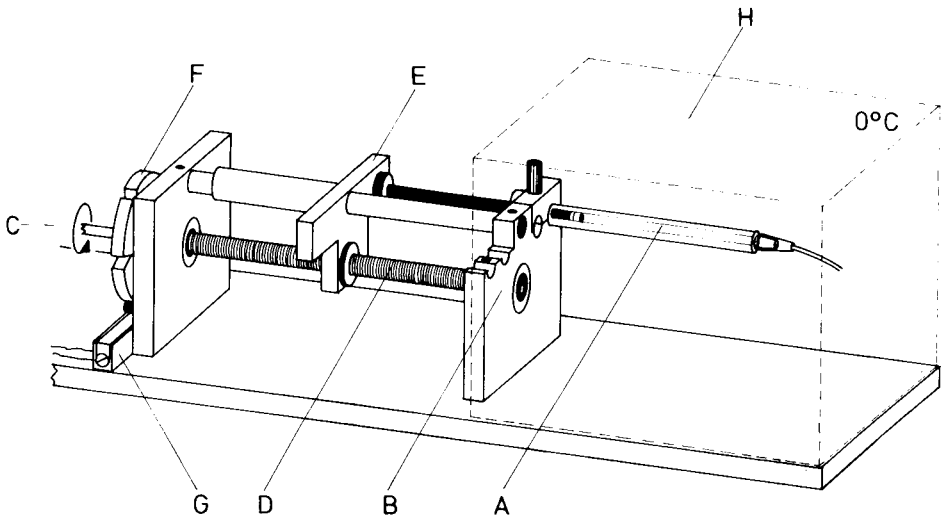


Fig. 3. Four-channel microdispenser. (A) 1 ml syringe, (B) syringe holder, (C) d.c. motor with gear box, (D) micrometer screw, (E) feed bar, (F) cam disc, (G) microswitch, (H) ice/water bath.

A specially built four-channel microdispenser (Fig. 3) permits the simultaneous addition of reproducible volumes of the four reagent solutions

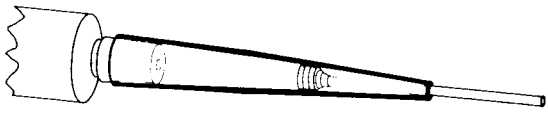
initiating the Landolt system [1]. Four gas-tight 1-ml syringes (A) (Hamilton 1001 LT) are fitted in parallel in a syringe holder (B). A d.c. motor with gear box (C) (ca. 12 rpm) drives a micrometer feed screw (D) with a bar (E) on it. By this means, the pistons of the four syringes can be moved simultaneously at 1 mm per revolution, which yields a delivery rate of about 8 μl per revolution from each syringe. The syringes are connected to polyethylene capillary tubes (0.5 mm i.d.; see Fig. 4), the tips of which end as a bunch in the reaction vessel (Fig. 2).

To guarantee delivery of reproducible volumes of the reagent solutions in the μl range, the feed of the syringe pistons must be reproducible, and capillary attraction and evaporation of the solutions between measurements must be overcome. Two cams on a disc (F) attached to the micrometer screw are set at an angle of about 45° and control a short-circuiting microswitch (G) for the d.c. motor (C); this provides two reproducible stops of the micrometer screw in one revolution. By this means, a 1-μl fraction of each reagent solution can be delivered and flushed previous to the 7-μl main fraction which is added to the reaction vessel. The volume delivered by this dispenser and its reproducibility were checked by weighing 82 fractions; the value found was 7.17 ± 0.02 μl. It must be emphasized that it is not the absolute volume that is critical but the reproducibility of the delivery.

The glass bodies of the syringes are immersed in an ice/water bath (H) at 0°C to prevent alteration or decomposition of the dilute reagent solutions [1]. For thermal control of the volume fractions delivered into the reaction vessel, about 1 cm of the bunch of capillaries is thermostated at 20°C by means of a simple water jacket connected to the thermostat (see Fig. 2).

Solutions and reagents

Zirconium stock solution, 10^{-2} M. Dissolve 322.25 mg of $ZrOCl_2 \cdot 8H_2O$ (Merck, p.a.) and dilute to 100 ml with 0.1 M HCl. Store at about 4°C for at



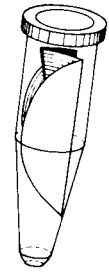


Fig. 4. Connection between a syringe with Luer cone and a capillary tube. A "yellow" tip of a microliter pipette is cut, the capillary is drawn through, and the end of it is widened by sticking a heated metal cone inside.

Fig. 5. Arrangement of a loaded filter (26 mm diam.) in a microtube for microdiffusion of fluoride.

least one month and then use for the preparation of the working solution [1].

Zirconium working solution, 1.5 × 10^{-4} M. Dilute 1.5 ml of the stock solution to 100 ml with 0.1 M HCl. Store in a refrigerator for at least 24 h before use [1].

Perborate solution, 0.01 M. Dissolve 154 mg of $NaBO_2 \cdot H_2O_2 \cdot 3H_2O$ (Riedel de Haen) and dilute to 100 ml with doubly-distilled water. Store in a polyethylene bottle at about 4°C. Discard after two weeks.

Ascorbic acid stock solution, 0.1 M. Dissolve 1.76 g of L(+)-ascorbic acid (Merck, p.a.) in doubly-distilled water and dilute to 100 ml. Transfer to a small gas washing borosilicate bottle and purge for 30 min with pure nitrogen to expel oxygen. The solution is stable for several months, when kept under nitrogen.

Ascorbic acid working solution, 5 × 10^{-4} M. Take 50 µl of the stock solution, excluding air, and dilute to 10 ml with doubly-distilled water. Purge the solution with pure nitrogen for 20 min in an ice/water bath. It is very sensitive to oxidation by air and must be kept at 0°C under nitrogen. Prepare daily.

Sodium fluoride standard solution, 2 × 10^{-5} M. Store in a polyethylene bottle.

Diffusion reagent. Dissolve about 0.1 g of Ag_2SO_4 (Merck) in 100 ml of 0.1 M $HClO_4$, made from 70% perchloric acid (Merck, p.a.) by dilution with doubly-distilled water. The amount of silver sulfate added must be sufficient to prevent diffusion of hydrogen chloride from the matrix. In a borosilicate glass separatory funnel this solution is extracted twice with ca. 5 ml of hexamethyldisiloxane (Fluka, puriss.), and stored under a layer of hexamethyldisiloxane. Before a fraction of the lower aqueous layer is taken for use, the aqueous and organic phases must be completely separated.

Receptor solution. Dilute 10 ml of 1 M NaOH to 100 ml with 2-propanol (Merck, p.a.) in a borosilicate glass flask.

Preparation of the receptor compartments for microdiffusion

Cut off the necessary number of lids from the original Eppendorf micro test tubes, to which they are attached by a plastic stem. To avoid contamination, do not touch the lids with the fingers. For coating with the receptor solution, place the lids upside down on a glass plate, and transfer 10 µl of the receptor solution carefully with a microliter pipette onto the pin in the centre of the little compartment. Contact of the solution with the outer walls of the compartments must be avoided. After filling a series of lids, quickly evaporate the 2-propanol by setting the glass plate with the lids on it for 5 min in a clean drying oven at 80°C. The dry receptor compartments are now coated with a thin uniform layer of NaOH (or Na_2CO_3) and must be protected against contamination by attaching the clean micro test tubes to them. A large number of receptor compartments may be prepared simultaneously and stored for some days until used.

Microdiffusion of fluoride

Sample volumes in the µl range. Fix a series of 1.5-ml micro test tubes in the vertical rotator disc in a nearly horizontal position. Transfer up to 200 µl of a sample or standard solution to each tube, so that the liquid lies as a drop in the angle of the tube wall (see Fig. 1). After addition of 50 µl of the diffusion reagent, immediately seal each tube with a receptor lid. To accelerate the microdiffusion process, rotate the disc slowly (ca. 10 rpm) in a position that allows the liquid drops to move round in the angle of the test tube wall. Original Eppendorf tubes are highly recommended for this process. Micro test tubes of other manufacturers were found to have a coarser surface structure which may lead to uncontrolled splashing of the drops. When diffusion is complete, tilt the rotator disc to the horizontal, so that the diffusion solutions are collected in the cones of the micro test tubes. Now the lids containing the fluoride can easily be removed from the tubes and used for the catalytic—kinetic procedure.

Sample volumes in the ml range. Transfer sample solutions and standard fluoride solutions to the polyethylene vials. Screw on the cap with the receptor carrier and pipet about an equal volume of the diffusion reagent into the vial. Immediately, close with a receptor lid (see Fig. 1). A series of diffusion cells can be prepared, fixed in a rotator disc, and rotated slowly until diffusion is complete [1].

Filter samples. Fill a micro test tube standing upright in a rack with 50—100 µl of the diffusion reagent. Push a loaded filter (see below) up to 26-mm diameter into the tube as shown in Fig. 5, using teflon-coated forceps. The exposed surface of the filter should be directed to the centre of the test tube. From the moment the filter material touches the diffusion reagent in the cone, the liquid will spread over the filter surface by capillary forces and initiate the diffusion process. Therefore the loaded tube must be sealed immediately with a receptor lid. Because of the large filter surface it is not necessary to rotate the diffusion cells.

Microdiffusion of fluoride is done at room temperature (20—25°C). Temperature drop along the diffusion devices (e.g. by draughts) must be avoided, in order to prevent distillation of water inside the cells. If this happens (mainly after long diffusion times), large droplets can be seen on the inner side of the receptor lids, causing variation of the liquid volume in the subsequent kinetic determination. This difficulty can be overcome either by drying the receptor lid in a clean drying oven at 80°C with careful exclusion of contaminants or by hanging the diffusion cells with their lower end in a cold water bath for redistillation of water from the receptor lids to the cold lower wall of the cell.

In all cases, suitable amounts of the sodium fluoride standard solution are treated in the same manner as the sample solutions. The receptor lids with the standard fluoride (standard lids) are used to prepare the calibration curve.

Fluoride determinations

Preparation. The four reaction solutions — 1.5×10^{-4} M zirconium, 0.01 M perborate, 5×10^{-4} M ascorbic acid and 0.1 M potassium iodide — are all sensitive to decomposition, though to different extents. Therefore each solution is allowed to contact only its storage vessel and one specially marked syringe and capillary. Syringes and capillary tubes are conditioned by storing them filled with the appropriate reagent solution in a refrigerator when not in use. For fluoride determinations they must be rinsed and filled with fresh solution, attached to the syringe holder, and then immersed in the ice/water bath. The water jacket, used for the thermal adjustment of the reagent solutions, is connected to the thermostat circulation pump. A few milliliters of 0.05 M HCl, for dissolution of the receptor contents, are stored in the thermostat at 20.0°C.

Measurements. Expel the first 1-μl fractions of all four reagent solutions from the capillary tips. Remove the drops with 0.01 M HCl using a wash bottle. Likewise rinse the platinum electrodes with 0.01 M HCl. Wipe the electrodes and the capillary tips with fluoride-free filter paper (e.g., Whatman Nos. 40, 41, 42), but be careful that no reagent solution is sucked out of the capillaries.

Fix a fluoride-loaded receptor lid on the stirrer spindle, lower the two platinum electrodes to nearly the bottom of the little compartment, and switch on the stirrer and the thermostated water supply. Dissolve the receptor containing the fluoride by adding 50 μl of thermostated 0.05 M HCl. Lower the capillary tips close to the surface of the solution in the rotating lid, and start the Landolt reaction by simultaneous addition of the 7-μl fractions of the four reagent solutions. At the same moment, the biamperometric detector with timer is initiated. After the addition of the reagents (ca. 4 s), remove the capillaries from the reaction solution. Appearance of free iodine in the reaction mixture characterizes the induction period of the Landolt reaction and results in stopping of the timer [1], from which the measured time (60—300 s) can be read off. Then raise the electrodes, reset the timer, and repeat the whole measurement cycle with a new receptor lid.

It is advisable to run some blank cycles with empty lids before the first readings are taken. This will ensure thermal equilibrium of the solutions and conditioning of the electrodes.

RESULTS AND DISCUSSION

Calibration graphs and their stability

In the previous paper [1] an empirical function was described relating the measured induction period, t, of the Landolt reaction to the molar ratio, MR, of the inhibitor fluoride to the catalyst zirconium: $t \simeq \exp(-MR)$, when $0.1 \leqslant MR \leqslant 1.0$. The same relation can be taken for the calibration graph in the working range described here, i.e. 0.1—1 nmol of fluoride with about 1 nmol of zirconium present in the reaction mixture.

Because the induction periods for different samples are measured consecutively, stability of temperature and of reagent solutions as well as the reproducible preparation of reaction mixtures are important prerequisites for the reliability of the results obtained. In order to establish how these parameters change during one day, two calibration graphs were prepared, one in the morning, the other one in the late afternoon, by using the same reagent solutions and applying standard amounts of 0.1, 1.0, 0.2, 0.8, 0.4 and 0.6 nmol of fluoride. In these experiments the standards were added directly to the lids from a microliter syringe without previous microdiffusion. The regression lines for the two sets of calibration points obtained exhibited no significant difference at a 99% confidence level:

(1) $t = (412.7 \pm 6.7) - (331.7 \pm 10.2)\ \exp(-MR)$; $n = 26$
(2) $t = (407.5 \pm 7.3) - (325.4 \pm 10.7)\ \exp(-MR)$; $n = 20$

Influence of the perborate concentration

The influence of the perborate concentration on the Landolt reaction can be derived from Fig. 6. Plots are shown of the inverse of the induction period, t, as a function of the molar ratio, BO_3^-: Zr, for different amounts of the inhibitor fluoride; t^{-1} is a measure of the rate of the overall reaction (catalysed + uncatalysed) between perborate and iodide [7]. At low BO_3^-:

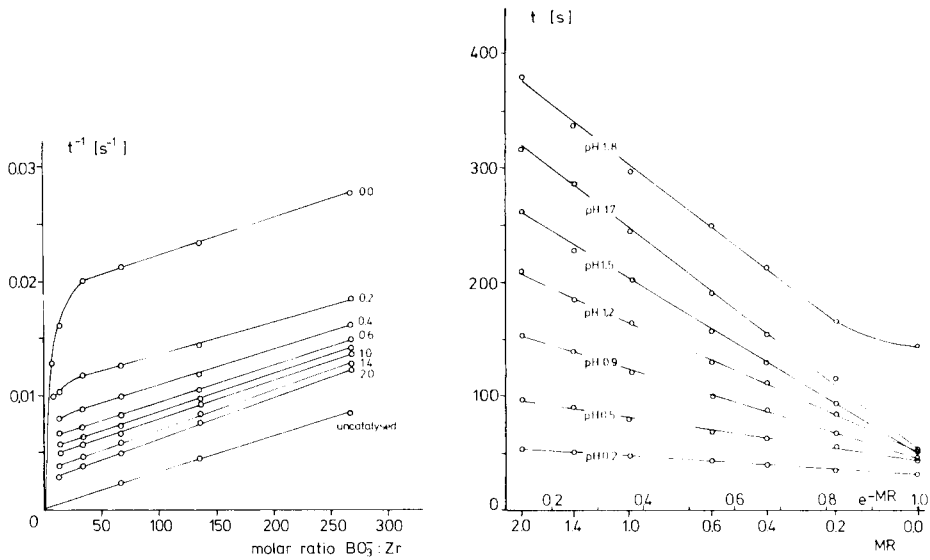


Fig. 6. Influence of the perborate concentration on the rate, t^{-1}, of the reaction between perborate and iodide with various amounts of fluoride present (1 nmol Zr, pH = 1.6). The numbers on the curves indicate the amount of fluoride in nanomoles.

Fig. 7. Influence of pH of the reaction mixture on the calibration graphs for fluoride (0.0—2.0 nmol F^-, 1 nmol Zr, 72 nmol BO_3^-).

Zr molar ratios, large variations in the reaction rate may be caused by small changes in the perborate concentration (especially at small levels of fluoride). At ratios above 40, there is a constant small increase in reaction rate with increasing molar ratio, which is nearly the same for all fluoride amounts. This increase can be interpreted as the contribution of the uncatalysed reaction between perborate and iodide, the rate of which shows a first-order dependence on the perborate concentration. Consequently, the rate of the catalysed reaction must have reached a "plateau" with respect to perborate; the height of the plateau depends on the concentration of fluoride. In this plateau region, the perborate concentration must be fixed only because of the contribution of the uncatalysed reaction, and the choice is not critical.

Influence of pH

Zirconium (IV) is present in dilute aqueous solutions as a polynuclear species, the size of which is pH-dependent. The change in the degree of polymerization with change in pH is slow [8]. Therefore the state of the zirconium catalyst during the measurements can be regarded as constant. Figure 7 shows calibration graphs obtained at different pH values in the range 0.2—1.8. The slope, i.e. the sensitivity of the catalytic—kinetic method, increases with increasing pH to a maximum at pH 1.7; further increase in pH only yields a parallel shift to longer induction periods. In the method described, the pH of the reaction mixture is about 1.6.

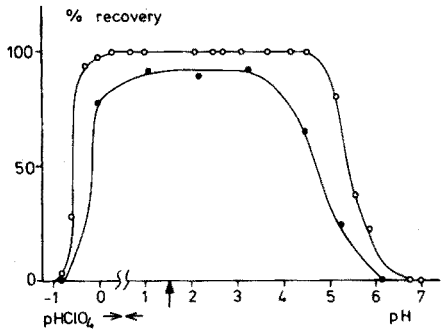
Rate of microdiffusion

The diffusion of fluoride from acidic media is accelerated by hexamethyldisiloxane (HMDSO) [2, 3] by formation of volatile (b.p. 16.4°C) trimethylfluorosilane [9]:

$$HF + (CH_3)_3SiOSi(CH_3)_3 \rightarrow (CH_3)_3SiF + (CH_3)_3SiOH$$
$$HF + (CH_3)_3SiOH \rightarrow (CH_3)_3SiF + H_2O$$

The reaction rate is pH-dependent. To examine this effect 1 nmol of fluoride (pure NaF solutions) was diffused by addition of equal volumes of perchloric acid or hydrogenphthalate buffer solutions of different pH, saturated with HMDSO. One series of experiments was finished after a diffusion time of 30 min and the other after 2 h. The amount of fluoride diffused to the receptor was determined and the pH of the diffusion solution measured with a micro glass electrode. Reaction mixtures without fluoride added showed no fluoride blank. pH measurements of some of the receptors used for microdiffusion from strong acidic medium showed that their capacity was not depleted by neutralization. Figure 8 shows a plot of fluoride recovery against pH of the diffusion solution. After 2-h diffusion times, the recovery is not pH-dependent in the pH range 0.5--4.5. Acid concentrations greater than 1 M reduce the recovery, and 6 M perchloric acid prevents diffusion of fluoride almost completely.

The diffusion rate at room temperature (ca. 20°C) under the conditions



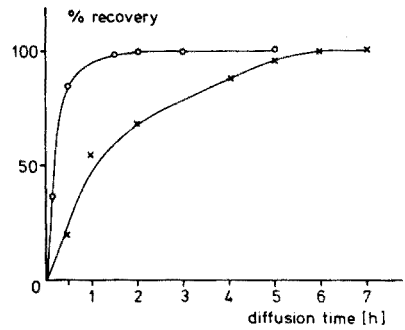


Fig. 8. Recovery of 1.0 nmol of fluoride at various pH values of the diffusion mixture (rotated cells, room temperature). Diffusion times: (•) 30 min, (○) 2 h. The arrow indicates the pH selected.

Fig. 9. Recovery of 1.0 nmol of fluoride after diffusion from aqueous solutions at room temperature. (○) Rotated cells; (x) stationary cells.

given here was investigated with and without rotation of the diffusion devices, by using pure sodium fluoride solutions (1 nmol F^-). The amounts of fluoride transported to the receptor lid after different times were determined; the calibration graph used was obtained with receptor lids loaded with known amounts of sodium fluoride. Figure 9 shows that diffusion starts rapidly and comes to completion after 2 h in the rotated devices and after about 6 h for stationary samples.

Interferences

Serious interferences with the catalytic—kinetic determination of fluoride derive from low amounts of ions which form strong complexes with the fluoride ion or the zirconium catalyst, e.g. Al^{3+} and PO_4^{3-} or SO_4^{2-} [10]. It is possible, however, to overcome these difficulties by the microdiffusion step. The influence of Al^{3+}, Fe^{3+}, Ca^{2+}, $H_2PO_4^-$ and SO_4^{2-} on the diffusion process was investigated by adding solutions containing the interfering ions to 0.0 and 0.5 nmol of fluoride. No interference could be observed for a 25000-fold molar amount of phosphate, a 200000-fold amount of sulphate or a 5000-fold amount of calcium or iron(III). The influence of aluminium shows a strong dependence on the acidity of the diffusion solution. Mixtures of 0.5 nmol of fluoride and 1.25 μmol of aluminium(III) containing different amounts of perchloric acid (total volume 1.1 ml) were diffused for 16 h with rotation. Figure 10 shows a plot of the fluoride recovery (corrected for the fluoride blank of the Al^{3+} solution) against the acid concentration. The decelerating effect of aluminium in samples can be overcome by using larger amounts of perchloric acid and longer diffusion times.

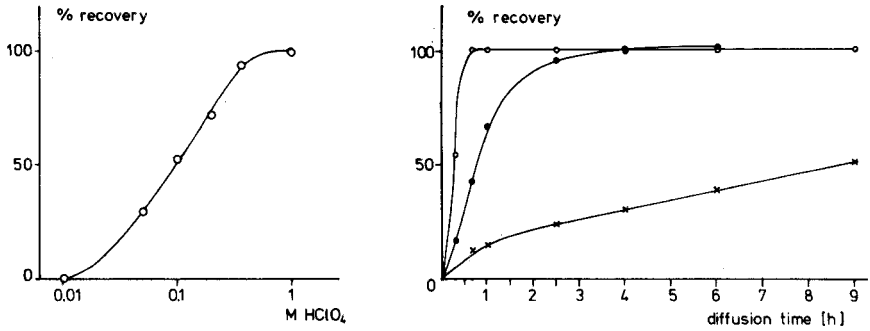


Fig. 10. Recovery of 0.5 nmol of fluoride from solutions containing 1.25 μmol Al^{3+} and various concentrations of perchloric acid (total volume 1.1 ml) after a diffusion period of 16 h (room temperature, rotated cells).

Fig. 11. Recovery of 1 nmol F^- from Whatman No. 40 filters (26 mm diam.) after microdiffusion: (x) at ca. 18°C, (•) at 40°C, (○) at 70°C.

TABLE 1

Results of an interlaboratory analysis of geochemical samples for fluoride

Sample	Fluoride content (ppm) found by				$\bar{x}^b$
	Lab. 4	Lab. 9	Lab. 25[a]	Lab. 27	
Crystal-Granite I	1078	1398/1124	1170	1128	1180
Granite KA-I	759	976/772	810	780	819
Humus Pseudogley	494	598/538	460	506	519
Dacite KA-2	345	464/324	382	413	387
Andesite KA-3	235	354/238	250	278	271

[a]Lab. 25: method described, mean value of 5 measurements, standard deviation ± 4%.
[b]$\bar{x}$: mean value of all laboratories.

Analysis of geochemical samples

In an interlaboratory study [11], the trace element contents of five samples of rocks and soils have been determined. Four laboratories contributed results for fluoride (Table 1). In this laboratory (Lab. 25), milligram portions of the samples were fused with sodium hydroxide in platinum micro crucibles and dissolved in 2 ml of doubly distilled water under ultrasonic agitation. Fractions (50 μl) were analysed for fluoride as described above, including a 16-h diffusion period (overnight). At present, chondritic inclusions with sample weights of about 0.1 mg are being investigated. This is a typical application of the microtechnique.

Analysis of rain waters

Rain-water samples were collected daily between May 1975 and December 1976 at background stations in West Germany, Schauinsland, and El Salvador, Central America, Cerro Verde, in open polyethylene collectors for total deposition [12]. The samples were analysed for several ionic species [13, 14] and also for their total fluoride contents with the method described. The sample volumes required for analysis were 2 ml or less; this suffices for an estimate of whether the sample fits in the working range, and duplicate determinations. Results in the working range of the method (2—19 ppb F^-) were found for 67.3% of the 440 samples analysed. A fluoride content of less than 2 ppb was found for 15% and 19—130 ppb for 17.7% of the samples. The reliability of the method described was checked by analysis of synthetic rain-water samples distributed for collaborative studies by ECN, Petten, The Netherlands. The mean values and standard deviations at a 95% confidence level obtained from 5 replicate measurements of 50—200 μl of the different samples are listed in Table 2.

Analysis of filter samples

It was intended to separate fluoride by microdiffusion directly from airborne dust collected on filters. Various filter materials were investigated for their fluoride impurities and for their applicability to the diffusion process without previous extraction. Whatman No. 40, 41, and 42 cellulose filters and Schleicher and Schüll BA 85 cellulose nitrate membrane filters gave low and reproducible fluoride blanks below 1 ng F^- per filter (26 mm diam.). They were wetted by the aqueous diffusion reagent and produced no interfering products at higher acid concentrations. Storage under a protective atmosphere is recommended to prevent the filters from being contaminated by laboratory air.

Sodium fluoride aerosol particles (μm range) were generated by nebulizing methanolic 1×10^{-4} M and 5×10^{-4} M sodium fluoride solutions in a Berglund—Liu Monodisperse Aerosol Generator [15], collected on Whatman No. 42 filters and analysed for fluoride with the method described; a 6-h diffusion period at room temperature was used. Under constant operating

TABLE 2

Analysis of synthetic rain water for fluoride

Sample	Fluoride content (ppb)		
	Given by ECN	Found by ECN [17]	Present method
3-79 Anion V	118	117	120.5 ± 9.0
3-79 Anion W	27.8	28.0	30.0 ± 4.6
3-79 Anion X	86	83	90.0 ± 6.4
3-79 Anion Y	57	58	60.7 ± 4.6
3-79 Anion Z	222	230	221.2 ± 6.9

conditions (orifice 20 μm, 70 kHz, feed rate 0.3 ml min^{-1}, dispersion air 2 l min^{-1}, dilution air 5 m^3 h^{-1}) and sampling conditions (flow 40 l h^{-1}, filter holder inlet velocity 35 cm min^{-1}), 0.13 ± 0.01 and 0.63 ± 0.03 nmol of fluoride per minute of sampling time were found on the filters in sampling periods of 1—5 min. This corresponds to a sampling yield of about 50%, which is characteristic for the apparatus used [16].

If airborne dust is sampled day-by-day on filters, the filter samples normally have to be stored until a sufficient number has been accumulated for economic analysis. It may be advantageous to use the lag between sampling and analysis for the microdiffusion separation of the fluoride from the filter matrix. Such a possibility was verified by the following experiment. Whatman No. 40 filters (26 mm diam.) spiked with 0.5 nmol of fluoride (NaF) as well as pure unloaded filters were pushed into micro test tubes containing 50 μl of the diffusion reagent. The tubes were closed with prepared receptor lids and stored upright in a cabinet at room temperature. This was done every day during a period of nine days. On the tenth day all the sample lids as well as the blank lids were analysed for their fluoride contents. No statistically significant differences were observed within the sample and blank series, though the mixtures of the first day had about 9 days for microdiffusion and the mixture of the ninth day about 16 h. Because the diffusive separation of fluoride from filter discs exhibits nearly the same time dependence as unrotated solutions (cf. Fig. 9), a diffusion time of about 6 h will normally be sufficient for the investigation of filter samples.

An important parameter affecting the diffusion rate is the temperature. Total recovery of 1 nmol of fluoride from Whatman No. 40 filters (26 mm diam.) was obtained after a 1-h diffusion time at 70°C. From the viewpoint of contamination or losses of analyte it is advisable, however, to work at 20—25°C. A further decrease in temperature will lead to incomplete recoveries even after diffusion time of one day (cf. Fig. 11).

The authors thank the "Arbeitsgemeinschaft Industrieller Forschungsvereinigungen", Köln, F. R. G., for financial support.

REFERENCES

1 D. Klockow, J. Auffarth and Ch. Kopp, Anal. Chim. Acta, 89 (1977) 37.
2 D. R. Taves, Talanta, 15 (1968) 969.
3 R. J. Hall, Talanta, 16 (1969) 129.
4 R. Sara and E. Wänninen, Talanta, 22 (1975) 1033.
5 M. Yoshida, M. Kitami, N. Murakami and T. Katsura, Anal. Chim. Acta, 106 (1979) 95.
6 G. Tölg, Talanta, 21 (1974) 327.
7 G. Svehla, Analyst, 94 (1969) 513.
8 Gmelins Handbuch der Anorganischen Chemie, Zirkonium (System-Nr. 42), Verlag Chemie, Weinheim, 8. Aufl., 1958, p. 306.
9 C. Eaborn, Organosilicon Compounds, Butterworths, London, 1960, p. 171.
10 D. Klockow, H. Ludwig and M. A. Giraudo, Anal. Chem., 42 (1970) 1682.
11 Point-of-main-effort program, Geochemie umweltrelevanter Spurenstoffe, sponsored by the German Research Society.

12 G. Rönicke, K. Dirnagl and D. Klockow, Luftchemische Meß- und Analysenverfahren, Mitteilung IV der Kommission zur Erforschung der Luftverunreinigung, Deutsche Forschungsgemeinschaft, H. Boldt Verlag, Boppard, 1979.
13 D. Klockow, H. Denzinger and G. Rönicke, VDI-Ber., 314 (1978) 21.
14 G. T. Guzman Lopez, in Air Pollution Measurement Techniques, Special Environmental Report No. 10, World Meteorological Organization, Geneva, 1977, p. 70.
15 R. N. Berglund and B. Y. H. Liu, Environ. Sci. Technol., 7 (1973) 147.
16 A. Teckentrup, Thesis, University of Dortmund (1979).
17 J. Slanina, F. Bakker, C. Lautenbag, W. A. Lingerak and T. Sier, Mikrochim. Acta, (1978) 519.

Analytica Chimica Acta, 111 (1979) 103—109

EVALUATION OF NEW HIGH-FREQUENCY DISCHARGE LAMPS FOR ATOMIC ABSORPTION AND ATOMIC FLUORESCENCE SPECTROMETRY OF CADMIUM, LEAD AND ZINC

K. TSUJII*, K. KUGA, S. MURAYAMA and M. YASUDA

Central Research Laboratory, Hitachi Ltd., Kokubunji, Tokyo (Japan)

(Received 22nd May 1979)

SUMMARY

High-frequency discharge lamps with a hollow electrode are successfully utilized as the spectral line sources for atomic absorption and atomic fluorescence spectrometry of cadmium, lead and zinc. The sensitivities for atomic absorption spectrometry are superior to those obtained with commercially available hollow-cathode lamps by factors of 1.5 (Cd), 1.4 (Pb) and 1.6 (Zn). Detection limits for non-dispersive atomic fluorescence spectrometry with graphite furnace atomization are 1×10^{-13} g (Cd), 3×10^{-11} g (Pb) and 2×10^{-13} g (Zn). The linear analytical range covers over four (Cd, Zn) and three (Pb) decades of concentration above the detection limits.

It is well known that the sensitivity of atomic absorption spectrometry depends on the spectral line width of the atomic resonance line. This has spurred the use of narrow line sources such as hollow-cathode lamps, but such lamps often provide relatively poor intensity at the atomic resonance line; this results in a poor signal-to-noise ratio. In such cases, a high-intensity light source such as an electrodeless discharge lamp can be utilized to provide sufficient spectral output. High spectral intensity of the light source is even more desirable in atomic fluorescence spectrometry where fluorescence intensity is directly proportional to source intensity [1—3]. High-intensity hollow-cathode lamps and electrodeless discharge lamps have been commonly used in atomic fluorescence spectrometry.

A new spectral line source with a hollow electrode, which is operated by high-frequency power, was recently developed in this laboratory. In a previous report [4], the radiances of the new lamps were compared with those of hollow-cathode lamps for aluminum, cadmium, chromium, nickel, titanium and vanadium, and an application to coherent forward scattering measurement was described.

This paper reports the successful application of the new lamp as the radiation source for atomic absorption and atomic fluorescence spectrometric measurement of cadmium, lead and zinc.

EXPERIMENTAL

Apparatus

The construction of the high-frequency discharge lamp is shown in Fig. 1. The hollow electrode (3 mm i.d., 8 mm o.d., length, 15 mm; hollow depth, 10 mm) is made of a cadmium—silver alloy for cadmium, a lead—silver alloy for lead and a zinc—silver alloy for zinc. The nickel ring electrode is located 5 mm from the hollow electrode. High-frequency (20 MHz) alternating voltage is supplied between the hollow and ring electrodes. The lamp is surrounded by a copper cylinder (37 mm i.d.), which prevents the radiation of high-frequency power from the lamp. The copper cylinder and the ring electrode are electrically connected and grounded. The lamp is designed so that the high-frequency alternating current flows mainly through the inner surface of the hollow cylinder. This was achieved by surrounding the hollow cylinder with a double-walled glass tube through which cooling water flows; the inner diameter of this glass tube must be made with a tolerance of only ± 100 μm. Argon was used as the filler gas, the pressure being maintained at 4 torr for each lamp.

The atomic absorption spectrometer utilized was a Perkin-Elmer Model 403 equipped with a three-slot air—acetylene burner.

Atomic fluorescence measurements were made in the non-dispersive mode with a graphite furnace atomizer as described previously [5]. The beam from the radiation source was mechanically modulated at 115 Hz and focused immediately above the graphite tube by a quartz lens (30 mm diameter, 50 mm focal length). The beam diameter just above the graphite furnace and the distance between the center of the beam and the top of the furnace were 3 mm and 2 mm, respectively. The fluorescence was focused by a quartz lens (50 mm diameter, 100 mm focal length), positioned at 90° to the excitation beam, and was detected by a solar blind photomultiplier (HTV R166). A John Fluke power supply (Model 412B) was used to provide

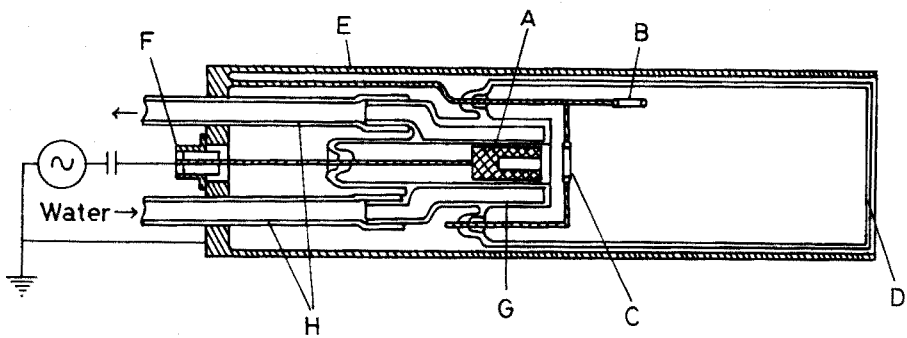


Fig. 1. Construction of high-frequency discharge lamp. A, hollow electrode; B, getter; C, ring electrode; D, quartz window; E, copper cylinder; F, connector; G, double-walled glass tube; H, poly(vinyl chloride) tube.

high voltage (700 V) to the photomultiplier, signals from which were fed into a lock-in amplifier (Princeton Applied Research Corp., Model HR-8); the peak heights displayed on a recorder (Hitachi Model 056) were measured as the fluorescence intensity. A mirror system was not used in the optical alignment. The sample solution was added by an Eppendorf pipette (10 μl) with a plastic tip.

The experimental conditions utilized in atomic absorption and atomic fluorescence measurements are shown in Table 1. The furnace temperatures given for atomic fluorescence measurements are the values at the exit of the graphite tube [5], which were measured with a Pt/PtRh thermocouple for drying and ashing, and with an optical pyrometer for atomizing. The ashing temperature chosen was 100°C lower than the lowest value at which significant loss of the particular element occurred. The atomizing temperature was chosen so as to provide the maximal signal-to-noise ratio for each element.

Reagents

Cadmium, lead and zinc stock solutions (1000 ppm) were prepared by dissolving 99.99% cadmium, lead or zinc metal in nitric acid. Each stock solution was stored as a 2 M nitric acid solution. More dilute standard solutions were prepared as required. All other reagents were of analytical-reagent grade.

RESULTS AND DISCUSSION

Lamp stability

The time dependence of the emission intensity was measured for each lamp. After the initial break-in period, only a 3-min warm-up period was required to achieve stability after initiation of the discharge. A typical recorder tracing of the variation of cadmium emission intensity at 228.8 nm with time is shown in Fig. 2. Similar tracings were obtained for lead and zinc lamps. The short term (about 1 min) intensity variation was less than 1% over several

TABLE 1

Experimental conditions

Atomic absorption		Atomic fluorescence	
Acetylene flow	13 l min^{-1}	Argon flow	0.1 l min^{-1} (carrier gas)
Air flow	21 l min^{-1}		1.0 l min^{-1} (sheath gas)
Distance from burner top to beam center	15 mm	Drying (Cd, Pb, Zn)	120°C, 30 s
Wavelength (Cd)	228.8 nm	Ashing (Cd)	300°C, 20 s
(Pb)	283.3 nm	(Pb)	500°C, 20 s
(Zn)	213.9 nm	(Zn)	400°C, 20 s
Spectral bandwidth	0.7 nm	Atomizing (Cd)	1700°C, 5 s
		(Pb)	2000°C, 5 s
		(Zn)	1800°C, 5 s

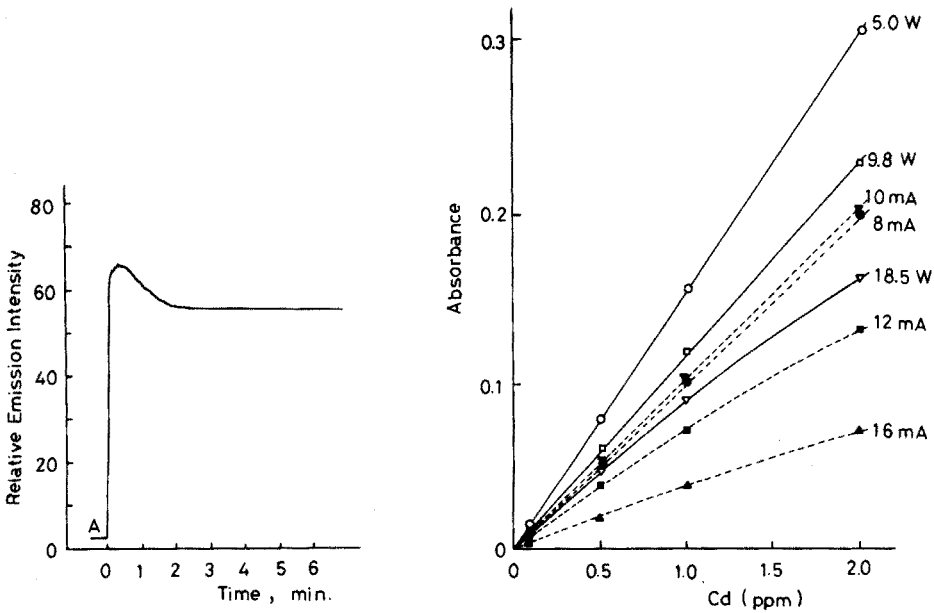


Fig. 2. Time dependence of cadmium emission intensity from the high-frequency discharge lamp. *A* denotes 14-W power on.

Fig. 3. Effect of lamp operating conditions on cadmium sensitivity by atomic absorption spectrometry: (----) hollow-cathode lamp (Westinghouse WL36016); (—) high-frequency discharge lamp.

hours for each lamp. The time constant of the recorder was kept at 0.5 s in the stability measurements. The high-frequency power quoted in this paper means the net power for the discharge, i.e. the reflected power is subtracted from the incident power.

Atomic absorption measurements

The sensitivities obtainable for the atomic absorption spectrometric measurements of the elements with the new lamps and commercially available hollow-cathode lamps were compared. The calibration graphs for cadmium, lead and zinc obtained are shown in Figs. 3, 4 and 5, respectively. In these measurements no special care was taken to secure maximal signal-to-noise ratio, and only the lamp operating conditions were changed. The sensitivities for cadmium, lead and zinc increase with decreasing power to the high-frequency lamps, probably because of the decreasing line widths emitted from the light sources. However, the power has a lower limit for steady lamp operation, and maximal sensitivities were obtained at 5 W in all cases, as compared with 10 mA (Cd), 6 mA (Pb) and 24 mA (Zn) for the hollow-cathode lamps. The sensitivities achieved by the new lamps were superior to those for the hollow-cathode lamps by factors of 1.5 (Cd), 1.4 (Pb) and 1.6 (Zn), respectively.

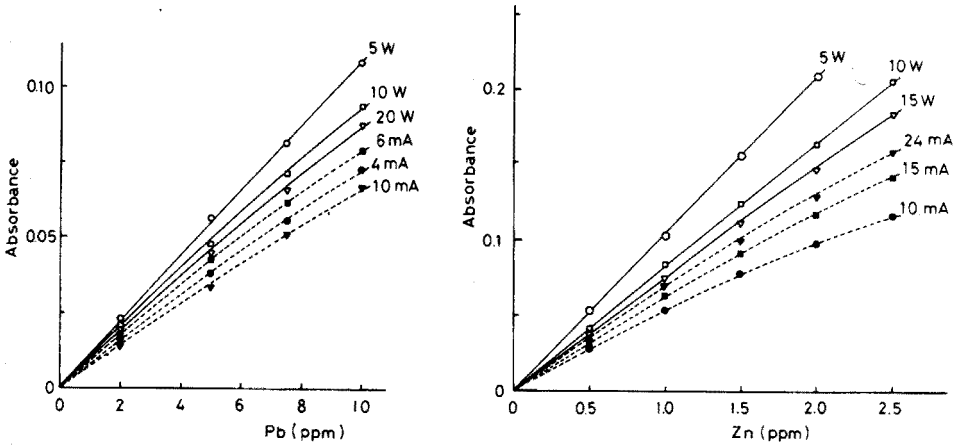


Fig. 4. Effect of lamp operating conditions on lead sensitivity by atomic absorption spectrometry: (----) hollow-cathode lamp (Westinghouse WL36039); (—) high-frequency discharge lamp.

Fig. 5. Effect of lamp operating conditions on zinc sensitivity by atomic absorption spectrometry: (----) hollow-cathode lamp (Perkin-Elmer 8129); (—) high-frequency discharge lamp.

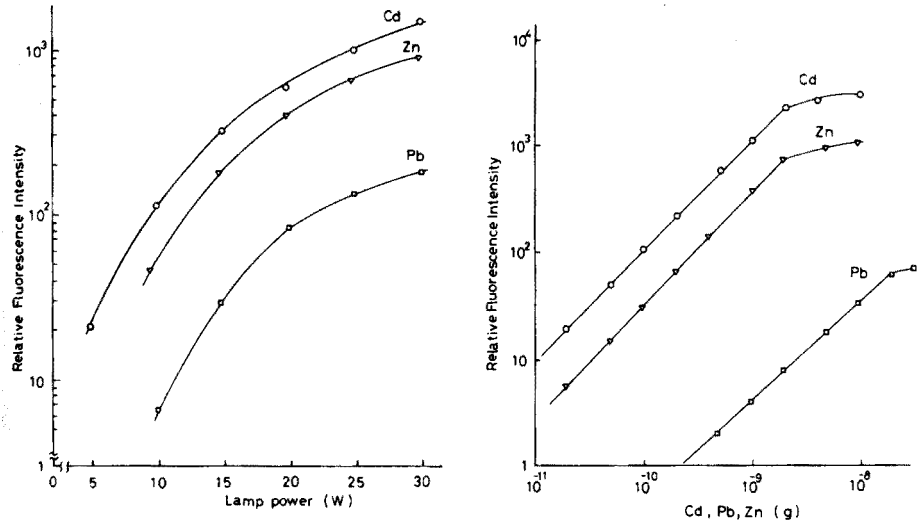


Fig. 6. Dependence of atomic fluorescence intensity on high-frequency power to the lamp.

Fig. 7. Calibration graphs for atomic fluorescence measurements of cadmium, lead and zinc.

Atomic fluorescence measurements

The dependence of atomic fluorescence intensity on high-frequency power was measured for each lamp. The fluorescence intensities increased with

increasing power as shown in Fig. 6. The detection limits (signal-to-noise ratio of 2) were 1×10^{-13} g (Cd), 3×10^{-11} g (Pb) and 2×10^{-13} g (Zn), when the lamps were operated at 25 W (Cd), 16 W (Pb) and 25 W (Zn). The calibration graphs were linear over about four (Cd and Zn) and three (Pb) decades of concentrations above the respective detection limits (Fig. 7). The inferior detection limit and linear analytical range for lead possibly arise because the most sensitive fluorescence spectral line of lead (405.8 nm) [6, 7] is out of the spectral response range of the solar blind photomultiplier (160—320 nm).

Applications to practical analyses by non-dispersive atomic fluorescence spectrometry

The high-frequency cadmium lamp was applied to the determination of cadmium in a waste water, which was taken at the inlet of the waste-water treatment facility of this laboratory. A 2-ml sample of 1-ppm cadmium solution was transferred to a 100-ml volumetric flask and diluted to the mark with a waste water in which no cadmium was detected. A 10-μl aliquot was taken and the atomic fluorescence signal measured under the conditions given in Table 1. The results are shown in Table 2, along with results obtained by using the hollow-cathode lamp. Similar precision was obtained with both sources, indicating that the precision limitation is mainly determined by the pipetting and atomizing processes.

The new zinc lamp was applied to the determination of zinc in gallium arsenide crystals. A sample (0.1 g) of gallium arsenide was weighed exactly and dissolved in 8 ml of aqua regia. The solution was evaporated to dryness and the residue redissolved in 2 ml of 6 M hydrochloric acid. A 5-ml aliquot of 50% (w/v) ammonium citrate and 5 ml of 2 M ammonium acetate solution were added and the pH was adjusted to 8.0—8.5 by adding aqueous 1.5 M ammonia solution. A 10-ml amount of chloroform, 0.002% in dithizone, was added and zinc was extracted as its dithizonate. The extraction was repeated three times with 10-ml portions of chloroform, before back-extracting the combined extracts with 5 ml of 0.05 M hydrochloric acid. Prior to the atomic fluorescence measurements, a new graphite tube was installed and the tube was heated repeatedly until no significant light scattering signals caused by carbon particles were observed. A 10-μl aliquot of the back-extracted solution

TABLE 2

Determination of cadmium added to a waste water

Radiation source[a]	Mean cadmium found (n = 10) (ppm)	R.s.d. (%)
A	0.020	7.2
B	0.020	7.1
C	0.020	7.5

[a](A) Hollow-cathode lamp (Westinghouse WL36016) operated at 10 mA; (B) high frequency lamp operated at 10 W; (C) high-frequency lamp operated at 25 W.

TABLE 3

Determination of zinc in gallium arsenide crystals

No.	Zinc (μg)		R.s.d. (%)	Recovery (%)
	Added	Found[a]		
1	0	0.65	6.9	—
2	1.0	1.61	5.2	96
3	5.0	5.72	2.9	102

[a]Mean of 5 determinations.

was injected and the atomic fluorescence signal was measured under the conditions given in Table 1. The results of these experiments (Table 3) show that recoveries of added traces of zinc are satisfactory.

Conclusion

The new high-frequency discharge lamps have been successfully utilized as radiation sources for both atomic absorption and atomic fluorescence spectrometry. Further experiments are in progress to construct high-frequency discharge lamps for a number of other elements.

We are grateful to Dr. Kazuo Yasuda for supporting this work, and to Dr. Manabu Yamamoto and Messrs. Konosuke Oishi and Masaru Ito for their encouragement.

REFERENCES

1 J. D. Winefordner, M. L. Parsons, J. M. Mansfield and W. J. McCarthy, Spectrochim. Acta, Part B, 23 (1967) 37.
2 P. J. T. Zeegers, R. Smith and J. D. Winefordner, Anal. Chem., 40 (1968) 26A.
3 J. D. Winefordner and R. C. Elser, Anal. Chem., 43 (1971) 24A.
4 S. Murayama, M. Yasuda, M. Ito, K. Oishi and M. Yamamoto, Spectrochim. Acta, Part B, 34 (1979) 159.
5 K. Kuga and K. Tsujii, Anal Chim. Acta, 81 (1976) 305.
6 V. Sychra and J. Matousek, Talanta, 17 (1970) 363.
7 R. F. Browner, R. M. Dagnall and T. S. West, Anal. Chim. Acta, 50 (1970) 375.

Analytica Chimica Acta, 111 (1979) 111—122

A STUDY OF THE DISPROPORTIONATION OF MERCURY(I) INDUCED BY GAS SPARGING IN ACIDIC AQUEOUS SOLUTIONS FOR COLD-VAPOR ATOMIC ABSORPTION SPECTROMETRY

RICHARD J. BALTISBERGER*, DAVID A. HILDEBRAND**, DAVID GRIEBLE***, and THOMAS A. BALLINTINE

Department of Chemistry, University of North Dakota, Grand Forks, North Dakota 58202 (U.S.A.)

(Received 21st June 1979)

SUMMARY

Instrumentation and procedures for the cold-vapor atomic absorption determination of mercury have been modified. Samples are analyzed by syringe injection under reducing and non-reducing conditions so as to allow mercury valence state differentiation. It is shown that chloride ion is effective in preventing mercury(I) disproportionation; in the absence of a strong mercury(II) complexing ligand, mercury(I) readily disproportionates when solutions are sparged with nitrogen. The data are consistent with the formation at the 10—500 ppb level of a Hg_2Cl_2 precipitate with a lower solubility product than the literature values.

The determination of mercury in environmental water samples is of interest because of its toxicological effect on plant and animal life. There is a continuous need to measure mercury quantitatively in its inorganic and organomercurial forms in various media. This is because of the extensive industrial and agricultural use of mercury compounds coupled with the ability of microorganisms to convert the inorganic forms to organomercurial compounds [1, 2]. Several analytical procedures have been used to differentiate the inorganic or organic forms including gas chromatography [3—6] and selective reduction conditions in atomic absorption work [7].

Determination of the concentrations of inorganic mercury according to oxidation state is of interest in understanding the role and transport of the element in environmental or biological samples. One of the major mechanisms proposed for the loss of mercury is that of volatilization of Hg(0) produced through reduction of Hg(II) with trace reductants (microorganisms or humic acid) to form Hg(I) which may in turn disproportionate to form volatile elemental mercury [8]. This study was undertaken to establish if quantitative measurements of the Hg(0) and Hg(I) oxidation

**Present address: Vulcan Materials Co., Wichita, Kansas, 67201, U.S.A.

***Present address: Department of Chemistry, Ohio University, Athens, Ohio 45702, U.S.A.

states of mercury were possible in pure water and to determine their possible analytical utility. It was found that in acidic chloride media the formation of the highly insoluble Hg_2Cl_2 species allowed the vaporization and measurement by cold-vapor atomic absorption techniques of elemental mercury dissolved in aqueous solution and prevented any disproportionation of mercury(I) present. The precipitate formed at 10—500 ppb mercury(I) levels does not undergo disproportionation in 0.001—0.01 M chloride media as predicted from thermodynamic calculations. The data suggest that the activity of the $Hg_2Cl_2(s)$ formed at these low concentrations is considerably less than when large quantities (10—100 mg) of $Hg_2Cl_2(s)$ are present in solution.

EXPERIMENTAL

Instrumentation

The instrumentation used is shown in Fig. 1. Solutions were analyzed by cold-vapour atomic absorption measurement of the elemental mercury collected by nitrogen sparging of the sample in the cell. The reaction cell consisted of a 20-mm fritted tube (A. H. Thomas Company type 4798-K10) to which a 5—8-mm side arm was added for attachment of the rubber septum (A. H. Thomas Company) above the medium-porosity glass frit. Below the frit the tube was tapered for connection to 6-mm glass tubing and two stopcocks. Ball and socket joints were added just above and below the cell to allow for cleaning and draining. A 24/40 joint was added 2 cm above the frit so that the cell could be further dismantled for cleaning. During analysis, the nitrogen carrier gas was regulated to a flow of 200 ml min^{-1} by means of a pressure reducing valve. Flow rates through the system were measured with a soap bubble flow meter attached to the end of the gas train (gas cell outlet). The drying tube was made of Teflon tubing, filled with dry sodium hydroxide and closed with quick-connect ends. The vaporized mercury was measured in a 30-cm gas cell as part of an LDC mercury analyzing system (Laboratory Data Control, Riviera Beach, Fla. model 1205). The absorbance read at 254 nm was recorded on an LDC model 330 flat-bed recorder.

Reagents and solutions

All reagents used were analytical grade except where stated. All water was triply distilled; the second distillation was made from alkaline permanganate solution followed by a third distillation in a glass still. Stock solutions of 10^{-3} M mercury(II) were prepared from mercury(II) acetate (Fisher Scientific Company). These solutions were standardized by titration with sodium thiocyanate and diluted to final concentrations with water on the day of their use. Mercury(I) and (0) solutions were standardized against these mercury(II) solutions by cold-vapor atomic absorption.

Elemental mercury used in the preparation of mercury(0) solutions was obtained by distillation from mercury(II) oxide under a nitrogen atmosphere. Solutions saturated in mercury(0) were prepared and stored under a nitrogen

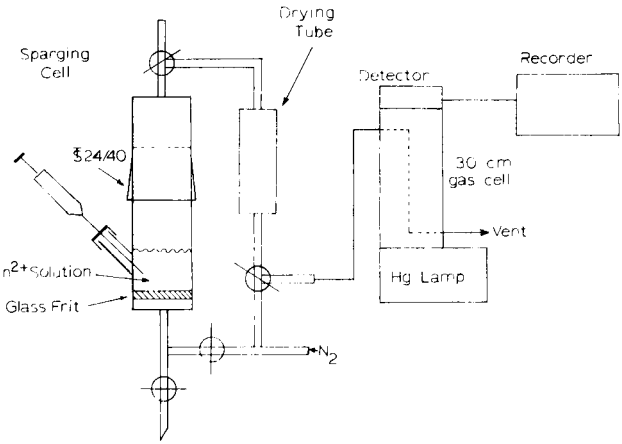


Fig. 1. Mercury analyzing system.

atmosphere in distilled water, 0.01 M sodium chloride or 0.1 M perchloric acid. In order to minimize oxidation, it was necessary to sparge the solutions with nitrogen for 45 min prior to the introduction of a drop of mercury. After addition of a drop of mercury, the solution was sparged with nitrogen for another 30 min. The solutions were stirred for 24 h before use with a glass-covered magnetic stirring bar. Samples of solution were then withdrawn by syringe with a nitrogen flow maintained to prevent oxygen diffusion into the solutions.

Mercury(I) solutions were prepared by the addition of a few drops of elemental mercury to a mercury(II) solution. In this preparation an aliquot of mercury(II) stock in 0.01 M sodium chloride, 0.01 M perchloric acid or 0.01 M sulfuric acid was syringed through a stopcock into a 250-ml round bottom flask which had been previously flushed with nitrogen. After the solution in the flask had been sparged for another 30 min with nitrogen, a few drops of freshly distilled mercury were added to the solution by syringe and the mixture was sparged for another 30 min. Stirring was again carried out with a glass-covered stirring bar. Teflon bars were found to adsorb ionic mercury. Reduction of the Hg(II) to Hg(I) by the elemental mercury required mixing for about 5 h. Samples for analysis were then removed by syringe with a flow of nitrogen to prevent introduction of air into the flask. Since the equilibration of the mercury(II) was carried out over a drop of mercury, the resulting solution would contain mercury(I) equal to the original mercury(II) concentration and a saturation level of elemental mercury. For example, a 200-ppb mercury(II) solution (10^{-6} M) would reduce to 400 ppb mercury(I) (10^{-6} M) and 63 ppb mercury(0) at 30°C. It was sometimes desirable to minimize the amount of mercury(0) in the equilibrium mixture. This could be done by equilibration of a 1000 or

2000 ppb mercury(II) solution with a drop of mercury. The resulting mercury(I) concentration was more than ten times larger than the mercury(0) which remained at its solubility value of near 60 ppb; Several investigators have found the solubility of mercury in water to be about 63 ppb at 30°C [9—13]. Dilution of this solution with distilled water after removal from the drop of mercury would give a solution of 10—200 ppb mercury(I) which contained only 1—2% mercury(0).

Glassware was tested by a.a.s. for mercury contamination prior to use. Mercury was found to be adsorbed and desorbed from glassware depending on the acidity, complexing ligand content and mercury concentration in the contact solution. Several types of plastic containers were tested, but were found to adsorb mercury to a greater extent than glassware (borosilicate). Glassware was soaked in a 0.001 M sodium chloride and 0.01 M perchloric acid mixture for 24 h and then washed with 0.01 M perchloric acid. The soaking in 0.001 M sodium chloride was repeated three times prior to use. At pH 2.0, sodium chloride solution was found to be very effective in removing mercury contamination from glassware. The last 0.001 M wash solution was analyzed for mercury content by a.a.s. If this solution contained less than 1 ppb total mercury, the glassware was used for preparation of mercury(II) solutions. Adsorption of mercury(II) from solution was evident from the time of preparation. It was found that a 10—100 ppb solution could be prepared and used within one day with less than 2% loss of mercury(II). Elemental mercury solutions were always prepared in the round-bottom flasks over a drop of mercury, as previously described. The solubility of mercury remained constant in these solutions and adsorption or desorption on the glassware was no problem. Mercury(I) solutions were prepared by addition of a mercury(II) solution to a mercury(0) solution under nitrogen. No adsorption problems were experienced here if the mixture was analyzed on the same day as that of preparation.

Procedures

The analytical procedure was first optimized for flow conditions and sample size. Next the reaction cell solution conditions were optimized so that dissolved elemental mercury, mercury(I) or mercury(II) could be determined. Total inorganic mercury content of a sample was determined by reduction with tin(II) in either alkaline or acidic solution [7]. Both solutions gave similar responses for the same concentration of inorganic mercury. Reduction under acid conditions, in which neither methylmercury(II) nor other organomercurials are reduced, provides a method of determining the total inorganic mercury. Reduction under alkaline conditions provides results for total (i.e. inorganic and organically bound) mercury.

The acidic tin(II) solution was prepared by dissolving 8.8 g of $SnCl_2.2H_2O$ and 2 ml of concentrated hydrochloric acid in water to give a final volume of 100 ml. For analysis, 10 ml of this solution was added to the reaction cell and up to fifteen injections of 0.2-ml samples were done before the reduction solution in the cell was replaced. The alkaline reducing solution was

prepared in two parts in order to minimize precipitation of tin. The first solution was prepared by dissolving 8.8 g of $SnCl_2 \cdot 2H_2O$ in 100 ml of water; the second solution of 5 g of cadmium chloride, 56 g of sodium hydroxide and 100 ml of water was prepared. Since cadmium hydroxide precipitates in alkaline solution, it must be shaken well before use. Equal portions (2.5 ml) of these two solutions were added to the reaction cell. Six analyses of 0.2-ml injections were done before replacement of the alkaline reducing solution.

Peak height or area response of the u.v. absorbance could be used for calibration in terms of concentration when the following procedures were observed. The flow rate of nitrogen sparge gas must be regulated to a constant flow rate; flow rates of 100—200 ml min^{-1} were used. When peak height was used for calibration then the sample injection volume and reactor solution volume had to be kept reasonably constant. For example, a 5-ml reaction solution gave a peak height of 60 chart units compared to 50 units for a 15-ml volume for the injection of a 0.2-ml sample of the same mercury(II) solution. Increased reaction volume increased the volume of the sparged mercury vapor resulting in a lower peak height. With a limit of fifteen 0.2-ml injections into 15 ml of reaction solution it was found that a decrease of less than 7% of the peak height occurred. Likewise the actual sample volume was limited to 0.5 ml or less so that the peak height would not be affected. These volume restrictions could be avoided by integration of the peak areas. The linear range of the system was 2×10^{-9}—4×10^{-7} g of mercury per sample injection. The standard deviation was ±3% for ten 0.2-ml injections of a 42-ppb mercury(II) solution into 15 ml of reducing solution.

Determinations of elemental mercury dissolved in solution or formed by induced disproportionation of mercury(I) were done in a reaction cell which had never contacted reducing agents. Mercury(0) was vaporized from a 0.01 M hydrochloric acid solution by injection of a solution of mercury(0) to the reaction cell. It was possible to prevent the disproportionation of Hg(I) to form Hg(0) in nonreducing solutions by the addition of chloride ion. The slow rate of mercury(I) chloride disproportionation allowed the measurement of the individual Hg(0) and Hg(I) species concentration. This point will be explained in the Results and Discussion. Extreme care must be taken not to contaminate the cell with reducing agents. Because these reagents were used in the determination of total mercury in solution, it was necessary to reserve for mercury(0) determinations a separate reaction cell which had never contacted reducing agents. Separate syringes were also used to eliminate the possibility of contamination. The peak-height response factor for the nonreducing mixture for determinations of mercury(0) was identical to that for the acidic reducing mixture. This response in chart units for 45 ppb mercury(0) was 49 ± 2 units for six analyses by each method. To demonstrate further that the comparison of peak heights between the reduction and nonreduction mixtures was valid, a series of experiments was done by a gold amalgamation procedure. In these experiments, 0.2 ml of 63 ppb mercury(0) solution was sparged in an acidic reducing solution and the

mercury was collected on gold. The mercury was then vaporized from the gold by heating, and its u.v. absorbance measured. In a second experiment, 0.2-ml samples of 63 ppb mercury(0) solution were vaporized from non-reducing mixtures, collected on gold, and analyzed in the same manner. The value obtained for the nonreducing analysis was 63 ± 2 ppb compared to 65 ± 2 ppb for the reducing mixture. The elemental mercury solution was prepared by equilibration of a drop of mercury with water at 30°C.

RESULTS AND DISCUSSION

Solubility of elemental mercury in water

The first attempts to measure the solubility of elemental mercury in water were made by Bonhoeffer and Reichardt in 1929 [14]. The most recent studies report the solubility of elemental mercury at 25°C to be near 60 ppb (Table 1). Each of the solubility values was obtained by a different technique. Choi and Tuck [9] measured the value using neutron activation analysis to quantify the mercury present in a vial of air-free purified water which had been equilibrated with mercury vapor. Sanemasa [10] prepared purified water samples equilibrated with mercury vapor under nitrogen. The dissolved mercury was then measured by injection of an aliquot of the solution to a tin(II)—sulfuric acid solution in a cold-vapor a.a.s. system. Onat [11] measured the solubility by conversion of the dissolved mercury(0) to mercury(I) by the addition of a stoichiometric excess of mercury(II) to oxidize all mercury(0) to mercury(I). The mercury(I) content was then measured by ultraviolet spectrophotometry at the 236.5-nm absorption band of the mercury(I) dimer, Hg_2^{2+}. Glew and Hames [12] equilibrated purified water with liquid mercury and a trace of reducing agent (sodium sulfite, hydrazine or sodium tetrahydroborate) under helium for 2 h and then measured the dissolved mercury by cold-vapor a.a.s. using tin(II)—hydrochloric acid reductants. Moser and Voigt [13] brought water in contact with mercury labelled with ^{203}Hg and measured the resultant dissolved mercury by counting techniques. These investigators found it necessary to add hypophosphorous acid to prevent radiolytic oxidation of the elemental mercury. In addition, Moser and Voigt [13] found the value of the equilibrium constant for the disproportionation reaction (1) to be 5.5×10^{-9} at 25°C in 0.01 M nitric acid.

$$Hg_2^{2+} \rightleftharpoons Hg^{2+} + Hg^0(aq) \qquad (1)$$

The value of K in this case includes the activity (solubility) of elemental mercury in the constant.

The solubility of mercury was measured in the present work by injection of an aliquot of an equilibrated solution of liquid mercury and purified water to a non-reducing cell as described in the experimental section. The only problem experienced was that solutions containing only water and mercury metal were quite prone to oxidation and on several occasions the

TABLE 1

Reported values for the solubility of mercury in pure water and sodium chloride

Temperature (°C)	Solubility (ppb)	Ref.	Temperature	Solubility	Ref.
25	61	11	40	81	12
25	60	13	30	63 ± 2[a]	b
25	64	10	23	59(0.1 M HCl)	b
25	63	9	23	59(0.01 M NaCl)	b
25	59	12	23	57(0.1 M NaCl)	b
30	64	12	25	55(sea water)	10

[a]Standard deviation, ten analyses. [b]This work.

total mercury sparged from solution was considerably larger than 63 ppb. This occurred via reaction (1) in which the ionic mercury formed by oxidation, present as Hg_2^{2+}, would disproportionate on sparging. Solutions equilibrated in HCl or NaCl media over a drop of mercury always gave values near 57—59 ppb at 23°C which agrees well with the literature value of 55 in sea water at 25°C [10]. The presence of chloride prevented the disproportionation of any mercury(I) through formation of the very insoluble Hg_2Cl_2 thus lowering the activity of Hg_2^{2+}. These data indicate that it is possible to determine the dissolved mercury(0) in an aqueous solution by direct sparging in the absence of reducing or oxidizing agents.

Sparging induced mercury(I) disproportionation

The solution conditions under which the disproportionation of mercury(I) can be induced by nitrogen sparging were investigated. The disproportionation equilibrium over a drop of mercury in the absence of complexing ligands slightly favors the stability of mercury(I):

$$Hg_2^{2+} \rightleftharpoons Hg^{2+} + Hg^0\,(l) \qquad (2)$$

The value of the equilibrium ratio, $[Hg^{2+}]/[Hg_2^{2+}]$, over a drop of mercury has been found to be 0.0077 at 25°C in a 0.5 M perchlorate medium [15]. The product of the solubility of elemental mercury (3×10^{-7} M) times this ratio should be equal to the disproportionation constant of 5×10^{-9} measured by Moser and Voigt in 0.01 M HNO_3 [13]. The slight difference between the values probably reflects a difference in activity coefficients between the two media. In the absence of complexing agents the vaporization of elemental mercury from a mercury(I) solution should shift the equilibrium towards a quantitative disproportionation. Table 2 shows the amount of mercury which was vaporized by sparging from a series of solutions containing Hg(I) and Hg(0). The analysis includes mercury in all forms in solution, Hg(II), Hg(I) and a saturation concentration (63 ppb) of Hg(0). The second column indicates the total mercury in these solutions as measured by the reduction—cold-vapor a.a.s. system. The third

TABLE 2

Analysis for volatile mercury in various solutions at 23°C

Solution	Total Hg (ppb)	Volatile Hg (ppb)	Expected solubility Hg(0) (ppm)
0.18 M H_2SO_4	288	166	55[a]
0.1 M $HClO_4$	600	97	55
0.01M $HClO_4$	600	268	55
0.01 M $HClO_4$ (dist.)	600	330	55
0.1 M HCl	600	59	55
0.001 M NaCl[c]	600	43	43[b]
0.01 M NaCl	600	57	55
0.01 M NaCl	172	62	60
0.1 M NaCl	600	57	55

[a]Solubilities taken from ref. [10]. [b]At 20°C. [c]0.001 M $HClO_4$ (distilled) added to NaCl solutions to prevent hydrolysis of Hg(I). [d]25°C.

column indicates the total quantity of vaporizable mercury measured in a nonreducing sparging cell. Vaporization from perchloric or sulfuric acid media approaches a 50% release of all the mercury in solution, suggesting that mercury(I) was quantitatively disproportionated under these conditions.

In the presence of complexing ligands, the position of equilibrium for reaction (1) or (2) is determined by the relative stability of the Hg(I) and Hg(II) species formed. The addition of hydroxide, cyanide or sulfide to 10^{-3} M mercury(I) solutions shifts the mercury to the more stable HgS, HgO and $Hg(CN)_2$ resulting in a production of mercury(0) [16]. The addition of a halide such as chloride or bromide results in the formation of very insoluble Hg_2Cl_2 or Hg_2Br_2 which minimizes disproportionation via the reaction

$$Hg_2Cl_2(s) \rightleftharpoons Hg + HgCl_2 \qquad (3)$$

Lindgren et al. [17] have measured the necessary equilibrium constants in 0.05 M perchlorate media to estimate the concentration of $HgCl_2$ over solid Hg_2Cl_2 and a drop of elemental mercury, giving the values $[HgCl_2] = K_{sp}K_1K_2K = 1.2 \times 10^{-7}$ M. Here K_{sp} is the solubility product of Hg_2Cl_2, K_1K_2 are the formation constants of $HgCl^+$ and $HgCl_2$, and K is the equilibrium ratio of $[Hg^{2+}]/[Hg_2^{2+}]$ over a drop of mercury. In addition, Lindgren et al. found K_3 for $HgCl_3^-$ formation to be 10, which would convert about 50% of $HgCl_2$ to $HgCl_3^-$ in 0.1 M chloride media. They did not find experimental evidence for the existence of $Hg_2Cl^+(aq)$ or $Hg_2Cl_2(aq)$ and, therefore, did not include terms for the formation of these species. These data, then, predict that appreciable disproportionation should result on sparging a 10—600 ppb mercury(I) (10^{-7}—10^{-5} M) solution in chloride media if the chloride concentration is greater than twice the Hg_2^{2+} concentration.

Table 2 shows that only the quantity of mercury equal to the solubility

of elemental mercury could be vaporized from chloride media. In these experiments, a Hg(I)—Hg(0) mixture was first equilibrated in 0.001 M perchloric acid. Enough sodium chloride or hydrochloric acid was added to produce 0.01 or 0.1 M chloride ion, and the mixture was then equilibrated for 1 h at 25°C. Analysis showed the concentration of total mercury in solution to be unchanged before and after the addition of chloride. This indicates there is no loss of mercury(0) by vaporization or precipitation from solutions of $Hg_2Cl_2(s)$. The results in columns 3 and 4 of Table 2 show that no disproportionation of the mercury(I) could be induced by the sparging in chloride media, i.e. reaction (3) did not occur. The observation that distillation of the perchloric acid prior to solution preparation increased the amount of disproportionation, a result that could also be obtained by decreasing the perchloric acid concentration, is consistent with the presence of chloride in the perchloric acid which, in turn, inhibited the disproportionation.

In the second type of experiment, mercury(I) was equilibrated with elemental mercury in 0.001 M perchloric acid, sodium chloride was added to prepare a 0.01 M chloride solution and the resulting solution was analyzed by nonreducing sparging as quickly as possible. After 5, 15, and 25 min, the analysis of a 240 ppb Hg(I)—60 ppb Hg(0) solution gave 110, 70, and 60 ppb of volatile mercury, respectively. Total mercury analysis remained at 300 ppb over this time period. This suggests a decrease in the activity of the Hg_2Cl_2 formed with time.

It is well known that rates of recrystallization of many freshly prepared precipitates are large, but diminish with time as the particles become perfected. For example, silver bromide ages in the presence of bromide ion and recrystallizes relatively slowly after 30 min [18]. Kolthoff and Von Fischer [19] have shown that radioactive labeled lead is exchanged rapidly between freshly prepared lead chromate and solution; but a 20-min-old precipitate required one hour of shaking to exchange the labeled lead. The stability of the mercury(I) in chloride media appears to be due to (1) formation of a colloidal suspension of Hg_2Cl_2 precipitate and (2) a slow rate of recrystallization of the Hg_2Cl_2 compared to the time of the vaporization step in the analysis. Rapid Hg^{2+}—Hg_2^{2+} exchange has been observed if the two cations are mixed immediately in HCl media; however, when labeled mercury(II) was added to a mercury(I) chloride precipitate which was more than a few minutes old, the rate of exchange was slow [20, 21].

Further indication of the decreased activity of the Hg_2Cl_2 is shown in the following experiment where a 92 ppb mercury(I) solution in 0.01 M sodium chloride and 0.001 M perchloric acid was continuously sparged with nitrogen. The sample was prepared and left to stand for 30 min before initiating the nitrogen sparge. Aliquots of 0.2 ml were withdrawn at various times and analyzed for total mercury content in the reducing cell of the a.a.s. system. Table 3 shows that there was little decrease after the 30 min induction period. This may be interpreted to mean that the rate of disproportionation of an aged chloride solution is slow and that the precipitate

TABLE 3

Aeration of a mercury(I) solution in 0.01 M sodium chloride at pH 3.0

Time (min)	Total Hg (ppb)	Time (min)	Total Hg (ppb)
0	92	142	86
10	92	206	90.8
16	92	222	86
26	90	279	80
40	89.5	325	85

[a]The analyses were done by injection of 0.2-ml sample aliquots into the aeration cell containing 5 ml of the alkaline reducing mixture.

remains suspended in solution since there is little decrease in total concentration with time.

A final series of experiments was carried out to demonstrate the decreased activity of colloidal $Hg_2Cl_2(s)$. A solution of mercury(II) in 0.01 M hydrochloric acid was prepared and sparged with nitrogen to remove any oxygen in the system. Five analyses gave a result of 76 ± 4 ppb; the uncertainty reported is the range of the results. A drop of mercury which had been repeatedly washed (under a nitrogen atmosphere) with 0.01 M perchloric acid to remove any ionic mercury was added to the solution. After a 3-h reaction period, the drop of mercury was removed and any dissolved mercury(0) was removed by sparging with nitrogen. The resulting solution gave a result of 150 ± 2 ppb total mercury for five analyses. A similar experiment was done starting with a 5 ppb mercury(II) solution (21 ± 3 chart units on analysis); the resulting solution gave 44 ± 2 chart units for five separate analyses. These experiments demonstrate that mercury(II) is converted quantitatively to $Hg_2Cl_2(s)$. If some $HgCl_2$ were present at equilibrium, the increase in mercury concentration would have been less than twice the original concentration.

Conclusions

The results of the experiments are consistent with either a kinetic effect where the rate of dissolution of the $Hg_2Cl_2(s)$ is slow after perfection of the crystal, or a thermodynamic effect in which case the product $K_{sp}K_1K_2K$ is less than 10^{-8}. This could occur if the value of K_{sp} is less than 10^{-18} when $Hg_2Cl_2(s)$ is formed at these low concentrations. The literature values of K_{sp} (ca. 10^{-17}) were obtained electrochemically in cases when large quantities of calomel were present [17]. The activity of the precipitate formed in very dilute solution could be less than the activity of larger particles formed at higher concentrations.

Chloride media have been found to be useful for the preservation of mercury samples in the parts per billion range [22, 23]. This may be partly

because reduction of mercury(II) by trace reducing agents converts the mercury to a chloro form quite stable with respect to disproportionation and subsequent loss of mercury(0). Controversy still exists over which preservation method is best. Agreement has been reached that a combination of low pH, high ionic strength and the presence of an oxidizing agent gives the best storage in solution [24, 25]. The present data suggest lowering of the activity of the Hg_2Cl_2 when formed at the ppb level. The Ostwald—Freundlich equation

$$RT \ln K_2/K_1 = (2\sigma/\rho)\,(1/r_2 - 1/r_1) \qquad (4)$$

predicts that the solubility of a precipitate should increase with decreasing particle size, r, if the surface tension, σ, at the solid—liquid interface remains constant. Langmuir and Whittemore [26] have shown with studies of $Fe(OH)_3$ precipitation that this behavior is followed, the pK_{sp} values varying from +44 to +33, depending on the particle size.

In the present studies the particle size of the precipitate was not measured and, therefore, the validity of the Ostwald—Freundlich equation was not assessed. The surface energy term, σ, may be considerably smaller when the precipitate is formed at 10^{-7}—10^{-8} M concentration, thus reducing the solubility relative to that of a larger particle formed at higher concentrations. This could also occur through the adsorption of an organic impurity or silica from the glass onto the surface of the $Hg_2Cl_2(s)$. Resolution of these questions may be aided by investigations of this system with respect to the effects of trace organic impurities on the rates of disproportionation of mercury(I) and by the determination of particle size formed in these dilute solutions.

The work shows the following features: (1) elemental mercury can be vaporized quantitatively from acidic chloride media in the presence of ionic mercury; (2) the physical form of the $Hg_2Cl_2(s)$ can change to the point where mercury(I) disproportionation does not occur and cannot be induced by sparging. At this time, the procedure is recommended for the determination of mercury(0) in the presence of ionic mercury in acidic solution only because it is unknown what occurs near neutral pH values. Preliminary studies indicate rapid disproportionation above pH 7.0 regardless of the medium.

This paper is based in part on the Ph.D. thesis of D. A. H. (May, 1977) and M.S. thesis of D. G. (December, 1976). Grateful acknowledgement is made to the Department of Interior, Office Research and Technology, which supported part of this work through Grant B-020-NDAK and A-050-NDAK.

REFERENCES

1 J. M. Wood, F. S. Kennedy and C. G. Rosen, Nature, 220 (1968) 173.
2 S. Jensen and A. Jernelov, Nature, 223 (1969) 753.
3 L. Fishbein, Chromatogr. Rev., 13 (1970) 83.
4 G. Westöö, Acta Chem. Scand., 22 (1968) 2277.
5 P. Zarnegar and P. Mushak, Anal. Chim. Acta, 69 (1974) 389.
6 C. J. Cappon and J. C. Smith, Anal. Chem, 49 (1977) 365.
7 L. Magos and T. W. Clarkson, J. Assoc. Off. Anal. Chem., 55 (1972) 966.
8 T. Y. Toribara, C. P. Shields and L. Koval, Talanta, 17 (1970) 1025.
9 S. S. Choi and D. G. Tuck, J. Chem. Soc., (1962) 4080.
10 I. Sanemasa, Bull. Chem. Soc. Jpn., 48 (1975) 1795.
11 E. Onat, J. Inorg. Nucl. Chem., 36 (1974) 2029.
12 D. N. Glew and D. A. Hames, Can. J. Chem., 49 (1971) 3114.
13 H. C. Moser and A. F. Voigt, J. Am. Chem. Soc., 79 (1957) 1837.
14 K. F. Bonhoeffer and H. Reichardt, Naturwissenschaften, 17 (1929) 933.
15 W. Forsling, S. Hietanen and L. G. Sillén, Acta. Chem. Scand., 6 (1952) 901.
16 F. A. Cotton and G. Wilkinson, Advanced Inorganic Chemistry, 3rd edn., Interscience, New York, 1972, pp. 507—510.
17 B. Lindgren, A. Johsson and L. G. Sillén, Acta. Chem. Scand., 1 (1947) 479.
18 I. M. Kolthoff and A. S. O'Brien, J. Am. Chem. Soc., 61 (1939) 3414.
19 I. M. Kolthoff and W. Von Fischer, J. Am. Chem. Soc., 61 (1939) 191.
20 E. L. King, J. Am. Chem. Soc., 71 (1949) 3553.
21 R. Wolfgang and R. Dodson, J. Phys. Chem., 56 (1952) 872.
22 M. H. Bothner and D. E. Robertson, Anal. Chem., 47 (1975) 592.
23 M. S. Masri and M. Friedman, Environ. Sci. Technol., 7 (1973) 951.
24 J. Carron and J. Agemian, Anal. Chim. Acta, 92 (1977) 61.
25 D. R. Christmann and J. D. Ingle, Jr., Anal. Chim. Acta, 86 (1976) 53.
26 D. Langmuir and D. O. Whittemore, Nonequilibrium Systems in Natural Water Chemistry, ACS Advances in Chemistry Series, 106, Washington D.C., 1971, pp. 209—233.

Analytica Chimica Acta, 111 (1979) 123—135

MOLEKÜLABSORPTIONSSPEKTROMETRIE BEI ELEKTROTHERMISCHER VERDAMPFUNG IN EINER GRAPHITROHRKÜVETTE
Teil 3. Möglichkeiten der Bestimmung von Fluoridspuren durch die Molekülabsorption von AlF-, GaF-, InF- und TlF-Molekülen

K. DITTRICH

Sektion Chemie der Karl-Marx-Universität Leipzig, Analytisches Zentrum, 701 Leipzig (Deutsche Demokratische Republik)

(Eingegangen den 25. Juni 1979)

SUMMARY

Molecular absorption with electrothermal volatilization in a graphite tube. Part 3. A study of the determination of fluoride traces by AlF, GaF, InF and TlF molecular absorption.

The use of the molecular absorption of thermally stable molecules such as AlF, GaF, InF and TlF, generated in a normal graphite tube, is assessed for determinations of fluoride. Optimal experimental conditions are discussed. The presence of Na^+ ions increases the signals. The influence of Na^+ ions and of other substances is discussed. The sensitivities (0.01 absorbance) are 0.4 ng F^- for AlF, 0.8 ng F^- for GaF, and 1.1 ng F^- for InF.

ZUSAMMENFASSUNG

Es wurde die Anwendbarkeit der Molekülabsorption thermisch stabiler Moleküle, wie AlF, GaF, InF und TlF, die in einer normalen Graphitrohrküvette erzeugt wurden, für die Bestimmung des Fluorids untersucht. Die experimentellen Bedingungen wurden optimiert. Es ist möglich, Fluoridspuren in Mikrovolumina zu bestimmen. Die Gegenwart von Na^+-ionen erhöht die Signale. Der Einfluß der Na^+-ionen und auch von anderen Substanzen auf die Molekülabsorption wird diskutiert. Die reziproken Empfindlichkeiten bezogen auf 0,01 Extinktion sind: AlF 0,4 ng F^-; GaF 0,8 ng F^-; InF 1,1 ng F^-.

Die in der 2 Mitteilung [1] gemachten Angaben über die Fluoridbestimmung durch Ausnutzung der Molekülabsorption (MA) von GaF-Molekülen und die von Tsunoda et al. [2] veröffentlichte Publikation über die Bestimmung des Fluorids durch Ausnutzung der MA der AlF-Moleküle waren für uns Anlaß, die Möglichkeiten für die Bestimmung von Fluoridspuren bei Einsatz folgender Elemente der 3. Hauptgruppe: Al, Ga, In, Tl zu prüfen. In der vorliegenden Arbeit werden die erhaltenen Ergebnisse mitgeteilt und vor allem unter dem Gesichtspunkt der analytischen Anwendung miteinander verglichen.

EXPERIMENTELLES

Apparatur

Zweikanal—Zweistrahl-AA-Spektrometer Typ 811 (Jarrell—Ash); Lichtquelle: H_2-Hohlkathodenlampe, 30 mA; Spektrale Bandbreite: 0,4 nm, Wellenlängen: vgl. Tabellen 1 und 2, Abb. 1; Untergrundkompensation nach der Zweilinienmethode (vgl. Tabelle 2). Graphitrohrküvette Typ 1268 (Beckman).

Reagenzien

Die Stammlösungen der zu untersuchenden Metalle (Me) wurden durch Auflösen der reinen Metalle (Gallium und Indium) bzw. der reinen Nitrate ($TlNO_3$ und $Al(NO_3)_3$) in Salpetersäure hergestellt (100 mg M ml^{-1} 1 M HNO_3.

Außerdem wurden folgende Lösungen eingesetzt: 0,1 M NaF; $LiNO_3$, $NaNO_3$, KNO_3, $Be(NO_3)_2$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$ (Stammlösungen zwischen 10 und 100 mg Me ml^{-1}); NaCl, NaBr, NaJ, $ZnCl_2$, $ZnBr_2$, ZnJ_2 (Stammlösungen 100 mg X^- ml^{-1}).

Verfahrensweise

Mikrovolumina von 10 bis 100 μl entsprechend zusammengesetzter Lösungen werden in die Graphitrohrküvette gegeben (Dosierung), getrocknet (1. thermische Phase: Trocknung), thermisch überarbeitet (2. thermische Phase: Veraschung oder thermische Überarbeitung) und verdampft (3. thermische Phase: Verdampfung oder Molekülbildung und bei der AAS Atomisierung). Die Aufnahme der Spektren erfolgte durch punktweises Messen bei ausgewählten Wellenlängen.

UNTERSUCHUNG UND OPTIMIERUNG DER EXPERIMENTELLEN BEDINGUNGEN

Spektrenaufnahme und Auswahl des Spektralgebietes

In der Abb. 1 wurden die Absorptionsspektren dargestellt, die bei der Verdampfung der metallischen Komponenten Al, Ga, In und Tl in Gegenwart und Abwesenheit von Fluorid in einer Graphitrohrküvette erhalten wurden. Die Identifizierung der Banden der Spektren erfolgte nach [3]. Für AlF-, GaF- und InF-Moleküle werden scharfe und intensive Absorptionsbanden festgestellt, deren Charakteristika in der Tabelle 1 angegeben werden. Die TlF-MA konnte auch nachgewiesen werden, jedoch ist die Extinktion verhältnismäßig niedrig und die erhaltene Absorptionsbande relativ breit.

Vergleicht man die in der Tabelle 1 angegebenen Dissoziationsenergien mit den bei der Absorption aufgenommenen Energiebeträgen, so stellt man fest, daß im Falle des TlF-Moleküls die aufgenommene Energie größer als die Dissoziationsenergie ist. Somit ist bei Absorption ein

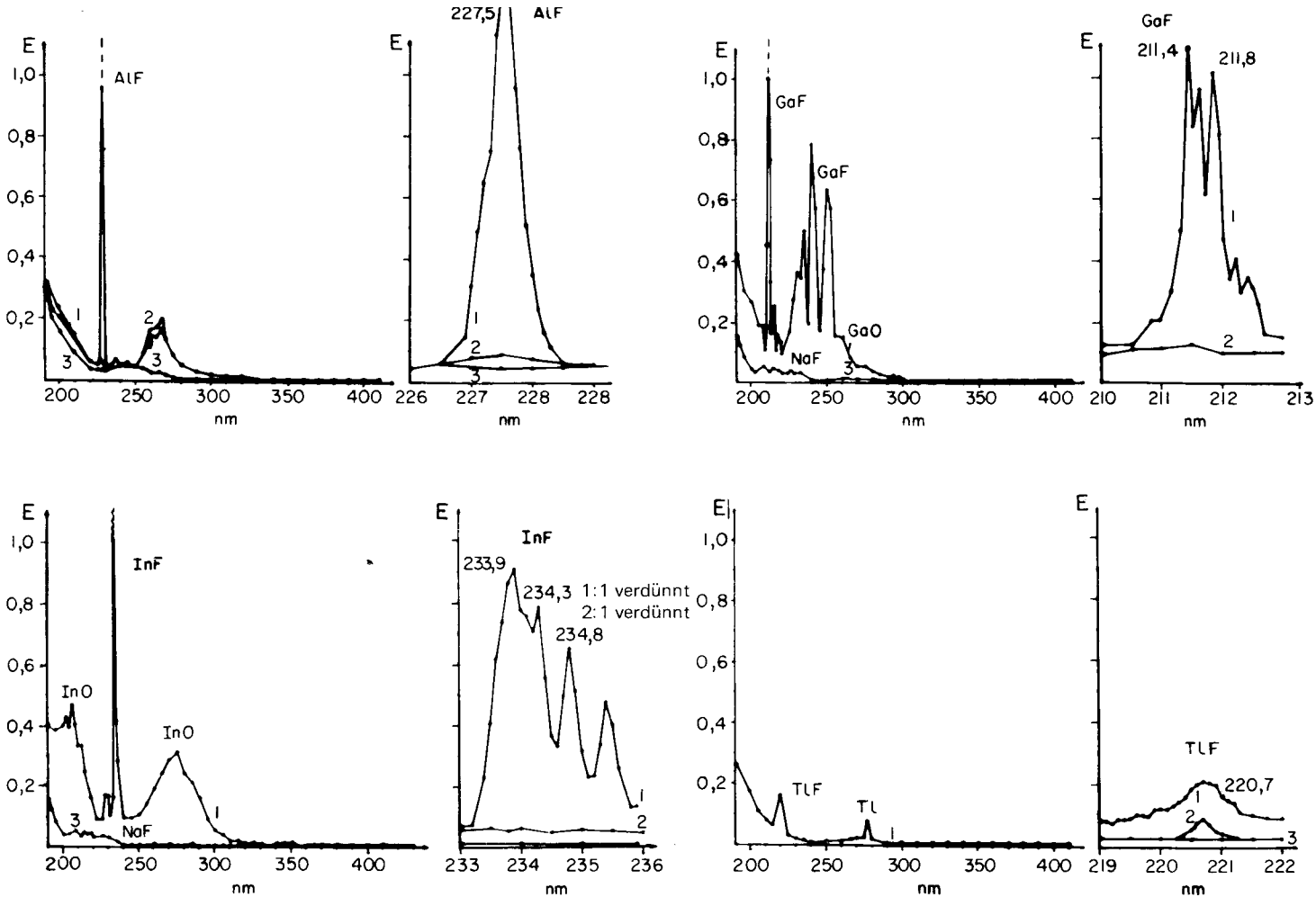


Abb. 1. Molekülabsorptionsspektren der AlF-, GaF-, InF-, TlF-, AlO-, GaO- und InO-Moleküle bei Verdampfung in einer Graphitrohrküvette. Kurven 1. Me^{3+}—F^-—Na^+; Kurven 2. Me^{3+}—Na^+; Kurven 3. Na^+—F^-.

TABELLE 1

Charakteristika der untersuchten Molekülbanden

Molekül	Intensivste Absorptionsbande			Dissoziationsenergie (eV)	Siedepunkt des Metalls (°C)
	Übergang	Wellenlänge (nm)	Energieaufnahme (eV)		
AlF	$A^1\Pi - X^1\Sigma^+$	227,6	5,4	6,8	2450
GaF	$C^1\Pi - X^1\Sigma^+$	211,4	5,9	6,0	2237
InF	$C^1\Pi - X^1\Sigma$	233,9	5,3	5,3	2000
TlF	$C^1\Pi - X^1\Sigma^+$	220.7	5,6	4,8	1457
AlF	$C^1\Sigma^+ - X^1\Sigma^+$	173,2			

Molekülzerfall zu erwarten. Neben der Tatsache, daß die Dissoziationsenergien in der Reihe vom AlF zum TlF abnehmen, sehen wir darin einen wesentlichen Grund für die relativ geringe Intensität der Bande der TlF-MA.

Trotz der nicht äquivalenten atomaren Konzentrationen der Metalle kann man die Schlußfolgerung ziehen, daß eine analytische Ausnutzung der AlF-, GaF- und InF-MA möglich sein müßte (vgl. auch [1, 2, 4]). Auf die analytische Anwendung der TlF-MA wurde aus den genannten Gründen verzichtet. Aus der Tabelle 1 ist weiterhin zu entnehmen, daß die intensiven Banden der GaF-, InF- und TlF-MA dem jeweiligen *C*-Bandensystem angehören. Die *A*- und *B*-Banden, die normalerweise in Emission am intensivsten sind und auch schon analytisch genutzt wurden [5], treten nicht auf. Im Fall der AlF-MA wurde jedoch eine *A*-Bande ausgenutzt. Die *C*-Bande des AlF-Moleküls liegt bei 173,2 nm und konnte deshalb nicht gemessen werden (vgl. [3]).

Neben den MeF-Absorptionsbanden treten noch andere Banden in den Spektren auf. Im Fall des Al konnte die Bande, die zwischen 250 und 280 nm erscheint, als AlO-MA-Bande identifiziert werden [3]. Die eingetragenen GaO- und InO-MA-Banden konnten mit Hilfe der Literatur nicht identifiziert werden, da sie noch nicht beschrieben wurden (vgl. auch [4]). Es besteht z.B. auch die Möglichkeit, daß es sich um GaOH- bzw. InOH-MA-Banden handeln könnte.

Ausgehend von den aufgenommenen Spektren wurden die Bedingungen für die Untergrundkompensation nach der Zweilinienmethode abgeleitet. Die experimentell überprüften Werte sind in der Tabelle 2 zusammengefaßt worden.

Abhängigkeit der AlF- und InF-MA von den thermischen Bedingungen

Sowohl die Veraschungstemperatur (2. Phase) als auch die Verdampfungstemperatur (3. Phase) können einen Einfluß auf die Größe des Signals haben. Eine zu hohe Veraschungstemperatur kann zur vorzeitigen Verdampfung entweder der Me- oder X-Komponente und damit zur Verminderung der Molekülkonzentration während der 3. Phase führen. Bei

TABELLE 2

Optische Bedingungen für die analytische Anwendung der AlF-, GaF- und InF-MA

Molekül	AlF	GaF	InF
Wellenlänge der Messung der Gesamtabsorption (nm)	227,6	211,4	233,9
Wellenlänge der Messung der Untergrundabsorption (nm)	226,45	215,5	232,5

der Verdampfungstemperatur sind zwei gegenläufige Effekte zu berücksichtigen: (1) eine zu hohe Verdampfungstemperatur bewirkt eine zu starke Molekülдissoziation und somit eine geringe Molekülkonzentration; und (2) eine hohe Verdampfungstemperatur bewirkt eine schnelle und gleichzeitige Verdampfung beider Komponenten und schafft damit die Voraussetzung für eine hohe Molekülkonzentration.

Ausgehend von den Siedetemperaturen der Elemente (Tabelle 1), den Dissoziationsenergien der Moleküle und den Ergebnissen, die bereits bei der Untersuchung der GaF-MA [1] erhalten wurden, konnte angenommen werden, daß die Plasmatemperaturen der 3. Phase möglichst hoch liegen sollten.

Die Ergebnisse der Untersuchungen sind in der Abb. 2 dargestellt. Im Fall der AlF-MA wurde für die 2. Phase (Veraschung) festgestellt, daß in Gegenwart thermisch stabilisierender Elemente wie Natrium oder Barium Veraschungstemperaturen bis zu 1870°C möglich sind. Erst bei noch höheren Temperaturen treten Substanzverluste auf (Teil A, Kurve 1). Die Abhängigkeit der AlF-MA von der Verdampfungstemperatur konnte nicht gemessen werden, da praktisch nur bei den maximalen Temperaturen der 3. Phase Al

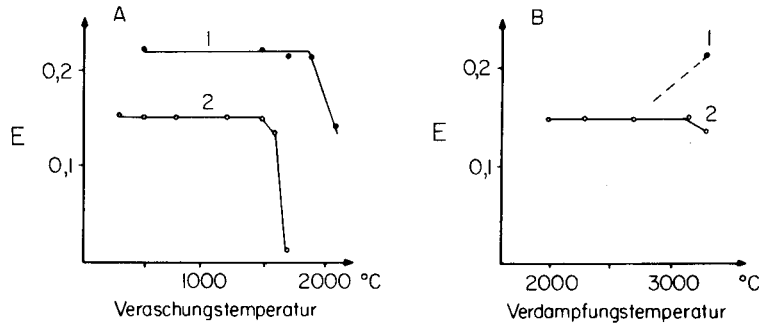


Abb. 2. Abhängigkeit der Intensität der AlF- und InF-MA von der Temperatur der Veraschungs- (A) und Verdampfungsphase (B). Kurven 1, 1 µg Al^{3+}, 7 µg Na^+, 38 ng F^-/10 µl. Kurven 2, 10 µg In^{3+}, 5 µg Na^+; 38 ng F^-/10 µl. Im Fall A: Messung der Extinktion in der nachfolgenden Verdampfungsphase bei 3300°C (10 s).

verdampft werden kann. Jegliche Temperaturverminderungen führten neben einem Intensitätsverlust auch zu schlechten Reproduzierbarkeiten.

Im Fall der InF-MA waren wegen des gegenüber Al und Ga geringeren Siedepunktes des In größere Einflüsse zu erwarten. Bereits bei Veraschungstemperaturen von 1500°C traten Substanzverluste in dieser Phase auf (Abb. 2, Teil A, Kurve 2). Der beginnende Einfluß der Molekül-dissoziation ist daran zu erkennen, daß die optimalen Extinktionswerte bereits ab 3150°C reduziert werden. Die gute Verdampfbarkeit des Indiums ist wiederum die Ursache dafür, daß bereits bei verhältnismäßig niedrigen Verdampfungstemperaturen maximale Extinktionswerte erhalten werden (Abb. 2. Teil B, Kurve 2). Die angegebenen Temperaturen sind von uns nicht gemessen worden, sie entsprechen den von der Firma geeichten Spannungswerten.

Einfluß der Me-Konzentration auf die AlF- und InF-MA

Ausgehend von den Ergebnissen, die bei der Untersuchung der GaF-MA erhalten wurden, wurde der Einfluß der Me-Konzentration auf die Intensität der MA überprüft. Die Ergebnisse sind in der Abb. 3 dargestellt. In der Abbildung ist zu erkennen, daß die MA mit zunehmender Me-Konzentration in beiden Fällen wächst. Es wird jeweils ein Grenzwert erreicht. Dieser Grenzwert bedeutet, daß entsprechend dem Gleichgewicht, Me + F $\rightleftharpoons$ MeF, nahezu das gesamte Fluorid gebunden ist. Somit bewirkt ein weiterer Me-Zusatz nur eine äußerst geringe Erhöhung der MeF-Konzentration. Da die Dissoziationsenergie des AlF größer als die des InF ist, wird im Fall der AlF-MA dieser Grenzwert schon bei relativ niedrigen Al-Konzentrationen erreicht. Dies ist für die praktische Analytik ein günstiger Umstand, denn es ist unmöglich, größere Mengen Al über einen längeren Zeitraum hinweg aus einem Graphitrohr zu verdampfen. Folgende optimalen Bedingungen wurden ermittelt: AlF-MA 1 µg Al/10 bzw. 100 µl, und InF-MA 10 µg In/10 bzw. 100 µl.

Einfluß der Na^+-Konzentration auf die AlF- und InF-MA

Bei der Untersuchung der GaF-MA hatten wir festgestellt [1], daß mit zunehmender Konzentration der Na^+-ionen in der Analysenlösung zuerst

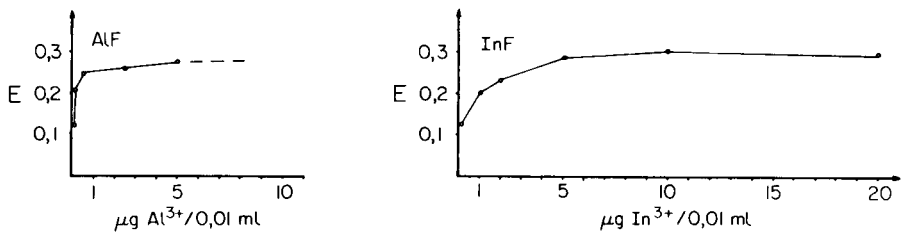


Abb. 3. Abhängigkeit der Intensität der AlF- und InF-MA von der jeweiligen Kationenmenge. AlF, 100 µg Na^+, 38 ng F^-/10 µl. InF, 5 µg Na^+, 38 ng F^-/10 µl.

eine Erhöhung und danach eine Erniedrigung der GaF-MA auftrat. Wir untersuchten deshalb auch den Einfluß dieses Ions auf die Intensität der AlF- und InF-MA. Das Ergebnis ist in der Abb. 4 dargestellt. Aus der Abb. 4 geht hervor, daß Na^+-ionen einen starken Einfluß auf die MA ausüben. Sowohl die AlF- als auch die InF-MA nehmen mit zunehmender Na^+-Konzentration stark zu. Lediglich im Fall der InF-MA ist bei sehr hohe Na^+-Konzentration eine beginnende Abnahme der Absorption zu erkennen. Diese Unterschiede lassen sich mit den verschiedenen Dissoziationsenergien der Moleküle und den dadurch gegebenen Gleichgewichtsbedingungen im Plasma erklären (s. auch Tabelle 1). Die hohe Stabilität des AlF-Moleküls bewirkt, daß sich trotz hoher Na^+-Konzentration praktisch kein NaF im Plasma bildet (Dissoziationsenergie des NaF 4,9 eV). Infolge der geringeren Stabilität der InF-Moleküle wirken Na-Atome gegenüber In-Atomen bei der Molekülbildung als Konkurrenten, d.h. mit steigender Na^+-Konzentration sinkt die InF-Konzentration auf Kosten einer steigenden NaF-Konzentration.

Der eigentliche, positive Effekt der Na^+-ionen besteht darin, daß das beim Trocknen gebildete NaF thermisch stabil ist, d.h. es tritt weder beim Trocknungsprozeß selbst, noch in der 2. Phase der thermischen Behandlung ein nennenswerter Verlust an HF durch Hydrolyse oder thermische Hydrolyse auf. Entsprechend der unterschiedlichen Basizitäten des Aluminiums und Indiums und der daraus folgenden unterschiedlichen Stabilität der hydratisierten Salze ergibt sich, daß die Na^+-ionen einen größeren Einfluß auf die AlF-MA ausüben.

Der Zusatz der Na^+-ionen kann im Fall des Al als Nitrat oder Hydroxid erfolgen. In Gegenwart von In ist ein Zusatz als Hydroxid wegen der möglichen Fällung des $In(OH)_3$ zu vermeiden. Die für analytische Bestimmungen optimalen Konzentrationen des Zusatzstoffes sind: AlF-MA 100 µg Na^+/10 bzw. 100 µl, und InF-MA 10 µg Na^+/10 bzw. 100 µl.

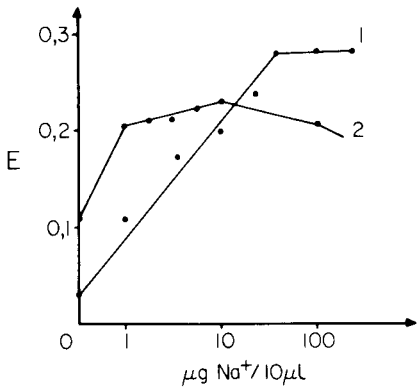


Abb. 4. Abhängigkeit der AlF- (Kurve 1) und InF-MA (Kurve 2) von der Na^+-Konzentration. Kurve 1, 1 µg Al^{3+}, 38 ng F^-/10 µl. Kurve 2, 10 µg In^{3+}, 38 ng F^-/10 µl.

Unter diesen Bedingungen wurde auch der Einfluß des pH-Wertes auf die Intensität der MA überprüft. Es wurde festgestellt, daß erst bei pH-Werten kleiner als 2 Signaldepressionen infolge von vorzeitiger HF-Verfüchtigung auftreten (vgl. auch [1]). In Gegenwart von HCl oder H_2SO_4 treten jedoch starke, nicht kompensierbare Blindwerte auf, deren Ursache bis jetzt noch nicht geklärt wurde.

Einfluß anderer Kationen auf die AlF- und InF-MA

Der Einfluß anderer Kationen auf die AlF- und InF-MA wurde sowohl in Abwesenheit als auch in Gegenwart des Zusatzstoffes Natrium untersucht. Die Ergebnisse sind in den Abb. 5 und 6 zusammengefaßt worden.

Die Abb. 5 (Abszissenmaßstab in vergleichbaren Atomkonzentrationen) zeigt, daß die Gegenwart von Alkali- und Erdalkalimetallen zu einer deutlichen Erhöhung der AlF- und auch der InF-MA führt. Damit wird die im vorhergehenden Abschnitt getroffene Feststellung, daß die Wirkung der Natriumionen in einer Stabilisierung des Fluorids während der Trocknungs- und Veraschungsphase besteht, bestätigt, denn auch die anderen Alkalien und auch Erdalkalien bilden thermisch stabile Fluoride.

Im Fall der InF-MA zeigt sich auch in diesen Fällen, daß sehr hohe Me-Konzentrationen dieser Metallionen einen depressiven Einfluß ausüben. Diese

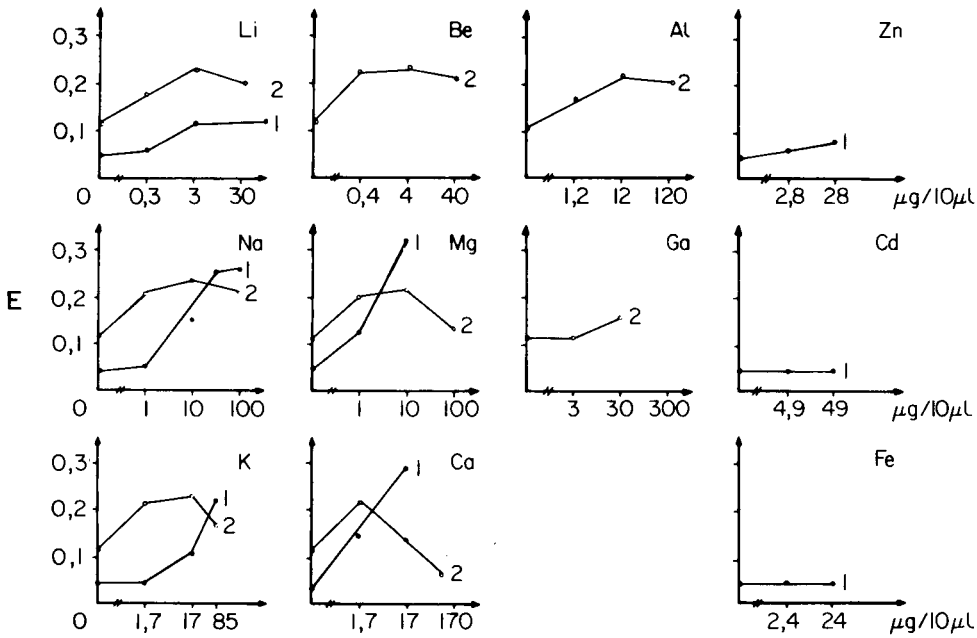


Abb. 5. Abhängigkeit der AlF- (Kurven 1) und InF-MA (Kurven 2) vom Zusatz anderer Kationen (als Zusätze wurden jeweils die Nitrate eingesetzt). Kurven 1, 1 µg Al^{3+}, 38 ng F^-/10 µl. Kurven 2, 10 µg In^{3+}, 38 ng F^-/10 µl.

Tatsache ist wiederum mit einer Konkurrenzreaktion im Plasma nach InF + Me ⇌ MeF + In, zu erklären. Diese Reaktion besitzt beim AlF-Molekül keine Bedeutung, weil die Dissoziationsenergie dieses Moleküls sehr hoch ist. Die Übergangsmetallionen Zn^{2+}, Cd^{2+} und Fe^{3+} üben praktisch keinen Einfluß aus. Mit diesen Ergebnissen konnten die von Tsunoda et al. [2] gemachten Angaben über den Einfluß der Alkalien und Erdalkalien auf die AlF-MA bestätigt werden. Die von Tsunoda et al. angegebene signalerhöhende Wirkung von Schwermetallionen konnte nicht bestätigt werden.

Die Gegenwart der bereits ermittelten optimalen Na^+-Konzentrationen bewirkt, daß der Einfluß der anderen Kationen zurückgedrängt wird (vgl. Abb. 6). In den meisten Fällen ist festzustellen, daß bei sehr hohen Konzentrationen der Zusatzstoffe eine Depression der MA-Signale auftritt. Dies ist unter anderem auf die bereits beschriebene Konkurrenzreaktion zurückzuführen, die natürlich mit höher werdender Fremdmetallkonzentration zunimmt.

Eine deutliche Ausnahme von dieser Tendenz ist lediglich im Fall des Ca^{2+}-Einflusses auf die AlF-MA zu beobachten. Die hier auftretende, signalerhöhende Wirkung konnte noch nicht aufgeklärt werden. Sie ist

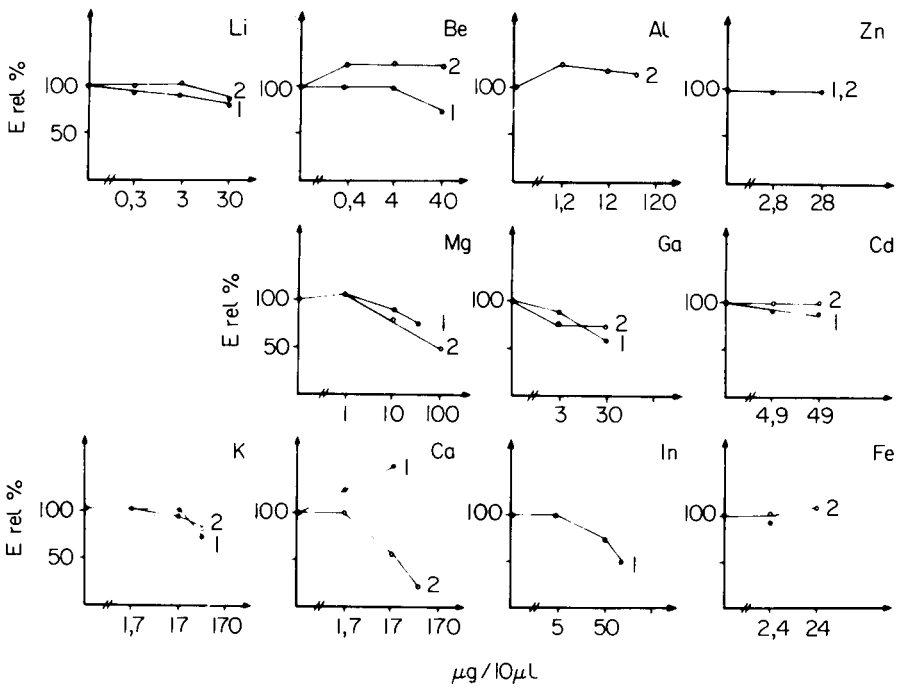


Abb. 6. Abhängigkeit der Intensität der AlF- (Kurven 1) und InF-MA (Kurven 2) vom Zusatz anderer Kationen in Gegenwart der optimalen Na^+-Konzentration. Kurven 1, 1 µg Al^{3+}, 38 ng F^-, 100 µg Na^+/10 µl. Kurven 2, 10 µg In^{3+}, 38 ng F^-, 10 µg Na^+/10 µl.

Gegenstand weiterer Untersuchungen über den Matrixeffekt. Zum besseren Vergleich der einzelnen Einflüsse wurden in der Abb. 6 die Ordinaten in relative Extinktionseinheiten unterteilt. Bezugsgröße (100%) war jeweils der Extinktionswert, der in Gegenwart von 100 μg Na^+ (AlF-MA) bzw. 10 μg Na^+ (InF-MA) erhalten wurde.

RESULTATE UND DISKUSSION

Analytische Bestimmung des Fluorids durch AlF- und InF-MA

Unter Anwendung der beschriebenen optimalen Bedingungen wurden sowohl für die AlF-MA als auch für die InF-MA analytische Bestimmungen für Fluoridspuren ausgearbeitet. Die Resultate sind in der Abb. 7 und der Tabelle 3 dargestellt worden. Aus der Abb. 7 geht hervor, daß in beiden Fällen leicht gekrümmte Eichkurven erhalten wurden. Die bei der InF-MA stärkere Krümmung ist auf die geringere Stabilität dieses Moleküls zurückzuführen. Es ist zu erkennen, daß Fluoridbestimmungen in einem Konzentrationsbereich von etwa 2 Zehnerpotenzen möglich sind.

In der Tabelle 3 wurden die reziproken Empfindlichkeiten bezogen auf 0,01 Extinktionseinheiten angegeben. Diese Werte sind in erster Näherung mit einer statistisch berechenbaren Nachweisgrenze (3 s — Kriterium) identisch. Auf der Basis dieser Werte wurden auch die relativen Angaben über das Nachweisvermögen gemacht. Zum besseren Vergleich wurden in diese Tabelle die bereits beschriebenen analytischen Resultate der Fluoridbestimmung unter Ausnutzung der GaF-MA mit aufgenommen (vgl. auch [1]). Aus der Tabelle 3 geht hervor, daß die reziproken Empfindlichkeiten bei Anwendung der AlF-MA am günstigsten sind. Die Verschlechterung des Nachweisvermögens in der Reihe Al—Ga—In— und auch Tl hängt offensichtlich mit der abnehmenden thermischen Stabilität dieser Moleküle infolge

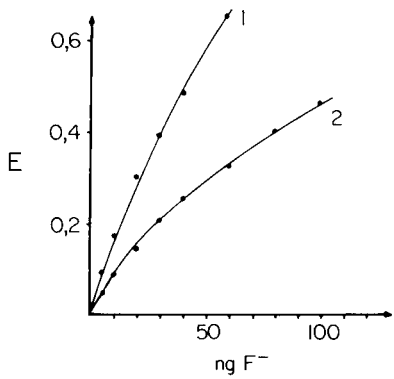


Abb. 7. Eichkurven für die Fluoridbestimmung durch AlF-MA (Kurve 1) und InF-MA (Kurve 2). Kurve 1, 1 μg Al^{3+}, 100 μg Na^+/10 μl. Kurve 2, 10 μg In^{3+}, 10 μg Na^+/10 μl. Trocknung 20 s, 150°C. Veraschung 15 s, 520°C. Verdampfung (1) 20 s, 3300°C; (2) 10 s, 3150°C.

TABELLE 3

Ergebnisse der analytischen Bestimmung des Fluoride durch AlF-, GaF- und InF-MA

Molekül	Reziproke Empfindlichkeit/0,01 Extinktion	
	Absolut (ng)	Relativ (Mol l^{-1})
AlF	0,4	2×10^{-6} [a]
		2×10^{-7} [b]
GaF	0,8	4×10^{-6} [a]
		4×10^{-7} [b]
InF	1,1	$5{,}5 \times 10^{-6}$ [a]
		$5{,}5 \times 10^{-7}$ [b]

[a]Probevolumen 0,01 ml. [b]Probevolumen 0,1 ml.

ihrer kleiner werdenden Dissoziationsenergien zusammen. Die für die AlF-MA in [2] angegebenen reziproken Empfindlichkeiten von 0,25 ng F/0,01 E konnten fast erreicht werden. Zu berücksichtigen ist dabei, daß Tsunoda et al. einen anderen Atomisator (CRA) benutzten, der einen steileren Temperaturanstieg ermöglicht.

Die erhaltenen Resultate beweisen, daß die Methode für die Spurenanalyse in Mikroproben geeignet ist.

Einfluß anderer Halogenide auf die Bestimmung des Fluorids durch MA

Für die Beurteilung der analytischen Möglichkeiten dieser Methode ist es vor allem wichtig, eine Aussage über den Einfluß anderer Halogenide zu treffen. Bereits bei den Untersuchungen über die GaF-MA wurde festgestellt, daß der Halogenideinfluß unterschiedlich ist. Aus der Abb. 8 ist zu entnehmen, daß alle Halogenide auf die InF-MA einen stärkeren Einfluß als auf die AlF-MA ausüben und daß die Wirkung in der Reihenfolge Cl > Br > J jeweils abnimmt. Da sich die Lösungsbedingungen durch die Addition der anderen Halogenide nur unwesentlich geändert haben, ist ihr Einfluß vor allem durch Plasmareaktionen, wie AlF + Cl $\rightleftharpoons$ AlCl + F, zu erklären. Dabei ist zu berücksichtigen, daß die im Plasma ablaufenden Reaktionen kaum zu exakten Gleichgewichtszuständen führen. Da jedoch die Stabilität der Moleküle in der Reihenfolge MF > MCl > MBr > MJ abnimmt (vgl. auch [4]), ist die stärkere Einflußnahme des Cl erklärt. Die größere Beeinflussung der InF-MA ist mit dem gleichen Argument zu erklären, d.h. die insgesamt geringere Stabilität der Moleküle des Indiums bewirkt eine stärkere Austauschbarkeit. Außerdem tritt bei der größeren Wellenlänge der InF-MA eine Untergrunderhöhung durch die Molekülabsorption der NaCl-, NaBr- und NaJ-Moleküle auf (vgl. auch [4, 6]).

Möglichkeiten für die analytische Bestimmung des Fluorids durch MA in Gegenwart anderer Stoffe

In der Tabelle 4 sind die Ergebnisse dieser Untersuchungen zusammengefaßt worden. Aus der Tabelle geht hervor, daß die Bestimmung des

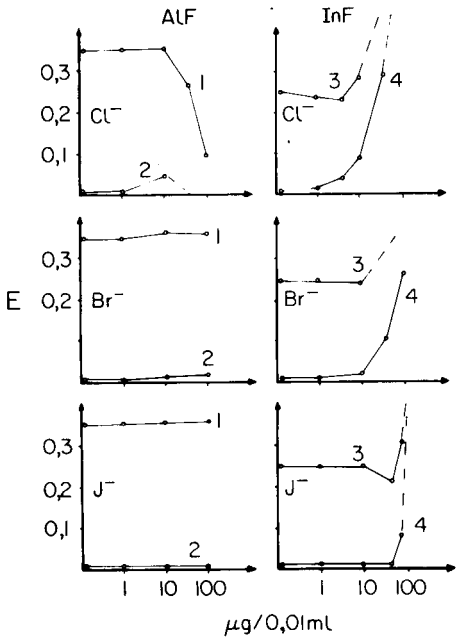


Abb. 8. Abhängigkeit der Intensität der AlF- und InF-MA von der Konzentration anderer Halogenidionen. Kurven 1 (AlF), 1 µg Al^{3+}, 100 µg Na^+, 38 ng F^-/10 µl. Kurven 2 (AlF), 1 µg Al^{3+}, 100 µg Na^+/10 µl. Kurven 3 (InF), 10 µg In^{3+}, 10 µg Na^+, 38 ng F^-/10 µl. Kurven 4 (InF), 10 µg In^{3+}, 10 µg Na^+/10 µl.

TABELLE 4

Analytische Möglichkeiten der Fluoridbestimmung durch AlF- und InF-MA in Gegenwart anderer Substanzen

Molekül	Maximaler Matrixüberschuß (Masseverhältnis)			
	10^5	5×10^4	10^4	5×10^3
AlF	Li, K, Be Mg, Ca, Sr, Ba, Zn, Cd, Br^-, J^-	In, Fe Cl^-, SO_4^{2-}	Ga	
InF	K, Zn, Cd	Li, Be, Mg Al, Fe, J^-	Ca, Ga, Cl^-, Br^-	SO_4^{2-}

Fluorids in Gegenwart eines Überschusses der verschiedensten Elemente möglich ist. Der in der Tabelle als Grenze angegebene Masseüberschuß bedeutet, daß in Gegenwart dieses Überschusses die beste relative Nachweisgrenze des Fluorids bezogen auf den hinzugefügten Stoff erhalten werden konnte, d.h. diese Angabe sagt nicht aus, daß bei diesen Konzentrationen keine Matrixeffekte auftreten.

Aus der Tabelle 4 geht weiterhin hervor, daß die Fremdionen in der Mehrzahl auf die AlF-MA einen geringeren Einfluß ausüben. Ein Vergleich mit den Resultaten der GaF-MA bestätigt diese Tendenz. Somit kann der Schluß gezogen werden, daß für die Bestimmung des Fluorids trotz der schlechten Verdampfbarkeit des Al die AlF-MA am besten geeignet ist. Die noch vorhandenen, bisher nicht exakt klärbaren Matrixeffekte beseitigt man sowohl bei der AlF- als auch der GaF- und InF-MA am besten durch Anwendung der Additionstechnik.

LITERATUR

1 K. Dittrich, Anal. Chim. Acta, 97 (1978) 69.
2 K. Tsunoda, K. Fujiwara und K. Fuwa, Anal. Chem., 49 (1977) 2035.
3 B. Rosen, Spectroscopic Data relative to Diatomic Molecules, International Tables of Selected Constants, Vol. 17, Pergamon, Oxford, 1970.
4 K. Dittrich, Anal. Chim. Acta, 97 (1978) 59.
5 N. Furuta, E. Yoshimura, H. Haraguchi und K. Fuwa, Spectrochim. Acta, Part B, 33 (1978) 715.

Analytica Chimica Acta, 111 (1979) 137—144

ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF SOME ELEMENTS FORMING VOLATILE HYDRIDES WITH A HEATED CELL ATOMIZER AND GAS HANDLING SYSTEM

J. F. CHAPMAN* and L. S. DALE

Chemical Technology Division, Australian Atomic Energy Commission Research Establishment, Lucas Heights, New South Wales, 2232 (Australia)

(Received 16th May 1979)

SUMMARY

A system is described which permits the atomic absorption spectrometric determination of antimony, arsenic, bismuth, lead, selenium, tellurium and tin after formation of their volatile hydrides. The apparatus consists of an electrically heated cell atomizer and a gas handling system which enables the gaseous hydrides either to be introduced directly into the cell (Sb, Pb, Te, Sn) or to be collected in a gasometer and subsequently transferred to the cell (As, Bi, Se). The latter elements are evolved more slowly, so that collection is necessary to achieve maximum sensitivity. The differences in the rates of hydride production are discussed.

The hydride evolution technique for determining antimony, arsenic, bismuth, lead, selenium, tellurium and tin by atomic absorption spectrometry is well established [1—5]. The use of a heated absorption cell as an atomizer has gained popularity since the original work of Chu et al. [5] because its advantages over chemical combustion flames include low noise, particularly below 200 nm, constant temperature and hence reproducible atomizing conditions, and the relative ease of construction and operation. One problem associated with the use of open-ended heated cells is ignition of the hydrogen—air mixture at the cell ends which can result in spurious absorption signals at below 200 nm. This can be overcome by fitting windows to the tube [6] or by directing nitrogen across the open cell ends [4, 7]. A simpler way is to maintain the centre section of the cell at a high temperature but leave the ends at a temperature below the ignition point of the hydrogen—air mixture.

In most procedures based on heated cells the hydrides are transferred directly to the atomizer. However, the present studies have shown that although maximum sensitivity can be achieved for a number of elements with this method, others form hydrides at a much slower rate even when excessive amounts of reductant are used. Consequently, direct transfer results in a loss of potential sensitivity. It is desirable, therefore, to collect the hydrides for an extended period. Methods available for gas collection in-

clude the use of freeze-out tubes [1] and balloons [2] but these are cumbersome to operate; because of this, it was decided to incorporate a gasometer in the gas-handling system to facilitate collection and storage of the evolved gases. The gasometer is easy to operate, durable and does not require replacements or auxiliary equipment.

A detailed investigation was made of this system to optimize the sensitivity for the individual elements. It was most important to determine the optimum reductant concentration that would produce the maximum yield of hydride so that the sensitivity was not reduced by excessive amounts of liberated hydrogen. The stability of the hydrides was also investigated; some of the hydrides are so unstable that collection is impracticable.

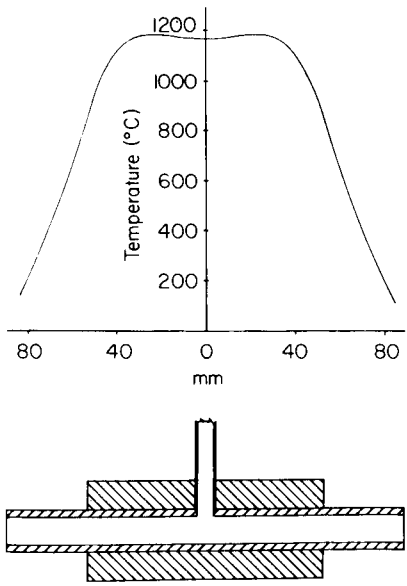
EXPERIMENTAL

Equipment

Atomizer. The cell is constructed from Vycor furnace tubing (12.5 mm i.d., 180 mm long). The silica inlet tube (150 mm long) is fused onto the Vycor cell, and is attached to the gasometer by a S19 ball joint. The centre section of the cell (90 mm) is wound with 2 m of Pt/13% Rh wire (0.5-mm diameter) and suitably insulated. The cell, mounted in a holder, is fitted into the receptacle of the adjustable burner mount of the instrument. Power is supplied through an autotransformer and the temperature is measured by an Inconel-sheathed Chromel—Alumel thermocouple. The cell is operated at 1150—1180°C. The cross-section of the cell is shown in Fig. 1, together with the temperature profile.

Gas-handling system. Figure 2 shows the cell and the gas-handling system. The system can be operated in two modes. For direct transfer, taps A and B are positioned so that the purge gas (nitrogen) is fed directly to the reaction vessel and all the gases bypass the gasometer (Fig. 2a). When the hydrides are to be collected, these taps are reversed (Fig. 2b); in this case, the purge gas bypasses the reaction vessel and is directed into the cell. In the collection mode, the gases are stored in the gasometer for a specified time and then, by turning tap A, the reaction vessel is purged to transfer more generated hydride until a predetermined volume is collected in the gasometer. When this volume is reached, tap C is opened and the stored gases pass into the cell. The volume of the reaction cell (100 ml) is not critical for the collection mode. However, for direct transfer a larger volume gives a broader peak while a smaller volume gives a larger, more erratic peak.

The collection procedure for arsenic and selenium varies slightly from the above. Since the resonance lines for these elements are below 200 nm, the transmittance of the cell to these wavelengths is decreased when air diffuses into it. This occurs when the reaction vessel is being purged. It is therefore necessary to turn tap A to the bypass position before tap C is opened. This re-establishes the transmittance of the cell which can be ascertained when the baseline absorbance returns to its original value.



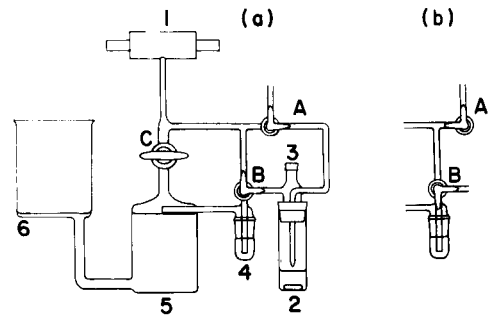


Fig. 1. Cross-section of the heated cell showing the position of the inlet tube. The temperature profile is indicated above.

Fig. 2. Schematic diagram of apparatus for hydride generation and gas handling. (a) Direct transfer mode; (b) collection mode. (1) Heated cell; (2) reaction vessel (capacity 100 ml); (3) septum; (4) moisture trap; (5) gasometer; (6) reservoir; (A, B) bypass taps; (C) gasometer release tap.

The gasometer reservoir (1-l capacity) contains a saturated sodium chloride solution to prevent reaction and subsequent loss of collected hydrides. The moisture trap is loosely packed with anhydrous magnesium perchlorate to prevent spray from reaching the gasometer.

Atomic absorption measurements. All measurements were made on a Varian-Techtron Model AA5 atomic absorption spectrometer. Signals were recorded on a 10-mV strip-chart recorder. Instrumental conditions were those generally recommended by the manufacturer.

Reagents

Standard solutions of all the elements except tellurium were obtained from BDH. The chemicals used in these standards are antimony potassium tartrate, arsenic trichloride, bismuth nitrate, tin(II) chloride, and selenous acid (selenium dissolved in nitric acid). A secondary lead standard was prepared by evaporating the lead nitrate standard solution to near dryness in the presence of perchloric acid and diluting the residue so that the final solution was 2% in perchloric acid. The tellurium standard was prepared by dissolving

tellurium dioxide (Specpure, Johnson and Matthey) in 6 ml of (1 + 1) nitric and hydrochloric acids, the final solution being 6 M in hydrochloric acid. All other reagents used were of A.R. quality.

The sodium borohydride (pellet form, Alpha Inorganics) was made up as a 5% (w/v) solution with up to 1.2 g of potassium hydroxide per 100 ml of solution to inhibit decomposition. The solution was filtered before use [8].

Hydride generation procedures

The conditions used are based on those found to produce highest sensitivity; they are similar to conditions used by other workers [3, 9, 10]. The nitrogen flow is held constant at 600 ml min^{-1}. For antimony, arsenic, bismuth and tellurium, 2.5 M hydrochloric acid solutions are used; for selenium and tin the solutions are 5 M and 0.05 M in HCl, respectively.

The procedure for lead is similar to that described by Fleming and Ide [10], in that 1 ml of 40% (w/v) tartaric acid is added to 7 ml of sample, then precisely 30 s later 1 ml of 10% (w/v) potassium dichromate solution is added, followed immediately by the sodium borohydride solution. For both lead and tin, only 0.4 g of potassium hydroxide is included in each 100 ml of sodium borohydride solution.

To generate the hydrides, the sample solution plus acid is stirred vigorously in the reaction vessel with a magnetic stirrer. The appropriate quantity of sodium borohydride solution is injected by syringe through the septum.

The kinetics of the hydride evolution for the individual elements was investigated by observing the absorbance as a function of time in the direct transfer mode for 500 ng of each element.

The absorbance values for the various elements were compared for both modes with varying quantities of sodium borohydride. In the collection mode, the evolved gases were collected for 30 s and then the reaction vessel was purged with nitrogen until a total volume of 300 ml was accumulated in the gasometer. The maximum volume of sodium borohydride solution was 2 ml (0.1 g $NaBH_4$).

RESULTS

Data obtained for the kinetic study of hydride evolution are shown in Fig. 3. The traces show that the hydrides of lead, tellurium and tin are evolved very rapidly and suggest that maximum sensitivity should be exhibited in direct transfer mode. The traces for antimony, arsenic, bismuth and selenium hydrides show slower evolution, which suggests that a collection procedure should give higher sensitivity for these elements. This was verified for arsenic, bismuth and selenium by the comparative results plotted in Fig. 4. These results show that, for the collection mode, there is an improvement in sensitivity for arsenic, bismuth and particularly selenium. They also indicate the dependence of the sensitivity for various elements on the sodium borohydride concentration. For arsenic and selenium, it is clear

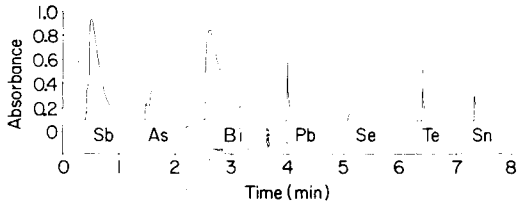


Fig. 3. Absorbance versus time plots obtained by the direct transfer mode of operation with 1 ml of 5% (w/v) sodium borohydride (0.05 g) for 500 ng of each element except bismuth (100 ng).

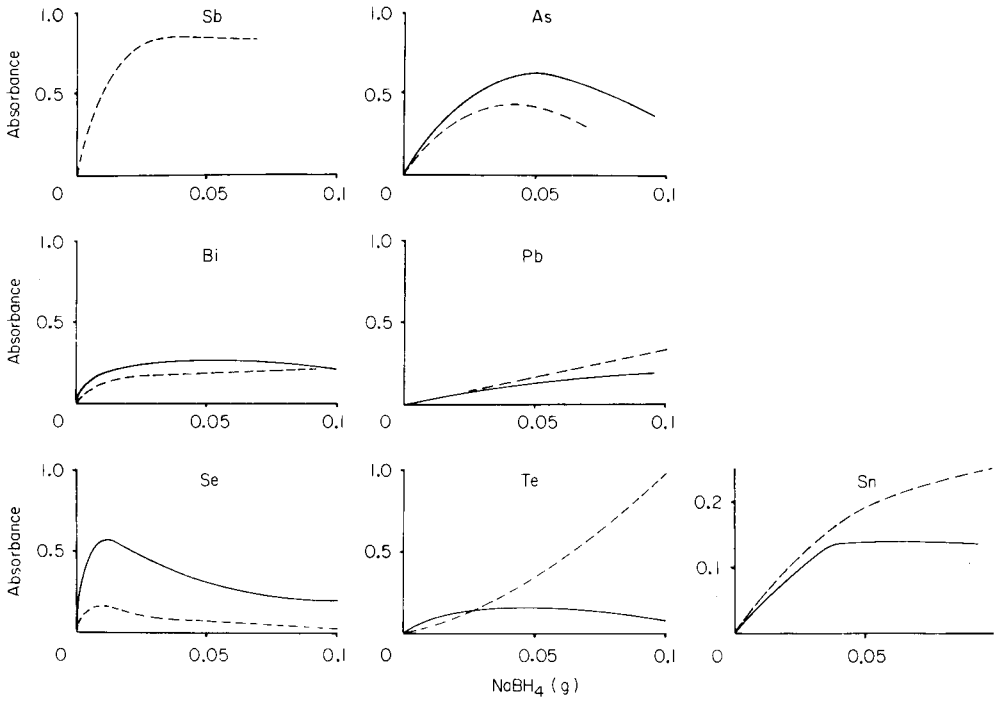


Fig. 4. Comparison of collection mode (——) and direct transfer mode (- - - -). Absorbance versus quantity of sodium borohydride for 500 ng of each element except bismuth (100 ng).

that reductant concentrations in excess of 0.05 g (1 ml of 5% $NaBH_4$) and 0.01 g, respectively, cause a decrease in sensitivity because of dilution of these hydrides by the hydrogen produced during the reaction.

Since a constant volume of 300 ml is collected, composed of hydrogen from the reaction and nitrogen from the purging, a large hydrogen component means very little purging and hence reduced sensitivity. This could

possibly be improved by using a longer nitrogen purge time and collecting a larger volume of gas.

Bismuth is not as dependent on the sodium borohydride concentration as is either arsenic or selenium; thus, although production of bismuthane is not instantaneous, it is considerably faster than that of arsine or hydrogen selenide.

The results for antimony were rather unusal, and only the direct transfer characteristics are shown in Fig. 4. Figure 3 shows the shape of the antimony peak; as more antimony samples were processed, the very small initial peak increased significantly in height while the secondary peak remained virtually unaffected. The height of the initial peak decreased when a series of blanks was processed. This indicated the existence of a memory effect in the tube which was probably due to the deposition of antimony in the cooler regions of the cell. In the collection mode, the two-peak phenomenon was far less pronounced and there was considerable overlapping of the two peaks. As a consequence, results for collection were somewhat erratic and are not included. Because of this, direct transfer and use of the second peak for measurement are preferred for this element.

The best values for lead, tellurium and tin (Fig. 4) resulted, as expected, from the use of direct transfer with higher concentrations of sodium borohydride. The lower sensitivity obtained in the collection mode for these elements could be due to the instability of their hydrides. This was tested by producing the hydrides in the normal collection mode and storing the gases for increasing periods before introducing them to the cell. Figure 5 shows that this instability is responsible for the decreased sensitivity in the collection mode, since the time elapsed between generation of the hydride and its subsequent introduction to the cell is about 1 min.

The conditions for maximum sensitivity for the elements tested are summarized in Table 1. Under these conditions, reproducibility was generally ±3—5%. When hydrochloric acid was added to neutral solutions containing tin and sodium borohydride, the sensitivity for tin was 6.5 ng. This system, although more sensitive, was impractical because the signals were very erratic; neutral tin(II) solutions are unstable.

DISCUSSION

The heated cell atomizer is a highly sensitive apparatus for determining a number of elements which form volatile hydrides. Particularly advantageous are the low noise characteristics of the cell; it is also unnecessary to use background correction. Although the apparatus gives high sensitivity, optimum performance depends on the manner in which the hydrides are introduced into the cell. To achieve the optimum performance for the different elements, it was necessary to devise a gas handling system which permits both direct transfer and collection. It is noteworthy that arsenic and selenium, the most sought-after of the elements investigated in environmental studies, require collection.

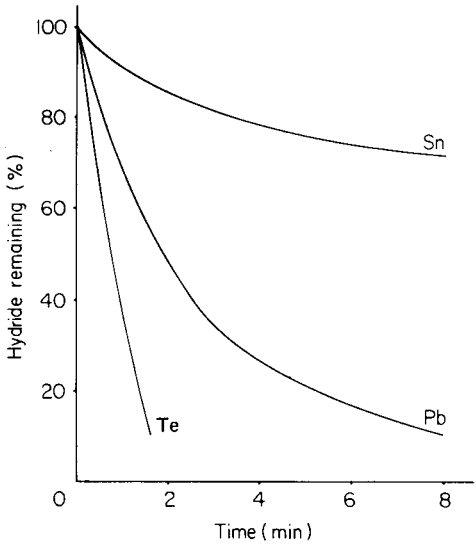


Fig. 5. Relative stability of lead , tellurium and tin hydrides (1 μg of lead, and tin, 0.5 μg of tellurium).

Various problems were encountered during the setting up of the equipment. Water and hydrochloric acid solutions were initially used as gasometer fluids but there were losses of the hydride by reaction or dissolution. Consequently, it was preferable to use a saturated sodium chloride solution although it was still necessary to condition the apparatus before analysis by running samples containing larger than normal amounts of the particular element. The apparatus could then be used successfully after running a series of blanks.

Problems were also encountered with the reproducible generation of the individual hydrides. Initially, rod-type magnetic stirrers (20 mm × 6 mm) were used but these gave erratic results. This was rectified by inserting the rods into Teflon rings. The stirrers produced a deep vortex in the reaction vessel and, provided that the sodium borohydride was added in a reproducible manner, there was good reproducibility. Another source of variation was the sodium borohydride solution. By increasing the potassium hydroxide concentration to 1.2 g per 100 ml, it was found that the solution was more stable and could be used for long periods. For lead and tin generation, which only requires a weakly acidic medium, the lower concentration of potassium hydroxide had to be retained so that the acidity was not disturbed. Another improvement in reproducibility was achieved by filtering the sodium borohydride solution. This is in agreement with the findings of other workers [8].

Recently, Evans et al. [11] have attributed the erratic response from a flame-heated tube atomizer to the condition of the inner surface of the tube. Preconditioning of the atomizer tube by formation of a catalytic film was necessary before consistent response was obtained. Over the past year of use

TABLE 1

Wavelengths, maximum sensitivities and corresponding operating conditions

Element	Wavelength (nm)	Sensitivity[a] (ng)	Conditions (20 ml vol. except Pb)
Antimony	217.6	2.6	2.5 M HCl — direct transfer, 1 ml $NaBH_4$
Arsenic	193.7	5.3	2.5 M HCl — collection, 1 ml $NaBH_4$
Bismuth	223.1	1.8	2.5 M HCl — collection, 1 ml $NaBH_4$
Lead	217.0	6.3	7 ml sample, 1 ml 40% tartaric acid + 1 ml 10% $K_2Cr_2O_7$ — direct transfer, 2 ml $NaBH_4$
Selenium	196.0	5.0	5.0 M HCl — collection, 0.25 ml $NaBH_4$
Tellurium	214.3	1.9	2.5 M HCl — direct transfer, 2 ml $NaBH_4$
Tin	224.6	9.2	0.05 M HCl — direct transfer, 2 ml $NaBH_4$

[a]The weight of the element that gives an absorbance of 0.0044 (1% absorption).

of the present electrically heated cell, similar observations were made, including a steady decrease in sensitivity. The problem was overcome by placing silica tube inserts into the cell so that a new surface was exposed to the hydrides. After a short preconditioning period sensitivity was fully restored to that obtained when the original tube was installed. This recent modification allows electrically heated cell atomizers to be used at optimum performance.

Germanium hydride could also be generated from acidic media by using sodium borohydride. However, the sensitivity was poor as a result of temperature limitations of the cell. The determination of germanium with a nitrous oxide—acetylene flame after generation of the volatile hydride will be reported later.

The authors thank M. Selby for assistance in this work.

REFERENCES

1 W. Holak, Anal. Chem., 41 (1969) 1712.
2 D. C. Manning, At. Absorpt. Newsl., 10 (1971) 57.
3 L. Duncan and C. Parker, Applications of Sodium Borohydride for Atomic Absorption Determination of Volatile Hydrides, Varian-Techtron Technical Topics, Varian-Techtron Pty. Ltd., Springvale, Victoria, Australia.
4 K. C. Thompson and D. R. Thomerson, Analyst, 99 (1974) 595.
5 R. C. Chu, G. P. Barron and P. A. W. Baumgarner, Anal. Chem., 44 (1972) 1480.
6 L. P. Greenland and E. Y. Campbell, Anal. Chim. Acta, 87 (1976) 323.
7 A. J. Thompson and P. A. Thoresby, Analyst, 102 (1977) 9.
8 J. R. Knechtel and J. L. Fraser, Analyst, 103 (1978) 104.
9 E. N. Pollock and S. J. West, At. Absorpt. Newsl., 12 (1973) 6.
10 H. D. Fleming and R. G. Ide, Anal. Chim. Acta, 83 (1976) 67.
11 W. H. Evans, F. J. Jackson and D. Dellar, Analyst, 104 (1979) 16.

Analytica Chimica Acta, 111 (1979) 145—153

DETERMINATION OF SULPHUR IN PETROLEUM PRODUCTS AFTER SULPHUR DIOXIDE GENERATION AND OF SULPHUR DIOXIDE IN AIR BY CHEMILUMINESCENT FLAME EMISSION

J. F. ALDER* and K. KARGOSHA

Chemistry Department, Imperial College, London S.W.7. (Gt. Britain)

(Received 16th May 1979)

SUMMARY

The determination of sulphur in petroleum products by combustion, concentration of the sulphur dioxide in sodium tetrachloromercurate solutions and cool flame molecular emission is described. Improvements in burner design and optimization of analytical conditions result in a minimum detectable amount of 6 ng of sulphur. The absorption of sulphur dioxide from air samples and its determination by a similar method gives a detection limit of 1.3 μg SO_2 m^{-3}.

The determination of sulphur by generation of hydrogen sulphide, sulphur dioxide or a sulphuric acid aerosol into a cool flame and measurement of the consequent S_2 band emission is well known [1, 2]. Previous work [3—5] has shown this to be a useful technique for the determination of sulphur in petroleum products giving a sensitive, selective and reasonably precise determination. Much of the previous work was based on the use of hydrogen sulphide as the intermediate and this has certain attractive features: storage of the gas in sodium hydroxide permits pre-concentration; the low solubility of hydrogen sulphide in acid solution ensures good and rapid recovery from the storage solution and formation of hydrogen sulphide by metal reduction can impart selectivity to the method permitting a degree of speciation [3].

The use of sulphur dioxide as intermediate, however, offers certain advantages over hydrogen sulphide in particular applications. For example, when fatty acids or long-chain esters are present, the use of sodium or acid/alloy as reductant to liberate H_2S is precluded by formation of soaps. Most organic materials can be burnt to give sulphur dioxide and trace-level atmospheric sulphur dioxide is of interest in pollution studies. In this study the generation, storage and release of sulphur dioxide as an intermediate in the determination of sulphur in petroleum products by flame chemiluminescent emission was investigated and the potential of the method for atmospheric sulphur dioxide determinations assessed.

EXPERIMENTAL

Apparatus and reagents

The flame photometric apparatus employed has been described elsewhere [3, 4]. The cool-flame burner design is discussed below. The light distillates were burned in a standard lamp apparatus (Townson Mercer) to produce sulphur dioxide. The generation cell was as described previously [3] but the gas inlet tube came very close to the bottom of the cell to permit bubbling through the solution.

Standard sodium sulphite solutions

Sodium sulphite solutions alone are unstable owing to oxidation of sulphite by oxygen catalysed by traces of heavy metals [6] particularly copper and iron. They can, however, be stabilized by sodium tetrachloromercurate (STCM) in the presence of EDTA at pH 5.0—7.6 [6—8]. A standard solution was prepared by making an aqueous 0.01 M solution of sodium sulphite heptahydrate and standardizing it iodimetrically [9]. A 5-ml aliquot was immediately diluted to 50 ml with 0.2 M STCM—0.002 M EDTA adjusted to pH 5—6 with sodium hydroxide. (The 0.2 M STCM—0.002 M EDTA solution was prepared by dissolving 2.34 g of NaCl, 5.44 g of $HgCl_2$ and 0.060 g of EDTA (disodium salt) in 100 ml of water.) All chemicals were of analytical-reagent grade. Stability tests were carried out on this stabilized solution (30 ppm S) in open and stoppered flasks over a 24-h period by using the analytical method described later. In both cases, no noticeable change in the sulphite concentration could be detected, whereas the levels in unstabilized solutions contained in unstoppered and stoppered flasks were reduced from 30 ppm to 0.6 ppm and 3.2 ppm S (as sulphite), respectively, after 24 h.

The stabilized solutions thus prepared were stable for a month if kept at 5°C. In the procedure for the determination of sulphur in petroleum distillates, it was found advantageous to add a pH buffer and glycerol as an antioxidant (see below).

DEVELOPMENT OF METHODOLOGY

Burner design

The chemiluminescent S_2 emission has been suggested to result from the combination of two sulphur atoms [10]. It is important, therefore, in order to achieve maximum emission intensity that the sulphur atom population be created initially, that the formation of S_2 should be favoured, and that the S_2 excited states should not be quenched. These criteria are best met in the cool parts of a hydrogen—argon diffusion flame and it is well established that the presence of carbon and hydrocarbon species quenches the emission markedly [10]. When hydrogen sulphide is employed as the intermediate gas in the determination of sulphur, it is passed directly into the cool cavity of an inward-burning diffusion flame and there is no contact with the hot com-

bustion gases. This is perfectly adequate for efficient formation of S_2 and the consequent emission of radiation. Sulphur dioxide, however, has a much higher free energy of formation than hydrogen sulphide ($\Delta H_F^0(SO_2) = -296.8$ kJ mol^{-1}; $\Delta H_F^0(H_2S) = -20.63$ kJ mol^{-1} [11]) and so the breakdown of sulphur dioxide to sulphur atoms requires much more energy. To improve this initial atomization step, the burner design was altered to permit passage of the sulphur dioxide through the hot combustion gases prior to their entering the cool region where chemiluminescence is greatest (about 390°C) [12]).

The burner employed is illustrated in Fig. 1. Argon and hydrogen pass up the inner tube and air, either a flow bypassing the SO_2 generation cell (to permit maintenance of the flame during cell cleaning) or one which purges the cell, passes up the outer tube. The burner is enclosed in a borosilicate glass tube with a restricting orifice at the open end. The actual mechanism of the influence of this sheath is not certain, but its effect is most important. Under the same gas flow conditions and with the same amount of sulphur dioxide going into the flame, altering the dimensions of the restricting tube had the effects shown in Table 1. The length of the tube makes little difference whereas its diameter and the diameter of the restricting orifice are crucial. This suggests that the efficiency of flame gas mixing is the parameter being affected, although the slight increase in pressure caused by the restricting orifice should help to maintain a cool flame by preventing ambient air being drawn into the burner assembly. Air cooling of the restricting tube did not

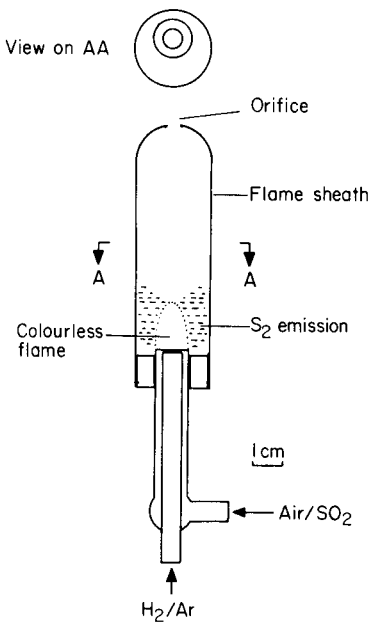


Fig. 1. The cool flame burner and sheath. The burner is off-set in the sheath as this reduces turbulence and flame noise.

TABLE 1

Effect of restricting tube dimensions on peak emission intensity (All dimensions in mm.)

Width (i.d.)	Length	Orifice diameter	Normalized peak height intensity, (60 ng S)
12	145	5	100
12	75	5	97
18	145	5	225
18	75	5	220
23	145	5	340
23	75	23	0
23	75	10	160
23	75	5	340
23	75	2.5	Flame extinguished

noticeably affect the signal intensity, nor did substitution of silica for the borosilicate glass tube. The argon acts as a flame gas diluent thus maintaining a cool flame. The optimum gas flow rates were: argon 980 ml min^{-1}; air 250 ml min^{-1}; hydrogen 150 ml min^{-1}. Purging the generation flask with argon and passing the $SO_2/Ar/H_2$ up the centre tube and air up the outside tube resulted in a five-fold decrease in peak intensities, which illustrates the effect of the sulphur dioxide passing through the hot flame gases prior to formation of S_2.

Generation of sulphur dioxide

The sulphur dioxide can be formed in several ways depending on the nature of the sample. The commonest way for light petroleum fractions is by combustion in a lamp apparatus [13] and for heavier fractions by hot tube combustion. However it is formed or occurs, the gas is first trapped in an 0.02 M STCM—0.002 M EDTA solution. The sulphur dioxide can be released by acidification with hydrochloric acid: $Hg(SO_3)_2^{2-} + 4H^+ + 4Cl^- \rightarrow HgCl_4^{2-} + 2SO_2 + 2H_2O$.

The rate of evolution is affected by the acid concentration and by the speed of stirring in the generation vessel. Normally 1 ml of acid was added to the empty generation cell and, when purged, 1 ml of sample was injected through the rubber (Subaseal) septum. (Reversing the procedure did not make any noticeable difference.) Maximum peak intensity was obtained at acidities exceeding 2 M with the maximum stirring speed obtainable without splashing. More reproducible and controllable stirring was achieved by using two 1-cm long stirrer bars rather than one. If the purging air was bubbled through the solution, rather than just sweeping the cell, the peak height for 80 ng of sulphur increased by 63%, at the expense of peak width. Heating the generation cell and hydrochloric acid to 50°C (with heating tape) before

introduction of the sample (and stirring), in order to increase the rate of reaction and decrease the solubility of sulphur dioxide, increased the peak height by 100% without affecting the peak area. Under these optimized conditions, the minimum detectable amount of sulphur was 6 ng of sulphur (as sulphur dioxide) in the 1-ml STCM sample aliquot; the signal was twice the blank signal from distilled water and STCM. The range of linearity of the log signal vs. log [S] plot was 0.08—1.8 μg in the 1-ml aliquots.

APPLICATIONS

Analysis of light distillates

In order to check the applicability of the method to light distillates, samples previously analysed by B.P. Research Ltd. by x-ray fluorescence (x.r.f.) were analysed for sulphur. The samples were burned in a lamp apparatus following the ASTM procedure D 1266 [13]. Air was drawn through the system at 250 ml min^{-1} and the sulphur dioxide collected. Passing the air and combustion products through the absorber solution containing sulphur dioxide is likely to oxidize it; Haller [14] has shown that the addition of glycerol inhibits the chain reaction mechanism by which sulphur dioxide is oxidized and its addition to the STCM—EDTA solution caused a noticeable increase in recovery (Table 2). In these recovery tests, 0.1 and 1 ppm of sulphur (as sulphite) were fixed in the STCM solution and the gases from a 40-min combustion of pure (60—80°C boiling range) petroleum ether in the lamp apparatus were drawn through the solution. The absorbing solution was then analysed.

Just as important as glycerol is the correct buffering of the absorbing solution; the addition of a sodium dihydrogenorthophosphate—disodium hydrogenorthophosphate buffer (pH 6.8—6.9) increased the recovery of sulphur dioxide markedly. Increasing the STCM concentration from 0.02 to 0.2 M

TABLE 2

Efficiency of absorbing solutions

Absorbing solution	pH of solution		Recovery (%)	
	before combustion	after combustion	0.1 ppm S added	1 ppm S added
0.1 M STCM	5.2	2.6	70	75
0.1 M STCM—0.002 M EDTA	4.6	2.6	80	80
0.1 M STCM—0.002 M EDTA—2% (v/v) glycerol	4.6	2.5	85	87
0.1 M STCM—0.002 M EDTA—2% (v/v) glycerol—phosphate buffer[a]	6.8	6.8	99	100
0.02 M STCM—0.002 M EDTA—2% (v/v) glycerol—phosphate buffer	6.8	6.8	94	96

[a]Increasing the STCM concentration to 0.2 M made no difference to the recovery, nor did increasing the glycerol level to 3% (v/v).

had some effect on the recovery (Table 2). Thus the absorbing solution finally adopted was 0.2 M STCM—0.002 M EDTA—0.02 M NaH_2PO_4—0.025 M Na_2HPO_4—2% (v/v) glycerol, at pH 6.8—6.9.

Samples (5 ml) of the light distillates diluted with the 60—80 petroleum ether, to bring them into the correct concentration range, were burned in the lamp apparatus and the combustion gases were scrubbed in 50 ml of the absorbing solution, which was then analysed. The sulphur content was calculated from peak emission intensity calibration curves prepared from solutions of t-butyl disulphide in 60—80 petroleum ether treated in the same manner. The results obtained are shown in Table 3 and are in good agreement with those obtained by x.r.f.

Analysis for atmospheric sulphur dioxide

There are many existing approaches to this analysis, e.g. conductimetry [15], amperometry [16] and spectrophotometry with *p*-rosaniline [8]. In all these methods there are potential interferences from reducing and oxidizing agents, particularly nitrogen oxides, and possibly from heavy metals [17—19]. Kouimtzis [20] used molecular emission cavity analysis for the determination and Crider [21] applied flame photometry.

The apparatus and method recommended above were examined for the determination of sulphur dioxide in atmospheric samples. In this work, which used 5-ml samples rather than the 1 ml recommended above, it was found better to heat the sulphur dioxide generation vessel to 80°C, in order to sharpen the analytical peaks. The steam produced was condensed from the gas flow by a small Liebig condenser at the outlet of the vessel to prevent it from reaching the flame and so reducing the signal.

In the absorption method, dissolved sulphur dioxide must not be decomposed or affected by air passing through the solution. In order to check this, solutions with different concentrations of STCM were made up and sodium sulphite was added. Air, previously scrubbed free of sulphur dioxide by passing it through a solution of STCM, was passed through these test solutions at 1 l min^{-1} for 30 min. The solutions were analysed as described. The results (Table 4) show that there were no detectable losses from solutions containing as little as 0.01 M STCM. Good recoveries were obtained without the addition of glycerol and buffer, perhaps because only clean air was passed through the solution rather than combustion gases; for heavily polluted atmospheres this

TABLE 3

Analysis of light distillates

Sample	7845	7846	7913	7935	7950	7957	7968	8053
% S given[a]	0.17	0.08	0.22	0.24	0.12	0.11	0.03	0.95
% S found[b]	0.14	0.06	0.19	0.22	0.15	0.12	0.023	1.05

[a]Data supplied by B.P. Research. [b]5% r.s.d. on 5 replicates.

TABLE 4

Effect of STCM concentration of stability of sulphur dioxide in aqueous solution

STCM present (M)	SO_2 content (μg/10 ml)	SO_2 recovered (%)	STCM present (M)	SO_2 content (μg/10 ml)	SO_2 recovered (%)
0.00	10	48	0.02	10	98
	20	42		50	99
	50	40		100	98
0.01	10	97	0.10	20	99
	30	98		50	98
	50	95		100	99

may not be true. The effect of air flow rate and volume treated was checked by passing previously scrubbed air through 10-ml aliquots of 0.02 M STCM containing sodium sulphite (10 μg of SO_2). The results (Table 5) show that there is some loss at high flow rates and large volumes of air treated.

Potential interferences

Dinitrogen oxide dissolves in water to give an equilibrium mixture of nitrous and nitric acid. The nitrous acid formed can be quantitatively destroyed by sulphamic acid without detriment to the dissolved sulphur dioxide [18]. Tests were made on 0.02 M STCM solutions containing various amounts (0—100 ppm) of nitrite and sodium sulphite corresponding to 10 μg of sulphur dioxide with and without addition of 1-ml aliquots of aqueous 0.6% (w/v) sulphamic acid solution. The samples were analysed by the usual flame procedure. The presence of sulphamic acid effectively removed the interference of nitrite. In the absence of sulphamic acid, as much as 85% of the sulphite was lost when 20 ppm of nitrite was added; in the presence of sulphamic acid, losses did not exceed 5% even when 100 ppm of nitrite was added.

TABLE 5

Effect of aeration time and air sample volume on the stability of STCM—sulphur dioxide solutions

Air flow rate (l min^{-1})	Aeration time (min)	Air sample volume (l)	SO_2 recovery (%)	Air flow rate (l min^{-1})	Aeration time (min)	Air sample volume (l)	SO_2 recovery (%)
0.2	30	6	100	1.00	30	30	99
	60	12	98		45	45	94
	90	18	99		60	60	90
					90	90	89
0.6	15	9	98				
	30	18	100	1.5	30	45	92
	60	36	98		60	90	87
	90	54	91		90	135	80

Heavy metals affect not only the stability of the sodium sulphitemercurate solution but also the regeneration of the sulphur dioxide. Representative salts were added to solutions in 0.02 M STCM with and without EDTA. A 10-ml portion of each solution was aerated with scrubbed air at 1 l min^{-1} in the sintered bubbler for 30 min. All the samples were analysed as described above after the addition of 1 ml of 0.6% sulphamic acid solution and shaking for 10 min. Table 6 shows that EDTA (disodium salt) at concentrations of 0.2—0.4 mg/10 ml is effective in removing the interferences. As the maximum reported values of these metals in the atmosphere are only (in $\mu g\ m^{-3}$) Fe, 22; Mn, 10; Cu, 10 and Cr, 0.24 [17], these metals should not interfere significantly. Where low levels of sulphur dioxide are to be determined and dust levels may be high, however, the use of EDTA is recommended.

TABLE 6

Effects of salts and their suppression by EDTA

Salt	Metal added (μg/10 ml)	EDTA added (mg/10 ml)	Signal suppression (%)
Iron(III) chloride	10	—	92
	20	—	98
	50	—	100
	10	2	4
	50	2	6
	200	2	23
	200	4	0
Iron(II) sulphate	20	—	2
	200	—	3
	20	1	2
	200	2	2
Chromium(III) chloride	10	—	15
	20	—	32
	50	—	50
	200	—	70
	20	1	3
	200	2	2
Potassium chromium(III) disulphate	20	—	2
	200	—	2
	20	1	0
	200	2	2
Manganese(II) chloride	20	—	2
	200	—	5
	20	2	0
	200	2	2
Copper(II) sulphate	20	—	2
	200	—	8
	20	1	2
	200	2	0

Sensitivity

When 10 ml of absorbing solution (0.02 M STCM—0.001 M disodium EDTA) was shaken with 1 ml of 0.6% sulphamic acid before analysis, a 5-ml aliquot containing 2 ppb of sulphur (as sodium sulphite) gave a signal twice that of the blank solution. Assuming that a 30-l air sample had been used, as little as 1.3 $\mu g\ m^{-3}$ sulphur dioxide in air could be determined.

CONCLUSION

These procedures provide other means of determining sulphur down to very low levels in organic matrices and in air. Compounds which, by nature of their reactive groups, are not amenable to analysis by metal reduction methods, can be analysed by the proposed method. Of equal interest is the application to ambient air, where air samples can be collected, stored and transported easily and then quickly analysed with little further sample preparation. The presence of nitrogen oxides and heavy metals can be readily overcome and the method provides good sensitivity and a reasonable linear response range.

The authors are grateful to B.P. Research, Sunbury, for the provision of the analysed samples.

REFERENCES

1 P. T. Gilbert, in R. Mavrodineanu (Ed.), Analytical Flame Spectroscopy, Springer-Verlag, 1970.
2 C. Veillon and J. Y. Park, Anal. Chim. Acta, 60 (1972) 393.
3 J. F. Alder and K. Kargosha, Anal. Chim. Acta, 107 (1979) 231.
4 J. F. Alder, M. A. Pinches, T. S. West and R. W. Williams, Lab. Pract., 25 (1976) 455.
5 P. L. Patterson, R. L. Howe and A. A. Shumays, Anal. Chem., 50 (1978) 339.
6 H. Pugh and W. R. Waterman, Anal. Chim. Acta, 55 (1971) 97.
7 F. Feigl, Chemistry of Specific, Selective and Sensitive Reactions, Academic Press, New York, 1949.
8 P. W. West and G. C. Gaeke, Anal. Chem., 28 (1956) 1816.
9 I. M. Kolthoff, R. Belcher, V. A. Stenger and G. Matsuyama, Volumetric Analysis, Vol. 3, Interscience, New York, 1957, p. 291.
10 S. A. Fredriksson and A. Cedergren, Anal. Chim. Acta, 100 (1978) 429.
11 J. R. W. Warn, Concise Chemical Thermodynamics, Van Nostrand, New York, 1977, p. 21.
12 R. M. Dagnall, K. C. Thompson and T. S. West, Analyst, 92 (1967) 506.
13 Annual Book of ASTM standards, ASTM D 1266-70, 1974.
14 P. Haller, J. Soc. Chem. Ind., 38 (1969) 52.
15 C. D. Hollowell, G. Y. Gee and R. D. McLaughin, Anal. Chem., 45 (1973) 63A.
16 J. Forrest and L. Newman, J. Air Control Assoc., 23 (1973) 761.
17 F. P. Scaringelli, B. E. Saltzman and S. A. Frey, Anal. Chem., 39 (1967) 1709.
18 P. W. West and F. E. Ordoveza, Anal. Chem., 34 (1962) 1324.
19 A. Attair, T. P. Igielski and B. Iaseleskis, Anal. Chem., 42 (1970) 1282.
20 T. A. Kouimtzis, Anal. Chim. Acta, 88 (1977) 303.
21 W. L. Crider, Anal. Chem., 37 (1965) 1770.

Analytica Chimica Acta, 111 (1979) 155—162

PULSE NEBULIZATION OF CHLOROFORM AND CARBON TETRACHLORIDE EXTRACTS IN FLAME ATOMIC ABSORPTION SPECTROMETRY

D. L. TSALEV*

Faculty of Chemistry, University of Sofia, Sofia 1126 (Bulgaria)

I. I. PETROV

Institute of Hygiene and Occupational Diseases, Sofia 1431 (Bulgaria)

(Received 25th May 1979)

SUMMARY

The determination of cadmium, cobalt, copper, nickel and lead after extraction with tetramethylenedithiocarbamate into various solvents is achieved by injection of 50-μl aliquots of extract by pulse nebulization into an air—acetylene flame. The technique is particularly suitable for cadmium and lead extracted into chlorinated solvents, which must not continuously be nebulized into the flame. The performance of the technique is compared for chloroform, carbon tetrachloride, butyl acetate, methyl isobutyl ketone, toluene and xylene.

Liquid—liquid extraction is a rapid and well-established method for trace preconcentration prior to atomic absorption spectrometry (a.a.s.) [1]. The choice of appropriate organic solvents for direct continuous spraying into the flame is limited by several important requirements: the solvent should have good nebulization and burning characteristics, not too low a boiling point, and low solubility in water. In addition, of course it should extract the analyte effectively. Allan [2] has pointed out that C_6 and C_7 ketones and esters are the most suitable solvents for flame a.a.s. At present, methyl isobutyl ketone (MIBK), butyl acetate and xylene are widely used for liquid—liquid extraction prior to flame a.a.s.

Some common and well-studied organic solvents such as chloroform, carbon tetrachloride and benzene are widely used for liquid—liquid extractions in general. Unfortunately, the continuous nebulization of these solvents into a flame gives an unstable, noisy flame, and toxic products such as hydrogen chloride and phosgene are evolved. If these organic solvents are used, tedious and time-consuming procedures are necessary before a.a.s., e.g. evaporation of the solvent and its replacement by MIBK [3], back-extraction [4, 5] or mineralization of the extract [6].

Gomišček and Špan [7] sprayed chlorinated solvents continuously into a turbulent air—hydrogen flame and found that chlorine influenced the magnesium and copper absorbances. Tsalev et al. [8] continuously nebulized

chloroform extracts into flames, using a specially designed spray chamber for aerosol drying; the sensitivity was improved by factors of 0.7—3.2.

The aim of this study was to develop a direct, simple and rapid method for flame a.a.s. applicable to chlorinated solvents and other "difficult" organic extractants, based on the pulse nebulization of small sample portions. This "injection" method has been proposed by Sebastiani et al. [9] and was recently reviewed by Berndt and Slavin [10]; it appears not to have been used for analysis of solvent extracts.

EXPERIMENTAL

Apparatus

The Perkin-Elmer Model 272 single-beam atomic absorption spectrometer used was equipped with a deuterium background corrector and a Model 056 recorder. The background corrector was used in all measurements except for part of the background study as indicated in Table 1.

Hollow-cathode lamp currents and slit widths were as recommended by the manufacturer. Electrodeless discharge lamps were used for cadmium and lead determinations. The lamps were heated for 20—30 min before the start of the measurements.

The variable nebulizer was adjusted to give maximum absorbance with an aqueous copper standard (4 μg ml^{-1}) continuously sprayed into a stoichiometric air—acetylene flame; the rate of liquid consumption was 1.8—2.0 ml min^{-1}.

A Perkin-Elmer teflon sampling cup was used for injection of small sample portions into the flame (pulse nebulization). The cup was attached to the end of the nebulizer capillary tubing. Standard solutions were introduced into the cup with 50-μl Eppendorf pipettes.

Reagents and standard solutions

Standard solutions (1000 μg ml^{-1}) of cadmium, copper, cobalt, nickel and lead (Hopkin & Williams Ltd, England) were diluted with redistilled water as required.

All reagents used were of analytical grade. Acetate buffer pH 5.0 was prepared from 55 g of sodium acetate dissolved in 80 ml of redistilled water; the pH was adjusted with acetic acid and the solution diluted to 100 ml.

Preliminary work

A 50-μl injection was found to give sensitivity and precision similar to those obtained by continuous nebulization. With 20-μl and 10-μl portions, the sensitivity (based on peak-height measurements) was less by factors of 1.6 and 3.4, respectively. All further experiments were done with 50-μl pulse-nebulized solutions.

Benzene gave a high irreproducible background absorbance and very poor precision; it was not studied further.

TABLE 1

Flame background (Bg) and noise (N) after pulse nebulization of different organic solvents (Standard deviations given in absorbance units.)

Element	Wavelength (nm)	Solvent	Without background correction		With background correction	
			Bg	N	Bg	N
Cd	228.8	$CHCl_3$	−0.008	0.002	0	0.002
		CCl_4	0.005	0.0004	−0.002	0.002
		BuOAc	−0.057	0.002	−0.007	0.002
		MIBK	−0.070	0.0005	−0.0059	0.0006
		Toluene	0.170	0.01	−0.024	0.01
		Xylene	−0.066	0.001	−0.006	0.001
Co	240.7	$CHCl_3$	−0.006	0.001	0	0.003
		CCl_4	0.003	0.001	0	0.004
		BuOAc	−0.031	0.0007	0	0.003
		MIBK	−0.037	0.002	0	0.002
		Toluene	0.143	0.02	−0.007	0.001
		Xylene	−0.034	0.002	0	0.002
Cu	324.7	$CHCl_3$	0.003	0.001	0	0.002
		CCl_4	0	0.001	0	0.003
		BuOAc	0	0.002	0	0.002
		MIBK	0	0.001	0	0.001
		Toluene	−0.035	0.004	−0.007	0.001
		Xylene	−0.003	0.001	0	0.002
Ni	232.0	$CHCl_3$	0	0.003	0	0.001
		CCl_4	0	0.003	0	0.001
		BuOAc	−0.034	0.002	0	0.002
		MIBK	−0.053	0.002	0	0.002
		Toluene	−0.051	0.003	0	0.004
		Xylene	−0.040	0.003	0	0.002
Pb	217.0	$CHCl_3$	−0.006	0.001	0.002	0.001
		CCl_4	0.012	0.0009	0.003	0.001
		BuOAc	−0.084	0.001	0	0.001
		MIBK	−0.103	0.003	0.005	0.0006
		Toluene	0.062	0.004	0.011	0.004
		Xylene	−0.092	0.002	0	0.001

An oxidizing (air, 21.5 l min^{-1}; acetylene, 2.4 l min^{-1}) flame was used for aqueous solutions as well as for butyl acetate, MIBK, toluene and xylene. This flame lifted-off when chloroform or carbon tetrachloride was injected. A higher acetylene flow rate (3.5 l min^{-1}) was used with these solvents. There was a memory effect when organic extracts were injected but not when aqueous solutions were used; accordingly, aqueous blanks were injected between organic extract samples.

Procedures

Standard solutions of cadmium, cobalt, copper, nickel and lead in MIBK, butyl acetate, chloroform and carbon tetrachloride were prepared by liquid—liquid extraction as follows: 10 ml of an appropriate aqueous standard solution (0.1—4 $\mu g\ ml^{-1}$) were placed in a separatory funnel and 1 ml of acetate buffer, 1 ml of aqueous 2% (w/v) ammonium tetramethylenedithiocarbamate and 10 ml of the organic solvent were added. After a 5-min extraction, the organic layer was allowed to separate completely and then tranferred to a glass tube. These extracts were used as standard organic extracts. The complete extraction of the analytes had previously been established in all instances. For a.a.s., 50 μl of the pure organic solvent was pulse-nebulized and the flame background was recorded. The background of the burning flame was used as the zero for metal determinations. For every organic solvent, 6—10 successive injections were done without and with background correction. The mean values of flame background absorbance and flame noise (expressed as standard deviations) are listed in Table 1. In some cases, e.g. at the cadmium and lead wavelengths, the background could not be completely compensated. Some organic solvents, such as benzene and toluene, gave a noisy flame.

Four organic solvents (chloroform, carbon tetrachloride, MIBK and butyl acetate) were studied further. Several series of measurements were done on different days to obtain the sensitivity and precision data. Standard solutions in water of MIBK and butyl acetate were continuously nebulized and calibration curves were plotted. These standard solutions, as well as chloroform and carbon tetrachloride extracts, were also pulse-nebulized into the flame. Typical calibration curves for cadmium in the various solvents are depicted in Fig. 1. Characteristic concentrations (absorbances = 0.0044) and detection limits (3 σ) are presented in Table 2. The relative standard deviations were calculated from 4—11 successive pulse-nebulization measurements (Table 3). The rate of liquid consumption was evaluated from the time necessary for continuous spraying of 1 ml of solvent (Table 4).

The efficiency of nebulization was measured as follows: a trap with glass wool was placed instead of the burner and 1 ml of a standard solution was continuously or pulse nebulized (20 successive 50-μl injections were made in the latter case). The amount of element found in the trap was divided by the amount introduced into the nebulizer to give the efficiency of nebulization (Table 4). Peak- height and peak- area measurements for calibration were compared (Table 4).

Other studies

In order to verify the validity of the results, some experiments were performed with two other atomic absorption spectrometers. A Perkin-Elmer Model 400 was employed with a stoichiometric air—acetylene flame (12 flow-meter divisions for air and acetylene). The nebulizer was adjusted to give a low rate of liquid consumption, ca. 3.0 ml min^{-1}. This reduction in

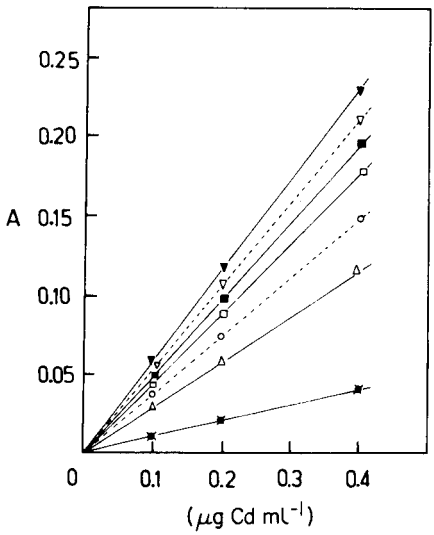


Fig. 1. Calibration curves for cadmium extracted with tetramethylenedithiocarbamate into different solvents. Continuous nebulization: (•) water, (○) BuOAc, (▽) MIBK. Pulse nebulization: (×) water; (△) carbon tetrachloride; (□) BuOAc; (■) MIBK; (▼) chloroform.

TABLE 2

Characteristic concentrations (c.c.) and detection limits (d.l., 3 σ) for aqueous solutions and organic extracts (in $\mu g\ ml^{-1}$)

Element		Continuous nebulization			Pulse nebulization[a]				
		Water	BuOAc	MIBK	Water	BuOAc	MIBK	$CHCl_3$	CCl_4
Cd	C.c.[b]	0.043	0.012	0.008	0.043	0.010	0.095	0.0075	0.014
	D.l.[c]	0.01	0.009	0.003	0.03	0.01	0.004	0.01	0.02
Co.	C.c.	0.31	0.075	0.067	0.31	0.075	0.072	0.08	0.11
	D.l.	0.5	0.2	0.07	0.4	0.1	0.08	0.2	0.3
Cu	C.c.	0.19	0.040	0.032	0.20	0.040	0.032	0.060	0.075
	D.l.	0.05	0.03	0.01	0.07	0.05	0.02	0.07	0.1
Ni	C.c.	0.30	0.005	0.055	0.32	0.60	0.065	0.13	0.18
	D.l.	0.2	0.06	0.07	0.2	0.08	0.09	0.09	0.1
Pb	C.c.	0.42	0.12	0.078	0.42	0.10	0.068	0.09	0.12
	D.l.	0.1	0.08	0.05	0.1	0.07	0.04	0.06	0.08

[a]50-μl injection.

sample consumption from 7.7 ml min^{-1} in the above studies gave only half the sensitivity obtained previously. Otherwise, similar results were obtained with this instrument (Table 4).

Pulse-nebulization experiments with a Pye-Unicam SP 1950 spectrometer were unsuccessful. When chloroform and carbon tetrachloride extracts were

TABLE 3

Relative standard deviation (s_r) for n successive 50-μl injections of extracts

Element	Conc. (μg ml^{-1})	Solvent	n	s_r (%)
Cd	0.1	$CHCl_3$	4	1.5
	0.4		4	3.0
	0.1	CCl_4	8	3.8
	0.4		10	2.4
Co	1	$CHCl_3$	7	5.2
	4		6	2.2
	2	CCl_4	5	3.9
	4		7	3.4
Cu	1	$CHCl_3$	11	4.7
	1	CCl_4	4	4.4
Ni	2	$CHCl_3$	7	1.7
	1	CCl_4	10	3.2
Pb	1	$CHCl_3$	7	4.6
	4		7	1.6
	1	CCl_4	5	5.1
	4		6	3.4

TABLE 4

Sensitivity enhancement for pulse-nebulized organic extracts compared to aqueous solutions

Element	Calibration mode	Organic solvent			
		BuOAc	MIBK	$CHCl_3$	CCl_4
Cd	Pk. ht.	4.3	4.5	5.6	3.1
	Pk. area	5.6	7.4	8.5	4.9
Co	Pk. ht.	4.1(3.2)[a]	4.3(3.7)	3.9(1.4)	2.8
Cu	Pk. ht.	5.0(3.4)	6.2(4.6)	3.3(2.8)	2.9
	Pk. area	4.8	5.7	3.9	3.6
Ni	Pk. ht.	5.3(3.2)	4.9(4.5)	2.4(2.1)	1.8
	Pk. area	5.3	6.5	4.1	3.2
Pb	Pk. ht.	4.2(3.3)	6.2(5.0)	4.7(4.6)	3.5(3.6)
	Pk. area	6.5	8.7	7.8	7.5
Efficiency of nebulization (%)		14	27	63	32
Rate of liquid consumption (ml s^{-1})		0.025	0.034	0.028	0.021

[a] Figures in parentheses were obtained with a Perkin-Elmer 400.

injected into the flame (air, 5.0 l min^{-1}; acetylene, 1.2 l min^{-1}), very high background absorption occurred and the noise from the photomultiplier increased. Because the uptake of solution by the nebulizer was not variable, it was not possible to decrease the rate of liquid consumption, which seemed to be high in relation to the total oxidant and fuel flow.

RESULTS AND DISCUSSION

The data in Tables 2 and 4 show that the sensitivity is improved in chloroform by a factor of 2.4—5.6, and in carbon tetrachloride by a factor of 1.8—3.5 as compared with pulse-nebulized aqueous solutions, values which are between 50 and 100% of those achieved with MIBK, the most suitable organic solvent for flame a.a.s. The detection limits for chloroform solution are between 50 and 100% of those achieved for aqueous solutions.

The efficiency of nebulization when chloroform and carbon tetrachloride were pulse-nebulized (Table 4) was very high, 63% and 32%, respectively. This could be explained by their low surface tension and low boiling points, which would lead to smaller aerosol droplets and more effective solvent evaporation in the spray chamber. The efficiency of nebulization was somewhat lower, 59% for continuously nebulized chloroform extracts. MIBK, a solvent with a higher boiling point and very low surface tension, gave the same efficiency of nebulization (27%) whether continuously nebulized or pulse nebulized.

Peak-area measurements (Table 4) were expected to throw more light on the processes involved in signal enhancement, since the peak area should not depend on the rate of liquid consumption. The sensitivity enhancement was the same or better with this mode of calibration. For carbon tetrachloride, which has a rate of liquid consumption lower than that of aqueous solutions, peak-area measurements were most favourable. The influence of the chlorine in chloroform and carbon tetrachloride could be responsible for the greater sensitivity observed for cadmium and lead in these solvents.

It seems that this technique can be easily adapted to any atomic absorption spectrometer equipped with a background corrector, a variable nebulizer, rapid electronics and a fast recorder. Ventilation is essential as toxic and aggressive gases are evolved in the flame.

The main result of this investigation is the development of a simple, fast and sensitive method for the analysis of chlorinated solvents and other organic extracts, which cannot continuously be sprayed into flames. The technique could lead to the use of a wider variety of organic solvents for preconcentration or sample dilution in flame a.a.s. A number of previously developed highly-efficient and/or selective extraction methods could then be adapted to flame a.a.s. Applications to elements with low atomization efficiency are worth further study.

REFERENCES

1 M. S. Cresser, Solvent Extraction in Flame Spectroscopic Analysis, Butterworths, London 1978.
2 J. E. Allan, Spectrochim. Acta, 17 (1961) 467.
3 D. G. Biechler and C. H. Long, At. Absorpt. Newsl., 8 (1969) 56.
4 K. Eckschlager Collect. Czech. Chem. Commun., 34 (1969) 1321.
5 E. M. Donaldson, D. J. Charette and V. H. E. Rolko, Talanta, 16 (1969) 1305.
6 G. H. Jamro and R. W. Frei, Mikrochim. Acta, (1970) 429.
7 S. Gomišček and M. Špan, Anal. Chim. Acta, 69 (1974) 49.
8 D. L. Tsalev, I. P. Alimarin and N. I. Tarasevich, Zh. Anal. Khim., 28 (1973) 19.
9 E. Sebastiani, K. Ohls and G. Riemer, Fresenius Z. Anal. Chem., 269 (1973) 105.
10 H. Berndt and W. Slavin, At. Absorpt. Newsl., 17 (1978) 109.

Analytica Chimica Acta, 111 (1979) 163—167

FLOW INJECTION SYSTEM FOR ATOMIC ABSORPTION SPECTROMETRY

NORIMASA YOZA*, YUJI AOYAGI and SHIGERU OHASHI

Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Higashiku, Fukuoka 812 (Japan)

AKIRA TATEDA

Chemistry Laboratory, College of General Education, Kyushu University, Ropponmatsu, Chuoku, Fukuoka 810 (Japan)

(Received 20th March 1979)

SUMMARY

A manifold is described which permits the analysis of less than 1 ml of sample solution. Water- and air-compensation methods for the introduction of the carrier stream into the nebulizer are effective in obtaining sensitive and reproducible measurements of magnesium, but air-compensation gives higher peak responses.

In flame atomic absorption spectrometry, 0.5—2 ml of sample solution is generally used for the determination of a single element by continuous aspiration [1]. However, it is often desirable to employ a technique that can handle less than 1 ml of sample solution when only a limited amount of sample is available. In such cases, the principle of flow injection analysis [2, 3] may be applicable, in which the concentration of an analyte is measured uninterruptedly in a moving stream of liquid. The sample solution is directly introduced into an unsegmented stream of water or a reagent solution via a sampling valve. When flow injection is used for flame atomic absorption spectrometry, it is important to design a suitable manifold for introducing the carrier stream into the nebulizer of the spectrometer [4]. Zagatto et al. recently reported [5] a successful manifold and its application to the determination of calcium, magnesium and potassium in plant material by atomic absorption and emission measurements. It was pointed out that the flow rate through the burner should not be less than the aspiration rate of the spectrometer under normal conditions (about 6 ml min^{-1}). Erratic results were obtained mainly because of air entering the injection system through the connectors if the flow rate through the burner was below the aspiration rate. The main purpose of this paper is to describe a manifold for flow injection atomic absorption analysis by which a carrier stream can be nebulized steadily even when the flow rate is less than the aspiration rate.

In connection with the development of liquid chromatographic detectors two types of manifolds [6, 7] have been proposed for interfacing an atomic

absorption spectrometer with a chromatographic column. As the pumping rate in most chromatographic operations is less than the nebulization rate, a manifold based on a "compensation method" [6, 8] has been extensively applied to the continuous monitoring of various metal ions and metal–polyphosphate complexes [9–11]. This paper describes the advantages and limitations of the compensation method when it is applied to flow injection atomic absorption analysis.

EXPERIMENTAL

Unless otherwise stated, an atomic absorption spectrometer (Perkin-Elmer 403) and a reciprocating pump (Kyowa KHU-52) were employed. The atomic absorption spectrometer was operated according to standard procedures, with an air–acetylene flame. A three-way connector (Technicon PT2) and polyethylene tubing (0.5 mm i.d.) were used for connecting a sample injector (Kyowa KS-M2) and the spectrometer. Flexible tubing (PVC, 2 mm i.d.) was used as a pre-coil.

The compensation method used is shown in Fig. 1. By using a three-way connector, the water in an open reservoir (W_2) is aspirated through a branch tubing (10 cm) into the spectrometer at a flow rate v_b just sufficient to compensate for the starvation of the nebulizer; v_b varies with the flow rate of the carrier stream v_c, but the total flow rate v_n ($= v_b + v_c$) into the nebulizer is automatically kept constant. A disadvantage of this system is that the carrier stream is diluted by the factor $v_c/(v_b + v_c)$.

The pre-coil in Fig. 1 damps the shock during sample injection. The sample solution is injected at S via a loop valve into a stream of water pumped by a reciprocating pump. The sample passes through a mixing coil (100 cm), a three-way connector and an aspiration tube (10 cm) to the nebulizer. The mixing coil was found to improve reproducibility, but with sacrifice of sensitivity. Dispersion of the sample zone to an appropriate extent in the mixing coil appears to be necessary to obtain a reproducible distribution of the sample zone.

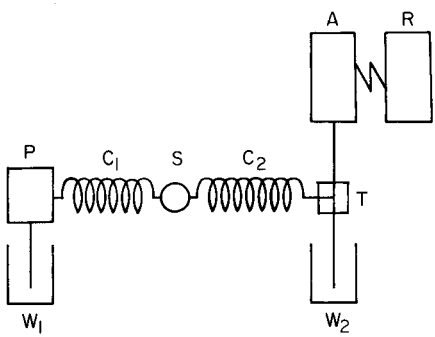


Fig. 1. Manifold for flow injection atomic absorption analysis. A, Atomic absorption spectrometer; R, recorder; P, pump; S, sample injector; T, three-way connector; C_1, pre-coil (2 mm i.d., 5 m long); C_2, mixing coil (0.5 mm i.d., 1 m long); W_1, carrier solution reservoir; W_2, water reservoir for compensation.

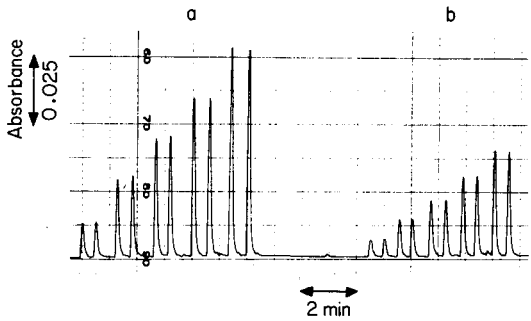


Fig. 2. Concentration profiles for magnesium obtained by (a) air-compensation and (b) water-compensation. Concentrations: 2, 4, 6, 8 and 10 × 10^{-6} M Mg^{2+} (from left to right for a and b). 285.2 nm; chart speed 10 mm min^{-1}. Each sample was injected in duplicate.

RESULTS AND DISCUSSION

Typical concentration profiles are shown in Fig. 2; 300-μl samples of magnesium solutions with different concentrations were injected successively. During the experiments with the water-compensation method (Fig. 2b), the introduction of air through the branch tubing in Fig. 1 for compensation in place of water was found to increase the sensitivity. The concentration profile shown in Fig. 2(a) was obtained by the air-compensation method under the same conditions as that in Fig. 2(b).

Effect of flow rate

The effect of the flow rate of the carrier stream (v_c) on the peak shape was examined; the sample size was kept constant, and water-compensation was used. As shown in Fig. 3, with increase in flow rate the peak width decreased while the peak height increased. The overall variation in peak height is shown in Fig. 4 (curve a) for flow rates of less than 6.3 ml min^{-1} (the maximum limit of pumping). At the lower flow rates, the peak height varied linearly with the flow rate, in accordance with the expected decrease

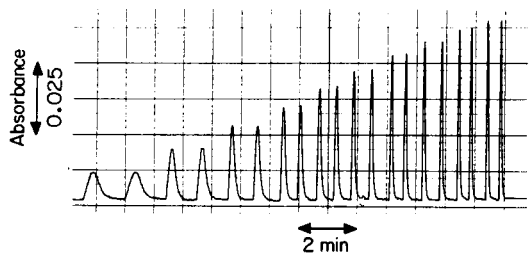


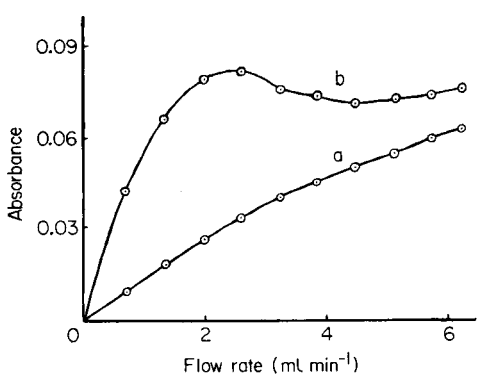
Fig. 3. Effect of flow rate (v_c) on the peak shape obtained by the water-compensation method. v_c: from 0.71 to 6.27 ml min^{-1} (left to right) at intervals of 0.62 ml min^{-1}; 300-μl injections of 1 × 10^{-5} M Mg^{2+} solution. All injections were done in duplicate.

in dilution by the carrier stream, by a factor of v_c/v_n. This result can also be correlated with a previous note that the peak area did not vary with the flow rate [8]. By using a separate pump, it was confirmed that the carrier stream tended to flow through the branch tubing in the reverse direction (overflow) when the pumping rate was greater than 9.5 ml min^{-1}, corresponding to the value of v_n. Quantitative evaluation of the effects of v_c and v_n on the sensitivity of detection is discussed below.

A similar experiment was carried out for the air-compensation method. As shown in Fig. 4 (curve b) the variation in peak height is somewhat complicated. The drastic increase in peak height at the lower flow rates is not simple to understand, because it may be complicatedly dependent not only on the hydrodynamic properties of the carrier stream, but also on the flow characteristics of the nebulizer. In fact, it was observed that a somewhat different pattern was obtained when a Jarrell-Ash AA8500 spectrometer was used in place of the Perkin-Elmer 403. At higher flow rates, curves (a) and (b) tended to approach each other and overlapped, as expected, at the extreme where $v_c = v_n = 9.5$ ml min^{-1}. In the region $v_c > v_n$, both curves showed decreasing peak heights with increase in the pumping rate, although this manifold was useful for quantitative measurements even under such conditions of "overflow".

Effect of sample volume

The effect of sample volume on peak height was examined at flow rates of 1.35 and 3.23 ml min^{-1}; the sample concentration was kept constant and both compensation methods were used. As shown in Fig. 5, the peak height increased with increasing sample volumes and tended to become constant at sample volumes greater than 0.5 ml. The slopes at smaller sample volumes can



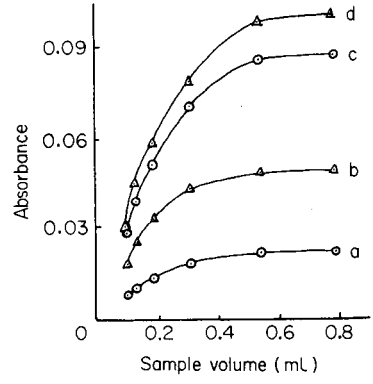


Fig. 4. Effect of flow rate on peak height. (a) Water-compensation; (b) air-compensation; 300-μl injections of 1×10^{-5} M Mg^{2+} solution.

Fig. 5. Effect of sample volume on peak heights for 1×10^{-5} M Mg^{2+} solution. Water-compensation at v_c values of (a) 1.35 ml min^{-1}; (b) 3.23 ml min^{-1}. Air-compensation at v_c values of (c) 1.35 ml min^{-1}; (d) 3.23 ml min^{-1}.

be explained by assuming that the sample zone spreads to such an extent that the variation in sample volume has a negligible effect on the band width. Volumes exceeding 0.5 ml are likely to be enough to affect the band width and result in the appearance of a peak with a plateau.

As mentioned above, the carrier stream is expected to be diluted by a factor of v_c/v_n, with a consequent decrease in sensitivity. The results in Fig. 5 for the water-compensation method can be semi-quantitatively explained on the basis of a v_n value of 9.5 ml min^{-1}. For example, v_c/v_n is 0.34 if v_c is 3.2 ml min^{-1} and the absorbance at the plateau of curve (b) in Fig. 5 corresponds to about 35% of the absorbance obtained when the same magnesium solution (10^{-5} M) is aspirated continuously. No explanation can be given for the increase in sensitivity to 70% for the air-compensation curve (d).

The concentration profiles in Figs. 2 and 3 were obtained by a student whose sample injection technique was not skillful. The appearance of irregular and small peaks at the base line level is ascribed to mistakes in operation. Even in such circumstances the relative standard deviation of measurements was less than 1.0% for the water-compensation method and less then 1.5% for the air-compensation method. The reproducibility can be improved by increasing the length of the mixing coil, though the sensitivity decreased correspondingly owing to the increase in band spreading.

In flame atomic absorption analysis, the addition of reagents such as lanthanum(III), EDTA and mineral acids to a sample solution is often necessary to eliminate chemical interferences. In flow injection analysis, this can easily be achieved by aspirating a reagent solution in place of water from the compensating reservoir. For example, a lanthanum(III) solution was successfully used to eliminate the interference of phosphorus compounds on the atomic absorption measurement of calcium and magnesium [12, 13]. It is surprising and interesting that the releasing activity by lanthanum is effective even in such a short time of mixing of the releasing solution with the sample. As has been suggested by Zagatto et al. [5], more effective releasing activity may be achieved when the releasing solution is introduced as a carrier solution from reservoir W_1. This problem will be discussed elsewhere.

REFERENCES

1 H. Berndt and W. Slavin, At. Absorpt. Newsl., 17 (1978) 109.
2 J. Růžička and E. H. Hansen, Anal. Chim. Acta, 99 (1978) 37.
3 D. Betteridge, Anal. Chem., 50 (1978) 832A.
4 A. S. Inglis and P. W. Nichols, Mikrochim. Acta, (1975) 553.
5 E. A. Zagatto, F. J. Krug, H. Bergamin F°, S. S. Jørgensen and B. F. Reis, Anal. Chim. Acta, 104 (1979) 279.
6 N. Yoza and S. Ohashi, Anal. Lett., 6 (1973) 595.
7 S. E. Manahan and D. R. Jones IV, Anal. Lett., 6 (1973) 745.
8 T. Miyajima, N. Yoza and S. Ohashi, Liquid Chromatography, Bunseki-Kiki, Zokan, 1974, p. 151.
9 N. Yoza, K. Kouchiyama, T. Miyajima and S. Ohashi, Anal. Lett., 8 (1975) 641.
10 N. Yoza, K. Kouchiyama and S. Ohashi, At. Absorpt. Newsl., 18 (1979) 39 and references therein.
11 F. J. Fernandez, At. Absorpt. Newsl., 16 (1977) 33.
12 T. Miyajima, Doctoral Dissertation, Kyushu University, 1978.
13 T. Miyajima and S. Ohashi, Bull. Chem. Soc. Jpn., 51 (1978) 2543.

Analytica Chimica Acta, 111 (1979) 169—176

SEMI-AUTOMATED METHOD FOR THE DETERMINATION OF BISMUTH IN ROCKS

C. Y. CHAN*, M. W. A. BAIG and A. E. PITTS

Geoscience Laboratories, Ontario Geological Survey, Ministry of Natural Resources, 77 Grenville Street, Toronto, Ontario (Canada)

(Received 20th June 1979)

SUMMARY

A rapid and accurate method for the determination of bismuth in rock samples is described. Automated equipment is used to generate bismuth hydride from solutions of rock samples prepared by digestion with a mixture of hydrofluoric and perchloric acids. The evolved hydride is carried to a heated quartz tube by a stream of argon, and the atomic absorption of bismuth recorded. Thiosemicarbazide and 1,10-phenanthroline are used as masking agents to minimize interferences from copper and nickel. As little as 20 ng Bi g^{-1} can be determined; the average r.s.d. is 5.4%. Results obtained for six USGS standard rocks are in close agreement with the recommended values obtained by an isotope dilution technique.

There has been growing demand in recent years for quantitative trace measurements of bismuth, a sulfide-associated element, in geological materials. The bismuth data are important in support of geochemical surveys, where bismuth plays a role as an indicator of various mineral deposits and in mineral deposit studies. In high-temperature hydrothermal deposits, bismuth is very often associated with gold and silver [1]. The occurrence of bismuth in lead sulfide ores is unique. The amount of bismuth in galena is usually about 10—20 ppm, in exceptional cases even 100—1000 ppm; it increases with increasing temperature of formation of the mineral deposits [1]. In the upper crust of the earth, bismuth concentrations are estimated to lie in the range 0.01—0.10 ppm [1]. In order to distinguish bismuth of high content from its background level in rocks, a sensitive analytical method is desirable.

Bismuth can be measured colorimetrically [2] based on its reaction with sodium diethyldithiocarbamate to form a yellow complex followed by solvent extraction; it can also be determined by conventional flame atomic absorption spectrometry. Neither procedure has enough sensitivity to measure the small enrichments of bismuth at the level near and slightly greater than the crustal abundance. The electrothermal atomization technique [3] is prone to matrix interferences although it provides better sensitivity. Ficklin and Ward [4] developed a flameless atomic absorption method which can determine as little as 50 ppb in 0.2g samples of rock or soil sample. In their procedure, the sample is fused with sodium hydrogensulfate and the

fusion product is leached with (1 + 4) HCl and treated with ammonium 1-pyrrolidinedithiocarbamate to form a complex which is extracted into MIBK. Aliquots of the solution are analyzed for bismuth by the graphite-furnace atomization technique. A similar approach was applied by Kane [5] who used aqueous rather than organic sample solutions for analysis with a graphite-furnace atomizer after the sample had been decomposed and bismuth extracted. The method is tedious, time-consuming and unsuitable for routine analyses of large numbers of samples. Thermal neutron activation analysis and the substoichiometric isotope dilution technique have been adopted successfully for accurate measurements of trace bismuth in rock samples; the results on USGS standard rocks have been reported on several occasions [6].

The hydride generation approach for determining bismuth in aqueous samples has been studied by several workers [7—9]. Fernandez [7] collected the generated bismuth hydride in a balloon reservoir and subsequently determined bismuth by atomic absorption spectrometry (a.a.s.). Thompson [8] introduced the hydride directly into a silica tube mounted in an air—acetylene flame for a.a.s. Both methods were sensitive, but few interferences were studied. Smith [9] investigated the chemical interferences inherent in the hydride generation method systematically; some elements, particularly copper and nickel, interfered strongly, but methods of avoidance were not elaborated. The masking effects of thiosemicarbazide on copper and of 1,10-phenanthroline on nickel are well known [10]. Kirkbright and Taddia [11] successfully used these compounds to minimize interferences from Cu, Ni, Pt, and Pd in the a.a.s. determination of arsenic after hydride generation. The semi-automated hydride generation- -atomic absorption method described in this paper for the determination of bismuth in rocks employs these masking agents and is based on previous experience with a semi-automated method for antimony determinations [12].

EXPERIMENTAL

Reagents

All reagents used were of analytical grade; water was glass-distilled. The acids used were hydrofluoric acid (Baker 49%), hydrochloric acid (Baker 38%), perchloric acid (Baker 60%) and nitric acid (Baker 70%).

Digestion mixture. Mix hydrofluoric acid, perchloric acid and water in the ratio of 2:2:1. Store the mixture in a polyethylene bottle.

Tetrahydroborate solution. Dissolve 3.0 g of sodium tetrahydroborate in 300 ml of water, adding one pellet of sodium hydroxide for each 100 ml of the solution. The solution is stable for at least a week in a refrigerator.

Masking reagent. Dissolve 0.5 g each of thiosemicarbazide and 1,10-phenanthroline in 100 ml of 0.1 M HCl solution.

Standard solutions. For the stock solution (1000 ppm Bi), dissolve 1.1148 g of Bi_2O_3 in (1 + 4) HCl, and dilute to 1 l with the same acid. Prepare working

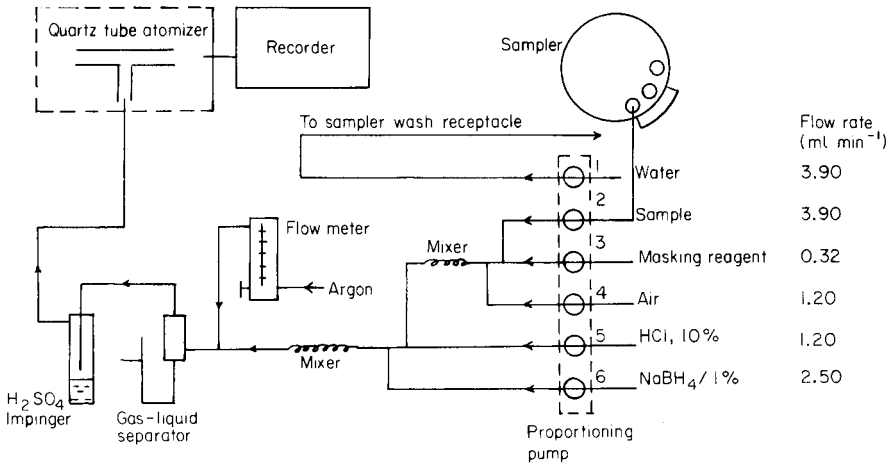


Fig. 1. Flow diagram of Autoanalyzer—a.a.s. system for determination of bismuth.

standards containing 1.0, 2.0, 3.0, 5.0 and 10.0 ng Bi ml^{-1} by serial dilutions of the stock standard with a solution containing (1 + 4) HCl and (1 + 9) $HClO_4$.

Apparatus

The flow diagram of manifold used is shown in Fig. 1.

The Varian Model AA6 atomic absorption spectrometer was equipped with a bismuth hollow-cathode lamp and a Model A-25, 1—10 mV variable-range, strip-chart recorder. A Technicon Sampler II and a Proportioning Pump I were used for sampling and mixing of the reagents. A gas–liquid separator separated the hydride and the waste solution. An impinger half-filled with concentrated sulfuric acid served to remove moisture and to homogenize the hydride—argon mixture. The quartz tube (10 cm long, 0.6 cm i.d. with an inlet tube fused into the centre), wound with a 22-gauge chromel A heating wire and insulated with a layer of wrapped asbestos string, was mounted on the burner of the spectrometer. The temperature of the quartz tube atomizer was controlled at 850 ± 20°C by a variable transformer.

The optimum operating parameters for a.a.s. are as follows: wavelength, 223.2 nm; lamp current, 8 mA; slit width, 50 μm; instrument damping, C (maximum); expansion, 6.0; recorder span, 1 mV full-scale; chart speed, 50 cm h^{-1}; atomizer temperature, 850 ± 20°C; argon flow rate, 300 ml min^{-1}; sampling time, 45 s; wash time, 45 s.

Procedures

Decomposition of samples. Weigh out 0.200 g of rock sample and transfer to a 30-ml Teflon beaker. Digest the sample with 5 ml of the acid digestion mixture on a hot plate at low heat for ca. 1 h until white fumes of perchloric

acid appear and the volume is reduced to 1—2 ml. Avoid heating the contents to dryness. Cool and dilute the contents with a little water; add 6 ml of (1 + 1) HCl. Transfer the contents with water, rinsing into a plastic test tube calibrated at 15 ml. Make up to volume with water. Mix the solution thoroughly and allow the residue to settle. Prepare a reagent blank simultaneously. Transfer the solution to a sample cup for subsequent a.a.s. determination as described below.

Determination of bismuth. Select the a.a.s. instrumental parameters indicated above and set up the hydride generation equipment as in Fig. 1 using the appropriate tube manifold. Mount the quartz tube on the burner, with its side arm connected to a tygon tubing leading to the hydride generator. After the bismuth lamp has warmed up, align the quartz tube with the light beam to allow maximum radiation to reach the detector. Obtain the required temperature of the quartz tube atomizer by switching on the pre-set variable transformer. Turn on the proportioning pump with all the reagent tubes dipped in the water. Introduce the argon gas immediately with its flow rate regulated by a flowmeter. As soon as the system has stabilized, insert the reagent tubes into their corresponding solutions, and establish a base-line signal. The standards, samples and blank solutions which have been loaded and held in the sampler can then be run continuously. Record the absorption signals. Measure the peak heights of the standards, and draw a calibration graph. The calibration curve is linear at least up to 10 ng Bi ml^{-1}. Typical recorder tracings on a series of standards are depicted in Fig. 2. Read the concentrations of bismuth in the samples from the graph after subtracting the peak height of the blank. For a sample weight of 0.200 g and a volume of 15 ml, ng Bi g^{-1} in rock corresponds to ng Bi ml^{-1} × 75 ml g^{-1}.

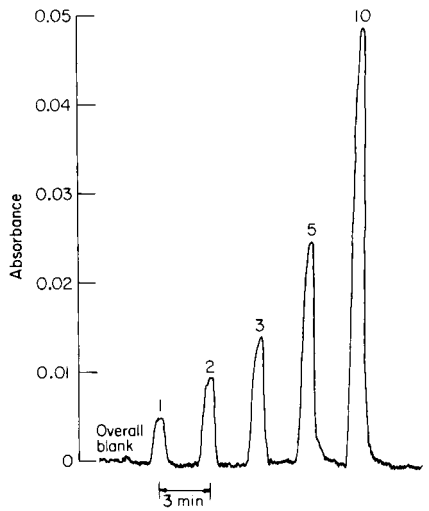


Fig. 2. Typical recorder tracings of bismuth. The numbers on the peak correspond to ng Bi ml^{-1}.

RESULTS AND DISCUSSION

Interferences

The influence of various ions was studied at concentrations equal to or greater than those found in the samples to be analyzed. The conclusions were as follows: the method is free of interference from large concentrations of the major constituents of rocks, namely Al, Fe, Ca, Mg, Na, K and Ti; anions such as chloride, fluoride, nitrate, sulfate and phosphate do not interfere; some trace elements exhibit severe interferences if the analysis is done without additional reagents. Although the tolerance limits and the corresponding concentrations of Te, Pt, Au and Ag are relatively low, the occurrence of these elements at interfering levels in natural rocks is very uncommon. Copper and nickel are potential interferences because they occasionally occur at high levels in natural rocks and minerals. Attempts were made to overcome these interferences by introducing chelating and complexing reagents. Reagents such as EDTA, thiocyanate, citrate and oxalate were found to be ineffective. Potassium iodide was effective for copper, but not for nickel. The application of thiosemicarbazide and 1,10-phenanthroline for controlling the interferences proved successful as can be observed from Table 1. The tolerance limit was increased tenfold for nickel, and the interferences of copper and platinum were eliminated completely.

Precision, accuracy, sensitivity and detection limit

Several geochemical standard reference samples were analyzed. The results are shown in Table 2, together with the recommended values reported in the literature [6]. The precision is satisfactory and the present results agree

TABLE 1

Elemental concentrations having no interference effect on bismuth (5 ng ml^{-1})

Element	Concentration[a] ($\mu g\ ml^{-1}$)	Equivalent content in rock[b]	Element	Concentration[a] ($\mu g\ ml^{-1}$)	Equivalent content in rock[b]
Ca	8000	60%	Mn	400	3%
Fe	8000	60%	Cu	400 (0.2)	3%
Mg	4000	30%	Pt	400 (0.2)	3%
K	4000	30%	Ni	60[c](6)	4500 ppm
Na	4000	30%	Co	100 (20)	7500 ppm
Al	4000	30%	As	40	3000 ppm
Li	1000	7.5%	Se	40	3000 ppm
Ba	1000	7.5%	Sb	40	3000 ppm
Zn	400	3%	Sn	40	3000 ppm
Pb	400	3%	Te	0.4[c](0.1)	30 ppm
Ti	400	3%	Au	0.03[c](0.01)	2 ppm
Cd	400	3%	Ag	1[c](0.1)	75 ppm
Cr	400	3%			

[a] Values in parentheses are the tolerance limits in the absence of masking reagents.
[b] Based on 0.2 g of sample in 15 ml of solution
[c] Tolerance limit.

TABLE 2

Results for bismuth (ppb) in standard reference rock samples

Sample		This work		Recommended value[a] (ppb)
		ppb[b]	R.s.d. (%)	
USGS	QLO-1	63	3.6	66.3
	RGM-1	273	2.9	283
	MAG-1	381	3.2	384
	SDC-1	272	2.6	276
	BHVO-1	22	12.1	18.8
	STM-1	172	13.1	250[c]
	SCo-1	384	3.4	—
	SGR-1	850	3.3	—
GSC	SY-2	154	2.5	—
	SY-3	293	1.9	—
	MRG-1	73	10.4	—

[a] Values quoted from ref. [6], which were obtained by isotope dilution.
[b] Mean of 5 determinations.
[c] The value is not recommended because of poor precision in results as described by Greenland et al. [6].

closely with the values recommended. The average relative standard deviation over the bismuth concentration range 20—900 ng g^{-1} is 5.4%. Samples SY-2, SY-3 and MRG-1 are reference materials supplied by the Geological Survey of Canada (GSC) and no recommended values are available.

Two reference mineral samples, supplied by GSC, with high bismuth content were analyzed by the present method and by a flame method. The results obtained (Table 3) show good agreement. Despite the high dilution involved, the deviation of the results from possible mechanical errors is minimized in the proposed method. Sample PR-1 was also analyzed by a colorimetric method with diethyldithiocarbamate with similar results.

A recovery study was done by adding known amounts of bismuth (as Bi_2O_3) to the rock samples and applying the entire procedure. The added

TABLE 3

Comparison of results for bismuth in two GSC reference samples

Sample	Bi (%)			
	Air—acetylene flame	Hydride generation	Colorimetric	Recommended value
Zn—Sn—Cu—Pb ore, MP-1	0.024	0.024	—	0.024
Molybdenum ore, PR-1	0.113	0.108	0.106	0.111

TABLE 4

Recovery of bismuth added as Bi_2O_3 to typical rock samples

Rock sample (0.2 g)	Bi (ng)			Recovery (%)
	Added	Found[a]	Recovery	
Amphibolite	0	25	—	
	75	102	77	102.6
Syenite, SY-3	0	65	—	
	75	138	73	97.3

[a]Average of 3 results.

bismuth was recovered quantitatively as shown in Table 4, and the matrix effect was negligible.

The sensitivity, defined as the concentration producing 1% absorption, of this procedure is 0.90 ng Bi ml^{-1}. The practical detection limit, defined as the concentration giving a signal three times the standard deviation of the overall blank, is 0.3 ng Bi ml^{-1} equivalent to 20 ng g^{-1} of rock.

General remarks

The absorbance does not vary appreciably with the following factors: (1) concentration and type of the acid in the sample solution; (2) concentration and type of the acid in the background solution fed in line 5 in Fig. 1; (3) concentration of sodium tetrahydroborate; (4) temperature and length of the quartz tube. Control of these variables need not be critical. In contrast, the absorbance does change significantly with change of flow rate and type of the carrier gas. Strict regulation of the gas flow is thus required. Argon as a carrier gas provided better sensitivity than nitrogen with a gain of about 20%. The impinger filled with concentrated sulfuric acid acts as a gas mixer and moisture absorber. It has the effect of homogenizing the gas mixture and hence reducing the noise of the signal. Perchloric acid in the digestion mixture can be replaced by sulfuric acid which is just as effective. To digest samples containing organic materials, a small amount of nitric acid should be added to the digestion mixture. A batch of 40 samples can be digested simultaneously on a hot plate with ease; complete digestion is normally attainable within an hour. It was observed that bismuth contamination occurred frequently if the sample solutions were kept in glass test tubes. Bismuth is present in most types of glassware as an additive in glass manufacturing, and if the digested samples are kept in contact with the glass tubes, the residual hydrofluoric acid that may occasionally exist in minute quantities will etch the glass and leach out bismuth, thus causing serious contamination. To prevent this from happening, plastic test tubes were used. When the system is ready to run, it is good practice to condition it first by repeatedly analyzing a sample solution or a bismuth standard solution containing

3000 μg Fe ml^{-1} until a constant signal is attained prior to the actual analyses. The analysis rate is 40 digested samples per hour.

This paper is published by permission of the Director, Ontario Geological Survey.

REFERENCES

1 V. M. Goldschmidt, Geochemistry, Oxford University Press, London, 1958, p. 480.
2 E. B. Sandell, Colorimetric Determination of Traces of Metals, Vol. 3, 3rd edn., Interscience, New York, 1959, p. 338.
3 B. R. Culver, Analytical Methods for Carbon Rod Atomizers, Varian Techtron Pty. Ltd., Springvale, Australia, 1975.
4 W. H. Ficklin and F. N. Ward, J. Res. U.S. Geol. Surv., 4 (1976) 217.
5 J. S. Kane, Anal. Chim. Acta, 106 (1979) 325.
6 L. P. Greenland, E. Y. Campbell and F. J. Flanagan, Descriptions and Analyses of Eight New USGS Rock Standards, Geological Survey Professional paper 840, 1976, pp. 45, 59.
7 F. J. Fernandez, At. Absorpt. Newsl., 12 (1973) 93.
8 K. C. Thompson, Analyst, 99 (1974) 595.
9 A. E. Smith, Analyst, 100 (1975) 300.
10 D. D. Perrin, Masking and Demasking of Chemical Reactions, Wiley-Interscience, New York, 1970, pp. 35, 37.
11 G. F. Kirkbright and M. Taddia, Anal. Chim. Acta, 100 (1978) 145.
12 C. Y. Chan and P. N. Vijan, Anal. Chim. Acta, 101 (1978) 33.

Analytica Chimica Acta, 111 (1979) 177—185

SIMULTANEOUS KINETIC DETERMINATION OF METAL ION MIXTURES BASED ON A LIGAND SUBSTITUTION REACTION

EDOARDO MENTASTI

Istituto di Chimica Analitica, Università di Torino, Via P. Giuria 5, 10125 Torino (Italy)

(Received 5th March 1979)

SUMMARY

The rates of the ligand substitution reactions between 2-(4-sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid (SPADNS) complexes of Co^{2+}, Ni^{2+}, Pb^{2+} and Cu^{2+} and EDTA (or CDTA) have been studied at 25.0°C, pH 8.0—9.3, by stopped-flow spectrophotometry. The reactions are first order in SPADNS complex and in incoming ligand. The absorbance change during the reaction progress can be kinetically separated for each component of a mixture, so that metal ions down to 10^{-6} M can be determined. Components of very high reactivity, such as Cd^{2+}, Mn^{2+} or Zn^{2+}, can also be determined as part of a multicomponent mixture.

Substitution reactions involving replacement of a ligand by another or of a metal by another in a metal complex, constitute important types of reaction that, together with double ligand exchange, have been the object of many mechanistic studies [1—5].

$MeX + Y \rightarrow MeY + X$ (nucleophilic substitution)

$MeX + Me' \rightarrow Me'X + Me$ (electrophilic substitution)

$MeX + Me'Y \rightarrow MeY + Me'X$ (double ligand exchange)

The different reactivities of various metals and ligands have suggested analytical applications of such reactions [6—9]. In the case of the nucleophilic substitution, if a mixture of metal ions is reacted with an excess of ligand X and a substitution reaction is started with a nucleophile Y that replaces X, each metal will react with a typical rate, which can be used as a qualitative parameter. Moreover, if a wavelength can be chosen where each MeX and MeY or free X have sufficiently different molar absorptivities, the variation of absorbance can be temporarily separated for each metal and used as a parameter for quantitative analysis. In this way, for example, gallium can be determined selectively in a mixture by its replacement from its Erio R complex by EDTA [10]. Mixtures of Co, Ni, Pb and Zn have been resolved by the reaction of MeEGTA with PAR (4-(2-pyridylazo)resorcinol) [11] and mixtures of Cd, Hg, Zn and Cu can be analyzed by displacing the metals from their zincon complexes by CDTA [12, 13].

In the work described here the following reaction was investigated:

$$MeSPADNS + EDTA \xrightarrow{k} MeEDTA + SPADNS$$

where Me = Co^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+} and Zn^{2+} and SPADNS is 2-(4-sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid.

EXPERIMENTAL

Reagents and apparatus

2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid (SPADNS; Eastman) was standardized spectrophotometrically against copper(II) perchlorate solutions standardized by EDTA titration. EDTA and CDTA (*trans*-1,2-diaminocyclohexane *N,N,N',N'*-tetraacetic acid) were reagent-grade chemicals (Merck).

Solutions of metal ion perchlorates were prepared by dissolution of the carbonates in perchloric acid and successive recrystallizations.

Kinetic measurements were carried out on Durrum-Gibson stopped-flow spectrophotometer (2.00-cm path length) equipped with Tektronix model 564 and 549 storage oscilloscopes.

Procedure

The metal ions (singly or in mixtures, usually 10^{-6}—10^{-5} M) were mixed with a 4—10-fold excess of SPADNS in order to ensure complete complexation, and adjusted to the desired pH with boric acid—NaOH solution (total buffer, concentration 0.01 M). The other reacting solution was prepared with the displacing ligand (EDTA or CDTA) and addition of the borate buffer. All the solutions were brought to ionic strength 0.1 M with $NaClO_4$. Equal volumes of the reacting solutions were mixed in the stopped-flow apparatus. All the concentrations reported refer to the final reaction mixture.

The reaction progress was monitored at 600 nm, the maximum absorption wavelength of the MeSPADNS complexes, by following the increase of transmittance as the complexes disappeared. Kinetic traces (% transmittance versus time) were photographed from the storage oscilloscopic display, and treated by weighted least-squares methods.

RESULTS AND DISCUSSION

Stoichiometry

Each investigated metal ion gives a 1:1 SPADNS complex, as indicated by spectrophotometric titrations done by adding to a known amount of metal ion additional amounts of ligand and monitoring the changes in absorbance in the range 550—650 nm. SPADNS has an absorption maximum at 500 nm ($\epsilon = 1.8 \times 10^4$ l mol^{-1} cm^{-1}) which decreases sharply at increased wavelengths. The presence of the metal shifts the absorption band toward longer wavelengths; 600 nm was found to be suitable for following the dis-

TABLE 1

Molar absorptivities for metal—SPADNS complexes at 600 nm (pH 8.0—9.3; 25.0°C, μ = 0.1 M)

Metal ion	ϵ (l mol^{-1} cm^{-1})	Metal ion	ϵ (l mol^{-1} cm^{-1})	Metal ion	ϵ (l mol^{-1} cm^{-1})
—	900	Zn^{2+}	3700	Ni^{2+}	14000
Mn^{2+}	6700	Co^{2+}	9600	Cu^{2+}	8500
Cd^{2+}	1800	Pb^{2+}	8300		

placement of SPADNS by EDTA from the complexes. Table 1 gives the molar absorptivities of the investigated complexes at this wavelength. The constancy of these values over the pH range 8.0—9.3 suggests that no protonation occurs in these conditions.

Kinetics

The disappearance of MeSPADNS at 600 nm was monitored at constant pH in the range 8.0—9.3. The following features were established. First, the reactions are first order in the original complex (MeSPADNS); plots of ln $(A - A_{eq})$ vs. time were linear up to at least 90% of complete displacement, which confirms the first-order dependence at low concentrations. (A is the absorbance at time t and A_{eq} is the absorbance at complete reaction). Secondly, the reactions are also first order with respect to the entering ligand, as shown by the data in Table 2, where all the kinetic parameters are collected. Thirdly, the rates are independent of the free SPADNS concentration (see Table 2). Finally, within experimental uncertainties the rates are independent of the entering ligand: the same rate constants, for Ni^{2+} and Pb^{2+} as examples, were found in the displacement by EDTA or by CDTA. All this means that if a sufficient driving force (negative free energy of reaction) is ensured by choice of a suitable entering ligand (i.e. the conditional stability constant of the entering ligand must be greater than that of the corresponding Me—SPADNS complex) the reaction rate depends mainly on bond-rupture steps rather than on bond-forming steps.

The following sequence accounts for the features observed (charges and protons are omitted for simplicity):

$$\text{Me—SPADNS} + \text{EDTA} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{SPADNS—Me—EDTA} \qquad K_1 = k_1/k_{-1} \tag{1}$$

$$\text{SPADNS—Me—EDTA} \xrightarrow{k_2} \text{Me—EDTA} + \text{SPADNS} \tag{2}$$

from which, by applying steady-state conditions to the intermediate,

$$\text{Rate} = -\text{d[Me—SPADNS]}/\text{d}t = k_1k_2[\text{Me—SPADNS}][\text{EDTA}]\,(k_{-1} + k_2)$$

The intermediate mixed complex SPADNS—Me—EDTA (where EDTA coordinates the metal without complete detachment of SPADNS) is rapidly

TABLE 2

Rate measurements for the investigated reactions, under different conditions [25.0°C; μ = 0.1 M ($NaClO_4$); k_{obs} is the slope of the plot of $\ln(A_t - A_{eq})$ vs. time (s^{-1}).]

COPPER						
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [Cu^{2+}] = 1×10^{-5} M)						
[EDTA] $\times 10^3$	1.0	2.0	5.0	10	20	
$k_{obs} \times 10^3$	1.62	2.85	7.60	15.1	32.7	
([EDTA] = 2×10^{-2} M; [SPADNS] = 1×10^{-4} M; [Cu^{2+}] = 1×10^{-5} M)						
pH	8.0	8.2	8.5	8.7	9.0	9.3
$k_{obs} \times 10^2$	3.12	3.40	3.27	3.30	3.19	3.30
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [EDTA] = 2×10^{-2} M)						
[Cu^{2+}] $\times 10^6$	5	10				
$k_{obs} \times 10^3$	34.7	32.7				
NICKEL						
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [Ni^{2+}] = 5×10^{-6} M)						
[EDTA] $\times 10^3$	0.1	1.0	5.0	10		
k_{obs}	0.268	3.04	12.3	26.2		
[CDTA] $\times 10^3$		1.0	5.0	10		
k_{obs}		2.84	11.8	25.7		
([EDTA] = 1×10^{-3} M; [SPADNS] = 1×10^{-4} M; [Ni^{2+}] = 1×10^{-5} M)						
pH	8.0	8.2	8.5	8.7	9.0	9.3
k_{obs}	3.32	3.28	3.08	3.00	2.86	2.75
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [EDTA] = 1×10^{-4} M)						
[Ni^{2+}] $\times 10^6$	10	8.0	6.0	5.0	4.0	2.0
k_{obs}	0.242	0.245	0.270	0.268	0.257	0.263
LEAD						
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [Pb^{2+}] = 2×10^{-5} M)						
[EDTA] $\times 10^3$	1.0	5.0	10			
k_{obs}	3.15	14.3	29.0			
([EDTA] = 1×10^{-3} M; [SPADNS] = 1×10^{-4} M; [Pb^{2+}] = 2×10^{-5} M)						
pH	8.0	8.2	8.5	8.7	9.0	9.3
k_{obs}	3.17	3.15	3.15	3.17	2.98	3.13
(pH = 8.5; [EDTA] = 1×10^{-3} M)						
[SPADNS] $\times 10^5$	10	10	5.0	5.0		
[Pb^{2+}] $\times 10^5$	4.0	1.0	2.0	1.0		
k_{obs}	3.09	3.12	2.99	3.23		
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [Pb^{2+}] = 2×10^{-5} M)						
[CDTA] $\times 10^4$	5.0	10	30	60	100	
k_{obs}	1.61	2.92	8.63	17.4	28.0	
COBALT						
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [Co^{2+}] = 5×10^{-6} M)						
[EDTA] $\times 10^5$	5.0	10	20			
k_{obs}	23.8	44.6	84			
([EDTA] = 5×10^{-5} M; [SPADNS] = 1×10^{-4} M; [Co^{2+}] = 5×10^{-6} M)						
pH	8.0	8.2	8.5	8.7	9.0	9.3
k_{obs}	25.7	25.1	23.8	24.2	27.0	26.4
(pH = 8.5; [SPADNS] = 1×10^{-4} M; [EDTA] = 5×10^{-5} M)						
[Co^{2+}] $\times 10^6$	5.0	10	20			
k_{obs}	23.8	25.3	26.0			

formed: in fact all the investigated metal ions have high coordinated water exchange rates [4, 14], and are made increasingly more labile by the presence of SPADNS in the coordination sphere. This intermediate then decomposes with release of SPADNS. The absence of kinetic effects on varying the concentration of SPADNS or on changing the structure of the entering ligand suggests that the rupture of the Me—SPADNS bonds (reaction 2) is the rate-determining step, i.e. that k_2 is the limiting rate constant. So, by assuming $k_2 \ll k_{-1}$, the second-order rate constant $k = k_2 K_1$ can be obtained.

A slight direct hydrogen ion dependence can be observed (Table 2) for nickel(II); since there is no evidence of a stable protonated Ni—SPADNS species in this pH range, protonation must be involved in the transition state. This effect tends to become constant as the pH is lowered. Thus, for nickel(II), the following additional reactions are involved.

$$\text{SPADNS—Ni—EDTA} + \text{H}^+ \overset{\text{fast}}{\rightleftharpoons} \text{SPADNS, H}^+\text{—Ni—EDTA} \qquad K_H \qquad (3)$$

$$\text{SPADNS, H}^+\text{—Ni—EDTA} \overset{k_3}{\rightarrow} \text{Ni—EDTA} + \text{SPADNS} + \text{H}^+ \qquad (4)$$

The overall rate is given by:

$$\text{Rate} = k_1(k_2 + k_3 K_H^{-1}[\text{H}^+])\,[\text{Ni—SPADNS}]\,[\text{EDTA}]\,/([k_{-1} + k_2 + k_3 K_H^{-1}[\text{H}^+])$$

where K_H is the protonation constant of reaction (3). From a plot of the second-order rate constant $k = k_{obs}/[\text{EDTA}]$ against $[\text{H}^+]$, it can be seen that at increasing acidity, protonation eventually has no further effect and the rate-determining step is shifted to reaction (1). Under these conditions, k_1 was calculated to be 3.4×10^3 l mol^{-1} s^{-1}; from the intercept of the plot ($[\text{H}^+] \to 0$), $k = k_1 k_2/(k_{-1} + k_2) \sim k_2 K_1 = 2.6 \times 10^3$ l mol^{-1} s^{-1}. It is worthwhile mentioning that this feature is found for the least labile metal ion, Ni^{2+}, for which reaction (2) must therefore be slowest [14, 15].

Table 3 collects the kinetic parameters derived for the investigated metal ions. While some metal ions (Cd, Mn, Zn) react very rapidly and their displacement is complete within the mixing time of the stopped-flow apparatus (1—3 ms) even at very low EDTA concentrations, the reactivities decrease in the order Co^{2+} ($t_{\frac{1}{2}} \approx 20$ ms for [EDTA] = 1×10^{-4} M) > Pb^{2+} ($t_{\frac{1}{2}} \approx 4$ s) ≈ Ni^{2+} > Cu^{2+} ($t_{\frac{1}{2}} = 1$ min for [EDTA] = 1×10^{-2} M).

By utilizing the different rates of reaction of these complexes, their concentrations in a multicomponent mixture can be determined by simultaneous kinetic analysis. The reactions have fairly short halflives and the stopped-flow technique is very useful for such determinations, allowing fast measurements and avoiding side-reactions. The molar absorptivity changes involved are sufficiently high to permit the evaluation of low metal concentrations.

Solutions containing one species of metal ion

At constant pH, temperature and EDTA concentration, a plot of $\ln(A_t - A_{eq})$ vs. time is linear and its slope, k_{obs}, is linearly related to the displacing ligand concentration. Thus $k_{obs}/[\text{EDTA}] = k_1$ is a characteristic par-

TABLE 3

Kinetic parameters for the investigated reactions
(25.0°C, μ = 0.1 M ($NaClO_4$))

Metal ion	$k = K_1 k_2$ ($l\ mol^{-1}\ s^{-1}$)	k_1 ($l\ mol^{-1}\ s^{-1}$)	Metal ion	$k = K_1 k_2$ ($l\ mol^{-1}\ s^{-1}$)
Cu^{2+}	1.57 ± 0.11		Cd^{2+}	$\geq 5 \times 10^6$
Ni^{2+}	$(2.6 \pm 0.2) \times 10^3$	$(3.4 \pm 0.3) \times 10^3$	Mn^{2+}	$\geq 5 \times 10^6$
Pb^{2+}	$(3.05 \pm 0.14) \times 10^3$		Zn^{2+}	$\geq 5 \times 10^6$
Co^{2+}	$(4.89 \pm 0.41) \times 10^5$			

ameter for each metal. The value of k_{obs} is also related, for pseudo-first order reactions (like the present ones), to the half-life of the reaction [16]: $t_{\frac{1}{2}} \times k_{obs} = \ln 2 = 0.693$. Thus, in a kinetic plot of absorbance versus time, an analogy can be made between the time corresponding to halving of the absorbance, as a readily attainable piece of information, and the half-wave potential in polarography. Moreover, in polarography the current can be related to the concentration of the electroactive species in the same way that the variation of absorbance can be used for quantitative purposes in kinetic experiments. The absorbance—time plots can be linearized in the form $\ln(A_t - A_{eq})$ as a function of time so that from the extrapolation to zero time (intercept = $\ln(A_{t=0} - A_{eq}) = \ln(\Delta A)$) the total absorbance variation ΔA can be evaluated with good accuracy, from which [metal] = $\Delta A/(\epsilon_{MeSPADNS} - \epsilon_{SPADNS}) \times l$, where l = light path-length.

Binary mixtures

For a binary mixture of metals (s, slow and f, fast) the above treatment applied to the slower cation enables k_{obs} and ΔA(s) to be evaluated for that ion. ($A_{eq} + \Delta A$(s)) gives the absorbance corresponding to completion of the faster reaction. From this value, a new plot of $\ln(A_t - A_{eq}(f))$ vs. time can be obtained, from which k_{obs} and ΔA(f) are evaluated. Figure 1 shows the oscilloscope recordings obtained for a typical Co—Ni mixture; the different time scales enable the different reactions to be separated. Figure 2 shows the plot of $\ln(A_t - A_{eq})$ vs. time for the reaction shown in Fig. 1a. Table 4 reports the analytical results for a series of Co—Ni mixtures. Mixtures such as Cu and Co (or Ni or Pb) and Co and Pb can also be analyzed by this method.

Multicomponent mixtures

Multicomponent mixtures such as Zn (or Mn or Cd), Co, Pb (or Ni) and Cu can be evaluated by following the above procedure. A very rapidly reacting metal ion (Zn, Mn or Cd) can be determined from the absorbance change during the mixing time of the stopped-flow apparatus, i.e. the difference between the absorbance at zero time (absorbance of the solution when none but the very fast ion has reacted) and the absorbance of the metals—SPADNS solution after mixing with a buffer solution without the displacing ligand.

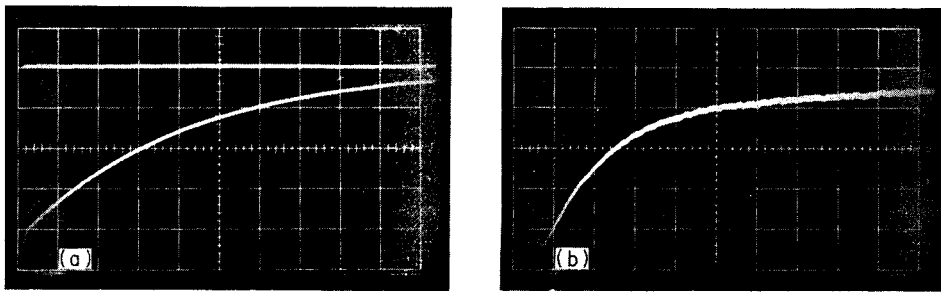


Fig. 1. Oscilloscope photographs (% transmittance vs. time) for a mixture of 6.0×10^{-6} M cobalt(II) and 4.0×10^{-6} M nickel(II). [SPADNS] = 1.0×10^{-4} M, [EDT] = 1.0×10^{-4} M, pH = 8.5, 25.0°C. Ordinates: 20% transmittance full scale (increasing transmittance upwards). Abscissae: (a) 1 s per major division; (b) 20 ms per major division.

TABLE 4

Determination of Co^{2+}—Ni^{2+} mixtures of 1.0×10^{-6} M total concentration ([SPADNS] = 1.0×10^{-4} M, [EDTA] = 1.0×10^{-4} M, pH = 8.5)

Co ($\times 10^{-6}$ M)		Ni ($\times 10^{-6}$ M)	
Added	Found	Added	Found
0	0	10.0	10.8
2.0	2.2	8.0	8.3
4.0	3.8	6.0	6.3
6.0[a]	6.1	4.0[a]	3.8
8.0	8.4	2.0	1.8
10.0	9.6	0	0
Mean accuracy (%)	5.1		6.4
R.s.d. (%)	5.0		5.5

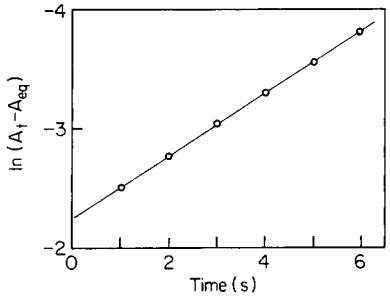
[a]A plot of absorbance vs. log (time) is shown in Fig. 2.

Figure 3 shows a plot of absorbance versus log (time) for a mixture of Mn, Co, Pb and Cu; Table 5 reports the results of analyses of such mixtures.

For these analyses, the precision is better than the accuracy; this can probably be ascribed to the effect of impurities present in the reagents.

Table 3 allows some possibilities of differential kinetic analysis of various combinations of metals to be detected and indicates the development of procedures for determining a metal ion in a multicomponent mixture. This is particularly important when other metal ions can give rise to serious interferences in equilibrium methods whereas the kinetic method offers the possibility of temporal separation from the interferences.

The present work was financially supported by the National Research Council of Italy (CNR-Rome).



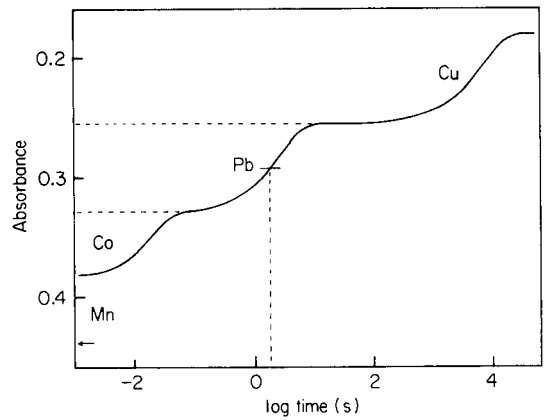


Fig. 2. Plot of $\ln(A_t - A_{eq})$ vs. time for the reaction of nickel(II), shown in Fig. 1 (upper display) (see also Table 4). From the slope of the plot, k_{obs} = 0.262 s^{-1}; from the intercept, $\ln(A_{t=0}(Ni) - A_{eq})$ = −2.30, ΔA = 0.100, [Ni] = 3.8 × 10^{-6} M)

Fig. 3. Variation of absorbance as a function of log (time) for a Mn^{2+}—Co^{2+}—Pb^{2+}—Cu^{2+} mixture (see Table 5); the arrow represents the absorbance of the metals—SPADNS mixture diluted 1:1 with a buffer solution at the same pH (see text).

TABLE 5

Determination of mixtures of Mn^{2+}, Co^{2+}, Pb^{2+} and Cu^{2+}
([SPADNS] = 1.0 × 10^{-4} M, [EDTA][a] = 1.0 × 10^{-4} M, pH = 8.5)

	Mn^{2+} (× 10^{-6} M)		Co^{2+} (× 10^{-6} M)		Pb^{2+} (× 10^{-6} M)		Cu^{2+} (× 10^{-6} M)	
	Added	Found	Added	Found	Added	Found	Added	Found
	5.0[b]	5.3	3.0	2.5	5.0	5.3	5.0	5.1
	8.0	7.7	4.0	3.8	4.0	3.6	8.0	8.2
	5.0	4.3	5.0	5.3	5.0	4.8	5.0	5.6
Mean accuracy		7.9%		12.6%		6.7%		5.5%
Relative standard deviation		7.0%		8.4%		6.0%		4.0%

[a]For the rapid measurement of Cu^{2+}, the displacement was carried out with 1 × 10^{-2} M EDTA.
[b]Plot of absorbance vs. log (time) is shown in Fig. 3.

REFERENCES

1 E. Mentasti and E. Pelizzetti, Inorg. Chem., 17 (1978) 3133.
2 R. E. Shepherd, G. M. Hadgson and D. W. Margerum, Inorg. Chem., 10 (1971) 989, and references therein.
3 D. C. Olson and D. W. Margerum, J. Am. Chem. Soc., 85 (1963) 297; D. W. Margerum and J. D. Carr, J. Am. Chem. Soc., 88 (1966) 1639.
4 R. G. Wilkins, in The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes, Allyn & Bacon, Boston, 1974.

5 D. Banerjea, J. Indian Chem. Soc., 54 (1977) 37.
6 H. B. Mark Jr., G. A. Rechnitz, in Kinetics in Analytical Chemistry, Interscience, New York, 1968.
7 M. K. S. Mak, C. H. Langford and T. R. Khan, J. Indian Chem. Soc., 54 (1977) 51.
8 J. B. Pausch and D. W. Margerum, Anal. Chem., 41 (1969) 226; D. W. Margerum, J. B. Pausch, G. A. Nyssen and G. F. Smith, Anal. Chem., 41 (1969) 233.
9 M. Tanaka (page 133), M. Kopanica and V. Starà (page 505), in E. Wanninen (Ed.), Analytical Chemistry. Essays in memory of Anders Ringbom, Pergamon, Oxford, 1977.
10 R. G. Gamon and C. N. Reilley, Anal. Chem., 34 (1962) 600.
11 M. Tanaka, S. Funshashi and K. Shirai, Anal. Chim. Acta, 39 (1967) 437.
12 G. M. Ridder and D. W. Margerum, Anal. Chem., 49 (1977) 2090 and 2098.
13 G. M. Ridder and D. W. Margerum, page 515, in E. Wanninen (Ed.), Analytical Chemistry. Essays in memory of Anders Ringbom, Pergamon, Oxford, 1977.
14 M. Eigen, Pure Appl. Chem., 6 (1963) 97; H. Diebler, M. Eigen, G. Maan and R. Winkler, Pure Appl. Chem., 20 (1969) 93; A. McAuley and J. Hill, Q. Rev. Chem. Soc., 23 (1969) 18.
15 E. Mentasti, E. Pelizzetti, F. Secco and M. Venturini, Inorg. Chem., 18 (1979) 2007.
16 For the treatment of the kinetic oscilloscopic traces and for details on $t_{\frac{1}{2}}$ and k_{obs}, see, for example, K. J. Laidler, in Chemical Kinetics, 2nd edn., McGraw-Hill, London, 1965, Ch. 1; E. S. Swinbourne, in Analysis of Kinetic Data, T. Nelson, London, 1971.

Analytica Chimica Acta, 111 (1979) 187—192

SPECTROPHOTOMETRIC DETERMINATION OF FUSICOCCIN

A. EVIDENTE, M. LASAPONARA, G. MARINO and G. RANDAZZO*

Instituto di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, 80134 Napoli (Italy)

(Received 12th March 1979)

SUMMARY

Fusicoccin incubated for 17 h in 6 M HCl at 37°C gives rise to a chromophoric system having an absorption maximum at 555 nm. A colorimetric method for the determination of pure fusicoccin and of fusicoccin in fermentation liquors and infected plant tissues is described. The method is useful for 50—800 μg of fusicoccin.

Fusicoccin(I) is the main phytotoxic metabolite produced by the fungus *Fusicoccum amygdali* Del. [1]. This diterpenoid glucoside is related chemically to other biologically active fungal metabolites having the same carbotricyclic system, e.g. ophiobolins [2] and cotylenins [3]. Extensive work on the chemical and biological properties of fusicoccin has been reviewed [4]. Fusicoccin shows pronounced plant-growth regulating properties and is a valuable tool for investigating physiological processes in higher plants [5]. Recent results [6] have shown its potential interest in agriculture.

On t.l.c. plates, fusicoccin and its derivatives give violet spots when sprayed with strong acids and heated at 100°C. This observation prompted an examination of the reaction in solution. A linear relationship was found between the amount of fusicoccin and the absorbance at 555 nm of the chromophore(s) obtained after treatment with hydrochloric acid and a simple spectrophotometric determination of fusicoccin was developed.

EXPERIMENTAL

F. amygdali was fermented [7] in 500-ml Erlenmeyer flasks containing 100 ml of medium. Fusicoccin was isolated [1] and derivatives were prepared [4, 8]. The amounts of toxin in the culture filtrates, determined by bioassay [9], were 21 ± 3 and 63 ± 7 μg ml^{-1} at the third and fourth stages of fermentation, respectively. The effect of strong mineral acids (e.g. HCl, $HClO_4$, H_2SO_4, H_3PO_4 (highest purity from commercial sources), was observed by adding appropriate dilutions to fusicoccin (or to its derivatives); spectra from 700 to 200 nm were recorded on a Cary 118 spectrophotometer equipped with a constant temperature bath.

To obtain a standard curve, weighed amounts of the crystalline toxin (5 mg) were dissolved in 10 ml of benzene and aliquots (0.1–1.5 ml) of this solution were evaporated to dryness in test tubes (1 × 20 cm) under nitrogen. After the addition of 5 ml of 6 M HCl, the tubes, lidded with glass balls, were kept at 37°C for 17 h. Absorbance at 555 nm was read against a 6 M HCl blank. T.l.c. was carried out on Kieselgel F_{254} 0.25-mm plates (Merck) with chloroform—propan-2-ol, 9:1 (v/v) and 1:1 (v/v), as eluents.

RESULTS

Formation and properties of the chromophore(s)

When fusicoccin was treated with 6 M HCl, a violet colour developed; Fig. 1 shows the absorption spectrum after incubation as described above. The time-dependence of the absorption maximum at 555 nm is shown in Fig. 2; absorbance was maximal during the period 14—21 h after incubation. However, the kinetics of the process were complex; several reactions seemed to occur simultaneously. The time-dependence was not influenced by the commercial origin of the acid used or by the addition of metal ions (Fe^{3+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}; 1 mM) to the 6 M HCl.

When the hydrochloric acid concentration was increased to 7 and 8 M, maximum absorbance was observed after 13 and 6 h, respectively, with an increase in the specific absorptivity. However, degradations were faster and the width of the maximum absorbance—time plateau was shortened. With 4 M HCl, maximum absorbance was observed only after 48 h. The effects of several mineral acids are compared in Table 1.

The effect of temperature on the reaction was tested on fusicoccin solutions in 6 M HCl at 18°C, 37°C and 60°C for 17 h. At 18°C, the

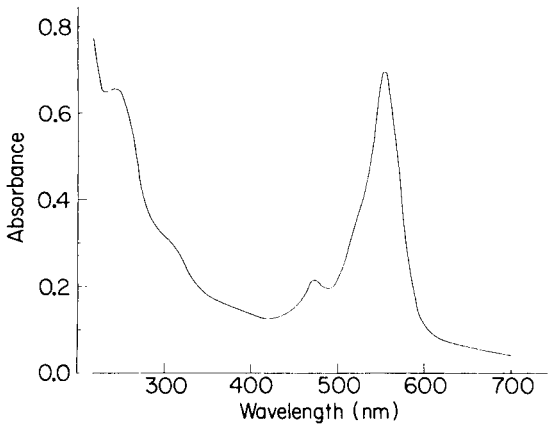


Fig. 1. Absorption spectrum (1-cm cell) of a solution of fusicoccin (120 μg ml^{-1}) incubated for 17 h in 6 M HCl at 37°C.

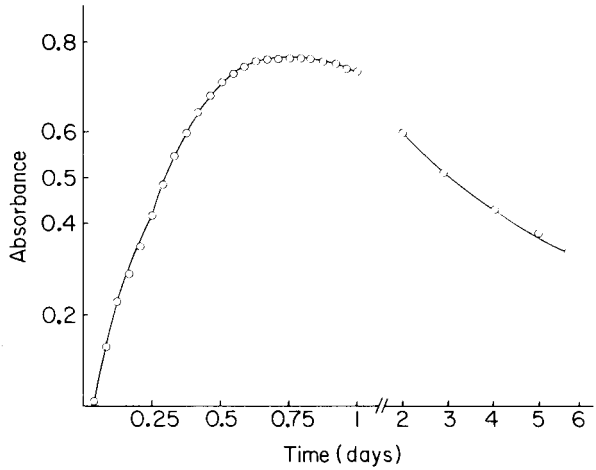


Fig. 2. Time dependence of the absorbance at 555 nm of a solution of fusicoccin (103 μg ml^{-1}) in 6 M HCl at 37°C.

TABLE 1

Effects of different acids (5 ml of 6 M) on the absorbance of fusicoccin (400 μg) at 555 nm after incubation at 37°C for 17 h

Acid	H^+ Activity	Ref.	Absorbance
HCl	22	[10]	0.510
H_2SO_4	3	[10]	0.126
$HClO_4$	94	[10]	0.280
H_3PO_4	0.52	[11]	0.0

maximum absorbance, reached after 72 h, was lower than that observed at 37°C. At 60°C the maximum absorbance occurred after 60 min but decreased after a few minutes. Differences were not observed when the reaction was carried out in sealed tubes under vacuum.

The chromophoric system is much more polar than fusicoccin and could not be extracted by common organic solvents. A sharp reversible colour change from violet to yellow occurred at ca. pH 1.3 when alkali was added to the acidic solution.

Examination of the 6 M HCl reaction mixture by t.l.c. showed that no fusicoccin remained after treatment at 37°C for 17 h; several unknown colourless products, more polar than fusicoccin, were formed but none of the degradation products formed in dilute acid solution at room temperature [12] was present under the conditions used.

When the same reaction mixture was chromatographed on a silica gel column, the coloured components were eluted only by polar solvents. A

sharp yellow band travelled through the stationary phase on addition of acetone, and a violet zone was then eluted by methanol. The latter contained at least three molecular species with different absorption spectra with maxima at 540, 555 (main component) and 570 nm, respectively.

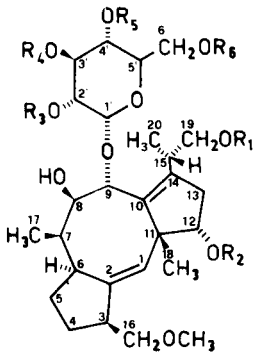
Standard curve

Under the optimal conditions established above for the colorimetric determination, there was a linear relationship between the absorbance at 555 nm and the amount of fusicoccin in the range 50—800 μg; the corresponding absorbance range was 0.040—1.000. The coefficient of variation observed in five separate experiments was less than 3% at the 600-μg level.

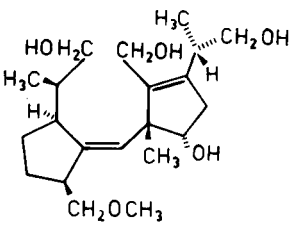
The recovery of various amounts of fusicoccin added to culture filtrates of *F. amygdali* was determined. The slope of the regression line, fitted to the experimental data by the least-squares method, was 0.98, i.e., 98% of the added fusicoccin was recovered on average.

Behaviour of fusicoccin derivatives

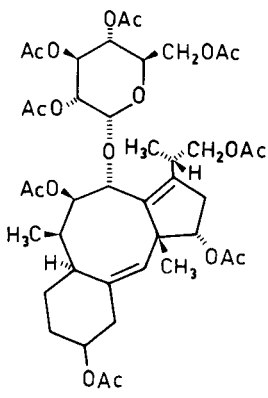
Several fusicoccin derivatives gave rise to chromophores with similar kinetics and colour yields to fusicoccin(I), e.g. dideacetylfusicoccin(II) [8], de-t-pentenyl-dideacetylfusicoccin(III) [12], deactylfusicoccin aglycone(I) and triacetylfusicoccin(IV) [1]. However, when the reaction was carried out with fusicoccin derivatives having a modified carbotricyclic skeleton, e.g. (V), (VI), or (VII) [13], there was no colour development.



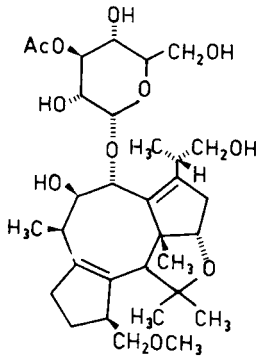
I	$R_1 = R_4 = COCH_3$	$R_2 = R_3 = R_5 = H$	$R_6 = C(CH_3)_2 - CH = CH_2$
II	$R_1 = R_2 = R_3 = R_4 = R_5 = H$		$R_6 = C(CH_3)_2 - CH = CH_2$
III	$R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$		
IV	$R_1 = R_2 = R_3 = R_4 = R_5 = COCH_3$		$R_6 = C(CH_3)_2 - CH = CH_2$



V



VI



VII

Chromophore(s) were produced when fusicoccin modified in the glucose moiety was covalently bound to macromolecular matrices, e.g. bovine serum albumin or lysine—Sepharose 4B. The colorimetric procedure represents the only direct method suitable for the determination of fusicoccin found to such macromolecules.

Determination of fusicoccin in crude extracts from fermentations and in plant tissues infected by Fusicoccum amygdali

Of wide interest is the determination of fusicoccin in *F. amygdali* culture filtrates or in fruit infected by Fusicoccum. The direct determination of fusicoccin in crude materials was found to be impossible, because of the presence of substances absorbing in the visible range of the spectrum. Preliminary purification was therefore essential.

For culture filtrates, a single extraction step was sufficient. The filtrate (25 ml) was extracted twice with 10 ml of chloroform. The extracts were pooled and dried over anhydrous sodium sulphate; the solvent was removed under reduced pressure. To the residue was added 5 ml of 6 M HCl. After incubation at 37°C for 17 h, the absorbance at 555 nm was measured. The amounts of fusicoccin found in the culture filtrates were 19 ± 1 and 67 ± 2 $\mu g\ ml^{-1}$ (mean of three determinations), as the third and fourth stages of fermentation [7] respectively, in agreement with results obtained by bioassay [9].

With infected peaches, a purification procedure [14] was followed to the silica gel column chromatography stage. The fractions containing fusicoccin-like (t.l.c.) material (which previously [14] had to be further purified by several carbon—Celite chromatographic steps followed by crystallization) were collected, dried and treated with hydrochloric acid as described above. The amount of fusicoccin (4.5 mg per 500 g of peach tissue) was in good agreement with that obtained (5.0 mg per 500 g) by the longer procedure [14].

DISCUSSION

The most effective acid for producing chromophore(s) from fusicoccin is hydrochloric acid. Although the chemical structure of the chromophore(s) is unknown, the absorption spectrum was reproducible (Fig. 1), with a maximum at 555 nm. The best conditions for the colorimetric determination of fusicoccin involved incubation at 37°C with 6 M HCl for 17 h.

The effect of acids other than hydrochloric acid, and the effects of different acid concentrations, suggest a dependency of chromophore(s) production on hydrogen ion activity. The results obtained with perchloric acid apparently contradict this, but are the consequence of a secondary reaction yielding the deacetylaglycone of (VI). This product, which lacks the features necessary for the formation of the chromophore(s), was detected by t.l.c. in the solvent-extractable fraction of the reaction mixture incubated for 30 min.

The selectivity of the above procedure for fusicoccin was evaluated by

testing structurally related compounds. Whereas fusicoccin aglycone, and derivatives differing from fusicoccin in the number and position of the acetyl groups, behaved similarly to the parent compound, two other classes of natural products having the same carbotricyclic skeleton, i.e. cotylenins [2] and ophiobolins [3], behaved differently. Cotylenol, the aglycone common to all the cotylenins, gave after treatment with 6 M HCl at 37°C an absorption spectrum identical to that obtained with fusicoccin or fusicoccin aglycone, but the overall reaction was very much faster. In contrast, solutions of both ophiobolin A and B remained colourless under the standard conditions of the assay. Thus the $\Delta^{1,2}$-$\Delta^{10,14}$-dicyclopenta[a,d]cycloctane moiety appears to be essential for formation of the chromophore. The negative results obtained with compounds (V), (VI) and (VII) support this suggestion.

The linear relationship between concentration and absorbance over a broad concentration range, the sensitivity of the assay (down to 50 μg), its good selectivity and simplicity, make this method acceptable for the determination of total fusicoccins in crude biological materials. The results obtained with culture filtrates of *Fusicoccum amygdali* and with plant tissues infected by this fungus agree well with those obtained by other procedures. The satisfactory results obtained by determining a fusicoccin derivative linked to high molecular weight substances (bovine serum albumin, lysine—Sepharose 4B) indicate further uses for this procedure.

The authors are grateful to Prof. A. Ballio for his advice and interest during the course of this work.

REFERENCES

1 A. Ballio, M. Brufani, C. G. Casinovi, S. Cerrini, W. Fedeli, R. Pellicciari, B. Santurbano and A. Vaciago, Experientia, 24 (1968) 631.
2 L. Canonica, A. Fiecchi, M. Galli Kienle, B. M. Ranzi and A. Scala, Tetrahedron Lett., (1968) 275.
3 T. Sassa, T. Tojyo and K. Munakata, Nature, 227 (1970) 379; subsequent literature quoted in T. Sassa and A. Takahama, Agric. Biol. Chem., 39 (1975) 2213.
4 A. Ballio, in E. Marré and O. Ciferri (Eds.), Regulation of Cell Membrane Activities in Plants, Elsevier/North-Holland, Amsterdam, 1977 217.
5 E. Marré, in E. Marré and O. Ciferri (Eds.), Regulation of Cell Membrane Activities in Plants, Elsevier/North-Holland, Amsterdam, 1977, 185.
6 J. W. Braun and A. A. Khan, J. Am. Soc. Hortic. Sci., 101 (1976) 716.
7 A. Ballio, A. Carilli, B. Santurbano and L. Tuttobello, Ann. Ist. Super. Sanità, 4 (1968) 317.
8 A. Ballio, C. G. Casinovi, G. Randazzo and C. Rossi, Experientia, 26 (1970) 349.
9 A. Bottalico, Phytopathol. Mediterr., 11 (1972) 77.
10 R. A. Robinson and R. H. Stokes, Electrolyte Solutions, Butterworths, London, 1959, p. 491.
11 R. P. Platford, J. Solut. Chem., 4 (1975) 591.
12 K. D. Barrow, D. H. R. Barton, E. Chain, C. Conlay, T. C. Smale, R. Thomas and E. S. Waight, J. Chem. Soc. C, (1971) 1259.
13 R. Capasso, A. Evidente, M. Lasaponara and G. Randazzo, Rend. Accad. Sci. Fis. Mat. Naples, 44 (1977) 69.
14 A. Ballio, V. D'Alessio, G. Randazzo, A. Bottalico, A. Graniti, L. Sparapano, B. Bosnar, C. G. Casinovi and D. Grivanovski-Sassu, Physiol. Plant Pathol., 8 (1976) 163.

Analytica Chimica Acta, 111 (1979) 193—199

DETERMINATION OF AN EXPERIMENTAL PLANT GROWTH REGULATOR ON WHEAT AND COTTON PLANTS BY REVERSED-PHASE ION-PAIR PARTITION HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY†

A. PRYDE*, A. SCHULER and F. P. A. VONDER MÜHLL

Dr. R. Maag A.G., CH-8157 Dielsdorf (Switzerland)

(Received 15th May 1979)

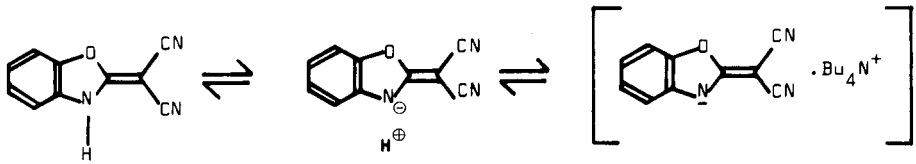
SUMMARY

After extraction from spiked wheat and cotton samples, and preliminary clean-up by anion-exchange chromatography, the plant growth regulator, 2-benzoxazolinylidenmalononitrile, was determined by reversed-phase ion-pair partition h.p.l.c. wih tetrabutylammonium as the pairing ion. In extracts, the solute could be separated from interferences by varying the tetrabutylammonium concentration; the average recovery of the compound from wheat plants (0.13—55.6 ppm) was 96.5% and from cotton plants (0.15—134.6 ppm) 104.4% by the h.p.l.c. method. These recoveries were confirmed by the scintillation counting method with the radiolabelled compound. In a dissipation study, a biological half-life of 1.5—2.0 days was found for the compound on cotton plants. Reversed-phase ion-pair partition h.p.l.c. is a promising technique for the determination of trace levels of ionic (or ionizable) compounds in environmental samples.

Reversed-phase ion-pair partition high-performance liquid chromatography (h.p.l.c.) is increasing in popularity in pharmaceutical and biochemical analysis [1—15], but the technique has not found wide application in pesticide residue analysis [1, 16, 17]. This is surprising as the method is applicable to ionic (or ionizable) compounds which are often difficult to determine (especially at trace levels) by other techniques. As described in detail elsewhere [10, 11, 18—21], ion-pair partition h.p.l.c. involves pairing the ion of interest with an ion of opposite charge (the pairing ion) to form an ion pair which behaves as a polar organic molecule and can thus be partitioned from an aqueous into an organic phase [22]. In reversed-phase ion pair partition h.p.l.c., a reversed-phase chromatographic support (e.g. a chemically-bonded alkyl-silica) is used in conjunction typically with an aqueous methanolic eluent containing the pairing ion; partitioning of the ion pair thus formed from the aqueous eluent into the organic stationary phase (the bonded alkyl groups enriched with the organic component of the eluent) forms the basis of the method. As the pairing ion is present in the eluent, its nature or concentration and the pH of the eluent are easily changed; also, aqueous ex-

†Presented in part at the Fourth International Congress of Pesticide Chemistry (IUPAC), Zürich, Switzerland, (July) 1978.

tracts can be directly injected into the h.p.l.c. column without prior lyophilization, so that the clean-up required is reduced. A particular advantage of the ion-pair approach is that in complex samples (where interferences may vary from sample to sample) the retention of the compound of interest can be controlled by varying the pairing ion concentration. This paper discusses the application of the method to the trace analysis of an experimental plant growth regulator (2-benzoxazolinylidenmalononitrile, I, Ro 10-3362) which ionizes in basic solution (II) and forms an ion pair (III) with tetra-n-butylammonium (TBA) ions.



(I) (II) (III)

At high concentrations of water in the eluent, an alternative dynamic ion-exchange process can occur [18, 23, 24] in which the hydrophobic, chemically-bonded packing material extracts the pairing ion from the eluent; the extracted pairing ion, which forms a monolayer on the support surface, can act as a dynamic ion-exchanging system.

EXPERIMENTAL

Chemicals

H.p.l.c. eluent. The h.p.l.c. eluent was methanol (40 ml)—water (60 ml)—tetra-n-butylammonium hydroxide (40%, 0.38 ml)—disodium hydrogenphosphate (0.15 g)—phosphoric acid to pH 7.5. (A sodium acetate buffer of pH 7.5 gave similar results.) At an eluent flow rate of 1.0 ml min^{-1}, the active ingredient was eluted in 7 min under these conditions.

Buffer pH 9.5. The buffer for anion-exchange column chromatography was prepared by adjusting 950 ml of 0.2 M ammonium chloride to pH 9.5 with ammonia and diluting to 1 l with distilled water.

Tetra-n-butylammonium hydroxide used was a 40% (w/v) solution in water (practical grade, Fluka). Other chemicals were analytical grade and used as received. All solvents were distilled from glass before use. Sephadex QAE A25 strong anion-exchange resin (40—120 μm) was obtained from Pharmacia Fine Chemicals, Uppsala.

Radioactive Ro 10-3362/010 (2-benzoxazolinylidenmalononitrile-1,3-^{14}C; $C_{10}H_5ON_3$; m.w. 183.16, specific activity 44.0 μCi mg^{-1}), ^{14}C-labelled in both cyano groups, was synthesized by Hoffmann-La Roche, Basel.

Equipment

High-performance liquid chromatograph. The liquid chromatograph was laboratory-assembled from a DMP AE 10-4 twin-headed reciprocating pump

(Orlita, Giessen, GFR) and a Perkin-Elmer LC55 variable-wavelength spectrophotometer operated at 310 nm and a sensitivity of 0.02 absorbance units, full scale. An electronic integrator, model CRS 208 (Infotronics Ltd) was used to determine h.p.l.c. peak areas. The h.p.l.c. column (100 × 5 mm i.d., stainless steel, internally polished), septum injector, column-packing reservoir (stainless steel, 30-ml volume), and SAS-Hypersil, 5-μm porous spherical silica particles containing chemically-bonded short-chain alkyl groups, were obtained from Shandon Southern Products Ltd. (Runcorn, Great Britain). Injection of 2—5-μl volumes was by syringe (Model 10A-RN-GP, SGE) against the operating pressure; flow rates were ca. 1.0 ml min^{-1}; columns were operated at ambient temperature.

Packing of h.p.l.c. column. The h.p.l.c. column was packed as follows: a slurry of 2 g SAS-Hypersil in 30 ml of a mixture of methanol—aqueous 0.1% sodium acetate (80%: 20%, v/v) was dispersed thoroughly by ultrasonic vibration and added to the combined analytical column—column packing reservoir assembly. The system was connected to a mini-Haskel pneumatic pump and the slurry was forced into the column at a pressure of 200 atm.; methanol—aqueous 0.1% sodium acetate (50% : 50%, v/v) was used as the follower liquid. When 100 ml of the latter had been pumped through the column, the pump was switched off and the pressure was allowed to dissipate completely before disconnecting the system. The analytical column was equilibrated on the liquid chromatograph with the required eluent prior to use. In routine use, the buffered mobile phase was flushed out each evening (by using a time switch) with ca. 100 ml of methanol—water (50%:50%, v/v) prior to overnight storage, to prevent degradation of the chemically bonded stationary phase.

Scintillation counting. Triplicate aliquots of the solution to be counted were pipetted into counting vials and 10 ml of Insta-Gel scintillation solution (Packard SA, Zürich) was added. The samples were counted twice in a Model 3375 liquid scintillation spectrometer (Packard SA, Zürich), and the counts were averaged; the automatic external standard ratio method was used to determine counting efficiency.

Homogenizer. An Omni-Mixer (J. Sorvall Inc., Norwalk, Conn.). was used with an adaptor for Mason jars.

Procedures

Extraction from wheat and cotton plants. The extraction is illustrated for the case where the plants were fortified additionally with ^{14}C-labelled Ro 10-3362 for confirmation of the recovery by scintillation counting. To avoid photochemical decomposition of the compound during work-up, the extraction, column chromatography and h.p.l.c. were conducted under artificial lighting.

To 25 g of the wheat or cotton plants in a one-pint Mason jar were added 0.5 ml of a standard solution (0.98 μg ml^{-1}) of the labelled compound I in methanol (≡47 900 dpm/0.5 ml) and the following quantities of a standard

solution (278 μg ml^{-1}) of the unlabelled compound I in methanol: for 0.13 ppm (μg per g of plant material), 10 μl; for 0.58 ppm, 50 μl: for 5.6 ppm, 0.5 ml; and for 55.6 ppm, 5.0 ml. Aqueous ammonia (25% w/v, 5 ml) and 90—95 ml of acetonitrile were added to give a final volume of 100 ml. The mixture was macerated for 5 min at 0°C on the Omni-Mixer and then filtered through a fluted filter paper. A 70-ml aliquot of the filtrate was transferred to a 250-ml separating funnel and shaken vigorously with 100 ml of hexane to remove lipids. The acetonitrile phase was transferred to a 250-ml round flask. The hexane layer was washed with acetonitrile (10 ml) and the combined acetonitrile phases were evaporated to 1—2 ml on a rotary evaporator (bath temperature 40°C; solutions tend to foam and bump). The distillation tube of the rotary evaporator was washed with methanol (10 ml) and the solution again evaporated to 1—2 ml.

Column chromatographic clean-up. About 1.0 g (dry weight) of a slurry of Sephadex QAE A25 in methanol—pH 9.5 buffer (75%:25%) was packed into a glass chromatography column (14 mm i.d.) to give a bed depth of ca. 30 mm. The residue from the extraction step above was dissolved (ultrasonic agitation) in 10 ml of the same solvent mixture and the solution was applied to the column. The flask was washed with a further 10 ml of the solvent followed by 40 ml of a methanol—aqueous 50% acetic acid (95%:5%) mixture. All washings were applied to the chromatography column. The product was eluted with 30 ml of a methanol—aqueous 0.5 M HCl (95:5%) mixture. The solution was carefully evaporated to dryness at 40°C on a rotary evaporator (solutions tend to foam and bump).

H.p.l.c. and scintillation counting. The residue from the clean-up step above was redissolved in the appropriate volume of a methanol—water—aqueous 25% (w/v) ammonia (45%:45%:10%) solution for direct injection onto the h.p.l.c. column (samples were diluted so that 10—50 ng of compound I could conveniently be injected in an injection volume of 2—5 μl, i.e., redissolved in 1.0 ml for the 0.13 ppm and 0.58 ppm samples, in 10 ml for the 5.6 ppm sample and in 100 ml for the 55.6 ppm sample). The solutions thus obtained were analyzed by h.p.l.c. and by scintillation counting.

Dissipation of the plant growth regulator on cotton plants. Pots containing three cotton plants at the 2-leaved stage were sprayed by hand to run-off with 10 ml per pot of the formulated product (1000 ppm compound I). The plants were maintained in a greenhouse (21—26°C) and harvested as required. Each sample for analysis comprised three plants taken at random from three different pots; duplicate samples were analyzed at each time interval. Work-up and h.p.l.c. analysis were as described above.

Dependence of the h.p.l.c. retention of compound I on the TBA concentration in the eluent. As interfering compounds may vary from extract to extract, the variation in the h.p.l.c. retention (k' value) of compound I with the TBA concentration in the eluent was studied for a series of eluents containing methanol (40%), water (60%), anhydrous sodium acetate (0.15%

w/v) and varying amounts of aqueous 40% TBA (0—1% v/v). The eluents were buffered at pH 7.5 with aqueous ammonia (no TBA) or glacial acetic acid (0.1—1.0% v/v TBA). Acetone was taken as the unretained peak.

RESULTS

Recoveries

The recoveries of compound I by the h.p.l.c. and scintillation counting methods are shown in Table 1. (Processing of unspiked wheat or cotton samples gave h.p.l.c. chromatograms with no interfering peaks in the region of the compound I peak.) In calculating the results, a water content of 90% was assumed for the wheat and cotton samples (i.e. 22.5 ml of water in a 25-g sample). Thus the amount of compound I found in the 70-ml aliquot (see Experimental) was scaled up to a total volume of 122.5 ml. An h.p.l.c. chromatogram of compound I from cotton plants at the 0.15 ppm level is shown in Fig. 1.

Dissipation on cotton plants

The fall in the concentration of compound I on cotton plants with time was followed by the h.p.l.c. method. Samples were analyzed at t = 0, 1, 4, 8, 15 and 36 days; a biological half-life of 1.5—2.0 days was found, suggesting a rapid dissipation of the compound on cotton plants.

Chromatographic behaviour of compound I

The dependence of the k' value of the peak for compound I on the TBA concentration in the eluent is shown in Fig. 2. A similar linear dependence of k' on the counter ion concentration at low concentrations, with a levelling off at higher concentrations, has been observed elsewhere [19].

DISCUSSION

Problems are frequently encountered in the determination of ionic or ionizable compounds at trace levels. Preliminary investigations with compound I ruled out gas chromatography (problems of volatility) and h.p.l.c. on adsorption columns (badly tailed peaks) as analytical techniques. The reversed-phase ion-pair partition method gave symmetrical peaks which could be satisfactorily quantified at trace levels. In addition, the method was robust; only one column was required for the whole of the study reported. To maintain column life and reproducibility, the methanol—buffer eluent was flushed out with aqueous methanol when the column was not in use (see Experimental) and any plant material adsorbed at the top of the column was removed occasionally by scraping off the top few millimeters of column packing material and adding an appropriate amount of a thick slurry of fresh packing material in methanol.

TABLE 1

Recovery values[a] of the plant growth regulator from wheat and cotton plants

Plant	Fortification level (ppm)	Recovery (%)	
		H.p.l.c.	Scintillation counting
Wheat	0.13	88.2 ± 9.4	97.1 ± 5.9
	0.58	99.7 ± 3.6	104.7 ± 3.6
	5.6	95.3 ± 3.7	104.6 ± 5.4
	55.6	102.9 ± 1.9	99.0 ± 2.0
Cotton	0.15	103.0 ± 20.4	103.0 ± 7.1
	6.97	107.1 ± 8.2	90.4 ± 2.4
	134.6	103.2 ± 5.3	84.3 ± 1.6

[a]Expressed as average % recovery ± s.d. from analyses in triplicate.

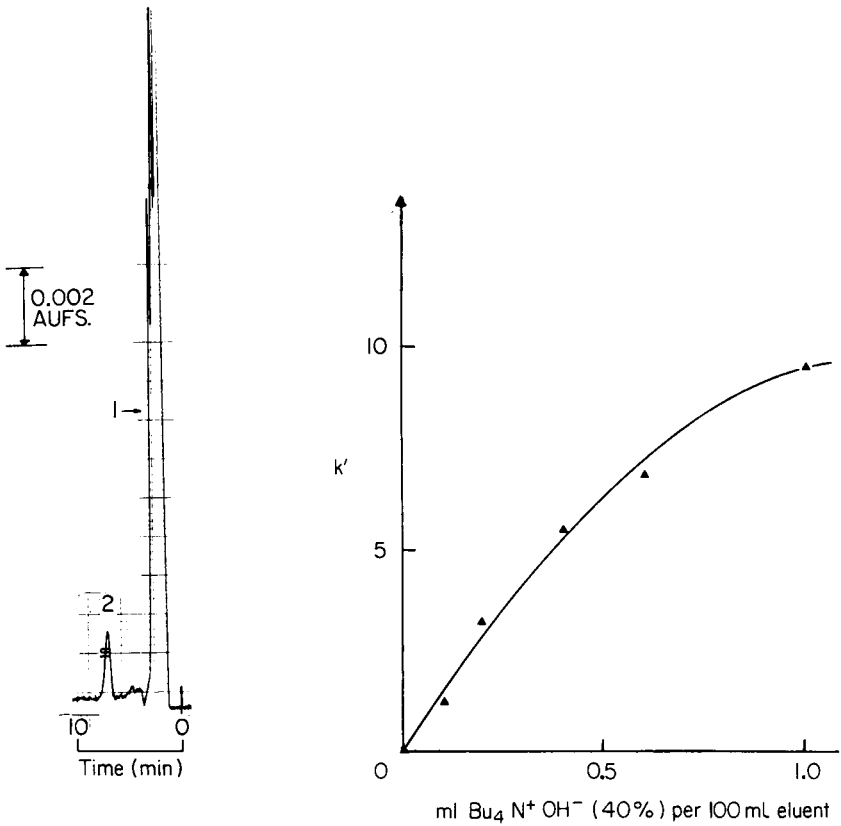


Fig. 1. H.p.l.c. of compound I from cotton plants at the 0.15 ppm level. Column, 100 × 5 mm i.d.; packing material, SAS-Hypersil; injection volume, 5μl (9.4 ng of compound I); other conditions as given in Experimental. Peak 1, interference; peak 2, compound I.

Fig. 2. Dependence of the k' value of compound I on the TBA concentration in the eluent.

The use of the ^{14}C-labelled compound was advantageous in optimizing the recovery at each stage of the analytical method and also confirmed the overall h.p.l.c. recovery values.

REFERENCES

1 A. Pryde and M. T. Gilbert, Applications of High Performance Liquid Chromatography, Chapman and Hall, London, 1979.
2 J. H. Knox and J. Jurand, J. Chromatogr., 103 (1975) 311.
3 D. Westerlund and A. Theodorsen, J. Chromatogr., 144 (1977) 27.
4 W. Santi, J. M. Huen and R. W. Frei, J. Chromatogr., 115 (1975) 423.
5 N. D. Brown, L. L. Hall, H. K. Sleeman, B. P. Doctor and G. E. Demaree, J. Chromatogr., 148 (1978) 453.
6 S. C. Su, A. V. Hartkopf and B. L. Karger, J. Chromatogr., 119 (1976) 523.
7 I. Lurie, J. Assoc. Off. Anal. Chem., 60 (1977) 1035.
8 B. Mellström and G. Tybring, J. Chromatogr. Biomed. Appl., 143 (1977) 597.
9 J. C. Kraak and P. Bijster, J. Chromatogr. Biomed. Appl., 143 (1977) 499.
10 B. Fransson, K.-G. Wahlund, I. M. Johansson and G. Schill, J. Chromatogr., 125 (1976) 327.
11 K.-G. Wahlund, J. Chromatogr., 115 (1975) 411.
12 B.-A. Persson and B. L. Karger, J. Chromatogr. Sci., 12 (1974) 521.
13 W. S. Hancock, C. A. Bishop, R. L. Prestidge, D. R. K. Harding and M. T. W. Hearn, Science, 200 (1978) 1168.
14 J. H. Knox and J. Jurand, J. Chromatogr., 142 (1977) 651.
15 J. H. Knox and J. Jurand, J. Chromatogr., 125 (1976) 89.
16 J. F. Lawrence and D. Turton, J. Chromatogr., 159 (1978) 207.
17 J. A. Sidwell, Med. Fac. Landbouww. Rijksuniv. Gent., 42 (1977) 1803.
18 J. H. Knox (Ed.), High-Performance Liquid Chromatography, Edinburgh University Press, Edinburgh, 1978, Ch. 6.
19 C. Horvath, W. Melander, I. Molnar and P. Molnar, Anal. Chem., 49 (1977) 2295.
20 R. Gloor and E. L. Johnson, J. Chromatogr. Sci., 15 (1977) 413.
21 C. P. Terweij-Groen and J. C. Kraak, J. Chromatogr., 138 (1977) 245.
22 G. Schill, in. J. A. Marinsky and Y. Marcus (Eds.), Ion Exchange and Solvent Extraction, Vol. 6, M. Dekker, New York, 1974, Ch. 1.
23 C. P. Terweij-Groen, S. Heemstra and J. C. Kraak, J. Chromatogr., 161 (1978) 69.
24 R. P. W. Scott and P. Kucera, J. Chromatogr., 142 (1977) 213.

Analytica Chimica Acta, 111 (1979) 201—213

THE DETERMINATION OF CHLORINATED LONG-CHAIN PARAFFINS IN WATER, SEDIMENT AND BIOLOGICAL SAMPLES

J. I. HOLLIES*, D. F. PINNINGTON and A. J. HANDLEY

I.C.I. Ltd., Research and Development Department, P O Box 8, The Heath, Runcorn, Cheshire WA7 4QD (Gt. Britain)

M. K. BALDWIN and D. BENNETT

Shell Research Ltd., Shell Toxicology Laboratory (Tunstall), Sittingbourne Research Centre, Sittingbourne, Kent ME9 8AG (Gt. Britain)

(Received 5th July 1979)

SUMMARY

Methods are described for the routine determination of traces of industrial chloro-n-paraffins having 13—30 carbon atoms and chlorine contents of 42—45% (w/w), in environmental samples of water, sediments and biota. The procedures are based on thin-layer chromatography detection and measurement. All samples are cleaned up by liquid—solid adsorption chromatography and thin-layer chromatography but those rich in lipids require preliminary solvent extraction. The methods distinguish between chloro-n-paraffins based on long carbon chains (C_{20}—C_{30}) and those based on shorter chains (C_{13}—C_{17}). The methods cover the ranges 500 ng l^{-1} to 8 μg l^{-1} for water (i.e. from about the solubility limit upwards) and 50 μg kg^{-1} to 16 mg kg^{-1} for sediments and biota. The precision of the methods ranges from ± 50% relative at the lowest concentrations to ± 12% relative at the highest. Recoveries are about 90% for water, 80% for sediments and between 80 and 90% for biota according to sample type.

Chloro-n-paraffins (CP) having at least 10 carbon atoms are widely used as plasticisers and extenders for PVC, as lubricating oil additives, as paint additives and for applications where good resistance to chemicals, water and fire is required. They are produced industrially with ranges of carbon chain lengths, commonly 13—17 or 20—30 atoms, and various degrees of chlorination. The principal producers are in Western Europe, North America and Japan, and the annual world consumption is estimated at 230,000 tonnes. In the U.K., they are produced solely by Imperial Chemical Industries Ltd. under the trade-name 'Cereclor'. Numerals following the trade-name indicate the approximate percentage of chlorine by weight in the product, e.g. 'Cereclor 42'.

The widespread use of these materials has prompted development of methods to detect them in the environment. The determination of CP is not straightforward. All grades contain many isomers and homologues and therefore their i.r. and n.m.r. peaks are broad and weak. CP are of low volatility and are thermally unstable, producing hydrogen chloride on decom-

position; hence direct gas chromatography is not attractive. Zitko [1] has devised a method based on column chromatography followed by microcoulometric detection. The procedure is not specific. Zitko has also described [2] a confirmatory method in which the CP are reduced to normal hydrocarbons which are then analysed by gas chromatography. Both methods lack sufficient sensitivity for trace (sub-ppm) analysis and the confirmatory method may be difficult to apply to biological samples because of interference. Friedman and Lombardo [3] have described a g.c. method applicable to CP that are slightly volatile; the method is based on microcoulometric detection and photochemical elimination of chlorinated aromatic compounds that otherwise interfere. However, in the study described here, their chromatography could not be reproduced, probably because of decomposition.

Choice of measurement technique

Three techniques were investigated for detecting CP. The first was liquid chromatography based on a Pye moving-wire transport system with a ^{63}Ni electron-capture detector. However, studies of this technique were discontinued because of its poor sensitivity to CP with low chlorine contents. The second technique was g.c. with a Coulson conductivity detector, but it proved impossible to avoid decomposition of the CP when they were volatilised, and the peaks from the decomposition products were broad and unrepeatable.

The third technique was thin-layer chromatography. Preliminary work was encouraging because sub-ppm concentration of CP could be detected by spraying the plate with silver nitrate. However, the aluminium oxide layers gave poor chromatography. Conversely, silica gel layers gave good chromatography but the sensitivity was poor. The key discovery in this investigation was that CP could be chromatographed on a silica gel plate from which an image of the chromatogram could be 'printed' on an aluminium oxide plate by heating the two face to face so that the high sensitivity of detection on aluminium oxide could be utilised. This detection procedure preceded by suitable preliminary clean-up and separation steps is the basis of the methods described here.

Development of the methods

For all samples liquid adsorption chromatography clean-up was essential and in the following methods a non-polar solvent, 60/80 petroleum spirit, is prescribed for separating mobile impurities from CP (the adsorbate). The latter are then desorbed with a polar solvent, toluene or carbon tetrachloride. According to sample type, two packings are prescribed. For water and sediments, aluminium oxide is effective and the column can be prepared simply by dry packing. However, lipidous samples are cleaned up more effectively on silica gel which must be slurry-packed to achieve satisfactory efficiency. Furthermore lipidous samples require pre-extraction based on solvent partition with dimethylformamide and 60/80 petroleum spirit. This procedure

has been established previously for chlorinated pesticides [4]. To get rid of any remaining impurities, which can spoil the subsequent t.l.c. stage by altering the chromatographic properties of the plate, a preliminary t.l.c. clean-up procedure is valuable: the impurities are separated from the CP and then discarded by cutting off that half of the plate which contains them. Omission of this procedure leads to t.l.c. spots that are distorted and therefore difficult to quantify.

The final t.l.c. stage is to separate the CP according to carbon chain length, Cereclor S45 and Cereclor 42 being used to calibrate the resulting chromatograms. These grades contain carbon chains in the ranges C_{13}—C_{17} and C_{20}—C_{30} respectively, and have chlorine contents of 45% and 42% (w/w), respectively. They were chosen because they give the poorest responses of all the Cereclors towards silver nitrate spray detection. Hence the methods are fail-safe: they may overestimate, rather than underestimate, CP concentrations in samples.

Quantification of the chromatogram is by visual comparison of sample and calibration spots. Discrimination between spots leads to the quoted precision which is acceptable for environmental survey work. Optionally, a densitometer may be used (Kontes, with Baseline Corrector accessory). This permits a detection limit similar to that of the visual method, a 50-ng spot of CP, and provides linear response up to 200 ng of CP.

Scope of methods

There are two methods, one for water and sediments and a second, incorporating additional clean-up, for lipidous samples such as eggs, animal tissues, fish, shellfish and edible oil.

Other halogenated compounds were examined by the t.l.c. procedure at concentrations above the highest Cereclor standard, but none was found to interfere. Compounds examined and their R_F values are given in Table 1. Certain compounds did not respond at the silver nitrate spray stage. These are recorded as 'not detected' (n.d.).

Precautions

Accidental contamination may occur during the analysis. In order to detect and allow for minor contamination by stray traces of CP which are often found in solvents, the methods include a blank procedure in which a portion of pure solvent is analysed in the same way. It is, however, essential that solvents should be rigorously tested before use. It is also advisable to discard sample bottles after use because although adsorption of CP on glass may not be observed, an unclean bottle may confuse results. Furthermore, the samples or their extracts must not be allowed to contact any plastic (especially PVC) container, stopper, cap liner, tubing etc. because these may incorporate CP. It is also recommended that neat CP should be excluded from the laboratory except when standard calibration solutions are being prepared.

TABLE 1

R_F values of halogenated compounds

Halogenated compound	R_F value	Halogenated compound	R_F value
Cereclor S45	0.74	Dichlorophen	n.d.
Cereclor 42	0.80	Dieldrin	0.49
Aldrin	0.88	DDD	0.69
Araclor 1254	n.d.	DDE	n.d.
α-BHC	0.67	DDT	0.65
β-BHC	0.71	Endrin	0.52
γ-BHC	0.69	Endosulphan	n.d.
Chlordane	0.64	Heptachlor	0.51
1-Chloroeicosane	0.54	Methoxychlor	0.47
1-Chlorohexadecane	n.d.	Mirex	n.d.
1-Chloro-octadecane	n.d.	Strobane	0.65
p-Dichlorobenzene	n.d.	Toxaphene	0.63

DETERMINATION OF TRACE CHLORO-n-PARAFFINS HAVING 13—30 CARBON ATOMS AND CHLORINE CONTENTS OF 42—45% (W/W) IN WATER AND SEDIMENTS BY THIN-LAYER CHROMATOGRAPHY (METHOD A)

Materials

Solvents. n-Hexane is used for t.l.c. plate development exclusively. Petroleum spirit (60/80) and toluene are used for extractions and column clean-up exclusively. Fisons' Distol grade is satisfactory for the three solvents but they must be checked as being free from interfering impurities. For this purpose, evaporate 200 ml of solvent to dryness, add 0.2 ml of the same solvent to dissolve any residue, apply the whole of this to a silica gel t.l.c. plate using a microapplicator, and detect any interfering impurity as recommended under 'Detection and measurement of t.l.c. spots'. Reject any batch of solvent that gives a spot.

Spray reagent. Use general-purpose laboratory reagents. Mix 45 ml of industrial methylated spirit, 2.5 ml of aqueous silver nitrate (10% w/v) and 2.5 ml of aqueous ammonia (d = 0.88). Use within two days.

Calibration compounds. Imperial Chemical Industries Cereclor grade S45 and Cereclor grade 42 are used. (Other manufacturers' CP have not been examined for use as standards but should be suitable.)

Prepare a standard solution (50 mg l^{-1}) in 60/80 petroleum spirit for each grade of Cereclor as follows: make a stock solution by dissolving 1 g in 50 ml of 60/80 petroleum spirit in a 100-ml volumetric flask, make up to the mark with petroleum spirit and mix. Dilute 0.5 ml of this solution to 100 ml with petroleum spirit. The stock solution is stable for several months, but the dilute standard must be prepared freshly each week.

Drying agent. Decontaminate anhydrous sodium sulphate (analytical-reagent grade) by heating at 300—350°C overnight.

Apparatus

T.l.c. plates. Silica gel F_{254} and aluminium oxide type E, F_{254}, both types 0.25 mm thick × 200 mm × 200 mm (Merck) are used without pretreatment.

Chromatographic columns. The glass column (10 mm bore × 300 mm long) is fitted with a glass sinter and tap (Gallenkamp, CR 12/30). Decontaminate by heating at 250°C for 24 h. To pack a column, half-fill it with petroleum spirit, add 10 g of aluminium oxide (Laporte Limited, grade UG) and allow to settle. Add 1 g of anhydrous sodium sulphate to form a layer on top of the aluminium oxide. Drain surplus petroleum spirit until the meniscus just touches the sodium sulphate layer. Retain the petroleum spirit and check that the column is free from interfering impurities by evaporating the petroleum spirit and chromatographing the residue as prescribed (see 'Solvents').

Glassware. For collection of samples, ground-glass stoppered bottles (1.5 l) and jars (250 ml) are suitable for water and sediment, respectively.

Other glassware comprises Soxhlet apparatus with 100-ml boiler; Soxhlet thimbles (glass microfibre, decontaminated by baking at 300°C for 1.5 h); vials, 2 ml; separating funnels, all glass, 250 ml, with graphite-lubricated taps; and conventional equipment.

Before use, decontaminate all glassware by heating at 250°C for 24 h.

Miscellaneous equipment. Vacuum oven; steam bath; t.l.c. tanks, 275 × 125 × 20 mm (Shandon Ltd); air blower; microapplicator (ISCO Model M) fitted with a No. 17 needle bent through 90°; t.l.c. spot drier (see Fig. 1; Uniscience Ltd); air oven settable to 240°C; t.l.c. plate drier (Desaga, No. 14/08/02); spray gun (Camlab No. 0411550); u.v. lamp (Camag No. TL-900) switched to 350 nm and with filter removed.

Sampling

Water. Take river or canal samples from as near mid-stream as possible, at about mid-depth and if possible near a weir or sluice. Fill the 1.5-l glass bottles by immersing neck downwards and then inverting; stopper. For shallow water, use a metal bucket as a scoop; swirl the contents and fill the bottle.

Sediment. Sample into the 250-ml jars with a clean wide spatula or scoop (metal, not plastic). Take several portions of sediment within a few metres of each other. Discard any large stones or debris. Do not sample above the low water level.

Storage of samples. Store water and sediment samples at ambient temperature and analyse them within a month of sampling. On no account filter the water.

Sample pretreatment

Water. Water from rivers, canals and reservoirs often contains suspended solids or sediment. Sometimes it is impracticable to apply the procedure for water directly to this suspension because the particles would interfere in the

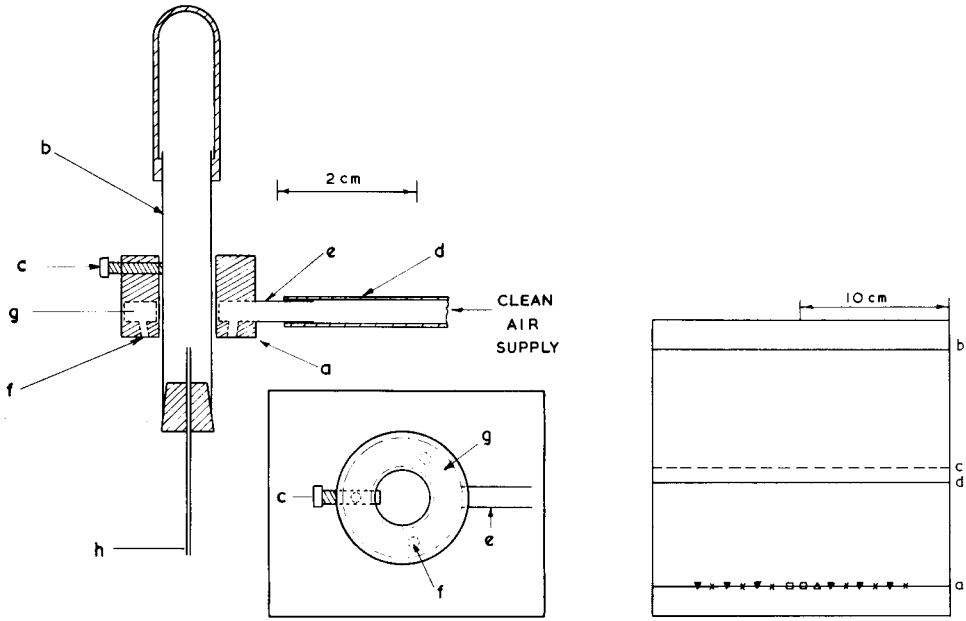


Fig. 1. The t.l.c. spot drier (a) in position on the Shandon micropipette assembly (b), and a plan of the spot drier (inset). The drier is made from Perspex and has a locking screw (c) to adjust its position on the pipette holder; clean air is fed in from 3 mm o.d. silicone rubber tubing (d) to side arm (e) and enters chamber (g); the air exits from three passages (f) which focus on the micropipette tip (h).

Fig. 2. Diagram of silica gel F_{254} t.l.c. plate. (a) Origin line, 20 mm from plate edge; (b) first solvent limit line, 160 mm from origin; (c) "cut line", 80 mm from origin; (d) second solvent limit line 70 mm from origin. (▾) Cereclor S45; (×) Cereclor 42; (□) sample; (△) blank.

solvent extraction stage. Therefore, settling must be allowed, followed by separation of the sedimentary and supernatant liquid layers. The CP contents of these two layers are determined separately and the results are combined, in relation to the amounts of the two layers, to give the overall concentration of CP in the original sample. Treat such water samples as follows: allow the bottle of sample to stand until any suspended solids or sediment has settled. Decant or syphon (glass tubing) off the supernatant water, note the volume and retain 1 l in a glass-stoppered conical flask for subsequent analysis and for the following operation. Pour the sedimentary layer into a tared 250-ml beaker, washing residual traces of sediment into the beaker with a little of the retained supernatant water. Reweigh the beaker to obtain the weight of the sedimentary layer.

Sediment. Thoroughly stir and mix the beaker or sample jar of sediment. Take the beaker of sediment, or take 20 g from the sample jar of sediment in a tared 250-ml beaker, place it in a vacuum oven at 70°C, and dry to constant weight. Calculate the weight of dried sediment.

Extraction of CP from water and sediment

Water. Extract a 100-ml aliquot of the supernatant water sample with 20 ml of petroleum spirit in a 250-ml separating funnel by shaking for a minute. Run the lower, aqueous, phase into a similar separating funnel. Run the organic phase into a 100-ml beaker via a filter funnel containing a 3-g bed of anhydrous sodium sulphate supported by a short plug of silica wool, freshly washed with 50 ml of petroleum spirit before use. Rinse the separating funnel and sodium sulphate bed with a further 5 ml of petroleum spirit and run into the 100-ml beaker. Complete the extraction by repeating this sequence of operations on the aqueous phase in the separating funnel. Discard the aqueous phase. Evaporate the bulked extract to about 5 ml on a steam bath.

At this stage, check for contamination by starting a blank determination in parallel with the sample analysis. Take 50 ml of petroleum spirit and, using duplicate apparatus, treat it in the same way as a sample extract in this and the subsequent stages.

Sediment. Weigh 10.0 ± 0.1 g (or as much as is available up to 10.0 g) of dried sediment into a tared Soxhlet thimble, enclose it with a pad of silica wool and transfer to the Soxhlet apparatus. Extract with about 60 ml of petroleum spirit for 24 h. Transfer the extract to a 100-ml beaker and evaporate to about 5 ml on a steam bath. At this stage, introduce as a blank 60 ml of petroleum spirit and starting with a dummy Soxhlet extraction, using duplicate apparatus, treat it in the same way as a sample extract in this and subsequent stages.

Adsorption chromatography clean-up

Transfer the petroleum spirit concentrate from the evaporation step into a prepared aluminium oxide column using a 10-ml pipette. Slowly run off the excess of solvent until the meniscus just touches the surface of the sodium sulphate plug. Wash the remains of the concentrate from the beaker with two consecutive 5-ml portions of petroleum spirit adding them to the adsorption column, as above. Pass 100 ml of petroleum spirit through the column to remove gross impurities from the CP that remains adsorbed. Discard this eluant. Desorb the CP by eluting with 50 ml of toluene and collect in a 100-ml beaker. Evaporate the toluene eluant on the steam bath, assisting the evaporation with a jet of clean nitrogen, and quantitatively transfer the concentrate to a 2-ml vial, using toluene for washing. Evaporate the concentrate to dryness, on a steam bath, cool, add 0.2 ml of petroleum spirit from a calibrated pipette and swirl to dissolve the residue. Carry out this clean-up stage on the 'blank'.

The t.l.c. separation

Use two t.l.c. tanks; into the first tank pour n-hexane to a depth of 10 mm and into the second pour toluene similarly. Line the ends of the tanks with filter paper wetted with the solvent in the tank. Replace the tank covers and

allow to equilibrate for 30 min. (It is very important that petroleum spirit is not used instead of n-hexane for this stage.)

Prepare a silica gel plate as follows (see Fig 2): mark the plate at lines (a) and (b) with a soft pencil. Rest the plate horizontally, clamp the air blower above it and allow a stream of cold air to play along the origin line.

Water extracts: Apply all the 200 μl of water extract and blank fractions from the adsorption chromatography clean-up stage with the microapplicator as shown in Fig. 2. On the same plate spot 4, 2 and 1-μl aliquots of the standard Cereclor S45 and 42 solutions progressively to the left of the sample spots (Fig. 2) from a 1-μl pipette using the spot drier (Fig. 1) and alternating the spots according to grade. Similarly, spot 8, 12 and 16-μl portions of the standard solutions to the right of the sample spots. With proper use of the spot drier, the spots should not exceed 3-mm diameter. The calibration spots cover the range 50—800 ng of Cereclor.

Sediment extracts: Apply 1-μl, 10-μl and 20-μl spots of sediment extracts and 20 μl of blank extract from the adsorption chromatography clean-up stage to the t.l.c. plate using 1-μl micropipettes and the spot drier. Apply calibration spots as described for water extracts.

For development, transfer the plate to the n-hexane tank and develop to the first solvent limit line (Fig. 2). Remove the plate and dry in the t.l.c. plate drier until no odour of solvent is apparent (ca. 10 min). Cut the plate in half and place the lower half in the toluene tank. Elute up to the second solvent limit line (Fig. 2). Remove the plate and dry as above. Reverse the plate by putting it back in the hexane tank with the cut edge dipping in the hexane. Elute back to the origin line, remove and dry as above.

Detection and measurement of the t.l.c. spots

Take an aluminium oxide half-plate and mark the positions of the origin and solvent limit lines in exactly the same way as for the silica gel half-plate. Clamp the two half-plates face to face with spring clips and 'print' the CP spots onto the aluminium oxide plate by heating the plates at 240°C for 8 min. Cool the half-plates to ambient temperature, unclamp them and discard the silica gel half-plate. Spray the aluminium oxide half-plate evenly with the silver nitrate reagent and then place it under the u.v. lamp for 10 min to develop the chromatogram. Remove the half-plate and inspect it under ordinary light. Any spots from chlorinated compounds will have a grey-to-black colour on a nearly-white background. Identify any CP in the sample by reference to the R_F values which are approximately 0.74 and 0.80 for C_{13}—C_{17} and C_{20}—C_{30} CP, respectively (see Table 1).

Within 30 min (the plate turns grey in strong light) estimate any CP in the sample by visual comparison between the sample and standard spot intensities. (Optionally, the spot intensities can be estimated with a densitometer.) However, if the blank has given a spot or if the plates show other serious interference, reject the results and look for contamination of the materials and equipment, as described below.

If the intensities of the sample spots are above the range of the calibration spots, repeat the t.l.c. procedure using a smaller aliquot of sample extract or a dilution of it as required. Store the half-plate in the dark but for a permanent record, photograph it within 30 min. Do not attempt to estimate the CP from the photograph.

Tracing contamination

Go back stage-by-stage through the method: start at 'Detection and Measurement of the t.l.c. Spots' using fresh plates and without applying spots. If the plates are blank, go back to 'The t.l.c. separation', take fresh plates and simulate the sample extracts using similar volumes of pretested petroleum spirit. Continue until the offending stage is found but watch for cumulative effects of different sources of contamination. Verify that all the relevant decontamination or quality-testing procedures have been done properly. If so, then the trouble should be pinned down by substituting, in turn, fresh items of apparatus and materials taken from different batches. When the contamination has been found, its source(s) can be eliminated considering each step in the handling and storage of the item(s).

Calculation of results

Water. Calculate the concentration (C_W) of CP in the water sample from

$$C_W = 1000\ W_W/V_W\ (\text{ng l}^{-1}) \qquad (1)$$

where V_W (ml) is the volume of water taken (100 ml as prescribed) and W_W is the weight of CP estimated in the t.l.c. spot (ng).

Sediment. Calculate the concentration (C_S) of CP in the sample expressed on a dried basis, from

$$C_S = 200 \times W_S/M_S \times v\ (\mu\text{g kg}^{-1}) \qquad (2)$$

where W_S is the weight of CP estimated in the t.l.c. spot (ng); M_S is the weight of dried sediment taken (g); v is the volume of extract spotted onto the t.l.c. plate (μl).

Water containing suspended sediment. Calculate the concentration expressed in terms of the predetermined concentrations in water and dried sediment by the equation:

$$(V \times C_W + M_{ST} \times C_S)/(M + 1000 \times V)\ (\mu\text{g kg}^{-1}) \qquad (3)$$

where V is the volume of supernatant water in the sample (l); M is the weight of wet sediment in the sample (g), M_{ST} is the total weight of dried sediment (g); C_W and C_S have the meanings above.

DETERMINATION OF TRACE CHLORO-n-PARAFFINS HAVING 13–30 CARBON ATOMS AND CHLORINE CONTENTS OF 42–45% (W/W) IN BIOTA BY THIN-LAYER CHROMATOGRAPHY (METHOD B)

Materials

Solvents. (Additional to those prescribed in Method A.) The grades used were as follows: acetone (Fisons' Distol Grade); dimethylformamide (Fisons AR Grade, No. F1601 or BDH Chemicals No. 27425); cyclohexane (BDH Chemicals No. 27867) redistilled in glass; carbon tetrachloride (Hopkin and Williams 'A Grade', No. 2854) which must be redistilled in glass and only fresh distillate used. Equivalent grades from other suppliers should be equally satisfactory. Dimethylformamide and carbon tetrachloride are toxic and must be handled in a well-ventilated fume cupboard.

For the 60/80 petroleum spirit—acetone used in extracting samples (mixture A) mix 2 volumes of 60/80 petroleum spirit with 1 volume of acetone.

For the 60/80 petroleum spirit—acetone used in chromatography clean-up (mixture B), mix 1 volume of 60/80 petroleum spirit with 4 volumes of acetone.

The solvents must be checked as being free from impurities that interfere with the method as described in Method A.

Sodium sulphate solution (2% w/v). Dissolve 40 g of anhydrous sodium sulphate (see Method A) in distilled water and make up to 2 l. Check the solution for interfering impurities.

Chromatography column packing. Pretreat silica gel (Sorbsil, grade M-60, J. Crosfield and Son, Ltd.) as follows: dry at 120°C overnight, cool and stir in distilled water to give 5% (w/w) of water, continue stirring until the powder is free-flowing. Store in an airtight jar (free from plastic) and use within two days.

Apparatus

The apparatus is as prescribed in Method A with the following exceptions.

Containers for storing samples. Keep eggs (fresh or dried), animal tissues and shellfish in ground-glass stoppered jars (see Method A); keep whole fish in knotted polythene (not PVC) bags a sample of which must be checked as being free from interfering impurities when used in the method described. Keep cod liver oil in the container in which it is supplied, but note whether there is any plastic present which may have contaminated the contents.

Miscellaneous equipment. Separating funnels, all-glass, 1 l, homogeniser (Ilado 10/20 Disperser); rotary evaporator (Büchi Rotavapor-R).

Adsorption chromatography columns. Clean the columns (14 mm bore × 200 mm long fitted with glass sinters and taps) as described in Method A. Pack with 10 g of prepared silica gel as a slurry in 20 ml of petroleum spirit. Allow to settle and continue as for Method A.

Sampling

Put freshly caught fish immediately into polythene bags and knot them. Do not wash or gut. Take shellfish from below water level, obtaining a mixture of individuals from an area of a few square metres. Put them directly into a glass jar, taking care not to contaminate the stopper. Put eggs temporarily into cardboard boxes. If any sample is obtained indirectly, check its history for possible contamination factors. Transport all perishable samples to the laboratory within a day if possible.

Storage of samples. Store fish, shellfish and animal tissues in a deep-freeze. Shell eggs into separate glass jars (see Method A) and store in a deep-freeze. (Whole eggs will burst if put into a deep-freeze. Some wild birds' eggs are so small that it is inconvenient to shell them into separate jars. Therefore it is best to analyse them straightaway.) Keep freeze-dried eggs in a refrigerator at 4°C or lower. Keep cod liver oil and non-perishables unopened at ambient temperature. Analyse samples within a month.

Sample pretreatment

Remove and discard the shells from shellfish and from fresh birds' eggs. Keep the samples separate unless there is less than 10 g of material when samples of the same species taken from the same place can be bulked. Homogenise the sample thoroughly. No pretreatment is necessary for cod liver oil.

Extraction of CP from samples

Weigh 10.0 ± 0.1 g of homogenate and mix with sufficient anhydrous sodium sulphate to produce a granular mass. Transfer to a Soxhlet thimble and extract in the Soxhlet apparatus with 150 ml of solvent mixture A for 6 h. After extraction, evaporate the solvents over a steam bath. Remove water from the sample by co-distillation with 30 ml of cyclohexane. Dissolve the dry residue in boiling petroleum spirit and make up to ca. 100 ml. For cod liver oil, simply weigh 10.0 ± 0.1 g and dissolve in petroleum spirit to give about 100 ml of solution.

Initiate a 'blank' at this stage: apply the above procedure using an empty Soxhlet thimble, or in the case of cod liver oil analysis, take 100 ml of petroleum spirit as the blank.

Liquid—liquid extraction

Separate the CP from the lipids as follows. Transfer the petroleum spirit sample solution to a 250-ml separating funnel and extract the CP with four 30-ml portions of dimethylformamide, running the lower dimethylformamide phase into a lower 250-ml separating funnel after each extraction. The petroleum spirit contains the bulk of the lipids: discard it. Shake the bulked dimethylformamide phase with 30 ml of petroleum spirit to remove traces of lipids. Allow the phases to separate and then run the dimethylformamide phase into a 1-l separating funnel. Shake the petroleum spirit with a further

15 ml of dimethylformamide to retrieve any traces of CP and run the dimethylformamide into the same 1-l separating funnel. Discard the petroleum spirit. Back-extract the CP into petroleum spirit by adding first 300 ml of sodium sulphate solution and then 30 ml of petroleum spirit solution to the dimethylformamide, shaking the mixture well and standing. Run off and discard the lower water—dimethylformamide phase and then run the petroleum spirit phase into a 100-ml beaker via a filter funnel containing a 3-g bed of anhydrous sodium sulphate supported by a short plug of silica wool, which has been previously rinsed with 50 ml of petroleum spirit. Rinse out the separating funnel and sodium sulphate bed with a further 5 ml of petroleum spirit. Finally reduce the petroleum spirit volume to about 5 ml by evaporation on the steam bath.

Adsorption chromatography clean-up

Use a procedure similar to that of Method A to purify the CP further by chromatographing the petroleum spirit concentrate from the liquid—liquid extraction stage on the silica gel column. First elute and discard 100—200 ml of petroleum spirit (which contains mobile impurities) and then desorb the CP from the column by eluting and retaining 200 ml of carbon tetrachloride. Remove the carbon tetrachloride using the rotary evaporator and retain the resulting residue. Dissolve the residue in 1—2 ml of acetone and wash into a 25-ml volumetric flask. Add 1 ml of solvent mixture B and make up to the mark with 2% sodium sulphate solution. Shake the flask well and allow to stand until the petroleum spirit phase separates in the neck of the flask. Sample from this phase in the subsequent t.l.c. procedure.

Preparation of calibration solutions and setting up the t.l.c. apparatus

Prepare the calibration solutions and set up two t.l.c. tanks as described in Method A.

T.l.c. separation, detection, measurement and calculations

Chromatograph 1 μl, 10 μl and 20 μl of the petroleum spirit solution from the adsorption chromatography clean-up alongside the blank and calibration spots, following the n-hexane—toluene elution procedure described in Method A for sediments, and estimate the CP. If the blank is unsatisfactory, reject the results and trace the trouble by procedures analogous to those of Method A: 'Tracing contamination'.

Calculate the concentration of CP in the sample from eqn. (2).

RESULTS AND CONCLUSIONS

The methods use inexpensive commercial equipment for the routine determination of CP in a wide range of environmental materials. Although the methods have been developed for CP containing 42—45% (w/w) of chlorine, they have also been applied successfully to CP containing 50—70% (w/w) of

chlorine. The precision of the methods ranges from ±50% relative at the lowest concentrations to ± 12% relative at the highest.

With regard to sample throughput, a practised analyst would be fully occupied given three simultaneous analyses and in a 40-h, five-day week should therefore average 15 single analyses of water, 10 of sediment or 8 of biota.

The results of some typical recovery tests are shown in Table 2; various types of sample were spiked with Cereclor 42 and Cereclor S45 as indicated in the footnotes. As can be seen, the recoveries vary with sample type, but can be regarded as entirely satisfactory for environmental survey work. A description of such work carried out by one of the co-author companies and a full list of results will be published elsewhere [5].

TABLE 2

The recovery of CP from various materials[a]

Material	Concentration of Cereclor added ($\mu g\ g^{-1}$)		Recovery efficiencies (%)	
	Cereclor 42	Cereclor S45	Cereclor 42	Cereclor S45
Hen egg	0.2, 0.5, 1.0	0.2, 0.5, 1.0	90	90
Cockle (*Cardium* sp.)	1.0, 2.0	1.0, 2.0	80	80
Cod	0.5, 1.0	0.5, 1.0	80	80
Human tissue	0.1, 1.0	0.1, 1.0	90	90
Sediments	0.2	0.2	80	80
Water	0.001	0.001	90	90

[a]Samples were spiked as follows. In each case, the whole sub-sample was used.

Hen eggs. A suitable sub-sample taken from the macerated whole sample was doped by pipetting into it a 1 or 10 $\mu g\ ml^{-1}$ solution of Cereclor in acetonitrile.

Cockle, cod, human tissue. A sub-sample taken from the macerated whole sample was doped by syringing into it a 10 $\mu g\ ml^{-1}$ solution of Cereclor in hexane.

Water. A sample of water from a reservoir shown to contain no CP was doped by syringing into it μl-quantities of a 10 $\mu g\ ml^{-1}$ solution of Cereclor in acetone. The whole of the doped sample was used in the test.

Sediment. A wet sediment was treated with μl-quantities of a 10 $\mu g\ ml^{-1}$ solution Cereclor in acetone.

REFERENCES

1 V. Zitko, J. Chromatogr., 81 (1973) 152.
2 V. Zitko, J. Assoc. Off. Anal. Chem., 57 (1974) 1253.
3 D. Friedman and P. Lombardo, J. Assoc. Off. Anal. Chem., 58 (1975) 703.
4 V. Zitko and E. Arsenault, Fish. Mar. Ser. Res. Dev. Tech. Rep., No. 491, 1974, 38 pp.
5 I. Campbell, G. McConnel, R. Madeley and R. D. N. Birtley, Environ. Sci. Technol., to be published.

Analytica Chimica Acta, 111 (1979) 215—226

COMPARISON OF PRECONCENTRATION PROCEDURES FOR TRACE METALS IN NATURAL WATERS

J. SMITS, J. NELISSEN and R. VAN GRIEKEN*

Department of Chemistry, University of Antwerp (U.I.A.), B-2610 Wilrijk (Belgium)

(Received 29th May 1979)

SUMMARY

The relative merits of eight procedures for preconcentrations of trace metal ions from natural water samples and synthetic solutions are evaluated. Spikes (100 $\mu g\ l^{-1}$) of Mn, Co, Zn, Eu, Cs and Ba and the corresponding radioactive tracers were added to batches of drinking water, estuarine water, sea water, ground water, twice-distilled water and a humic material solution. After equilibration for 2—5 months, the following techniques were applied: passage through columns of Dowex A1 chelating resin and of silylated silica gel, filtration through laminate membrane filters and chelating diethylenetriamine cellulose filters, precipitation with sodium diethyldithiocarbamate and 1-(2-pyridylazo)-2-naphthol, extraction with ammonium pyrrolidinedithiocarbamate, and chelation by 8-quinolinol (oxine) followed by adsorption on activated carbon. The quantitative characteristics of these techniques and the influence of the water matrix effects are discussed, as well as the applicability for x-ray fluorescence analysis.

The concentrations of dissolved trace elements in natural waters are so low that multi-element analytical techniques usually require a prior concentration step, which ideally enriches the transition and heavy metal ions exclusively and efficiently while leaving the abundant alkali and alkaline earth ions in solution. Several procedures have been presented in the literature for combination with x-ray fluorescence (x.r.f.), activation analysis, etc.

In natural waters, colloidal or organic compounds, e.g. humic material and abundant inorganic compounds, may exhibit strong complexation properties for trace metal ions. Little attention has been paid analytically to the influence of such interactions on the preconcentration techniques which are generally developed for trace metal ions and are tested only on synthetic ionic solutions. When, in natural waters, an important fraction of the trace metals is present in strong organic complexes that are difficult to decompose, or is adsorbed on colloids, the behaviour of the metals during the preconcentration step will not be the same as in synthetic solutions and the collection efficiency may vary significantly.

The work described here was aimed at comparisons of the efficiency and reliability of eight preconcentration techniques when applied not only to synthetic solutions but also to water doped with humic material and to natural waters of five different types. The applicability of the techniques in x.r.f. analyses is given particular consideration.

EXPERIMENTAL

Tracers and radioactivity counting

Experiments were carried out with ^{54}Mn, ^{60}Co, ^{65}Zn, ^{137}Cs, ^{133}Ba and $^{152\text{-}154}Eu$, all of high specific activity. For carriers, the following compounds were dissolved in twice-distilled water: $MnCl_2 \cdot 4H_2O$, $CoCl_2 \cdot 6H_2O$, $ZnCl_2$, CsCl, $BaCl_2 \cdot 2H_2O$, Eu_2O_3 and $FeCl_3 \cdot 6H_2O$. The γ-spectrometry was done with a Ge(Li) detector coupled to a 4096-channel analyser and a magnetic tape recorder for off-line computer spectrum evaluation.

Water samples

Twice-distilled water was taken from a quartz distillation unit.

The humic material solution was made by dissolving 52 mg of humic acids (Fluka 53680) in 1 l of 0.1 M sodium hydroxide, diluting to 10 l with twice-distilled water, adjusting the pH to 7 and filtering off the precipitate after an equilibration time of 2 days on a 0.4-μm Nuclepore membrane. The weight of the filter load indicated that 2.5 ppm of humic material remained in the solution.

Tap water, derived from surface water, was taken from the Antwerp drinking water supply. The tap was run for several hours before sampling was done. Typical concentration values are: 2.0 mg TOC l^{-1}, 35 mg Na l^{-1}, 60 mg Ca l^{-1}, 26 μg Fe^{-1}, 150 mg HCO_3^- l^{-1} and 35 μg Zn l^{-1}.

Estuarine water was taken near Valkenisse from the Easter Scheldt, at present an isolated rather unpolluted branch of the sea in the sedimentary Dutch estuarine region. The salinity of the sample was $20^{\circ}/_{\circ\circ}$.

Sea water was collected at a depth of 4 m, 100 m off the sandy beach of Blankenberge, Belgium.

Ground water was sampled from a borehole at Essen, Belgium. It was very rich in humic material and total iron. Typical analyses for this source are: 5.25 mg TOC l^{-1}, 11 mg Na l^{-1}, 60 mg Ca l^{-1}, 7.5 mg Fe l^{-1}, 200 mg HCO_3^- l^{-1} and 60 μg Zn l^{-1}.

All 6 samples were immediately filtered off on Nuclepore membranes (0.4-μm pore). To 10 l of the eluates, 100 ml of a tracer stock solution containing 10 ppm Mn, Co, Zn, Cs, Ba and Eu carriers was added; thus the final solutions each contained 100 ppb of these elements. The samples were left to equilibrate, stored in thoroughly precleaned plastic containers at room temperature for 2—5 months.

Preconcentration techniques

The preconcentration techniques tested were selected either because they are widely used or because they seem quite promising. The procedures were either copied from the original authors or slightly adapted. In all preconcentration experiments, 250-ml aliquots of sample were used.

Collection on Dowex A1 chelating resin. Dowex A1 or Chelex 100 is a chelating resin with iminodiacetate functional groups in a styrene—divinylbenzene copolymer matrix. Its use for trace metal preconcentrations from

sea water was initiated by Riley and Taylor [1] and recommended by Holynska [2]. In the procedure used here, 150 mg of Dowex resin, equilibrated in saturated NaCl solution and washed with a buffer of pH 7, served as the column; the flow rate was below 10 ml min^{-1}, and the solutions were at pH 6—7. The metal uptake capacity amounted to ca. 150 μeq.

Extraction with ammonium pyrrolidinedithiocarbamate (APDC). Marcie [3] suggested exploitation of the multi-element chelating properties of APDC for preconcentrations in combination with x.r.f. To 250 ml of sample were added 5 ml of a pH 5 buffer and 5 ml of a 2% (w/v) APDC solution. After a 5-min equilibration, the transition metals were extracted into three 5-ml portions of chloroform. The capacity was ca. 300 μeq of divalent transition metals.

Precipitation with sodium diethyldithiocarbamate (Na-DDTC). Na-DDTC has mainly been promoted as a broad coprecipitation agent by the work of Luke [4]; it has been used by Watanabe et al. [5] and by Holynska and Bisiniek [6]. To a 250-ml water sample, 20 ml of an aqueous 2% (w/v) Na-DDTC solution and 5 ml of a pH 5 buffer were added. After 10 min, the precipitate was filtered off on a Nuclepore membrane (0.4-μm pore). The coprecipitation capacity was about 900 μeq.

Chelation on silica gel with immobilized bisdithiocarbamate functional groups. Leyden et al. [7] have described pioneering thorough studies on the use of silica gel with various immobilized functional groups. Silylated silica gel with N-β-aminoethyl-γ-aminopropyl functional groups as provided by this author was treated with 100 ml of water, 25 ml of 0.25 M NaOH, 25 ml of 2-propanol and 20 ml of carbon disulphide. Some of the resulting bisdithiocarbamate product was filtered off, washed, dried and placed in a column (7.0 cm high, 0.5 cm i.d.). The preconcentration step consisted simply of passing the water sample at pH 7 through this column at a rate of about 1 ml min^{-1}, while taking care not to exceed the total capacity of ca. 75 μeq.

Filtration through Metrodisc preconcentration filters. Metrodisc filters type 1410 (Environmental Devices Corp., Marion, Ma. 02738) are compact filters containing a layered structure of activated carbon and an unspecified complexing agent [8]. Collection was done simply by filtering 250 ml of water at its natural pH, near pH 7, at a rate of about 0.5 ml min^{-1}. (Initial experiments at the 20-ml min^{-1} flow rate recommended by the authors seemed to yield less satisfactory results, maybe because some solute leaked around the active area of the filter under the higher vacuum.)

Filtration through chelating cellulose filters with diethylenetriamine functional groups (DEN). When DEN groups are introduced on Whatman-41 filters, as described by Smits and Van Grieken [9], the resulting chelating filter papers can be used for simple preconcentrations of cations and anions from natural waters [10]. The preconcentration step consisted of filtering through a 14-cm^2 DEN filter; usually, 250 ml of water at natural pH, containing less transition metals than the 35-μeq capacity was filtered at a rate of 10 ml min^{-1}.

Chelation by oxine and subsequent adsorption on activated carbon. This procedure, developed by Vanderborght and Van Grieken [11], consisted of adjusting a 250-ml water sample to pH 8, adding 25 mg of 8-quinolinol (oxine) dissolved in a minimum volume of acetone, equilibrating for 10 min, adding 100 mg of purified activated carbon, shaking for 1 h and filtering off. The collection capacity is ca. 100 μeq of divalent ions.

Co-crystallization on 1-(2-pyridylazo)-2-naphthol (PAN). Püschel [12] has discussed the co-crystallization of many trace ions on PAN. Vanderstappen and Van Grieken [13] have optimized the preconcentration procedure and studied its characteristics. The procedure consisted of adding 2 ml of a 1% PAN solution in ethanol to 250 ml of water, heating to 80°C, allowing to cool and filtering. A collection capacity of 50—100 μeq is attainable.

RESULTS AND DISCUSSION

Research involving tracer additions to natural water samples leads inevitably to a dilemma. The isotopic exchange process and the exchange between the added and the naturally present elements can be very slow. This has been discussed, e.g. for the case of zinc in sea water, by Bernhard et al. [14]. Therefore the equilibration time should be kept as long as possible. Prolonged storage should be avoided, however, because, especially at low concentration levels, chemical equilibria can shift considerably and chemical speciation can be altered by the activity of living organisms and adsorption/desorption on particles and on the container walls [15]. As a compromise, storage periods were kept to 2—5 months. Also, to reduce bacterial activity and sorption effects on particles, all samples were filtered off immediately after collection. To eliminate the effect of adsorption on the container walls, all recovery yields were calculated by comparing the radioactivity in solution immediately before the experiment to that remaining after the preconcentration step. To avoid the more important uncertainties at lower concentration levels, additions of 100 ppb of carrier were preferred for this preliminary work, although this will be well above the natural levels for several of the ions studied. It should thus be borne in mind that the conclusions below may not all be directly extrapolated to the preconcentration and subsequent analysis of trace metals in natural water. Still, the general trends indicate the analytical characteristics of the different procedures and allow their validation on a comparative basis.

Collection on Dowex A1 chelating resin

The collection yields for cesium(I) were very low in all cases. For barium(II), the recovery was complete from twice-distilled water and water doped with humic material, but low and erratic from the other waters containing more competing dissolved cations. Apparently Dowex A1 has only weak affinity for alkaline earth ions. This was also observed by Van Grieken et al. [16] for filters containing Chelex-100 resin and by Lee et al. [17] who found

TABLE 1

Collection yields for radioactive metal spikes by different procedures for six natural waters

Ion added	Recovery (%)					
	Twice-distilled water	Twice-distilled water doped with humic material	Potable water	Estuarine water	Sea water	Ground water
Collection on Dowex A-1 chelating resin						
Ba^{2+}	96	96	10	21	5	59
Mn^{2+}	91	86	83	48	68	90
Co^{2+}	89	93	92	67	83	89
Zn^{2+}	100	100	92	98	84	100
Eu^{3+}	82	32	68	96	95	100
Extractions with APDC						
Ba^{2+}	0	0	6	10	1	0
Mn^{2+}	11	77	39	62	55	53
Co^{2+}	74	32	44	90	96	97
Zn^{2+}	100	100	82	100	100	100
Eu^{3+}	4	1	87	80	58	100
Co-precipitation on Na-DDTC						
Ba^{2+}	0	0	3	7	3	0
Mn^{2+}	72	95	97	98	97	85
Co^{2+}	99	99	97	98	97	85
Zn^{2+}	100	100	82	91	100	78
Eu^{3+}	6	4	88	93	91	100
Collections on bisdithiocarbamate silica gel						
Ba^{2+}	38	22	3	15	7	0
Mn^{2+}	8	24	4	21	58	15
Co^{2+}	71	78	65	74	80	92
Zn^{2+}	90	90	100	91	100	100
Eu^{3+}	92	81	96	100	97	100
Filtrations on Metrodisc filters						
Ba^{2+}	31	3	—	0	5	11
Mn^{2+}	34	9	—	46	57	36
Co^{2+}	96	85	—	74	79	83
Zn^{2+}	94	74	—	87	94	89
Eu^{3+}	100	31	—	100	97	100
Filtrations on DEN filters						
Ba^{2+}	92	90	7	0	4	4
Mn^{2+}	95	96	39	31	8	38
Co^{2+}	97	88	98	85	84	95
Zn^{2+}	100	100	96	96	84	95
Eu^{3+}	78	63	98	100	95	100

TABLE 1 (continued)

Ion added	Recovery (%)					
	Twice-distilled water	Twice distilled water doped with humic material	Potable water	Estuarine water	Sea water	Ground water
Chelation by oxine and adsorption on activated carbon						
Ba^{2+}	61	10	5	3	7	10
Mn^{2+}	99	97	97	89	89	78
Co^{2+}	100	98	99	97	99	100
Zn^{2+}	100	100	100	84	93	100
Eu^{3+}	97	61	99	100	100	100
Co-crystallization on 1-(2-pyridylazo)-2-naphthol						
Ba^{2+}	41	24	7	0	4	0
Mn^{2+}	100	83	90	100	100	100
Co^{2+}	68	76	59	72	98	65
Zn^{2+}	100	100	95	100	100	100
Eu^{3+}	100	54	99	100	100	100

complete recovery of barium spikes from sea water while using a relatively large resin column.

The collection yields for the transition metals are listed in Table 1. For twice-distilled water the recoveries are rather high in all cases. Under similar conditions, Hirose et al. [18] noted quantitative recoveries for Mn^{2+}, Co^{2+}, Zn^{2+} and Eu^{3+}. The recoveries for twice-distilled water doped with humic material are similar except for the trivalent ions which form particularly stable complexes with humic material [19] and therefore may escape collection by Chelex-100 more easily. However, for the ground water, the europium(III) collection appears to be much higher although this water has a high content of humic material. Possibly an important fraction of the humic matter originally present precipitates with the iron hydroxide after collection and is subsequently removed during the filtration prior to storage, or the humic matter in ground water exhibits lower stability constants or forms more labile complexes with the trivalent metals. For highly saline estuarine water and for sea water, with its low organic content, satisfactorily high collection efficiencies were measured except for manganese(II). For tracers freshly added to sea water, Riley and Taylor [1] and Holynska [2] came to the same conclusion, as did Lee et al. [17] considering sea water that had been kept at pH 1 for several days. Florence and Batley [20] measured very low initial recoveries from sea water of, e.g., zinc in its natural speciation on Chelex resin (H^+-form) although fresh radioactive spikes were recovered fully. It is not clear whether the recoveries for sea water were generally higher in this work because the Na^+-form of Chelex resin was applied, or because natural complexes or colloids had decomposed during the 2—5 months equilibration period.

The Dowex A1 preconcentration procedure is quite simple but rather slow. It does not yield targets directly suitable for x.r.f. analysis. The resulting enrichment factor, defined as the original versus the final sample weight, amounts typically to 1500—2000.

Extraction by ammonium pyrrolidinedithiocarbamate (APDC)

In no case was significant collection of cesium(I) or barium(II) observed. Morris [21] found a 100% recovery of cobalt and zinc ions added to sea water; quantitative recoveries of manganese required a larger excess of APDC. When the same APDC concentration was used with single MIBK extractions, Florence and Batley [20] also observed quantitative extraction into MIBK of ^{65}Zn tracer freshly added to sea water, but only 23—59% recoveries for zinc in its natural form. Pakalns and Farrar [22] reported that the volume of MIBK is particularly critical in the recovery of manganese from distilled water, that the presence of humic material depressed the recovery for cobalt but not for zinc, and that added manganese and zinc ions, but not cobalt, could readily be extracted quantitatively from natural creek water.

For safe preconcentrations from natural waters several successive extractions with a larger volume of APDC appear necessary.

As can be seen in Table 1, the average collections for cobalt are less satisfactory than for zinc and the results for manganese are always erratic and rather low. The recovery of cobalt is poor from the humic material solution. Extremely low recoveries were noted for europium(III), not only in the humic material solution but also in distilled water. For the aged natural waters, the recoveries of cobalt and zinc are generally satisfactory.

For combination with x.r.f., a rather tedious sample preparation step [3] involving evaporation of the chloroform is needed; otherwise the enrichment factors are very low. The procedure was thought to be rather inconvenient and time- and labour-consuming.

Precipitation with sodium diethyldithiocarbamate (Na-DDTC)

As expected, cesium(I) and barium(II) were not collected by Na-DDTC; the dithiocarbamates are known not to form stable complexes with alkali and alkaline earth ions, but only with metal ions that lead to insoluble sulfides [23].

The recoveries for manganese(II) are quite high from all waters. The higher yield compared to the APDC extraction process may be attributed to the larger amount of available dithiocarbamate groups. The collection of manganese is probably due to co-precipitation; Watanabe et al. [5] found that manganese(II) alone does not precipitate satisfactorily with Na-DDTC. Cobalt and zinc ions are efficiently enriched; the results are higher for cobalt ions, as would be expected from the respective stability constants [5]. Luke [4] and Holynska and Bisiniek [6] reported excellent recoveries of divalent transition metals. As in the case of APDC, the collection of europium(III) is very poor from the twice-distilled water and humic matter solution. This low

recovery from waters of low ionic strength was confirmed by additional APDC and Na-DDTC preconcentrations on these solutions. The reasons for this effect are not clear.

Precipitation with Na-DDTC is simple and requires few operations. The target is directly suitable for x.r.f. analysis. For some of the natural waters, filtration of the finely dispersed precipitate was difficult. The enrichment factor, which depends on the metal content of the water, can be very high.

Filtration on silica gel with immobilized bisdithiocarbamate functional groups

No significant collection of cesium(I) was noted; barium(II) was collected to the extent of 20—40% from twice-distilled water and from humic material solution but not significantly from the other samples with higher ionic strength. The significant — although not competitive [7] — recovery of barium ions from solutions of low ionic strength is in contrast to the results discussed above for APDC and Na-DDTC preconcentrations and points either to unreacted diamine groups remaining after the bisdithiocarbamate synthesis or to some decomposition of the bisdithiocarbamate afterwards.

For the Z-6020 bisdithiocarbamate product, Leyden et al. [24] presented few comparable data. Quantitative comparison can be made only in the case of zinc, for which about 95% collection was claimed for batch experiments. In the present work, 90—100% collection was found in column experiments for all water types. For the dithiocarbamate form used at pH 7, Leyden et al. [24] observed poor recoveries for manganese and nearly quantitative collection for cobalt and zinc. The recovery of cobalt was reported to vary over 15% or less with the chloride content and ionic strength of the water [7]. In the present experiments, the recovery of zinc ions was high, essentially independent of the water type and quite reproducible. Manganese was collected poorly in all cases, and the cobalt recoveries were usually not quantitative, as could be expected if an important fraction of the column was in the diamine form [24]. For europium(III), a reasonable collection was achieved, even for the humic material solution. The remaining immobilized diamine functional groups, which are protonated at neutral pH, apparently collect the negatively charged humic material and therefore also the trivalent ions which are strongly complexed by the humic substances [19]. Collection of the humic matter results in distinctive colouring of the column. The procedure gives an enrichment factor around 3000 and the collected material cannot be measured directly by x.r.f. However, the method was judged to be very practical and fast and, as the data in Table 1 indicate, it is readily applicable to variable natural waters.

Filtration through laminate membrane adsorptive filters (Metrodisc 1410)

Kerfoot and Crawford [8] do not explicitly claim quantitative retention of transition and heavy metals by Metrodisc 1410 filters from sea water and other natural water matrices; they preferred standard additions, followed by acid elution and inductively coupled plasma emission spectrometry.

Hence, there are no quantitative data for comparison with the present results.

For cesium(I) and barium(II) the collection efficiencies were negligible except for twice-distilled water. For manganese(II), the recovery is not quantitative and differs for every water type. The collection of cobalt and zinc ions is reasonably high but is influenced somewhat by the ionic strength and humic matter content, respectively, of the water. For europium(III), the recovery is very satisfactory except from the humic material solution. This is probably due to competitive complexation between the chelating agent in the Metrodisc filter and the humic material, or to the significant adsorption of europium onto humic substances and the limited adsorptive capacity of the filter. This problem could probably be reduced, at the expense of the enrichment factor, by reducing the sample volume for waters rich in humic material. This preconcentration procedure is very simple and practical. However, the thickness and size of the Metrodisc filters are not optimal for x.r.f. determinations and the filters clog up quickly in natural water. Under the conditions used the enrichment factor is near 3000.

Filtration through chelating diethylenetriamine cellulose filters (DEN)

Cesium(I) is not collected by DEN filters, nor are the other alkali ions [10]. The barium tracer is only picked up from the twice-distilled water and humic material solution where the competition from other ions for the available sites is weak. Manganese(II) is quantitatively collected from these two water samples but not from the other samples. The influence of other ions and organic compounds on the collection of manganese by DEN filters is complex and puzzling. For quantitative uptake from simulated drinking water, a pH above 9 is necessary [10]. The collection of cobalt and zinc ions is complete or high for all samples. The effect of high ionic strength and humic matter content is minimal [10].

Europium(III) is quantitatively collected from all the natural water samples studied. The high charge of the trivalent cation apparently leads to more difficult collection from aqueous samples with few counter ions, as in the distilled water and humic matter solutions [25].

This extremely simple technique, consisting of a straightforward filtration at natural pH without any reagent addition, collects Co^{2+}, Zn^{2+}, Eu^{3+} and most other transition metals [10] from natural water samples. It yields an enrichment factor of typically 2000. The thin homogeneously loaded sample is very suitable for x.r.f. analysis. A major drawback is that the DEN filters are not commercially available.

Chelation by oxine and subsequent adsorption on activated carbon

Again cesium(I) and barium(II) were only partially collected from the distilled water. Vanderborght et al. [11, 26, 27] claimed recovery yields around 95% for Mn, Co and Zn at pH 7, independent of the sample salinity or hardness. These points are confirmed by the present results. Preliminary experi-

ments done after a 1-month equilibration time on aliquots of the same solutions also showed complete and reproducible collections. The lower values in Table 1 for manganese(II) in ground water and zinc(II) in estuarine water were not confirmed then and are probably artefacts.

The low recovery of the europium(III) in the humic material solution is not unexpected: Vanderborght and Van Grieken [26] observed a 23% recovery only for europium from a 2.5-ppm humic matter solution when 10 mg of activated carbon per 100 ml was used but complete recoveries for 60 mg of activated carbon. The present results obtained with 40 mg of activated carbon per 100 ml are intermediate. The incomplete collection can be attributed to the high stability of complexes between trivalent ions and humic matter, even compared with the oxinates, and to the competitive adsorption of the humic matter onto the activated carbon.

In general, this procedure gives reproducible quantitative recoveries, independent of the type of natural water, for divalent ions, and, if the humic matter content is low or if a large excess of activated carbon can be used, also for trivalent ions. Usually enrichment factors around 10000 are feasible. The loaded filters are quite suitable for x.r.f. analysis. The procedure involves two operations and is less simple than some of the other procedures. However, because it also collects naturally occurring organic metal complexes and colloids (at least up to its capacity of ca. 100 μeq per 100 mg of activated carbon), it appears very appropriate for preconcentrations from most natural waters.

Co-crystallization on 1-(2-pyridylazo)-2-naphthol (PAN)

At pH 9.5, there is no significant collection of cesium(I). Some collection of barium was observed only in the synthetic solutions of low ionic strength. Except for the humic material solution, the recoveries of Mn^{2+}, Zn^{2+}, and Eu^{3+} were nearly always at 95% or above. This agrees with earlier results [13]; complete collections and recoveries decreased by 15% for the divalent ions and more for trivalent ions in the presence of 2 ppm humic material were reported. Zinc is almost quantitatively collected by PAN from river waters [5, 13]. The collection of cobalt was not quantitative except for the sea water; the influence of counter ions is beneficial in this case. Preliminary experiments carried out after a 1-month equilibration yielded reproducible identical results, except for the humic material solution where losses were more severe. In general, this simple procedure seems directly applicable to most waters including sea water; it yields targets that are suitable for x.r.f. and enrichment factors of 15000 or more.

CONCLUSIONS

Any conclusions from the above results must be considered as tentative only, in view of the complexity of trace element behaviour in natural waters, and in view of the uncertainties in experiments involving equilibration of tracers and excess carrier with natural substances during prolonged storage.

The few trace elements involved may not be representative of the overall capabilities of the preconcentration procedures. From the available results, however, it seems that chelation by oxine and adsorption on activated carbon gives the highest and most reproducible collection yields for all the waters and elements studied, followed by DDTC and PAN precipitation; the results of APDC extractions were least satisfactory. If one considers the average variation of the collection yields from the different water samples studied, then filtration through silylated silica gel and oxine chelation followed by activated carbon adsorption are least influenced by the water characteristics, while APDC extraction and DDTC precipitation suffer most. On average, it appeared that zinc is most easily collected, followed by Co, Eu and Mn.

As far as simplicity is concerned, DEN and Metrodisc filters and silylated silica gel columns are most satisfactory. These methods do not require any previous addition of chemicals, and can even be used for on-line collections at the natural pH. In view of its higher speed, preconcentration on silylated silica gel columns is more suitable for field work. APDC extractions were found to be the most tedious.

Targets that are ideally suitable for x.r.f. analysis are obtained from the use of DEN filters, DDTC and PAN precipitation, and oxine chelation — activated carbon adsorption. PAN and DDTC precipitation generally gives the highest enrichment factor, and since the detection limit in x.r.f. is roughly proportional to the target thickness, these techniques will allow the lowest x.r.f. detection limits.

These results give some indication of the relative merits of the different preconcentration procedures. Yet, the selection of an optimal preconcentration procedure will always depend on many parameters, and the quantitative character of the method chosen must be tested separately for every application.

REFERENCES

1 J. P. Riley and D. Taylor, Anal. Chim. Acta, 40 (1968) 479.
2 B. Holynska, Radiochem. Radioanal. Lett., 17 (1974) 313.
3 F. J. Marcie, Environ. Sci. Technol., 1 (1967) 164.
4 C. L. Luke, Anal. Chim. Acta, 41 (1968) 237.
5 H. Watanabe, S. Berman and D. S. Russell, Talanta, 19 (1972) 1363.
6 B. Holynska and K. Bisiniek, J. Radioanal. Chem., 31 (1976) 159.
7 D. E. Leyden, G. H. Luttrell, A. E. Sloan and N. J. De Angelis, Anal. Chim. Acta, 84 (1976) 97.
8 W. B. Kerfoot and R. L. Crawford, ICP Inf. Newsl., 2 (1977) 289.
9 J. Smits and R. Van Grieken, Angew. Makromol. Chem., 72 (1978) 105.
10 J. Smits, Bepaling van sporenelementen in water, Ph.D. dissertation, University of Antwerp, 1979, 218 pp.
11 B. M. Vanderborght and R. E. Van Grieken, Anal. Chem., 49 (1977) 311.
12 R. Püschel, Talanta, 16 (1969) 351.
13 M. G. Vanderstappen and R. E. Van Grieken, Talanta, 25 (1978) 653.
14 M. Bernhard, E. D. Goldberg and A. Piro, in E. D. Goldberg (Ed.), Report of the Dahlem Workshop on the Nature of Seawater, Abakon Verlaggesellschaft, Berlin, 1975, p. 43.

15 G. E. Batley and D. Gardner, Water Res., 11 (1977) 745.
16 R. E. Van Grieken, C. M. Bresseleers and B. M. Vanderborght, Anal. Chem., 49 (1977) 1326.
17 C. Lee, N. B. Kim, I. C. Lee and K. S. Chung, Talanta, 24 (1977) 241.
18 A. Hirose, K. Kobori and D. Ishii, Anal. Chim. Acta, 97 (1978) 303.
19 B. Kribek, J. Kaigl and V. Oruzinsky, Chem. Geol., 19 (1977) 73.
20 T. M. Florence and G. E. Batley, Talanta, 23 (1976) 179.
21 A. W. Morris, Anal. Chim. Acta, 42 (1968) 397.
22 P. Pakalns and Y. J. Farrar, Water Res., 11 (1977) 145.
23 A. Hulanicki, Talanta, 14 (1967) 1371.
24 D. E. Leyden and G. H. Luttrell, Anal. Chem., 47 (1975) 1612.
25 J. Dingman, Jr., S. Siggia, C. Barton and K. B. Hiscock, Anal. Chem., 44 (1972) 1351.
26 B. M. Vanderborght and R. E. Van Grieken, Int. J. Environ. Anal. Chem., 5 (1978) 221.
27 B. Vanderborght, J. Verbeeck and R. E. Van Grieken, Bull. Soc. Chim. Belg., 86 (1977) 23.

Analytica Chimica Acta, 111 (1979) 227—234

ANALYSIS OF THE COMPONENT ALDEHYDE AND ALCOHOLS IN BOROHYDRIDE-REDUCED DIALDEHYDES FROM HEXOSAMINE DERIVATIVES†

SUSUMU HONDA*, KAZUAKI KAKEHI and KAZUNORI MUKAI

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae, Higashi-osaka (Japan)

(Received 30th May 1979)

SUMMARY

Unlike the dialdehydes formed from neutral monosaccharide residues, the dialdehydes from hexosamine residues are resistant to acidified ethanethiol, leaving the hemiacetal bond intact. However, the borohydride-reduced dialdehydes are readily split into the component aldehyde and alcohols; gas chromatographic analysis of their derivatives is useful for structural studies of hexosamine-containing carbohydrates. Investigation by these sequential derivatization reactions indicated that anomeric methyl 2-acetamido-2-deoxy-D-glucopyranosides were oxidized in normal Malapradian fashion, whereas 2-acetamido-2-deoxy-D-glucose was over-oxidized. The rate of oxidation of 2-acetamido-D-glucitol was very rapid; the oxidation product can also be analyzed by the direct dithioacetal method (mercaptalation followed by trimethylsilylation).

The structures and the proportions of the aldehydes in the dialdehydes formed in the periodate oxidation of carbohydrates depend on the structures of the carbohydrate chains involved. A convenient gas chromatographic method [1—3] for the simultaneous determination of these aldehydes as trimethylsilated dithioacetals was applied successfully to the determination of the positions of attachment of the glycosidic linkages in oligosacharide glycosides from plants [4]. However, there are some difficulties in applying this method to the oxidation products of hexosamine-containing carbohydrates. This paper describes a solution to this problem and discusses the mode of oxidation of model hexosamine derivatives on the basis of the results obtained.

EXPERIMENTAL

Chemicals

All chemicals were of the highest grade commercially available. Pyridine, dehydrated by heating under reflux with barium oxide, was distilled before use. The samples of 2-acetamido-2-deoxy-D-glucose [5], and methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides [6, 7] were synthesized

†Part 15 of the Series: Periodate Oxidation Analysis of Carbohydrates. Part 14: J. Chromatogr., 177 (1979) 109.

by published methods. 2-Acetamido-2-deoxy-L-glyceraldehyde was prepared by oxidation of 2-acetamido-2-deoxy-D-glucitol with 0.05 M sodium metaperiodate for 24 h at 25°C, followed by deionization of the reaction mixture. 2-Acetamido-2-deoxyglycerol was prepared by reduction of 2-acetamido-2-deoxy-L-glyceraldehyde with an excess of sodium borohydride in water, followed by removal of boric acid by repeated evaporation of the methanolic solution of the decationized product. All the synthetic samples of hexosamine derivatives and 2-acetamido-2-deoxyglycerol gave single gas chromatographic peaks for their trimethylsilyl derivatives. The trimethylsilylated dithioacetal of 2-acetamido-2-deoxy-L-glyceraldehyde gave a single peak when analyzed by the dithioacetal method [1].

Apparatus

Gas chromatography was performed on a Shimadzu 4BMPF instrument equipped with a hydrogen flame ionization detector. A glass column (0.3 cm i.d., 2 m long) packed with Chromosorb W (AW-DMCS) coated with 3% silicone OV-1 was used; the carrier gas flow rate (nitrogen) was regulated at 50 ml min^{-1}. A gradient of 100—250°C (5°C min^{-1}) was applied after the injection of each sample. Peaks were integrated by a Shimadzu ITG-2A integrator.

Oxidation of hexosamine derivatives with periodate

Equal volumes of 0.01 M aqueous solution of a hexosamine derivative and 0.1 M sodium metaperiodate were mixed, and the mixture was kept at 25°C in darkness. For direct application of the dithioacetal method, a 100-μl aliquot was removed at intervals, and deionized by passage through a column of Amberlite CG-120 (H^+, 0.5 ml) and Amberlite CG-400 (OAc^-, 0.5 ml). The combined eluate and washing fluids (20 ml) were concentrated to a small volume, transferred to a small reaction tube (0.5 cm i.d., 5 cm), and evaporated to dryness. For reduction with borohydride, a 500-μl aliquot was deionized in the same manner, and the combined eluate and washing fluids were concentrated to 5 ml. The amounts of periodate reduced were determined as described by Fleury and Lange [8].

Reduction of the periodate-oxidized hexosamine derivatives with borohydride

To the concentrate obtained above was added sodium borohydride (10 mg), and the resultant solution was kept at 25°C overnight. The solution, acidified with acetic acid, was passed through a column of Amberlite CG-120 (H^+ form, 2 ml). The combined eluate and washing fluids (20 ml) were evaporated to dryness, the residual syrup was dissolved in a small volume of methanol, and the solution was evaporated to dryness. This process was repeated several times to eliminate boric acid. The syrup finally obtained was dissolved in aqueous acetone to make the volume to 1.00 ml, and a 200-μl aliquot was evaporated in a small reaction tube. The residue was subjected to component analysis.

Procedure for component analysis by the dithioacetal method

This procedure was essentially as described previously [3]. A mixture (2 + 1; 20 μl) of ethanethiol and trifluoroacetic acid was added to the residue of a periodate-oxidized (or periodate-oxidized and borohydride-reduced) hexosamine derivative contained in a reaction tube; the tube was closed tightly with a polyethylene stopper, the sample was dissolved by gentle swirling, and the solution was kept for 10 min at 25°C. A solution (50 μl) of D-xylitol (internal standard) in pyridine was added, followed by hexamethyldisilazane (100 μl) and chlorotrimethylsilane (50 μl). The mixture was incubated for 30 min at 50°C and centrifuged; a 1-μl sample of the supernatant solution was injected into the gas chromatography column.

The component alcohols in the oxidized and reduced hexosamine derivatives, as well as the remaining hexosamine derivatives, were converted to their trimethylsilyl derivatives, whereas 2-acetamido-2-deoxy-D-glyceraldehyde in the oxidized and reduced hexosaminide, and its enantiomorph in the oxidized hexosaminitol, were converted to their trimethylsilylated diethyldithioacetal derivatives. The retention times and molar response factors of the peaks of these derivatives, relative to the trimethylsilylated D-xylitol, were as follows: 2-acetamido-2-deoxy-D-glucose, 1.41 and 0.93; methyl-2-acetamido-2-deoxy-α-D-glucopyranoside, 1.38 and 0.63; methyl-2-acetamido-2-deoxy-β-D-glucopyranoside, 1.34 and 0.63; 2-acetamido-2-deoxy-D-glucitol, 1.39 and 0.93; glycerol, 0.40 and 0.63; 2-acetamido-2-deoxy-glycerol, 0.66 and 0.41; 2-acetamido-2-deoxy-L-glyceraldehyde, 1.07 and 0.64. The trimethylsilylated diethyldithioacetal of the dialdehyde from methyl-2-acetamido-2-deoxy-α-D-glucopyranoside gave a peak at a relative retention time of 1.78 with a molar response factor of 0.42.

The yields of the component aldehyde and alcohols were corrected by the average recoveries of the oxidized or oxidized and reduced hexosamine derivatives.

RESULTS AND DISCUSSION

In a previous paper [9] it was reported that methyl-α-D-glucopyranoside was oxidized with periodate initially at the C-3—C-4 bond, and finally at both the C-2—C-3 and C-3—C-4 bonds, to yield a dialdehyde composed of D-glyceraldehyde and glyoxal. These compounds were converted quantitatively to the trimethylsilylated diethyldithioacetal and the bis(diethyldithioacetal) derivatives, respectively, by the dithioacetal method [1—3], and the derivatives were detected by gas chromatography as peaks 3 and 6, respectively (Fig. 1a). In contrast, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was oxidized only at the C-3—C-4 bond to give a dialdehyde composed of D-glyceraldehyde and 2-acetamidomalonaldehyde. Replacement of the hydroxyl group at C-2 by the acetamido group resulted in stabilization of the acetal bond, which remained almost intact under these conditions. The resultant trimethylsilylated dithioacetal derivative of the dialdehyde was

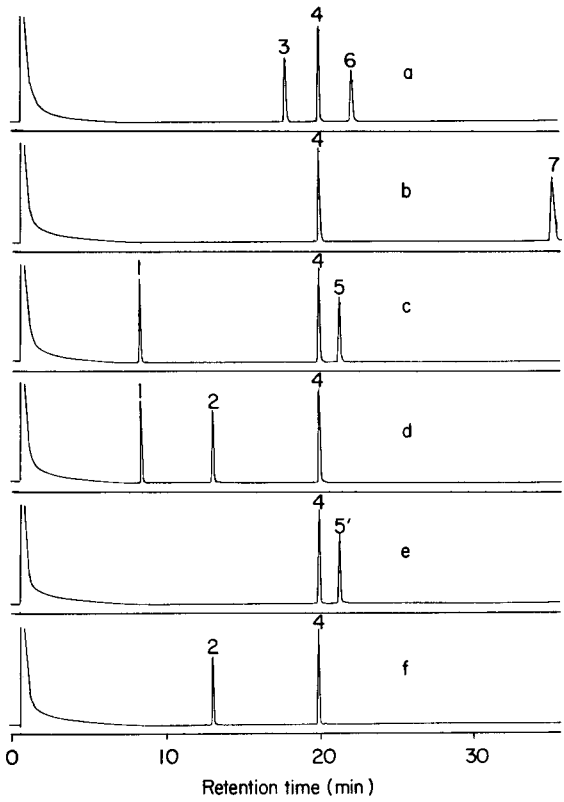


Fig. 1. Gas chromatograms obtained by applying the dithioacetal method to: (a) the dialdehyde from methyl-α-D-glucopyranoside [9]; (b) the dialdehyde from methyl 2-acetamido 2-deoxy-α-D-glucopyranoside; (c) the borohydride-reduced dialdehyde from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside; (d) the borohydride-reduced dialdehyde from 2-acetamido-2-deoxy-D-glucose; (e) the product of periodate oxidation of 2-acetamido-2-deoxy-D-glucitol; and (f) the product of borohydride reduction of (e). Peak assignments: (1) trimethylsilylated glycerol; (2) trimethylsilylated 2-acetamido-2-deoxy-glycerol; (3) trimethylsilylated D-glyceraldehyde diethyldithioacetal; (4) trimethylsilylated D-xylitol (internal standard); (5) trimethylsilylated 2-acetamido-2-deoxy-D-glyceraldehyde diethyldithioacetal; (5′) the enantiomorph of 5; (6) glyoxal bis(diethyldithioacetal); (7) trimethylsilylated diethyldithioacetal of the dialdehyde from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside.

eluted slowly, giving peak 7 (Fig. 1b). The borohydride-reduced dialdehyde was readily split into its components, however, and trimethylsilylated glycerol and trimethylsilylated 2-acetamido-2-deoxy-D-glyceraldehyde diethyldithioacetal were detected as peaks 1 and 5, respectively (Fig. 1c). The retention time of the latter peak was almost identical with that of peak 5′ from its enantiomorph, produced by sequential mercaptalation and trimethylsilylation on the periodate-oxidized 2-acetamido-2-deoxy-D-glucitol (Fig. 1e).

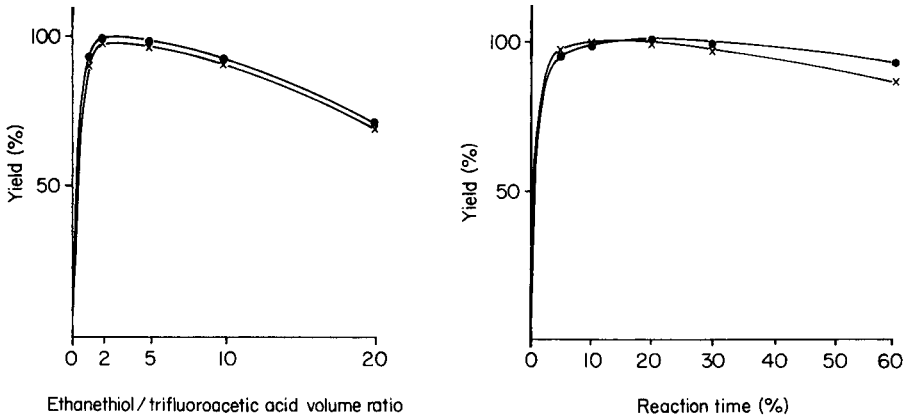


Fig. 2. Effect of the volume ratio of ethanethiol to trifluoroacetic acid on the mercaptalation of the borohydride-reduced dialdehyde from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside. (X) trimethylsilylated glycerol; (•) trimethylsilylated 2-acetamido-2-deoxy-D-glyceraldehyde diethyldithioacetal.

Fig. 3. Course of mercaptalation of the borohydride-reduced dialdehyde from methyl 2-acetamido-2-deoxy-α-D-glucopyranoside with a 2 + 1 mixture of ethanethiol and trifluoroacetic acid at 25°C. (X) trimethylsilylated glycerol; (•) trimethylsilylated 2-acetamido-2-deoxy-D-glyceraldehyde diethyldithioacetal.

Figure 2 shows the effect of the volume ratio of ethanethiol to trifluoroacetic acid (catalyst) on the yields of the derivatives of glycerol and 2-acetamido-2-deoxy-D-glyceraldehyde from the reduced dialdehyde; the maximal yield was obtained for the volume ratio of 2:1. Figure 3 shows the course of mercaptalation of the reduced dialdehyde with a 2 + 1 mixture of ethanethiol and trifluoroacetic acid at 25°C. Approximately quantitative conversion was observed for reaction times between 5 and 20 min; the optimum conditions were almost the same as those reported for the rapid analysis of the periodate oxidation products of glycoproteins [3].

As the above conditions allow the simultaneous determination of the component aldehyde and alcohol in the reduced dialdehyde, the course of periodate oxidation of methyl 2-acetamido-2-deoxy-α-D-glucopyranoside was followed by determining these compounds at intervals. In an unbuffered medium, the yields of the derivatives of glycerol and 2-acetamido-2-deoxy-D-glyceraldehyde increased rapidly to reach the theoretical value in 5 h (Fig. 4a) but decreased slowly thereafter, although the values after 24 h were more than 90% of the theoretical values. The corresponding decrease of the starting hexosaminide in 5 h indicates normal Malapradian oxidation as reported [10] on the basis of periodate reduction. The present values for the reduction of periodate (1.32 mole per mole of the hexosaminide after 5 h; 1.34 mole per mole of the hexosaminide after 24 h) were higher than those reported earlier [10], probably because of non-specific oxidations in this un-

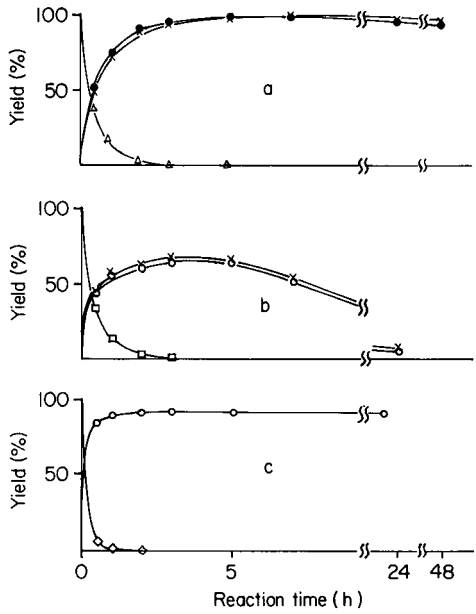


Fig. 4. Course of periodate oxidation of (a) methyl 2-acetamido-2-deoxy-α-D-glucopyranoside; (b) 2-acetamido-2-deoxy-D-glucose; and (c) 2-acetamido-2-deoxy-D-glucitol. (•) Trimethylsilylated 2-acetamido-2-deoxy-D-glyceraldehyde diethyldithioacetal; (×) trimethylsilylated glycerol; (○) trimethylsilylated 2-acetamido-2-deoxyglycerol; (△) trimethylsilylated methyl 2-acetamido-2-deoxy-α-D-glucopyranoside; (□) trimethylsilylated 2-acetamido-2-deoxy-D-glucose; (◇) trimethylsilylated 2-acetamido-2-deoxy-D-glucitol.

buffered reaction medium. The β-anomer of this hexosaminide gave a similar result, though the reaction rate was slightly lower. As the formation of 2-acetamido-2-deoxy-D-glyceraldehyde is characteristic of unsubstituted *N*-acetylhexosaminide residues, examination of the chromatographic peak given by its derivative serves to estimate the hexosamine residues at the non-reducing end of glycolipids and glycopeptides. *N*-Acetylhexosaminide residues in which the hydroxyl group at C-6 is substituted by an oxidizable aldosyl group may also give these peaks, but such carbohydrate chains are rarely found in nature.

The gas chromatogram for the borohydride-reduced dialdehyde from 2-acetamido-2-deoxy-D-glucose (Fig. 1d) gave the trimethylsilylated glycerol peak (peak 1) and also peak 2, assignable to trimethylsilylated 2-acetamido-2-deoxyglycerol as its retention time was identical with that of the trimethylsilyl derivative of periodate-oxidized, borohydride-reduced 2-acetamido-2-deoxy-D-glucitol. The same chromatogram was obtained when the mercaptalation process was omitted from these sequential derivatization reactions. These results are reasonable, as these component alcohols arise by reductive cleavage of the hemiacetal bond in the dialdehyde, but not by

hydrolytic nor mercaptolytic cleavage of this bond. At an early stage of oxidation, the derivative of the residual hexosamine was also detected. A study of the course of oxidation indicated that the maximal yield of the derivative of glycerol was only ca. 70% of the theoretical value, and that of 2-acetamido-2-deoxyglycerol was a little lower (Fig. 4b). These derivatives decomposed rapidly after 3 h, and their yields were less than 10% after 24 h, indicative of rapid hydrolysis of the hemiacetal bond in the dialdehyde and subsequent over-oxidation of the resultant oxy—oxo compound. The amount of periodate consumed after 24 h was 4.88 mol per mole of the hexosamine derivative, consistent with the result reported [11]. The mode of oxidation observed for this hexosamine derivative is also expected for the hexosamine residues at the reducing ends of carbohydrate chains; for such residues the hydroxyl groups at C-6 are glycosylated.

These results suggest that the derivative peak for the unique component aldehyde, 2-acetamido-2-deoxy-D-glyceraldehyde, may arise from the hexosaminide residues at the non-reducing ends and the interior hexosaminide residues in which the hydroxyl groups at C-6 are substituted by oxidizable aldosyl groups, by sequential borohydride reduction, mercaptalation, and trimethylsilylation reactions of the products of periodate oxidation of hexosamine-containing carbohydrates for 24 h. Other hexosamine residues are either immune to periodate oxidation or are decomposed almost completely under the same conditions.

In relation to the analytical studies of the products from a hexosaminide and a hexosamine derivative, the products from 2-acetamido-2-deoxy-D-glucitol were also analyzed. For the periodate-oxidized hexosaminitol, the dithioacetal method could be applied directly to obtain peak 5′ given by trimethylsilylated 2-acetamido-2-deoxy-L-glyceraldehyde (Fig. 1e). When the oxidized hexosaminitol was reduced, and analyzed by the same method (or merely trimethylsilylated) the derivative of 2-acetamido-2-deoxyglycerol (peak 2) appeared at a shorter retention time (Fig. 1f). Figure 4c shows the course of periodate oxidation of 2-acetamido-2-deoxy-D-glucitol, as observed from the yields of the derivatives of glycerol and 2-acetamido-2-deoxyglycerol. The reaction was very rapid, and the product of oxidation was stable for at least 24 h. The shift of the peak, corresponding to the change of the derivative from trimethylsilylated 2-acetamido-2-deoxy-L-glyceraldehyde to -glycerol by reduction with borohydride is characteristic of hexosaminitol residues which are glycosylated at C-6. Examination of this shift for borohydride-reduced carbohydrate materials may be useful for determining the position of substitution of the hexosamine residue at the reducing end.

REFERENCES

1 S. Honda, Y. Fukuhara and K. Kakehi, Anal. Chem., 50 (1978) 55.
2 S. Honda, Y. Ohkaru and K. Kakehi, Anal. Chim. Acta, 98 (1978) 85.

3 S. Honda, Y. Takai and K. Kakehi, Anal. Chim. Acta, 105 (1979) 153.
4 S. Honda, K. Takeda and K. Kakehi, Carbohydr. Res., 73 (1979) 135.
5 D. Horton and M. L. Wolfrom, J. Org. Chem., 27 (1962) 1974.
6 K. Freudenberg, H. Eich, C. Knoevenagel and M. Westphal, Ber., 73B (1940) 441.
7 R. Kuhn and W. Kirschenlor, Chem. Ber., 86 (1953) 1331.
8 P. F. Fleury and J. Lange, J. Pharm. Clin., 17 (1933) 107.
9 S. Honda, N. Hamajima and K. Kakehi, Carbohydr. Res., 68 (1979) 77.
10 R. W. Jeanloz and E. Forchielli, J. Biol. Chem., 188 (1951) 361.
11 S. A. Barker, A. B. Foster, M. Stacey and J. M. Webber, J. Chem. Soc., (1958) 2218.

Analytica Chimica Acta, 111 (1979) 235—241

GEL PERMEATION CHROMATOGRAPHY OF OXYGENATED COMPONENTS OF CIGARETTE SMOKE CONDENSATE†

W. J. CHAMBERLAIN*, M. E. SNOOK, J. L. BAKER and O. T. CHORTYK

Tobacco Laboratory, Science and Education Administration/Agricultural Research, U.S. Department of Agriculture, P.O. Box 5677, Athens, Georgia 30604 (U.S.A.)

(Received 23rd April 1979)

SUMMARY

Oxygenated neutral constituents of a tumor-inhibiting fraction of cigarette smoke condensate were separated from interfering phenolic compounds by a combination of silicic acid and gel chromatography. Silicic acid adsorption and Bio-Beads gel chromatography separated aliphatic from aromatic ketones, and gel chromatography on Sephadex LH-20 resolved aromatic ketones from phenols. Identifications were achieved by studying individual fractions by g.c. and g.c.—m.s.

Neutral oxygenated compounds have been characterized in a recent review as major contributors to the overall flavor and aroma of cigarette smoke [1]. A fraction of cigarette smoke condensate (CSC) containing these compounds and showing tumor-inhibiting properties has been examined [2]; ketones were one of the major classes of compounds present [3]. Characterization of these ketones and other constituents of this fraction has been undertaken to obtain further information on its tumor-inhibiting properties. The development of a chromatographic method for isolating a purified ketone fraction free of phenols and sterols is reported here; a gel chromatographic step separated the aromatic ketones from the phenols and facilitated the identification of both classes of compounds.

EXPERIMENTAL

An ether-soluble neutral (ESN) fraction from 1 kg of cigarette smoke condensate (CSC) was obtained as previously described [4, 5]. This fraction represented 11% of the original condensate weight. One half of this material (54 g) was chromatographed in two portions on methanol-washed, activated silicic acid (150°C for 17 h, 750 g) in a large glass column (6 × 85 cm). The column was eluted successively with different mixtures of diethyl ether and petroleum ether (E, PE). Fraction A (0.6 g) was eluted with 5% E—95% PE

†Presented in part at the 30th Southeastern Regional Meeting of the American Chemical Society, Savannah, Georgia, November 1978.

(5 l); fraction B (10.1 g) with 10% E—90% PE (7 l); fraction C (34.4 g) with 50% E—50% PE (8 l); and fraction D (8.4 g) with 100% diethyl ether (10 l).

Fractions A and B were then chromatographed on a 4-column system (four 1.25 × 109-cm columns of Bio-Beads SX-12 gels) with benzene at a flow rate of 120 ml h^{-1}. The eluate was collected in 8-ml fractions and monitored at 280 nm.

Chromatography of the gel fractions from fraction B on Sephadex LH-20 was performed on a single column (1.25 × 58 cm) with chloroform as eluting solvent at a flow rate of 120 ml h^{-1}. The eluate was collected in 5-ml fractions and monitored at 254 nm.

Analytical gas chromatography (g.c.) was conducted with a Varian 2800 flame ionization instrument; preparative g.c. for sample collection was performed on a Varian 2700 instrument. Both instruments were equipped with glass columns (2-mm i.d., 1.8-m long) containing 6% OV-17 on Chromosorb G-HP, 100—120 mesh; the temperature program was 90—250°C at 2° min^{-1} (20 ml min^{-1} He; injector 290°C; detector, 350°C). Confirmatory identifications were made with a Hewlett-Packard 5930A mass spectrometer—5700A g.c. system containing an identical column. U.v. spectra were obtained on a Beckman Acta CIII spectrophotometer. Individual g.c. peaks were collected in capillary tubes and washed into microcuvettes (3 mm × 1 cm) with 300 μl of cyclohexane.

RESULTS AND DISCUSSION

The complete separation scheme for the ether-soluble neutral (ESN) fraction is given in Fig. 1. CSC dissolved in diethyl ether was first extracted with aqueous sodium hydroxide and dilute hydrochloric acid to separate acids and bases. The remaining "neutral fraction" was placed on a silicic acid column and eluted with the solvents shown. The fraction eluted with ethyl ether was investigated. In an initial study, this ESN fraction was directly subjected to gel chromatography on Bio-Beads SX-8, and the individual gel fractions were characterized by g.c. [3]. However, phenolic compounds were eluted with the ketones of interest and interfered with identification. Apparently the phenols were carried over into the neutral fraction during the original acid—base fractionation step. The presence of alkylated phenolics in a neutral fraction has been documented [6].

To eliminate such interference, the ESN fraction was rechromatographed on silicic acid with mixtures of ethyl ether and petroleum ether. Fractions A and B, containing the ketones, were further fractionated by gel filtration on Bio-Beads SX-12. Figure 2 shows that the bulk of the weight of fraction A was recovered in gel fractions (GF) 32—40 which accounted for much of the u.v. absorbance. Individual gel fractions were analyzed by g.c. on an OV-17 column. In preparative g.c., compounds corresponding to individual peaks were collected in glass capillary tubes and washed into cuvettes with cyclohexane for u.v. spectral analysis. A combination of the g.c., u.v., and m.s. data

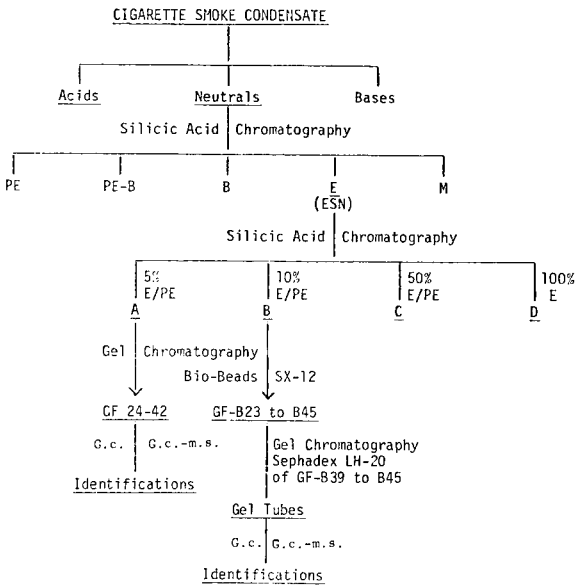


Fig. 1. Separation scheme for constituents of the ether-soluble neutral (ESN) fraction (PE, petroleum ether; E, ethyl ether; B, benzene; M, methanol).

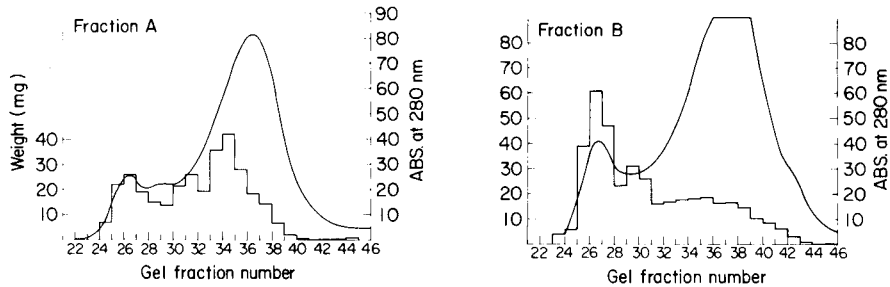


Fig. 2. Elution characteristics of fractions A and B on Bio-Beads SX-12 (curved line is absorbance trace at 280 nm, bar graph is GF weight).

allowed the identifications shown in Table 1. The major compound was solanone. The mass spectrum of this compound gave a molecular peak of 194 and fragments of 136, 121, 93, and 43, in excellent agreement with published data [7]. Smaller amounts of geranyl acetone, nonanone and decanone were also found in these fractions.

Fraction B was similarly analyzed. Its gel elution characteristics are also shown in Fig. 2. The large amount of material eluted in the early gel fractions, GF 23—30, consisted of sterols and solanesol, a C-45 terpenoid alcohol. Components found in GF 31—38 consisted of aliphatic ketones (solanone, geranyl acetone, etc.) which resulted from incomplete separation on the silicic acid column. The large absorbance in subsequent gel fractions was mainly due to

TABLE 1

Carbonyl components identified in fraction A of cigarette smoke neutrals

	Gel tube number						
	32	33	34	35	36	37	38
Solanone	X	X	X	X			
Geranyl acetone		X	X	X	X		
Tetramethylbenzoquinone					X	X	X
Nonanone			X	X			
Decanone		X	X	X			

phenolic material. To separate the aromatic ketones from the phenols, the separation of individual gel fractions on a single column of Sephadex LH-20 was studied. Previous work had shown that very polar aromatic compounds, such as phenols, are retained strongly by the column via hydrogen bonding and that aliphatic and aromatic ketones are eluted more rapidly [8]. Similar results were obtained with the gel fractions from fraction B. Thus, for example, separation of B-41 (GF-41 from fraction B), on Sephadex LH-20 gave an almost pure ketone fraction in the early tubes (6—12) whereas the phenols were eluted, with partial separation, in tubes (26—67 (Fig. 3). The gas chromatogram for materials in combined tubes 6—12 is shown in Fig. 4A. The predominant compounds were acetophenone, *m*- and *p*-methylacetophenone, dimethyl-1-indanone, fluorenone, and methylfluorenones. Adjacent GF fractions were also examined and contained similar compounds. The gas chromatogram for material in combined tubes 6—10 of B-39 is shown in

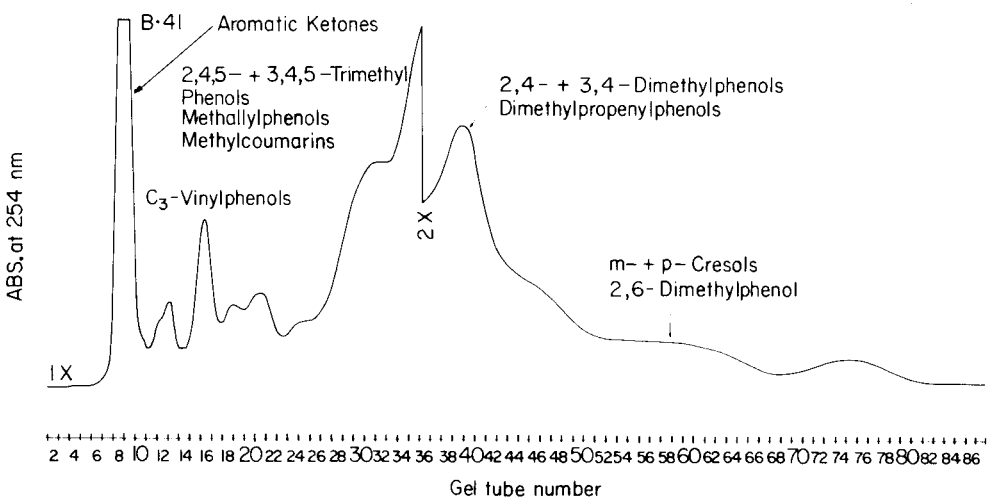


Fig. 3. Absorbance trace (254 nm) for the elution and separation of B-41 (GF-41 from fraction B) on Sephadex LH-20.

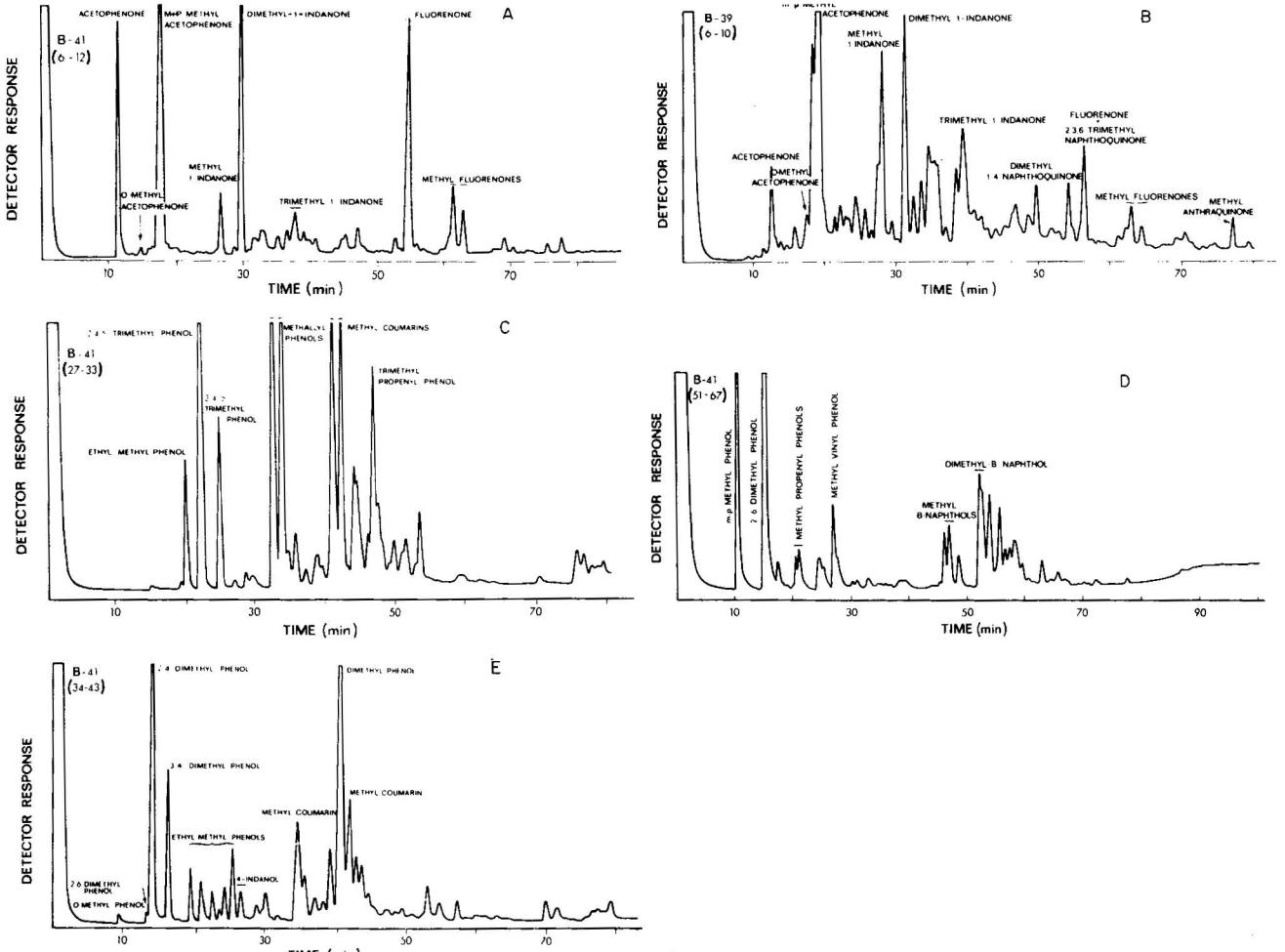


Fig. 4. Gas chromatogram of combined gel tubes on an OV-17 column. (A) Tubes 6—12 of B-41; (B) tubes 6—10 of B-39; (C) tubes 27—33 of B-41; (D) tubes 34—43 of B-41; (E) tubes 51—67 of B-41.

Fig. 4B. In addition to the above ketones, trimethyl-1-indanone, dimethyl-1,4-naphthoquinone, 2,3,6-trimethylnaphthoquinone, and 2-methyl-9,10-anthraquinone were also found. The 2,3,6-trimethylnaphthoquinone is the major quinone in CSC and is present at levels of about 220 ng/cigarette, whereas anthraquinone is the major quinone of tobacco leaf [9]. The ketones identified in fraction B are summarized in Table 2.

Analysis of the higher-numbered tubes from the Sephadex LH-20 separation of B-41 indicated that the phenols were also being resolved to some extent. Tri-alkylated phenols were eluted earlier than mono- and di-alkyl phenols, and larger phenols, such as coumarin and naphthols, were eluted later (Fig. 4C—E). The identified phenols are summarized in Table 3. Gel chromatography on Sephadex LH-20 with chloroform appears to be an acceptable method for fractionating phenolic mixtures. However, the key to successful identifications was the examination of the individual gel fractions. Examination of the individual gel tubes by g.c. showed the separating power for phenols of Sephedex LH-20. All of the components to date in this tumor-inhibiting fraction have previously been reported in CSC. The compounds in fractions C and D are being examined.

TABLE 2

Carbonyl components in GF 39—41 of fraction B from cigarette smoke ether-soluble neutrals

Compound	Compound
Acetophenone	Trimethyl-1-indanone
o-Methylacetophenone	Dimethyl-1,4-naphthoquinone
m-Methylacetophenone	Fluorenone
p-Methylacetophenone	2,3,6-Trimethyl-1,4-naphthoquinone
Methyl-1-indanone	Methylfluorenones
Dimethyl-1-indanone	Methyl-9,10-anthraquinone

TABLE 3

Phenolic components in GF 39—45 of fraction B from cigarette smoke condensate

Compound	Compound
Methyl-β-naphthols	4-Indanol
Dimethyl-β-naphthols	Ethylmethylphenols
Methylvinylphenol	*o*-Methylphenol
Methylpropenylphenols	2,4-Dimethylphenol
3,4-Dimethylphenol	Trimethylpropenylphenol
m- and *p*-Methylphenol	Methallylphenols
3,5-Dimethylphenol	3,4,5-Trimethylphenol
Methylindanol	2,4,5-Trimethylphenol
Dimethylpropenylphenol	Trimethylvinylphenol
Methylcoumarins	2,6-Dimethylphenol

CONCLUSIONS

The combination of adsorption and gel chromatography is effective for separating and resolving neutral organic compounds. Highly complex mixtures should be subfractionated before attempts are made to identify the constituents by g.c. or even by g.c.—m.s. In studies of the polynuclear aromatic hydrocarbons [10] and phenolics [11] of tobacco smoke, identifications of all the components were possible only after fractionation by silicic acid and gel chromatography.

REFERENCES

1 C. R. Green, Recent Adv. Tob. Sci., (1977) 94.
2 F. J. Akin and W. J. Chamberlain, J. Nat. Cancer Inst., 52 (1977) 613.
3 W. J. Chamberlain, M. E. Snook and A. F. Haeberer, J. Anal. Toxicol., 2 (1978) 138.
4 A. P. Swain, J. E. Cooper and R. L. Stedman, Cancer Res., 29 (1969) 579.
5 W. J. Chamberlain, F. J. Akin, M. E. Snook, D. B. Walters and O. T. Chortyk, Beitr. Tabakforsch., 8 (1975) 132.
6 R. L. Miller and R. L. Stedman, Phytochemistry, 10 (1971) 1135.
7 E. Stenhagen, S. Abrahamsson and F. W. McLafferty, Registry of Mass Spectral Data, Vol. 2, Wiley-Interscience, New York, 1974, p. 898.
8 M. E. Snook, R. F. Severson, R. F. Arrendale, P. J. Fortson and O. T. Chortyk, Tob. Sci., in press.
9 I. Schmeltz, J. Tosk, G. Jacobs and D. Hoffmann, Anal. Chem., 49 (1977) 1924.
10 M. E. Snook, R. F. Severson, R. F. Arrendale, H. C. Higman and O. T. Chortyk, Beitr. Tabakforsch., 9 (1978) 222.
11 M. E. Snook, W. S. Schlotzhauer and W. J. Chamberlain, Tob. Sci., XXII (1978) 106.

Analytica Chimica Acta, 111 (1979) 243—249

ANALYTICAL BEHAVIOUR OF HYDROPHILIC GLYCOLMETHACRYLATE GELS WITH BOUND THIOL GROUPS

Z. SLOVÁK*, M. SMRŽ, B. DOČEKAL and S. SLOVÁKOVÁ

Research Institute of Pure Chemicals, Lachema, 621 33 Brno (Czechoslovakia)

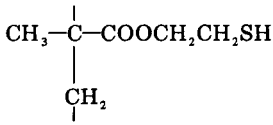
(Received 25th June 1979)

SUMMARY

Hydrophilic glycolmethacrylate gels with side chains containing thiol groups show high sorption selectivity for Hg, Sb, Bi, As, Ag, Cu and Pt, even in 1—3 M solutions of sulfuric or hydrochloric acid. The preparation of the sorbent Spheron Thiol and the distribution coefficients for 13 ions as a function of pH are described; within the optimal pH range the distribution coefficients exceed 10^4. The sorption capacity of the resin reached ca. 0.5—1.0 mmol Hg g^{-1} in 0.05 M HCl and equilibrium was achieved within 5 min, except for arsenic and platinum in insufficiently acidic solutions.

Recently, extensive attention has been given to thiol-containing polymers which can be used as selective sorbents of heavy metal ions and some other elements. In most papers published previously, the preparation and properties of various polystyrene-based thiol derivatives were described. However, the hydrophobic nature of these materials affects unfavourably the sorption process. When the sorption of heavy metals on thiol derivatives of the polystyrene and methacrylate types was compared, better results were obtained for the latter [1].

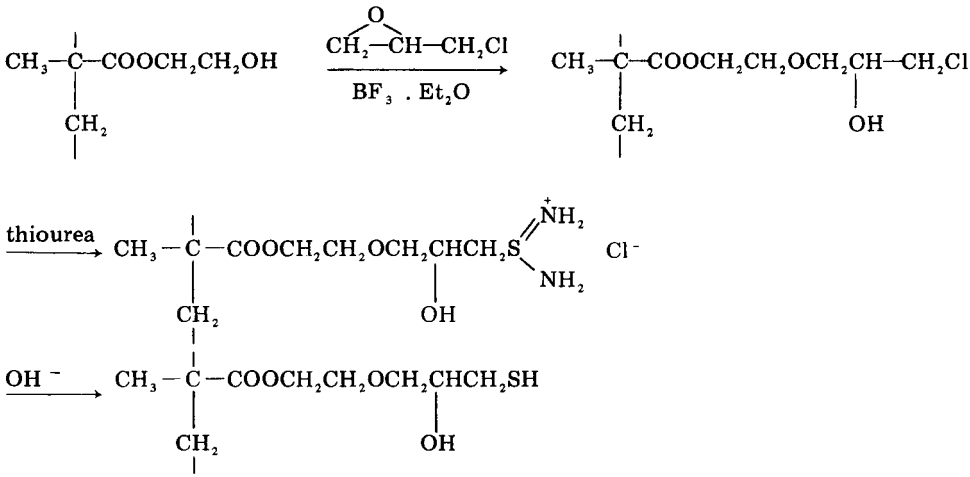
In a systematic study of chelating resins derived from macroporous hydrophilic hydroxyethyl methacrylate gels (Spheron) [2, 3], some thiol derivatives were prepared [4, 5]. The first material used for the study of sorption properties was a modified Spheron gel containing β-mercaptoethyl methacrylate units(I) [4].



(I)

When the time-dependence of the sorption of very small quantities of mercury on this material was investigated under static conditions, only 95% sorption of the metal was observed after 10 min and prolonged shaking of the

mixture resulted in a decrease in the amount of metal sorbed (see Fig. 2, curve 4). This behaviour, which can be explained by β-elimination of the mercaptoethyl group [6], led to the preparation of another type of sorbent — Spheron Thiol — according to the following scheme:



All the results discussed here were obtained with this sorbent. The analytical behaviour of Spheron Thiol was studied for the sorption of elements from dilute solutions.

EXPERIMENTAL

Chemicals and instrumentation

Hydroxyethyl methacrylate gels with thiol groups in the side chains (Spheron Thiol) of particle size 40—63 μm and average pore diameter ca. 370 nm, were prepared as described below. For the determination of distribution coefficients, sorption isotherms, etc., solutions of analytical-grade chemicals were prepared.

A Perkin-Elmer Model 420 atomic absorption spectrometer was used with both flame and electrothermal (HGA-74 graphite furnace) atomization; a Hitachi Perkin-Elmer Model 200 u.v.-visible spectrophotometer was also used.

Preparation of Spheron Thiol 1000

To the suspension of Spheron 1000 beads of particle size 40—63 μm (100 g) in dry benzene (500 ml), epichlorhydrin (15.6 ml, 200 mmol) and boron trifluoride etherate (2 ml) were added and the mixture was refluxed with stirring for 30 min. The chlorohydrin intermediate was filtered off, washed with benzene, and dried. This intermediate (100 g) was suspended in water (500 ml). After addition of thiourea (500 g) and 12 M hydrochloric acid (40 ml), the mixture was heated at reflux, with stirring, for 7 h. The

polymeric isothiouronium salt obtained was filtered off, washed with hot water, and resuspended in 2 M NaOH. The mixture was then stirred for 20 min, and the product was filtered off, washed with water, 1 M hydrochloric acid, water and methanol, and dried. The product contained neither chlorine nor nitrogen; the sulfur content was 4.3%. In a similar way, thiol gels with sulfur contents varying from 0.96 to 9.12% were prepared.

Determination of elements

Elements were determined by atomic absorption spectrometry (a.a.s.), except for arsenic which was determined spectrophotometrically with silver diethyldithiocarbamate after distillation as arsine [7], and for platinum which was determined spectrophotometrically by treatment with tin(II) chloride [8]. The a.a.s. procedures with flame atomization were those recommended by the instrument manufacturer. Low concentrations of mercury bound on the sorbents were determined by sampling the suspensions into a graphite tube as described earlier [9].

Distribution coefficients, equilibrium rate, sorption isotherms

Batch sorption procedures were generally applied. For determinations of the distribution coefficients (K_d) shown in Fig. 1, suspensions of 50 mg of the sorbent in 5 ml of acid or buffer solution containing the test ion in the quantities given in Table 1 were shaken for 60 min; after sedimentation the concentration of the element in the clear supernatant liquid ($[Me]_l$, $\mu g\ ml^{-1}$) was determined, and its concentration in the solid phase ($[Me]_s$, $\mu g\ g^{-1}$) and K_d values, $[Me]_s[Me]_l^{-1}$, were calculated. Sodium acetate–acetic acid–KCl buffer solutions of ionic strength 0.1 were used.

The equilibration rate was investigated for the sorption of 10 μg of arsenic from 20 ml of 1M sulfuric acid on 100, 30 and 10-mg portions of Spheron Thiol (Fig. 2, curves 1–3). Immediately after shaking, small samples of the solution were obtained for analysis by means of a pipette with a microfilter tip. The sorption of 10 ng of mercury on 1 mg of thiol material (I). (Fig. 2, curve 4) and on Spheron Thiol from 1 ml of 0.01 M HCl was done in 1-ml Eppendorf reaction tubes [9]. After shaking and centrifugation, the main part of the solution was thrown away and all the sorbent was placed in the HGA-74 graphite tube [9].

For the determination of the sorption isotherms of mercury in suspensions of 10 mg of Spheron Thiol in 5 ml of 0.05 M HCl containing 1–5 mg Hg (i.e., 0.5–2.5 mmol g^{-1}), the concentration of mercury in the clear supernatant liquid was determined by flame a.a.s. after shaking for 60 mins. Similarly, the sorption from an aqueous solution of mercuribenzoic acid (0.2–0.84 mmol g^{-1}) at pH 4.5–5.5 was studied.

DISCUSSION

The physical properties of Spheron Thiol beads are similar to the other

TABLE 1

Experimental conditions for the determination of K_d values

Element	Loading (mmol g^{-1})	Sorption from
Hg	0.010	0.005—1.5 M H_2SO_4; 0.1—2 M HCl
As	0.013	0.005—1.5 M H_2SO_4
Bi	0.005	0.005—0.5 M H_2SO_4
Sb	0.008	0.005—0.5 M H_2SO_4
Ag	0.009	0.005—0.5 M H_2SO_4
Cu	0.015	0.005—0.5 M H_2SO_4
Pt	0.010	0.02—2 M HCl; buffer solutions
Cd	0.009	Buffer solutions
Co	0.015	Buffer solutions
Fe	0.018	Buffer solutions
Ni	0.017	Buffer solutions
Pb	0.005	Buffer solutions
Zn	0.015	Buffer solutions

chelating ion exchangers derived from hydroxyethyl methacrylate gels, namely Spheron Oxin [2] and Spheron Salicyl [3]. These are semi-rigid macroporous materials showing practically no changes in swelling in aqueous solutions over a wide range of pH and ionic strength values. The chemical stability of the gel is adequate in neutral and acid solutions.

The selectivity of Spheron Thiol was studied from the distribution of ions between the sorbent and solutions of differing acidities at minimal loading (2% or less with respect to the sorbent capacity for mercury in 0.05 M HCl, see Table 1). Quantitative sorption of Hg, Sb, Bi and As from 1—2 M H_2SO_4 or HCl was observed (Fig. 1); the K_d values were so high (above 10^4) that they could not be estimated under the conditions of these experiments. In addition to these ions, silver and copper showed considerable sorption from acidic solutions. The use of Spheron Thiol for the concentration of these elements from very dilute solutions and their separation from complicated matrices should be advantageous.

Noble metals differed significantly in their behaviour. Whereas palladium was completely sorbed from dilute acids and acidic buffer solutions, the sorption of platinum from acetate buffers was not quantitative and its rate was slow; for rhodium, practically no sorption was found when chloride solutions were applied. These phenomena, and the incomplete and slow sorption of arsenic from acidic solutions of pH above zero (see Fig. 2) may be due to complicated hydrolytic processes. Other elements which form insoluble sulfides in acidic or neutral solutions (Cd, Pb, Ni, Fe, Zn, Co) were sorbed from solutions of pH above 3. From the practical point of view, however, Spheron Thiol can be considered less useful for the sorption of Ni, Fe, Zn, and Co, than other chelating sorbents, such as Spheron Oxin.

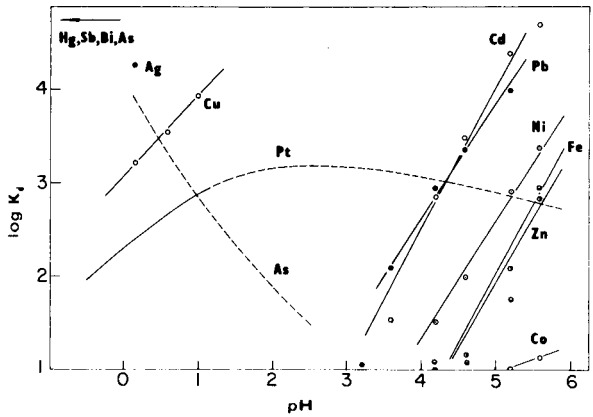


Fig. 1. Distribution coefficients for various ions on Spheron Thiol.

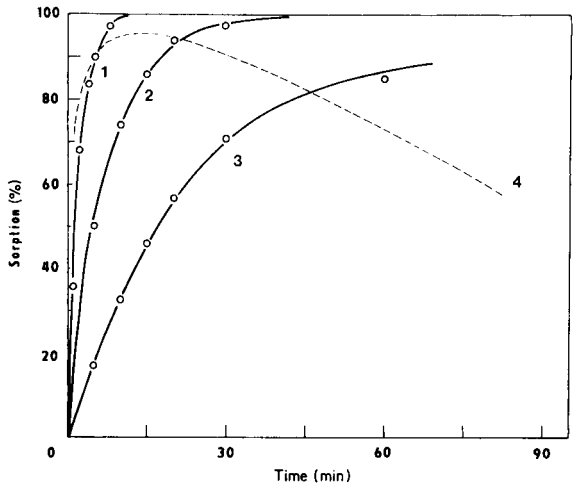


Fig. 2. Rate of equilibration. Curves 1—3 relate to sorption of 10 μg As in 20 ml of 1 M sulfuric acid on 100 mg, 30 mg, and 10 mg of Spheron Thiol, respectively. Curve 4 relates to sorption of 10 ng Hg in 1 ml of 0.01 M hydrochloric acid on 1 mg of thiol sorbent I.

One of the principle features of Spheron Thiol is the fact that ions are sorbed exclusively by interaction with the thiol groups of the material with no possibility of any unselective sorption. Therefore, even high concentrations of elements which do not react with thiol groups under the given reaction conditions cannot interfere.

If not affected by other reactions, e.g. hydrolysis, the rate of sorption equilibration was very high. The results shown in Fig. 2 are similar to those obtained for the sorption of heavy metals on related resins derived from

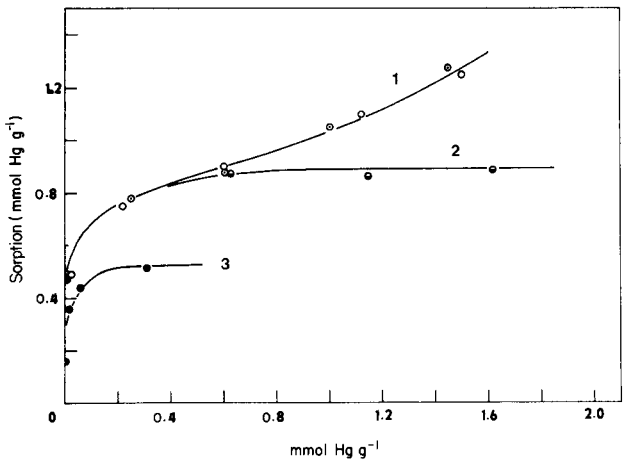


Fig. 3. Sorption isotherms for mercury from 0.05 M HCl. Curve 1, immediately and 1 month after the synthesis; curve 2, after storage for 1 year; curve 3, aqueous solutions of mercuribenzoic.

macroporous and hydrophilic hydroxyethyl methacrylate gels [2, 3]. The rapidity of sorption permits Spheron Thiol to be used with advantage for analytical batch sorption methods with a sorbent:sample solution ratio of up to 1:1000 g ml^{-1} [10]. The sorption of 500 μg of mercury from 10 ml of 0.05 M HCl on 100 mg of Spheron Thiol was complete after shaking for 1 min; the sorption of 10 ng of mercury from 1 ml of 0.05 M HCl on 1 mg of Spheron Thiol (conditions similar to those in the experiment with the thiol gel I, Fig. 2, curve 4) was complete after 15 min: mercury "bleeding" after prolonged periods of shaking was not observed with Spheron Thiol.

The sorption capacity of Spheron Thiol is sufficient for most analytical applications. Attention was paid mainly to the sorption of mercury (Fig. 3) from solutions of mercury(II) chloride and of mercuribenzoic acid as a model for other organomercurials.

The long-term stability of Spheron Thiol was tested by the sorption of mercury; significant changes in the sorption efficiency of a sorbent preparation were not observed under the conditions used in analytical practice (i.e., low loading of the sorbent with respect to its maximal sorption capacity) either immediately after synthesis, after 1 month, or even after storage for 1 year at room temperature (Fig. 3). It is also possible to regenerate the sorbent, if slight oxidation has decreased its capacity, by treatment with reducing agents, e.g., sodium dithionite or dithiothreitol.

The desorption of elements which can be sorbed from strongly acidic solutions is rather difficult. For their elution it is necessary to use complexing or thiol agents, e.g., a fresh 10% (w/v) thiourea solution in 0.1 M HCl, which releases almost all bound mercury and regenerates the resin for further

use. For the analytical determinations of small amounts of elements, especially when batch sorption methods are used, the direct sampling of aqueous suspensions of Spheron Thiol, after sorption, into the a.a.s. electrothermal atomizer proved to be advantageous [9] and, hence, desorption is not needed.

REFERENCES

1 M. J. Beneš, J. Štamberg, J. Peška, M. Tichý and M. Cikrt, Angew. Makromol. Chem., 44 (1975) 67.
2 Z. Slovák, S. Slováková and M. Smrž, Anal. Chim. Acta, 75 (1975) 125.
3 Z. Slovák, S. Slováková and M. Smrž, Anal. Chim. Acta, 87 (1976) 149.
4 M. Smrž and J. Hradil, Czechoslov. Pat., AO 190 171 (1978).
5 M. Smrž, Z. Slovák and J. Borák, Czechoslov. Pat., AO 190 189 (1978).
6 T. K. Dykstra and D. A. Smith, Makromol. Chem., 134 (1970) 209.
7 J. Meyer, Fresenius Z. Anal. Chem., 229 (1967) 409.
8 O. G. Koch and G. A. Koch-Dedic, Handbuch der Spurenanalyse, Vol. 2, Springer-Verlag, 1974, p. 971.
9 Z. Slovák, Anal. Chim. Acta, 110 (1979) 301.
10 Z. Slovák and S. Slováková, Fresenius Z. Anal. Chem., 292 (1978) 213.

Analytica Chimica Acta, 111 (1979) 251—256

COATING OF BOROSILICATE GLASS CONTAINERS FOR PREVENTING CONTAMINATION IN TRACE ELEMENT ANALYSIS

ATSUSHI MIZUIKE* and AKIRA IINO

Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464 (Japan)

(Received 22nd June 1979)

SUMMARY

Pyrex glass dishes are coated with amorphous 78 SiO_2—21 ZrO_2—1 Na_2O (mol %) films ca. 50 nm thick. The coated dishes possess reasonable chemical resistance and can be successfully used in the dry ashing of a standard botanical sample for boron determinations and in the evaporation needed for aluminum determination in nitric acid.

Materials suitable for containers used in trace element analysis at temperatures above 100°C include platinum, vitreous silica, and polyfluorocarbons, the maximum service temperatures being ca. 1500, 1100, and 250°C, respectively. All of these materials are, however, rather expensive. Borosilicate glasses are cheap and usable up to at least 500°C, but they contain boron, aluminum, sodium, etc., which may contaminate the samples for the determination of traces of these elements.

The coating of borosilicate glass containers with organic films, such as silicones and polyfluorocarbons, has been proposed [1]. This treatment, however, seriously lowers the maximum service temperature of the containers.

Recently, a novel technique for preparing glasses from mixed alcoholic solutions of alkoxides has been proposed by Dislich [2]. This technique can be applied to the preparation of various kinds of glasses or amorphous substances, including those which cannot be manufactured by conventional melting techniques because of the high temperatures required. It has been reported that amorphous SiO_2—ZrO_2 films prepared by this technique are highly resistant to alkaline solutions [3]. In the present work, pyrex glass dishes are coated with amorphous SiO_2—ZrO_2 films ca. 50 nm thick. The coated dishes can be successfully employed in the determination of traces of boron in a botanical standard reference material (Orchard Leaves) and of traces of aluminum in reagent-grade nitric acid. Contamination with the elements to be determined, which occurred during the ashing of the organic matter and the evaporation of the acid in pyrex glass dishes, was not observed in the coated dishes. The coating procedure is simple, easy and inexpensive.

EXPERIMENTAL

Apparatus

For secondary ion mass spectrometric (s.i.m.s.) measurements, a Hitachi IMA-2 ion microanalyzer was operated under the following conditions: primary ion, Ar^+; primary-ion accelerating voltage, 5 kV; primary-ion current, 0.1 μA; spot diameter, 250 μm; secondary-ion accelerating voltage, 1.5 kV; sample chamber pressure, 3×10^{-5} Pa; and electron-multiplier voltage, 2 kV. The electric charges accumulated on the sample surfaces were eliminated by the electron spray method. An Olympus Model MF microscope (magnification 100—400 ×) with a Model MF-NIC Nomarski interference contrast attachment and a Hitachi-Akashi Model MSM-2 scanning electron microscope (magnification 2000—10000 ×) were used for the observation of surfaces.

The thickness of the SiO_2—ZrO_2 films on the glass plates was measured with a Mizojiri Kogaku Model II multiple beam interferometer (Hg 546.1 nm, magnification 40 ×), with a maximum error of ±3 nm. For inductively coupled plasma—atomic emission spectrometry (ICP—a.e.s.), a computer-controlled programmable monochromator (0.5-m Ebert) [4] and a Nippon Koshuha 27.12-MHz generator (output power, 1.4 kW) were employed. A Nippon Jarrell-Ash AA-1 Mark II atomic absorption/flame emission spectrometer was used with an FLA-10 electrothermal atomizer (graphite tube furnace) and a Yanaco Model YR 101 recorder.

Reagents

As alkoxides, silicon tetraethoxide (Nakarai Chemicals, Ltd.) and zirconium tetrapropoxide (Ventron Corp.) were used. 1-Butanol was dehydrated with molecular sieve 4A. Other chemicals were of reagent grade and were employed without further purification. Water was purified by ion exchange. Standard boron and aluminum solutions were prepared from boric acid and aluminum potassium sulfate 24-hydrate, respectively.

Coating procedure

In a 100-ml round-bottomed flask with a reflux condenser, 20 ml of silicon tetraethoxide, 15 ml of zirconium tetrapropoxide, and 65 ml of 1-butanol were mixed with a magnetic stirrer for 3 h at 85 ± 5°C in a stream of nitrogen (dried with calcium chloride, 10 ml min^{-1}). The resulting mixed alkoxide solution was stored in a glass-stoppered bottle in a desiccator.

A portion (25 ml) of the solution was diluted with 75 ml of 1-butanol, and a pyrex glass round-bottomed dish (45 mm in diameter, 20 mm deep) was entirely immersed in it. The inverted dish was raised at a speed of 4 mm min^{-1} by a motor to the position illustrated in Fig. 1, and the solution surface inside the dish was lowered slowly to the edge of the dish by introducing dried nitrogen at a rate of 10—20 ml min^{-1} to form a solution film of uniform thickness. The above operations were carried out at 20 ± 3°C (relative humidity, 40—60%).

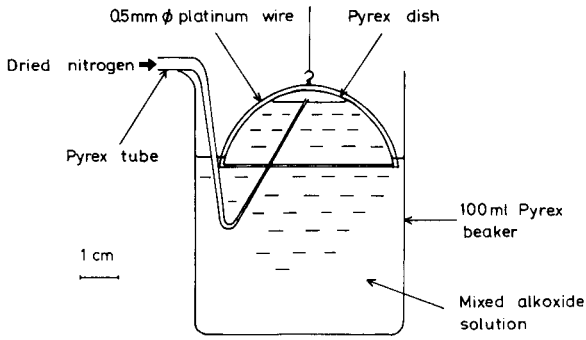


Fig. 1. Coating of pyrex glass dishes with mixed alkoxide solution.

The dish was removed from the solution by the motor drive (4 mm min^{-1}) and placed in a desiccator containing a saturated potassium sulfate solution (relative humidity, 97%) at 20 ± 3°C for 1 h to effect hydrolysis. It was then heated in an electric muffle furnace at 150°C for 1 h. The temperature was raised to 550°C at a rate of 10°C min^{-1}, kept at 550°C for 1 h, and lowered to 350°C at a rate of 1—2°C min^{-1}. The dish was then cooled to room temperature.

Cleaning of containers

Before each use, containers were cleaned with the following acids: a 1 + 1 mixture of sulfuric and nitric acids (1 day, 20°C) for pyrex glass dishes; 1 M nitric acid (1 day, 20°C) for SiO_2—ZrO_2 coated pyrex glass dishes; 6 M hydrochloric acid (1 h, 70°C) for platinum crucibles; and 0.1 M sulfuric acid (5 min, ultrasonics) for Teflon beakers.

RESULTS AND DISCUSSION

Characterization of the films

The SiO_2—ZrO_2 films on the surfaces of pyrex glass dishes were colorless and transparent. Under the optical and electron microscopes, the surfaces of the films were found to be smooth and without cracks and pinholes. The thickness of the films was estimated to be 30—50 nm, by comparing the s.i.m.s. depth profiles with those obtained for films of known thickness (measured with an interferometer) on glass plates (flat substrates) (Fig. 2). When the films were thicker, cracks appeared during the hydrolysis step. The films were not damaged by rubbing with the fingers, but they were destroyed by rubbing with a sharpened pyrex glass rod. Repeated cycles (5 times) of heating to 450°C and cooling to room temperature (similar in temperature change to dry ashing) did not produce any cracks in the films.

The chemical composition of the films, determined by s.i.m.s., was 77 SiO_2—22 ZrO_2—1 Na_2O mol % [5]. The sodium originated from the pyrex glass substrate, but neither boron nor aluminum was found in the films.

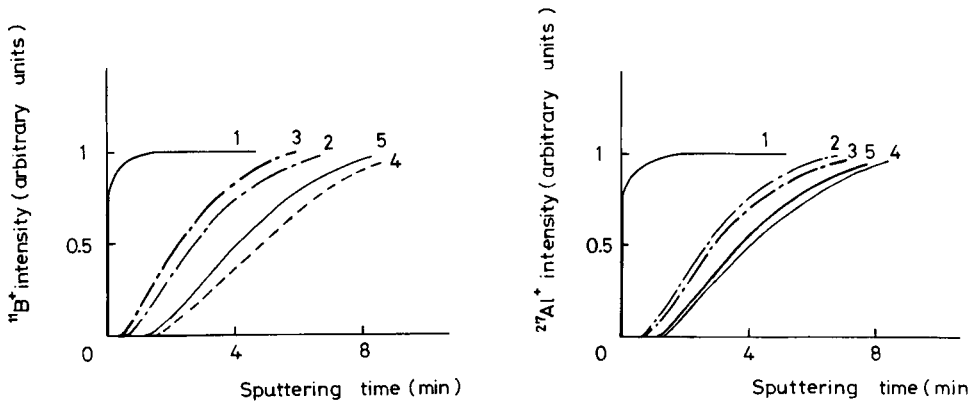


Fig. 2. S.i.m.s. depth profiles. (1) Pyrex glass plate; (2) 30-nm SiO_2—ZrO_2 film on pyrex glass plate; (3) 30-nm SiO_2—ZrO_2 film on pyrex glass plate (after dry ashing of orchard leaves); (4) 50-nm SiO_2—ZrO_2 film on pyrex glass plate; (5) SiO_2—ZrO_2 film on pyrex glass dish.

Fig. 3. S.i.m.s. depth profiles. (1) Pyrex glass plate; (2) 30-nm SiO_2—ZrO_2 film on pyrex glass plate; (3) 30-nm SiO_2—ZrO_2 film on pyrex glass plate (after evaporation of nitric acid); (4) SiO_2—ZrO_2 film on pyrex glass dish; (5) SiO_2—ZrO_2 film on pyrex glass dish (after evaporation of nitric acid five times).

Application to dry ashing of the Orchard Leaves standard reference material for the determination of boron

The SiO_2—ZrO_2 coated dishes were compared with pyrex glass dishes and platinum crucibles in the dry ashing of the U.S. National Bureau of Standards standard reference material SRM-1571 (orchard leaves) for the determination of boron, one of the essential micronutrients for plants. The analytical procedure was as follows.

The sample was dried in an air oven at 85°C for 5 h. A 0.5-g aliquot was weighed into a dish (or crucible) and 2 ml of saturated calcium hydroxide solution (0.02 M at 20°C) was added to prevent the volatilization of boron. The dish was heated at 100°C for 30 min in an electric muffle furnace. The temperature was then raised to 450°C at a rate of 7°C min^{-1}, and kept at 450°C for 5 h. After cooling to room temperature, the ash was dissolved in 1 ml of 2 M hydrochloric acid. To stabilize the boron concentration (for up to 5 days), 2 ml of 0.001 M mannitol was added and the solution was diluted with water to 0.1--1 μg B ml^{-1}. The supernatant liquid was submitted to ICP- a.e.s. at 249.8 nm.

The working curve was linear over the range 0—1 μg B ml^{-1}, with a maximum deviation of ±0.02 μg B ml^{-1}. The analytical results are listed in Table 1. A small amount of insoluble siliceous residue (probably the result of external sand or soil contamination of the Orchard Leaves [6]) was disregarded, because no boron was found in it (analyzed by ICP—a.e.s. after alkali fusion). There was very significant contamination of boron from pyrex glass dishes, but no contamination occurred in SiO_2—ZrO_2 coated dishes or in platinum

TABLE 1

Determination of boron in SRM-1571 (Orchard Leaves)

Containers used in dry ashing	Boron[a] ($\mu g\ g^{-1}$)
Platinum crucible	34, 33, 31 (av. 33)
Pyrex dish coated with SiO_2—ZrO_2 film	34, 33, 35 (av. 34)
Pyrex dish	40, 41, 39 (av. 40)

[a]Certified values: 33 ± 3 $\mu g\ g^{-1}$.

crucibles. Microscopic observations showed that the surfaces of the used coated dishes were appreciably etched, although no boron on the surfaces was detected by s.i.m.s. (Fig. 2). The coated dishes should not be used repeatedly.

Application to evaporations for the determination of aluminum in nitric acid

In the determination of aluminum in reagent-grade 14 M nitric acid (62%) by graphite-furnace atomic absorption spectrometry, direct injection of the sample is not applicable because of severe depression of the aluminum signal and damage to the furnace; both are due to nitric acid. Therefore, the following procedure was employed.

Nitric acid (1 ml) was evaporated to dryness in a dish (or beaker) in a polymethacrylate evaporation chamber (flushed with nitrogen filtered through a 0.1-μm membrane filter) on a hot plate at 100°C. The residue was dissolved in 1 ml of 0.1 M sulfuric acid in an ultrasonic chamber (29 kHz, 150 W) for 3 min. A 20-μl aliquot was injected into a graphite furnace, dried at ca. 200°C for 15 s followed by a second drying at ca. 700°C for 20 s, and then atomized at ca. 2400°C for 10 s for the absorbance measurement at 309.3 nm. The peak height was measured three times and averaged.

The working curve was linear over the range 0—2 ng of aluminum, with a maximum deviation of ±0.06 ng of aluminum. The analytical results are listed in Table 2. Both airborne contamination and loss of aluminum were negligible. The results show that the SiO_2—ZrO_2 coating is effective in reducing the aluminum contamination originating from pyrex glass. No

TABLE 2

Determination of aluminum in 14 M nitric acid

Containers used in evaporation	Aluminum (ng ml^{-1} HNO_3)
Teflon beaker	50, 48, 49, 48 (av. 49)
Pyrex dish coated with SiO_2—ZrO_2 film	51, 53, 50, 49 (av. 51)
Pyrex dish	62, 74, 65, 57 (av. 65)

change was observed under the microscope on the surfaces of the coated dishes before and after use. S.i.m.s. depth profiles (Fig. 3) showed that a coated dish can be used at least five times repeatedly. The coated dishes may also be applied to wet ashing of organic samples for the determination of aluminum.

The authors thank Messrs. T. Ito and H. Hirota for their help in the experimental work.

REFERENCES

1 P. B. Adams, in M. Zief and R. M. Speights, Jr. (Eds.), Ultrapurity: Methods and Techniques, M. Dekker, New York, 1972, p. 309.
2 H. Dislich, Glastech. Ber., 44 (1971) 1.
3 M. Nogami and Y. Moriya, Yogyo Kyokai Shi, 85 (1977) 448.
4 H. Kawaguchi, M. Okada, T. Ito and A. Mizuike, Anal. Chim. Acta, 95 (1977) 145.
5 A. Iino and A. Mizuike, Bull. Chem. Soc. Jpn., 52 (1979) 2433.
6 E. J. Maienthal, J. Assoc. Off. Anal. Chem., 55 (1972) 1109.

Analytica Chimica Acta, 111 (1979) 257—263

A DIFFERENTIAL MODIFICATION OF THE THERMAL MAXIMUM METHOD FOR EVALUATING THE ENTHALPIES AND RATE CONSTANTS OF HOMOGENEOUS REACTIONS

EDWARD W. SCHINDLER, Jr.** and LOUIS MEITES*

Department of Chemistry, Clarkson College of Technology, Potsdam, New York 13676 (U.S.A.)

(Received 2nd July 1979)

SUMMARY

Both the change of enthalpy associated with a reaction and the rate constant of the reaction can be found from the dependence on time of the difference between the temperatures of a reaction mixture and a reference solution contained in identical non-insulated vessels submerged in a thermostat. Heats of dilution and fluctuations of the temperature of the thermostat are cancelled by the differential arrangement, and the heat-transfer constant for the reaction vessel need not be known or measured separately, which is an important advantage over the classical thermal maximum method. For half-times between about 9 and 30 s and overall changes of temperature between 25 and 90 mK, relative standard deviations of a few per cent in both ΔH and k are easily attainable.

Probably the simplest and most widely applicable way of following the rate of a reaction that occurs in solution is to follow the change of temperature that accompanies the reaction. Very few reactions are accompanied by changes of enthalpy too small to give rise to appreciable variations of temperature, even in quite dilute solutions, and the natures of the reactants, products, and solvent are immaterial instead of being constrained in various ways as they are with most other techniques.

The "thermal maximum" technique devised by Bell et al. [1—3] is a remarkably simple and ingenious technique for evaluating the rate constant of a reaction. The reactants are mixed in a thin-walled flask or other poorly insulated vessel that is submerged in a thermostat, and the time t_{max} that is required to attain the maximum on the temperature—time curve is measured. The Newtonian constant ϵ for heat exchange through the walls of the vessel is evaluated separately, either under identical conditions or in the same experiment after a time so long that the reaction has certainly reached equilibrium. If the order of the reaction is known, the value of the rate constant can be calculated from the values of t_{max} and ϵ; if the order is not

**This paper is based in part on a Thesis submitted by Edward W. Schindler, Jr. to the Faculty of Clarkson College of Technology in partial fulfilment of the requirements for the degree of B.S. in Chemistry in May, 1978.

known, it may be found from data showing how t_{max} depends on the initial concentrations of the reactants. It is further possible to evaluate ΔH if T_{max}, the change of temperature that has occurred at the time t_{max}, is measured as well and if the thermal equivalent (in, say, J K^{-1}) of the reaction vessel is known.

Soon after the technique was first described, it was adopted by a number of different groups [4—8], but none used it twice and it has since been almost forgotten. One can discern several possible reasons for its failure to gain acceptance. The necessity of evaluating ϵ separately complicates the measurements, and attempting to employ in one experiment a value obtained in another leads to errors because values of ϵ are not closely reproducible even under very carefully controlled conditions. Some experimenters encountered difficulty from the fact that there is no way to test the adherence of the data in any particular experiment to the equation employed for calculating the value of k. Trouble arises, unless the solutions are rather concentrated or ΔH is unusually large, from unavoidable periodic fluctuations in the temperature of the thermostat, and also from heats of dilution and other uncompensated thermal effects.

All these problems may be minimized, or even eliminated, by two expedients. One is to measure not the temperature of the reaction mixture but the difference between that temperature and the temperature of a reference solution contained in a similar vessel nearby in the same thermostat. This is easily done by means of a simple Wheatstone-bridge arrangement employing two standard resistors and two matched thermistors, one in each vessel. Heats of dilution, stirring, and evaporation are almost perfectly compensated by the differential arrangement, and the effect of fluctuations in the temperature of the thermostat is decreased by approximately two orders of magnitude. The other expedient is to improve the efficiency of experimentation by employing non-linear regression analysis [9] of the data obtained in each experiment. In this way ϵ and k can be evaluated simultaneously, so that there is no doubt about the relevance of ϵ to the conditions of the particular experiment. Moreover, it then becomes a simple matter to make sure that the data do obey the equation employed.

EXPERIMENTAL

Equipment

A glass-walled rectangular tank containing approximately 100 dm^3 of water was used as a thermostat. A refrigeration coil, electrical heater, and efficient dual-propeller stirrer were so arranged that the temperature at any point in the bath underwent fluctuations with a period of approximately 80 s and an amplitude of approximately ± 14 mK (milliKelvin). Much closer regulation is of course easily secured, but would have made it more difficult to detect the amplitude of the corresponding fluctuations of the differential temperature that was actually of concern in the experiments.

The reaction and reference vessels were made from ordinary 125-cm^3 Pyrex erlenmeyer flasks. Each carried a vertical side tube of about 6 mm i.d. and about 3 cm long, and had a hole about 1 cm in diameter opposite the side tube. One of a pair of carefully aged and matched [10] 100 000-ohm thermistors was inserted into each side tube. The top of the thermistor was wrapped with Parafilm so that it fitted snugly into the side tube, and the tip was positioned about 5 mm from the bottom of the flask and about 15 mm from its side. A tightly fitting rubber stopper was inserted into each hole, and a Teflon tube, whose tip was submerged beneath the solution in the flask and whose other end was attached to a 3-cm^3 plastic syringe, was led through a small hole in the stopper. The neck of each flask carried a rubber stopper fitted with a Teflon sleeve bearing, through which there passed the shaft of a glass propeller-type stirrer whose propeller was just small enough to enter the neck. Each stirrer was driven by a cone-drive stirring motor fitted with a flexible shaft. It was easy to adjust the motors so that the rates of stirring, and the corresponding rates of evolution of heat, were so nearly equal that no drift of the difference of temperature could be detected.

The two flasks were placed about 15 cm apart, with the syringes between them so that both could be actuated simultaneously. The flasks were immersed in the thermostating liquid to the bottoms of their necks, and the syringes were immersed to a depth sufficient to submerge the entire contents of each.

The thermistors constituted two of the arms of a d.c. Wheatstone bridge whose output voltage was measured with a Hewlett-Packard Model 3450 digital voltmeter interfaced to a PDP 8/I minicomputer provided with a 10-kHz crystal clock. The output voltage passes through a maximum and then returns to zero because of heat exchange through the walls of the reaction vessel, and data acquisition was continued until it had decayed to approximately one-fourth of its maximum value. Values of the output voltage and time were stored in core memory until the experiment was complete, and were then analyzed by non-linear regression as described below.

Procedures

At the beginning of an experiment the reaction and reference vessels contained equal volumes (usually 40 cm^3) of the same solution, which was aqueous 0.3 M hydrochloric acid for some experiments or an aqueous 0.1—0.8 M solution of ethyl acetate for others. The syringe attached to the reaction vessel contained approximately 0.5 cm^3 of 0.4 M sodium hydroxide, which was drawn up through the Teflon tube in such a way that the entire portion of that tube inside the reaction vessel was filled with air to prevent premature mixing of the reactants. The syringe attached to the reference vessel contained as nearly as possible the same volume of water, and was loaded in the same way. At the concentrations used in these experiments the heat of dilution of sodium hydroxide is insignificant; if it were not, compensation of it could have been achieved by placing water in the reference vessel and sodium hydroxide in the corresponding syringe.

When thermal equilibrium was thought to have been achieved, 400 measurements of the unbalance voltage were made over a 12-min period, and the average and standard deviation of these values were computed. At the next instant when the voltage was within one-half standard deviation of the average, the data-acquisition program rang a bell on a Model 33 ASR Teletype as a warning that measurements would begin 2 s later. The plungers of the syringes were depressed manually so that about half of the contents of each had been delivered into the corresponding vessel when the bell rang again as the measurements began. Synchronization within a fraction of a second was easily achieved at a cost much lower than a solenoid-actuated system would have entailed. The time interval between data points was made to depend on the total elapsed time, and varied from about 1 s at the start of the reaction to about 2 s in the vicinity of the maximum and about 15 s at the end. The syringe attached to the reaction vessel was weighed, together with the Teflon tube attached to it, both before and after each experiment, and the volume of reagent added to the reaction vessel was calculated from the measured density of the solution and the difference of weight.

Non-linear regression was effected with a PDP 8/I minicomputer and a general program used for many other purposes in this laboratory [9, 11].

RESULTS AND DISCUSSION

As was stated above, the temperature of the liquid surrounding the reaction and reference vessels underwent fluctuations having an amplitude of approximately ± 14 mK. In either vessel alone the fluctuations had an amplitude of only about ± 2 mK, and were so nearly equal that the difference between the temperatures of the solutions in the two vessels varied over a range of only about ± 150 μK. Since all these variations are clearly proportional, controlling the bath temperature within ± 1 mK, which is not at all difficult to do, should provide constancy of the differential temperature within about ± 10 μK. The variation of −150 μK obtained in this work made it possible to obtain useful results from experiments in which the total variation of temperature was between 10 and 50 mK; one of ± 10 μK would serve if the total variation of temperature were as small as about 200 μK. That would make it possible, if 0.5 cm^3 of an 0.1 M solution of a reagent is added to 40 cm^3 of a solution containing an excess of the substance with which it reacts, to study a reaction for which the change of enthalpy is only about 1 kJ mol^{-1}, or, if ΔH^0 is 40 kJ mol^{-1}, to work with as little as one micromole of material. The variations are roughly sinusoidal, but their period is fairly irregular, and attempts to correct for them by extrapolating the baseline obtained before adding the reagent were deemed unlikely to succeed.

To evaluate the effective heat capacity of the reaction vessel and its contents, about 0.5 cm^3 of 0.400 M sodium hydroxide was added to 40.00 cm^3 of 0.3 M hydrochloric acid in the reaction vessel while 0.5 cm^3 of water was added to 40.00 cm^3 of the same acid in the reference vessel.

About 30 data points were acquired at 2-s intervals, and their coordinates were fitted to the Newtonian equation

$$\Delta T = \Delta T^0 \, e^{-\epsilon t} \quad (1)$$

where ΔT is the observed difference of temperature at time t, ΔT^0 is the constant difference of temperature that would have been observed in the absence of heat exchange, and ϵ is the effective heat-transfer constant. The resulting values of ϵ were in the vicinity of 0.013 s^{-1}; those of ΔT^0 were used to compute the total effective heat capacity Q_{tot} from the familiar equation

$$\Delta T^0 = -(\Delta H)N/Q_{tot} \quad (2)$$

where ΔH is the change of enthalpy accompanying the reaction (−55.73 kJ mol^{-1}). The effective heat capacity Q_{app} of the apparatus alone was calculated from the equation

$$Q_{app} = Q_{tot} - 4.184\,(V_{HCl} + V_{NaOH}) \quad (3)$$

where the quantity inside the parentheses represents the total volume of the reaction mixture. Three experiments gave $Q_{app} = 30.2 \pm 0.8$ J K^{-1}; had all the values of V_{NaOH} been identical, the corresponding relative precision of Q_{tot} would have been ± 0.5%.

In the experiments with ethyl acetate, 40.00 cm^3 of a freshly prepared 0.1—0.8 M aqueous solution of the ester was placed in each flask, and 0.15—0.7 cm^3 of 0.400 M sodium hydroxide was placed in the syringe attached to the reaction vessel while a nearly equal volume of water was placed in the syringe attached to the reference vessel. Approximately an hour was allowed to elapse so that thermal equilibrium would be attained, and data acquisition was then begun in the manner described above.

When the experiment was complete the data were fitted to the equation

$$\Delta T = k\Delta T^0 \, (e^{-k't} - e^{-\epsilon t})/(\epsilon - k) \quad (4)$$

where k' is the pseudo-first-order rate constant. Values of ΔT^0, ϵ and k were obtained without difficulty as long as k/ϵ exceeded about 2, as it did in experiments in which the concentration of ethyl acetate was at least 0.2 M. In 0.1 M solutions, however, k and ϵ must have been nearly equal, and satisfactory fits could not be obtained. Conditions under which k/ϵ is between about 0.7 and 1.5 should be avoided.

The value of k' is related to that of the second-order rate constant by the equation $k' = k[\mathrm{EtOAc}]$; that of ΔT^0 is related to ΔH by eqns. (2) and (3), with V_{EtOAc} in place of V_{HCl} in the latter. Table 1 summarizes the results obtained. The mean value of k is in reasonable agreement with the results of prior investigations under similar conditions [12—15], and that of ϵ is very close to the value (0.013 s^{-1}) obtained from experiments on the neutralization of hydrochloric acid in the same apparatus. The values of ΔT^0 ranged from 27 to 87 mK but, because of loss of heat through the walls of the reaction flask, the maximum actual values of ΔT were only about half as

TABLE 1

The alkaline hydrolysis of ethyl acetate

[EtOAc] (M)	NaOH (mmol)	k' (s^{-1})	k ($dm^3\ mol^{-1}\ s^{-1}$)	$-\Delta H$ (kJ mol^{-1})	ϵ (s^{-1})	Standard deviation from regression (mK)
0.801	0.206	0.0783	0.0978	51.6	0.0121	0.12
0.701	0.189	0.0657	0.0937	52.6	0.0127	0.25
0.600	0.210	0.0578	0.0963	50.7	0.0127	0.23
0.504	0.0987	0.0487	0.0966	55.0	0.0128	0.27
0.503	0.1165	0.0480	0.0953	56.9	0.0126	0.18
0.501	0.177	0.0476	0.0950	54.9	0.0125	0.14
0.500	0.215	0.0549	0.1098	52.2	0.0116	0.07
0.497	0.322	0.0492	0.0990	54.2	0.0124	0.27
0.400	0.209	0.0431	0.1078	48.4	0.0123	0.15
0.300	0.203	0.0319	0.1061	47.7	0.0119	0.13
0.200	0.215	0.0238	0.1190	43.6	0.0109	0.10
Mean:			0.1015	51.6	0.0122	0.17
Standard deviation:			0.0080 (8.0%)	3.8 (7.5%)	0.0006 (4.7%)	—

large as the corresponding values of ΔT^0. The mean value of ΔH is in reasonable agreement with the one (—54.8 kJ mol^{-1}) obtained by Papoff and Zambonin [16] but is substantially larger than that (—44.3 kJ mol^{-1}) obtained by Wadsö [17].

Finally a deviation plot was constructed for each experiment by combining the best values of the parameters with eqn. (4) to compute a value of ΔT at each experimental point, subtracting these values from the corresponding measured ones, and plotting the differences against time. If there is no systematic error, and if the data do indeed conform to eqn. (4), there would normally be a random scatter of the differences around the time axis, but a systematic error of either experiment or interpretation would produce a random scatter around some smooth curve [9]. The data from these experiments always yielded a random scatter around a roughly sinusoidal curve having an amplitude of 10—15 mK, which is indistinguishable not only from the average standard deviation from regression but also from the amplitude of the variations observed in blank experiments with water in both flasks. It may be concluded that baseline variations are the only significant errors in the execution and interpretation of those experiments.

CONCLUSIONS

It is well known that the second-order rate constant k for the reaction of ethyl acetate with hydroxide ion appears to be larger in solutions containing excess of hydroxide ion than in solutions containing excess of ester. The relative standard deviation of all eleven of the values of k given in Table 1 is

8.0%. However, there appears to be a systematic decrease of k as the concentration of ester increases, and the best index of the precision actually attained is probably the relative standard deviation of 6.1% in the five values secured with 0.5 M ethyl acetate. As the necessary equipment includes nothing more than two flasks, two syringes, two stirrers, two thermistors, a simple d.c. Wheatstone bridge that could be constructed for about $50, a strip-chart recorder, and a thermostat of no very exceptional characteristics, this is certainly one of the simplest and least expensive techniques available for investigating the rate of a reaction.

In principle the standard deviation from regression could be greatly decreased, and the precision with which k and ΔH could be evaluated could be correspondingly improved, by controlling the temperature of the thermostat within narrower limits. That would be easy to do, but the average standard deviation from regression (0.17 mK) in these experiments was well under 0.5% of the average value of ΔT at the peaks of the curves, and it would probably be difficult to make a substantial improvement in that ratio, especially if a strip-chart recorder were used. A far more important inducement to improve the precision of the thermostat would be to permit work with more dilute solutions, or with reactions having smaller values of ΔH.

This work was supported by grant number CHE-7727751 from the National Science Foundation.

REFERENCES

1 R. P. Bell and J. C. Clunie, Proc. Roy. Soc., A212 (1952) 16.
2 R. P. Bell, V. Gold, J. Hilton and M. H. Rand, Discuss. Faraday Soc., 17 (1954) 151.
3 R. P. Bell, M. H. Rand and K. M. A. Wynne-Jones, Trans. Faraday Soc., 52 (1956) 1093.
4 P. Baumgartner and P. Duhaut, Bull. Soc. Chim. Fr., (1960) 1187.
5 N. H. Ray, J. Chem. Soc., (1960) 4023.
6 F. Becker and F. Spalink, Z. Phys. Chem. (Frankfurt am Main), 26 (1960) 1.
7 C. H. Lueck, L. F. Beste and H. K. Hall, Jr., J. Phys. Chem., 67 (1963) 972.
8 L. T. Eremenko, U. R. Kolesov and L. V. Kustova, Zh. Fiz. Khim., 38 (1964) 1259.
9 L. Meites, CRC Crit. Rev. Anal. Chem., 8 (1979) 1.
10 T. Meites, L. Meites and J. N. Jaitly, J. Phys. Chem., 73 (1969) 3801.
11 L. Meites, The General Multiparametric Curve-Fitting Program CFT4, Computing Laboratory, Department of Chemistry, Clarkson College of Technology, Potsdam, N.Y., 1976.
12 J. Walker, Proc. Roy. Soc. London, A78 (1960) 157.
13 B. Stead, F. M. Page and K. G. Denbigh, Discuss. Faraday Soc., 2 (1947) 263.
14 K. J. Laidler and D. Chen, Trans. Faraday Soc., 54 (1958) 1026.
15 H. Tsujikawa and H. Inoue, Bull. Chem. Soc. Jpn., 39 (1966) 1837.
16 P. Papoff and P. G. Zambonin, Talanta, 14 (1967) 581.
17 I. Wadsö, Acta Chem. Scand., 12 (1958) 630.

Analytica Chimica Acta, 111 (1979) 265—274

DETERMINATION OF PLUTONIUM IN SEDIMENTS BY SOLVENT EXTRACTION AND α-SPECTROMETRY

NARAYANI P. SINGH*†, PAUL LINSALATA, RICK GENTRY and McDONALD E. WRENN†

Institute of Environmental Medicine, New York University Medical Center, Box 817, Tuxedo, N.Y. 10987 (U.S.A.)

(Received 10th April 1979)

SUMMARY

A simple technique for the determination of environmental levels of plutonium in a highly complex matrix (sediments containing very high amounts of iron and other metals) is reported. The sediments, collected from the Hudson River Estuary with an Emory dredge, were hand-homogenized before a sample aliquot was taken. Samples were air-dried, weighed, spiked with ^{242}Pu tracer, and heated at 400°C for 24 h. Plutonium was leached from the sediment with an acid mixture. The leachate was filtered, and plutonium coprecipitated with iron by adding ammonia solution. After dissolution, plutonium was extracted with 20% trilaurylamine in xylene, the extracts were thoroughly acid-washed to remove uranium and thorium traces, and plutonium was then back-extracted with 2 M sulfuric acid prior to electrodeposition onto a platinum planchet. The isotopic composition of plutonium was determined by α-spectrometry. Tracer yield and plutonium concentrations determined on aliquots of the same samples by this method and by an ion-exchange technique were not significantly different.

Many different biological and environmental samples, e.g. air, food, water, excreta, fused rocks and soil, have been analyzed for plutonium for monitoring purposes [1—8]. Plutonium has often been separated from other elements by an ion-exchange technique [9—11]. To extend a study of the isotopic distribution of plutonium in the Hudson River Estuary along with other γ-emitting radionuclides, a method reported by Chu [12] was tried. Although the method gave satisfactory tracer recovery, it was time-consuming on a routine basis. Therefore, a method involving solvent extraction with trilaurylamine was undertaken. Veselsky [13] has reported a technique for the determination of plutonium in environmental samples by solvent extraction with trilaurylamine including back-washing with hydrochloric and hydrofluoric acids. This paper deals with certain modifications, particularly the complete removal of thorium-228, which has an α-energy spectrum overlapping that of plutonium-238.

†Present address: Radiobiology Division, University of Utah, Salt Lake City, Utah 84112, U.S.A.

Trilaurylamine (TLA) was chosen because of its high distribution coefficient for plutonium within a wide range of nitric acid concentrations (2—8 M) and also because of its low extraction coefficient for uranium [14]; it has been used successfully to determine plutonium [15] and thorium [16] in soft tissues.

EXPERIMENTAL

Reagents and apparatus

All reagents used were of analytical grade.

Electrodeposition apparatus. The plating cell consisted of an elongated 22-mm cap which held a 1-oz. polyethylene bottle from which the bottom had been removed. The cap and space for a platinum plating disc (17.6 mm diameter, 0.005 in. thick, mirror-finished on one side) supported by a nickel backing disc (17.6 mm diameter) and could be firmly screwed into the polyethylene bottle forming a leak-tight plating cell. A threaded brass bushing was molded into the cap, thus making the electrical contact with the platinum disc cathode by clip leads. The cell was supported on a lucite pedestal which rested in an ice-water bath. The anode was a platinum—iridium rod (4 in. long, 1/16 in. diameter) with a circular beading on the bottom end. It was connected through a constant-speed stirrer to the positive outlet of the power supply [1]. The power supply furnished a constant current in the range 0—10 A and a constant voltage in the range 0—36 V.

Sample preparation

Sediment samples were collected during the summer and fall of 1976 at a variety of locations occurring between 19.3 miles north (Newburgh Bay) and 7 miles south (Buoy 14 East) of Indian Point nuclear power station. Sediment samples, collected on board a 22-foot Aqua-Sport with an Emory dredge, were immediately hand-mixed to obtain homogeneity and placed inside 500-ml polyethylene containers. The Emory dredge is believed to capture the upper 2—4 inches of bottom sediment, the area thought to contain the highest concentration of plutonium and other radionuclides [17]. After collection, sediment samples were air-dried, crushed to obtain uniform particle size, and stored. An aliquot (20 g) was weighed and transferred to fused quartz baking dishes, and an aliquot (2—3 dpm) of plutonium-242 tracer (by weight) was dropped on the surface of the sediment sample. The advantages of ^{242}Pu instead of ^{236}Pu as tracer have been adequately discussed [18, 19]. After the tracer had dried, the sample was heated at 400°C for 24 h in a muffle furnace to remove organic matter. The sample was cooled to room temperature, and plutonium was leached with 400 ml of a mixture of concentrated nitric and hydrochloric acids (3 + 1). After the sample had been filtered through Whatman no. 42 paper, the sediment portion was again leached, the leachates were combined, and the remaining sediment residue and filter paper were discarded.

Procedures

After plutonium had been leached from the sediment sample with two 400-ml portions of aqua regia, filtered twice, and the two leachates combined, the solution was boiled down to 100 ml. The solution was diluted to 300 ml with distilled—deionized water, and iron was precipitated by the slow, careful addition of ammonia liquor until precipitation was complete. After centrifugation, the aqueous portion was discarded and the precipitate washed with a dilute ammonia solution (1 + 20). Washing and centrifugation were repeated at least twice to insure the removal of the sulfate moiety (confirmed by adding $BaCl_2$ to a few ml of solution). After the aqueous portion had been discarded, the precipitate was dissolved in a minimum volume of concentrated nitric acid and divided into two equal portions.

Solvent extraction. The acidity of both portions of the solution was adjusted to 8 M following the addition of ca. 100 mg of sodium nitrite and gentle heating of the solution. The sample was cooled to room temperature (ice bath) and transferred to a 250-ml separatory funnel. An equal volume of 20% TLA in xylene, pre-equilibrated with ca. 10 ml of 8 M HNO_3, was added to the separatory funnel, which was shaken gently for 10 min. After the two phases had separated, the aqueous phase was removed and discarded. (To enhance recovery, a second and third extraction proved necessary.) An equal volume of 10 M HCl was added and the separatory funnel was shaken gently for 5 min to remove traces of thorium. After the aqueous phase had been discarded, the organic phase was shaken twice (5 min each time) with an equal volume of nitric acid (1 + 1) to remove uranium. The aqueous phase was discarded and plutonium was back-extracted with 2 M H_2SO_4 by shaking gently for 10 min. Back-extraction with 2 M H_2SO_4 repeated twice, further enhances recovery.

Anion-exchange separation. The other portion of the solution was processed by the ion-exchange technique reported by Chu [12]. The plutonium solution, previously adjusted to 8 M in nitric acid and heated in the presence of sodium nitrite, was loaded onto a 20-ml column containing Bio-Rad AG1-X4 (100—200 mesh) anion-exchange resin pre-equilibrated with nitric acid (1 + 1). Plutonium was eluted from this column with 100 ml of 0.4 M HNO_3—0.01 M HF solution. This solution, after evaporation and subsequent additions of 8 M nitric acid, was loaded onto a smaller (10 ml) column containing the pre-equilibrated AG1-X4 resin. This column was then rinsed consecutively with 10 M HCl and 8 M HNO_3 to remove traces of thorium and uranium, respectively. Plutonium was again eluted with 100 ml of 0.4 M HNO_3—0.01 M HF solution. The solution was evaporated to dryness and the residue was converted to the chloride form prior to electrodeposition by adding several 1-ml portions of concentrated hydrochloric acid and evaporating to dryness after each addition

Electrodeposition. The solution obtained after back-extraction of plutonium with 2 M H_2SO_4 in the extraction procedure was evaporated to dryness. If black organic particles floated during the evaporation, several drops

of concentrated nitric acid and 30% hydrogen peroxide were added to decompose them (during the back-washing of plutonium from the TLA phase, some organic matter was usually entrained with the aqueous phase). The solution thus obtained was evaporated to dryness. The residue containing plutonium was dissolved in 1 ml of 2 M H_2SO_4 and transferred to the plating cell. The beaker was washed twice with 1-ml portions of 2 M H_2SO_4 and the washings were transferred to the cell. Following the addition of one drop of methyl red indicator, concentrated ammonia followed by (1 + 1) ammonia was added dropwise until a yellow color appeared. A few drops of 2 M H_2SO_4 were added to restore the red color and finally 3—4 drops of 2 M H_2SO_4 were added to give the required pH of the plating solution. The platinum anode was positioned 1—2 mm above the plating disc and the plutonium was electroplated at a current of 1.2 A for 1 h. The electrolyte was quenched with 3—4 drops of ammonia solution (1 + 1) at the completion of the plating process. The cell was dismantled and the platinum disc was rinsed with distilled—deionized water followed by ethanol and heated to redness with a Bunsen burner. Plutonium tracer recovery and the isotopic composition were determined by α-spectrometry.

In the case of the ion-exchange technique, plutonium was electroplated from hydrochloric acid solution. The residue containing plutonium was dissolved in 1 ml of 1 M HCl and transferred to the plating cell. The beaker was washed twice with 1-ml portions of 1 M HCl and transferred to the plating cell. The desired pH was achieved as described earlier except that 1 M HCl was used in place of 2 M H_2SO_4. Plutonium was electroplated as described above.

A superior final electroplating procedure [20] is currently being used. Basically, the procedure involves the complete removal of organic compounds entrained in the back-extract by treating with nitric acid and hydrogen peroxide in 1—2 ml of 18 M H_2SO_4; the volume is then reduced to 0.5 ml and the beaker and watch glass are rinsed with 3 ml of distilled—deionized water. After addition of 2 drops of 0.1% thymol blue solution, the solution is adjusted to a yellowish color by addition of concentrated ammonia vapor, which has the advantage of avoiding both an increased plating volume and the possible formation of any local polymeric plutonium at this critical stage. The solution is then placed in the electroplating cell along with three washings of the watch glass and beaker with 0.18 M H_2SO_4. Plutonium is electroplated for up to 2 h at 1.2 A, and then 10 ml of 1.5 M ammonia solution are added to quench the solution. The electrode is removed after a quenching period of 1 min with the power supply still on. The electroplating cell is rinsed with an additional 10 ml of 1.5 M ammonia solution. The planchet is then rinsed, flamed, and counted as usual.

Procedure modifications

Attempts to improve chemical yield were made by extracting plutonium from 3 M HNO_3 solution; by back-extracting plutonium with different

agents including 0.03 M hydroxylammonium chloride in 0.01 M HCl, 0.04 M HNO_3—0.01 M HF, and with 2 M H_2SO_4. Finally, changes were made in the percentage of TLA used, the number of HNO_3 and HCl scrubbings, and the number of back-extractions.

In an accompanying experiment, the leachability of fallout plutonium with aqua regia was tested by splitting one sample into two aliquots. One aliquot was analyzed after being treated only with aqua regia (i.e., by the usual procedure) and the other aliquot was analyzed after being treated with aqua regia followed by dissolution in hydrofluoric acid. The results from the aqua-regia leach and the hydrofluoric acid dissolution were compared for plutonium-239, 240 content.

Standardization

Counting efficiency for the 1000-channel Nuclear Data analyzer supplied with a 300-mm Ortec surface barrier detector was determined by counting a platinum planchet standardized with known amounts of ^{242}Pu (4.90 MeV), ^{239}Pu (5.16 MeV) and ^{238}Pu (5.50 MeV).

The average counting efficiency was 17%; the analyzer energy calibration averaged 5.6 (keV/channel), and background activity in the energy regions of interest was usually less than 0.001 cpm. A further reduction in counter background and simultaneous increase in counting efficiency (26%) was achieved by counting samples in a 16,384-channel Ortec 6240B multichannel analyzer equipped with 8 dual-port α-spectrometric modules.

RESULTS AND DISCUSSION

Comparison of two techniques

The results of repeating the split sample experiment 11 times are shown in Table 1; mean recoveries were not significantly different (two sided "*T*-test" with $\alpha = 0.05$), averaging 35% for solvent extraction and 37% for the ion-exchange procedure. Although the mean recoveries for the two techniques were very similar, the superiority of solvent extraction, in terms of time, cannot be overlooked.

The low variable recoveries from both methods may be due to the very complex matrix of these sediments, including a very high iron concentration as well as other metals [23]. The plutonium-239,240 concentrations reported for the two techniques, treated as paired data, showed no significant difference in the sign test ($p \leqslant 0.01$). The ratios of the plutonium 239,240 contents given by each procedure are sufficiently close to 1.0 (mean ratio = 1.004) to indicate an absence of systematic bias between the two procedures.

The precision of the solvent extraction method was checked by analyzing two or three aliquots of the same sediment specimen separately. Four specimens were analyzed; the individual results (Table 2) fall within one standard deviation (representing the counting error) of each other, indicating that the samples were sufficiently homogenized prior to analysis and

TABLE 1

Comparison of plutonium-242 tracer recoveries and sediment concentrations by TLA and ion-exchange techniques

Sample	Date of collection	TLA extraction		Ion exchange		Ratio of Pu fo TLA/ion excha
		Recovery (%)	Pu found[a] (pCi kg^{-1}, dry)	Recovery (%)	Pu found[a] (pCi kg^{-1}, dry)	
1	11/19/76	9 ± 1	48 ± 9	11 ± 1	49 ± 8	0.98
2	11/19/76	7 ± 1	29 ± 6	61 ± 3	29 ± 3	1.0
3	10/15/76	9 ± 1	13 ± 4	77 ± 4	16 ± 2	0.81
4	11/19/76	50 ± 3	14 ± 3	10 ± 1	23 ± 7	0.61
5	11/19/76	71 ± 3	32 ± 3	34 ± 2	22 ± 4	1.45
6	11/19/76	31 ± 2	26 ± 4	28 ± 2	27 ± 4	0.96
7	8/4/76	69 ± 2	195 ± 11	25 ± 2	178 ± 12	1.10
8	11/19/76	68 ± 2	71 ± 6	38 ± 3	64 ± 8	1.11
9	8/4/76	16 ± 2	236 ± 16	52 ± 5	208 ± 9	1.13
10	8/4/76	12 ± 2	214 ± 18	46 ± 4	221 ± 12	0.97
11	4/29/77	42 ± 2	11 ± 1	28 ± 2	12 ± 2	0.92
		Mean = 35%		Mean = 37%		Mean ratio = 1

[a]Plutonium-239, 240.

TABLE 2

Plutonium concentrations in Hudson River sediments from aliquots of the same sediment sample by TLA extraction

Sample	Sampling station	Date of collection	$^{239,240}Pu$ (pCi kg^{-1}, dry)	^{238}Pu (pCi kg^{-1}, dry)
1A	5	8/4/76	214 ± 18	15 ± 6
1B			195 ± 11	3 ± 1
1C			236 ± 16	9 ± 3
			Mean ± 1σ = 215 ± 21	
2A	2	11/19/76	48 ± 9	2 ± 2
2B			32 ± 7	0.0
			Mean ± 1σ = 40 ± 11	
3A	6	10/15/76	28 ± 7	7 ± 3
3B			18 ± 5	0.0
3C			18 ± 4	2 ± 2
			Mean ± 1σ = 21 ± 6	
4A	3	10/15/76	13 ± 4	1 ± 1
4B			11 ± 6	7 ± 5
			Mean ± 1σ = 12 ± 1	

that the method yields precise results. The overall precision varied between 8 and 28%; clearly most of this could result from counting error.

Repeated analyses by the two techniques showed some interesting characteristics. The major observation was that ca. 10 times as much uranium [α (^{238}U) = 4.2 MeV and α (^{234}U) = 4.77 MeV] found its way through the

solvent extraction process compared to the ion-exchange procedure. Assuming a sediment uranium concentration of 1 pCi g^{-1} [21], the discrimination factor for solvent extraction, incorporating three 7 M HNO_3 washes of the TLA phase, is greater than 90%. As the α-energy difference between ^{234}U and the tracer ^{242}Pu (4.902 MeV) is only 130 keV, it is essential to add sufficient tracer (3—10 dpm) and to obtain a sample planchet with good resolution (<70 keV) in order to minimize any recovery error caused by the spectral interference of uranium-234.

Although traces of polonium-210 (α-energy $\simeq$ 5.30 MeV) survived the ion-exchange procedure, causing some overlap with plutonium 239,240, it was not present in sediments treated by solvent extraction.

Although the presence of thorium-228 (α-energy $\simeq$ 5.43 MeV) has been observed in ion exchange [22] and solvent extraction [13] techniques, it was not found in either of the techniques described here. It appears that washing the TLA phase twice with 10 M or 11 M HCl is sufficient to remove all thorium traces. Figures 1a and 1b provide typical α-spectra from sediment samples analyzed by the solvent extraction and ion-exchange procedures, respectively.

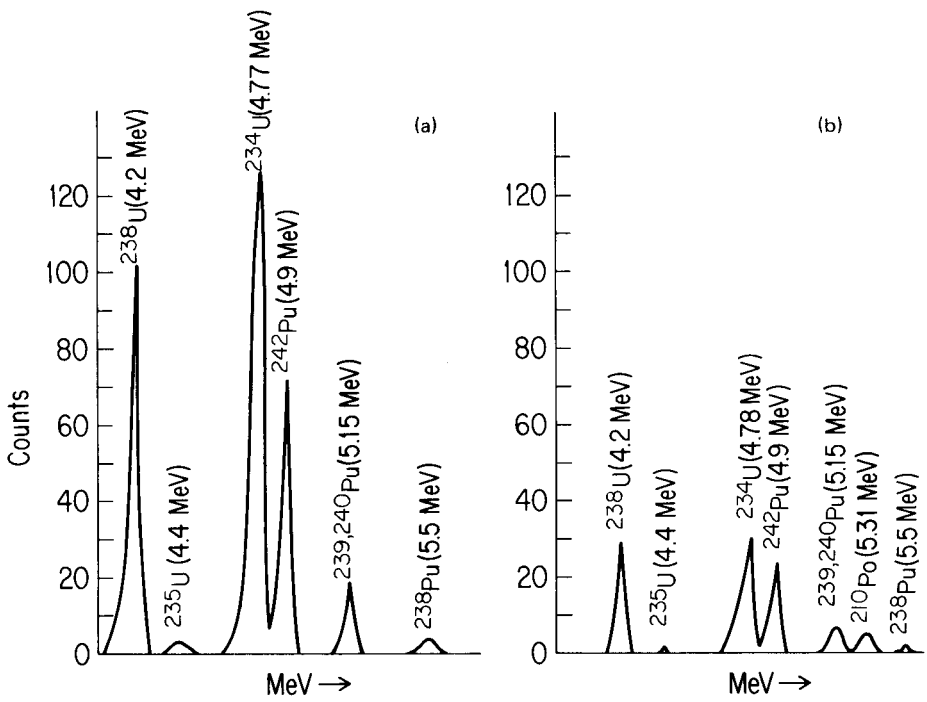


Fig. 1. Typical α-spectra for a sediment sample: (a) after TLA extraction (count time 3784 min, chemical yield 42 ± 2%); (b) after ion-exchange separation (count time 1271 min, chemical yield 25 ± 2%).

The leachability of fallout plutonium

Conflicting views have been presented regarding the physical characteristics of plutonium in the environment. Concern has arisen that some of the plutonium may be in a refractory form which may not be completely leachable from the sediment, thus giving erroneous results. Results of the split sample experiment described, in which one portion of a sample was analyzed by the usual procedure (i.e., leaching) while the remaining portion was analyzed by leaching with aqua regia followed by hydrofluoric acid dissolution, indicated plutonium-239, 240 concentrations of 13 ± 2 and 9 ± 5 pCi kg^{-1} (dry weight), respectively. This preliminary result indicates that both methods are equally efficient for leaching plutonium from these sediments. Similar results were found by Chu [12] when the plutonium concentrations after leaching with aqua regia were compared with an aqua-regia leach followed by a fusion method.

Factors affecting recovery

Unsuccessful attempts were made to improve the recovery of plutonium (single extractions) by extracting from 3 M HNO_3 solutions. The results, summarized in Table 3, indicate that the mean tracer recovery was slightly less (28%) when the extraction was carried out from 3 M HNO_3 instead of 7 M HNO_3 (35%), and more uranium survived the procedure when the extraction was done from 3 M HNO_3.

A further attempt was made to improve tracer recovery by back-extracting plutonium from the TLA phase with a reducing agent such as 0.03 M hydroxylamine hydrochloride in 0.01 M hydrochloric acid medium; for 3 samples the highest recovery was only 34% with no improvement in the variability of percentage recovery (Table 3). With this technique a lot of material persisted even after heating with sulfuric acid—nitric acid mixtures, eventually yielding a planchet with considerable mass and poor resolution. The choice of 2 M H_2SO_4 for back-extracting plutonium was based on the fact that the distribution coefficient for plutonium in 20% TLA from 2 M H_2SO_4 is 0.004 [14], insuring quantitative recovery from the TLA phase; an additional advantage is that entrained organic materials are usually easily decomposed by adding only a few drops of concentrated nitric acid before electrodeposition.

Because chemical yields were highly variable and relatively low, a simple experiment was done which allowed the yield to be checked after each experimental step. It was found that negligible amounts of tracer were lost during the leaching and precipitation phases, and the majority of the plutonium-242 tracer was lost during the extraction procedure. Approximately 3% of the tracer activity that survived the extraction procedure was lost during the combined washings with 8 M HNO_3 and 11 M HCl, and 7% of the tracer activity that survived the entire procedure (i.e., that up to and including back-extraction) was lost during electrodeposition. The final recovery during this experiment was 33%.

TABLE 3

Plutonium tracer recovery and sediment concentrations found by extraction with TLA under different conditions (see text)

Sample	Sample station	Tracer recovery (%)	$^{239,240}Pu$ (pCi kg^{-1}, dry)	^{238}Pu
Extraction from 3 M HNO_3				
1	1	61 ± 3	7 ± 1	0.45 ± 0.05
2	1	19 ± 2	19 ± 4	3.3 ± 2.10
3	6	15 ± 2	18 ± 2	0.0
4	2	18 ± 3	32 ± 7	0.0
		Mean = 28%		
Addition of 0.03 M hydroxylammonium chloride				
1	6	34 ± 4	18 ± 4	1.5 ± 1.6
2	1	9 ± 2	125 ± 20	4.8 ± 6.0
3	3	7 ± 2	11 ± 6	6.6 ± 5.4

There is reason to believe that the high iron concentration (up to 20% w/w) in Hudson River sediments [23] along with other metals may interfere in TLA extraction and ion-exchange techniques.

The presence of organic material persisting after heating at 400°C for 12 h and that contributed from the various filtrations may also be responsible for large tracer losses during extraction.

The current experimental procedure calls for additional ashing of the sediments with nitric acid and hydrogen peroxide after the muffle furnace treatment and prior to leaching. Ashing must be continued before the second leach to remove any organic contribution from the filter paper. Following precipitation and adjustment to a 7 M HNO_3 solution, three extractions of 20% TLA in xylene are recommended, followed by three washings with 7 M HNO_3, to remove uranium and two washings with 10 M HCl to remove thorium. Plutonium is then back-extracted twice with a slightly more selective agent, 0.4 M HNO_3—0.01 M HF, which, compared with the 2 M H_2SO_4 previously used, slightly decreased the amount of uranium and iron stripped from the organic phase. The back-extracting solution was evaporated and ashed completely with H_2SO_4, HNO_3 and H_2O_2 before electroplating according to the previously outlined procedure utilizing thymol blue. The resulting planchets were clean and free of mass. This modified technique has reduced the recovery variability and increased the overall mean recovery to about 40%.

REFERENCES

1 Health and Safety Laboratory, Manual of Standard Procedures, 1976.
2 H. Levine and A. Lamanna, Health Phys., 11 (1965) 117.

3 P. J. Mango, P. E. Kauffman and B. Schleien, Health Phys., 13 (1967) 1325.
4 E. E. Campbell and W. D. Moss, Health Phys., 11 (1965) 737.
5 K. Wolfsberg, W. R. Daniels, G. P. Ford and E. T. Hitchcock, Nucl. Appl. Technol., 3 (1967) 375.
6 M. C. deBortoli, Anal. Chem., 39 (1967) 375.
7 K. C. Pillai, R. C. Smith and T. R. Folson, Nature, 203 (1964) 568.
8 E. L. Geyer, Health Phys., 1 (1959) 405.
9 D. B. James, Los Alamos Scientific Laboratory Ref. TID 4500, January 1967.
10 I. K. Kressin and G. R. Waterbury, Anal. Chem., 34 (1962) 1598.
11 R. F. Buchanan, J. P. Ferris, K. A. Orlandini and T. P. Hughes, U.S.A.E.C. Ref. TID 7560 (1958) 179.
12 N. Y. Chu, Anal. Chem., 43 (1971) 449.
13 J. C. Veselsky, Int. J. Appl. Radiat. Isot., 27 (1976) 499.
14 N. Srinivasan, M. V. Ramaniah, C. L. Rao, P. K. Khopkar, G. M. Nair and N. P. Singh, Bhabha Atomic Research Centre, Report no. 374 (1968).
15 N. P. Singh, S. A. Ibrahim, N. Cohen and M. E. Wrenn, Anal. Chem., 50 (1978) 357.
16 N. P. Singh, S. A. Ibrahim, N. Cohen and M. E. Wrenn, Anal. Chem., 51 (1979) 207.
17 D. N. Edgington and J. A. Robbins, Proceedings of an International Symposium on Radiological Impacts of Release from Nuclear Facilities in Aquatic Environment, held by IAEA, IAEA/SM-198/40 (1975) 245.
18 C. W. Sill, Health Phys., 29 (1975) 619.
19 I. D. Kressin, W. D. Moss, E. E. Campbell and H. F. Schulte, Health Phys., 28 (1975) 41.
20 N. A. Talvitie, Anal. Chem., 43 (1971) 1827.
21 New York State Department of Environmental Conservation, Annual Report of Environmental Radiation in New York State, 1975.
22 H. J. Simpson and S. C. Williams, Annual Technical Progress Report prepared for ERDA, Doc. COO-2529-1 (1975).
23 A. W. McCrone, Geogr. Rev., LVI(2) (1966) 175.

Analytica Chimica Acta, 111 (1979) 275—279

Short Communication

POLAROGRAPHIC ANALYSIS FOR CORTICOSTEROIDS
Part 3. Determination of Corticosteroids in Single-Component Tablets

H. S. DE BOER, P. H. LANSAAT, K. R. KOOISTRA and W. J. VAN OORT*

Department of Analytical Pharmacy, Faculty of Pharmacy, State University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht (The Netherlands)

(Received 23rd April 1979)

Summary. The differential pulse polarographic determination of corticosteroids in single-component tablets is described. The active compounds are extracted from the excipients with a solvent with similar lipophilicity as the polarographic solvents, with a yield of about 100%. The corticosteroid esters and alcohols are determined by standard addition methods. The standard deviations range from 0.5 to 2.5% depending on the excipients and on the method of extraction.

In part 2 [1] of this series, the differential pulse polarographic (d.p.p.) determination of corticosteroids in solutions, suspensions, ointments and creams was described. This Part deals with the determination of corticosteroids in tablets. In pharmacopoeias, a colorimetric method based on extraction of an aqueous solution (or suspension) of the tablet with chloroform and derivatization of the corticosteroids with tetrazolium blue is usually recommended. The absorbance is determined at 485 nm and compared with the absorbance of standard solutions. In practice, only experienced analysts can achieve acceptable standard deviations (see, e.g. [5]). Even in its improved forms [6—8], the method is time-consuming. Brower [9] semi-automated the procedure and obtained standard deviations of about 1%. A direct photometric determination based on the absorption band at 240 nm has been described [10] but its poor specificity has been mentioned by Görög and Szász [11].

In this work, an attempt was made to apply the minimal clean-up procedure described for the assay of "liquid" corticosteroid formulations [1] to single-component tablets. The lipophilicity of the polarographic solvents described previously [1, 12] and of the corticosteroids (3 hydroxyl groups and 2 carboxyl groups) allows preliminary procedures similar to those in reversed-phase liquid chromatography to be used, i.e. dissolution and centrifugation of the sample; the supernatant liquid can then be used directly for the determination [13—15]. In liquid chromatography an internal standard is needed. The main difference from the assay of "liquid" formulations is the necessary phase transference of the corticosteroids. The desorption and dissolution depend on the adsorptive forces, the kind and size of the granules [16], and the solubility of the steroid and excipients. Complete disintegration and/or dissolution

of the tablet need an aqueous solution, whereas complete dissolution of corticosteroids needs a non-aqueous solution. In practice, the optimum is methanol or ethanol. Yadav and Teare [17] dissolved the tablet mass in ethanol or methanol before mixing with Sørensen buffer pH 5.6 for d.p.p. Cantin et al. [18] applied a two-phase extraction of progesterones in tablets before d.p.p. Chatten et al. [19] determined progesterones in tablets by d.p.p. in DMF or ethanol, but needed a time-consuming pre-concentration step. Only Deys and van Pinxteren [20] dissolved the tablet directly in the (aqueous) polarographic solution before d.c. polarography.

In tablet analysis, either several tablets, e.g. 20, are powdered and vigorously mixed and a portion is analysed, or one tablet is powdered and the whole is analysed. In the latter method the variation per tablet can be measured for content uniformity tests. In the former method, the standard deviations are influenced only by chemical and instrumental parameters [17, 19] whereas in the second method the standard deviation includes the deviation per tablet [20, 21]. The necessity of procedures suitable for single tablets is illustrated by the results of Steinigen and Brüne [22] on the uniformity of prednisolone, prednisone, and dexamethasone tablets. In the single-tablet assay proposed here, the standard addition method applied avoids the addition of the (mostly unknown) excipients to the calibration solutions [21], and differential pulse polarography offers the sensitivity required.

Experimental

Apparatus and chemicals. The d.p.p. curves were recorded with a PAR 174 polarograph and a Brucker E310 modular electrochemical system, both of which were equipped with a drop timer, a Houston 2200-3-3 X-Y recorder, and a Metrohm Polarecord E506 with polarographic stand E505. The instrument settings were: scan rate 2 mV s^{-1}, drop time 2 s, modulation amplitude 100 mV.

A water-jacketed (20 ± 0.2°C) 10-ml polarographic cell (Metrohm EA880-T-5) was employed with a dropping mercury electrode, a Metrohm EA432 Ag/AgCl reference electrode and a platinum wire auxiliary electrode. The salt bridge of the reference electrode was filled with the supporting electrolyte. A piston microburette (Metrohm E457) was used for standard additions.

The reagents for the supporting electrolytes were methanol (Nanograde, Mallinckrodt), methanol (zur Analyse) and tetramethylammonium hydroxide (TMAH, 10% zur Polarographie, Merck). All reagents were used without further purification.

Procedures. A d.p.p. curve of 10 ml of 0.03 M TMAH in methanol was recorded after deaeration with oxygen-free nitrogen [23] for 15 min. Then a suitable amount of the sample (usually in 1 ml of solvent) was added, to give a concentration of 10^{-3}—10^{-5} M steroid. After deaeration for 1 min, the polarogram was recorded. A small volume of a standard solution of the pure steroid in methanol (containing about the same amount of steroid as the sample) was then added and, after deaeration for 1 min, the polarogram was

again recorded. Dilution on addition of the standard should not exceed 1%, otherwise a correction is needed.

Two methods were tested for extraction of the active compounds from the tablet. In Method 1, one tablet was mixed with a suitable amount of methanol (usually 10.00 ml) and disintegrated by ultrasonic vibration for 5 min. Most of the commercially available tablets disintegrated completely and the yield of the corticosteroid was about 100%. A suitable aliquot of the solution was used for polarography.

In Method 2, the tablet was mixed with 1.00 ml of water and, after disintegration, the slurry was mixed with 9.00 ml of methanol to dissolve the corticosteroid. A correction was necessary for contraction on mixing, because the final volume was only 9.85 ml.

Results and discussion

Table 1 shows the results of the analysis of corticosteroid-containing tablets. The tablets disintegrate in water but the corticosteroids do not dissolve to any great extent. For complete desorption, a solvent such as methanol is needed but then all the granular material may not disintegrate completely; relatively large particles can remain in sample solutions even after ultrasonic vibration for 30 min. Of the two extraction methods described under Experi-

TABLE 1

Assay of the dosage forms and standard deviations based on 5 determinations with one tablet per determination

Name	Source	Active constituent	Content (mg)	Found (mg)	Method	R.s.d. (%)
Adreson	Organon	cortisone acetate[a]	25	25.0	1	1.8
Cortisone Acetate	Organon	cortisone acetate[a]	25	24.7	1	1.4
			25	24.9	2	0.8
Hydrocortisone	Organon	hydrocortisone[a]	20	20.2	2	0.8
Betnelan	Glaxo	betamethasone[a]	0.5	0.50	1	1.9
				0.51	2	1.7
Prednisolone	Organon	prednisolone[a]	5	5.0	1	1.5
				4.8	2	1.7
Prednisone	Organon	prednisone[a]	5	5.0	1	0.9
				4.8	2	2.2
Prednisone WSG	Organon	prednisone[a]	5	5.0	1	0.5
				4.8	2	1.2
Decadron	MSD	dexamethasone[a]	0.5	0.50	1	2.7
				0.43	2	7
Ledercort	Lederle	triamcinolone[b]	2	1.9	1	12
				1.4	2	10
Ledercort	Lederle	triamcinolone[b]	8	8.0	1	0.7
				6.9	2	1.9
Kenakort	Squibb	triamcinolone[b]	4	3.9	1	1.1

[a]Obtained in pure form from Nogepha. [b]Obtained in pure form from Lederle.

mental, Method 1 is preferable, if applicable. Method 2 needs extra manipulations and the extraction seems to be less complete after contact of the corticosteroids with water (see Table 1). Ultrasonic vibration after mixing methanol and water sometimes increases the yield [24].

Analysis of different types of tablet

Triamcinolone tablets. The Ledercort tablets containing 2 mg of triamcinolone gave a large standard deviation which included instrumental and chemical parameters as well as content uniformity. To reduce the effect of content non-uniformity, 10 tablets were powdered and 5 samples of this powder were analysed by Method 1; the standard deviation was then 0.9%. Obviously, the content uniformity of these tablets was not good. The high adsorptivity of triamcinolone was illustrated by the results of a vigorous powdering procedure with a mortar and pestle of triamcinolone 2-mg tablets: Method 1 gave a content of only 1.6 mg. A similar experiment with Ledercort 8-mg tablets gave a result of 6.5 mg per tablet. It seems that mechanical forces can make the adsorption of this rather hydrophilic steroid to the tablet excipients irreversible [11].

Hydrocortisone tablets. The linear diffusion current—concentration range of hydrocortisone is shorter than that of other corticosteroids: 10^{-4}—10^{-5} M hydrocortisone. The sample and standard solutions were adjusted to lie within this range. This limitation is being investigated.

Betamethasone and dexamethasone tablets. The differential pulse polarogram of dexamethasone in 0.03 M TMAH in methanol in the range of 10^{-6}—10^{-5} M have been presented [12]. The first reduction peak, caused by pinacol formation, decreases with decreasing concentration; the second peak, caused by alcohol formation, which in the lower concentration range slightly overlaps the first peak, is scarcely affected by decreasing concentration. Consequently, the calibration plot of the first peak deviates slightly from linearity in this range. The fluorine-containing corticosteroids betamethasone and dexamethasone are administered in such small amounts that the standard addition method can be applied only with an experimentally determined slightly bent calibration plot.

Cortisone acetate tablets. Formulations of cortisone acetate containing aerosil gave lower results than those without aerosil (about 12%). Addition of aerosil to pure cortisone acetate confirmed this. The highly adsorptive character of the surface of the aerosil particles (silicon oxides) must be the cause.

The authors thank Organon International B.V., for kindly supplying some of the preparations and Dr. H. Hindriks and Mr. J. van Gorp (Organon) for helpful discussions.

REFERENCES

1 H. S. de Boer, P. H. Lansaat and W. J. van Oort, Anal. Chim. Acta, 108 (1979) 389.
2 British Pharmacopoeia 1973, The Pharmaceutical Press, London.
3 The United States Pharmacopoeia, 19th rev., Mack Publishing, Easton, 1975, p. 622.
4 Nederlandse Farmacopee, 8th edn., Staatsuitgeverij 's-Gravenhage, 1978.
5 J. P. Bauwens and G. N. Logghe, Pharm. Weekbl., 111 (1976) 633.
6 R. E. Graham, E. R. Biehl and C. T. Kenner, J. Pharm. Sci., 67 (1978) 792.
7 R. E. Graham, E. R. Biehl and C. T. Kenner, J. Pharm. Sci., 65 (1976) 1048.
8 F. M. Kunze and J. S. Davis, J. Pharm. Sci., 53 (1964) 1259.
9 J. F. Brower, J. Assoc. Off. Anal. Chem., 52 (1969) 842.
10 Pharmacopoeia of Japan VIII, English edn., Soc. Jpn. Pharmacopoeia, 1973, part I.
11 S. Görög and G. Szász, Analysis of Steroid Hormone Drugs, Elsevier, Amsterdam, 1978.
12 H. S. de Boer, J. den Hartigh, H. H. J. L. Ploegmakers and W. J. van Oort, Anal. Chim. Acta, 102 (1978) 141.
13 M. D. Smith and D. J. Hoffman, J. Chromatogr., 168 (1979) 163.
14 R. E. Huettemann and A. P. Schroff, J. Chromatogr. Sci., 13 (1975) 357.
15 K. R. Bagon and E. W. Hammond, Analyst, 103 (1978) 156.
16 P. H. Cox, T. J. G. Ambaum and H. P. Wijnand, J. Pharm. Pharmacol., 20 (1968) 238.
17 R. N. Yadav and F. W. Teare, J. Pharm. Sci., 67 (1978) 436.
18 D. Cantin, J. Alary and A. Coeur, J. Pharm. Belg., 32 (1977) 255.
19 L. G. Chatten, R. N. Yadav, S. Binnington and R. E. Moskelyk, Analyst, 102 (1977) 323.
20 H. P. Deys and J. A. C. van Pinxteren, Pharm. Weekbl., 93 (1958) 760.
21 L.-N. Opheim, Anal. Chim. Acta, 89 (1977) 225.
22 M. Steinigen and B. Brüne, Pharm. Ztg., 121 (1976) 990; 122 (1977) 1978.
23 L. Meites, Polarographic Techniques, 2nd edn., Interscience, New York, 1964, p. 87.
24 H. Hindriks, personal communication.

Analytica Chimica Acta, 111 (1979) 281—285

Short communication

FLOW-INJECTION DETERMINATION OF MEPTAZINOL WITH ELECTROCHEMICAL DETECTION

H. K. CHAN*

Wyeth Laboratories, Huntercombe Lane South, Maidenhead, Berkshire, SL6 0PH (Gt. Britain)

A. G. FOGG

Department of Chemistry, Loughborough University of Technology, Loughborough, Leicestershire, LE11 3TU (Gt. Britain)

(Received 16th May 1979)

Summary. A flow-injection analysis system incorporating a glassy carbon voltammetric detector cell is described. Meptazinol (0.01—10 μg ml^{-1}) can be determined by electrochemical oxidation in a carrier stream of 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol at sampling rates up to 80 samples per hour.

Previous studies [1, 2] have shown that several phenolic analgesics and two anti-inflammatory drug compounds undergo electrochemical oxidation at a glassy carbon electrode in 0.1 M sodium acetate—0.1 M acetic acid in 98% ethanol. Although the voltammetric procedure developed for the determination of these drugs and their pharmaceutical dosage forms was simple and rapid, it lacked sensitivity for the determination of one of the phenolic analgesics, meptazinol [*m*-(3-ethyl-1-methyl-hexahydro-1H-azepin-3-yl)-phenol hydrochloride], in biological fluids where concentrations are usually at the pg ml^{-1} level.

A gas—liquid chromatographic procedure has been described [3] for determination of the drug in plasma after extraction, but this requires at least 3 ml of plasma to achieve a detection limit of 30 ng ml^{-1}. A liquid scintillation counting procedure [4] has been used for pharmacokinetic and metabolic studies of the drug but this gives a detection limit of only 20 ng ml^{-1}.

In view of the lack of a simple, selective and sensitive method of determining meptazinol, it was decided to investigate an adaptation of the static voltammetric method [2] to hydrodynamic voltammetry [5] by using the flow-injection technique of Růžička and co-workers [6, 7]. A commercially available system [8] was modified; this system comprises a flow-injection block and a glassy carbon electrochemical detector based on the wall-jet principle [9, 10]. Small volumes (5—10 μl) of sample solution are injected into a carrier stream of supporting electrolyte, and the anodic current is monitored as the sample passes the electrode surface and the depolarizer is oxidized at a suitable applied potential. As most of the sample solution

hits the surface of the glassy carbon electrode, approximately 80% of the depolarizer is oxidized and the method is extremely sensitive: picogram amounts of an electroactive substance can be detected at detector volumes of only about 2 μl.

The present communication describes a flow injection—voltammetric procedure for the determination of meptazinol at the 100-pg level. Modifications to the manufacturer's instructions are described which improve the reliability of the technique.

Experimental

Apparatus. A commercially available Flow-Injection Stand (Metrohm E634) was used with some modification. The electrochemical detector cell (EA 1069/2) was housed separately in a metal container (30-cm^3 capacity) to cut out external signals. A hole was drilled to introduce an 80-cm length of Teflon tubing (1.5/0.3 mm) between the injection block and the detector cell to ensure a sufficient time-lag between injection of the sample and the appearance of the corresponding current peak. The glass bottle containing the supporting electrolyte was connected directly to a nitrogen cylinder which provided a constant pressure of 1 bar to give a steady flow of supporting electrolyte of 1.2 ml min^{-1} through the detector cell.

A PAR 174A polarographic analyser (Princeton Applied Research Corp.) was used to control the applied potential and to measure the resulting current with a sensitivity of 10 V output for a 20-nA signal. A Servoscribe recorder (Model RE511.20) fitted with an attenuator (two 10 kΩ resistors in series across the terminals) was used to give a full scale deflection of 10 V on the 5-V range; the chart speed was 600 mm h^{-1}.

The conventional three-electrode configuration used comprised a working glassy carbon electrode (EA 286/1, nominal surface area 0.18 cm^2), a platinum counter electrode (EA 286/2) and a silver—silver chloride reference electrode (EA 442). The reference electrolyte was 1 M lithium chloride in ethanol presaturated with silver chloride; with this electrolyte the potential for oxidation of the drug in the recommended supporting electrolyte was sufficiently reproducible over long periods of time. In order to achieve smooth baselines, the reference electrode had to be placed downstream adjacent to the effluent outlet of the detector cell.

Injections were made with a 10-μl microsyringe (Scientific Glass Engineering Pty. Ltd.).

Reagents. The purity of meptazinol hydrochloride (Wyeth Laboratories) was confirmed by thin-layer chromatography. Other reagents were of analytical-reagent grade.

For the supporting electrolyte, dissolve 8.2 g of anhydrous sodium acetate in 20 ml of distilled water, add 5.8 ml of glacial acetic acid and dilute to 1 l with absolute ethanol; filter through a Fluoropore 0.2-μm filter prior to use.

For the standard meptazinol hydrochloride solution, dissolve 100 mg of meptazinol hydrochloride in 100 ml of 95% ethanol.

Calibration graphs. Prepare working solutions of meptazinol hydrochloride (0.01—10 μg ml^{-1}) by dilution of the 1 mg ml^{-1} standard with 95% ethanol. Inject 10-μl aliquots under the instrumental conditions described below, and plot graphs of peak height (nA) versus concentration of the injected samples (ng).

Procedure. Potentiostat the working electrode at +1.2 V versus the recommended silver—silver chloride electrode for 20 min in a flowing stream of 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol (1.2 ml min^{-1}) at a current sensitivity of 5 μA. Set the detector potential at +1.03 V before adjusting to the required current sensitivity in the range 0.05—0.5 μA f.s.d. Inject 10 μl of meptazinol solution and measure the peak heights.

Results and discussion

In flow-injection analysis with this wall-jet electrochemical detector, it is important to ensure that the flow rate is constant and pulse-free. A constant flow rate of 1.2 ml min^{-1} was achieved by using Teflon tubing of 0.3 mm i.d. at a constant nitrogen pressure of 1 bar.

Adsorption occurs at the glassy carbon electrode surface after repeated injections of meptazinol solutions, and the surface was reconditioned by cycling the potential between cathodic and anodic limits until the original characteristics were restored. The working electrode was first potentiostated at −0.2 V for 5 min at a current sensitivity of 5 μA and then at +1.2 V for 5 min in a flowing stream of the recommended supporting electrolyte. Subsequently, the potential of the detector was set at +1.03 V, and the peak heights obtained for meptazinol were identical to those obtained initially. Adsorption effects were apparent after about 40 injections.

A linear-sweep voltammogram for the oxidation of meptazinol (50 μg ml^{-1}) in a quiescent solution of 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol with the recommended three-electrode system is shown in Fig. 1. The current—potential curve shown in Fig. 2 depicts a typical sigmoidal hydrodynamic voltammogram with a well-defined plateau region, analogous to classical d.c. polarography and rotating electrode voltammetry. This was constructed from the peak currents obtained by 10-μl injections of meptazinol solution (20 μg ml^{-1}) into the flow stream at each applied potential. The optimum potential of +1.03 V was determined from this curve.

Calibration graphs of peak current (nA) against the amount of depolarizer (ng) in the injected samples were rectilinear over the range 0.01—10 μg ml^{-1} under the conditions recommended.

The stability of the detector cell was checked; neither the potential of the reference electrode nor the performance of the working electrode changed during the course of the experiments. This is in contrast to initial results obtained by using the manufacturer's instructions. It is essential that the reference electrode be placed after the detector electrode in the flowing stream. Further, large drifts in potential occurred when the internal reference solution in the reference electrode was not presaturated with silver chloride.

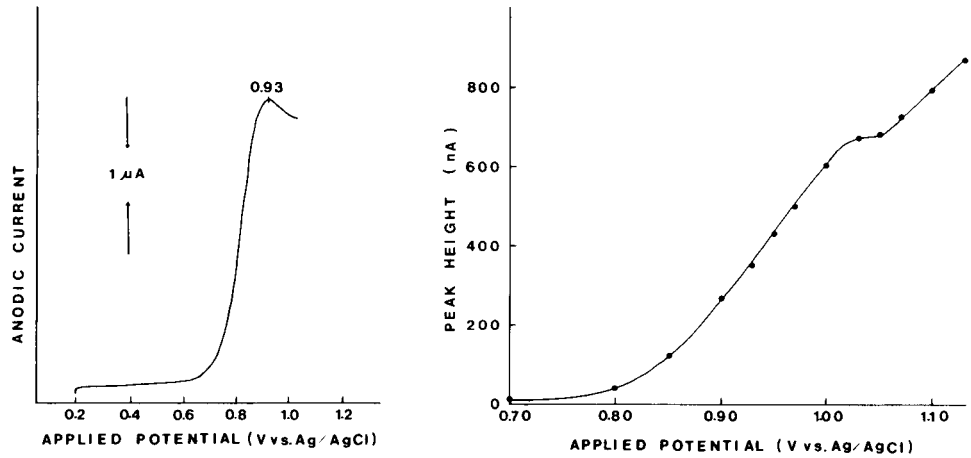


Fig. 1. Typical linear-sweep voltammogram of meptazinol (50 μg ml^{-1}) in 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol at the glassy carbon electrode. Scan rate, 5 mV s^{-1}.

Fig. 2. Hydrodynamic current—potential curve for meptazinol (20 μg ml^{-1}) in a flowing stream of 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol. Flow rate 1.2 ml min^{-1}; sample injected 10 μl..

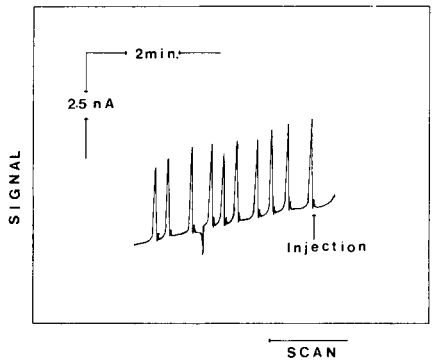


Fig. 3. Current vs. time curves for meptazinol (0.01 μg ml^{-1}) in a flowing stream of 0.05 M sodium acetate—0.1 M acetic acid in 98% ethanol. Potential + 1.03 V vs. Ag/Ag Cl; flow rate 1.2 ml min^{-1}; current sensitivity 2.5 nA cm^{-1}; sample injected 10 μl (i.e. each peak represents 100 pg of meptazinol); chart speed 60 cm h^{-1}.

Figure 3 shows ten 10-μl injections of 0.01 μg ml^{-1} meptazinol solution (i.e. 100 pg per injection) at a sampling rate of up to 80 injections per hour; the coefficient of variation was 7.2%. The high sensitivity is due to efficient mass transfer and the fact that at constant applied potential charging current is eliminated. The application of this procedure to the determination of meptazinol in biological fluids following a simple clean-up procedure, is under study, but low concentrations require a chromatographic separation prior to electrochemical detection.

REFERENCES

1 H. K. Chan and A. G. Fogg, Anal. Chim. Acta., 105 (1979) 423.
2 H. K. Chan and A. G. Fogg, Anal. Chim. Acta, 108 (1979) 205.
3 M. T. Rosseel, M. G. Bogaert, F. M. Belpaire and W. Oosterlinck, Curr. Med. Res. Opin., 3 (1975) 181.
4 R. A. Franklin, A. Aldridge and C. de B. White, Br. J. Clin. Pharmac., 3 (1976) 497.
5 V. G. Levich, Physicochemical Hydrodynamics, Prentice-Hall, Englewood Cliffs, N.J., 1962.
6 J. Růžička and E. H. Hansen, Anal. Chim. Acta, 78 (1975) 145.
7 J. Růžička and J. W. B. Stewart, Anal. Chim. Acta, 79 (1975) 79.
8 P. Gilgen and P. Rach, Chimia, 32(9) (1978) 345.
9 J. Yamada and H. Matsuda, J. Electroanal. Chem., 44 (1973) 189.
10 B. Fleet and C. J. Little, J. Chromatogr. Sci., 12 (1974) 747.

Analytica Chimica Acta, 111 (1979) 287—289

Short Communication

INTERFERENCE EFFECT IN THE ATOMIC ABSORPTION SPECTROMETRIC DETERMINATION OF ARSENIC IN FILTER-COLLECTED AIR SAMPLES

GIOVANNI CARELLI*, ANGELO IANNACCONE, ROBERTO LA BUA and VALENTINO RIMATORI

Institute of Occupational Medicine, Faculty of Medicine, Università Cattolica del Sacro Cuore, Rome (Italy)

(Received 20th June 1979)

Summary. The arsenic response obtained by arsine generation is decreased by the presence of the filter digest; a period of 25 min after mixing is needed before added arsenic gives a constant response.

Atomic absorption spectrometry has become the method of choice for the determination of arsenic in many matrices such as water, soil, food and air. Several authors have shown that significant interfering effects occur in many instances, for many atomic absorption techniques involving flame [1] or graphite-tube atomization [2—4] and hydride generation with argon—hydrogen flames [5] or heated quartz-tube [6] atomization.

A particular depressive interference has been found to occur during the determination of airborne particulate arsenic collected on cellulose membrane filters; this is caused by degradation products of the filter on alkaline digestion. This communication describes a study of this effect based on the addition of arsenic to the alkali-digested filter solution. Every three minutes a portion of this sample was reduced by sodium tetrahydroborate and analyzed by heated quartz-tube atomic absorption spectrometry.

Experimental

Apparatus and reagents. A Perkin-Elmer Model MHS-1 was used for hydride generation. The analysis program selected was Hyd I and the quartz tube temperature was 1000°C. A Perkin-Elmer Model 403 atomic absorption spectrometer was used (at 193.7 nm) with a Hitachi Model 56 potentiometric recorder (2.5 mV f.s.d.). The radiation source was an arsenic electrodeless discharge lamp run at 10 W.

The sodium tetrahydroborate solution was prepared by dissolving 5 g of the salt and 2 g of sodium hydroxide in water and diluting to 100 ml. Demineralized water was used throughout. A 1000 μg ml^{-1} arsenic (III) solution was used for the standard additions.

Samples. The following samples were prepared for analysis:

(*A*) The standard arsenic solution (10 μl) was diluted to 10 ml with water.
(*B*) Sodium hydroxide solution (5 ml of 5% w/v), 10 μl of standard arsenic solution and a membrane filter were introduced into 100 × 30 mm quartz, polypropylene or Pyrex glass test-tubes. The tubes were transferred to a heated block at 100°C, and digestion was continued at 100°C for 1 h, before dilution to 10 ml with water.
(*C*) As for (*B*) but excluding the arsenic addition.
(*D*) As for (*B*) but excluding the filter.
(*E*) As for (*D*) but excluding the arsenic addition.
(*F*) Standard arsenic solution (10 μl) was added to 10 ml of solution (*C*). The resulting arsenic concentration was 1 μg ml^{-1} plus the arsenic concentration of solution (*C*).

Arsenic determination. A 25-μl aliquot of a sample was placed in the hydride generator vessel, and 10 ml of 3% hydrochloric acid and 2.5 ml of 2% sodium hydroxide—5% sodium tetrahydroborate were added. The arsine evolved was measured by the recommended atomic absorption procedure.

In order to study the effect of storage time with the membrane filter digest, 20-μl aliquots of solution (*F*) were analyzed every 3 min. The signal was corrected for the blank obtained by analyzing 25 μl of solution (*C*).

Results and discussion

Figure 1 shows the decrease of arsenic peak heights as a function of time elapsed from arsenic addition. The initial absorbance was obtained by analyzing solution (*A*). The graph shows that the peak heights become constant at 65% of the initial absorbance after 25 min for all three tube materials tested. The final value is the same as that obtained by analyzing solutions (*B*). The analysis of solutions (*D*) and (*A*) confirms that this effect is due only to the filter and does not depend on the test-tube material.

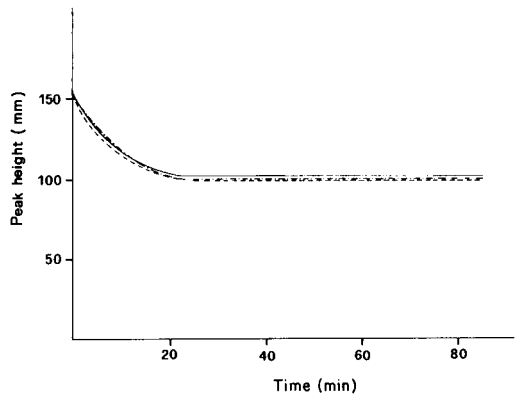


Fig. 1. Typical absorbance decrease for arsenic; 25 μl of solution (*F*) was analyzed in quartz (···), polypropylene (—) and Pyrex (---) glass.

The results clearly show that the standard addition method for determination of arsenic on membrane filters is reliable only if a period of at least 25 min has elapsed after arsenic addition to the sample digest. In order to understand better the matrix interference shown here, further studies are in progress.

REFERENCES

1 H. L. Kahn and J. E. Schallis; At. Absorpt. Newsl., 7 (1968) 5.
2 B. B. Mesman and T. C. Thomas. Anal. Lett., 8 (1975) 449.
3 M. McDaniel, A. D. Shendrikar, K. D. Reiszner and P. W. West, Anal. Chem., 48 (1976) 2240.
4 H. Freeman, J. F. Uthe and B. Flemming; At. Absorpt. Newsl., 15 (1976) 49.
5 A. E. Smith, Analyst, 100 (1975) 300.
6 F. D. Pierce and H. R. Brown, Anal. Chem., 49 (1977) 1417.

Analytica Chimica Acta, 111 (1979) 291—295

Short Communication

ELECTROTHERMAL ATOMIC ABSORPTION DETERMINATION OF TOTAL ALUMINIUM (INCLUDING ZEOLITE TYPE A) IN WATERS AND WASTE WATERS

M. J. T. CARRONDO, J. N. LESTER and R. PERRY*

Public Health Engineering Laboratory, Imperial College, London SW7 2AZ (Gt. Britain)

(Received 6th April 1979)

Summary. A previous procedure involving sample homogenization (where necessary) followed by direct electrothermal atomic absorption determination of aluminium is extended to accommodate the presence of an aluminosilicate, zeolite type A, used as a detergent builder, in sewage, sewage sludge, water or waste-water samples.

Among the commonly occurring elements, aluminium is one of the most difficult to determine chemically because of the lack of specific reagents and the common incidence of interfering elements [1]. Surfactants have been shown to interfere in the generally accepted spectrophotometric methods [2]. Atomic emission and absorption determinations are more selective and less prone to interference. Because of their increased sensitivity and capability for in situ sample pretreatment, electrothermal atomic absorption procedures are increasingly being applied to the determination of aluminium in environmental samples [3—5]. Such a procedure, involving homogenization as the only pretreatment, has been applied to the determination of aluminium in sewage sludges [6], sewages and final effluents [7]. The total concentrations of aluminium in aqueous samples may further be increased by the introduction of zeolite type A, an aluminosilicate, as a detergent builder [8]. This communication describes the extension of the electrothermal atomization procedure to the determination of total aluminium in waters and waste waters containing zeolite type A.

Experimental

Instrumental. For flame analyses, a Perkin-Elmer model 603 atomic absorption spectrometer was used with a deuterium background corrector and a nitrous oxide—acetylene reducing flame. In order to suppress the ionization of aluminium, samples and standards were made upto 2 mg ml^{-1} in potassium chloride [9]. For electrothermal analyses, the same spectrometer was employed in conjunction with a Perkin-Elmer HGA 76 heated graphite atomizer, with argon as the inert gas. The conditions were: sample injected, 20 μl; drying, 100°C for 30 s; two stage chaining with ramping from 100°C to 400°C in 45 s followed by isothermal ashing at 1200°C for 30 s; atomization

at 2770°C for 8 s. The insensitive line at 257.5 nm, and a spectral bandwidth of 0.2 nm were generally used for electrothermal analysis. For low concentrations of aluminium and for flame analysis, the line at 309.3 nm and a spectral bandwidth of 0.7 mm were used.

Reagents. Water distilled in a glass system was used throughout. Aristar-grade reagents were used to minimize blank values.

A standard stock solution of aluminium (1000 mg l^{-1}) was made up by dissolving analytical-reagent grade aluminium metal in a minimum amount of hydrochloric acid and diluting to volume. Working standards were prepared by suitable dilution with 1% (v/v) nitric acid.

Zeolite type A (Degussa, Wasseleing, F.R.G.) has a typical oxide formula of $Na_2O{\cdot}Al_2O_3{\cdot}2SiO_2{\cdot}4.5H_2O$. However, because of possible occlusion of $NaAlO_2$ in the structure, the Si:Al ratio can vary from 0.7 to 1.2 [10]. The composition of the batch used was 17.8% Na_2O, 31.3% SiO_2, 28.5% Al_2O_3 (15.1% Al) and 22.4% H_2O.

Sample pretreatment. Samples to be analyzed by the electrothermal procedure were homogenized as described previously [6, 7]. Calibration was carried out with the standards in 1% (v/v) nitric acid.

A nitric—perchloric—hydrofluoric acid digestion method [6, 7] was used to dissolve the samples for flame analyses.

Results and discussion

Influence of graphite tube age on sensitivity. Initial results obtained by electrothermal analysis indicated that variations in sensitivity occurred when peak heights were measured. It was thought that these were due to tube age and that the sensitivity increased with age. Under otherwise identical conditions, absorbances for 10 μg Al ml^{-1} solutions measured at 257.5 nm varied between 0.22 and 0.25 for a new tube and between 0.34 and 0.36 with a tube that had had 60 to 70 firings. This meant that standards had to be run after every 4—5 injections, rather than the usual 10—15 injections for other elements. This effect can probably be explained by Fuller's kinetic theory of atomization [11]. Usually, 100 to 120 firings were obtained from each tube.

The calibration curve obtained with triplicate determinations of aqueous acidic standards in an old tube is presented in Fig. 1. The sensitivity (i.e. the concentration giving 0.0044 absorbance) was 0.1 μg ml^{-1} for the 20 μl injected (or 2 ng) when an old tube was used.

Zeolite dissolution. Some zeolites, in particular zeolite type A, are readily decomposed by acids [10]. Therefore, zeolite type A was dissolved in 1% (v/v) nitric acid, which is used in this laboratory to stabilize metals in wastewater samples. The electrothermal atomic absorption procedure was used to determine whether complete recoveries of aluminium were achieved, by using various additions of zeolite dissolved in 1% (v/v) nitric acid. Additions of zeolite corresponding to 0.5, 1.0, 2.0, 3.0 and 4.0 μg Al ml^{-1} gave recoveries of 96, 96, 101, 99 and 98%, respectively.

Analysis of sewage sludge, water and waste water. It has previously been

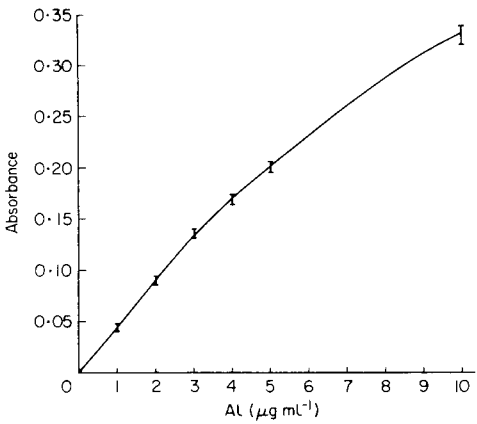


Fig. 1. Calibration curve for aluminium at 257.5 nm. Vertical bars indicate the range for three 20-μl injections.

proven that the electrothermal atomic absorption procedure using homogenization as the only pretreatment works well for determination of aluminium in sewage sludge [6] and final effluents [7]. However, because the zeolite had also been added, the procedure was compared with a flame atomic absorption procedure following digestion with a nitric—perchloric—hydrofluoric acid mixture. Mixed primary sludge (total solids 3.04%) was collected in a previously leached polythene container, acidified to 1% (v/v) with nitric acid, and divided into two. Zeolite type A was added to one subsample at the concentration (1 g l^{-1}) to be expected if zeolite type A was introduced into detergent formulations. Each portion was analyzed by both procedures; five replicates and two blanks were analyzed in each instance.

The initial concentration of aluminium in the sludge was 226 μg ml^{-1}, and 148 μg ml^{-1} of aluminium was added as zeolite. The mean aluminium concentrations found were 367 μg ml^{-1} by digestion and flame analysis and 372 μg ml^{-1} by the homogenization—electrothermal procedure. A *t*-test detected no statistical difference at the 95% significance level; however, the relative standard deviation for the flame method (4.4%) was lower than that for the electrothermal procedure (6.5%).

Analyses were also undertaken with electrothermal atomization after homogenization of samples of tap water, raw sewage (suspended solids 277 mg l^{-1}), settled sewage (suspended solids 138 mg l^{-1}) and final effluent (suspended solids 17.2 mg l^{-1}). Zeolite type A was added at the maximum levels to be expected in waste waters (ca. 60 mg l^{-1} for raw sewage, 30 mg l^{-1} for settled sewage and 10 mg l^{-1} for final effluent) and at 7 mg l^{-1} in tap water. Five replicates were used for all samples. The sensitive line at 309.3 nm was used for the determination of aluminium in tap water and final effluent, before zeolite addition. The results are presented in Table 1. Recoveries always exceeded 95%, i.e. quantitative within experimental error.

TABLE 1

Recovery of aluminium from waters and waste waters containing added zeolite type A by the electrothermal procedure after sample homogenization

Sample	Al present[a]			Al added ($\mu g\ ml^{-1}$)	Al found			Reco (%)
	Conc. ($\mu g\ ml^{-1}$)	S.d. ($\mu g\ ml^{-1}$)	R.s.d. (%)		Conc.[a] ($\mu g\ ml^{-1}$)	S.d. ($\mu g\ ml^{-1}$)	R.s.d. (%)	
Tap water	0.02	0.005	25	1.05	1.03	0.049	4.8	96
Raw sewage	1.49	0.09	6.0	9.19	10.8	0.62	5.7	101
Settled sewage	0.48	0.046	9.6	4.46	4.74	0.25	5.3	96
Final effluent	0.09	0.011	12.2	1.47	1.48	0.075	5.1	95

[a]Average of 5 replicates (duplicate analysis of each replicate) with standard deviation (s.d.) and relative standard deviation (r.s.d.).

TABLE 2

The determination of aluminium in samples containing added zeolite

Sample	Dilution (times)	Within batch[a]			Day-to-day[b]		
		Al found ($\mu g\ ml^{-1}$)		R.s.d. (%)	Al found ($\mu g\ ml^{-1}$)		R.s.d. (%)
		Mean	S.d.		Mean	S.d.	
Sewage sludge	100	3.74	0.24	6.4	3.66	0.25	6.8
Raw sewage	5	2.12	0.12	5.7	2.18	0.12	5.5
Settled sewage	2	2.31	0.12	5.2	2.32	0.13	5.6
Final effluent	nil	1.49	0.075	5.0	1.48	0.08	5.3

[a]Ten samples at each concentration. [b]Duplicate analyses each day, on six different days.

The within-batch and day-to-day precision for various samples to which zeolite type A was added are shown in Table 2. The relative standard deviations were always less than 7%, the higher values indicating the increasing difficulties of sampling and homogenizing samples with higher solids contents. Relative standard deviations previously reported include 10% for biological tissue [3] and 8% for blood [4].

Conclusion

The precision of the electrothermal procedure described is somewhat poorer than that obtained for other metals. The more frequent injections of standard made this method more time-consuming than is usual for electrothermal atomic absorption analysis. However, considering the chemical nature of aluminium and, since preliminary acid digestion or separation can be avoided, this is a useful specific method. The procedure can also be used to estimate zeolite type A in any of the samples analyzed provided that the

background aluminium concentrations are known or, in laboratory or pilot plant experiments, a control unit without zeolite is used.

Contamination can be a severe problem. Randomly high concentrations of aluminium were obtained, mainly at the initial stages of this work, unless great care was exercised with cleanliness of all apparatus.

The authors acknowledge the financial support provided for this work by the Centre Européen D'Etudes des Polyphosphates E. V. M. J. T. Carrondo thanks the Instituto Nacional de Investigação Científica, Lisboa, Portugal, for the award of a postgraduate scholarship.

REFERENCES

1 G. H. Farrah and M. L. Moss, in I. M. Kolthoff and P. J. Elving (Eds.), Treatise on Analytical Chemistry, Part II, Vol. 4, Interscience, New York, 1966, pp. 367—439.
2 P. Pakalns and Y. J. Farrar, Water Res., 11 (1977) 387.
3 S. S. Krishnan, S. Quittkat and D. R. Crapper, Can. J. Spectrosc., 21 (1976) 25.
4 F. J. Langmyhr and D. L. Tsalev, Anal. Chim. Acta., 92 (1977) 79.
5 D. A. Lord, J. W. McLaren and R. C. Wheeler, Anal. Chem., 49 (1977) 257.
6 M. J. T. Carrondo, J. N. Lester and R. Perry, Talanta, in press.
7 M. J. T. Carrondo, J. N. Lester and R. Perry, The Analyst, in press.
8 W. D. Hopping, J. Wat. Pollut. Control Fed., 50 (1978) 433.
9 G. F. Kirkbright and M. Sargent, Atomic Absorption and Fluorescence Spectroscopy, Academic Press, London, 1974.
10 D. W. Breck, Zeolite Molecular Sieves, Wiley, New York, 1974.
11 C. W. Fuller, Electrothermal Atomisation for Atomic Absorption Spectrometry, The Chemical Society, London, 1977.

Analytica Chimica Acta, 111 (1979) 297—300

Short Communication

DETERMINATION OF Ca, Er, Eu, Ga, In, K, Na, Mo and W BY ATOMIC ABSORPTION SPECTROMETRY WITH AN ELECTROTHERMAL GRAPHITE BRAID ATOMIZER

AKBAR MONTASER* and A. A. MEHRABZADEH

Department of Chemistry, Arya Mehr University of Technology, Tehran (Iran)

(Received 11th June 1979)

Summary. The limits of detection lie in the range 10^{-10}—10^{-12} g and are usually similar to the values achieved with other electrothermal atomizers. Except for molybdenum and tungsten, calibration graphs are linear over two orders of magnitude.

Electrothermal atomization [1] achieved by devices such as the tantalum boat, carbon rod, and graphite furnace has greatly improved the detection limits of atomic absorption spectrometry (a.a.s.) and atomic fluorescence spectrometry. Resistively-heated atomizers constructed from graphite have found widespread acceptance. Some problems associated with electrothermal atomizers have included complexity of design, the requirement of several kW to achieve temperatures in the range 2500—3000°C, and the relatively high atomizer cost. In an effort to overcome some of these problems Montaser et al. [2—4] investigated the use of a graphite braid atomizer for the a.a.s. determinations of some 30 elements. In addition to its low cost (about 1 U.S. cent for a braid 30 mm long and 1.5—2 mm in diameter) and relatively low power consumption (about 350 W is needed to reach 2500°C), the atomizer provides a furnace-type environment and no cooling system is necessary. The present study is an extension of an earlier report [4]. The use of the braid atomizer for the determination of Ca, Er, Eu, Ga, In, K, Na, Mo and W is evaluated.

Experimental

Apparatus. The graphite braid atomizer [2—4] was mounted on a workhead described earlier [4] and was used without further modification. The hollow-cathode lamps were operated at their optimum lamp current and were modulated at 285 Hz. The analysis wavelengths (in nm) chosen for absorbance measurements were 422.8(Ca), 400.8(Er), 459.4(Eu), 287.4(Ga), 303.9(In), 766.7(K), 589.2(Na), 313.3(Mo) and 255.1(W).

Reagents and methodology. Ultrapure concentrated nitric and hydrochloric acids and deionized twice-distilled water were used for making solutions. Stock solutions (1000 ppm) of each element were prepared from

analytical-reagent-grade chemicals. Aqueous standards were prepared by dilution of the stock solutions.

Atomic absorption signals were measured in the following manner. The nitrogen flow rate around the atomizer was adjusted to its optimum values. The braid was decontaminated by heating it to about 2500°C for 1—2 s. The cleaning procedure was checked by running a blank through the heating cycle for the element being determined. The sample solution (1 μl) was placed on the braid, dried (120°C) and atomized for 40 and 2—3 s, respectively, and the absorbance measured during the atomization step. After the first sample measurement, a blank cycle served to check for memory effects. A higher atomization temperature was used if a residue was indicated. The braid was allowed to cool for 30 s between analyses.

Results and discussion

In order to avoid the loss of atoms by chemical reaction or the transport of atoms outside the light path by diffusion, all atomic absorption measurements were conducted at 1 mm above the braid. The optimum monochromator spectral bandpass for In, Er, Eu and Ga was 0.2 nm, while those for Ca, K, Na, Mo and W were 0.33, 1.0, 0.66, 0.33 and 0.17 nm, respectively.

The braid temperature influenced the absorption signals of the elements. Atomization temperatures exceeding 1400°C did not increase absorbance for Na and K, whereas a temperature of 1700°C was required for calcium determination. The optimal atomization temperatures for Ga, In, Eu and Er were found to be 2100, 2200, 2250 and 2350°C, respectively. For these elements, the atomization process was completed in 2 s and the use of peak height and peak area measurements was equally applicable. An atomization temperature of 2700°C for 3 s was necessary for Mo and W determinations. The relative standard deviation for integrated absorption signals was 5—7%, while the corresponding value for peak absorbance measurements was 7—10%. Because of the high atomizer temperature required for Mo and W, the braid condition had seriously deteriorated after only 15 determinations.

The optimum nitrogen flow rate was between 1 and 2 l min^{-1} for all elements. At higher flow rates, turbulence set in, which adversely affected the reproducibility, the precision changing from 5—7% to 7—15% as the nitrogen flow rate was increased from 2 to 6 l min^{-1}. A high nitrogen flow rate cooled the braid causing the absorbance for molybdenum and tungsten to decrease sharply. The use of a nitrogen—hydrogen diffusion flame enhanced the absorbance of In, Er, Eu, Ga, Mo and W and improved their detection limits by a factor of about 5, but it had no effect on the determinations of Na, K, and Ca. Since all measurements were conducted 1 mm above the braid the hydrogen diffusion flame was not only protecting the metal-atom cloud from oxide formation, but also might be assisting in metal atom production from metal oxides.

The limit of detection for the present studies is defined as that concentration of analyte resulting in a signal-to-noise (r.m.s.) ratio of 2. The detection limits with the braid atomizer are compared with reported results from

TABLE 1

Comparison of detection limits obtained by atomic absorption with different atomizers

Element	Maximum determinable amount (g)[a]	Limit of detection (g)			
		Braid atomizer	Tantalum boat [5]	Carbon rod [6]	Graphite furnace [8]
Ca	8×10^{-10}	2×10^{-12}	1×10^{-12}	3×10^{-13}	4×10^{-13}
Er	3×10^{-7}	8×10^{-10}	—	—	3.7×10^{-11} [c]
Eu	4×10^{-8}	1×10^{-10}	3×10^{-11}	1×10^{-10}	—
Ga	8×10^{-9}	3×10^{-11}	1×10^{-10}	2×10^{-11}	1×10^{-12}
In	3×10^{-8}	1×10^{-10}	1×10^{-10}	2×10^{-10} [b]	4×10^{-13}
K	6×10^{-10}	1×10^{-12}	1×10^{-12}	9×10^{-13}	4×10^{-11}
Na	2×10^{-9}	7×10^{-12}	1×10^{-12}	1×10^{-13}	—
Mo	9×10^{-10}	1×10^{-10}	—	1×10^{-11}	3×10^{-12}
W	7×10^{-8}	1×10^{-8}		—	—

[a]With the graphite braid atomizer. [b]Reference [7]. [c]Reference [9].

three other electrothermal atomizers in Table 1. The graphite furnace had a lower detection limit in all instances, but those for the carbon rod and tantalum boat were similar to those achieved with the braid. The calibration graphs for the braid atomizer were linear for about 2 decades of concentration for Ca, Er, Eu, Ga, In, K and Na. The relative standard deviations of 10 consecutive determinations at a concentration one order of magnitude greater than the detection limits, were usually 5—7%. The linear ranges for Mo and W were less than one decade of concentration. This may be attributed to oxide and carbide formation. One of the major differences between the braid and the graphite furnace is the residence time of atoms in the high temperature zone. This is of the order of seconds for the furnace, while with a braid, it can be only 10^{-2} s. It might be postulated that a furnace retains the metal compounds until its temperature is high enough to rupture the metal—carbon or metal—oxygen bond. In this respect the use of a radiation method of programmed heating [10] may improve the detection limits and dynamic ranges for these elements when the braid atomizer is used.

REFERENCES

1 See, e.g., C. W. Fuller, Electrothermal Atomization for Atomic Absorption Spectrometry, Chemical Society, London, 1978.
2 A. Montaser, S. R. Goode and S. R. Crouch, Anal. Chem., 46 (1974) 599.
3 A. Montaser and S. R. Crouch, Anal. Chem., 46 (1974) 1817.
4 A. Montaser and A. A. Mehrabzadeh, Anal. Chem., 50 (1978) 1697.
5 J. Y. Hwang, C. J. Mokeler, and P. A. Ullucci, Anal. Chem., 44 (1972) 2018.
6 M. D. Amos, P. A. Bennett, K. G. Brodie, P. W. Y. Lung and J. P. Matousek, Anal. Chem., 43 (1971) 211, and Analytical Methods for Carbon Rod Atomizer, from Varian-Techtron Corp., Melbourne, Australia.
7 H. Massmann, Spectrochim. Acta, Part B, 23 (1968) 215.

8 B. V. L'vov, Spectrochim. Acta, Part B, 24 (1969) 53.
9 R. Woodriff, R. W. Stone and A. M. Held, Appl. Spectrosc., 22 (1968) 408.
10 A. Montaser and S. R. Crouch, Anal. Chem., 47 (1975) 38.

Analytica Chimica Acta, 111 (1979) 301—306

Short Communication

SIMULTANEOUS KINETIC DETERMINATION OF PHOSPHATE AND SILICATE BASED ON HETEROPOLY BLUE FORMATION

KOUSABURO OHASHI* HITOSHI KAWAGUCHI and KATSUMI YAMAMOTO
Department of Chemistry, Faculty of Science, Ibaraki University Mito 310 (Japan)
(Received 1st May 1979)

Summary. Small amounts of phosphate (0.08—1.16 $\mu g\ ml^{-1}$) and larger amounts of silicate (12—60 $\mu g\ ml^{-1}$) can be determined simultaneously by a kinetic method based on the difference in the rates of the heteropoly blue formation with molybdenum(V)—molybdenum(VI) mixtures in 0.28 M perchloric acid. The interference of large amounts of iron(III) on the determination of phosphate can be eliminated by masking with sodium hydrogen sulfite; this method is applicable to reagent-grade iron(III) chloride.

Many methods for the determination of phosphate and silicate are based on reduction of molybdophosphoric acid and molybdosilicic acid with reagents such as chlorostannous acid, 1-amino-2-naphthol-4-sulfonic acid, and hydrazine dihydrochloride to heteropoly blue [1]. Selectivity for phosphate can be obtained by control of the acidity, e.g., at high acidity small amounts of silicate do not interfere. Many different applications of these reactions have been proposed [1] but difficulties are often encountered when two or more ions forming heteropoly blue are present simultaneously. Takagi et al. [2] determined small amounts of silicate in reagent-grade phosphoric acid based on heteropoly blue formation after separation of silicate by ion-exchange chromatography.

In a previous report [3], small amounts of phosphate were determined without interference from similar amounts of silicate based on the development of heteropoly blue with molybdenum(V) ($Mo_2O_4^{2+}$) and molybdenum(VI). Large amounts of silicate interfered badly. In the work described here, the rate of formation of the heteropoly blue from silicate and molybdenum(V) with molybdenum(VI) was found to be strikingly small compared with that formed from phosphate. Small amounts of phosphate and larger amounts of silicate could be determined simultaneously by kinetic measurements based on the difference in the rates of formation of the two heteropoly blues. The elimination of large amounts of iron(III) in the determination of phosphate was also studied.

Experimental

Materials and apparatus. Molybdenum(VI) solution (1.00×10^{-1} M) was prepared by dissolving 24.196 g of sodium molybdate dihydrate in 1 l of

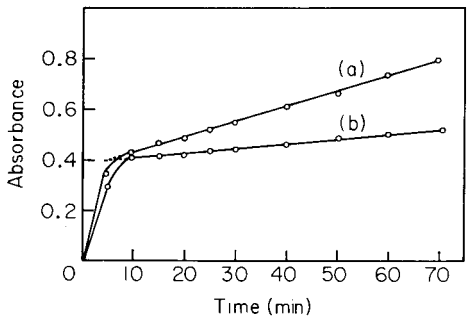
water. Working solutions were prepared by suitable dilution. Molybdenum-(V) perchlorate was prepared and analyzed as reported by Sasaki et al. [4]. The hydrogen-ion concentration of the molybdenum(V) solution was found by a cation-exchange method to be 2.0 M. Phosphate solution (2.00×10^{-2} M) was prepared from sodium dihydrogen phosphate dihydrate, and silicate solution (3.34×10^{-3} M) by fusing 0.100 g of pure dry silica with anhydrous sodium carbonate.

All the chemicals were of analytical grade. Twice-distilled water was used throughout.

A Hitachi EPS-3 spectrophotometer was used for all measurements with 1-cm cells.

General procedure for the simultaneous kinetic determination of phosphate and silicate. For each run, 2 ml of 1.0 M perchloric acid, 8 ml of 2.00×10^{-2} M molybdenum(VI), 6 ml of 1.60×10^{-2} M molybdenum(V), and the appropriate quantities of 4.00×10^{-4} M phosphate and 3.34×10^{-3} M silicate solutions were pipetted into 50-ml volumetric flasks. After dilution to 50 ml with water, the solutions were thermostated at 80°C and the absorbances at 840 nm were measured every 5 min. Absorbances were then plotted against time (Fig. 1). The heteropoly blue formation from silicate started 3 min after thermostating at 80°C. Because of the difference in formation rates, phosphate could be determined from the absorbance obtained by extrapolating the slope of the absorbance vs. time plot to 3 min.

As shown in Fig. 2, the rate of formation of the heteropoly blue from silicate decreased as the phosphate concentration increased. For example, the rate in the presence of 1.55×10^{-5} M phosphate was 18% less than that in the absence of phosphate. The consumptions of molybdenum(V) and molybdenum(VI) in the reaction with phosphate caused the decreased rate in the reaction with silicate. Accordingly, when both silicate and phosphate were present, phosphate was first determined by the above general procedure, and then the calibration curve for silicate was prepared with solutions containing this amount of phosphate.



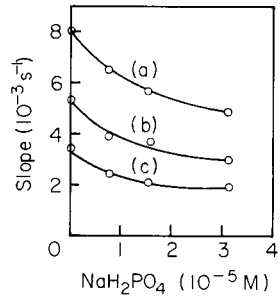


Fig. 1. Plots of absorbances vs. time under the recommended conditions for mixtures of 1.56×10^{-5} M phosphate and (a) 1.00×10^{-3} M silicate or (b) 3.34×10^{-4} M silicate.

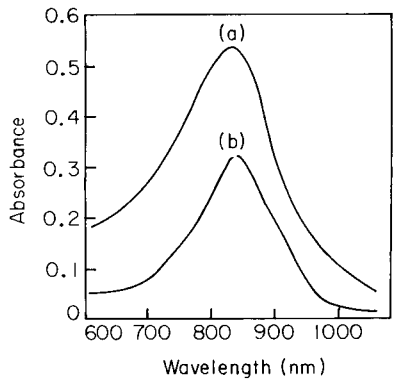
Fig. 2. Effect of the phosphate concentration on the rate of formation of heteropoly blue from silicate: (a) 1.00×10^{-3} M; (b) 6.68×10^{-4} M; (c) 3.34×10^{-4} M.

Results and discussion

Absorption spectra of heteropoly blue from phosphate and silicate. The absorption spectrum of the heteropoly blue formed by the reaction of silicate with a mixture of molybdenum(V) and molybdenum(VI) in 0.28 M perchloric acid is shown in Fig. 3; the spectrum was measured after the solution had been thermostated for 80 min at 80°C. The molar absorptivity was not determined because of the small rate of heteropoly blue formation. The absorption spectrum of the heteropoly blue formed from phosphate is also shown in Fig. 3; this solution was prepared as reported previously [3]. These absorption spectra differ slightly; the absorption maxima appear at 840 nm and 825 nm for the phosphate and silicate products, respectively.

Calibration graphs for phosphate and silicate. In each experiment under the solution conditions recommended above, a given quantity of 4.00×10^{-4} M phosphate solution was pipetted into a 50-ml volumetric flask. Perchloric acid was always added first to avoid the formation of isomolybdenum blue from the reaction of molybdenum(V) with molybdenum(VI). The solution was diluted to 50 ml with water, and heated for 15 min at 80°C, before measurement of the absorbances at 840 nm. The calibration graph was linear in the phosphate range 0.08—1.16 μg ml^{-1} (absorbance range 0.050—1.08). The molar absorptivity was 2.4×10^4 l mol^{-1} cm^{-1}.

The earlier study of the phosphate reaction [3] showed that the dependence of the formation rate on experimental parameters was complicated.



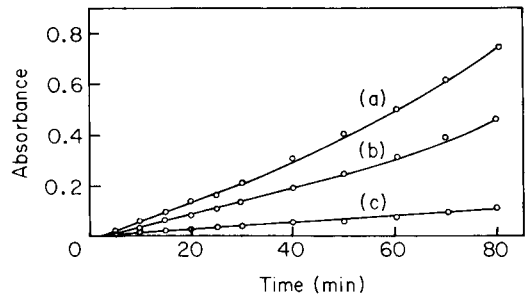


Fig. 3. The absorption spectra of the two heteropoly blues (a) 1.45×10^{-5} M phosphate; (b) 4.28×10^{-4} M silicate; both with 3.20×10^{-3} M Mo(VI) 1.80×10^{-5} M Mo(V), 0.28 M $HClO_4$.

Fig. 4. Plots of the absorbances of the heteropoly blue from silicate in the absence of phosphate. Silicate concentration: (a) 1.00×10^{-3} M; (b) 6.68×10^{-4} M; (c) 2.00×10^{-4} M; all with 3.20×10^{-3} M Mo(VI), 1.81×10^{-3} M Mo(V), 0.28 × M $HClO_4$. After thermostating at 80°C, absorbance is measured at 840 nm.

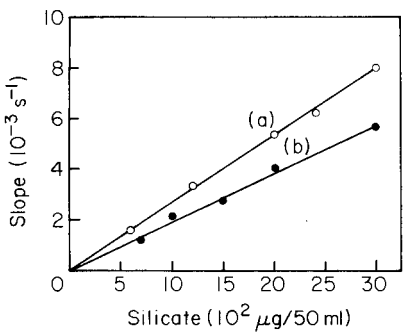
Therefore, almost the same experimental conditions as used previously [3] for phosphate were adopted for the determination of silicate. The absorbances for silicate increased linearly with time for the initial reaction (Fig. 4), and the slopes of the absorbance vs. time plots were used for the determination of silicate. The plot of the initial slope vs. the silicate concentrations was linear in the silicate concentration range 12.0—60.0 $\mu g\ ml^{-1}$ (Fig. 5).

As indicated in the general procedure, the amount of phosphate present affected the rate of formation of the heteropoly blue from silicate, and calibration plots for silicate had to be prepared after establishment of the phosphate content in the sample.

The accuracies for the determinations of phosphate and silicate are indicated in Table 1.

The effect of the perchloric acid concentration on the rate of the heteropoly blue formation from silicate is illustrated in Fig. 6. For concentrations below 0.20 M perchloric acid, molybdenum(V) reacts with molybdenum(VI) to provide isomolybdenum blue. Obviously, the acidity requires careful control.

Effect of the iron(III) on the phosphate determination. Iron(III) in amounts up to 4 $\mu g\ ml^{-1}$ does not affect the determination of phosphate, but the larger amounts interfere [3], because some molybdenum(V) is oxidized by iron(III); the rate of this oxidation is quite high [5]. Sodium hydrogensulfite has been used to eliminate the interference of iron(III) in an earlier determination of phosphate based on molybdenum blue formation [6]. In the present work, the interference of high concentrations of iron(III) (up to $5.72 \times 10^2\ \mu g\ ml^{-1}$) could also be eliminated by reducing iron(III) with hydrogensulfite (Table 2).



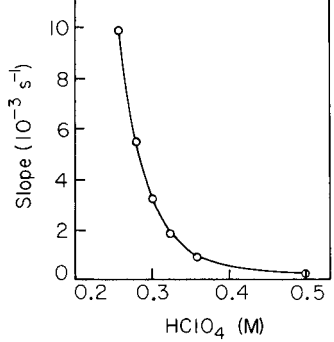


Fig. 5. Calibration curves for silicate. (a) In the absence of phosphate; (b) in the presence of 1.60×10^{-5} M phosphate. Conditions as for Fig. 4.

Fig. 6. Effect of the perchloric acid concentration on the formation rate of heteropoly blue from 6.68×10^{-4} M silicate. Other conditions as for Fig. 4.

TABLE 1

Accuracy of the determinations of phosphate and silicate under the recommended conditions

Absorbance at 840 nm	Slope ($\times 10^{-3} s^{-1}$)	Phosphate or silicate ($\mu g\ ml^{-1}$)		Error (%)
		Taken	Found	
Phosphate in presence of silicate (29.5 $\mu g\ ml^{-1}$)				
0.19	—	0.74	0.79	+5.8
0.39	—	1.48	1.52	+2.7
0.40	—	1.48	1.57	+5.8
0.77	—	2.97	3.01	+1.7
Silicate in presence of phosphate (1.5 $\mu g\ ml^{-1}$)				
—	2.2	20.0	21.0	+5.0
—	2.7	29.0	28.0	−3.4
—	4.0	40.0	41.5	+3.8
—	5.6	60.0	57.5	−4.2
Silicate in absence of phosphate				
—	2.5	20.1	21.0	+4.8
—	3.3	24.2	25.5	+2.2
—	3.5	40.2	39.5	−1.9
—	6.2	48.2	47.1	−2.2
—	6.3	60.1	59.4	−1.2

TABLE 2

Effect of iron(III) concentration on the determination of 1.56×10^{-5} M NaH_2PO_4 under the recommended conditions

$Fe(ClO_4)_3$ ($\times 10^{-3}$ M)	2.76	7.68	10.2	2.76	7.68	10.2
$NaHSO_3$ ($\times 10^{-2}$ M)	0	0	0	3.84	3.84	3.84
Absorbance	0.41	0.02	0.01	0.40	0.42	0.42

Determination of phosphate in reagent-grade iron(III) chloride. A sample (4.366 g) of iron(III) chloride hexahydrate (Nakarai Co. Ltd) was dissolved in 30 ml of 8 M hydrochloric acid. Iron(III) was extracted with 40 ml of methyl isobutyl ketone and the organic phase was washed twice with 10 ml of 7 M hydrochloric acid to recover phosphate from the organic phase. Perchloric acid (3 ml of 10 M) was added to the aqueous phase which was then evaporated to 1 ml, and diluted to 50 ml with water. Phosphate was determined in a suitable aliquot of this solution (5 or 10 ml) by addition of 4 ml of 2.00×10^{-2} M molybdenum(VI), 3 ml of 1.51×10^{-2} M molybdenum(V), 1 ml of 0.91 M $NaHSO_3$, dilution to 25 ml, heating at 80°C for 15 min, and measurement at 840 nm. The reagent-grade iron(III) chloride used is stated to contain 0.01% P as impurity. The result found here was 0.002% P with a relative standard deviation of 10% (n = 4).

REFERENCES

1 See, e.g., D. F. Boltz and J. A. Howell (Eds.), Colorimetric determination of Nonmetals, 2nd edn., Wiley-Interscience, New York, 1978.
2 T. Takagi, T. Hashimoto and M. Sasaki, Bunseki Kagaku, 12 (1962) 618.
3 K. Ohashi, C. Suzuki and K. Yamamoto, Bull. Chem. Soc. Jpn., 50 (1977) 3202.
4 Y. Sasaki, R. S. Taylor and A. G. Sykes, J. Chem. Soc., Dalton Trans., (1975) 396.
5 K. Ohashi, unpublished data.
6 S. Maekawa and K. Kato, Bunseki Kagaku, 17 (1968) 597.

Analytica Chimica Acta, 111 (1979) 307—309

Short Communication

DETERMINATION OF RHENIUM IN THE URe_2 BINARY COMPOUND BY DIFFERENTIAL SPECTROPHOTOMETRY

B. N. JAGTAP, S. S. DESAI and S. R. DESAI*

Analytical Chemistry Division and M.D.R.S., Bhabha Atomic Research Centre, Trombay, Bombay-400 085 (India)

(Received 27th March 1979)

Summary. A differential spectrophotometric determination of rhenium in its binary compound with uranium is described; the method is based on the action of tin(II) chloride on perrhenate in the presence of dimethylglyoxime with measurement at 445 nm. Uranium does not interfere. For solutions containing about 1 mg of rhenium, the coefficient of variation was 0.08%.

A precise and accurate method was required for the determination of rhenium in its binary compound with uranium (URe_2). The macro methods available [1—3] are not sufficiently accurate, and are inconvenient and time-consuming. In addition, a prior separation of the rhenium from uranium would be necessary. The differential spectrophotometric method described here avoids these shortcomings; the method is based on the action of tin(II) chloride on perrhenate in the presence of dimethylglyoxime (DMG) [1]. The spectrophotometric determination of rhenium with DMG has been described frequently [4—6]. The optimum experimental parameters necessary for Re—DMG colour development in a differential spectrophotometric method are reported here.

Experimental

A Beckman spectrophotometer Model DU was used and all reagents were analytical grade.

Rhenium stock solution. Prepare a solution containing 5 mg of Re ml^{-1} in hydrochloric acid (1 + 9). Dissolve the metal in 25 ml of a mixture of nitric and hydrochloric acids (1 + 4). Take the solution nearly to dryness on a water bath and evaporate repeatedly with small aliquots of hydrochloric acid to ensure complete removal of nitric acid. Prepare working solutions of rhenium in 2.5 M HCl by appropriate dilution.

Tin(II) chloride solution. Prepare a 10% solution by treating 10 g of $SnCl_2$ with 40 ml of NaOH solution (25% w/v). Dissolve the precipitated hydrated tin(II) oxide in 30 ml of 12 M HCl and dilute to 100 ml with distilled water.

Dimethylglyoxime solution. Prepare a solution (0.5% w/v) of the reagent in redistilled ethanol.

Uranium solution. Prepare a 1.0% (w/v) solution by dissolving the metal in a mixture of HCl and HNO_3 (1 + 4). As for the rhenium solution, remove all nitric acid by repeated evaporation with hydrochloric acid and adjust the solution finally to contain 5% HCl.

Procedure. Weigh 0.2 g of the alloy, transfer to a 250-ml beaker and cautiously dissolve it in 25 ml of a mixture of nitric and hydrochloric acids (1 + 4). After the initial reaction has subsided, place the beaker on a water bath and evaporate nearly to dryness. Add 5 ml of 12 M HCl and evaporate again nearly to dryness. Repeat twice to remove traces of nitric acid completely. Take up the residue in 50 ml of 12 M HCl, transfer to a 500-ml volumetric flask, and make up to the mark with distilled water. Place a 5-ml aliquot into a 50-ml standard flask. Add 1.0 ml of 12 M HCl and 12.5 ml of dimethylglyoxime solution followed by 5 ml of tin(II) chloride solution. Adjust to the mark with distilled water. Stopper the flask, shake well, and allow to stand. Simultaneously run the standard reference solution of rhenium in the same way. After 1 h measure the absorbance of the sample solution against the reference solution at 445 nm. Obtain calibration curves by running standards in the range 1—1.5 mg Re per 5 ml in the same way.

Establishment of optimum conditions for normal spectrophotometry

For these experiments, 100 μg of rhenium was taken and the final volume was made up to 50 ml. The absorption spectrum of the complex shows a single broad band with maximum absorbance at 445 nm. All measurements were therefore made at this wavelength.

The acid concentration was varied from 0.1 to 1 M; maximum absorbance was obtained for 0.1—0.4 M acid solutions. The amount of 10% (w/v) $SnCl_2$ solution was varied from 1 to 5 ml; there was no change in the absorbance of the Re—DMG complex within this range. The rather high concentration of tin(II) was selected to ensure complete reduction of rhenium when milligram quantities were taken for differential spectrophotometry. The concentration of DMG was varied from 1 to 100-fold mole ratio excess over the concentration of rhenium. There was a marked increase in absorbance when a 50-fold excess of DMG was used, but higher DMG concentrations did not cause further increases.

The ethanol concentration in the solution was varied from 10% to 40% (v/v). The absorbance was almost constant for ethanol concentrations of 20—40%, decreased for solutions containing less than 20% ethanol, and at lower ethanol concentrations the DMG precipitated out on standing. The concentration of ethanol was therefore fixed at 25%.

At room temperature, 45 min were required for the Re—DMG reaction to come to a steady state. Thereafter, the colour was stable for 24 h. In all further experiments readings were taken 1 h after adding the reagent.

Interferences. The effects of the following ions in 10-fold excess over the rhenium concentration were studied: Cu, Co, Al, La, Mo, Mn, V, Fe, Tl, Zn, Ni, U, W; and F^-, ClO_4^-, PO_4^{3-}, NO_3^- and SO_4^{2-}. Of these, only copper(II)

gave a positive interference (6%); nitrate reduced the absorbance by 20%. The effect of the other ions on the absorbance was marginal and within experimental error (Mo, Ni, W and U, 0.6% decrease; Tl, 0.6% increase; La, SO_4^{2-}, 1.2% increase; F^-, PO_4^{3-}, 1.2% decrease; Co, V, Fe, 1.8% increase; Zn, 2.4% increase; ClO_4^-, 2.4% decrease; Al, 3% increase).

Differential spectrophotometry

On the basis of these results the procedure for the differential spectrophotometric determination of rhenium was established. The concentration of rhenium in the reference solution was varied from 0.5 mg to 2.0 mg per 50 ml. With 2.0 mg Re, it was not possible to adjust the slit width for 100% transmittance. Though the slit width could be adjusted for 1.5 mg Re per 50 ml, the concentration of rhenium in the reference solution was fixed at 1.0 mg per 50 ml.

Results and discussion

Of the numerous colorimetric reagents [1—3, 5, 7] available for the determination of rhenium, DMG was selected because of its ready availability and stability; in differential spectrophotometry, stability and reproducibility are more important than sensitivity [8]. The additional advantage of DMG is that it does not react with uranium, hence rhenium can be determined without prior separation of these metals.

A linear calibration graph for the Re—DMG complex for the range 1—1.5 mg of rhenium per 50 ml was obtained when the reference solution contained 1 mg Re per 50 ml. For a mixture containing 61.01% rhenium, a mean value of 61.0% (n = 11; range 60.95—61.07) was obtained with a standard deviation of 0.049 and a coefficient of variation of 0.08%. The absorbance measurements were made at room temperature (24 ± 2°C). With close temperature control and the use of grade A volumetric glassware, better accuracy and precision could probably be obtained.

REFERENCES

1 I. M. Kolthoff and P. J. Elving (Eds.). Treatise on Analytical Chemistry, Part II, Vol. 7, Interscience, New York, 1961, pp. 519—530.
2 B. N. Gonser (Ed.), Rhenium, Elsevier, Amsterdam, 1962, pp. 191—210.
3 A. I. Busev, V. G. Tiptsova and V. M. Ivanov, Handbook of Analytical Chemistry of Rare Elements, Ann Arbor—Humphrey, London, 1970, pp. 230—245.
4 B. T. Kenna, Anal. Chem., 33 (1961) 1130.
5 S. Stefanev, N. Jordanov and M. Pavlova, Mikrochim. Acta, (1976) 449.
6 E. N. Pollock and L. P. Zopatti, Anal. Chim. Acta, 32 (1965) 418.
7 P. K. Gangopadhyay and S. C. Shome, Anal. Chim. Acta, 75 (1975) 235.
8 C. V. Banks, K. E. Burke, J. W. O'Laughlin and J. A. Thompson, Anal. Chem., 29 (1957) 998.

Analytica Chimica Acta, 111 (1979) 311—314

Short Communication

THE SPECTROPHOTOMETRIC DETERMINATION OF NITRITE IN WATER WITH 8-QUINOLINOL

JAGADEESAN NAIR and V. K. GUPTA*

Department of Chemistry, Ravishankar University Raipur (M.P.) (India)

(Received 19th April 1979)

Summary. The method is based on the formation of a purple azoxine dye by coupling diazotized *p*-nitroaniline with 8-quinolinol. Beer's law is obeyed at 550 nm in the range 2—28 μg NO_2^- per 25 ml. The molar absorptivity and Sandell sensitivity are 3.88×10^4 l mol^{-1} cm^{-1} and 0.0012 μg cm^{-2}, respectively.

Nitrite occurs in water as an intermediate during the nitrogen cycle; traces of nitrite in environmental samples give an excellent indication of the extent of pollution and eutrophication. Many spectrophotometric methods [1—7] have been reported for the determination of small amounts of nitrite. Most of these involve diazotization of an aromatic amine with nitrite and subsequent coupling of the azo compound with an aromatic amine or phenol to form highly coloured azo dyes. Many of these methods offer excellent sensitivity and selectivity, but quite often require close control of pH and temperature during the diazotization step as well as relatively long coupling times.

The present communication deals with the use of 8-quinolinol as a coupling reagent for the determination of nitrite in water. *p*-Nitroaniline is used as the diazotizing reagent. An intense purple azoxine dye [8], extractable into organic solvents, is formed in alkaline medium. The colour reaction is very sensitive and reproducible results can be obtained without rigorous control of experimental conditions.

Experimental

Apparatus. An ECIL spectrophotometer Model GS 865 or a Carl Zeiss Spekol was used with matched 1-cm glass cells; pH measurements were made on an ECIL pH meter, Model PH 821.

Standard sodium nitrite solution (1 mg NO_2^- ml^{-1}). A stock solution of sodium nitrite was prepared by dissolving 150 mg of dried analytical-grade reagent in 100 ml of deaerated, doubly-distilled water. A little chloroform was added as stabilizer. Working standards were prepared by appropriate dilution.

p-*Nitroaniline solution.* The commercially available reagent was crystallized twice before use; a 1×10^{-3} M solution was prepared in 2 M hydrochloric acid.

Procedure. Transfer an aliquot (not more than 15 ml) of the water sample containing 2—28 μg NO_2^- to a 25-ml volumetric flask and add 2 ml of *p*-nitroaniline reagent. Shake for 1 min and add 1 ml of ethanolic 0.2% (w/v) 8-quinolinol solution and 1 ml of aqueous 10% (w/v) disodium—EDTA solution. Make alkaline (to about pH 12) with 2 M sodium hydroxide solution (ca. 3 ml) and dilute to the mark with distilled water. Measure the absorbance at 550 nm against a reagent blank. Calculate the amount of nitrite from a calibration graph prepared from measurements done in the same way.

Results and discussion

The reactions involved are

$$O_2NC_6H_4.NH_2 + NO_2^- + 2H^+ \rightarrow O_2N.C_6H_4N^+ \equiv N + 2H_2O$$

$$O_2N.C_6H_4.N^+ \equiv N + C_9H_7NO \rightarrow O_2N.C_6H_4.N = N.C_9H_6NO$$

The final product in alkaline medium gives an intense purple colour. The coupling of the *p*-nitrophenyldiazonium ion may take place at either the 5- or 7-position of 8-quinolinol, so that the formation of two isomeric dyes may be expected, but at ca. 30°C coupling takes place only at the 5-position [9]. Paper chromatographic examination of the butanol extract of the dye confirmed that only one component is present.

The azoxine dye has a maximum absorption at 550—555 nm; the absorption of the reagent blank in this region is negligible. Under the recommended conditions, the dye shows no appreciable change in absorbance up to 30 h, and the absorbance remains constant in the temperature range 20—40°C.

Effects of varying reaction conditions. The effect of acidity on the diazotization reaction was studied in the range 0—4 M hydrochloric acid. At least 0.02 M hydrochloric acid is necessary for complete diazotization; in 0.02—4 M acid, constant absorbance was observed. The time necessary for complete diazotization was determined; the absorbance is constant for development periods of 1—90 min. The diazotization reaction is fast at 30°C and the *p*-nitrophenyldiazonium ion is stable under the experimental conditions used. The time required for coupling the *p*-nitrophenyldiazonium ion with 8-quinolinol was investigated; the minimum time for full colour development was 1 min. Although the colour started to form at pH 9, full colour development was obtained only above pH 11.

The effects of varying the molar ratios of *p*-nitroaniline and 8-quinolinol were examined. For *p*-nitroaniline, the absorbance was constant at molar ratios of 1:1 and above. For a 10-fold excess of *p*-nitroaniline, λ_{max} remained the same.

A molar ratio for 8-quinolinol of 6:1 or greater was needed for full colour development. There was no significant change in the absorbance on adding a very large excess of 8-quinolinol.

Beer's law, optimum range, sensitivity, molar absorptivity and reproducibility. The system obeyed Beer's law in the range 2—28 μg of nitrite

TABLE 1

Spectral characteristics of the dye in various solvents

Solvent	λ_{max} (nm)	ϵ ($\times 10^4$ l mol^{-1} cm^{-1})	Stability
Water	550	3.88	30 h
n-Butanol	560	3.50	20 min
n-Hexanol	565	2.90	5 min
Chloroform	530	3.45	Very unstable
Benzene—butanol (1 + 1)	555	3.50	10 min

TABLE 2

Effects of some ions and compounds on the determination of 0.4 ppm nitrite

Ion (tolerance limit in ppm)
PO_4^{3-} (1000), NO_3^- (1000), SO_4^{2-} (1000), Br^- (400), I^- (1.6), SO_3^{2-} (10[a]), K^+ (1000), Be^{2+} (80), Ca^{2+} (160[b]), Mg^{2+} (40[b]), Ba^{2+} (200[b]), Cr^{6+} (1.6), Cr^{3+} (8), Mo^{6+} (40), W^{6+} (40), Mn^{2+} (20), Fe^{3+} (40[c]), Co^{2+} (40[b]), Ni^{2+} (60[b]), Zn^{2+} (40[b]), Cd^{2+} (80[b]), Hg^{2+} (80[b]), Al^{3+} (60[b]), Pb^{2+} (80[b]), NH_4^+ (1000), SCN^- (200), Bi^{3+} (60[c]), As^{3+} (20), Sn^{2+} (40), analine (40), formaldehyde (40), phenol (40)

[a]In the presence of 0.5 ml of 1% H_2O_2 (20 vol). [b]In the presence of 1 ml of 10% EDTA. [c]In the presence of 1 ml of 10% sodium potassium tartrate. All masking agents were added after 8-quinolinol.

per 25 ml (0.08—1.12 ppm) at 550 nm. The optimal concentration range, evaluated from the Ringbom curve [10], was 6—24 μg per 25 ml. The Sandell sensitivity [11] and molar absorptivity calculated from Beer's law data were 0.0012 μg cm^{-2} and 3.88 $\times$ 10^4 l mol^{-1} cm^{-1}, respectively.

The reproducibility of the method was checked by replicate analyses of a standard sodium nitrite solution over a period of 7 days. The standard deviation and relative standard deviation were ±0.099 and ±0.99% respectively (n = 7) for a solution containing 10.00 μg of nitrite per 25 ml (mean value found, 9.99 μg; range, 9.85—10.15).

Extraction with organic solvents. After extraction into butanol, hexanol, chloroform, and benzene—butanol (1 +1) the dyestuff was less stable than in water (stability > 30 h). A shift in λ_{max} was also observed with different solvents. The data are given in Table 1.

Effect of foreign ions. Because this method was developed for the analysis of water samples, the effects of the foreign ions commonly present in water were studied. Metal ions forming hydroxides in alkaline medium were expected to interfere but a large number of these ions were masked with EDTA. Copper(II), iron(II) and sulphide ions caused serious interference. The tolerance limits of the foreign ions shown in Table 2 are the amounts that caused not more than 2% change in the absorbance during the determination of a fixed amount of nitrite.

This method is satisfactory for the rapid determination of nitrite in water samples. The rapid colour development, excellent reproducibility, and independence of the colour intensity from pH, temperature, and reagent concentrations make the method versatile and useful. The reagents are cheap and easily available.

The authors express their thanks to the Head, Department of Chemistry, Ravishankar University, Raipur, for providing laboratory facilities and J. N. thanks the University Grants Commission, New Delhi, for the award of a fellowship.

REFERENCES

1 Standard Methods for the Examination of Water, Sewage and Industrial Waste, American Public Health Association, 13th edn., 1971.
2 I. M. Kolthoff and P. J. Elving, Treatise on Analytical Chemistry, Part II, Vol. 5, Interscience, New York, 1961, p. 275.
3 C. A. Streuli and P. R. Averell, The Analytical Chemistry of Nitrogen and its Compounds, Part I, Wiley-Interscience, New York, 1970, p. 121.
4 A. K. Babko and A. T. Pilipenko, Photometric Analysis, Methods of Determination of of Non-metals; translated from Russian by A. Rosinkin, Mir Publishers, Moscow, 1976, p. 35.
5 F. Celardin, M. Marcantonatos and D. Monnier, Anal. Chim. Acta, 68 (1974) 61.
6 K. Tôei and T. Kiyose, Anal. Chim. Acta, 88 (1977) 125.
7 M. Roman, A. Fernadez-Gutierrez and M. C. Mahedero, Bull. Soc. Quim. Peru, 43 (1977) 16.
8 J. S. Fritz, W. J. Lane and A. S. Bystroff, Anal. Chem., 29 (1957) 821.
9 A. Badrinas, Talanta, 10 (1963) 704.
10 A. Ringbom, Fresenius Z. Anal. Chem., 115 (1938) 332.
11 E. B. Sandell, Colorimetric Determination of Traces of Metals, Interscience, New York, 3rd edn., 1959, p. 80.

Analytica Chimica Acta, 111 (1979) 315—319

Short Communication

FACILE DETERMINATION OF THE SPECIFIC ACTIVITY OF CARBONYL COMPOUNDS REDUCED BY TRITIATED BOROHYDRIDE

GAD AVIGAD

Department of Biochemistry, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, New Jersey 08854 (U.S.A.)

(Received 6th April 1979)

Summary. Three procedures are described for microliter samples of glucose 6-phosphate or lactose as model compounds. After the reduction with [^{3}H]-$NaBH_4$ and suitable treatment, specific activity is calculated from the ratios ^{3}H activity/total phosphorus, ^{3}H/^{14}C activity, or ^{3}H activity/galactoside concentration.

Tritiated borohydrides, particularly the sodium salt [borate(1-)tetrahydro-t_4-sodium] are extensively employed in biological research for introducing a radioactive label into cellular components by reduction of carbonyl or imino Schiff's base groups in the molecule. This procedure facilitates the detection and tracing of a reduced molecule during its purification or in evaluating its distribution in cellular structures. It also permits quantitative measurement of the absolute amount of a reduced molecule. For this purpose the specific activity of the tritium used in the reduction must be established. The values supplied with commercial preparations of tritiated borohydride are usually only approximate. Factors such as natural decay, exchange of protons with the solvent, self-radiolysis, spontaneous decomposition and formation of non-volatile tritium products which do not participate in reduction [1—6] contribute to the difficulty of direct evaluation of the specific activity of the tritium which participated in the reduction. An effective approach is based on the isolation of the reduced, tritiated product. Such procedures [7—12] may be lengthy, often involving time-consuming chromatographic steps, or are designed for a preparative scale production of tritium-labeled compounds.

Recent studies required the introduction of tritium into complex carbohydrates oxidized by periodate or by galactose oxidase [13—18]; three simple procedures devised for the rapid determination of the specific activity of very small samples of carbonyl compounds reduced by tritiated borohydride are described below.

Experimental

Materials and methods. Chromatographically pure D-[U-^{14}C]-glucose 6-phosphate (240 mCi mmol^{-1}) and [^{3}H]-$NaBH_4$ (172 mCi mmol^{-1}) were purchased from the Amersham Corporation. A primary stock solution (0.052 M) of [^{3}H]-$NaBH_4$ (10 mCi ml^{-1} of 0.01 M NaOH) was kept at −20°C and used

in reduction and labeling experiments during ca. 3 weeks. Before use samples of this stock solution were diluted 1:100 or higher with carrier 0.05 M $NaBH_4$ in 0.01 M NaOH to obtain a reagent with lower levels of specific activity. Reagent-grade chemicals were used throughout. Glass microfiber filters (Reeve Angel No. 934AH or Whatman GF/B) and ashless filter papers Whatman No. 540 or No. 40) were used for absorption.

Glucose 6-phosphate concentrations were estimated spectrophotometrically [19]. Radioactivity was measured by liquid scintillation counting with the Aquasol scintillating cocktail (New England Nuclear) with 25% and 80% counting efficiency for ^3H and ^{14}C, respectively.

Procedure I. (a) Glass or paper filters were soaked in 0.2 M $BaCl_2$ solution at pH 8.0 and then dried. (b) A 40-μl sample of 0.1 M glucose 6-phosphate in 0.1 M Tris buffer, pH 8.0, was reduced with 200 μl (0.01—0.02 mCi) of 0.05 M [^3H]-$NaBH_4$ for about 15 min at room temperature; glacial acetic acid (40 μl) was then added to the solution to decompose the excess of borohydride. (c) Samples (10—30 μl) of the reduced sugar solution were applied to barium-impregnated filters as spots (5—10 mm diameter) and their circumference was marked by pencil. A series of 10 or more duplicate spots, as well as samples of a control solution which contained [^3H]-$NaBH_4$ but no sugar, were applied to the filter. Spots were air-dried and the paper was washed with 0.01 M NaOH in acetone—absolute methanol (1:9). The filters were then dried in a stream of warm air. (d) Areas corresponding to the applied spots were cut out and transferred to conical test tubes containing 0.5 ml of 0.01 M HCl. After 3 min at 90°C, the tubes were spun in a bench-top clinical centrifuge. Samples of the eluate (100 μl) were measured by scintillation counting. Another series of samples from this eluate (25 μl) was analyzed for total phosphate after ashing [20]. Control samples, obtained from a solution containing [^3H]-$NaBH_4$ without sugar, were analyzed in the same manner to provide blank values for both the tritium counting and the phosphorus determination. (e) For each individual sample the ratio of counts/phosphorus provides a value of the specific activity of the tritium which reacted in the reduction of glucose 6-phosphate. One mole equivalent of ^3H, of the four originally present in [^3H]-$NaBH_4$, is theoretically expected to appear in the [^3H]-glucitol 6-phosphate product.

Procedure II. A reaction mixture was prepared exactly as in Procedure I but with [^{14}C]-glucose 6-phosphate (0.01 μCi μmol^{-1}). Reduction and application of samples (20—50 μl of the reaction mixture) to the impregnated filter was done as in steps (b) and (c) above. After washing with alkaline methanol as in (c), the spots were cut out and the filter discs transferred to a scintillation vial containing 0.2 ml of water. After heating for 3 min at 90°C cold scintillation fluid (10 ml) was added and the ^3H and ^{14}C activities were measured. The ratio of $^3H/^{14}C$ for each sample was calculated after correction (8%) for spillover counts from the ^{14}C-channel to the ^3H-channel and after subtracting counts of ^3H present in control spots sampled from a sugar-free reaction system. The $^3H/^{14}C$ ratio, and the specific activity of the [^{14}C]-glucose

6-phosphate stock solution used, provide a means of calculating the specific activity of the ^3H which participated in the reduction.

Procedure III. A 40-μl sample of 0.1 M lactose solution in 0.1 M Tris buffer, pH 8.0, was reduced by [^3H]-$NaBH_4$ as described in Procedure I(b). Series of samples (20—40 μl) of the reaction mixture were spotted on barium-impregnated glass microfiber filters and air-dried. An aliquot (50 μl) of 1 M acetic acid in absolute methanol was applied to each spot and then air-dried; this was repeated four more times. The spots were cut out, transferred to conical tubes, eluted with 1.0 ml of water for 3 min at 90°C, and then centrifuged for several minutes to remove the filter. Samples (0.5 ml) of the eluate were assayed colorimetrically for galactoside [21] with galactose or lactitol as the standard. A second series of samples of the eluate (0.4 ml) was counted for radioactivity. The counts for each sample (after correction for blank values obtained for control spots) furnishes the specific activity of the [^3H]-lactitol present. Lactitol reacts in the phenol—sulfuric acid colorimetric method as a monosaccharide; its glucitol residue does not react [22]. In order to determine the specific activity of the [^3H]-$NaBH_4$ in the primary stock solution, the experimental value obtained should be multiplied by the dilution factor used to prepare the [^3H]-$NaBH_4$ reagent used in the three procedures described.

Discussion

The methods described are based on the fact that complete reduction of the sugar present occurs in the presence of a molar excess of borohydride, as verified by colorimetric, enzymatic, t.l.c. and g.l.c. examinations of the reaction mixtures. The three procedures described yield identical and reproducible values (±5%) for the specific activity of a given [^3H]-$NaBH_4$ solution used for reduction. Examples obtained for two [^3H]-$NaBH_4$ preparations are presented in Table 1. Circumstances and the reagents available determine which procedure should be used in a particular case and the dilutions of the primary reagent stock solution required. For better accuracy, it is advisable to analyze several samples of a given reaction mixture in order to obtain an average value. The conditions of assay, e.g. pH range and type of buffer used, can be modified to approximate more closely to the conditions used for reduction and tritiation in a particular experiment. Whereas specific activity determinations with the same [^3H]-$NaBH_4$ stock solution and buffer solution yielded similar values, they were usually only 30—80% of the values expected from the data supplied by the manufacturer for different batches of [^3H]-$NaBH_4$ tested. This indicates the unreliability of using the label values for quantitative measurements. In the reduction of various functional groups by [^3H]-$NaBH_4$, some isotope effect may occur and the specific activity values may vary slightly from the values obtained for the sugars used as model substrates in the reduction.

It is well recognized, but unfortunately often ignored, that most commercial ^3H-borohydride preparations contain a ^3H-"blank" which is acid-stable and non-volatile [1, 7, 11]. Some of this material, which may account

TABLE 1

Examples of the determination of the specific acitivity of tritium incorporated after reduction by [^{3}H]-$NaBH_4$

[^{3}H]-$NaBH_4$ [a] sample	Procedure[b]	Specific activity of product (dpm[^{3}H] μmol^{-1})	Specific activity[c] of 0.05 M [^{3}H]-$NaBH_4$ stock solution dpm[^{3}H] μmol^{-1} × 10^4)	
			Found	Expected from manufacturer's label value
1	I	419,920	8,398	13,200
	II	405,240	8,105	13,200
	III	430,418	8,608	13,200
2	I	193,300	3,866	11,100
	II	197,860	3,937	11,100
	III	184,350	3,687	11,100

[a]Samples of commercial product of different batches and age.
[b]See Experimental; the specific activity of the [^{14}C]-glucose 6-phosphate used in assay II was 24,000 dpm μmol^{-1}.
[c]Value given corresponds to 25% of the total radioactivity of [^{3}H]-$NaBH_4$, assuming random distribution of the tritium in the molecule and lack or minimal isotope effect during reduction of the glycose.

for up to 3% of the total radioactivity present, is non-dialyzable and can introduce significant errors in evaluating the amount of tritium incorporated into macromolecules or cellular structures. In such experiments, to establish the blank values for the acid-stable, non-volatile tritium present in the reagent is of paramount importance [17]. In the procedures described, most of this contaminant is not retained by the filters and the small residual background values for non-volatile tritium are obtained from control samples.

This work was supported by a grant from the College of Medicine and Dentistry of New Jersey-Rutgers Medical School General Research Fund.

REFERENCES

1 E. A. Evans, Tritium and its Compounds, 2nd edn., Butterworths, London, 1974, Ch. 4.
2 E. A. Evans, Self-decomposition of radiochemicals, Review No. 16, The Radiochemical Centre, Amersham, Gt. Britain, 1976.
3 R. H. Cornforth, Tetrahedron, 26 (1970) 4635.
4 R. H. Cornforth, Tetrahedron, 30 (1974) 3933.
5 S. Chaykin, K. Chakravarty, L. King and J. G. Watson, Biochim. Biophys. Acta, 124 (1966) 1.
6 D. A. Werner, C. C. Huang and D. Aminoff, Anal. Biochem., 54 (1972) 554.
7 H. E. Conrad, J. R. Bamburg, J. D. Epley and T. J. Kindt, Biochemistry, 5 (1966) 2808.
8 G. Avigad, Carbohydr. Res., 3 (1967) 430.
9 M. A. Paz, E. Henson, R. Rombauer, L. Abrash, O. O. Blumenfeld and P. M. Gallop, Biochemistry, 9 (1970) 2123.

10 G. N. Richards and W. J. Whelan, Carbohydr. Res., 27 (1973) 185.
11 H. E. Conrad, E. Varboncouer and M. E. James, Anal. Biochem., 51 (1973) 486.
12 H. C. Robinson, A. A. Horner, M. Höök, S. Ogren and V. Lindahl, J. Biol. Chem., 253 (1978) 6687.
13 A. G. Morell and G. Ashwell, in Methods in Enzymology, Vol. 28, Academic Press, New York, 1972, pp. 205—208.
14 L. V. VanLenten and G. Ashwell, in Methods in Enzymology, Vol. 28, Academic Press, New York, 1972, pp. 209—211.
15 T. H. Liao, P. M. Gallop and O. O. Blumenfeld, J. Biol. Chem., 248 (1973) 8247.
16 C. G. Gahmberg, K. Itaya and S. I. Hakomori, Methods Membr. Biol., 7 (1976) 179.
17 C. G. Gahmberg, in G. Poste and G. L. Nicolson (Eds.), Dynamic Aspects of Cell Surface Organization, Vol. 3, North-Holland, Amsterdam, 1977, pp. 371—422.
18 C. G. Gahmberg, in Methods in Enzymology, Vol. 50, Academic Press, New York, 1978, pp. 204—206.
19 G. Lang and G. Michal, in H. J. Bergmeyer (Ed.), Methods of Enzymatic Analysis, 2nd edn., Academic Press, New York, 1974, pp. 1238—1242.
20 B. N. Ames, in Methods in Enzymology, Vol. 8, Academic Press, New York, 1966, pp. 115—118.
21 M. Dubois, I. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Anal. Chem., 28 (1956) 350.
22 C. Asensio, G. Avigad and B. L. Horecker, Arch. Biochem. Biophys., 103 (1963) 299.

Analytica Chimica Acta, 111 (1979) 321—325

Short Communication

APPLICATION OF HYDRIDE GENERATION FOR THE DETERMINATION OF ANTIMONY AND ARSENIC IN BIOLOGICAL MATERIAL BY NEUTRON ACTIVATION ANALYSIS

D. HOEDE and H.A. VAN DER SLOOT*

Netherlands Energy Research Foundation, Postbus 1, 1755 ZG Petten (The Netherlands)

(Received 28th June 1979)

Summary. A rapid and sensitive procedure for the determination of antimony and arsenic in biological material is described. It is based on thermal neutron activation to ^{122}Sb ($t_{1/2}$ = 2.7 d) and ^{76}As ($t_{1/2}$ = 26.4 h), dry ashing with magnesium nitrate as the oxidizing agent and volatilization of the hydrides which are collected on an active carbon trap. This carbon adsorber is counted. The limit of determination is 5 ng g^{-1} for both elements.

For the determination of arsenic and antimony in biological materials, many procedures have been described based on distillation, hydride generation and adsorption on inorganic adsorbents [1—7]. Of these methods, hydride generation is attractive because of its speed, but the yield may be variable because of incomplete destruction of the biological material [4, 5].

Siemer et al. [5] described a procedure involving dry ashing with magnesium nitrate, which was quite effective and suffered no losses of arsenic in either valency form. In the work reported here, this approach was followed by neutron activation analysis instead of atomic adsorption. Collection of the hydrides on active carbon and counting this fraction resulted in pure ^{76}As and ^{122}Sb spectra. The 559-keV photopeak of ^{76}As and the 564-keV photopeak of ^{122}Sb were evaluated. The computer program used for calculation compensated for partial overlap of these peaks.

Experimental

Reagents and apparatus. The hydride generation apparatus with a septum for injection of sodium tetrahydroborate is shown in Fig. 1.

Hydrochloric acid was of Suprapur grade; all other reagents were of analytical grade.

Preliminary tracer experiments. Tracer experiments with ^{74}As and ^{124}Sb were carried out to check the recovery of arsenic and antimony from the mineralization with magnesium nitrate and the yield of the hydride generation. Both steps gave quantitative yields within experimental error. Previous experiments with a sulfuric—nitric acid mixture in a teflon-lined pressure decomposition vessel resulted in incomplete hydride generation with variable recoveries of 70—90%, probably because of incomplete destruction.

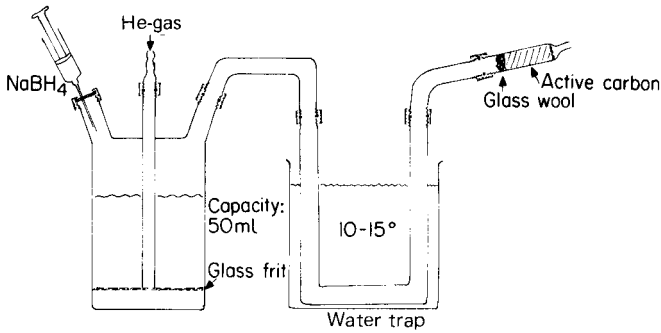


Fig. 1. The hydride generation vessel.

As arsenic(V) is reduced more slowly by sodium tetrahydroborate than arsenic(III) [6], a reduction with potassium iodide and l-ascorbic acid is required to convert all forms of arsenic present in the sample to the tervalent state. Granular active carbon is used for collection of the hydrides. A segmented adsorber was used to check the collection efficiency; 1 g of carbon proved to be quite sufficient.

Sample preparation and irradiation. Approximately 300 mg of sample is dried according to NBS specifications or in a freeze-drier, transferred to a polythene capsule and weighed. Standards of arsenic and antimony are prepared by pipetting 50-μl aliquots of standard solutions (200 μg As ml^{-1}, 200 μg Sb ml^{-1}) on pure active carbon (home-made quality) in vials as used for the samples. Thin iron rings, used as flux monitors, are mounted on top of each vial. Samples and standards are irradiated for 2 h in a rotating facility at a thermal neutron flux of 2×10^{12} n cm^{-2} s^{-1}.

Mineralization of samples. After cooling for ca. 12 h, the samples are transferred to 100-ml beakers, and 5 ml of saturated magnesium nitrate solution is added. Care should be taken to cover the samples completely. The beakers are placed on a hot plate and the solutions evaporated to dryness by heating slowly to 200°C. After addition of a further 5 ml of the magnesium nitrate solution to cover any incompletely mineralized parts of the sample, the beakers are again heated to 200°C. When the samples are dry, the beakers are heated in a muffle oven at ca. 450°C for 30 min. The white residue obtained is dissolved in 30 ml of 6 M hydrochloric acid.

Hydride generation. The solution is transferred to the hydride generation vessel, which is then sealed. A stream (200 ml min^{-1}) of helium is passed through the solution. Through the septum, 2 ml of 0.1 M KI—2% (w/v) l-ascorbic acid solution is added followed by 5 ml of 10% (w/v) sodium tetrahydroborate solution. After 2 min, a second 5-ml portion is injected. After a total reaction time of 4 min, the helium flow is stopped and the carbon adsorber is transferred to a test tube for counting.

Counting. Samples and standards are counted on a Ge/Li detector coupled

to a multichannel analyzer. The spectra are recorded on tape and processed by a computer program which compensates for partial peak overlap, and corrects for pulse pile-up by a pulser, for decay and sample weights.

The thin iron rings are measured in a 3 × 3-in. NaI detector coupled to a 400-channel analyzer. The 1292-keV photopeak area is normalized against previously determined count-rates for this ^{59}Fe peak.

The specific count-rates for the 559-keV peak of ^{76}As ($t_{1/2}$ = 26.4 h) and the 564-keV peak of ^{122}Sb ($t_{1/2}$ = 2.68 d) at the end of a 2-h irradiation at 2×10^{12} n cm^{-2} s^{-1} are 3800 cpm per μg As and 1650 cpm per μg Sb, respectively. This results in a limit of determination of 5 ng As g^{-1} and 5 ng Sb g^{-1} for a 300-mg sample when a 3-h counting period is used. If the Sb/As ratio is very unfavourable, a second counting after a decay period of a week is necessary.

Results and discussion

The results obtained for arsenic and antimony in NBS and IAEA standard reference materials are presented in Table 1. For comparison, literature data are given. The agreement with literature values is excellent. The method is rapid, sensitive and free of radiochemical interferences. The γ-spectrum of arsenic and antimony hydrides collected on a carbon adsorber obtained from IAEA animal blood is shown in Fig. 2. In comparison with the work of

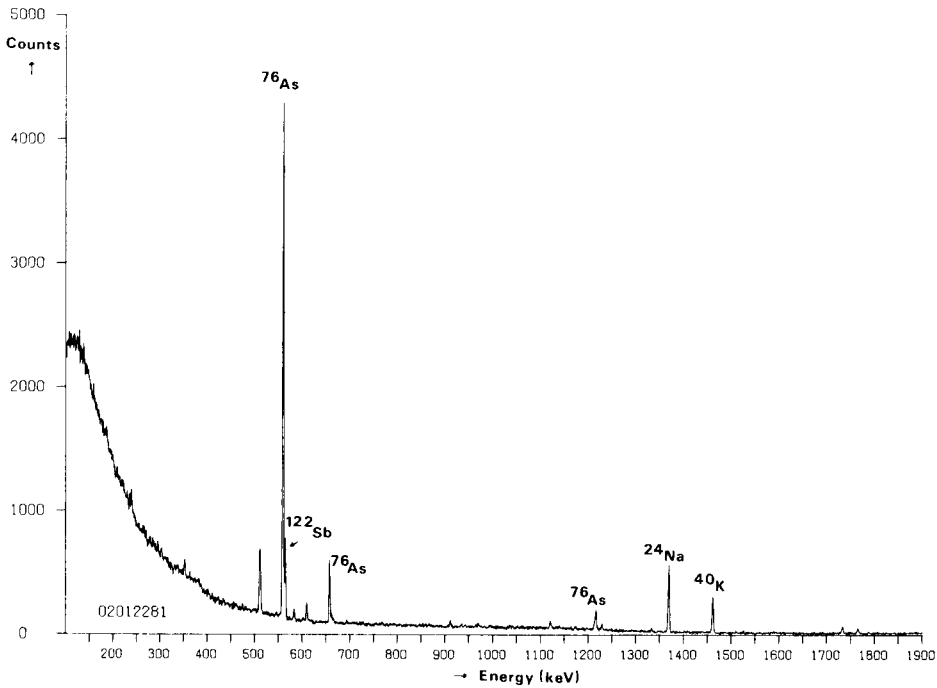


Fig. 2. The γ-spectrum of the carbon adsorber obtained for IAEA animal blood.

TABLE 1

Results (in ppm) for NBS and IAEA biological reference materials

SRM/RM	Arsenic	
	This work	Literature[a]
Orchard Leaves SRM 1571	9.76 ± 0.17	10*, 9.94 [1], 9.68 [8]
Bovine Liver SRM 1577	0.046 ± 0.002	(0.055)*, 0.046 [1], 0.053 [7], 0.08 [2]
Spinach SRM 1570	0.147 ± 0.001	0.150*, 0.120 [1]
Tomato Leaves SRM 1573	0.225 ± 0.003	0.27*, 0.26 [10]
Pine Needles SRM 1575	0.181 ± 0.003	0.21*, 0.20 [10]
Wheat Flour V2/1-74	0.020 ± 0.001	0.024**, 0.035 [1], 0.013 [2]
Animal Muscle H4-13	0.005 ± 0.001	0.0074**, 0.0051 [7]
Animal Bone A3-74	1.039 ± 0.010	—
Animal Blood A2-74	0.198 ± 0.005	0.212 [2]
Fish Solubles A6-75	15.80 ± 0.30	14.5**, 17 [10]
Bowen's Kale	0.098 ± 0.004	0.101 [1], 0.111 [2], 0.14 [9]
SRM/RM	Antimony	
	This work	Literature[a]
Orchard Leaves SRM 1571	2.90 ± 0.09	2.9*
Bovine Liver SRM 1577	0.005 ± 0.002	0.015 [2]
Spinach SRM 1570	0.038 ± 0.003	(0.040)*, 0.027 [10]
Tomato Leaves SRM 1573	0.030 ± 0.001	0.040 [10]
Pine Needles SRM 1575	0.185 ± 0.003	(0.200)*, 0.180 [10]
Wheat Flour V2/1-74	0.005 ± 0.002	0.008 [2]
Animal Muscle H4-13	<0.002	<0.02 [10]
Animal Bone A3-74	0.170 ± 0.005	—
Animal Blood A2-74	0.045 ± 0.002	0.045 [2]
Fish Solubles A6-75	0.154 ± 0.006	0.072 [10]
Bowen's Kale	0.039 ± 0.001	0.070 [2]

[a]NBS certified (or proposed) values and IAEA specifications are asterisked and double-asterisked, respectively.

Orvini and Delfanti [7], the present method is less time-consuming and allows for the measurement of both arsenic and antimony.

As mentioned above, digestion with an acid mixture in a pressure decomposition vessel was often found to lead to incomplete destruction of plant material, which causes incomplete volatilization of the hydrides.

In the proposed method, some 40 samples can be irradiated simultaneously. Mineralization of 20 samples takes about an hour and the generation of the hydrides, including rinsing of the apparatus, takes 6—8 min per sample. The simultaneous use of five hydride generation vessels reduces the total time-requirement to about 1.5 h. By splitting the mineralized sample into two equal portions and using one aliquot for the generation of hydrides (As, Sb) and the other for the separation of some 25 trace elements by successive adsorption on active carbon as described elsewhere [11], a very profitable and fast combination is obtained.

Thanks are due to Prof. Dr. H. A. Das for his fruitful suggestions in preparing the manuscript.

REFERENCES

1 M. Ihnat and H. J. Miller, J. Assoc. Off. Anal. Chem., 60 (1977) 1414, 813.
2 P. S. Tjioe, J. J. M. de Goey and J. P. W. Houtman, J. Radioanal. Chem., 37 (1977) 511.
3 F. Girardi, R. Pietra and E. Sabbioni, J. Radioanal. Chem., 5 (1970) 141.
4 J. A. Fiorino, J. W. Jones and S. G. Gapar, Anal. Chem., 48 (1976) 120.
5 D. D. Siemer, R. K. Vitek, P. Koteel and W. C. Houser, Anal. Lett., 10 (1977) 357.
6 A. U. Shaikh and D. E. Tallman, Anal. Chim. Acta, 98 (1978) 251.
7 E. Orvini and R. Delfanti, Radiochem. Radioanal. Lett., 37 (1979) 199.
8 K. Heydorn, NBS Spec. Publ., 422 (1976) 127.
9 H. J. M. Bowen, J. Radioanal. Chem., 19 (1974) 2150.
10 R. A. Nadkarni, Radiochem. Radioanal. Lett., 30 (1977) 329.
11 H. A. v.d. Sloot, G. D. Wals, C. A. Weers and H. A. Das, Anal. Chem., in press.

Analytica Chimica Acta, 111 (1979) 327—331

Short Communication

TITRIMETRIC DETERMINATION OF MICROGRAM AMOUNTS OF LEAD BY THIOCYANATE AMPLIFICATION AFTER PRECIPITATION AS $Pb(SCN)_3[N(C_4H_9)_4]$ †

J. HERNÁNDEZ-MÉNDEZ*, A. ALONSO-MATEOS and E. J. MARTIN-MATEOS

Department of Analytical Chemistry, Faculty of Science, University of Salamanca (Spain)

(Received 4th June 1979)

Summary. Lead (15—120 μg) is precipitated as $Pb(SCN)_3[N(C_4H_9)_4]$. After filtration, the precipitate is treated with hydrogencarbonate solution and the thiocyanate released is determined by a titrimetric procedure which provides 114 iodine atoms for each original lead ion.

Organic precipitants for inorganic ions generally offer the advantage of favourable gravimetric factors. This is especially true for the lead(II) ion, whose inorganic precipitates (sulphate, chromate, dioxide, chloride, sulphite, etc.) exhibit exceptionally unfavourable gravimetric factors. Many organic reagents have been proposed for the gravimetric determination of lead, including 8-quinolinol, dimethylglyoxime, anthranilic acid, salicylaldoxime, dibromo-8-quinolinol, picrolonic acid, mercaptobenzothiazole, mercaptobenzimidazole and thio-5-nitrooxime [1]. However, the use of these reagents requires a very critical control of the experimental conditions to ensure quantitative precipitation and to avoid excessive contamination.

Amplification methods were first used over 100 years ago, but the term only came into general use during the development of microanalytical techniques. As illustrated in Belcher's review [2], most known amplification procedures are based on iodide amplification. For example, Belcher et al. [3] have described a titrimetric method for the determination of iodide involving a 24-fold amplification by using the iodide—periodate reaction. Shveikina [4] used both a kinetic method and a colorimetric method to determine 0.1—0.5 μg of iodide in water. The kinetic method is based on the loss of colour in the oxidation of iron(III) thiocyanate by sodium nitrite in the presence of iodide. The colorimetric method involves toluene extraction of the complex of brilliant green with I_2Cl^-. Belcher et al. [5] recently developed an indirect method for the determination of small amounts of thiocyanate (2.7—90 μg) and thiosulphate (4.5—90 μg) ions, by oxidation with iodine in alkaline solution to give sulphate. After acidification, the excess of iodine was extracted into chloroform, and the iodide ions formed in the

†Taken in part from the Ph.D. work of E. J. Martin Mateos.

redox reaction were amplified. Either a titrimetric or a spectrophotometric finish could be used. Each thiocyanate and thiosulphate ion results in the ultimate production of 19 and 24 iodine molecules, respectively.

Belcher et al. [6] also recently developed an indirect method for the determination of small amounts of bismuth. After precipitation as bismuth hexathiocyanatochromate(III), the thiocyanate was determined by the above amplification reaction. Some quaternary ammonium compounds have been used as precipitants of large anionic complexes. In this communication, the application of the above thiocyanate amplification to the determination of lead [15—120 μg] after precipitation as $Pb(SCN)_3[N(C_4H_9)_4]$ is reported.

Experimental

Reagents. All solutions were prepared in twice-distilled water. Unless otherwise specified, all reagents were of analytical grade. A 0.1024 M ammonium thiocyanate solution was prepared by dissolving the solid in water, and standardizing by titration with 0.1026 M silver nitrate solution. Other solutions were prepared by direct weighing of the solid on the basis of this assay. Tetrabutylammonium chloride solutions were prepared by dissolving the solid (Ega-Chemie) in water. Lead(II) nitrate pentahydrate, for which a purity of 99.92% was found by gravimetry as chromate, was used to prepare lead(II) solutions for the amplification reactions containing 19.4, 47.5 and 193.5 μg Pb ml^{-1}, by dissolution of the solid in water. Sodium thiosulphate (2.028 mM and 4.056 mM) solutions were standardized by titration with standard iodate solution.

Determination of lead by indirect amplification. To 3—4 ml of cold solution containing 15—120 μg of lead in a test-tube, add 0.5—1 ml of 1.25 M potassium thiocyanate solution and 1 ml of 0.05 M tetrabutylammonium chloride solution. After precipitation is complete (see Table 4), filter off the precipitate on a porosity-5 sintered-glass filter and wash with 1-ml portions of 0.01 M tetrabutylammonium chloride solution and then with 2-ml portions of cold twice-distilled water. Dissolve the precipitate in 10 ml of saturated sodium hydrogencarbonate solution, transfer the solution to a separating funnel, add 1 ml of a saturated solution of iodine in chloroform and shake well. After 5 min, acidify with 4 ml of 2 M sulfuric acid to pH 2.5; extract the iodine with 10-ml portions of chloroform, shaking for 1 min each time (normally 4—6 extractions are sufficient). Transfer the aqueous layer to a 100-ml stoppered flask, add 1 ml of saturated bromine water, shake gently for 5 min, destroy the excess of bromine with 3—4 ml of 90% formic acid, removing any bromine vapour by suction and add 1—1.5 g of potassium iodide. Leave to stand in the dark for 5 min, and titrate the liberated iodine with standard sodium thiosulphate solution, using starch as indicator. Run a hydrogencarbonate blank through the whole process.

Results and discussion

Precipitation of lead as $Pb(SCN)_3[N(C_4H_9)_4]$. Preliminary tests showed

TABLE 1

Determination of the gravimetric factor for precipitation of lead as $Pb(SCN)_3[N(C_4H_9)_4]$

Pb^{2+} (mg)	Precipitate (mg)	Gravimetric factor	Pb^{2+} (mg)	Precipitate (mg)	Gravimetric factor
186.8	860.6	0.3333	712.5	2144.5	0.3322
566.3	1697.8	0.3335	824.5	2471.7	0.3336
640.4	1927.4	0.3323	906.2	2727.0	0.3323
569.8	1977.3	0.3337			Mean 0.3330

that the white precipitate obtained when thiocyanate and tetrabutylammonium chloride are added in excess to a solution which contains lead ions may be oven-dried to constant weight at low temperatures without decomposition. The gravimetric factor found (0.3330) is close to the value of 0.3322 (Table 1) corresponding to anhydrous $Pb(SCN)_3^-[N(C_4H_9)_4]^+$. The thiocyanate ion content of this compound was confirmed by a Volhard's titration. An excess of thiocyanate and of the quaternary salt is necessary to obtain an easily filterable precipitate with a large particle size.

Determination of lead by indirect amplification. On treatment with sodium hydrogencarbonate solution, the precipitate releases thiocyanate with formation of lead carbonate:

$$Pb(SCN)_3[N(C_4H_9)_4] + HCO_3^- \rightarrow PbCO_3 + 3SCN^- + H^+ + [N(C_4H_9)_4]^+$$

According to the reaction sequence described by Belcher et al. [5], thiocyanate is oxidized to sulphate by an excess of iodine, and ultimately from each original thiocyanate ion, 19 molecules of iodine are obtained; therefore from each lead ion, 57 molecules of iodine are obtained.

The effect of lead ions and tetrabutylammonium chloride on the amplification of 39.9 μg of thiocyanate was investigated. The results, summarized in Table 2, show that the amounts of these reagents present do not influence the determination at the levels studied.

Dissolution of a known amount of the precipitate in a hydrogencarbonate medium and titration of aliquots of this standard solution by the amplification procedure showed that as little as 3.9 μg of lead could be titrated satisfactorily (Table 3). However, in practice, when the amount of lead is less than 20 μg, precipitation is very slow and the method becomes impractical (Table 4).

It proved possible to precipitate quantitatively 15—250 μg of lead in 3—4 ml of sample solution by using the precipitation times given in Table 4. The results in Table 5 show recoveries ⩾ 99.5% for 15—120 μg of lead; for more than 120 μg, the recovery decreases as the amount of lead increases. The blank was always within the range 0.45—0.65 ml of 2.028 mM thiosulphate and 0.20—0.35 ml of 4.056 mM thiosulphate solutions.

TABLE 2

Effect of lead and tetrabutylammonium chloride on the amplification reaction

Pb^{2+} added (μg)	SCN^-:Pb^{2+}	SCN^- found[a] (μg)	$[R_4N]Cl$ added (μg)	SCN^-:$[R_4N]^+$	SCN^- found[a] (μg)
39.9	1:1	39.8	1995	1:50	39.8
399	1:10	39.9	7980	1:200	39.9
798	1.20	39.8	15960	1:400	39.9
1995	1.50	40.1	19950	1:500	39.8
2793	1:70	39.6			

[a]39.9 μg SCN^- taken.

TABLE 3

Determination of lead by titration of thiocyanate released from $Pb(SCN)_3[N(C_4H_9)_4]$

Pb taken (μg)	3.9	7.8	9.7	19.4	38.7	71.3	96.9	136	155	194
Pb found (μg)[a]	3.9	7.8	9.7	19.4	38.5	71.1	96.5	135	154	190

[a]Mean of three determinations.

TABLE 4

Effect of time on extent of precipitation

Pb^{2+} taken (μg)	Pb^{2+} found (μg)						
	Precipitation time						
	5 min	15 min	30 min	2 h	3 h	5 h	8 h
9.7	2.4	5.3	6.8	7.4	8.1	9.3	9.6
19.4	14.2	17.7	18.0	18.6	19.0	19.4	—
38.7	34.7	38.0	38.5	—	—	—	—
71.3	70.2	71.1	—	—	—	—	—
96.9	96.6	—	—	—	—	—	—

TABLE 5

Results obtained for the indirect determination of lead

Pb taken (μg)	15.5	23.8	47.5	71.3	155.0	193.7	251.8
Pb found (μg)	15.4	23.7	47.4	71.0	153.6	189.9	247.0
Difference (%)	0.6	0.4	0.2	0.4	0.9	3.8	4.8

The presence of Ca^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+} and Ni^{2+} in 10-fold amounts had no effect on the determination of 96.9 μg of lead. However, the following will interfere: Ag^+, Hg_2^{2+}, Hg^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, Sb(III), Sn^{2+},

Mo(VI), Al^{3+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Ti(IV), V(IV), Mn^{2+} and Zn^{2+} because they form similar compounds with thiocyanate and tetrabutylammonium chloride.

REFERENCES

1 See, e.g., L. Erdey, Gravimetric Analysis, Part 2, Pergamon, Oxford, 1965, p. 27.
2 R. Belcher, Talanta, 15 (1968) 357.
3 R. Belcher, J. W. Hamya and A. Townshend, Anal. Chim. Acta, 49 (1970) 570.
4 R. V. Shveikina, Zh. Prikl. Khim., 41 (1968) 1212.
5 R. Belcher, S. S-T. Liao and A. Townshend, Talanta, 23 (1976) 541.
6 R. Belcher, S. Liawruangrath and A. Townshend, Talanta, 24 (1977) 590.

Analytica Chimica Acta, 111 (1979) 333—339

Book Reviews

J. Veselý, D. Weiss and K. Stulík, *Analysis with Ion-selective Electrodes* (Translated by M. Stulíkova), Ellis Horwood, Chichester — J. Wiley, New York, 1978, 245 pp., price £16.00.

Ion-selective electrodes have become conventional laboratory equipment for many areas of science in recent years. The detailed theory of various facets of their behaviour remains a matter of argument but there can now be no doubt of their practical value for rapid and on-line analysis if they are used sensibly with a proper appreciation of their limitations and possibilities. This essentially practical book will prove of significant value to potential and actual users who are more concerned with practice than with theory.

After the introductory chapter, which contains about the right amount of theory for the purpose, chapters are devoted to instrumentation, experimental techniques, and applications. In this final chapter, the use of the electrodes available for direct and indirect determinations is reviewed. The chapters on general instrumentation and techniques are both good; the latter contains the clearest exposition of standard addition, standard subtraction, Gran plot and analogous procedures that this reviewer has encountered. The appendix contains eleven tables of useful data. The reference list contains 839 citations, indicating the selectivity of the treatment. The translation and production are both very good.

This is an eminently sensible book which can be recommended warmly not only to students and their teachers but to those who require a succinct account of the utilization of ion-selective electrodes for practical applications.

H. Freiser (Ed.), *Ion-selective Electrodes in Analytical Chemistry*, Vol. 1, Plenum Press, New York, 1978, xiii + 439 pp., price $51.00.

This is the first volume of a series intended to provide well-rounded, up-to-date reviews of important aspects in the exciting field of ion-selective electrodes (i.s.e.). It contains six chapters: theory and principles of membrane electrodes by R. P. Buck; precipitate-based i.s.e. by E. Pungor and K. Tóth; i.s.e. based on neutral carriers by W. E. Morf and W. Simon; poly-(vinyl chloride) matrix membrane i.s.e. by G. J. Moody and J. D. R. Thomas; sources of error in i.s.e. potentiometry by R. A. Durst; and applications of i.s.e. by G. J. Moody and J. D. R. Thomas. Thus, the editor has selected his authors from among the leading exponents of the art, and has been rewarded with excellent reviews.

Theory is concentrated in the first chapter (141 pp.) but most other chapters also contain theoretical sections, and the different approaches set together provide interesting reading. The chapters on the different types of electrodes also contain much valuable descriptive material. Those who think still that using these electrodes simply involves sticking in a probe and taking a meter reading will find Durst's short chapter a splendid education, whereas anyone who considers that i.s.e. are rather esoteric items of dubious practical value should read the chapter on applications (94 pp.)

The book is quite capable of standing alone, as intended by its editor, who may indeed be rather pushed to maintain this standard of contribution in subsequent volumes. It is properly printed and well-produced, so that it can be regarded as cheap at the price. As an up-to-date survey of the i.s.e. field, this volume is strongly recommended to all analytical chemists.

C. Clark Westcott, *pH Measurements*, Academic Press, New York, 1978, x + 172 pp., price $16.00 (£10.40).

Measurement of pH with glass combination electrodes is now such a commonplace laboratory operation that inexperienced workers tend to forget even that there are such things as reference electrodes and that standardisation is essential. Such workers will find this book invaluable. It is a practical manual, offering minimal essential theory and maximal practical instruction. The seven chapters cover principles, a description of various pH meters, electrodes and how to treat them, standard buffer solutions, measurement techniques, applications, and troubleshooting. Useful appendices give tables of temperature effects, dielectric constants, Clark and Lubs buffers, solvent conductivity, Debye—Hückel constants and liquid-junction potentials. The author is from Beckman Instruments, California, and obviously has many years of experience in answering silly questions about pH measurements. The very useful chapter on applications deals with non-aqueous solutions, solid samples, soils, slurries, high-purity water, fluoride-containing solutions and biological samples.

This is not a text for electroanalytical chemists but for practical workers who know little and care less about the niceties of electrochemistry yet need to produce meaningful pH measurements. Ideally, the book should be slightly condensed and a paperback version placed beside every pH meter. The general standard of laboratory pH measurements would then improve spectacularly.

E. Pungor (Ed.), *Ion-selective Electrodes.* Akademiai Kiado, Budapest and Elsevier, Amsterdam, 1978, ix + 609 pp., price Dfl. 180.00 (U.S. $75.00 approx.).

The papers included in this volume were presented at the Conference on ion-selective electrodes held in Budapest in September, 1977. These inter-

national conferences organized by Professor Pungor and his colleagues are deservedly popular and well-attended. There were seven plenary lectures and 47 discussion papers, covering most aspects of i.s.e. theory and application. For the specialist, there is much of interest in this volume, although a significant amount of it has appeared elsewhere in the literature. However, the production is very poor; the typescripts have been directly reproduced complete with handwritten corrections, and the figures and tables are bundled together at the end of the paper in most cases. This sort of thing does nothing for the general stature of analytical conferences or their proceedings. Only very enthusiastic i.s.e. researchers who like to have their files complete are likely to part with the outrageous price asked for this collection.

J. F. K. Huber (Ed.), *Instrumentation for High-performance Liquid Chromatography (J. Chromatogr. Library, Vol. 13)*, Elsevier, Amsterdam, 1978, xii + 204 pp., price Dfl. 80.00 (U.S. $34.75 approx.).

This book provides a survey of general design features and technical considerations for instrumentation in h.p.l.c. Professor Huber has brought together authors with extensive experience in h.p.l.c. and has ensured that there is negligible overlap between their contributions. The text is introduced by a short chapter on systems theory (J. F. K. Huber). M. Martin and G. Guiochon then deal with pumps systems and solvent gradient systems; J. C. Kraak discusses sample introduction systems and column design; A. Wehrli copes with preparative h.p.l.c. Three chapters are devoted to detector systems: those based on optical and electrical or electrochemical properties are described by H. Poppe and radiometric detectors by P. Markl. Liquid chromatography—mass spectrometric systems, both actual and possible, are reviewed by E. Kenndler and E. R. Schmid. Finally, R. R. Becker lists the specifications of commercial instruments. The literature is covered up to September, 1977.

Essentially a practical guide, this volume provides a wealth of authoritative information on h.p.l.c. and is strongly recommended.

R. P. W. Scott, *Liquid Chromatography Detectors, (J. Chromatogr. Library, Vol. 11)*, Elsevier, Amsterdam, 1978, vii + 248 pp., price Dfl. 84.00 (U.S. $34.50 approx.).

Even the best liquid chromatographic column is useless without a suitable detection system. Yet far less attention has been given to detectors than to almost any other aspect of the general technique. Dr. Scott's book draws attention to the theoretical and practical advantages and deficiencies of the systems available up to about 1975. The text is divided into four parts which deal with general characteristics, bulk property detectors, solute property

detectors, and the use of detectors. The method of organization means that the same sort of detector is discussed at different parts of the book, but this is rarely clear from the index, which is poor. The book is marred by grammatical and/or proofreading errors and carelessnesses such as the attribution of Professor Kemula's well-known early work on polarographic detectors to a character named Varmula. If the reader proceeds with a certain wariness, however, there is a great deal of extremely useful information to be gleaned from the text, based on Dr. Scott's vast experience of the field.

R. J. Hamilton and P. A. Sewell, *Introduction to High-performance Liquid Chromatography*, Chapman and Hall, London, 1977, xii + 183 pp., price £8.00.

Despite the importance of high-performance liquid chromatography in research and industrial applications, there has been, until recently, a great shortage of books dealing with the technique at a level suitable for use by beginners. With the increasing sophistication of commercial h.p.l.c. equipment, it is vital that users have an appreciation of the fundamental principles and practice of the method, for instruments can only follow instructions and these should be given on a well-informed basis. This book is intended to provide the basics and, on the whole, it succeeds very well.

After a brief introduction in which the confusing nomenclature and the different sorts of h.p.l.c. are properly defined, the subsequent seven chapters are devoted to chromatographic theory, equipment, stationary phases, mobile phases, development of chromatograms, preparative h.p.l.c. and trace analysis, and applications.

As with all rapidly-developing techniques, the refinements date quickly, but any student well versed in the contents of this book will be in a good position to tackle more advanced texts. The text is what it claims to be: it provides an introduction useful both to students and their teachers.

N. A. Parris, *Instrumental Liquid Chromatography (J. Chromatogr. Library, Vol. 5)*, Elsevier, Amsterdam, 1977, x + 328 pp., price Dfl. 100.00 (U.S. $41 approx.).

This book is subtitled: A Practical Manual on High-Performance Liquid Chromatographic Methods. After brief introductory chapters on basic principles, terminology, and chromatographic supports and columns, there are detailed descriptions of instrumentation, and detection systems. The major portion of the text is devoted to factors influencing selectivity in the different varieties of chromatography (l.s.c., l.l.c., ion-exchange, steric exclusion) and to the special features which must be considered in qualitative and quantitative applications as well as in trace analysis and preparative work.

Finally, there are reference lists to illustrate the broad applicability of h.p.l.c., and useful appendixes. This should be a very useful text for readers who have a reasonable basic knowledge of h.p.l.c. without claiming any great expertise on the subject. It merits browsing as well as reading by anyone concerned with improving the quality of their h.p.l.c. results.

D. M. Hercules, G. M. Hieftje, L. R. Snyder and M. A. Evenson (Eds.), *Contemporary Topics in Analytical and Clinical Chemistry, Vol. 3*, Plenum Press, New York, 1978, xi + 306 pp., price U.S. $39.00

Volume 3 of this series includes six reviews, five of which deal with different aspects of chemical analysis. In the first review (50 pp.), surface characterization of biological materials by x-ray photoelectron spectroscopy is discussed by M. M. Millard; general principles are reviewed briefly and the bulk of the review is devoted to the difficult problems posed by polymer coatings on wool fibres and by tissue culture cells. In the second review (30 pp.), J. A. Borders gives a short survey of surface analysis with energetic ions in the range 100 keV--2.0 MeV, and provides a useful evaluation of the applicability of the available methods ranging from Rayleigh back-scattering to secondary ion mass spectrometry. The third article, by J. P. Walters (60 pp.), is concerned with the construction of a spectrochemical system suitable for graduate teaching and research; anyone involved with the juggling acts necessary to maintain both expensive equipment and a viable large research program will find this article of interest. The longest review (63 pp.) deals with correlation methods in chemical data measurement. G. Horlick and G. M. Hieftje pitch this review at the beginner level and explain in relatively simple terms the great value of these techniques, particularly in spectroscopic analysis. D. M. Peterson and J. M. Hayes discuss signal-to-noise ratios in mass spectroscopic ion-current measurements. The final review deals with analytical techniques for studies of biological membranes; this is the only one which is really concerned with clinical chemistry.

All these reviews are authoritative and critical, and can be recommended for general reading in the topics concerned.

T. Kuwana (Ed.), *Physical Methods in Modern Chemical Analysis, Vol. 1*, Academic Press, New York, 1978, x + 320 pp., price U.S. $33.00.

Berl's *Physical Methods in Chemical Analysis* is well known to analytical chemists of the older generation at least. This series is intended as a modern version, and will include descriptions of selected techniques and methodologies. The first volume contains articles on gas chromatography (J. P. Okamura and D. T. Sawyer), mass spectrometry instrumentation (B. N. Colby), applications of mass spectrometry (C. Fenselau), atomic fluorescence

and atomic absorption spectroscopy (T. J. Vickers), and flame and plasma emission analysis (P. N. Keliher). The book is well printed and produced.

The series is aimed at chemists who wish to expand their working knowledge or update their background. Each chapter is intended to cover fundamental principles, instrumentation and typical applications. In this first volume, this general design has led to an excessive amount of common textbook material in several chapters, but this aspect may improve as the series moves on to less securely established techniques. There is certainly a need for treatment of instrumental methods at a level between student textbooks and specialized monographs. The general impression given by this volume, however, is that editor and authors should raise their sights a bit. The series could turn out to be as useful as Berl's was, but a proper verdict cannot be given on the basis of this first volume.

A. R. West (Ed.), *Molecular Spectroscopy*, Heyden, London, 1977, xx + 578 pp., price £30.00 U.S. ($60.00).

These Proceedings of the Sixth Conference on Molecular Spectroscopy organized by the Institute of Petroleum and held in Durham in 1976 contain much of value. The introductory lecture by Professor Sir Harold Thompson is a personal 40-year perspective of chemical spectroscopy. The subsequent twenty-six papers are concerned with n.m.r., i.r. and Raman spectroscopy, electron spectroscopy (mainly e.s.c.a.), and assorted applications. Most of them are reviews written by established experts in the particular area and provide excellent state-of-the-science surveys at the time of the Conference. The emphasis is generally placed on the instrumentation and on the sort of results that have been and can be achieved by molecular spectroscopic techniques.

This volume is very well produced, and is printed properly, which makes a welcome change from the reproduced typescripts now so frequently seen in conference proceedings. The book can be recommended, not only to those spectroscopic specialists who were unable to attend the Conference but also to the general chemist, as an easily read survey of modern progress in this area.

T. Y. Toribara, J. R. Coleman, B. E. Dahneke and I. Feldman (Eds.), *Environmental Pollutants — Detection and Measurement*, Plenum Press, New York 1978, xi + 500 pp., price U.S. $51.00.

This large volume contains the full texts and discussions of the twenty-three papers read at the Tenth Rochester Conference on Environmental Toxicity, held at Rochester, N.Y., in May 1977. The papers are divided between five sections: specification of analytical problems; familiar principles;

methods for field use; high spatial resolution microprobe methods; physical analytical methods. All are of the review type. The book ends with the names, addresses and photographs of the 23 lecturers and all five (sic!) participants. No doubt the participants will find this book a splendid memento. Others would be well advised to spend the money on proper textbooks or on journal subscriptions. It can be recommended only to fervent environmentalists.

Proceedings of the Sixth Ceramic Chemists' Conference on Silicate Analysis, Spec. Publ. No. 98, British Ceramic Research Association, Stoke-on-Trent, England, 1979, vi + 153 pp., price £8.00.

This paperback contains the six papers presented at the Sixth Ceramic Chemists' Conference held in April 1978, as well as the introductory speeches and discussions. The papers are mostly concerned with applications of spectroscopic technique (e.g. infrared, x-ray fluorescence, optoacoustic) to materials of industrial concern, and make interesting reading from the practical viewpoint.

R. S. Young, *The Chemical Analysis of Manganese*, Manganese Centre, 17 Avenue Hoche, 75008 Paris, 32 pp., free on request.

This pamphlet provides a survey of methods for the separation and determination of manganese; chemical phase analysis for manganese and its carbonates and oxides, and analysis for other elements in manganese-containing materials are also summarized. There is little information here that is not readily available from other sources.

H. J. Boniface, *Analytical Notes: A Summary of Inorganic Methods of Chemical Analysis*, Sigma Technical Press, Wolverhampton, England, 1978, 60 pp., price £3.50.

This booklet is designed as a guide to chemical methods available for the determination of the commoner elements (30 in all), two pages being devoted to each element. The information listed includes standard solutions, reaction equations, separation methods, determinations by gravimetric, titrimetric, spectrophotometric, fluorimetric, flame spectrometric and, sometimes, electrometric techniques, and applications to typical materials. References are given but too often they are neither the best nor the most recent for a particular procedure. The booklet is intended as a guide for analysts, lecturers and students. It cannot, unfortunately, be recommended to any of them. For analysts, it is a listing, not a guide; for pedagogic use, there are far too many errors, misprints and inconsistencies; for all, the information given is so inadequate that it is misleading and many common elements have been omitted.

ANALYTICA CHIMICA ACTA, Vol. 111 (1979)

AUTHOR INDEX

Announcement

The Internatinal Winter Conference1980 on Developments in Atomic Plasma Spectrochemic: Analyses with inductively coupled (ICP), microwave (MIP, MWP), and D.C. plasma (DCP) discharches will be held at the Hilton Condado Beach-La Concha Hotels and Convention Cen in San Juan, Puerto Rico (U.S.A.) from January 7—11, 1980.

Papers describing original work on applications, fundamentals, and instrumentation developments of atomic plasmas (ICP, MIP, DCP) in spectrochemical analysis will be presented in general and invited symposia. Special application symposia organized and chaired by recognized experts will be held in topical areas which include agriculture and food; biology, medicine, and industrial hygiene; geology and mining; energy production and energy-related materials; environmental monitoring; metals and industrial chemicals; oceanography; petroleum products and fuels, and water quality monitoring. Other special symposia will feature recent developments in sample treatment and introduction; element-specific plasma detectors for chromatography, and new plasma generators, sources, and spectrometer systems.

Further information can be obtained from: Winter Conference 1980, ICP Information Newsletter, Chemistry — GRC Tower I, University of Massachusetts, Amherst, Mass. 01003. Telephone: (413)-545-2294.

ANALYTICA CHIMICA ACTA
(including COMPUTER TECHNIQUES AND OPTIMIZATION)

INFORMATION FOR AUTHORS

Analytica Chimica Acta publishes original papers, short communications, preliminary communications, and reviews dealing with every aspect of modern chemical analysis, both fundamental and applied. The section on *Computer Techniques and Optimization* is devoted to new developments in chemical analysis by the application of computer techniques and by interdisciplinary approaches, including statistics, systems theory and operation research.

Reviews are written by invitation of the editors, who welcome suggestions for subjects. Short communications are usually complete descriptions of limited investigations, and should generally not exceed four printed pages. Preliminary communications of important urgent work can be printed within 4 months of submission, if the authors are prepared to forego proofs.

Submission of papers

Authors should submit three copies of the manuscript in double-spaced typing on one side of the paper only, with a margin of 4 cm, on pages of uniform size. If any variety of machine copying is used (e.g. xerox), authors should ensure that all copies are easily legible and that the paper used can be written on with both ink and pencil. Authors are advised to retain at least one copy of the manuscript. Manuscripts should be preceded by a sheet of paper carrying (a) the title of the paper, (b) the name and full postal address of the person to whom proofs are to be sent, (c) the number of pages, tables and figures.

Manuscripts should be sent to the editorial addresses given on the covers of current issues; submission to the publisher leads to delays. Submission of a manuscript implies that the work described has not been, and will not be, published elsewhere (except as an abstract, or as part of a lecture, review or academic thesis). On acceptance of the manuscript, the copyright passes to the publishers, if it has not been previously reserved by a government institution or company.

The preferred language of the journal is English, but French and German manuscripts are also acceptable. For authors whose first language is not English, French or German, linguistic improvement is provided as part of the normal editorial processing.

Notes on the preparation of manuscripts

Authors are given every latitude, consistent with clarity and brevity, in the style and form of their papers. Very useful advice is provided in the Handbook for Authors issued by the Chemical Society and American Chemical Society.

Title and initial layout. All manuscripts should be headed by a concise but informative title. This is followed by the names of the authors, and the address of the laboratory where the work was carried out. The author to whom correspondence should be addressed must be indicated by an asterisk (without a footnote). If the present address of an author is different from that mentioned, it should be given in a footnote. Acknowledgements of financial support should not be made in footnotes but at the end of the paper.

Summary. Research papers and reviews begin with a Summary (50—250 words) which should comprise a brief factual account of the contents of the paper, with emphasis on new information. Uncommon abbreviations, jargon and reference numbers must not be used. The Summary should be suitable for use by abstracting services without rewriting. Papers in French or German require a Résumé or Zusammenfassung followed by a Title and Summary in English; authors are encouraged to provide translations where necessary. Short communications and preliminary communications require summaries, which should not exceed 50 words.

Introduction. The first paragraphs of the paper should contain accounts of the reasons for the work, any essential historical background (as briefly as possible and with key references only) and preliminary experimental work.

Experimental. The experimental methods may be described after the introductory material, or after the discussion of results, depending on the nature of the paper. Detailed experimental descriptions should, however, be restricted to one section of the paper, and not scattered throughout the text. Working procedures should be given in the imperative mood; sufficient detail should be given to allow any reasonably experienced worker to carry out the procedure. Detailed descriptions of well-known techniques and equipment are unnecessary, as are simple preparations of reagents or solutions, and lists of common chemicals. Manufacturers should be named only if the product differs essentially from that of other manufacturers. Local suppliers for multi-national concerns should not be named. In writing, complete sentences should be used, and procedural steps should not be numbered.

Results and Discussion. These may be treated together or separately. In discussing results, unnecessary repetition of experimental detail, unsupported elaboration of hypotheses, and verbose exposition of ideas should be avoided. Chemical formulae should not be used in the text unless confusion is likely to arise from the use of names. Formulae should, however, be used for brevity in Tables and Figures. Calculations well known to specialists are unnecessary. Conclusions should be added only if needed for interpretation; they should not be used as extended summaries.

Acknowledgements. These should be kept as short as possible, and placed, without a heading, at the conclusion of the text.

References

The references should be collected at the end of the paper, numbered in the order of their appearance in the text (*not* arranged alphabetically), and typed on a separate sheet. If the paper forms part of a series, the reference to the previous part should appear as the first reference, the number being cited at the title of the paper. References given in Tables should be numbered according to the position of the Table in the text. Every reference listed must be cited in the text. Reference numbers in the text are set in square brackets on the line. Numerals referring to equations are placed in parentheses.

In the list of references, the following forms should be adopted.

Journals

1 W. Lund and M. Salberg, Anal. Chim. Acta, 76 (1975) 131.

2 M. McDaniel, A. D. Shendrikar, K. D. Reizneir and P. W. West, Anal. Chem., 48 (1976) 2240.

The title of the journal must be abbreviated as in the Bibliographic Guide for Editors and Authors.

Books

1 D. D. Perrin, Masking and Demasking of Chemical Reactions, Interscience-Wiley, New York, 1970, p. 188.

2 S. Hofmann, in G. Svehla (Ed.), Wilson and Wilson's Comprehensive Analytical Chemistry, Vol. 9, Elsevier, Amsterdam, 1979, p. 89.

Titles of papers are unnecessary. Citations of reports which are not widely available (e.g. reports from government research centres) should be avoided if possible. Authors' initials should not be used in the text, unless real confusion could be caused by their omission. If the reference cited contains three or more names, only the first author's name followed by et al. (e.g. McDaniel et al.) should be used in the text; but the reference list must contain the initials and names of *all* authors.

Tables, Computer Programs and Figures

Tables and Figures must be essential for the clear and concise presentation of the material. The same information should not be given in Tables and Figures, and material from the published literature should not be reproduced.

Tables. All Tables should be numbered with Arabic numerals, and have brief descriptive headings; they should be typed on separate pages. The layout should be given serious thought, so that the significance of the results can be grasped quickly. Column headings should be brief.

Tables with only two or three headings are best printed horizontally, e.g.

Hg^{2+} added (μg)	1.0	2.0	3.0	5.0
Extraction (%)	95.0	99.8	99.5	89.0

Experimental information which is relevant to all the results in the Table is best given in parentheses immediately after the heading. No column should contain the same number or unit throughout its length. Footnotes to Tables are denoted by superscript a, b, c.... The units used should be clearly stated. Confusion can arise from the use of powers in column headings. The following usage is recommended: e.g., if molar absorptivities are listed, the heading should be $\epsilon \times 10^{-4}$ l mol^{-1} cm^{-1}) so that a number 2.32 in the column signifies 23 200.

Alphanumeric computer output is usually unsuitable for reproduction and should therefore be retyped and presented as Tables; capitals can be used to simulate computer output if such simulation is essential for illustration.

Computer programs. Computer algorithms should be described clearly; a standard high-level programming language or a suitable algorithmic notation should be used as necessary. Complete program listings, however, are not normally admissible. Extensive flow charts should be avoided if the material can equally well be given in descriptive or tabular form. Statements on the portability of the software described to other computer systems, as well as on its availability to interested readers, should be given.

Figures. Figures should be prepared in black waterproof drawing ink on drawing or tracing paper of the same size as that on which the manuscript is typed. One original (or sharp glossy print) and two photostat (or other) copies are required. Attention should be given to line thickness, lettering (which should be kept to a minimum) and spacing on axes of graphs, to ensure suitability for reduction during printing. Axes of a graph should be clearly labelled, along the axes, and outside the graph itself.

The following standard symbols should be used in graphs:

▼ ▽ ■ □ + × ● ○ ▲ △

Simple straight-line graphs are not acceptable, because they can readily be described in the text by means of an equation or a sentence. Explanatory information should be placed not in the figure, but in the legend, which should be typed on a separate sheet of paper. All Figures should be numbered with Arabic numerals, and require descriptive legends.

Photographs should be glossy prints and be as rich in contrast as possible; colour photographs cannot be accepted. In general, line diagrams are more informative and less liable to dating than photographs of equipment, which are therefore not usually acceptable.

Computer outputs for reproduction as Figures must be of good quality on blank paper, and should preferably be submitted as glossy prints.

Nomenclature, abbreviations and symbols

In general, the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) should be followed, and attention should be given to the recommendations of the Analytical Chemistry Division in the journal *Pure and Applied Chemistry.* (see also *IUPAC Compendium of Analytical Nomenclature*, 1978).

Basic SI and other accepted metric nomenclature are given in the Appendix. In accordance with IUPAC rules, the mass number, atomic number, number of atoms and ionic charge should be designated by a left upper index, a left lower index, a right lower index and a right upper index, respectively, placed round the atomic symbol. For example, the phosphate ion should be designated as PO_4^{3-} (*not* PO_4^{-3} or PO_4^{---}), and phosphorus-32 as ^{32}P (*not* P^{32} or P-32).

The Stock notation for the indication of stoichiometric valency states (and indirectly the proportion of the constituents) should be used. Examples are iron(III) chloride rather than ferric chloride, and potassium hexacyanoferrate(II) rather than potassium ferrocyanide. These rules are valid for French and German as well as English usage.

The use of nanometre (nm) and micrometre (μm), for the expression of analytical wavelengths has long superseded mμ or Å or μ, all of which should be avoided, although Å is sensibly retained in crystallographic work.

Natural or Naperian logarithms should be denoted by ln and decadic logarithms by log.

In analytical chemistry, the term normality (N) serves many useful purposes and will be retained. It should not, however, be used if no ambiguity is introduced by the use of molarity (M). The term formality (F) should be avoided.

Unusual abbreviations require definition when first used. Abbreviations for long chemical names (e.g. EDTA, HEDTA, TBAH, en, pn, Tris) are useful, especially in equations, Tables or Figures. For ease of distinction, well-known techniques should be abbreviated by using lower-case letters and full stops, such as, g.c.-m.s., u.v., i.r., a.a.s., n.m.r., a.s.v., d.p.p., etc.

Ambiguity in expressing dilution can be avoided by the use of e.g. (1 + 2) rather than 1:2 which could mean either one part diluted with two parts or one part diluted to twice its volume.

Symbols, formulae and equations should be written with great care, capitals and lower-case letters being distinguished where necessary. Greek letters and unusual symbols should be defined by name in the left-hand margin beside their first appearance in the paper. Wherever possible, mathematical expressions should be typed on one line, by using brackets, e.g., $\{[()]\}$, and the solidus, e.g., $A/b = x^{1/2}/(u + v)^{5/6}$, which is valuable in conserving vertical space. Particular attention should be given to the correct sequence of brackets and to the correct placing of superscripts and subscripts in complicated equations; careful proof-reading of such equations is essential. Short equations should not be numbered unless required for subsequent reference.

Decimal points should be indicated by full stops in papers written in English and by commas in French and German papers.

Appendix

Basic SI units

metre	m	candela	cd
kilogram	kg	mole	mol
second	s	(an Avogadro number of any	
ampere	A	particle: atoms, molecules,	
degree Kelvin	K	ions, electrons, etc.)	

Derived SI units

joule	J	$kg\ m^2\ s^{-2}$	farad	F	$A\ s\ V^{-1}$
newton	N	$J\ m^{-1}$	weber	Wb	V s
watt	W	$J\ s^{-1}$	henry	H	$V\ s\ A^{-1}$
coulomb	C	A s	tesla	T	$V\ s\ m^{-2}$
volt	V	$J\ A^{-1}\ s^{-1}$	hertz	Hz	s^{-1}
ohm	Ω	$V\ A^{-1}$	degree Celsius	°C	K — 273.15

Other units

litre	l	$10^{-3}\ m^3$	hour	h	3.6×10^3 s
gram	g	10^{-3} kg	dyne	dyn	10^{-5} N
poise	P	$10^{-3}\ m^{-1}\ s^{-1}$	atmosphere	atm	101.325 kN m^{-2}
electron volt	eV	1.6021×10^{-19} J	molar	M	mol l^{-1}
calorie	cal	4.184 J	molal	*m*	mol kg^{-1}
minute	min	60 s	curie	Ci	$3.7 \times 10^{10}\ s^{-1}$

Prefixes to abbreviations for the names of units indicating

Multiples		Sub-multiples			
tera ($\times 10^{12}$)	T	milli ($\times 10^{-3}$)	m	pico ($\times 10^{-12}$)	p
giga ($\times 10^9$)	G	micro ($\times 10^{-6}$)	μ	femto ($\times 10^{-15}$)	f
mega ($\times 10^6$)	M	nano ($\times 10^{-9}$)	n	atto ($\times 10^{-18}$)	a
kilo ($\times 10^3$)	k				

(continued from outside of cover)

Short Communications

CONTENTS

Printed in The Netherlands.

(continued on inside page of cover)