

ANALYTICA CHIMICA ACTA

International Journal devoted to all branches of analytical chemistry

EDITORS

A. M. G. MACDONALD (Birmingham, Great Britain)

HARRY L. PARDUE (West Lafayette, IN, U.S.A.)

ALAN TOWNSHEND (Hull, Great Britain)

J. T. CLERC (Bern, Switzerland)

Editorial Advisers

F. C. Adams, Antwerp
H. Bergamin F², Piracicaba
G. den Boef, Amsterdam
A. M. Bond, Waurin Ponds
D. Dyrssen, Göteborg
J. W. Frazer, Livermore, CA
S. Gomisček, Ljubljana
S. R. Heller, Bethesda, MD
G. M. Hieftje, Bloomington, IN
J. Hoste, Ghent
A. Hulanicki, Warsaw
G. Johansson, Lund
D. C. Johnson, Ames, IA
P. C. Jurs, University Park, PA
J. Kragten, Amsterdam
D. E. Leyden, Fort Collins, CO
F. E. Lytle, West Lafayette, IN
D. L. Massart, Brussels
A. Mizuike, Nagoya
E. Munk, Tempe, AZ

M. Otto, Freiberg
E. Pungor, Budapest
J. P. Riley, Liverpool
J. Růžička, Copenhagen
D. E. Ryan, Halifax, N.S.
S. Sasaki, Toyohashi
J. Savory, Charlottesville, VA
W. D. Shultz, Oak Ridge, TN
W. I. Stephen, Birmingham
M. Thompson, Toronto
G. Tölg, Schwäbisch Gmünd
W. E. van der Linden, Enschede
A. Walsh, Melbourne
H. Weiss, Freiburg i. Br.
P. W. West, Baton Rouge, LA
T. S. West, Aberdeen
J. B. Willis, Melbourne
E. Ziegler, Mülheim
Yu. A. Zolotov, Moscow

ELSEVIER

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 1985

	J	F	M	A	M	J	J	A	S	O	N	D
Analytica Chimica Acta	167	168	169	170/1 170/2	171	172	173	174	175	176	177	178

Scope. *Analytica Chimica Acta* publishes original papers, short communications, and reviews dealing with every aspect of modern chemical analysis both fundamental and applied.

Submission of Papers. Manuscripts (three copies) should be submitted as designated below for rapid and efficient handling:

Papers from the Americas to: Professor Harry L. Pardue, Department of Chemistry, Purdue University, West Lafayette IN 47907, U.S.A.

Papers from all other countries to: Dr. A. M. G. Macdonald, Department of Chemistry, The University, P.O. Box 36 Birmingham B15 2TT, England. Papers dealing particularly with computer techniques to: Professor J. T. Cle Universität Bern, Pharmazeutisches Institut, Baltzerstrasse 5, CH-3012 Bern, Switzerland.

Submission of an article is understood to imply that the article is original and unpublished and is not being considered for publication elsewhere. Upon acceptance of an article by the journal, authors will be asked to transfer the copyright of the article to the publisher. This transfer will ensure the widest possible dissemination of information.

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. 170 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 7DH (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 Science Publishers B.V., Journals Department, P.O. Box 211, 1000 AE Amsterdam, The Netherlands. Tel: 5803 911, Telex: 18582.

Publication. *Analytica Chimica Acta* appears in 12 volumes in 1985. The subscription for 1985 (Vols. 167-178) Dfl. 2400.00 plus Dfl. 264.00 (p.p.h.) (total approx. US \$986.70). All earlier volumes (Vols. 1-166) except Vols. 1 and 28 are available at Dfl. 231.00 (US \$85.56), plus Dfl. 15.00 (US \$5.56) p.p.h., per volume.

Our p.p.h. (postage, packing and handling) charge includes surface delivery of all issues, except to subscribers in the U.S.A., Canada, Japan, Australia, New Zealand, P.R. China, India, Israel, South Africa, Malaysia, Singapore, South Korea, Taiwan, Pakistan, Hong Kong and Brazil who receive all issues by air delivery (S.A.L. — Surface Air Lifted) at no extra cost. For the rest of the world, airmail and S.A.L. charges are available upon request.

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

For further information, or a free sample copy of this or any other Elsevier Science Publishers journal, readers in the U.S.A. and Canada can contact the following address: Elsevier Science Publishing Co. Inc., Journal Information Center, 52 Vanderbilt Avenue, New York, NY 10017, U.S.A., Tel: (212) 916-1250.

© 1985, ELSEVIER SCIENCE PUBLISHERS B.V.

0003-2670/85/90

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher, Elsevier Science Publishers B.V., P.O. Box 1000 AH Amsterdam, The Netherlands. Upon acceptance of an article by the journal, the author(s) will be asked to transfer copyright of the article to the publisher. The transfer will ensure the widest possible dissemination of information.

Submission of an article for publication entails the author(s) irrevocable and exclusive authorization of the publisher to collect any sums or considerations for copying or reproduction payable by third parties (as mentioned in article 17 paragraph 2 of the Dutch Copyright Act of 1912 and in the Royal Decree of June 20, 1974 (S. 351) pursuant to article 16b of the Dutch Copyright Act of 1912) and/or to act in or out of Court in connection therewith.

Special regulations for readers in the U.S.A. — This journal has been registered with the Copyright Clearance Center, Inc. Consent is given for copying articles for personal or internal use, or for the personal use of specific clients. This consent is given on the condition that the copier pays through the Copyright Clearance Center, Inc., 27 Congress Street, Salem, MA 01970, U.S.A. If no code-line appears, broad consent to copy has not been given and permission must be obtained directly from the author(s). All articles published prior to 1980 may be copied for a per-copy fee of US \$ 2.25, also payable through the Center. This consent does not extend to other kinds of copying, such as for general distribution, resale, advertising and promotion purposes, or creating new collective works. Special written permission must be obtained from the publisher for such copying.

CHEMOMETRICS AND INTELLIGENT LABORATORY SYSTEMS

An International Journal

Editor-in-Chief: D.L. Massart, Brussels, Belgium

This international journal publishes articles about new developments on laboratory techniques chemistry and related disciplines, which are characterized by the application of statistical and computer methods.

Special attention is given to emerging new technologies and techniques for the building of intelligent laboratory systems i.e. artificial intelligence and robotics.

One of the main aims of the journal is to be an interdisciplinary journal; more particularly intends to build bridges between chemists, statisticians, and designers of laboratory systems. The journal deals with the following topics:

- * **chemometrics:** the chemical discipline that uses mathematical and statistical methods
 - to design or select optimal procedures and experiments
 - to provide maximum chemical information by analyzing chemical data
- * **computerized acquisition, processing and evaluation of data**
- * **developments in statistical theory destined to be used in chemistry**
- * **intelligent laboratory systems including self-optimizing instruments and the application expert systems and robotics in the laboratory**
- * **techniques for the modelling of chemical processes such as environmental models and industrial processes including quality control**
- * **new software to implement the methods described above**

The journal will be of interest to chemists, as well as pharmacists, statisticians and information specialists working in the different fields of chemistry such as analytical chemistry, organic chemistry, environmental chemistry, food chemistry and pharmaceutical chemistry.

There will be no page charges; fifty reprints of each article will be supplied free of charge.

Instructions for the preparation of manuscripts can be obtained from the publisher.

Volume 1 — four issues — will cover 1986/1987. The subscription price is 242 Dutch guilders US \$ 83.50.

Requests for further information and orders may be sent to the publishers.

ELSEVIER SCIENCE PUBLISHER

A NEW title in Elsevier's Analytical Chemistry Symposia Series

Volume 18:
**MODERN TRENDS IN
ANALYTICAL CHEMISTRY**

**Proceedings of Two Scientific
Symposia held in Matrafüred,
Hungary, October 17-20 and
October 20-22, 1982**

*Edited by E. PUNGOR, I. BUZAS and
E. VERESS, Institute for General and
Analytical Chemistry, Technical
University, Budapest, Hungary*

Two symposia were held in Matrafüred,
Hungary, in October 1982 and the pro-
ceedings of both are contained in this
book.

The first was the Symposium on Electro-
chemical Detection in Flow Analysis, the
aim of which was to define the physical
parameters of electrochemical detectors
that are most important in flow applica-
tions, and to study how and under what
conditions these detectors can be used in
other fields in addition to direct flow
analysis, e.g. in chromatography or
clinical analysis.

The other meeting was the first interna-
tional symposium to be held on Pattern
Recognition in Analytical Chemistry. This
is a particularly successful meeting and
the lectures and discussions are presented
in the second part of the book. The volume
will provide much information and food for
thought for many workers in various fields
of analytical chemistry.

(Due to limitations of space, only the
plenary and keynote lectures from the first
conference and the topics of the second
conference are listed below.)

CONTENTS

Part A - Electrochemical Detection in Flow Analysis

Plenary Lectures: Amperometric flow-
through detection in liquid chromato-
graphy (W. Kemula, W. Kutner).
Potentiometric flow-through detectors and
their clinical applications (W.E. Morf,
W. Simon). Potentiometric and amperomet-
ric detection in flow injection enzymatic
determinations (H.A. Mottola et al.).
Behaviour of solid electrodes in anodic
flow-through systems with respect to noise
and stability (H. Poppe, H.W. van
Rooijen).

Keynote Lectures: Enzyme reactors in
analytical flow systems (G. Johansson et
al.). Fundamentals of the electrochemical
high sensitivity sensors for the detection of
various contaminants in atmosphere
(U. Palm). Some characteristics of flow
and continuous analysis with ion-selective
electrodes (J.D.R. Thomas). Automated
polarographic and photometric system for
serial analysis (K. Tóth et al.). Some
aspects of application of ion-selective
electrode detectors in flow analysis
(M. Trojanowitz). Discussion Lectures (15
papers). Panel discussion. Subject Index.

Part B - Pattern Recognition in Analytical Chemistry

COBAC and Chemometrics (2 papers).
Pattern Recognition and Structure
Elucidation (3 papers). Applications of
Pattern Recognition and Cluster Analysis
Methods (4 papers). Characterization and
Comparison of Different Methods of Pattern
Recognition (1 paper). Subject Index.

1984 xxii + 628 pages
US \$ 129.75 / Dfl. 350.00
ISBN 0-444-99631-1

ELSEVIER

P.O. Box 211
1000 AE Amsterdam
The Netherlands

P.O. Box 1663
Grand Central Station
New York, NY 10163

QSAR in Toxicology and Xenobiochemistry

Proceedings of a Symposium, Prague, Czechoslovakia,
September 12-14, 1984

edited by **M. TICHÝ**, *Institute of Hygiene and Epidemiology, Prague, Czechoslovakia*

This symposium provided an opportunity for experimental toxicologists, hygienists and xenobiochemists to exchange ideas and experiences with specialists in the field of QSAR analysis. All the papers at the symposium are contained in this volume. Sections I to VI contain papers that apply methods or are connected in some way with methods of QSAR analysis. Those in Section VII are of a purely experimental nature and cover several fields in toxicology and xenobiochemistry. The closing lectures present perspectives in estimating hazardous chemicals before their use according to chemical structure. Thus the volume reflects all areas of toxicology where QSAR analysis may find its applications.

CONTENTS: Introductory Lecture. Section I. Nonspecific Acute Toxicities (7 papers). Section II. Long-Term Effects (6 papers). Section III. Metal Intoxications (3 papers). Section IV. Clinical and Therapeutical Applications (4 papers). Section V. Distribution and its Kinetics: Experiment and Theory. (6 papers). Section VI.

Calculation Methods and Molecular Parametrization (8 papers). Section VII. Toxicological Experimental Varia (15 papers). Closing Lectures - Predictive Toxicology (2 papers). Author Index. Subject Index.

PHARMOCHEMISTRY LIBRARY, 8

1985 x + 474 pages
Price: US \$ 109.25
(in USA & Canada)
Dfl. 295.00
(Rest of World)



ORDER FORM

To: Elsevier

P.O. Box 211

1000 AE Amsterdam

The Netherlands

P.O. Box 1663

Grand Central Station

New York, NY 10103

Please send me _____ copy(ies) of

**QSAR in Toxicology
and Xenobiochemistry**

by M. Tichý

Price: US \$ 109.25 (in USA & Canada)

Dfl. 295.00 (Rest of World)

☐ I enclose

☐ personal cheque

☐ bankdraft

☐ Unesco coupons

☐ Please bill my credit card

☐ MasterCard ☐ Eurocard ☐ Access ☐ Visa ☐ Amex

Card No.: _____ Exp. Date: _____

Signature: _____

Name: _____

Address: _____

Zip/Post code _____

ELSEVIER

ANALYTICA CHIMICA ACTA
VOL. 176 (1985)

ANALYTICA CHIMICA ACTA

International journal devoted to all branches of analytical chemistry

EDITORS

A. M. G. MACDONALD (Birmingham, Great Britain)

HARRY L. PARDUE (West Lafayette, IN, U.S.A.)

ALAN TOWNSHEND (Hull, Great Britain)

J. T. CLERC (Bern, Switzerland)

Editorial Advisers

F. C. Adams, Antwerp
H. Bergamin F^º, Piracicaba
G. den Boef, Amsterdam
A. M. Bond, Waurin Ponds
D. Dyrssen, Göteborg
J. W. Frazer, Livermore, CA
S. Gomisček, Ljubljana
S. R. Heller, Bethesda, MD
G. M. Hieftje, Bloomington, IN
J. Hoste, Ghent
A. Hulanicki, Warsaw
G. Johansson, Lund
D. C. Johnson, Ames, IA
P. C. Jurs, University Park, PA
J. Kragten, Amsterdam
D. E. Leyden, Fort Collins, CO
F. E. Lytle, West Lafayette, IN
D. L. Massart, Brussels
A. Mizuike, Nagoya
E. Munk, Tempe, AZ

M. Otto, Freiberg
E. Pungor, Budapest
J. P. Riley, Liverpool
J. Růžicka, Copenhagen
D. E. Ryan, Halifax, N.S.
S. Sasaki, Toyohashi
J. Savory, Charlottesville, VA
W. D. Shults, Oak Ridge, TN
W. I. Stephen, Birmingham
M. Thompson, Toronto
G. Tölg, Schwäbisch Gmünd
W. E. van der Linden, Enschede
A. Walsh, Melbourne
H. Weisz, Freiburg i. Br.
P. W. West, Baton Rouge, LA
T. S. West, Aberdeen
J. B. Willis, Melbourne
E. Ziegler, Mülheim
Yu. A. Zolotov, Moscow



ELSEVIER Amsterdam—Oxford—New York—Tokyo

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the publisher, Elsevier Science Publishers B.V., P.O. Box 330, 1000 AH Amsterdam, The Netherlands. Upon acceptance of an article by the journal, the author(s) will be asked to transfer copyright of the article to the publisher. The transfer will ensure the widest possible dissemination of information.

Submission of an article for publication entails the author(s) irrevocable and exclusive authorization of the publisher to collect any sums or considerations for copying or reproduction payable by third parties (as mentioned in article 17 paragraph 2 of the Dutch Copyright Act of 1912 and in the Royal Decree of June 20, 1974 (S. 351) pursuant to article 16b of the Dutch Copyright Act of 1912) and/or to act in or out of Court in connection therewith.

Special regulations for readers in the U.S.A. — This journal has been registered with the Copyright Clearance Center, Inc. Consent is given for copying of articles for personal or internal use, or for the personal use of specific clients. This consent is given on the condition that the copier pays through the Center the per-copy fee for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. The per-copy fee is stated in the code-line at the bottom of the first page of each article. The appropriate fee, together with a copy of the first page of the article, should be forwarded to the Copyright Clearance Center, Inc., 27 Congress Street, Salem, MA 01970, U.S.A. If no code-line appears, broad consent to copy has not been given and permission to copy must be obtained directly from the author(s). All articles published prior to 1980 may be copied for a per-copy fee of US \$ 2.25, also payable through the Center. This consent does not extend to other kinds of copying, such as for general distribution, resale, advertising and promotional purposes, or for creating new collective works. Special written permission must be obtained from the publisher for such copying.

CAPACITIVE-DISCHARGE-HEATED ANISOTROPIC PYROLYTIC GRAPHITE FURNACE USED FOR ATOMIC ABSORPTION SPECTROMETRY

Part 1. Theoretical Modelling of the Heating Characteristics of the Furnace Surface and of the Gas Phase

S. B. CHANG, C. L. CHAKRABARTI*, T. J. HUSTON and P. C. BERTELS

Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6 (Canada)

J. T. ROGERS

Department of Mechanical and Aeronautical Engineering, Carleton University, Ottawa, Ontario K1S 5B6 (Canada)

R. DICK

ELPA Engineering, Ottawa, Ontario K2C 3L5 (Canada)

(Received 21st February 1985)

SUMMARY

A model of the temperature distribution in the tube wall and in the gas phase of an anisotropic pyrolytic graphite furnace heated by capacitive discharge technique is proposed. The heat loss from the graphite tube by conduction via the contact electrodes to the water-cooled electrode supports and by radiation to its surroundings are the main processes considered. In calculating the gas temperature, heat transfer by conduction from the tube wall to the gas phase is the only mode taken into account. The instantaneous temperature of a graphite furnace is expressed in the form of a differential equation. A finite-difference form of the differential equation is used in a computer program to calculate the temperature at each time step. The computer simulation offers the capability of studying the factors affecting the characteristics of temperature/time profiles and the distribution of surface and gas phase temperatures of the furnace. The results of simulation studies of the effect of the capacitance and the initial voltage of the capacitor bank on the heating characteristics of the furnaces show a reasonable agreement with those obtained experimentally.

The importance of the study of temperature distribution lies not only in knowledge of temperature/time characteristics of the heating of a graphite furnace, but also in the application of the above knowledge in predicting absorption pulse characteristics and in their experimental verification. Several researchers [1–9] have made theoretical and/or experimental studies of surface-temperature gradients across the contact electrodes of conventional graphite furnaces used for atomic absorption spectrometry.

In this paper, the predominant factors which affect the instantaneous temperature of an anisotropic pyrolytic graphite furnace heated by the

capacitive discharge technique [5, 6, 10] is expressed as a differential equation. A finite-difference form of the differential equation is used in a computer program to calculate the temperature at each time step.

The graphite contact electrodes and the anisotropic pyrolytic graphite tubes are arranged to give three configurations of the furnace: types 1, 2 and 3 furnaces [11]. The types 1 and 2 furnaces are similar to each other except for the design of one of the contact electrodes. The surface temperature distribution of these two furnaces is assumed to be unaffected by the difference in the configuration of the contact electrodes because of high thermal conductivity and low electrical resistivity in the *ab*-plane of anisotropic pyrolytic graphite relative to its *c*-direction. Therefore, only two types of furnaces were selected for the present study: types 1 and 3. The results of the simulation studies of the effect of the capacitance and the initial voltage of the capacitor bank on the heating characteristics of the furnace are compared with those experimentally measured by optical pyrometry.

THEORETICAL MODELLING

Two factors are included in the mathematical formulation of the model to describe the resistive heating of a cylindrical graphite tube and the heat transfer to the gas inside the tube from the interior surface of the tube towards its centre. The first is the geometrical description of the furnace and is important when a numerical solution with explicit finite-difference approximation is used in the mathematical formulation of the model. The second concerns the heat transfer, and boundary conditions (for the differential equation) which allow simplification of finite-difference equations.

Temperature distribution in the interior surface of the graphite furnaces

The type 1 furnace. The type 1 furnace was constructed with the lamellar planes (*ab*-plane) of the anisotropic pyrolytic graphite tube parallel to the optical axis, with two contact electrodes in horizontal position across the diameter of the tube [11]. Because the tube is symmetrical about a vertical plane, only half of the cross-section needs to be considered. Figure 1(a) illustrates the nodal geometry for the finite-difference approximation of the temperature calculation for the type 1 furnace. Because regular (isotropic) graphite electrodes and the *ab*-plane of the anisotropic pyrolytic graphite have high thermal conductivities [12–16], it is assumed that the temperature is uniformly distributed over the sectional planes around each node, i.e., that there is no temperature gradient along the length of the tube of the type 1 furnace at any particular node in Fig. 1(a). The two-dimensional view of the furnace is therefore reduced to a line of nodes, as shown in Fig. 1(b). There are j nodes across the external radius of the graphite tube. The region from node $j - m + 1$ to node j forms the contact between the regular graphite electrode and the anisotropic pyrolytic graphite tube. At each node on the line, the true volume and surface area are used in the finite-difference approximation.

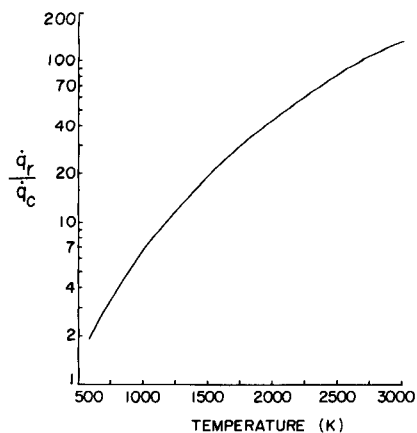
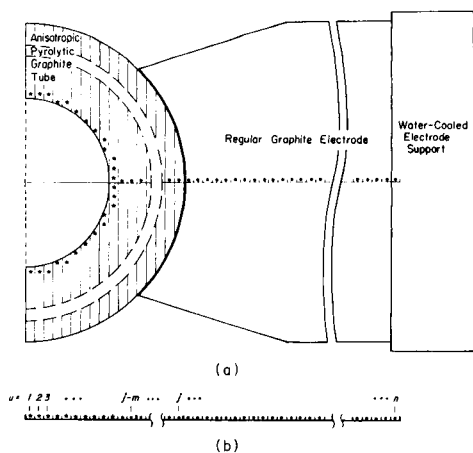


Fig. 1. Nodal geometry of the type 1 furnace for the finite-difference approximation of the surface temperature calculation. Dashed co-axial lines mean that the external radius of the tube is a variable. For further explanation, see text.

Fig. 2. The estimated ratio of the rate of heat loss by radiation, $\dot{q}_r$, and by free convection, $\dot{q}_c$, from the heated graphite surface as a function of a uniformly-distributed surface temperature. $\dot{q}_r$ is calculated as radiational heat transfer from the heated graphite surface to the surroundings at 300 K. $\dot{q}_c$ is calculated on the basis of the free convection heat loss from a horizontal cylinder [24]. Parameters used in the estimation such as the emissivity of graphite and the properties (viscosity, Prandtl number, thermal conductivity) of argon at atmospheric pressure as a function of temperature were taken from [6].

In considering the boundary conditions for modelling the surface temperature distribution in the type 1 furnace, it is assumed that the electrode support and the bulk environment of the argon-filled furnace housing (which resembles the Varian-Techtron CRA-63 atomizer head) remain at a temperature of 300 K. This is because the brass electrode support is water-cooled to prevent it from overheating, and a large amount of argon is flushed into the housing to prevent the oxidation of the graphite tube. Both tend to keep the temperature at 300 K.

Lack of intimate physical contacts between the contact electrodes and the tube would result in enhanced thermal resistance which would tend to insulate the tube from the cooling effect of the water-cooled electrodes. The same lack of intimate physical contacts between the contact electrodes and the tube would result in enhanced electrical resistance which would tend to heat the surface of the graphite tube. These two effects would reduce the temperature gradient around the tube, but would increase the possibility of electrical arcs when a high voltage is applied from the capacitor bank. Although absence of electrical arcs does not necessarily mean a perfect contact between the electrodes and graphite tube, a loud electric spark would result from a poor contact. Because no electrical arcs were noticed

nor tolerated during the heating of the furnace, it is assumed that a perfect contact is made between the electrodes and the graphite tube, i.e., the thermal and the electrical contact resistance are negligible.

As summarized in Table 1, the properties (thermal conductivity, electrical resistivity, and emissivity) of both regular graphite and anisotropic pyrolytic graphite are temperature-dependent. However, these property values at any node are considered to be constant over each time step. At any instant of time, t , the total resistance across the electrode supports, $R(t)$, equals twice the resistance from the centre of the graphite tube to the electrode support, and is expressed as follows

$$\begin{aligned}
 R(t) &= 2 \sum_{u=1}^n R_u^p(T) = 2 \left[\sum_{u=1}^{j-m} R_u^p(T) + \sum_{u=j-m+1}^j R_u^p(T) + \sum_{u=j+1}^n R_u^p(T) \right] \\
 &= 2\Delta x \left\{ \sum_{u=1}^{j-m} [\phi 1_u^p(T)/A 1_u] + \sum_{u=j-m+1}^j [\phi 1_u^p(T)\phi 2_u^p(T)/(A 2\phi 1_u^p(T) \right. \\
 &\quad \left. + A 1\phi 2_u^p(T))] + \sum_{u=j+1}^n [\phi 2_u^p(T)/A 2_u] \right\} \quad (1)
 \end{aligned}$$

TABLE 1

Property values of regular (isotropic) graphite, anisotropic pyrolytic graphite and argon as a function of temperature

Material	Property	Unit	Function	Ref
Regular (isotropic) graphite	Density, ρ	g cm^{-3}	$\rho(T) = 1.87$	
	Heat capacity, c_p	$\text{J g}^{-1} \text{K}^{-1}$	$c_p(T) = 6.71 \times 10^{-2} + 2.60 \times 10^{-3} T - 1.11 \times 10^{-6} T^2 + 1.58 \times 10^{-10} T^3$	17,
	Thermal conductivity, κ_{cond}	$\text{W cm}^{-1} \text{K}^{-1}$	$\kappa_{\text{cond}}(T) = 1.73 - 1.67 \times 10^{-3} T + 6.84 \times 10^{-7} T^2 - 9.41 \times 10^{-11} T^3$	19
	Electrical resistivity, ϕ	ohm-cm	$\phi(T) = 1.17 \times 10^{-3} - 2.70 \times 10^{-7} T + 7.51 \times 10^{-11} T^2 + 1.50 \times 10^{-14} T^3$	19
	Emissivity, ϵ		$\epsilon(T) = 0.788 + 1.88 \times 10^{-5} T$	20
Anisotropic pyrolytic graphite	Density, ρ	g cm^{-3}	$\rho(T) = 2.20$	
	Heat capacity, c_p	$\text{J g}^{-1} \text{K}^{-1}$	$c_p(T)$ for anisotropic pyrolytic graphite is taken to be the same as $c_p(T)$ for regular graphite	12,
	Thermal conductivity in the c -direction, κ_{cond}	$\text{W cm}^{-1} \text{K}^{-1}$	$\kappa_{\text{cond}}(T) = 2.18 \times 10^{-2} - 9.67 \times 10^{-6} T + 1.24 \times 10^{-9} T^2$	19
	Electrical resistivity in the c -direction, ϕ	ohm-cm	$\phi(T) = 0.808 - 7.91 \times 10^{-4} T + 3.22 \times 10^{-7} T^2 - 4.51 \times 10^{-11} T^3$	15,
	Emissivity at the edge of ab -plane, ϵ		$\epsilon(T) = 0.978 + 1.47 \times 10^{-5} T - 2.47 \times 10^{-8} T^2$	20
Argon ^a	Density, ρ	g cm^{-3}	$\rho(T) = 1.784 \times 10^{-3} \times (273.15/T)$	
	Heat capacity, c_p	$\text{J g}^{-1} \text{K}^{-1}$	$c_p(T) = 0.52$	22
	Thermal conductivity, κ_{cond}	$\text{W cm}^{-1} \text{K}^{-1}$	$\kappa_{\text{cond}}(T) = 1.774 \times 10^{-4} \times (T/303.15)^{0.68}$	23

^a $c_v = c_p - R$ where R is gas constant.

where $R_u^p(T)$, $\phi 1_u^p(T)$ and $\phi 2_u^p(T)$ are resistance and resistivity of the anisotropic pyrolytic graphite along the c -direction, and resistivity of the regular graphite, respectively, all as functions of temperature, T . The subscript, u , denotes the position of a node. The superscript p is the time step; time t is the product of the time step and the step size, Δt ; $A1$ and $A2$ represent the average area of two cross-sectional planes on each side of the node which is associated with the anisotropic pyrolytic graphite and the regular graphite, respectively; Δx is the distance between nodes. Because the electrical resistivity of the anisotropic pyrolytic graphite in the ab -plane is about 1000 times less than that along the c -direction and is similar to that of the regular graphite, uniform currents through each area ($A1$ and $A2$) in the region of $j - m + 1$ to j are implicitly assumed in Eqn. 1. Furthermore, because it is assumed that the resistance of any particular node is constant in a finite time interval, Δt , the power input to that node by ohmic heating, P_u , can be described by the following equation

$$P_u = i^2 R_u^p \quad (2)$$

where i is the instantaneous current passing through the furnace having a resistance, $R(t)$, and R_u^p is the resistance of the node at time $t = p\Delta t$.

Figure 2 shows the estimated ratio between the rate of heat loss by radiation and that by free convection from the heated graphite outer surface at various uniformly-distributed surface temperatures. Although heat loss by forced convection may also take place, as purge gas is continuously flushed into the atomizer housing, it is estimated that there is only about 40% increase in the heat loss by convection because of the low purge gas velocity (ca. 6 cm s⁻¹). The results indicate that heat loss by radiation dominates at high surface temperatures (>1000 K). Because the observed temperature gradients along the tube and the electrode [11] result in regions of low temperature, convection will have some effect on heat loss. However, the present model neglects heat loss by convection from the outer surface of the tube and the electrodes. Therefore, the modes of heat loss from the type 1 furnace are considered to be thermal radiation from the outer surface to the surroundings and conduction of heat to the water-cooled electrode support. Because the heat capacity of the gas volume inside the cylindrical tube is much smaller than the heat capacity of the furnace housing, the amount of heat dissipated from the interior surface of the graphite tube is not taken into account.

Around any particular node, u , the rate of heat storage, Q_u , can be described as

$$Q_u = P_u + Q_u(\text{cond}) + Q_u(\text{rad}) \quad (3)$$

where P_u is the power of ohmic heating given by Eqn. 2, $Q_u(\text{cond})$ is the rate of heat transfer by conduction from adjacent nodes, and $Q_u(\text{rad})$ is the rate of heat transfer by radiation between the node and its surroundings. The rate of heat conduction, $Q_u(\text{cond})$, can be expressed [24] by a partial differential

equation (Eqn. 4a), and then approximated in a finite-difference form as Eqn. 4b

$$Q_u(\text{cond}) = A_u(\text{cond})\kappa_u [(\partial^2 T/\partial x^2) \Delta x]_{x=(u-1/2)\Delta x} \quad (4a)$$

$$\approx [A_u(\text{cond})\kappa_u/\Delta x](T_{u+1} + T_{u-1} - 2T_u) \quad (4b)$$

where $A_u(\text{cond})$ is the average area of the interfacing cross-sectional plane between nodes u and $u + 1$ and that between nodes u and $u - 1$, and κ_u stands for the thermal conductivity of the graphite around node u . Because the area surrounding the tubular furnace (i.e., the interior surface area of the furnace housing) is much larger than the outer surface area of any particular node on the furnace wall, so that the surroundings act effectively as a black-body, the rate of heat loss from the node to its surroundings by radiation, $Q_u(\text{rad})$, can be described as follows [24, p. 337]

$$Q_u(\text{rad}) = \sigma \epsilon_u A_u(\text{rad})[T_s^4 - T_u^4] \quad (5a)$$

$$= h_u A_u(\text{rad})(T_s - T_u) \quad (5b)$$

where σ is the Stefan-Boltzmann constant ($5.699 \times 10^{-12} \text{ W cm}^{-2} \text{ K}^{-4}$), ϵ_u is the emissivity of graphite at node u (dimensionless), $A_u(\text{rad})$ is the radiating outer surface area around the node, T_s is the surrounding temperature (K), and h_u is the equivalent radiation heat transfer coefficient

$$h_u = \sigma \epsilon_u (T_s^4 - T_u^4)/(T_s - T_u) \quad (5c)$$

The rate of the heat storage at any node which results in the time rate of change in the temperature, $\partial T_u/\partial t$, can be described by

$$Q_u = V_u \rho c_p \partial T_u/\partial t \quad (6)$$

where V_u is the volume of node u (cm^3), c_p is the heat capacity ($\text{J g}^{-1} \text{ K}^{-1}$), and ρ is the density (g cm^{-3}). The time derivative is expressed in a finite-difference form as

$$\partial T_u/\partial t \approx (T_u^{p+1} - T_u^p)/\Delta t \quad (7)$$

where superscript $p + 1$ denotes the time step explicitly in terms of the previous time step, p , by a finite increment of time, Δt .

Substituting and rearranging Eqns. 2–7, the temperature at node u after a finite time increment can be described by

$$T_u^{p+1} = (\Delta t/V_u c_p \rho) \{ i^2 R_u^p + [A_u(\text{cond})\kappa_u^p/\Delta x] (T_u^{p+1} - T_u^{p-1}) + h_u^p A_u(\text{rad}) T_s \} + T_u^p [1 - 2Fo - Bi Fo A_u(\text{rad})/A_u(\text{cond})] \quad (8)$$

where $V_u \approx A_u(\text{cond})\Delta x$, $Fo = \kappa_u^p \Delta t/c_p \rho (\Delta x)^2$ and $Bi = h_u^p \Delta x/\kappa_u^p$. The mathematical stability of the finite difference formulation of Eqn. 8 is governed by the coupling of the two independent variables, Δx and Δt . This stability criterion is that the coefficient of the T_u^p term in Eqn. 8 be positive [24–26].

The change in the total resistance across the electrode support with increasing temperature of the furnace and of the electrode, as described

earlier in Eqn. 1, implies a variation of electric current during the heating cycle if a constant voltage across the assembly is considered. With the capacitive-discharge heating technique, the instantaneous electric current passing through the heating element by discharge of a capacitor bank is further complicated by a progressive drop in the voltage of the capacitor bank during the heating of the furnace. However, it is assumed that the resistance across the electrode support remains constant in the finite time interval, Δt . The voltage drop in the discharging RC-circuit within this finite time interval can be described [27] by

$$\Delta V = V^{p+1} - V^p = V^p \{ \exp [-\Delta t / (R(t)C)] - 1 \} \quad (9)$$

where C is the capacitance of the capacitor bank. The instantaneous current passing in such a circuit can be expressed by

$$i = -C \, dV/dt \approx -C \, \Delta V / \Delta t = -(CV^p / \Delta t) \{ \exp [-\Delta t / (R(t)C)] - 1 \} \quad (10)$$

Because the resistance of the type 1 furnace is considered to be the sum of the resistance of each node in series, current passing through each node at a given instant is therefore the same. Once the preset atomization temperature has been attained in the first node, i.e., $u = 1$ in Fig. 1(b), the electric current required to maintain this temperature was considered to be the current passing through the tube and electrode assembly at that moment. This current can be determined from Eqn. 8 by setting $T_{u=1}^{p+1} = T_{u=1}^p$, i.e., for the rate of heat storage of zero in Eqn. 6.

The type 3 furnace. The type 3 furnace was constructed with lamellar planes of the anisotropic pyrolytic graphite perpendicular to the optical axis, with two contact electrodes across the graphite tube length [11]. Figure 3 shows the nodal geometry for the finite-difference approximation of the surface temperature calculation in the type 3 furnace. Under the same assumptions and boundary conditions as for the type 1 furnace, the surface temperature calculation of the type 3 furnace is formulated in essentially the same way as Eqn. 8. However, it should be noted that only the first and third terms of Eqn. 1 will be needed for the type 3 furnace, because there is no region in this furnace where the electric current flows parallel through different graphite material.

Temperature distribution in the gas phase in the type 1 and type 3 furnaces

The type 1 furnace. The geometry of the gas-filled space of the type 1 furnace is approximated by an equally-spaced, orthogonal grid of nodes shown as the solid dots and open circles in Fig. 4. Heating of the gas inside the furnace is the result of heat dissipation from the interior graphite surface. The points positioned near the interior graphite wall and shown as asterisks in Fig. 4 are considered to be at the temperature of the graphite surface. Only one quadrant needs to be considered because of symmetry, if the effects of the sample-injection hole are neglected. The actual circular cross-sectional plane of the tube is approximated by the saw-shaped dashed line as shown in

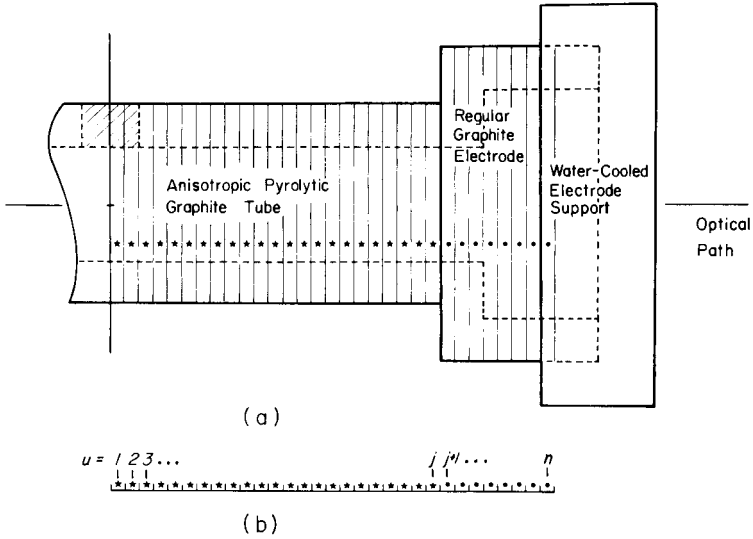


Fig. 3. Nodal geometry of the type 3 furnace for the finite-difference approximation of the surface temperature calculation.

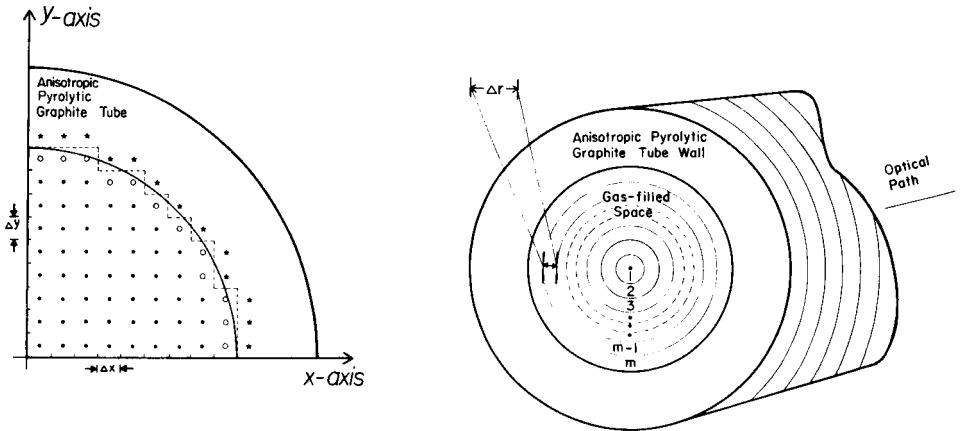


Fig. 4. Nodal geometry of the gas-filled space of the type 1 furnace for the finite-difference approximation of the gas temperature calculation. For further explanation, see text.

Fig. 5. Nodal geometry of the gas-filled space of the type 3 furnace for the finite-difference approximation of the gas temperature calculation.

Fig. 4. Although a cylindrical coordinate system would more accurately describe the geometry of a cylindrical tube, non-uniform surface-temperature distribution around the circumference of the type 1 furnace would be more difficult to model.

As to the modes of heat transfer from the graphite wall to the stagnant argon gas inside the tube, Chakrabarti et al. [28] have demonstrated that

conduction dominates the overall heat-transfer process. The small diameter of the cylindrical tube inhibits heat transfer by free convection. The thermal spectrum shows that the radiation lies in the region of 0.1–100 μm , and the shift of the peak of the thermal spectrum follows Wien's Displacement Law. Because monatomic argon, which is the predominant species inside the furnace, has no strong characteristic emission and absorption spectral lines around the peak, heating of the argon inside the graphite furnace by radiational heat transfer is unlikely and is not taken into account in the model. Because gas conduction is the only mode of heat transfer within the furnace, it is assumed that there is no thermal boundary layer between the surface and the adjacent gas. The assumption is valid because of the low velocity of the gas along the furnace length throughout the heating cycle [7]. The temperature of the gas next to the surface, indicated by open circles in Fig. 4, is therefore the same as the adjacent surface temperature (indicated by asterisks) or the average of two adjacent surface temperatures (see Fig. 1).

Two-dimensional transient heat transfer in the gas phase by conduction alone is given [24] by

$$\kappa [(\partial^2 T / \partial x^2) + (\partial^2 T / \partial y^2)] = \rho c_v \partial T / \partial t \quad (11)$$

where c_v is the heat capacity of the argon at constant volume, and x and y represent the two independent space coordinates shown in Fig. 4. The heat capacity at constant volume, c_v , is used in the energy storage term of Eqn. 11 even though the gas in the furnace remains at essentially constant pressure during the transient. The reason for the use of c_v instead of c_p has been given by Chakrabarti et al. [7]. Because the gas-filled space inside the tube is approximated by an equally-spaced, orthogonal grid of nodes, Eqn. 11 can be reexpressed in its finite-difference form with these approximations:

for conduction along the x -axis,

$$\partial^2 T / \partial x^2 \approx [1/(\Delta x)^2] (T_{u+1,v} + T_{u-1,v} - 2T_{u,v}) \quad (12)$$

for conduction along the y -axis,

$$\partial^2 T / \partial y^2 \approx [1/(\Delta y)^2] (T_{u,v+1} + T_{u,v-1} - 2T_{u,v}) \quad (13)$$

for temperature change with time,

$$\partial T / \partial t \approx (1/\Delta t) (T_{u,v}^{p+1} - T_{u,v}^p) \quad (14)$$

where subscripts u and v denote the node position on the x and y coordinates, respectively. When Δx equals Δy , and if the temperatures of the nodes are known at any particular time, the temperature at an arbitrary node in the gas phase after a time increment, Δt , can be evaluated from the equation obtained by rearranging and substituting Eqns. 12–14 into Eqn. 11

$$T_{u,v}^{p+1} = Fo_{u,v}^p (T_{u+1,v}^p + T_{u-1,v}^p + T_{u,v+1}^p + T_{u,v-1}^p) + T_{u,v}^p (1 - 4Fo_{u,v}^p) \quad (15a)$$

$$Fo_{u,v}^p = \xi_{u,v}^p \Delta t / (\Delta x)^2 \text{ and } \xi_{u,v}^p = (\kappa / \rho c_v)_{u,v}^{t=p} \cdot \Delta t \quad (15b)$$

where Fo and ξ are the Fourier number and volumetric thermal diffusivity, respectively. The mathematical stability criterion of Eqn. 15a is that the

coupling of the two independent variables, Δx and Δt , yields a positive coefficient for the $T_{u,v}^p$ term in Eqn. 15a, i.e., $Fo < 0.25$ [24–26]. The property values of the gas around any node in the gas-filled space of the furnace are constant over each time step. These property values of argon as a function of temperature are summarized in Table 1.

The increase in the gas phase temperature inside the furnace causes expansion of the gas when the pressure inside the furnace is kept constant (1 atm). Because the gas-filled volume inside the furnace is also constant for a given tube, the increase in the volume occupied by the gas with increasing temperature means that part of the gas is expelled from the furnace. Chakrabarti et al. [7] have theoretically investigated the rate of gas expulsion as a function of time during pulse-heating of a commercial graphite furnace with sophisticated analysis of mass and energy conservation law by assuming no temperature gradient along the axial length of the furnace. However, the temperature gradient on the interior surface as well as the non-uniform temperature distribution of the gas phase in the type 1 furnace makes the analysis of the rate of gas expulsion more complex. In the model considered here, the volumetric expulsion rate is simply estimated on the basis of the ideal gas law. This has been calculated by using the increase in the volume occupied by the gas based on the increase in the weighted-average gas-phase temperature, T_{ave} , over each time step. T_{ave} is calculated from

$$T_{ave} = \frac{\sum_{u=1}^n \sum_{v=1}^n m_{u,v} T_{u,v}}{\sum_{u=1}^n \sum_{v=1}^n m_{u,v}} \quad (16)$$

where $m_{u,v}$ is the mass of gas inside the control volume of node (u, v) .

The type 3 furnace. Gradients in the surface temperature along the axial length of the type 3 furnace have been observed. However, the surface temperature around the circumference at any node along the axial length of the tube is uniform because of the high thermal conductivity in the ab -plane of the anisotropic pyrolytic graphite.

The modes of heat transfer inside the gas-filled space of the furnace by free convection and radiation are neglected using similar considerations as in the modelling of gas phase temperature in the type 1 furnace. The gas expansion with increasing gas temperature causes axial transfer of thermal energy. However, this axial heat transport becomes less significant with increasing temperature because of decreasing gas density [7]. Heating of the gas inside the furnace is therefore attributed to only radial conduction of the thermal energy from the surface of a given axial node.

Figure 5 shows the nodal geometry of the gas-filled space of any node along the axial length of the tube of the type 3 furnace for finite-difference approximation of the gas temperature calculation. Transient heat transfer inside the gas-filled space of any node by gas conduction alone in the radial direction, using cylindrical coordinates, may be expressed [24, 25] by

$$(\kappa/r) \partial(r \partial T / \partial r) / \partial r = \rho c_v \partial T / \partial t \quad (17)$$

Re-expressing Eqn. 17 in its finite-difference form, the temperature around each annular node, $T_{u,v}$, after a finite time increment can be derived as

$$T_{u,v}^{p+1} = Fo_{u,v}^p [T_{u,v+1}^p (1 + \Delta r/2r) + T_{u,v-1}^p (1 - \Delta r/2r)] + T_{u,v}^p (1 - 2Fo_{u,v}^p) \quad (18)$$

where $\Delta r = r_0/n$, $2 \leq v \leq n-1$, $1 \leq u \leq j$, $Fo_{u,v}^p = \xi_{u,v}^p \Delta t / (\Delta r)^2$, and $\xi_{u,v}^p = (\kappa/\rho c_v)_{u,v}^{t=p} \cdot \Delta t$. The subscripts u and v of the above equation represent the position of a node along the axial length of the type 3 furnace and the position of the annular node in the radial direction, respectively. The mathematical stability criterion of the above equation is $Fo < 0.5$. However, the problem of approximating the temperature at the central zone, $T_{u,1}$, with the cylindrical coordinate finite-difference equation (Eqn. 18) should be noted. This equation cannot be used to solve for the central node because the radius, r , is zero. In the model considered here, $T_{u,1}$ is assumed to be the same as $T_{u,2}$. In order to minimize the propagated error, Δr should be a small value so that the total heat capacity involved in the central zone does not significantly alter the simulated results.

The average gas temperature of a cross-section at an axial node along the tube length is calculated by the weighted-average gas temperature of all annular nodes in that section. The average gas-phase temperature over the entire gas-filled space of the graphite tube is obtained by taking the weighted-average of these average sectional gas temperatures.

Initial condition

The time corresponding to charring and atomization temperatures of the furnace is that when the surface temperature of the first node (i.e., $u = 1$ in Figs. 1 and 3) reaches these preset temperatures. As mentioned earlier, the boundary conditions of the model are that the surrounding temperature and the temperature of node n in Figs. 1 and 3 are held constant at 300 K. In order to initiate the simulation of the temperature distribution on the surface and in the gas phase with the finite-difference equations, the initial temperatures of all the nodes of the electrode, the tube, and the gas are required.

A non-uniform temperature distribution is expected once the furnace has been heated above room temperature. The temperature distribution at the initiation of the atomization stage is set equal to the quasi-steady-state temperature at the termination of the charring stage. This quasi-steady-state temperature distribution is first obtained using 300 K as the initial temperature of all the nodes except that of the first node in Figs. 1 and 3. The temperature of the first node is set equal to the charring temperature. The electric current passing through the furnace is therefore calculated as the current required to maintain the temperature of the first node at the charring temperature. This distribution of the quasi-steady-state temperature is further used as the initial temperature of each node at the initiation of the atomization stage.

EXPERIMENTAL

Apparatus

Computation was done with a Honeywell CP-V computer which has a Sigma-9 central processing unit. The program was written in FORTRAN IV.

The capacitive-discharge-heated furnace made of anisotropic pyrolytic graphite and the atomic absorption spectrometer described earlier were used [5, 6, 10, 11]. The surface temperature of the graphite furnace was measured by optical pyrometry. Optical pyrometers (Ircon series 1100, model 11 $\times$ 30, and Ircon series 300, model 300) were used. The output voltage from the pyrometer (Ircon series 1100, model 11 $\times$ 30) was recorded with a Nicolet 2090-III, dual-channel digital oscilloscope fitted with a model 201 plug-in unit and a disk recorder (Nicolet Instrument Co., Madison, WI). Oscilloscope traces were converted to temperature profiles by using the pyrometer calibration curve supplied by the manufacturer.

RESULTS AND DISCUSSION

Geometry of the type 1 and type 3 furnaces

Unless otherwise stated, the dimensions of graphite tube used for both the type 1 and type 3 furnaces in the present studies are 3.0 mm i.d., 6.0 mm o.d., and 15.0 mm long. To calculate the surface temperature of the type 1 furnace with a finite-difference approximation, the nodal geometry in Fig. 1 was set with $n = 60$ across the assembly half (15.0 mm long). When a graphite tube of internal radius 1.5 mm was used, the interior surface of the tube was described by 6 nodes. The surface temperature distribution of the type 3 furnace was calculated using $n = 35$, $j = 15$ (Fig. 3); the total length of the assembly was 35.0 mm (with a tube length of 15.0 mm).

The heating rate of the furnace was calculated from the time taken for the temperature of the first node ($u = 1$ in Figs. 1 and 3) of the furnace to reach the preset, steady-state value from the initial temperature (i.e., the charring temperature).

Temperature/time curves of the furnace heated by the capacitive discharge technique

When the furnace is heated by the capacitive discharge technique, the instantaneous input power can be described [5, 6] by

$$P_t = [V_0^2/R(t)] \exp \left\{ - \int_0^t [2/R(t)C] dt \right\} \quad (19)$$

where P_t , V_0 , $R(t)$ are the instantaneous input power, initial voltage, and instantaneous resistance of the furnace, respectively. Because the heating of the furnace is directly related to its input power, Eqn. 19 shows that factors such as the initial voltage, the total capacitance of the capacitor bank, and

the resistance of the furnace, will affect the characteristics of the temperature/time curve of the furnace.

Figure 6 shows the results of the effect of the capacitance of the capacitor bank on the simulated temperature/time curves. Curves a–d are the characteristic temperature/time curves of the first node of the type 1 and type 3 furnaces which are simulated with the capacitor bank having capacitance values of 2.0–0.25 F. Curves 1–4 represent the instantaneous voltage of the capacitor bank having capacitance values of 2.0, 1.0, 0.5 and 0.25 F, respectively, during the heating of the furnace. Figure 6 shows that the temperature/time curve bends towards the time axis when a capacitor bank of low capacitance is used, whereas it bends towards the temperature axis for a capacitor bank of high capacitance. The large voltage drop in the low capacitance case results in a decrease in the power level, and hence, a decrease in the heating rate. However, the bending of the temperature/time curve towards the temperature axis in the high capacitance case is mainly a result of a decrease in the furnace resistance with increase in temperature of the graphite tube.

Figure 7 shows the theoretical results of the effect of the initial voltage on the characteristic temperature/time curves of the first node of the type 1 and type 3 furnaces. The capacitor banks having capacitances of 2.0 and 0.3 F were used for calculating the temperature of the type 1 and type 3 furnaces, respectively. As can be seen from Eqn. 19, when the product of the capacitance and resistance is very high, the value of the exponential term on the right-hand side of the equation is approximately one when the duration of the discharge of the capacitor bank is very short. The instantaneous output power from the capacitor bank, hence the heating rate of the furnace, is mainly governed by the initial voltage and the reciprocal of the instantaneous resistance across the furnace. Because the electrical resistance of the type 3 furnace is higher than that of the type 1 furnace, for the type 3 furnace the

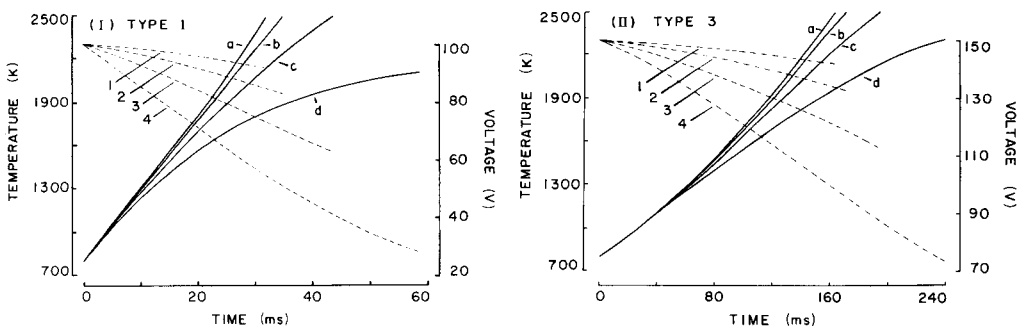


Fig. 6. Effect of the capacitance of the capacitor bank on the temperature/time curves of the type 1 (I) and type 3 (II) furnaces predicted by the model. (—) Voltage/time curves; initial voltages used in the calculations for the type 1 and type 3 furnaces were 100 and 150 V, respectively. (—) Temperature/time curves of the first node, i.e., $u = 1$ in Figs. 1 and 3. Capacitance: (a, 1) 2.0 F; (b, 2) 1.0 F; (c, 3) 0.5 F; (d, 4) 0.25 F.

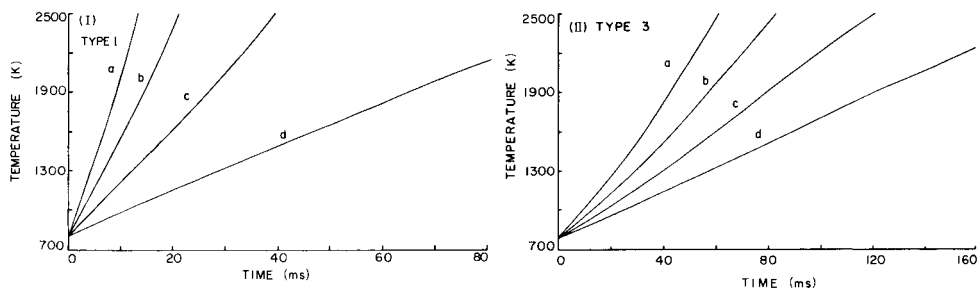


Fig. 7. Effect of the initial voltage on the temperature/time curves predicted by the model for the type 1 (I) and type 3 (II) furnaces at the first node, i.e., $u = 1$ in Figs. 1 and 3. For I, initial voltage: (a) 150 V; (b) 120 V; (c) 90 V; (d) 60 V; capacitance, 2.0 F. For II, initial voltage: (a) 250 V; (b) 220 V; (c) 190 V; (d) 160 V; capacitance, 0.3 F.

initial voltage of the capacitor bank is higher than that of the type 1 furnace in order to achieve the same heating rate.

Figures 8 and 9 illustrate the temperatures of the type 1 and type 3 furnaces that were measured experimentally at the central interior surface of the graphite tube through the sample injection hole by optical pyrometry by using various initial voltages as well as various capacitances of the capacitor bank. The simulation results of the model shown in Fig. 7 are in agreement with the experimental results. For a given capacitance and initial voltage, the slight difference between the theoretical and the experimental heating rate for the type 1 furnace may be attributable to the difference in the apparent contact area (between the electrodes and the graphite tube) in the experimental set-up and the contact area used in the simulation. In the present design of type 1 furnace, the increase in contact area decreases the resistance across the electrodes, and hence, changes the input power level (Eqn. 19). However, the difference between the theoretically predicted and experimentally measured heating rates for the type 3 furnace is greater than that for the type 1 furnace. At a given capacitance and initial voltage, the heating rate of the type 3 furnace predicted by the model is consistently lower than the experimentally measured heating rate. Because electrical resistance is the major variable in determining the input power level, the results suggest that the electrical resistivity of the anisotropic pyrolytic graphite in Table 1 may have been over-estimated. The effect is not shown by the type 1 furnace, but is shown by the type 3 furnace. Because the resistance of the furnace is proportional to the path length of the current and inversely proportional to the cross-sectional area (Eqn. 1), the above difference may be attributable to shorter path length and larger cross-sectional area of the type 1 furnace relative to those of the type 3 furnace.

Conclusions

A capacitor bank having a high capacitance shows less drop in voltage as the discharge proceeds, and hence, less change in the energy level. In this

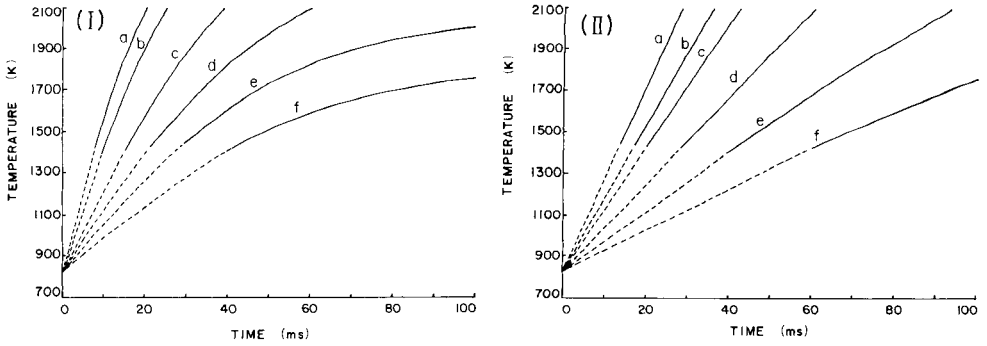


Fig. 8. Temperature/time curves of the type 1 furnace when the capacitor bank is discharged at various initial voltages. For (I), capacitor bank A, 0.30 F, with initial voltage: (a) 130 V; (b) 120 V; (c) 110 V; (d) 100 V; (e) 90 V; (f) 80 V. For (II), capacitor bank B, 1.94 F, with initial voltage: (a) 100 V; (b) 90 V; (c) 80 V; (d) 70 V; (e) 60 V; (f) 50 V.

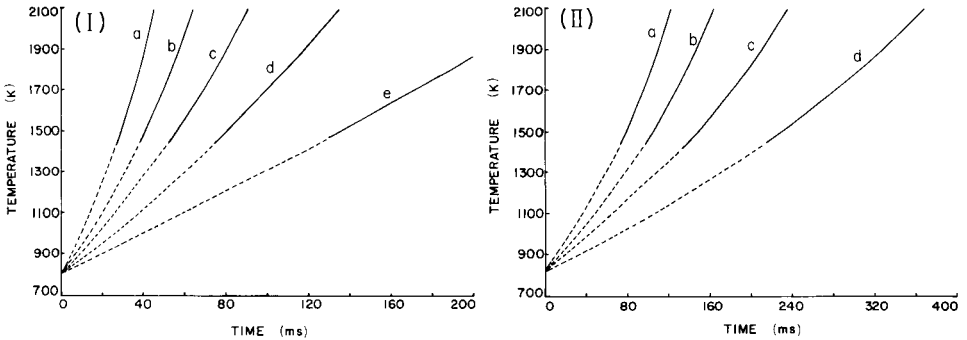


Fig. 9. Temperature/time curves of the type 3 furnace when the capacitor bank is discharged at various initial voltages. For (I), capacitor bank A, 0.30 F, with initial voltage: (a) 248 V; (b) 210 V; (c) 180 V; (d) 150 V; (e) 120 V. For (II), capacitor bank B, 1.94 F, with initial voltage: (a) 150 V; (b) 130 V; (c) 110 V; (d) 90 V.

case, the transient input power to the furnace depends on the furnace resistance. The observed nonlinear heating characteristic of the anisotropic pyrolytic tube furnace used for capacitive discharge may be attributed to the decrease in resistance as the temperature of the furnace increases. The high resistance of the type 3 furnace requires a very high initial voltage to achieve a high heating rate. This high initial voltage also compensates for high capacitance because the total energy in the capacitor bank increases with the square of the initial voltage. The effects of the initial voltage and the capacitance of the capacitor banks on the instantaneous rate of heating of the furnaces predicted by the model show a reasonable agreement with the experimental results obtained by optical pyrometry.

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support of this research project. The authors also thank S. M. Wong for her assistance with the computer simulation work.

REFERENCES

- 1 W. Slavin, S. A. Myers and D. C. Manning, *Anal. Chim. Acta*, 117 (1980) 267.
- 2 H. G. C. Human, N. P. Ferreira, C. J. Rademeyer and P. K. Faure, *Spectrochim. Acta*, Part B: 37 (1982) 593.
- 3 L. A. Pelieva, E. V. Prostetsova and V. G. Bukhantsova, *Zh. Prikl. Spectrosk.*, 36 (1982) 898.
- 4 L. A. Pelieva, V. G. Bukhantsova and O. A. Davidyuk, *Zh. Prikl. Spectrosk.*, 38 (1983) 533.
- 5 S. B. Chang, Ph.D. Thesis, Department of Chemistry, Carleton University, Ottawa, Ontario, Canada, 1985.
- 6 S. B. Chang and C. L. Chakrabarti, *Prog. Anal. At. Spectrosc.*, 8 (1985) 83.
- 7 C. L. Chakrabarti, S. Wu, R. Karwowska, J. T. Rogers and R. Dick, *Spectrochim. Acta*, Part B: 40 (1985) in press.
- 8 H. Falk, A. Glismann, L. Bergann, G. Minkwitz, M. Schubert and J. Skole, *Spectrochim. Acta*, Part B: 40 (1985) 533.
- 9 W. M. G. T. Van den Broek, L. de Galan and J. P. Matousek, *Anal. Chim. Acta*, 100 (1978) 121.
- 10 C. L. Chakrabarti, H. A. Hamed, C. C. Wan, W. C. Li, P. C. Bertels, D. C. Gregoire and S. Lee, *Anal. Chem.*, 52 (1980) 167.
- 11 C. L. Chakrabarti, S. B. Chang, S. R. Lawson and P. C. Bertels, *Spectrochim. Acta*, Part B: 39 (1984) 1195.
- 12 W. C. Riley, in I. E. Campbell and E. M. Sherwood (Eds.), *High Temperature Materials and Technology*, Chap. 7, Wiley, New York, 1967.
- 13 A. Moore, *Chem. Phys. Carbon*, 11 (1972) 69.
- 14 B. T. Kelly, *Physics of Graphite*, Applied Science Publishers, London, 1981.
- 15 J. Pappis and S. L. Blum, *J. Am. Ceram. Soc.*, 44 (1961) 592.
- 16 D. Schiff, *Metals Engineering Quarterly*, 2(4) (1962) 32.
- 17 A. R. Ubbelohde and F. A. Lewis, *Graphite and Its Crystal Compounds*, Oxford University Press, London, 1960.
- 18 Y. S. Touloukian and E. H. Buyco, *Thermophysical Properties of Matter — Vol. 5, Specific Heat of Nonmetallic Solid*, IFI/Plenum, New York, 1970.
- 19 Y. S. Touloukian, *Thermophysical Properties of High Temperature Solid Matter — Vol. 1, Elements*, Macmillan, New York, 1967.
- 20 Y. S. Touloukian and D. P. DeWitt, *Thermophysical Properties of Matter — Vol. 8, Thermal Radiative Properties of Nonmetallic Solids*, IFI/Plenum, New York, 1972.
- 21 K. Aoki and S. Yajima, *J. Mater. Sci.*, 6 (1971) 1338.
- 22 Y. S. Touloukian and C. Y. Ho, *Properties of Nonmetallic Fluid Elements*, McGraw-Hill Book Co., New York, 1981.
- 23 J. C. Bailar, H. J. Emeleus, S. R. Nyholm and A. F. Trotman-Dickenson, *Comprehensive Inorganic Chemistry*, Vol. 1, Pergamon Press, Oxford, 1973.
- 24 J. P. Holman, *Heat Transfer*, 5th Edn., McGraw-Hill, New York, 1981, pp. 115, 337, 265–304.
- 25 C. L. Smith, R. W. Pike and P. W. Murrill, *Formulation and Optimization of Mathematical Models*, International Textbook Co., Scranton, PA, U.S.A., 1970.
- 26 C. F. Gerald, *Applied Numerical Analysis*, 2nd Edn., Addison-Wesley, New York, 1978.
- 27 H. W. Jackson, *Introduction to Electronic Circuits*, 5th Edn., Prentice-Hall, NJ, U.S.A., 1981.
- 28 C. L. Chakrabarti, S. Wu, R. Karwowska, J. T. Rogers, L. Haley, P. C. Bertels and R. Dick, *Spectrochim. Acta*, Part B, 39 (1984) 415.

CAPACITIVE-DISCHARGE-HEATED ANISOTROPIC PYROLYTIC GRAPHITE FURNACE USED FOR ATOMIC ABSORPTION SPECTROMETRY

Part 2. Experimental Verification of Temperature Distribution of the Furnace Surface and of the Gas Phase

C. L. CHAKRABARTI*, S. B. CHANG, T. J. HUSTON and P. C. BERTELS

Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6 (Canada)

J. T. ROGERS

Department of Mechanical and Aeronautical Engineering, Carleton University, Ottawa, Ontario K1S 5B6 (Canada)

R. DICK

ELPA Engineering, Ottawa, Ontario K2C 3L5 (Canada)

(Received 21st February 1985)

SUMMARY

The predictions of a theoretical model, embodied in a computer program, describing the heating characteristics of the furnace surface and the gas phase of an anisotropic pyrolytic graphite furnace heated by the capacitive discharge technique are compared with the experimental results obtained by optical pyrometry and by two-line atomic absorption spectrometry, respectively. The surface temperature gradient around the circumference of the type 1 furnace and along the optical axis of the type 3 furnace are calculated and compared with the measured temperatures. The weighted-average of the theoretically predicted gas temperature is in reasonable agreement with the effective vapour temperature measured by two-line atomic absorption method. The heating rate of the furnace does not have a significant effect on the temperature distribution of either the furnace surface or the gas phase. The effect of the difference in the temperature distribution of the type 1 and type 3 furnaces on the atomic absorption signals is also discussed.

Van den Broek et al. [1] and Chakrabarti et al. [2] have theoretically modelled the temperature of the graphite surface and the vapour phase of conventional graphite furnaces and compared the theoretical temperatures with the experimentally measured temperatures. Non-uniform temperature distribution of conventional graphite furnaces has also been reported [3–7]. Although the effect of non-uniform distribution of temperature of graphite furnaces on chemical reactions and physical processes involved in the atomization process is not yet fully understood, matrix interferences and reduced sensitivities in conventional graphite-furnace atomic absorption spectrometry have frequently been attributed to non-uniform distribution of temperature.

In the present studies, the predictions of a model describing the heating of the anisotropic pyrolytic graphite furnace by the capacitive discharge

technique, embodied in a computer program developed by Chang et al. [8], hereinafter called Part 1, are compared with experimental results of the instantaneous temperature distribution of the type 1 and type 3 furnaces. The effect of the characteristic difference in the temperature distribution of the type 1 and type 3 furnaces on the atomic absorption signals is also studied. Because the surface temperature was measured by optical pyrometry, the systematic errors in the measurement are also discussed.

EXPERIMENTAL

Unless otherwise stated, the capacitive-discharge-heated graphite furnace atomic absorption spectrometer used was the same as described in Part 1. Optical pyrometers, Ircon series 1100, model 11 $\times$ 30, and Ircon series 300, model 300, were used for the measurement of the surface temperature.

The experimental set-up for measurement of the temperature distribution in the type 1 furnace is shown in Fig. 1. The temperature at two places (90 degrees apart on the interior tube surface), specified as zones A and B in Fig. 1, were measured using two identical, automatic, optical pyrometers, series 1100, model 11 $\times$ 30 (Ircon). The positions of zones A and B were defined as the interior surfaces of the type 1 furnace below the sample injection hole and that nearest to the contact electrode, respectively. Temperature distribution in the type 3 furnace was measured sequentially with the optical pyrometer along the axial length on the outer surface of the graphite tube. The voltage output of pyrometers was recorded with a Nicolet 2090, model 201 plug-in, dual-channel digital oscilloscope (Nicolet Corporation).

The gas temperature was determined by the two-line atomic absorption method [9–11]. The line pairs used were 368.3/280.2 nm for lead, 286.3/284.0 nm for tin and 229.0/232.6 nm for nickel. The working range of these thermometric species was discussed by Chakrabarti et al. [12]. The gas temperature was calculated from two separate atomic absorption measurements of each line pair in the same way as done by others [1, 6, 12, 13].

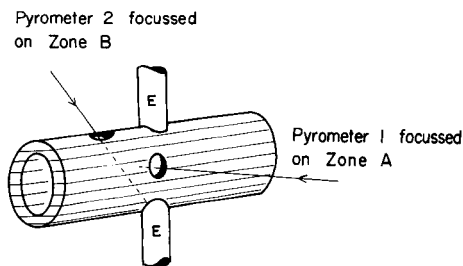


Fig. 1. The instrumental set-up for simultaneous temperature measurement at two zones, 90° apart, of the type 1 furnace with two automatic optical pyrometers. Zone A, the central lamellar plane across the two contact electrodes; zone B, the interior surface nearest to one of the contact electrodes.

RESULTS AND DISCUSSION

The settings of nodal geometry of the type 1 and type 3 furnaces for finite-difference approximation were the same as used in Part 1. For the type 1 furnace, $n = 60$ across the assembly half (15.0-mm long, Fig. 1 in Part 1). A graphite tube of 1.5-mm internal radius was used, and the interior surface of the graphite tube was described by 6 nodes. One face of the control volume about the seventh node is tangential to the interior surface of the graphite tube. The calculated temperature distribution of the first seven nodes represents the theoretical temperature distribution of the interior surface of the type 1 furnace around the circumference. Because the internal radius of the furnace was divided into 6 segments, the nodal geometry of the gas-filled space inside the furnace is approximated by a 6×6 grid.

The surface temperature distribution of the type 3 furnace was calculated using $n = 35$, $j = 15$ (Fig. 3 in Part 1); the total length of the assembly was 35.0 mm (with a tube length of 15.0 mm). The temperature distribution of the gas-filled space of each axial node was calculated using $m = 10$ (Fig. 5 in Part 1).

Errors in the measurement of surface temperature by optical pyrometry and their correction

When the furnace temperature is measured through the sample injection hole by optical pyrometry, systematic errors in the measured temperature are associated with the ratio of the half-length and the radius of the graphite tube, the operational wavelength of the pyrometer, the emissivity of the graphite surface, the final temperature, and the temperature distribution [14]. In the experimental conditions used in this study, the ratio of the half-length and the radius of the graphite tube is 5.0 (7.5 mm/1.5 mm). The operational wavelength of the narrow-band spectral pyrometer (Ircon series 1100, model 11 $\times$ 30) is $0.85 \mu\text{m}$, as determined from its calibration graph. Because the graphite surface is not an ideal blackbody radiator, i.e., the emissivity is not unity, the error in each single measurement is mainly attributable to nonuniform temperature distribution along the graphite surface. When the temperature difference between zones A and B of the type 1 furnace (see Fig. 1) is determined by optical pyrometry, the systematic error in each of the two simultaneously-measured temperatures is not expected to be the same. The higher amount of reflected radiation from zone B, due to its higher surrounding temperature, increases the systematic error in the measured temperature of zone B.

According to the principles of temperature measurement with narrow-band spectral pyrometers [15, 16], the measured temperature, T_m , its true temperature, T_t , and the surrounding temperature, T_s , are related as follows:

$$\begin{aligned} 1/[\exp(c_2/\lambda_c T_m) - 1] &= \epsilon_\lambda(T_t)/[\exp(c_2/\lambda_c T_t) - 1] \\ &+ [1 - \epsilon_\lambda(T_t)]/[\exp(c_2/\lambda_c T_s) - 1] \end{aligned} \quad (1)$$

where c_2 , λ_c and $\epsilon_\lambda(T_t)$ are the second universal radiation constant (1.4388 cm K), the characteristic effective central wavelength, and the spectral emissivity of the object as a function of temperature, respectively. However, the above equation is derived on the assumption that the surrounding temperature is uniform.

Figure 2 shows the correlation of the measured temperature and the true temperature of the object at various surrounding temperatures and with the emissivities at $\lambda_c = 0.85 \mu\text{m}$. As can be seen, the higher the emissivity of the object, the less the relative error in the measurement because a good emitter is also a good absorber, and hence less radiation is reflected. In the temperature range 1000–2800 K, the relative error between the measured temperature and the true temperature of the object is small when the temperature of the object is higher than that of the surroundings. However, a large error in the temperature measurement, caused by reflected radiation, is expected when the temperature of the object is much lower than that of the surroundings. The horizontal part of the curves in Fig. 2 (I and II) indicates a potential difficulty in correcting for the measured temperature using the above equation when the true temperature of the object is much lower than the surrounding temperature. A slight variation in the temperature measurement in such circumstances results in a variation of a few hundred degrees in the corrected temperature. Comparison of Fig. 2-I with Fig. 2-II shows that uncertainties in the measurement increase with decreasing emissivity.

To minimize error in measuring the temperature of an object with low emissivity and/or when the temperature of an object is much lower than its surroundings, a long-operational-wavelength pyrometer is better than a short-operational-wavelength one because an increase in the characteristic central wavelength of the pyrometer significantly decreases the ratio of the radiance reflected from the object and the radiance emitted by the object, and hence,

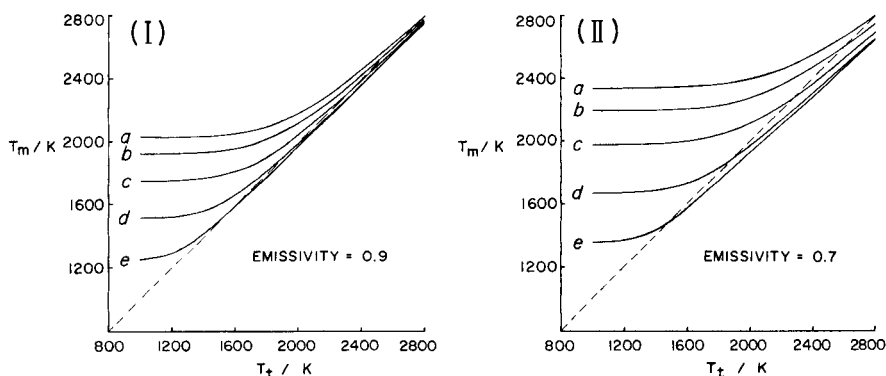


Fig. 2. Correlation of the measured temperature, T_m , with the true temperature, T_t , of the object at various surrounding temperatures, T_s , and with various emissivities: (I) 0.9 (II) 0.7. Dashed lines represent perfect correlation. $\lambda_c = 0.85 \mu\text{m}$. T_s value: (a) 2800 K (b) 2600 K; (c) 2300 K; (d) 1900 K; (e) 1500 K.

decreases the error in measuring the temperature. This is illustrated in Fig. 3. Also, when the temperature of the object is much lower than its surroundings, the smaller error in the temperature measurement with a long-operational-wavelength pyrometer means a correspondingly smaller correction factor and less uncertainty in the corrected value of the temperature. Figure 3 also predicts a great increase in error in the temperature measured with a long-operational-wavelength pyrometer when the surrounding temperature is below the true temperature of the object; hence, a long-operational-wavelength pyrometer should not be used in such a case.

The following conclusions are drawn for the type 1 furnace: (i) to obtain high precision in the temperature measurement of zone A, a short-operational-wavelength pyrometer should be used; (ii) to achieve a smaller error in the measurement or a correctable error in temperature measurement of zone B, a long-operational-wavelength pyrometer should be used. However, long-operational-wavelength pyrometers normally suffer from slow response. Fast heating of the furnace by the capacitive discharge technique required that the measurement be made with a short-operational-wavelength pyrometer, such as the Iacon series 1100, model 11 $\times$ 30, which had a faster response. This pyrometer was therefore used.

Temperature distribution in the type 1 furnace

Distribution of the interior surface temperature. As mentioned earlier, the anisotropic pyrolytic graphite tube used for the type 1 furnace was manufactured in such a way that the lamellar plane (*ab*-plane) of the graphite was parallel to the length of the tube. Because the thermal conductivity of the anisotropic pyrolytic graphite in the *ab*-plane is about 150 times greater than that along the *c*-direction, there is a negligible temperature gradient along the length of the tube. However, heat flow by conduction from the central

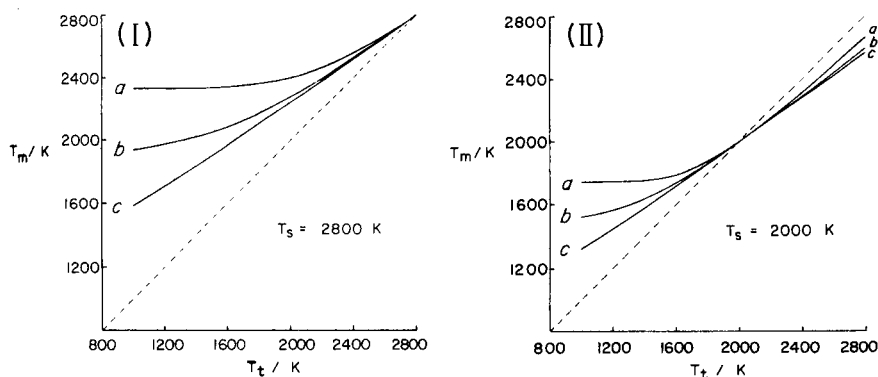


Fig. 3. Effect on the correlation of the measured temperature, T_m , with true temperature, T_t , of an object having emissivity of 0.7 at a given surrounding temperature, T_s , with effective central wavelength, λ_c , as a parameter. Dashed lines represent perfect correlation. (I) $T_s = 2800$ K; (II) $T_s = 2000$ K. λ_c values: (a) $0.85 \mu\text{m}$; (b) $2.0 \mu\text{m}$; (c) $8.0 \mu\text{m}$.

lamellae of the tube to the water-cooled electrode support results in a non-uniform temperature distribution across the assembly.

Figure 4 shows theoretical temperature/time curves of the first, fifth, and seventh nodes which are denoted by $T_{u=1}$, $T_{u=5}$ and $T_{u=7}$, respectively. The dots in Fig. 4 represent the temperature of other nodes (i.e., $u = 2, 3, 4$ and 6) at the time when the temperature of the first node reaches its steady-state, final value. Figure 5 shows an example of the temperature measured simultaneously with two identical, automatic, optical pyrometers (Ircon 1100). The oscilloscopic traces a and b of the temperature of zones A and B, respectively, measured with the two pyrometers, were converted into the temperature profiles A and B, respectively, using the pyrometer calibration curve. In Fig. 5, the temperature difference, ΔT , is the difference between the temperatures of zones A and B at the instant of time when zone A reaches its final, steady-state temperature.

Similar to the pyrometrically measured temperature/time curves of zones A and B in Fig. 5, the temperature of the seventh node in Fig. 4 shows a slow increase after the temperature of the first node attains its steady-state, final temperature. When the temperature was measured with an optical pyrometer, the target area of the pyrometer was defined by the optical arrangement. The Ircon series 1100, model 11×30 , pyrometer has a minimum target area of 0.15 mm^2 . The coverage of the target area indicates that the temperature measurement is restricted effectively to the region corresponding to the sixth node described in the model, as the seventh node is tangential

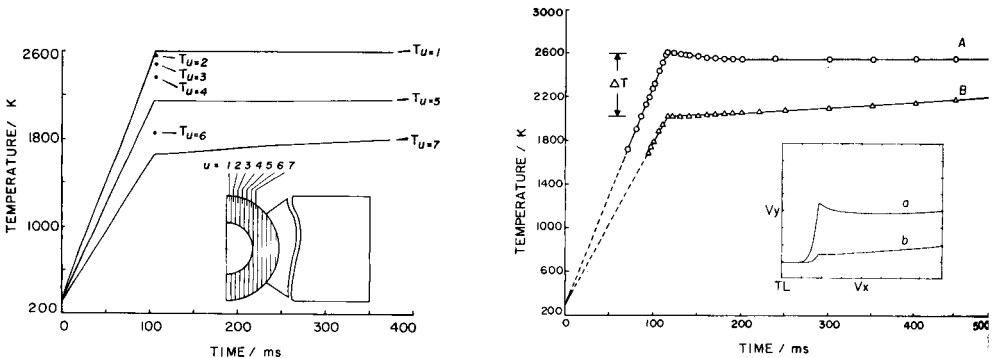


Fig. 4. Theoretically predicted distribution of the temperature of the interior surface of the type 1 furnace. Capacitance, 2.0 F; initial voltage, 70 V; number of nodes within the contact region, 3 (i.e., value of m in Fig. 1 of Part 1); tube dimension, 3.0 mm i.d., 6.0 mm o.d., and 15.0 mm long.

Fig. 5. Simultaneously-measured temperature profiles of zones A and B of the type 1 furnace. Temperature profiles A and B are derived for the oscilloscopic traces (a) and (b), respectively. ΔT is the temperature difference at the instant of time when the temperature of zone A attains its pre-established, final temperature. Dimensions of the tube are the same as in Fig. 4.

to the interior surface of the graphite tube. In order to compare the results of the temperature difference, ΔT , measured pyrometrically, as shown in Fig. 5, with that calculated theoretically as shown in Fig. 4, the latter is taken as the difference between the temperatures of the first and sixth nodes. The results in Fig. 4 show that the difference in the theoretically calculated temperature is ca. 750 K for the combination of final temperature of 2600 K and heating rate of 21 K ms^{-1} , both determined at the first node. However, the experimentally measured temperature difference between zones A and B in Fig. 5, was ca. 550 K for the combination of final temperature of 2600 K and heating rate of 20 K ms^{-1} , both measured at zone A. The difference between the heating rates is not sufficient to account for the 200 K difference. This difference may be attributable mainly to the systematic error involved in the temperature measurement by optical pyrometry.

It is reasonable to assume that the pyrometrically measured temperature of T_A is the true temperature of zone A because of the high emissivity of anisotropic pyrolytic graphite at the edge of the *ab*-plane viewed by the short-operational-wavelength of the pyrometer. Because Eqn. 1 is derived on the assumption that the object is completely enclosed in a uniform temperature, the maximum error in the temperature measurement of zone B caused by reflected radiation from the high-temperature surroundings can be estimated by assuming that the spectral emissivity is 0.85–0.90 at $\lambda_c = 0.85 \mu\text{m}$. In this case, at the temperature of 2600 K at zone A, the maximum difference between the pyrometrically measured temperature (2050 K) and the corrected temperature of zone B is 370–170 K (depending on the emissivity value chosen for the correction). Because the spectral emissivity of anisotropic pyrolytic graphite is highly dependent on the surface [17], quantitative corrections for the complex temperature distribution of the type 1 furnace can hardly be achieved by a simple method. However, both theoretical and experimental results agree qualitatively on the temperature distribution as well as the heating performance of the type 1 furnace.

As discussed in Part 1, an increase in the initial voltage of the capacitor bank increases the input power and hence the heating rate. Figure 6 shows both the theoretical and experimental results for the effect of heating rate on the temperature difference between zones A and B at the moment zone A reaches its steady-state, final temperature. The pyrometrically measured temperature difference is consistently lower than the temperature difference predicted by the model. However, both experimental and theoretical results agree that, for a given heating rate, an increase in the final temperature of zone A will increase the temperature difference. Also, an increase in the input power, and hence in the heating rate, will tend to enhance the difference between the temperatures of zone A and zone B slightly. A low heating rate increases the time taken for the temperature to reach a given value. A low heating rate also tends to heat the electrodes to a higher temperature by conduction, and hence decreases the temperature difference.

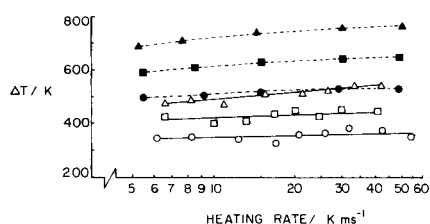


Fig. 6. Theoretical and experimental results for the effect of the heating rate on the temperature difference, ΔT , of the type 1 furnace. (—) Temperature difference between zones A and B experimentally measured by optical pyrometry; (---) difference in temperature simulated between the first and sixth nodes. Dimensions of tube are the same as in Fig. 4. (Δ) $T_A = 2600$ K; ($\blacktriangle$) $T_{u=1} = 2600$ K; ($\square$) $T_A = 2300$ K; ($\blacksquare$) $T_{u=1} = 2300$ K; ($\circ$) $T_A = 2000$ K; ($\bullet$) $T_{u=1} = 2000$ K.

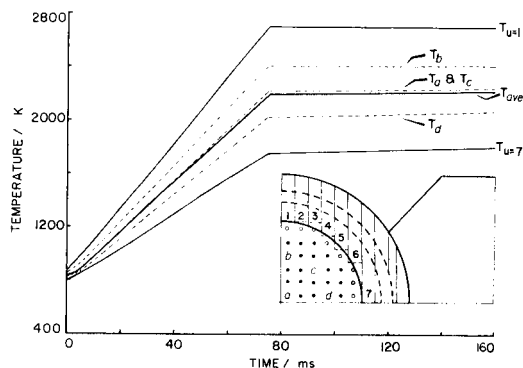


Fig. 7. Theoretical distribution of the gas temperature of the type 1 furnace. Temperature/time curves: (—) $T_{u=1}$ and $T_{u=7}$ are the surface temperature at nodes 1 and 7, respectively; (---) T_a , T_b , T_c and T_d are the temperatures at the selected positions a , b , c and d in the gas-filled space of the furnace, respectively, as a function of time. The T_{ave} line is the weighted-average of gas temperature inside the type 1 furnace. Dimensions of tube are the same as in Fig. 4.

Further results predicted by the model are as follows. A decrease in the contact areas between the tube and the graphite electrodes tends to make the tube temperature distribution uniform because of a decrease in the rate of heat loss to the electrode by conduction. In the model considered, the change in contact area is made by changing the value of m in Fig. 1 of Part 1, i.e., by changing the number of nodes in the region of $j - m + 1$ to j . When $j = 12$ is set for a tube of 3.0 mm i.d. and 6.0 mm o.d., a change in the value of m from 1 to 5 corresponds to an increase in the contact area from 4.7 to 21.8 mm².

The theoretical results show a significant change in the temperature distribution across the assembly with variations in the contact area. Only a slight increase in the temperature difference (<100 K) of the interior surface of the graphite tube between the first and seventh nodes is predicted by the model when the contact area increases from 4.7 to 21.8 mm²; the plot of ΔT vs. contact area is a straight line almost parallel to the abscissa. The above theoretical results agree with the experimental results (not shown). Although the temperature difference would be expected to be much greater because of conduction loss through the larger contact area, the low thermal conductivity of the anisotropic pyrolytic graphite in the direction of heat flow and the high input power effectively counteract this.

Additionally, an increase in the contact area means that more nodes are involved in the parallel path of low resistance of the regular (isotropic)

graphite electrode, and hence the resistance across the assembly decreases. Conversely, a decrease in the contact area increases the resistance. When the furnace is heated from 300 to 2600 K using a 2.0 F capacitor bank charged to 70.0 V, the theoretical results predict that the change in contact area from 4.7 to 21.8 mm² will produce a linear increase in the average heating rate from 14.4 to 32.0 K ms⁻¹. The experimental results agree with those theoretically predicted.

The model also predicts a decrease in, but not elimination of, the temperature difference between the first and seventh nodes caused by an increase in the thickness of the graphite tube wall. This may be attributable to isolation of the central region of the graphite tube of low thermal conductivity from the regular (isotropic) graphite electrodes of high thermal conductivity, resulting in a decrease in the rate of heat loss. However, the great increase in the mass of the graphite tube with increase in its external radius would drastically decrease the heating rate of the furnace with the same initial conditions.

Optimization of the geometrical shape of the anisotropic pyrolytic graphite tube and the regular (isotropic) graphite contact electrodes may make the temperature distribution of the type 1 furnace more uniform, but it is not the subject of the present theoretical model.

Distribution of the gas temperature and the rate of expulsion of gases from the furnace. Figure 7 shows the results for the simulation of the gas temperature distribution inside the type 1 furnace of dimensions 3.0 mm i.d. and 6.0 mm o.d. In these temperature/time curves, $T_{u=1}$ and $T_{u=7}$ correspond to the surface temperatures at nodes 1 and 7, respectively, and T_a , T_b , T_c and T_d are the theoretical temperature/time curves for selected positions within the furnace. The line T_{ave} represents the weighted-average gas temperature. As can be seen, during the first few milliseconds of the heating cycle, the rate of increase of the T_{ave} is much lower than that of the interior surface temperature at any node. Thereafter, it increases nearly linearly with increase in the temperature of the surface. Throughout the entire heating cycle, T_{ave} lies between the surface temperatures of the first and seventh nodes which the theory predicts to be the highest and the lowest temperature, respectively, of the interior surface of the type 1 furnace.

Physically, the transport of the gaseous species inside the furnace is governed by thermal flux, diffusion flux, and expansion force. The transport of the gaseous species is from the high temperature region to the low temperature region. As can be seen from Fig. 7, positions a and c have the same temperature. The line through these two positions is an isothermal one. The theoretically predicted isothermal lines in the gas temperature inside the type 1 furnace are generally parallel to the line through points a and c , but bend away from the centre of the tube. The direction of thermal flux, therefore, is such that the gaseous species travel perpendicular to isothermal lines down the temperature gradient from the highest temperature region near the surface of the first node to the lowest temperature region around the seventh node.

Figure 8 shows both the experimentally determined gas temperature (by the two-line atomic absorption method) and the theoretically-simulated, weighted-average gas temperature of the type 1 furnace at different heating rates. Because the gas temperature measured by the two-line atomic absorption method is affected by factors such as the spatial distribution of the thermometric species, radiation-beam geometry relative to the tube geometry, etc., the measured gas temperature is called “effective temperature” [9, 10]. The experimentally determined “effective temperature” agrees reasonably well with the theoretically-simulated, weighted-average gas temperature when the surface temperature of zone A > 2300 K. However, Fig. 8 shows that the “effective temperatures” are consistently lower than the theoretically-simulated, weighted-average temperatures when the surface temperatures of zone A are < 2300 K. The lower “effective temperature” may be due to insufficient contribution from the thermometric species that have diffused to the cooler parts of the graphite tube. The theoretical model predicts that the

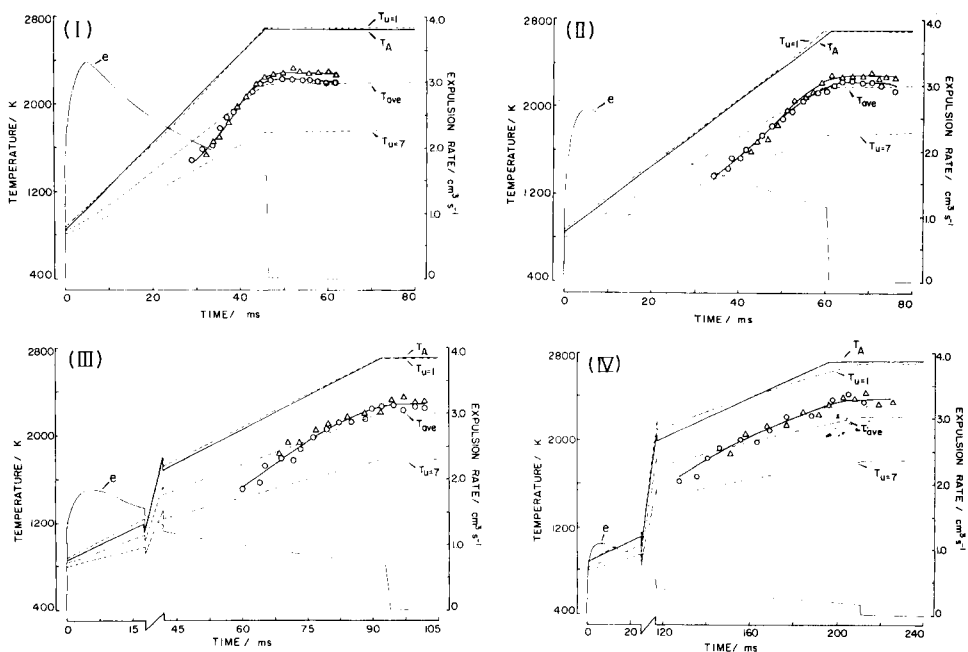


Fig. 8. Effect of the heating rate of the type 1 furnace on the gas temperature and the rate of vapour expulsion. Line T_{ave} is the theoretical weighted-average gas temperature. (Δ , $\circ$) Experimentally measured “effective temperature” by the two-line atomic absorption method with Sn (Δ), and Pb ($\circ$) as the thermometric species. Curve e is the rate of volumetric expulsion as a function of time. (---) Theoretically predicted surface temperature at the first node ($T_{u=1}$) and seventh node ($T_{u=7}$), respectively. (—) Experimentally measured temperature of zone A, T_A . Heating rate of zone A: (I) 39.0 K ms^{-1} ; (II) 27.3 K ms^{-1} ; (III) 18.5 K ms^{-1} ; (IV) 8.5 K ms^{-1} .

surface temperature around zone B is ca. 1500 K when the surface temperature of zone A is 2000 K.

Figure 8 also shows the theoretically-simulated instantaneous expulsion rates which increase with increasing heating rates of the furnace. At a given heating rate and initial temperature, the maximum rate of expulsion is independent of the final furnace temperature because the attainment of this maximum rate occurs shortly after the temperature begins to increase from its initial value. However, the results of the theoretical model suggest that when a furnace is operated at a high heating rate, an increase in the initial temperature of the furnace reduces the instantaneous expulsion rate. This agrees with results reported by Chakrabarti et al. [2].

Temperature distribution in the type 3 furnace

Distribution of the interior surface temperature. Unlike the type 1 and type 2 furnaces, the type 3 furnace was constructed in such a way that the c-axis of the anisotropic pyrolytic graphite was collinear with the optical axis. Temperature was uniformly distributed over the tube circumference because of high thermal conductivity of the *ab*-plane.

Figures 9 and 10 show the theoretically simulated and experimentally measured temperature distributions, respectively, along the length of the tube

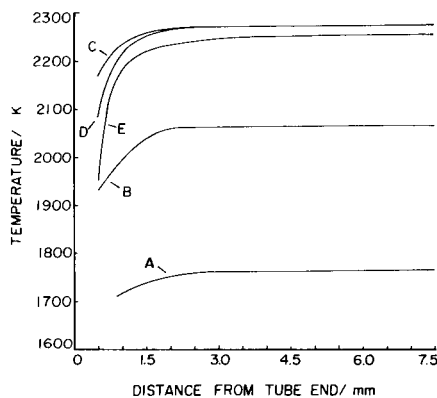
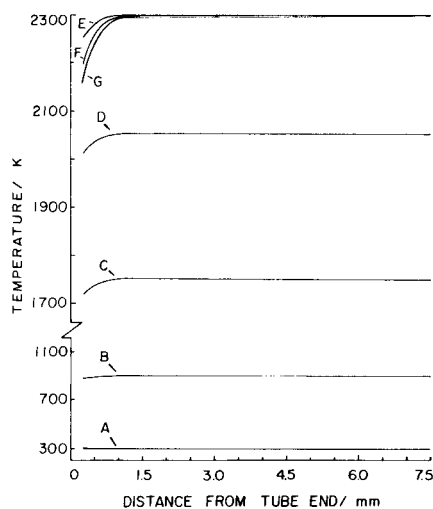


Fig. 9. Theoretically simulated distribution of the surface temperature of the type 3 furnace. Heating rate of the first node (see Fig. 4, Part 1) is 13.6 K ms^{-1} . Dimensions of tube: 3.0 mm i.d., 6.0 mm o.d., 15.0 mm long. Time: (A) 0.0 ms; (B) 45.6 ms; (C) 107.8 ms; (D) 129.8 ms; (E) 147.3 ms; (F) 294.6 ms; (G) 441.8 ms.

Fig. 10. Distribution of the surface temperature of the type 3 furnace measured by optical pyrometry. Heating rate, 12.8 K ms^{-1} ; initial temperature, 300 K; dimensions of tube as in Fig. 9. Time: (A) 125 ms; (B) 140 ms; (C) 155 ms; (D) 300 ms; (E) 500 ms.

at various times during a heating cycle. Comparison of Fig. 9 with Fig. 10 shows qualitative agreement in the temperature distribution of the furnace as a function of time. During the temperature ramping stage, temperature is uniformly distributed over the length of the graphite tube because of low thermal conductivity in the direction of heat flow (*c*-direction) and the high input power level. However, once the temperature at the centre of the graphite tube has reached the preset value, less power is required to maintain this temperature. Also, a large difference between the temperature at the ends of the tube and that of the electrodes at that time is both experimentally observed and theoretically predicted. This results in a drop in the temperature at the ends of the graphite tube with time, which agrees with the reported temperature distribution of a conventional, end-contacted graphite tube furnace [7]. However, the drop in temperature at the ends of the anisotropic pyrolytic graphite tube is much smaller than that at the ends of the conventional graphite-tube furnace because of the lower thermal conductivity of the anisotropic pyrolytic graphite relative to that of regular (isotropic) graphite in the direction of heat flow. Furthermore, the temperature at the ends of the graphite tube predicted by the model is consistently higher than that measured by optical pyrometry. The over-estimation of the temperature at the ends of the graphite tube by the model may be due to omission of heat transfer by internal surface radiation. The low temperature of the contact electrodes of the type 3 furnace results in a large heat loss by radiation from the internal surface at the ends of the graphite tube relative to its centre.

Increasing the heating rate by increasing the input power level does not change the temperature distribution along the length of the graphite tube of the type 3 furnace.

It is interesting to note that the presence of an injection hole increases the local resistance. In this study, the experimentally measured (outside surface) atomization temperature (2300 K) at the injection hole was observed to be 100–200 K higher than that measured at either side of the hole along the length of the tube. Thus, the corresponding heating rates of the type 3 furnace calculated from the measured temperature at the injection hole are higher by 1–3 K ms⁻¹ than would be obtained from most of the tube length.

Gas temperature and the rate of expulsion of gases from the furnace. Figure 11 shows the simulated surface temperatures and weighted-average gas temperatures as well as experimentally-measured surface temperatures and “effective temperatures” of the gas phase at various heating rates of the type 3 furnace. The “effective temperature” measured by the two-line atomic absorption method is consistently lower than the weighted-average gas temperature predicted by the model because of over-estimation of the temperature at the ends of the graphite tube, as mentioned earlier. No significant change in temperature difference between the surface and the gas phase caused by an increase in the heating rate from 6 to 40 K ms⁻¹ was observed experimentally, nor predicted theoretically.

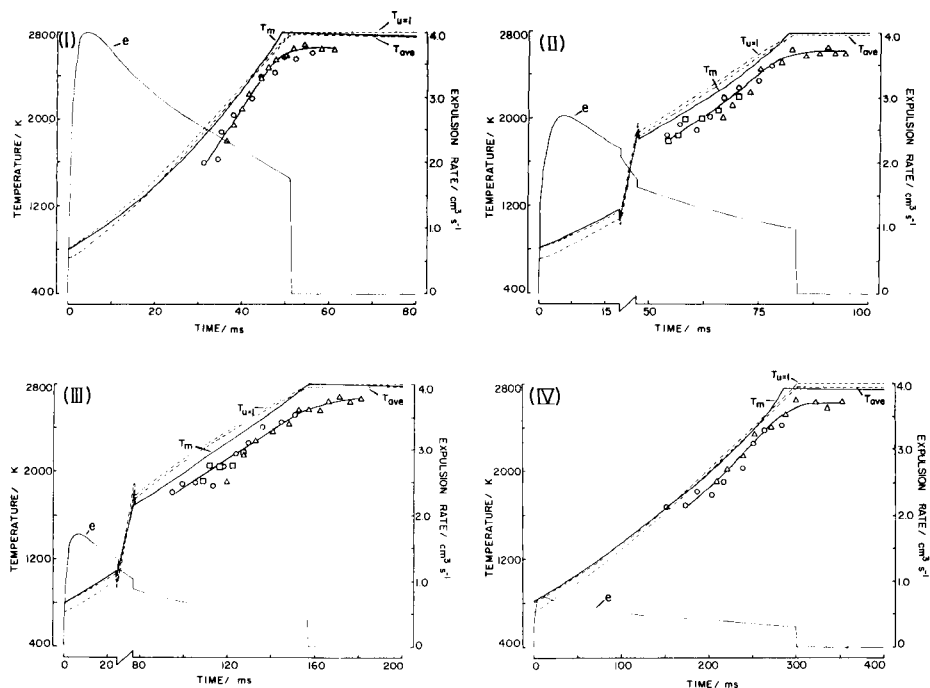


Fig. 11. Effect of the heating rate of the type 3 furnace on the gas temperature and the rate of vapour expulsion. Line T_{ave} is the theoretical weighted-average gas temperature. (Δ , $\circ$, $\square$) Experimentally measured "effective temperature" by two-line atomic absorption method with Sn (Δ), Pb ($\circ$) and Ni ($\square$) as the thermometric species. Curve e is the rate of volumetric expulsion as a function of time. (---) Theoretically predicted surface temperature at the first node, $T_{u=1}$. (—) Experimentally measured surface temperature at the centre of the graphite tube, T_m . Heating rate: (I) 39.0 K ms^{-1} ; (II) 23.8 K ms^{-1} ; (III) 12.3 K ms^{-1} ; (IV) 6.0 K ms^{-1} .

Curve (e) in Fig. 11 shows simulated results for the volumetric expulsion rate of the type 3 furnace at various heating rates. Similarly to the phenomenon observed in the type 1 furnace, the volumetric expulsion rate increases with increasing heating rate. At the same heating rate, the type 3 furnace shows a higher instantaneous volumetric expulsion rate than the type 1 furnace.

Effect of temperature distribution on the atomic absorption signals

Figures 12 and 13 show the atomic absorption signals of lead at different heating rates of the type 1 and type 3 furnaces, respectively. The atomic absorption pulses are superimposed on the temperature profiles of the graphite surface. Temperatures of the furnaces were measured by focussing the optical pyrometer through the sample injection hole (on zone A for the type 1 furnace). Table 1 summarizes the characteristics of the atomic absorption pulses and the corresponding temperature profiles shown in Figs. 12 and 13.

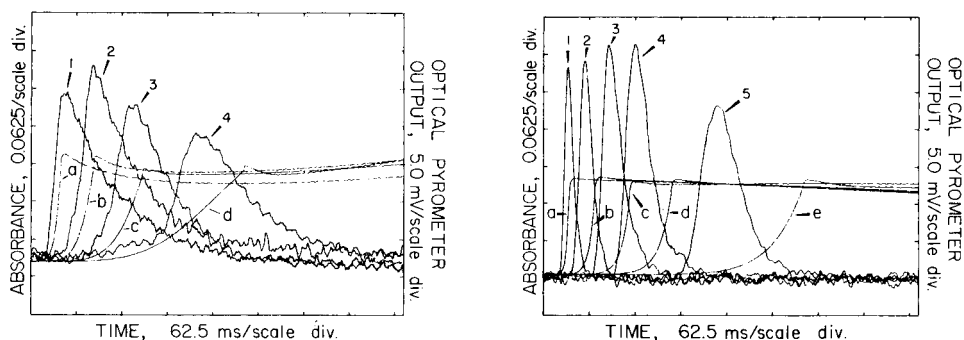


Fig. 12. Atomic absorption pulses of lead (1.6×10^{-10} g taken as nitrate) obtained with the type 1 furnace at various heating rates, superimposed on the temperature profiles of zone A, plotted as the output voltage of the optical pyrometer. Analytical wavelength, 283.3 nm; charring temperature, 770 K; atomization temperature, 2470 K; tube dimensions, 3.0 mm i.d., 6.0 mm o.d., 15.0 mm long. Atomic absorption pulses 1, 2, 3 and 4 were obtained using the temperature profiles a, b, c and d, respectively.

Fig. 13. Atomic absorption pulses of lead (3.2×10^{-10} g taken as nitrate) obtained with the type 3 furnace at different heating rates, superimposed on the surface temperature profiles of the centre of the graphite tube, plotted as the output voltage of the optical pyrometer. Atomic absorption pulses 1, 2, 3, 4 and 5 were obtained using the temperature profiles a, b, c, d and e, respectively. Tube, 10.0 mm long; other parameters as for Fig. 12.

TABLE 1

Characteristics of the atomic absorption pulse of lead and the corresponding temperature profile of the type 1 and type 3 furnaces^a

Furnace	Heating rate (K ms ⁻¹)	T_{app} (K)	t_{app} (ms)	Peak-height absorbance	Integrated absorbance (Abs. $\times$ ms)	T_{peak} (K)	t_{peak} (K)
Type 1 ^b	5.7	1560	105	0.203	21.6	2270	232
	11.0	1520	64	0.250	21.0	2310	139
	19.5	1550	40	0.303	26.4	2470	86
	40.5	1520	18	0.272	24.6	2470	44
Type 3 ^c	4.8	1300	180	0.288	20.2	1670	236
	9.6	1280	93	0.388	16.9	1690	123
	14.2	1320	64	0.390	13.1	1750	88
	22.8	1400	37	0.360	8.5	1933	56
	44.1	1400	20	0.351	5.5	2223	33

^a T_{app} , t_{app} , T_{peak} and t_{peak} are appearance temperature, appearance time, temperature at the peak of the atomic absorption pulse and time to reach the peak of the atomic absorption pulse, respectively. ^bData obtained from Fig. 12. Temperature measured by focussing the optical pyrometer on zone A. ^cData obtained from Fig. 13. Temperature measured by focussing the optical pyrometer on the centre of the furnace.

Although the difference in the tube lengths affects the absorption measurement with the peak-height and the peak-area mode, the atomic absorption signal profiles for lead show a significant difference between the type 1 and type 3 furnaces. At a similar heating rate, the rise time of the signal generated by type 1 furnace is longer than that generated by the type 3 furnace. Also, Figs. 12 and 13 show that signals from the type 1 furnace have a slower decay relative to that from the type 3 furnace. The peak area remains nearly constant for the type 1 furnace, but drastically decreases for the type 3 furnace as heating rate of the furnaces increases. The difference in the atomic absorption pulses from different furnace configurations may be due to differences in their temperature distribution and in their nature (open vs. semi-enclosed). Because the temperature of zone B is much lower than that of zone A for the type 1 furnace, some analyte vapour might condense on zone B following its vaporization from zone A, on which the sample had been deposited. The longer rise time and the slower decay of the tail of the atomic absorption signal pulse generated by the type 1 furnace is due to its relatively low heating rate and the lower final temperature of zone B. The latter leads to a slow revaporization of the analyte from zone B. The great decrease in the peak area with increasing heating rates of the type 3 furnace suggests that a large fraction of the analyte vapour is lost with the expelled purge gas during the period of rapid increase in the temperature.

Conclusions

The temperature distribution of the anisotropic pyrolytic graphite furnace heated by the capacitive discharge technique is non-uniform over the circumference of the type 1 furnace; this is theoretically predicted and experimentally observed. A decrease in the contact area between the graphite tube and the support electrodes does not significantly alter the distribution of the interior surface temperature of the type 1 furnace, but increases the initial voltage required to achieve the same heating rate of the furnace at its central lamellar plane.

Surface temperature distribution of the type 3 furnace does not show a significant temperature gradient during the ramp of the heating cycle. This may be attributed to the low thermal conductivity of the anisotropic pyrolytic graphite tube in the direction of the heat loss (*c*-direction) and the high input power level. Once the temperature at the central lamellae of the type 3 furnace has attained the predetermined steady-state, final value, there is a gradual drop in the temperature at the ends of the tube while the temperature at the tube centre remains practically constant.

The gas temperature distribution inside both the type 1 and type 3 furnaces showed that experimentally measured "effective temperature" of the gas at different heating rates were in reasonable agreement with the theoretically predicted, weighted-average gas temperatures. Increase in the heating rate of the type 1 furnace from 8 to 40 K ms⁻¹ and of the type 3 furnace from 6 to 40 K ms⁻¹ produced no significant change in the difference between

the surface temperature and the weighted-average gas temperature at the instant when the central lamellae of the graphite tube attained the predetermined, final temperature. This might be due to the small internal radius of the graphite tube used. The model also predicts that an increase in the heating rate of the furnace increases the instantaneous rate of vapour expulsion. The maximum rate of volumetric expulsion is dependent on the initial temperature and the heating rate, but independent of the final temperature of the furnace.

The difference in the temperature distribution between the type 1 and type 3 furnaces results in a significant difference in the vaporization/atomization processes, and hence in a significant difference in the atomic absorption signals generated by these two furnaces.

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support of this research project. The authors also thank S. M. Wong for her assistance with the computer simulation work.

REFERENCES

- 1 W. M. G. T. Van den Broek, L. de Galan and J. P. Matousek, *Anal. Chim. Acta*, 100 (1978) 121.
- 2 C. L. Chakrabarti, S. Wu, R. Karwowska, J. T. Rogers and R. Dick, *Spectrochim. Acta*, Part B: 40 (1985) in press.
- 3 W. Slavin, S. A. Myers and D. C. Manning, *Anal. Chim. Acta*, 117 (1980) 267.
- 4 H. G. C. Human, N. P. Ferreira, C. J. Rademeyer and P. K. Faure, *Spectrochim. Acta*, Part B: 37 (1982) 593.
- 5 C. J. Rademeyer, H. G. C. Human and P. K. Faure, *Spectrochim. Acta*, Part B: in press.
- 6 R. E. Sturgeon and C. L. Chakrabarti, *Prog. Anal. At. Spectrosc.*, 1 (1978) 5.
- 7 H. Falk, A. Glismann, L. Bergann, G. Minkwitz, M. Schubert and J. Skole, *Spectrochim. Acta*, Part B: 40 (1985) 533.
- 8 S. B. Chang, C. L. Chakrabarti, T. J. Huston, P. C. Bertels, J. T. Rogers and R. Dick, *Anal. Chim. Acta*, 176 (1985) 1.
- 9 I. Reif, V. A. Fassel and R. N. Kniseley, *Spectrochim. Acta*, Part B: 28 (1973) 105.
- 10 B. V. L'vov and N. A. Orlov, *Zh. Anal. Khim.*, 30 (1975) 1653.
- 11 R. F. Browner and J. D. Winefordner, *Anal. Chem.*, 44 (1972) 247.
- 12 C. L. Chakrabarti, S. Wu, R. Karwowska, J. T. Rogers, L. Haley, P. C. Bertels and R. Dick, *Spectrochim. Acta*, Part B: 39 (1984) 415.
- 13 D. D. Siemer, E. Lundberg and W. Frech, *Appl. Spectrosc.*, 38 (1984) 389.
- 14 H. Falk, *Spectrochim. Acta*, Part B: 39 (1984) 387.
- 15 W. Helmann and U. Mester, in B. F. Billing and T. J. Quinn (Eds.), *Temperature Measurement*, 1975-Inst. Phys. Conf. Ser. No. 26, p. 219, The Institute of Physics, London, 1975.
- 16 J. D. Ray, Report on the Steel Industry/Research Workshop, p. 99, The Holiday Inn, Burlington, Ontario, Canada, May 8-9, 1984.
- 17 Y. S. Touloukian and D. P. DeWitt, *Thermophysical Properties of Matter - Vol. 8, Thermal Radiative Properties of Nonmetallic Solids*, IFI/Plenum, New York, 1972.

SPECTRAL INTERFERENCES AND BACKGROUND OVERCOMPENSATION IN INVERSE ZEEMAN-CORRECTED ATOMIC ABSORPTION SPECTROMETRY

Part 2. The Effect of Cobalt, Manganese and Nickel on 30 Elements and 53 Element Lines

G. WIBETOE* and F. J. LANGMYHR

*Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, 0315 Oslo 3
(Norway)*

(Received 30th April 1985)

SUMMARY

The background compensation performance of a transversal alternating-current Zeeman corrector system with the magnet acting on the graphite atomization cell was assessed for 30 elements and 53 element lines in the presence of relatively large amounts of cobalt, manganese or nickel. The study revealed three cases of background overcompensation, all being caused by a cobalt line adjacent to the analytical line. When the magnetic field is on (and the background is measured), a σ -component of the cobalt lines overlaps the emission lines of boron (249.7 nm), mercury (253.7 nm) and gold (267.6 nm). The interfering effect on boron is small, but mercury and gold are more seriously affected; for both elements a serious negative systematic error is introduced. Manganese and nickel did not give any overcompensation effects on the elements and lines studied. When gold and mercury were measured with the use of the same experimental parameters and a conventional deuterium-arc background corrector, only mercury suffered from spectral interference. The spectral interference of cobalt on mercury, with either type of background correction, can be avoided by selecting a proper furnace program. When gold is to be measured in the presence of cobalt and with the present Zeeman background-correction system, the 267.6-nm line should not be used; the more sensitive 242.8-nm line is recommended.

In atomic absorption spectrometry (a.a.s.) the conventional continuum-source background correctors are now being replaced by improved concepts, such as the Zeeman and Smith-Hieftje systems. The different Zeeman corrector systems offer a number of advantages; however, one disadvantage inherent in designs based on applying the magnetic field to the atomization cell is that the field produces a multitude of atom lines which may introduce spectral interference.

In the literature, some cases of spectral interferences in direct or inverse Zeeman a.a.s. have been reported [1–4]; however, to the authors' best knowledge, systematic studies have not been reported. It is the purpose of the present series of papers to investigate spectral interferences in inverse Zeeman a.a.s. In the first paper [4], the interfering effect of iron on 30 elements and 49 element lines was investigated. The present paper reports on

the effects of cobalt, manganese and nickel on 30 elements and 53 element lines.

EXPERIMENTAL

Instruments, apparatus, reagents and standard solutions

The instruments and apparatus were as described in Part 1 [4]. The hollow-cathode and electrodeless lamps were all (as in the previous study) delivered by Perkin-Elmer.

Stock solutions (10 mg ml^{-1}) of cobalt, manganese and nickel were prepared from high-purity metals dissolved in minimum volumes of nitric acid (Suprapur, Merck). The 1 mg ml^{-1} stock solutions of gold and mercury were obtained from BDH Chemicals. Nitric acid was added to the dilute gold and mercury standard solutions to obtain the same concentration as in the 0.1% (w/v) cobalt solution, i.e., ca. 1.0% (w/v). The potassium dichromate was of reagent-grade quality (Merck).

Measurements

The effect of cobalt, manganese and nickel on the 30 elements and 53 element lines listed in Table 1 was studied with the model 5000/Zeeman

TABLE 1

Elements tested for background overcompensation effect with 0.1% solutions of cobalt, manganese and nickel

(The furnace parameters are given in Table 2; except where mentioned, hollow-cathode lamps were used.)

Element	Wave-length (nm)	Slit width (nm)	Element	Wave-length (nm)	Slit width (nm)	Element	Wave-length (nm)	Slit width (nm)
Ag	328.1	0.7	Fe	248.3	0.2	Pb	283.3	0.7
	338.3	0.7		248.8	0.2		217.0	0.7
Al	309.3	0.7		271.9	0.2	Pt	265.9	0.7
	237.3	0.2	Ga	287.4	0.7	Sb ^a	217.6	0.2
As ^a	193.7	0.7		294.4	0.7		206.8	0.2
	189.0	0.7	Ge ^a	265.1	0.2		231.2	0.2
	197.2	0.7		259.2	0.2	Se ^a	196.0	2.0
Au	242.8	0.7		271.0	0.2		204.0	0.7
	267.6	0.7	Hg	253.7	0.7	Si	251.6	0.2
B	249.7	0.7	In	303.9	0.7	Sn ^a	286.3	0.7
Bi	222.8	0.2		325.6	0.2		224.6	0.2
	223.1	0.2	Mg	285.2	0.7		235.5	0.7
	306.8	0.7	Mn	279.5	0.2	Te ^a	214.3	0.2
Cd ^a	228.8	0.7		279.8	0.2	Ti	365.3	0.2
Co	240.7	0.2	Mo	313.3	0.7	Tl ^a	276.8	0.7
Cr	357.9	0.7	Ni	232.0	0.2	V	318.4	0.7
Cu	324.8	0.7	P ^a	213.6	0.2	Zn	213.9	0.7
	327.4	0.7		214.9	0.2			

^aElectrodeless discharge lamp used.

instrument. Once the appropriate element lamp had been installed, the power and slit width adjusted, and the wavelength selected, 5 μ l of the 0.1% metal solution was pipetted into the graphite tube and the element was atomized. The furnace parameters are given in Table 2.

At three of the wavelengths tested, the resonance lines of boron, gold and mercury, negative signals appeared when cobalt was atomized. The negative signal at the boron line was very small and this interference was not further investigated. Gold and mercury were then atomized in the absence and presence of cobalt; the furnace programs for these measurements are given in Table 3.

RESULTS AND DISCUSSION

Among the 30 elements and the 53 element lines studied, three cases of spectral overcompensation were discovered, all originating from cobalt. No overcompensation effects were detected from manganese and nickel.

Cobalt gave a considerable negative signal at the 267.595-nm gold line and at the 253.652-nm mercury line; both interferences are explained by the presence of adjacent cobalt lines, the 267.598-nm and 253.649-nm lines. Although the two interfering cobalt lines are both positioned at the same distance (0.003 nm) from the two analyte lines, only mercury exhibited a distinct positive spectral interference from the overlapping 253.649-nm cobalt line when measurements were made with the deuterium-arc corrector; only to a small extent did the 267.598-nm cobalt line overlap with the 267.595-nm gold line. The explanation of the different behaviour of the two sets of adjacent lines is probably that the many isotopes of natural mercury produce a relatively broad emission line around the peak at 253.652 nm. The interferences on gold and mercury will be discussed below in more detail.

Cobalt gave a small negative absorbance signal at the 249.678-nm boron line, which is 0.007 nm from the 249.671-nm cobalt line. As this interference was found to be very small and as boron is not frequently determined in the graphite furnace, further studies of this system were not conducted.

TABLE 2

Furnace parameters for studying the effects of cobalt, manganese or nickel on the elements listed in Table 1. The Zeeman-effect background corrector was used. Keyboard entries are shown (pyrolytically-coated graphite tube; 5- μ l samples)

Step	1	2	3	4 ^a
Temp. (°C)	120	600	2200	2600
Ramp (s)	10	10	0	1
Hold (s)	20	20	5	4
Read (s)		29	— ^b	
Int. flow (ml min ⁻¹)			gas stop	

^aClean step. ^bRead step.

TABLE 3

Furnace and other parameters for determining gold and mercury. Two furnace programs were used, one with maximum power heating and the other with ramp time in the atomization step. The Zeeman-effect or the deuterium-arc background corrector was used

Step	Program (a)				Program (b)			
	1	2	3	4 ^b	1	2	3	4 ^b
<i>Parameters for gold^a</i>								
Temp. (°C)	120	600	1600	2600	120	600	2100	2600
Ramp (s)	10	10	0	1	10	10	1	1
Hold (s)	20	20	5	4	20	20	4	4
Read (s)		29	— ^c			29	— ^c	
Int. flow (ml min ⁻¹)			gas stop				gas stop	
<i>Parameters for mercury^d</i>								
Temp. (°C)	120	1000	2600 ^b		120	2000	2600 ^b	
Ramp (s)	10	0	1		10	1	1	
Hold (s)	20	5	4		20	4	4	
Read (s)	29	— ^c			29	— ^c		
Int. flow (ml min ⁻¹)		gas stop				gas stop		

^aWavelength, 267.6 nm or 242.8 nm; radiation source, gold hollow-cathode lamp; lamp current, 15 mA; graphite tube, pyrolytically-coated; sample volume, 5 μ l. ^bClean step. ^cRead step. ^dWavelength, 253.7 nm; radiation source, mercury hollow-cathode lamp; lamp current, 10 mA; graphite tube, pyrolytically-coated; sample volume, 5 μ l. Step 4 was not required for mercury, for which step 3 was the clean step.

Gold

The gold lines most frequently used in a.a.s. are the resonance lines at 242.8 nm and 267.6 nm. The latter line is less sensitive, but because of its higher intensity [5] it is often preferred for a.a.s. purposes. Figure 1 shows the absorption signals obtained for gold and cobalt atomized separately and together and measured at the wavelengths of the two gold lines. As is apparent from Fig. 1, the overcompensation effect was only observed at the 267.6-nm gold line; consequently, when measurements are made with inverse Zeeman-corrected instruments and in matrices containing 100 μ g ml⁻¹ (5 μ l sample volume) cobalt or more, it is recommended that the measurements be done at 242.8 nm. Figure 2 (curve b) gives peak-area signals for cobalt as measured at the 267.6-nm gold line and with Zeeman background correction.

As is apparent from Fig. 1B, gold is more easily evaporated than cobalt. When the two metals are atomized separately, gold is practically completely atomized at the appearance time of cobalt. It was considered of interest to examine the possibility of determining both elements in the same liquid or solid sample by stepwise atomization. Unfortunately, when the metals were together, the gold peak appeared later and the two signals overlapped in time. Different settings of temperature and time were tested, as well as standard

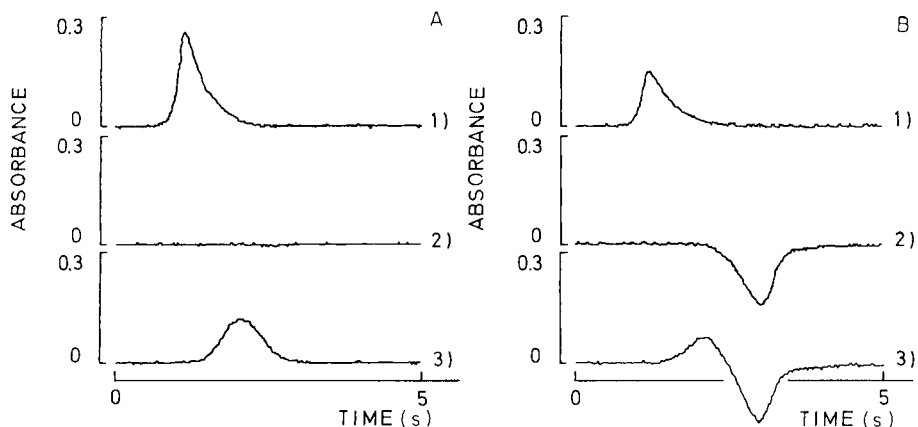


Fig. 1. Absorbance profiles showing the influence of cobalt at the wavelengths of two gold resonance lines, with Zeeman background correction: (A) the 242.8-nm line; (B) the 267.6-nm line. Profiles: (1) $0.05 \mu\text{g ml}^{-1}$ Au; (2) 0.1% (w/v) Co; (3) $0.05 \mu\text{g ml}^{-1}$ Au in 0.1% Co. For experimental conditions, see Table 3, program b.

and pyrolytically-coated graphite tubes; however, the two signals could not be separated.

Cobalt was found also to affect the sensitivity of gold. With the furnace parameters listed in Table 3, the sensitivity was reduced in pyrolytically-coated tubes and increased in standard graphite tubes.

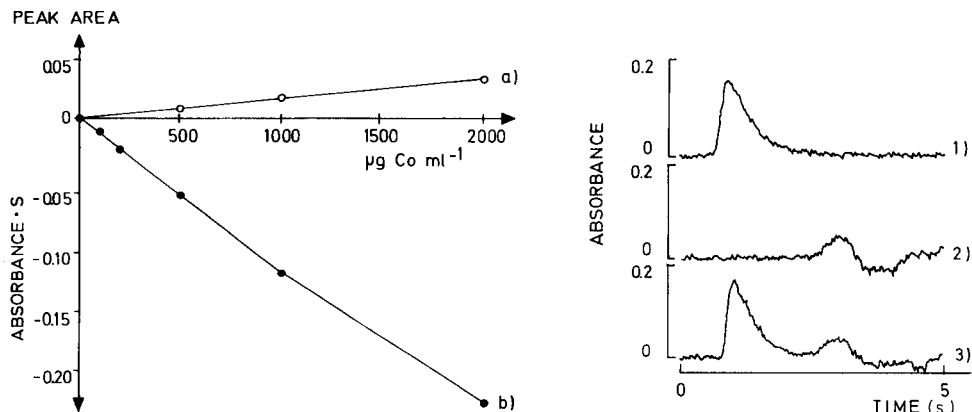


Fig. 2. Integrated absorbances for cobalt at the 267.6-nm gold line: (a) with the deuterium-arc background corrector; (b) with the Zeeman background corrector. For experimental conditions, see Table 3, program b, except the atomization temperature, 2600°C , used with the deuterium-arc corrector.

Fig. 3. Absorbance profiles showing the influence of cobalt on mercury at the 253.7-nm mercury line with the Zeeman background corrector. Profiles: (1) $2 \mu\text{g ml}^{-1}$ Hg; (2) 0.1% (w/v) Co; (3) $2 \mu\text{g ml}^{-1}$ Hg in 0.1% Co. All solutions contained 0.05% (w/v) potassium dichromate as matrix modifier; for experimental conditions, see Table 3, program b.

With a deuterium-arc background-correction instrument and the furnace parameters of Table 3, 5 μ l of 0.1% cobalt solution did not exhibit any spectral interference when measured at the 267.6-nm gold line. However, when cobalt was atomized at higher temperatures (2600°C), a small positive signal appeared (Fig. 2, curve a), which indicated that the cobalt line is overlapping only at the far end wing of the gold emission peak, while the σ -component of the cobalt line is overlapping closer to the maximum of the gold emission peak. When the deuterium arc is used as the background corrector, the presence of cobalt causes overcompensation also when gold is measured at the 242.8-nm line with slit width of 0.7 nm [6].

Mercury

Mercury is not normally determined in the graphite furnace, the main reason being the high volatility of the element and many of its compounds. However, the volatility of the element can be reduced by the addition of a matrix modifier. Potassium dichromate in nitric acid has been recommended [7] as the modifier, the reagent permitting heating up to 250°C without any loss of mercury. Direct determination of mercury in solid samples has been described [8]; the measurements were made with a direct Zeeman a.a.s. instrument.

The present work was done with the two furnace programs given in Table 3 for mercury. The best sensitivity was obtained with pyrolytically-coated tubes and these were used throughout. Figure 3 shows the absorption profiles for mercury and cobalt atomized separately and together with potassium dichromate as the matrix modifier. With an integration time of 2 s, the peak area of the mercury signal was recorded without any contribution from cobalt. The furnace parameters of Table 3, program a, also ensured the complete atomization of mercury before cobalt started to evaporate. In Table 4, a heating program is given for consecutive determinations of the two metals in the same sample portion.

Figure 4 reproduces a series of absorbance signals obtained for increasing concentrations of cobalt; the measurements were made at the 253.7-nm

TABLE 4

A furnace program with two atomization steps (step 2 and 4) applicable for the determination of mercury and cobalt in the same sample portion with the deuterium-arc background corrector^a

Step	1	2	3	4
Temp. (°C)	120	1000	800	2600
Ramp (s)	10	0	5	1
Hold (s)	20	5	5	4
Read (s)	29	— ^b	9	— ^b

^aWavelength, 253.7 nm; radiation source, mercury hollow-cathode lamp; lamp current, 10 mA; graphite tube, pyrolytically-coated; sample volume, 5 μ l. ^bRead step.

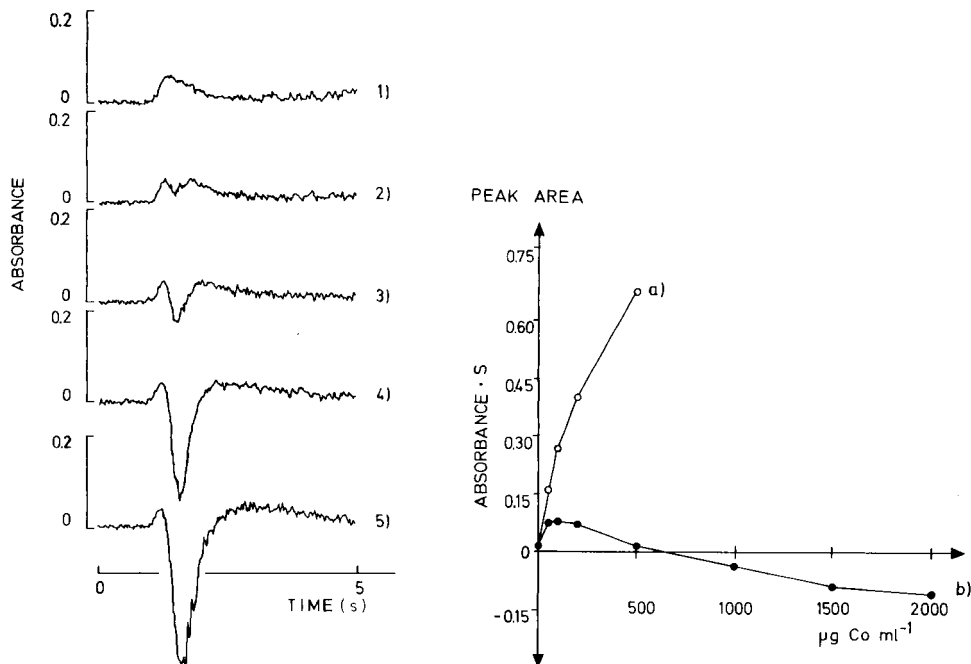


Fig. 4. Absorbance profiles of different cobalt concentrations as measured at the 253.7-nm mercury line with Zeeman background correction. Cobalt concentrations ($\mu\text{g ml}^{-1}$): (1) 50; (2) 100; (3) 200; (4) 500; (5) 1000. For experimental conditions see Table 3, program b (except the atomization temperature, 2600°C).

Fig. 5. Integrated absorbances for cobalt at the 253.7-nm mercury line: (a) with the deuterium-arc background corrector; (b) with the Zeeman background corrector. For experimental conditions, see Table 4 (second atomization step, step 4). Gas stop was used during the atomization step.

mercury line. As is seen from Fig. 4, the lowest cobalt concentration gives a positive signal; at increasing concentrations a small constant cobalt signal remains, but this is succeeded by increasing negative signals. At higher concentrations the absorption line profile is changed (probably broadened) thus causing an increased σ -overlap compared to the direct overlap.

The spectral interference of cobalt on mercury has been recognized for a long time [9]. A 1 mg ml^{-1} cobalt solution gives about 10% absorption when measured in the air/acetylene flame at the 253.7-nm mercury line. In flame a.a.s. the interference is more serious because the mercury and cobalt signals cannot be separated by changing the atomization temperature. Figure 5 shows the peak areas obtained for cobalt at the 253.7-nm mercury line. The measurements were made with deuterium arc (curve a) and Zeeman background-correction (curve b). When mercury and cobalt are to be determined consecutively in the same sample portion, the analysis should preferably be done with a deuterium-arc background-correction system.

Conclusions

The present cases of spectral interference may occur with both direct (source-shifted) and inverse (analyte-shifted) Zeeman background-correction systems. Thus the interference of iron on zinc and gallium encountered with an inverse Zeeman instrument [4], was confirmed by other workers [10] operating direct Zeeman instruments.

Cobalt has been found [3] to interfere in the determination of mercury by flame a.a.s.; a direct Zeeman instrument was used in these measurements, the effect of cobalt depending on the strength of the magnetic field. The present type of interference can be expected to occur when foreign emission/absorption lines are less than 0.01 nm from the analyte line. These interferences may be avoided by studying tables of atom lines [11] before the determinations are made.

REFERENCES

- 1 U. Kurfürst, *Fresenius Z. Anal. Chem.*, 315 (1983) 304.
- 2 H. Massmann, *Talanta*, 29 (1982) 1051.
- 3 R. Stephens and G. F. Murphy, *Talanta*, 25 (1978) 441.
- 4 G. Wibetoe and F. J. Langmyhr, *Anal. Chim. Acta*, 165 (1984) 87.
- 5 See, e.g., B. Welz, *Atomic Absorption Spectroscopy*, Verlag Chemie, Weinheim, 1976.
- 6 F. J. Fernandez and R. Giddings, *At. Spectrosc.*, 3 (1982) 61.
- 7 F. G. Kirkbright, S. Hsiao-Chuan and R. D. Snook, *At. Spectrosc.*, 1 (1980) 85.
- 8 U. Kurfürst and B. Rues, *Fresenius Z. Anal. Chem.*, 308 (1981) 1.
- 9 D. C. Manning and F. Fernandez, *At. Absorpt. Newsl.*, 7 (1968) 24.
- 10 B. Radziuk, Bodenseewerk, Perkin-Elmer & Co, GmbH, B.R.D., and U. Kurfürst, Grün-Optik Wetzlar, GmbH, B.R.D., personal communications.
- 11 See, e.g., P. W. J. M. Boumans, *Line Coincidence Tables for Inductively-Coupled Plasma Atomic Emission Spectroscopy*, Pergamon Press, Oxford, 1980.

SYNTHESIS OF BIDENTATE PYRIDYLAZO AND THIAZOLYLAZO REAGENTS AND THE SPECTROPHOTOMETRIC DETERMINATION OF COPPER IN A FLOW-INJECTION SYSTEM

K. OHSHITA

Laboratory of Chemistry, Daido Institute of Technology, Minami-ku, Nagoya (Japan)

H. WADA* and G. NAKAGAWA

Laboratory of Analytical Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya (Japan)

(Received 12th April 1985)

SUMMARY

Some pyridylazo and thiazolylazo compounds were synthesized as spectrophotometric reagents for copper(II). The water-soluble bidentate ligand, 4-(3,5-dibromo-2-pyridylazo)-*N*-ethyl-*N*-(3-sulfopropyl)aniline (3,5-diBr-PAESA), provides the greatest sensitivity, forming a 1:2 Cu:L in the presence of sodium dodecylsulfate. The molar absorptivity of the complex is $1.24 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 638 nm. Copper(II) ($10\text{--}200 \mu\text{g l}^{-1}$) is easily and quickly (60 h^{-1}) determined in a flow-injection system. Application to the determination of copper(II) in serum is described.

Recently, 2-(3,5-dibromo-2-pyridylazo)-5-diethylaminophenol (3,5-diBr-PADAP) was applied to the spectrophotometric determination of silver(I) in the presence of an anionic surfactant [1]. Generally, silver(I) does not react sensitively with such heterocyclic azo-phenols. In the presence of an anionic surfactant, a stable complex (ML_2) between silver(I) and 3,5-diBr-PADAP was formed. It is doubtful that 3,5-diBr-PADAP behaves as a terdentate ligand in the formation of the silver(I) complex. Thus, in this study some bidentate ligands such as pyridylazobenzenes and thiazolylazobenzenes were synthesized, and the reactions of these reagents with various metal ions were examined. Silver(I) and copper(II) ions were found to react most sensitively with 4-(3,5-dibromo-2-pyridylazo)-*N,N*-diethylaniline (3,5-diBr-PAEA) and 4-(3,5-dibromo-2-pyridylazo)-*N*-ethyl-*N*-(3-sulfopropyl)aniline (3,5-diBr-PAESA) in the presence of an anionic surfactant. The molar absorptivities of silver(I) chelates and the copper(II) chelates are larger than the corresponding values for the chelates formed with 3,5-diBr-PADAP.

This paper deals with the flow-injection determination of copper(II) with water-soluble 3,5-diBr-PAESA and an application to the determination of copper(II) in serum.

EXPERIMENTAL

Synthesis of reagents

Reagents 4, 5, 7 and 8 in Table 1 were synthesized in this study.

Preparation of 3,5-diBr-PAEA (4). 2-Amino-3,5-dibromopyridine was diazotized as described previously [5]. The diazonium compound was added to *N,N*-diethylaniline in methanol with stirring, and the mixture was stirred overnight at room temperature. Carbon dioxide was bubbled through for 3 h, and then the mixture was left for 5 days with occasional stirring. The precipitate was filtered off and recrystallized from ethanol. [Yield, 10%. Calcd. for $C_{15}H_{16}N_4Br_2$, 43.7% C, 3.9% H, 13.6% N; found 42.85% C, 3.7% H, 13.4% N.]

Preparation of 3,5-diBr-PAESA (5). This was synthesized from 2-amino-3,5-dibromopyridine and *N*-ethyl-*N*-(3-sulfopropyl)aniline by a method similar to that for 3,5-diBr-PAEA. The reagent was purified as follows. The precipitate was dissolved in a little 0.1 M acetic acid solution, and then filtered. A large amount of ethanol was added to the filtrate. The solution was kept in a refrigerator until a precipitate was obtained (about 3 days). [Yield, 10%. Calcd. for $C_{16}H_{17}N_4SO_3Br_2Na \cdot H_2O$, 35.2% C, 3.5% H, 10.25% N; found 35.2% C, 3.4% H, 9.9% N.]

Preparation of TAEA (7). 2-Aminothiazole was diazotized with sodium nitrite solution in the usual way. The diazonium compound obtained was coupled with *N,N*-diethylaniline in 2 M hydrochloric acid at 0°C. The solution was allowed to stand for 1 h. Addition of sodium acetate precipitated the crude dye which was recrystallized from ethanol. [Yield, 80%. Calcd. for $C_{13}H_{16}N_4S$, 59.95% C, 6.2% H, 21.5% N; found 59.75% C, 6.15% H, 21.3% N.]

Preparation of TAESA (8). The diazotized 2-aminothiazole was coupled with *N*-ethyl-*N*-(3-sulfopropyl)aniline in aqueous solution at 0°C. After 2 h, the crude dye was filtered off and dissolved in water. The solution was filtered, and by acidification with sulfuric acid, the pure TAESA was obtained. [Yield, 80%. Calcd. for $C_{14}H_{18}N_4S_2O_3 \cdot H_2O$, 45.15% C, 5.4% H, 15.0% N; found 44.9% C, 5.3% H, 15.1% N.]

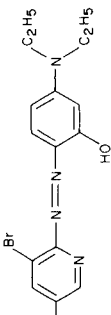
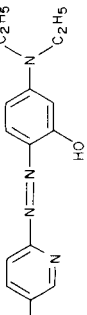
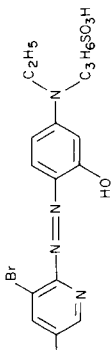
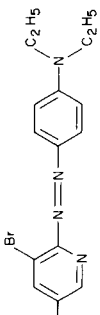
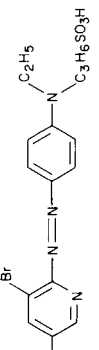
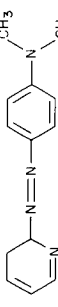
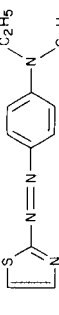
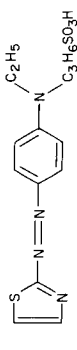
The coupling component, *N*-ethyl-*N*-(3-sulfopropyl)aniline was synthesized by heating a mixture of propanesultone and *N*-ethylaniline, and recrystallized from water. [Calcd. for $C_{11}H_{17}NSO_3 \cdot H_2O$, 50.55% C, 7.3% H, 5.4% N; found 50.4% C, 7.45% H, 5.3% N.]

Reagents

The 3,5-diBr-PAESA and 3,5-diBr-PAEA were dissolved in water and ethanol, respectively. The surfactant solutions were prepared by dissolving the appropriate sodium salts in water. Sodium decylsulfate (SDeS) and sodium tetradecylsulfate (STDS) were synthesized from sulfuric acid and decanol or tetradecanol (myristyl alcohol), and recrystallized from ethanol several times. Other surfactants were used as received.

TABLE 1

Reaction characteristics of silver(I) and copper(II) with some pyridylazo and thiazolylazo compounds (pH 4-6)

Reagent	$\lambda_{\max}$ (nm) (in H ₂ O or 20% EtOH)	$\lambda_{\max}$ (nm) (in 0.1% SDS)	ϵ (l mol ⁻¹ cm ⁻¹)	Ref.
(1) 	$\left\{ \begin{array}{l} \text{HR} \ 454 \\ \text{AgR} \ 458 \end{array} \right\}$ CuR 568	$\left\{ \begin{array}{l} 460 \\ 527, 557 \end{array} \right\}$ AgR ₂ 570 CuR 578	$\left\{ \begin{array}{l} 77000 \\ 81000 \end{array} \right\}$	2
(2) 	$\left\{ \begin{array}{l} \text{HR} \ 446 \\ \text{AgR} \ 448 \end{array} \right\}$ CuR 558	$\left\{ \begin{array}{l} 446 \\ 525 \\ 550 \\ 568 \end{array} \right\}$ AgR ₂ 550 CuR 568	$\left\{ \begin{array}{l} 60000 \\ 80000 \end{array} \right\}$	2
(3) 	$\left\{ \begin{array}{l} \text{HR} \ 454 \\ \text{AgR} \ 454 \\ \text{CuR} \ 560 \end{array} \right\}$	$\left\{ \begin{array}{l} 455 \\ \text{AgR}_2 \ 523, 557 \\ \text{CuR} \ 570 \end{array} \right\}$	$\left\{ \begin{array}{l} 60000 \\ 73000 \end{array} \right\}$	3
(4) 	$\left\{ \begin{array}{l} \text{R} \ 509 \\ \text{AgR} \ 541 \end{array} \right\}$ CuR 632	$\left\{ \begin{array}{l} 506 \\ 560 \end{array} \right\}$ AgR ₂ 600 CuR ₂ 638	$\left\{ \begin{array}{l} 80000 \\ 110000 \end{array} \right\}$	This work
(5) 	$\left\{ \begin{array}{l} \text{R} \ 504 \\ \text{AgR} \ 490-550 \end{array} \right\}$ CuR 632	$\left\{ \begin{array}{l} 504 \\ 550 \end{array} \right\}$ AgR ₂ 590 CuR ₂ 638	$\left\{ \begin{array}{l} 80000 \\ 124000 \end{array} \right\}$	This work
(6) 	$\left\{ \begin{array}{l} \text{R} \ 468 \\ \text{AgR} \ 502 \end{array} \right\}$	$\left\{ \begin{array}{l} 465 \\ 502 \end{array} \right\}$		4
(7) 	$\left\{ \begin{array}{l} \text{R} \ 519 \\ \text{AgR} \ 523 \end{array} \right\}$	$\left\{ \begin{array}{l} 511 \\ 528 \end{array} \right\}$		This work
(8) 	$\left\{ \begin{array}{l} \text{R} \ 515 \\ \text{AgR} \ 508 \end{array} \right\}$	$\left\{ \begin{array}{l} 511 \\ 526 \end{array} \right\}$		This work

The standard solution of copper(II) was prepared by dissolving copper (99.99%) in dilute nitric acid. Other metal ion solutions were prepared by dissolving the metals in nitric acid or the appropriate nitrates, sulfates or chlorides in dilute nitric acid.

Acetic acid/sodium acetate buffers (2 M, pH 3–7) were prepared.

All reagents used were of analytical grade. The water was redistilled from a hard-glass vessel.

Apparatus

A Union Giken Model SM-401 spectrophotometer and a Hitachi-Horiba Model F-7 pH meter were used.

A diagram of the flow-injection manifold is shown in Fig. 1. The teflon tubing used was of 0.5 mm i.d. except for the back-pressure coil which was of 0.25 mm i.d. (50 cm long). The peristaltic pump was a Gilson HP-4 model. Samples were injected via a rotary valve (Oyo-Bunko Kiki) to which a loop of appropriate volume was attached. The mixing coil and the reagent solutions were kept in a thermostat at 20°C. The flow cell (Oyo-Bunko Kiki; 10-mm light path, 20- μ l volume) was situated in a spectrophotometer (Oyo-Bunko Kiki; Type UVILOG 7), and a recorder (Rika Denki, Type R-31) was used for recording the absorbance.

Recommended procedure for the determination of copper

The manifold used is shown in Fig. 1. The buffer solution (0.1 M acetic acid/sodium acetate buffer, pH 4.5) and the 4×10^{-5} M 3,5-diBr-PAESA solution in the same acetate buffer but with 0.4% (w/v) SDS added, were each delivered at 1.0 ml min⁻¹. The samples (60 μ l) were injected into the buffer stream. Sample and reagents were mixed in the 200-cm coil, and the absorbance measured at 638 nm was recorded.

RESULTS AND DISCUSSION

Reactions with metal ions

The terdentate reagents 1–3 react with various metal ions whether surfactant is present or not. Copper(II) forms stable complexes with these ligands

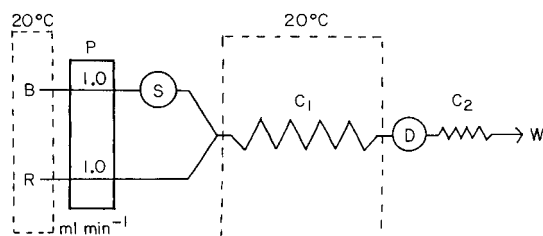


Fig. 1. Flow diagram: P, peristaltic pump; S, sample injector (60 μ l); C₁, mixing coil (200 cm, 0.5 mm i.d.); C₂, back-pressure coil (50 cm, 0.25 mm i.d.); D, spectrophotometer (638 nm); B, acetate buffer (0.1 M, pH 4.5); R, 3,5-diBr-PAESA solution (4×10^{-5} M) in acetate buffer (0.1 M, pH 4.5) containing 0.4% SDS.

in aqueous solution, and addition of SDS produces little change in the absorption spectra. In contrast, the bidentate reagents 3,5-diBr-PAEA and 3,5-diBr-PAESA (4 and 5) react with only a few metal ions, e.g., copper(II) and palladium(II) in aqueous solution, and the presence of SDS greatly enhances the molar absorptivities of their copper(II) chelates. Silver(I) reacts with reagents 1–5 in aqueous solution. The molar absorptivities are small but are greatly improved in 0.1% SDS solution. Reagents 6–8 did not behave like 3,5-diBr-PAESA or 3,5-diBr-PAEA.

The absorption spectra of 3,5-diBr-PAESA and of the copper(II) chelate in SDS solution and in aqueous solution are shown in Fig. 2. Other reagents not described in Table 1, such as 1-(2-pyridylazo)-2-naphthol and its bromopyridyl and sulfonaphthol derivatives, or 2-(2-thiazolylazo)-5-dimethylaminophenol and its analogs [2], did not show appreciable changes in the color reaction with silver(I) or copper(II) in SDS solution compared with aqueous solution. The monodentate ligand, 4-(4-pyridylazo)diethylaniline, reacts with neither silver(I) nor copper(II). As 3,5-diBr-PAESA is the most sensitive for copper(II) and the chelate is water-soluble, its application in the determination of copper was studied.

Acidity constants of 3,5-diBr-PAEA and 3,5-diBr-PAESA

The dissociation equilibria of the bidentate reagents 4 and 5 can be written as follows, the latter reagent being taken as the example:

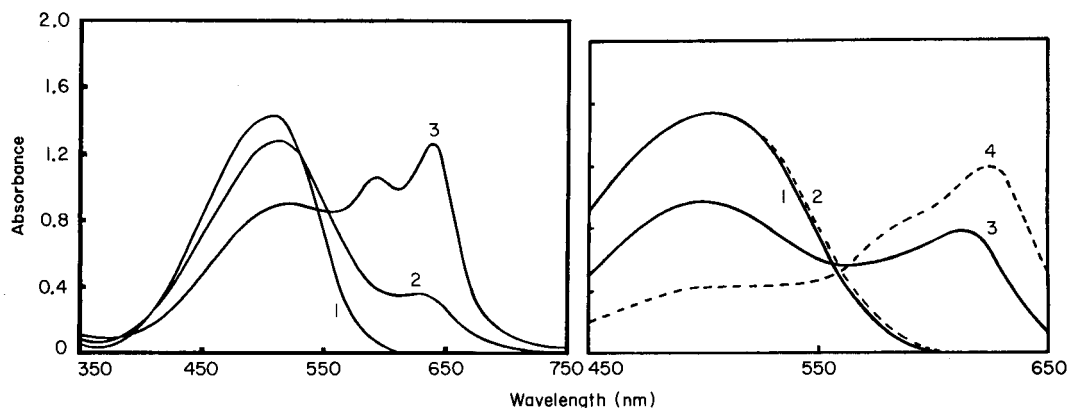
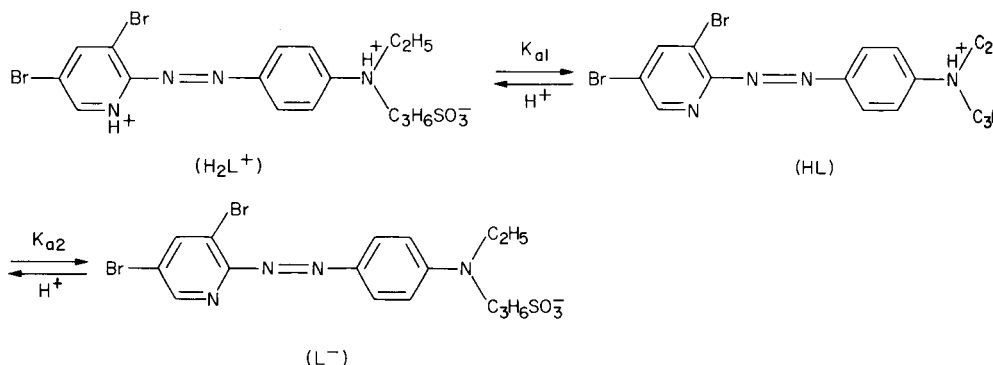


Fig. 2. Absorption spectra: (1) 3,5-diBr-PAESA (4×10^{-5} M) in aqueous or 0.1% SDS solution; (2) Cu(II) chelate (10^{-5} M) in aqueous solution; (3) Cu(II) chelate in 0.1% SDS solution. All at pH 5.0 and 20°C.

Fig. 3. Absorption spectra of 3,5-diBr-PAESA (4×10^{-5} M): (—) in aqueous solution; (---) in 0.1% SDS solution. Curves: (1, 2) pH 5 corresponding to L⁻; (3, 4) pH 1 corresponding to HL.



The two successive acidity constants are then defined by $K_{a1} = a_{H^+}[HL]/[H_2L^+]$ and $K_{a2} = a_{H^+}[L^-]/[HL]$. The absorption spectra of both reagents were almost the same. The absorption spectra of 3,5-diBr-PAESA in aqueous and 0.1% SDS solutions are shown in Fig. 3; as can be seen, the spectra are little affected by the surfactants. Being less than 1, the K_{a1} values of the reagents were not determined. The K_{a2} values were determined by Hildebrand and Reilley's method [6]. The results are given in Table 2.

Composition of the copper(II) chelates

The composition of the copper(II)/3,5-diBr-PAESA chelate in 0.1% SDS solution was shown to be 1:2 by the mole ratio method. With terdentate reagents 1–3, a 1:1 chelate was formed in both the aqueous and 0.1% SDS solutions. This is the reason for the much higher molar absorptivity of the copper(II)/3,5-diBr-PAESA chelate compared with those from reagents 1–3. It is possible that the bidentate 3,5-diBr-PAESA is locally concentrated in a SDS micelle so that it easily forms a ML_2 chelate with copper(II).

Optimization of experimental conditions

Optimum reaction conditions were examined by the batch method. The surfactants examined were SDS, SDeS, STDS, sodium dodecylbenzenesulfonate (SDBS), Triton X-100 and quaternary ammonium salts. In the presence of an alkylsulfate, the absorbance increased greatly. The effect of the

TABLE 2

Acidity constants of the reagents (pK_{a2}) in different media

Reagent	pK_{a2} values		0.1% SDS
	Water	Water/ethanol	
3,5-DiBr-PAEA	—	2.75 ^a	3.90
	—	2.15 ^b	
3,5-DiBr-PAESA	2.45	—	2.10 ^c

^a 20% ethanol. ^b 40% ethanol. ^c The value is minimum around 0.1% SDS.

SDS concentration on the absorbance at 638 nm is shown in Fig. 4. For SDS, the critical micelle concentration (c.m.c.) in 0.1 M sodium acetate is 1.8×10^{-3} M. Over the range $3\text{--}6 \times 10^{-3}$ M SDS, the absorbance was constant but decreased at higher concentrations. In the case of SDeS, no concentration region with constant absorbance could be found, and the absorbances were generally lower than those in SDS solution. In SDBS solution, the absorbance was only slightly lower than that in SDS solution. Triton X-100 had no effect on the absorbance. Quaternary ammonium salts such as tetradecyldimethylbenzylammonium chloride (zephiramine) decomposed copper(II)/3,5-diBr-PAESA chelate. Consequently, SDS was used in the flow-injection system for copper.

The absorbances of copper(II)/3,5-diBr-PAESA chelate at different pH values in the presence of SDS were measured at 638 nm. As shown in Fig. 5, the absorbances were constant and maximum over the pH range 3.5–4.5 at 25°C. The pH range in which the absorbances were constant expanded with decreasing temperature. Temperature also affected the absorbance of the copper(II) chelate. The absorbance increased with decrease in temperature, and the absorbance at 18°C was higher than that at 25°C by 8%. At 18°C, the absorbance was constant at pH 3.5–5.5. The flow-injection system was run at pH 4.5 and 20°C, which was near room temperature.

The absorbance was also influenced by the concentration of salts such as sodium chloride, decreasing with increasing salt concentration. If sodium chloride (≤ 0.3 M) was added to both the buffer and the reagent solutions

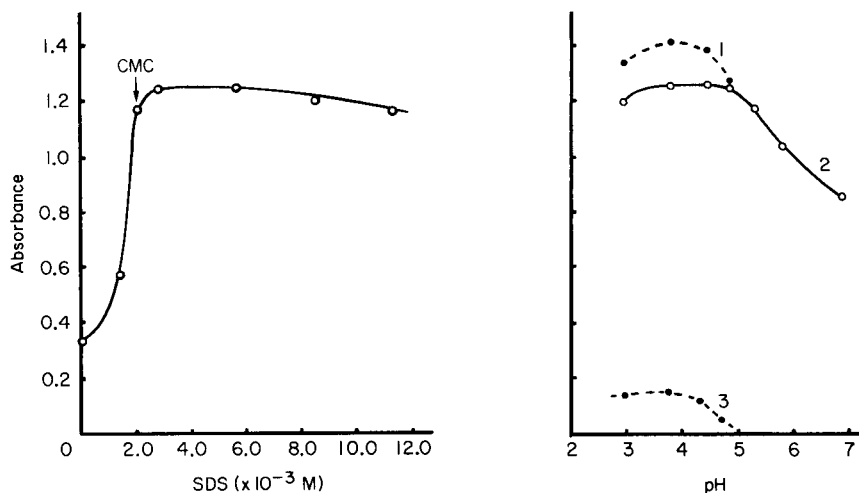


Fig. 4. Effect of SDS concentration on the absorbance of the copper/3,5-diBr-PAESA chelate. Conditions: 10^{-5} M Cu^{2+} , 4×10^{-5} M 3,5-diBr-PAESA, pH 5.0, 18°C.

Fig. 5. Effect of pH on the absorbance of the copper(II)/3,5-diBr-PAESA chelate. Conditions: 10^{-5} M Cu^{2+} , 8×10^{-5} M 3,5-diBr-PAESA (L), 0.16% SDS, 25°C. Curves: (1) CuL_2 against water; (2) CuL_2 against reagent blank; (3) blank.

in the flow-injection system, the same concentration of the salt in the sample did not influence the results, but SDS precipitated at higher salt concentrations.

Application in flow-injection analysis

For flow-injection analysis, the optimum conditions were examined. On increasing the concentration of SDS in the reagent solution from 0.3% to 0.8%, the peak heights decreased by 10%. For good reproducibility, a 0.4% SDS solution was used. When the concentration of 3,5-diBr-PAESA was increased from 10^{-5} M to 6×10^{-5} M, the peak heights became constant for concentrations exceeding 4×10^{-5} M.

The effect of mixing coil length on the peak height was examined for the range of 1–4 m. The peak height decreased with increasing coil length, but the reproducibility was poor with a 1-m coil. A 2-m coil is recommended. The same flow rates were used for both the buffer and the reagent channels. When the flow rate for each solution was varied over the range 0.5–1.1 ml min⁻¹, the peak heights increased with increasing flow rate and became constant over 0.8 ml min⁻¹. A flow rate of 1.0 ml min⁻¹ was used. For the apparatus employed, 60 μ l was the most suitable sample volume. As described above, the mixing coil and reagent solutions were thermostated at 20°C.

The salt concentration in the standard copper solution was adjusted to be the same as that in the buffer and reagent solutions (0.1 M acetic acid/sodium acetate, pH 4.5) for the analysis of serum.

A calibration output obtained under the recommended conditions is shown in Fig. 6. The calibration graph was linear for 20–200 μ g l⁻¹ copper.

Interferences. With 3,5-diBr-PAESA, several metal ions such as Pd(II), Ag(I), Hg(II), Ni(II), Co(II) and Co(III) produced colour reactions in the presence of SDS. Suitable masking agents to eliminate the interferences of these metal ions could not be found, but these ions are scarcely present in serum. The tolerance limits for various metal ions are listed in Table 3.

Determination of copper in serum. To the glass bottle containing freeze-dried normal control serum (S₁), 3 ml of 1 M hydrochloric acid was added and the bottle was heated in a water-bath; then 2 ml of 20% (w/v) trichloroacetic acid containing potassium periodate was added and the solution was filtered. A 1.0-ml aliquot of the filtrate was pipetted into a 10-ml volumetric flask, the pH was adjusted to about 4 by adding 1 M sodium acetate, and the solution was diluted to the mark with water. A 60- μ l aliquot of this solution was injected into the flow-injection system. Control serum I (S₂) was treated in the same manner.

The results (94 mg l⁻¹ for S₁, 112 mg l⁻¹ for S₂) shown in Fig. 6 were in good agreement with the certified values (93 ± 10 mg l⁻¹ for S₁, 124 ± 19 mg l⁻¹ for S₂). A sample throughput of 60 h⁻¹ was possible.

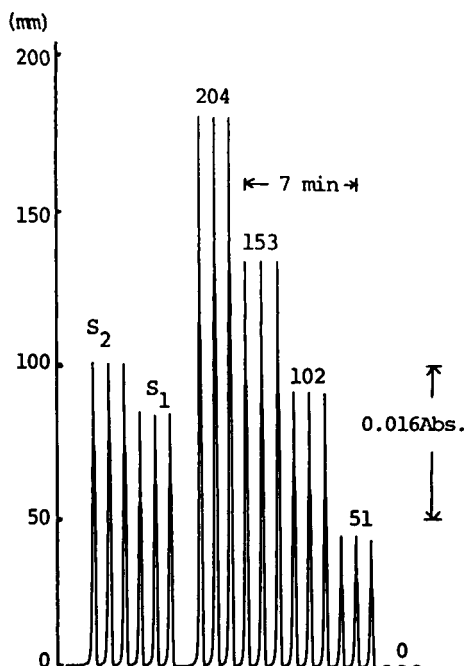


Fig. 6. Calibration graph for 0–204 $\mu\text{g l}^{-1}$ copper and the determination of copper in serum. All standards and samples are injected three times each. (S_1) Normal control serum; (S_2) control serum I (treated by the procedure in the text).

TABLE 3

Effect of diverse ions on the determination of copper

Ion	Tolerance limit ^a (μg) (Batch method)	Tolerance limit ^b ($\mu\text{g l}^{-1}$) (F.i.a.)
Fe(II)	20	300
Fe(III)	150	1500
Ni(II)	2	15
Co(II), Co(III)	7	50
Zn(II)	150	650
Hg(II)	5	40

^aWith 16 $\mu\text{g Cu}^{2+}$ in 25 ml. ^bWith ca. 200 $\mu\text{g l}^{-1}$ Cu^{2+} .

Conclusion

The novel reagent, 3,5-diBr-PAESA, provides excellent sensitivity for copper(II), because it forms a CuL_2 chelate in the presence of SDS. The absorbance of the copper(II) chelate is affected by the concentration of surfactant, pH, temperature and salt concentration, but these features are readily standardized in the flow-injection system for satisfactory routine work.

REFERENCES

- 1 Shui-Chieh Hung, Chan-Ling Qu and Shui-Sheng Wu, *Talanta*, 29 (1982) 85.
- 2 S. Shibata, *Chelates in Analytical Chemistry*, Vol. 3, M. Dekker, New York, 1972.
- 3 K. Ohshita, H. Wada and G. Nakagawa, *Anal. Chim. Acta*, 149 (1983) 269.
- 4 R. W. Faessinger and E. V. Brown, *J. Am. Chem. Soc.*, 73 (1951) 4606.
- 5 K. Ohshita, H. Wada and G. Nakagawa, *Anal. Chim. Acta*, 124 (1981) 193.
- 6 G. P. Hildebrand and C. N. Reilley, *Anal. Chem.*, 29 (1957) 258.

VARIATIONS OF THERMAL-LENS SPECTROPHOTOMETRY WITH HELIUM-CADMIUM LASER EXCITATION FOR THE DETERMINATION OF TRACES OF PHOSPHORUS

V. I. GRISHKO, I. G. YUDELEVICH* and V. P. GRISHKO

*Institute of Inorganic Chemistry, Siberian Branch of the U.S.S.R. Academy of Sciences,
630-090 Novosibirsk (U.S.S.R.)*

(Received 22nd January 1985)

SUMMARY

Single-beam, differential single-beam and dual-beam thermal-lens measurements are compared for the determination of phosphorus traces by means of a helium-cadmium laser. Phosphate is determined as the molybdophosphate/auramine ion-pair which is extracted into 1:2 isobutanol/hexane; acetone is added to dissolve any ion-pair precipitate. In dual-beam thermal lens experiments, a low-power He–Ne laser is used as the probe laser. The detection limit, restricted by variable blank measurement, is 0.6 ng cm^{-3} phosphorus in all cases. The advantages and drawbacks of each measurement technique are discussed. The dual-beam system of measurement is more sensitive than the single-beam system and is appropriate for measurements on samples of small volume. The dual-beam system is applied for layer-by-layer determination of phosphorus in semiconductor silicon over the concentration range 5×10^{-8} – $5 \times 10^{-4}\%$. The data obtained by the dual-beam method compare well with those obtained by a neutron activation method.

The great possibilities of many spectrophotometric methods widely used for the determination of low concentrations are often limited because of the poor sensitivity of routine instrumentation. The indirect measurement of a signal by monitoring the difference between the intensities of the initial radiation and the emerging radiation from an absorbing specimen is traditional but, forbids any significant increase in sensitivity simply by increasing the power of the light source. The so-called calorimetric methods avoid this shortcoming; among these methods, thermal-lens spectrophotometry is the most sensitive [1].

All modifications of the well known thermal-lens instrumentation, may be categorized as single-beam, differential single-beam, intracavity, intracavity based on correlated cavity systems, and dual-beam [2]. Intracavity measurements were described in detail monitoring traces of phosphorus and arsenic [3]. Comparative consideration of the single-beam, differential single-beam and dual-beam techniques applied to the determination of traces of phosphorus is the subject of this paper.

A helium-cadmium laser ($\lambda = 441.6 \text{ nm}$) was used for inducing the thermal lens in all the optical systems tested. The chemical procedure for phosphorus

determination was modified. Phosphorus was monitored as the ion-pair of molybdophosphate with auramine (C.I. Basic Yellow 2). The instrumental system for the single-beam thermal-lens measurements is shown in Fig. 1. The situation of the thermal lens in relation to the waist of the Gaussian laser beam is influenced considerably by changes in the laser beam parameters. A negative thermal lens situated before the waist of the laser beam (the left cell) produces a decrease in the divergence of the laser beam in the remote region of propagation; a cell situated after the waist (the right cell) increases the beam divergence. A change in the beam intensity at its center, I_{bc} , which is proportional to the absorbance of the specimen of interest, is detected at a long distance from the waist in the aperture position (Fig. 1).

The influence of the thermal lens on the beam divergence is greatest when the sample cell is placed at a distance $3^{1/2} z_c$ from the waist [4]. The confocal distance, z_c , can be calculated from $z_c = \pi w_0^2 / \lambda$, where λ is the wavelength of the laser and w_0 is the radius of the beam waist. The relative change in intensity at the centre of the laser beam is related to the absorbance A of the sample, placed at a distance $3^{1/2} z_c$ from the waist, by the simple expression $\Delta I_{bc} / I_{bc} = (I_0 - I_\infty) / I_0 = 0.524 EA$, where $E = -P(dn/dT) / \lambda k$, P is the laser power, (dn/dT) is the temperature gradient of the refractive index, k is the thermal conductivity of the solvent, and I_0 and I_∞ are the laser beam intensities before and after thermal lens generation in solution, respectively.

The sensitivity of single-beam measurements is limited by the high background absorbance of the matrix (e.g., solvent). Dovichi and Harris [5] faced this problem in determining copper. They proposed differential measurements [6] which do not have this drawback and allow a direct difference between the unknown sample absorbance and that of the reference to be measured. During differential measurements, the samples are placed at the different sides of the waist. Two thermal lenses, situated symmetrically with respect to the laser beam waist, cancel out the effects on the divergence of the laser beam.

To obtain the greatest effect of a thermal lens on beam divergence, the right cell (Fig. 1) is placed at a distance $(z_1 + z_2) = -3^{1/2} z_c$ after the waist

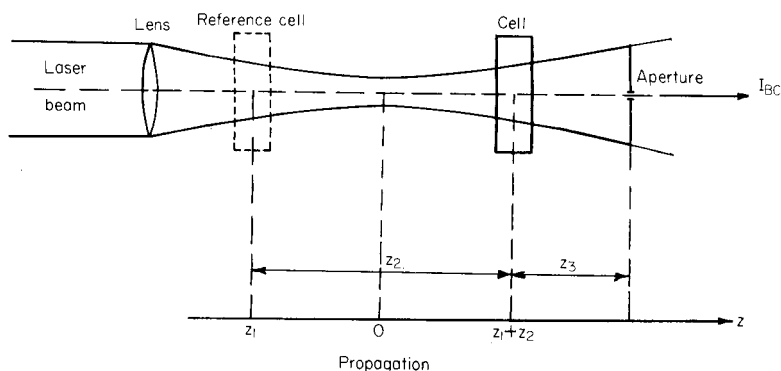


Fig. 1. Diagram of a single-beam thermal-lens spectrophotometer.

during differential measurements. If the same solvent is used for preparation of unknown and reference solutions, and if the laser output power does not change after the radiation has passed through the left cell, then $\Delta I_{bc}/I_{bc} = 0.524 E(A_2 - A_1)$, provided that the left cell is placed at the distance $z_1 = 3^{1/2} z_c$ from the waist. However, reflection and absorption losses at the left cell decrease the power of the radiation incident on the right cell. Coefficient E may also change because of the dependence of the thermal conductivity of the solvent on the solute. All such differences can be avoided by transferring the left cell.

The waist of the laser beam is usually inside the laser cavity; in thermal-lens spectrophotometry, the waist can be adjusted via a positive lens. The radius of the waist can be calculated [7] from the equation $w_{01} = F_1\theta$, where θ is the beam divergence (in radian) and F_1 is the focal length of the positive lens. In order to obtain the largest signal in these differential measurements (with the cells placed on both sides of the waist) it is necessary that $3^{1/2} z_c < F_1$ or $F_1 < \lambda/3^{1/2} \pi\theta^2$. The possibilities of the differential technique were demonstrated for determination of the absorbances of iodine in carbon tetrachloride [6] and for the determination of sulfides [8].

The special nature of the dual-beam thermal-lens method is that the thermal lens is generated inside a sample by a high-power laser and the absorbance is monitored via the change in the beam divergence of a continuous-wave (c.w.) low-power laser at a wavelength different from that of the inducing laser. As a rule, the sample must not absorb the probe-laser radiation. Dual-beam measuring systems were successfully exploited for the investigation of thermal lenses induced by impulse lasers (e.g., for the determination of nitrogen dioxide in the atmosphere with a limit of determination of 0.8 ppm [9], and uranium(VI) in aqueous solutions with a determination limit of 4×10^{-6} M [10]) and for the analysis of gaseous media under excitation from a c.w. laser. For example, a CO_2 laser ($b = 1$ m) was used to determine benzene in air down to 0.5 ppm [11], and an argon laser ($b = 1$ m) to determine nitrogen dioxide in air down to 0.005 ppm [12]). The original optical arrangement was described by Perry et al. [13]. The second harmonic of the dye laser inducing the thermal lens was used as the monitoring radiation. A comparison of the single-beam and dual-beam techniques was described by Carter and Harris [14], who discussed the absorbances of iodine solution in carbon tetrachloride.

Especially to take advantage of the ready availability of laser sources for the determination of traces in the present work, a chemical procedure was developed for monitoring phosphorus in which the helium-cadmium laser ($\lambda = 441.6$ nm) could be used. The determination of phosphorus with a helium-neon laser ($\lambda = 632.8$ nm) was described earlier [3].

Of the many spectrophotometric procedures for the determination of phosphorus based on heteropoly acids, the most sensitive are the extraction procedure based on formation of molybdophosphate/basic dye ion-pairs [15]. Molybdophosphoric acid is known to form an ion-pair with auramine

$[(\text{CH}_3)_2\text{NC}_6\text{H}_4]_2(\text{CNH})\cdot\text{HCl}$; C.I. Basic Yellow 2) [16]; the proposed procedure for the determination of traces of phosphorus is based on this selective extraction. Molybdophosphoric acid formed in aqueous solution is separated from the large excess of molybdate by extraction into isobutanol. Then hexane is added to give a 2:1 volume ratio to isobutanol and finally an acidic solution of auramine is introduced. The isobutanol/hexane (1:2) mixture extracts the ion-pair well; free dye remains in the aqueous phase. The addition of hexane is necessary solely because isobutanol extracts both the ion-pair and the free dye. Acetone is needed to dissolve the ion-pair precipitate. The manual procedure has been described [17].

EXPERIMENTAL

Apparatus

The optical system for the single-laser thermal-lens measurement is outlined in Fig. 2. The thermal lens was generated by radiation from a single-mode He—Cd laser (LG-62; $\lambda = 441.6$ nm); the radius of the beam on the output mirror (w_p) was 0.15 cm, the beam divergence θ was 9×10^{-4} rad and the power was 100 mW. The laser beam was chopped by an electromagnetic shutter opening for 0.2 ms. The outward waist was created with a quartz achromatic lens (f 7.5 cm). Quartz cells (0.5 cm pathlength) were used. The cell holder allowed the cells to be switched relative to the waist. The change in the intensity at the beam center was detected by a photodiode (PD-10GB) limited by its own aperture. The distance from the cells to the photodiode was magnified by a mirror M, and could be varied if necessary. The signal from the photodiode was monitored by a digital voltmeter and a recording system. A peak detector was used to measure the initial intensity at the beam center. An oscillogram of the thermal-lens generation in an auramine solution in 1:1 acetone/hexane is shown in Fig. 3. It can be seen

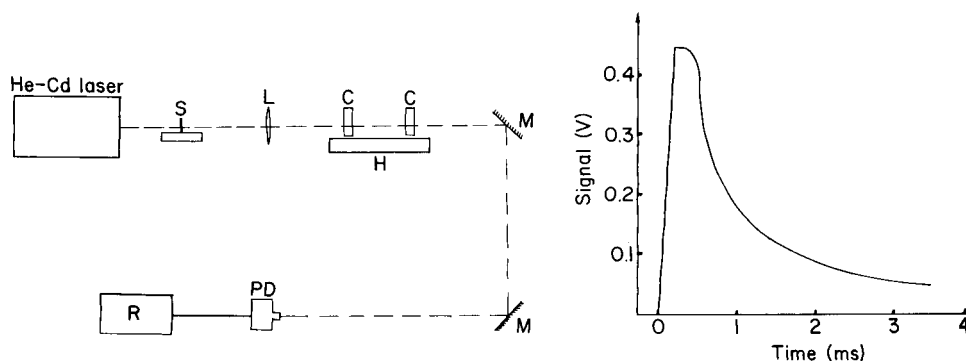


Fig. 2. Diagram of the device for single-beam thermal-lens measurements: S, shutter; L, lens; C, cells in cell holder H; M, mirror; PD, photodiode; R, recording system.

Fig. 3. Oscillogram of thermal-lens generation in auramine solution.

that the response time of the peak detector was enough for recording the initial intensity I_0 at the laser beam center (i.e., the plateau after full opening of the shutter). In this case, the value of the recorded signal was directly proportional to the radiation power P . The intensity at the center of the laser beam was also measured by a broad-band amplifier after generation of the thermal lens (I_∞).

The arrangement for dual-beam measurement of the thermal lens is shown in Fig. 4. In this system, the thermal lens was produced by the same He—Cd laser as above (LG-62). The radiation of the He—Ne probe laser was directed towards that of the He—Cd laser, as described by Higashi et al. [12]; a single-mode He—Ne laser (LG-77) was used ($\lambda = 632.8$ nm, $P = 0.25$ mW, $\theta = 2.4 \times 10^{-3}$ rad). The parameters of each laser beam were defined by measuring its spot size at various distances from the waist. The He—Ne laser was focused by lens 2 (lenses with 3.0 or 7.5-cm focal length were used). In front of the He—Ne laser, the radiation of the He—Cd laser was blocked by an optical glass filter (F3). To avoid optical feedback, a 60-degree quartz prism was used. Photodiode PD2 served to monitor the stability of the He—Cd laser. The signal was recorded in two ways. In the absence of quartz plate QP and mirror M3, the system of recording described for single-laser measurements was used. A broad-band amplifier was used without placing mirror M2 in the optical system; I_0 was detected with the shutter closed, and I_∞ was detected after opening the shutter and generating the thermal lens in the solution. The relation $(I_0 - I_\infty)/I_0$ served as the analytical signal S .

Recommended procedure

All chemicals were of analytical grade and triple-distilled deionized water was used. To a 10-ml separatory funnel, add 0–1 ml of neutral orthophosphate solution and adjust the volume to 3 ml with water. Then add 1 ml of ammonium molybdate solution (0.08 M in 1 M hydrochloric acid), mix

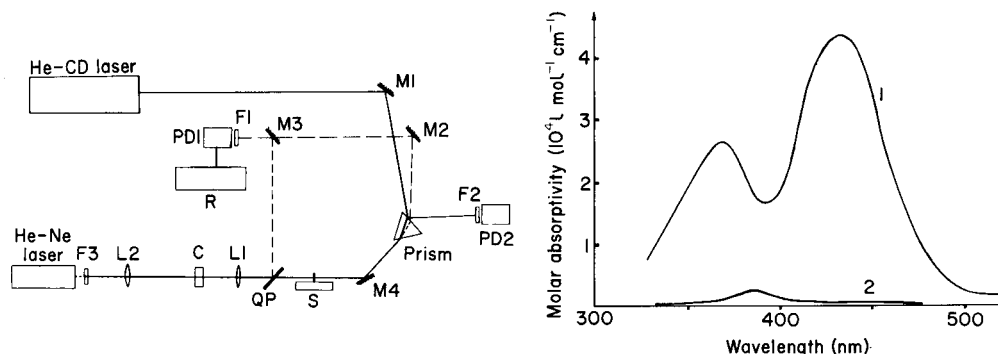


Fig. 4. Diagram of the device for dual-beam thermal-lens measurements: F, optical glass filter; QP, quartz plate; other lettering as for Fig. 2.

Fig. 5. The absorption spectra: (1) ion-pair; (2) blank. Both were measured against acetone.

carefully, and leave for 10 min for completion of the reaction. Add 1 ml of isobutanol and shake the funnel for 0.5–1 min to extract molybdophosphate. When the phases have separated, wash the organic layer with 2 ml of water to remove the molybdate extracted into the organic phase. To the organic layer add 2 ml of hexane and a little isobutanol to bring the volume to 3 ml. Add 20 μl of 0.05 M hydrochloric acid and 1 ml of auramine solution (0.2 g L^{-1}) and mix for 5 min. Separate the aqueous layer carefully to avoid loss of the ion-pair precipitate floating on the phase boundary. Wash the organic layer twice with 3 ml of hydrochloric acid (pH 3). Add 2 ml of acetone to dissolve the ion-pair precipitate, shake the mixture vigorously and measure the absorbance within 5 min against the blank.

Simultaneously, when it was possible, the absorbance was measured in a SPh-26 spectrophotometer at 441.5 nm ($b = 0.5$ cm). The blank solution with necessary reagents was prepared as in the recommended procedure. The absorption spectrum of the ion-pair and that of the blank compared to acetone are shown in Fig. 5. The calculated value of the molar absorptivity was $4.6 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 441.5 nm.

RESULTS AND DISCUSSION

Differential measurements

An important drawback of the differential thermal-lens technique is that measurements of the absorbances of unknown solutions against a single blank are imprecise because of blank fluctuations. This can be avoided in two ways. In the first, a series of blank solutions is measured and thereafter all results obtained relative to a single blank are corrected to the average value. In the second, which is more convenient, all blanks are mixed to provide an "average blank" which is used as a sample for comparison. This blank is also checked by measurements against a solution with known concentration. The measurement relative to a series of blanks is imprecise because a change in value of the left thermal lens produces a change in the laser beam parameters (w_0 and z_c). For reasons given above, the application of the differential technique is thought to be indispensable. To simplify the optical instrumentation, however, it is better to use a non-differential measurement with automatic correction for the "average blank". No special demands are then placed on selection of the positive lens for the optical system. Furthermore, the radius of the laser beam at its waist increases when there is a negative thermal lens behind the positive one. This increase in the size of the waist of the laser beam does not affect the sensitivity, which is evident from considering the expression $\Delta I_{bc}/I_{bc} = 0.524 EA$. But this factor becomes very significant in measuring samples with small volumes, because the minimum volume of the specimen is defined by the radius of the laser beam [18].

Moreover, the change in divergence of the laser beam ($\Delta\theta$) reaches a limiting value $\Delta\theta_{\text{lim}} = (dn/dT)P/\pi n_0 w_0 k$ as the absorbance increases; n_0 is the refractive index of the solvent for the laser wavelength at room temperature

[3]. This $\Delta\theta_{\text{lim}}$ defines the upper limit of the dynamic range of measurement. For the differential modification, the maximum detectable absorbance drops as w_0 increases, thus the dynamic range is narrower than that for single-beam measurements, as has been noted by Dovichi and Harris [6]. For thermal-lens measurements, the highest detectable absorbance (A_{max}) can be evaluated from the expression $S = (I_0 - I_\infty)/I_0 = 0.524 EA$ for the analytical signal. When the cell is placed at the distance $3^{1/2} z_c$ from the waist, I_∞ decreases as the absorbance increases, approaching zero, i.e., $S \rightarrow 1$. Hence $A_{\text{max}} = (0.524 E)^{-1}$. It is possible to increase the highest detectable absorbance by using a laser with a greater wavelength. For example, A_{max} with a CO_2 laser is about 16.8 times the value with a He-Ne laser. Alternatively, of course, a cell with smaller optical length can be used.

As the radius of the laser beam at its waist and inside the cell is in the micrometer range, special attention has to be given to the purity of the chemicals, cells and environment. During measurements, the laboratory atmosphere must be free from dust and the cell space must be protected. It is recommended to filter the sample and to use hermetically sealed cells. The differential method is less prone to noise effects than the single-beam method, particularly for small differences in absorbance [6].

Differential measurements surpass single-beam consecutive measurements in reproducibility because the time between measurements is shorter and so the influence of long-term instability of the laser output (σ_P/P) is decreased. The measurement error [6] for the single-beam modification is $\sigma_{A_2-A_1} = (\sigma/P)(A_1^2 + A_2^2)^{1/2}$, and that for the differential modification is $\sigma_{A_2-A_1} = (\sigma/P)/(A_2 - A_1)$. As mentioned above, the differential measurement depends strongly on the spot size of the beam within the cell and on the blank absorbance. To observe linearity of the signal vs. ΔA , the absorbances of the samples must be small. All this significantly diminishes the principal advantage of the differential modification, i.e., the possibility of determining a small absorbance against a large background. Nevertheless, differential measurements with an "average blank" are useful for reducing the time of analysis and for lowering the influence of the long-term fluctuations in laser output. This is necessary in measuring extremely small absorbances.

In the present experiments, the calibration plots were linear over the concentration range 0.60–70 ng ml⁻¹ phosphorus; the linear range for single-beam measurements was 0.60–200 ng ml⁻¹. The data were averaged from ≥ 5 parallel determinations. The absorbance of each specimen was measured thirty times; the relative standard deviation of measurements was < 0.01 . The detection limit for phosphorus (3σ) was 0.6 ng ml⁻¹. The absolute detection limit was 8.1×10^{-14} g, calculated from the cell volume irradiated with the laser beam.

The accuracy of the method was confirmed by parallel measurements with a conventional spectrophotometer over the range 5–1000 ng ml⁻¹ phosphorus (as orthophosphate). Beer's law was obeyed up to 550 ng ml⁻¹, and the detection limit (3σ) was 5 ng ml⁻¹. The average error was 5.3% for the range

10–1000 ng ml⁻¹. For the concentration range 5–200 ng ml⁻¹, it was possible to obtain calibration graphs for both the conventional spectrophotometric system and the thermal-lens system. The determination limit for phosphorus as the molybdophosphate/auramine ion-pair is restricted by blank fluctuations, which depend in turn on the concentration of auramine. Extension of the calibration plots for the conventional spectrophotometer to the range of very low concentrations was achieved by diluting the stock solution of auramine and by applying the thermal-lens system, because the sensitivity of the normal spectrophotometer was insufficient.

Dual-beam measurement

The sensitivity of thermal-lens spectrophotometry based on a c.w. laser can be improved via design of the optical arrangement only when the dual-beam system is used. For the single-beam modification, the species must be situated at a distance $3^{1/2} z_c$ from the waist of the laser beam to obtain the maximum signal. But to produce maximum power density and a thermal lens with as short a focus as possible, the cell must be placed at the waist of the main laser beam and also at a distance $3^{1/2} z_c$ from the waist of the probe laser beam. If the origin of the optical system corresponds to the waist, the radius of the laser beam will increase as distance Z (the distance from the waist) grows [19]: $w^2 = w_0^2[1 + (Z/z_c)^2]$. Evidently, the laser power density will increase four times, if a species is transferred from the point $3^{1/2} z_c$ to the waist of the main laser beam.

In these experiments, the analytical signal was found to increase 2.34 times if lens 2 was of 7.5-cm focal length (Fig. 4) and 2.19 times if lens 2 was of 3.0-cm focal length, when the cell was transferred from point $3^{1/2} z_c$ to the waist of the He–Cd laser. (A 0.5-cm cell with a model solution of auramine in acetone/hexane/isobutanol was placed at the distance $3^{1/2} z_c$ from the waist of the beam of He–Ne laser in both cases.) The disagreement of the experimental data with the predicted increase in the signal is probably connected with a change in the spot size of the probe-laser beam over the pathlength of the cell.

Comparison of the data obtained from the two systems used for signal recording showed that the peak detector was sufficiently fast to monitor the original intensity of the laser beam at its center; the signal values obtained with the recording systems were identical. However, it should be noted that the broad-band amplifier made it possible to monitor the current output power of the probe laser and such a system is also simpler to exploit.

Figure 6 shows the dependence of the thermal-lens effect on the position of the cell. The distance from the cell to the waist was varied by changing the position of lens 2 (7.5-cm focal length). The continuous line corresponds to the data calculated from the equation [4]: $S = (I_0 - I_\infty)/I_0 = EA \tan^{-1} [2zz_0/(3 + z^2/z_c^2)]$, where z is the distance from the cell to the waist. The confocal distance, $z_c = \pi w_0^2/\lambda$, was 16.1 cm. Experimental and theoretical curves are similar in shape and have a maximum at the point $3^{1/2} z_c$. The

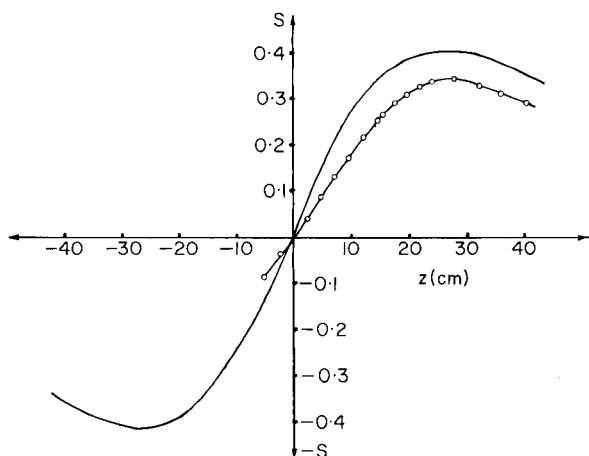


Fig. 6. The dependence of the thermal-lens effect on cell position. See text for explanation.

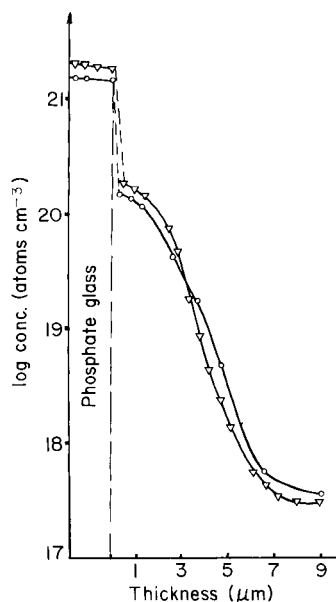


Fig. 7. Layer-by-layer determination of phosphorus in phosphate glass and semiconductor silicon: (○) neutron-activation method; (▽) thermal-lens spectrophotometry.

greater height of the calculated curve can be explained by the approximate estimate of coefficient E . In further experiments, the distance from the cell to the waist was 28 cm.

Carter and Harris [14] emphasized that if the beam radius of the inducing laser within the cell was significantly smaller than that of the probe laser, then the part of the probe-laser beam affected by changing refractive index would be small, and so the effect on the signal would also be small. Conversely, some small part of the probe-laser beam must be affected by changes in refractive index. The greatest effect must be obtained when these radii are equal. But the thermal lens induced inside the sample is essentially wider than the laser beam [20]. Thus, even if the radius of the probe-laser beam is slightly greater than that of the inducing (main) laser, the signals will not be affected. In the present experiments, the signals were of the same value whether the radius of the probe-laser beam within the cell was more than 5.3 times the radius of the main laser for lens 2 with a 7.5-cm focal length, or more than 2.1 times for lens 2 with 3.0-cm focal length. If the radius of the probe-laser beam is significantly larger, the signal will change, probably because of diffraction of the probe beam on microlenses. But there are no effects on the signal provided that the radius of the probe beam is smaller than that of the inducing beam. On the contrary, the effect of any aberration in the thermal-lens border will decrease.

The comparison of thermal-lens measurements with conventional spectrophotometry was discussed [14] for the single-beam and dual-beam modifications. Coefficients of enhancement, E_p and E_s for dual-beam and single-beam measurements, respectively, can be related by the expression $E_p/E_s = (dn/dT)_p \lambda_s / (dn/dT)_s \lambda_p$, where $(dn/dT)_p$ and $(dn/dT)_s$ are the temperature gradients of the refractive index for irradiation with the probe laser (λ_s) and the inducing laser (λ_p). Clearly, the dual-beam system will outdo the single-beam system in sensitivity if the wavelength of the probe laser is shorter than that of the main laser.

The detection limits of thermal-lens measurements are restricted by the short-term instability of the lasers. For the dual-beam system, it is necessary to stabilize both lasers or to monitor power fluctuations continuously. Measurement errors are larger for the dual-beam variation than for the single-beam system, because of the additional contribution of fluctuations in the probe-laser output. Because the optical design for single-beam measurements is simpler, the dual-beam variation should be used only when the single-beam method is useless.

The calibration graph for phosphorus (as orthophosphate) obtained with the dual-beam system was linear over the range 0.60–400 ng ml⁻¹. The detection limit (3σ) was 0.6 ng ml⁻¹, the absolute detection limit being 1.8×10^{-14} g. The wider linear range can be attributed to the E_p coefficient being smaller than E_s . The average error was 7.5% and the relative standard deviation of the data was <0.03 ($n = 30$).

Application to semiconductor silicon

The procedure developed and the dual-beam arrangement were used for phosphorus determinations in semiconductor silicon. The analytical procedure with differential thermal-lens measurements was described earlier for phosphorus determinations in high-purity hydrochloric and nitric acids, indium and bismuth [21]. Scrupulous precautions were taken in the preparation of solutions and glassware. For layer-by-layer analysis of phosphate glass and semiconductor silicon, various mixtures of hydrofluoric acid (40%), concentrated nitric acid and water were used as etching reagent. The thickness of the layers was monitored with a laser ellipsometer (LEF-2) and by weighing. To analyze the bulk sample, the specimen was first dissolved in a hydrochloric/nitric acid/water mixture in a teflon beaker and the solution was evaporated to dryness under an infrared lamp at 60–70°C. The operations were similar for the layer-by-layer analysis. The residue in the beaker was dissolved in a little water, the solution was transferred to a separatory funnel, and then the recommended procedure was applied. Corresponding blanks were also prepared.

To check the silicon separation and to investigate any influence of silicon, layer-by-layer analysis of semiconductor silicon containing less than $5 \times 10^{-8}\%$ phosphorus was conducted. At high concentrations, the accuracy of the analysis was checked by parallel measurements with conventional spec

trophotometry; at low concentrations, comparison was with a neutron-activation method. The data were in good agreement with each other for bulk silicon samples containing 5×10^{-8} — $5 \times 10^{-4}\%$ phosphorus. Figure 7 shows the profiles of phosphorus distribution obtained by the dual-beam thermal-lens spectrophotometry and the neutron-activation method.

REFERENCES

- 1 T. D. Harris, *Anal. Chem.*, 54 (1982) 741A.
- 2 V. I. Grishko, I. G. Yudelevich and V. P. Nikitina, *Zh. Anal. Khim.*, 39 (1984) 129.
- 3 V. I. Grishko, I. G. Yudelevich and V. P. Grishko, *Anal. Chim. Acta*, 160 (1984) 159.
- 4 S. J. Sheldon, L. V. Knight and J. M. Thorne, *Appl. Opt.*, 21 (1982) 1663.
- 5 N. J. Dovichi and J. M. Harris, *Anal. Chem.*, 51 (1979) 728.
- 6 N. J. Dovichi and J. M. Harris, *Anal. Chem.*, 52 (1980) 2338.
- 7 J. R. Arnaud, *Beam and Fiber Optics*, Academic Press, New York, 1976.
- 8 V. I. Grishko, I. G. Yudelevich, V. P. Grishko and L. Kh. Kravchenko, In: *Organic Reagents in Analytical Chemistry*, Naukova Dumka, Kiev, 1983, Vol. 2, p. 134.
- 9 K. Mori, T. Imasaka and N. Ishibashi, *Anal. Chem.*, 55 (1983) 1075.
- 10 T. Berthoud, P. Maunchien, N. Omenetto and G. Rossi, *Anal. Chim. Acta*, 153 (1983) 265.
- 11 T. Higashi, T. Imasaka and N. Ishibashi, *Bunseki Kagaku*, 31 (1982) 680.
- 12 T. Higashi, T. Imasaka and N. Ishibashi, *Anal. Chem.*, 55 (1983) 1907.
- 13 J. W. Perry, E. A. Ryabov and A. H. Zewail, *Laser Chem.*, 1 (1982) 9.
- 14 C. A. Carter and J. M. Harris, *Anal. Chem.*, 55 (1983) 1256.
- 15 S. Motomizu, T. Wakimoto and K. Toei, *Anal. Chim. Acta*, 138 (1982) 329.
- 16 A. K. Babko, Yu. F. Shkaravsky and V. I. Kulik, *Zh. Anal. Khim.*, 21 (1966) 196.
- 17 V. P. Grishko, I. G. Yudelevich and V. I. Grishko, *Zav. Lab.*, 50 (1984) N9, 13.
- 18 C. A. Carter and J. M. Harris, *Anal. Chem.*, 56 (1984) 922.
- 19 H. Kogelnik, *Bell Systems Techn. J.*, 44 (1965) 455.
- 20 I. Glatt, Z. Karny and O. Kafri, *Appl. Opt.*, 23 (1984) 274.
- 21 V. I. Grishko, I. G. Yudelevich and V. P. Grishko, *Zh. Anal. Khim.*, 39 (1984) 1813.

SYNTHESIS OF 2-[2-(8-HYDROXYQUINOLYL)AZO]-1-NAPHTHOL AND ITS USE IN THE SPECTROPHOTOMETRIC DETERMINATION OF CALCIUM IN POTABLE WATER

T. ISHIZUKI, H. WADA*, K. KODAMA and G. NAKAGAWA

Laboratory of Analytical Chemistry, Nagoya Institute of Technology, Showa-ku, Nagoya (Japan)

(Received 13th March 1985)

SUMMARY

The synthesis of a novel reagent, 2-[2-(8-hydroxyquinolyl)azo]-1-naphthol (HQAN), is described. The acidity constants of the reagent and its reactions with various metal ions are reported. The HQAN reagent forms a soluble calcium chelate in 50% (v/v) dioxane/water at pH > 8.5 ($\epsilon = 24\,000\text{ l mol}^{-1}\text{ cm}^{-1}$ at 570 nm). It also forms chelates with magnesium, other alkaline earth metals, and divalent Mn, Fe, Ni, Cu, Zn, Cd and Pb. These interferences (except for Mn) can be avoided by pH adjustment or by adding masking agents, and a very simple procedure for the determination of calcium in potable waters is obtained.

For the spectrophotometric determination of calcium, glyoxal bis-(2-hydroxyanil), chlorophosphonazo-III and *o*-cresolphthalein complexone are generally used as the chromogenic reagent. The glyoxal derivative and its calcium chelate are not stable, and the pH, temperature and standing time affect the absorbance of the chelate [1, 2]. With chlorophosphonazo-III, calcium can be determined at pH 2.2 without interference from magnesium, but the sensitivity is not good and the reagent blank is large [3]. *o*-Cresolphthalein complexone is a highly sensitive reagent and is widely used in clinical analysis; but the reagent blank is again large and magnesium interferes [4, 5].

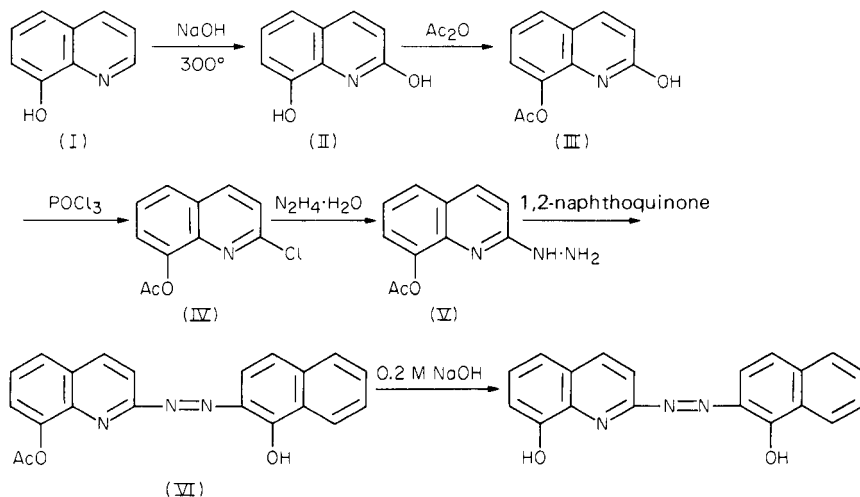
Previously, a highly sensitive *o,o'*-dihydroxyazo compound, 2-(2-hydroxy-3,6-disulfo-1-naphthylazo)-5-(*N,N*-diethylamino)phenol, was synthesized and applied to the determination of calcium and magnesium in a flow injection system [6]. With this reagent, the blank is so high that it can only be used in a flow-injection procedure.

8-Quinolinol reacts with many metal ions, but the sensitivity of the spectrophotometric methods is usually not good. Previously, 5- or 7-azo dye derivatives of 8-quinolinol have been synthesized, whereas the 2-azo dye derivatives have not. In a continuation of the search for a simple and reliable method for the determination of calcium, a 2-azo dye derivative of 8-quinolinol, 2-[2-(8-hydroxyquinolyl)azo]-1-naphthol (HQAN), was synthesized. It was expected that HQAN would react with calcium as a quadridentate reagent.

EXPERIMENTAL

Preparation of 2-[2-(8-hydroxyquinolyl)azo]-1-naphthol

The steps involved in the synthesis are as follows:



2,8-Dihydroxyquinoline (II). The procedure for the synthesis described previously [7] was modified as follows. Sodium hydroxide (60 g) was placed in a nickel beaker, and 4 ml of water was added. The beaker was heated to about 140°C. 8-Quinolinol (15 g) was added little by little with stirring using a nickel bar. Then the temperature was raised to ca. 300°C, which temperature was maintained for 3 h with occasional stirring. The color of the mixture changed from clear yellow to brown. After cooling until the product solidified, 200 ml of water was added to dissolve the precipitate. The pH of the solution was adjusted to 1–2 with (1 + 4) sulfuric acid, and a pale brown precipitate was obtained. The precipitate was filtered off and washed with ethanol to remove 8-quinolinol. (Yield 80%, m.p. 284.5°C.)

2-Hydroxy-8-acetoxyquinoline (III) [7]. Acetic anhydride was added little by little to compound II in 200 ml of pyridine. Stirring was continued for 2 h, and the solution was allowed to stand overnight. Compound III was precipitated by adding 200 ml of water. (Yield 80%, m.p. 247–248°C.)

2-Chloro-8-acetoxyquinoline (IV). This was prepared with phosphorus oxychloride in the usual way. (Yield 35%, m.p. 80–81°C.)

2-Hydrazino-8-acetoxyquinoline (V). Compound IV was added to 50 ml of hydrazine hydrate and the solution was refluxed for 3 h. Addition of water after cooling caused the product to crystallize from solution. (Yield 80%, m.p. 180°C.)

2-[2-(8-Acetoxyquinolyl)azo]-1-naphthol (VI). Compound V (2.2 g) and 1,2-naphthoquinone were dissolved in a mixture of glacial acetic acid (40 ml) and dioxane (60 ml), and the solution was heated on a boiling waterbath for

4 h. After cooling, 50 ml of water was added, and a dark-red precipitate was obtained. (Yield 40%.)

2-[2-(8-Hydroxyquinolyl)azo]-1-naphthol. Compound VI was dissolved in 0.1–0.2 M sodium hydroxide and the solution was filtered. The filtrate was acidified to pH 1–2 by adding 6 M hydrochloric acid. Crude HQAN precipitated and was filtered off. Crude HQAN was then dissolved in dioxane and filtered. Addition of water to the filtrate precipitated the HQAN. This recrystallization was repeated four times. Reddish-brown needles were obtained (m.p. 212°C). The results of elemental analysis of HQAN were as follows: calcd. for $C_{19}H_{13}N_3O_2 \cdot 1/2H_2O$, 70.4% C, 4.35% H, 12.95% N; found 70.9% C, 4.4% H, 12.35% N.

Reagents and apparatus

The HQAN reagent was dissolved in dioxane, and a 6×10^{-4} M solution in aqueous 70% (v/v) dioxane was prepared by adding water.

Metal ion solutions were prepared in various ways. For calcium, calcium carbonate was dissolved in a small excess of hydrochloric acid and the solution was diluted with water, so that the pH adjustment was not necessary. Copper(II), nickel(II) and zinc(II) solutions were prepared by dissolving the metals (99.99% purity) in dilute nitric acid. Magnesium solution was prepared from magnesium sulfate, and standardized by EDTA titration with eriochrome black T as indicator. Other metal ion solutions were prepared from the appropriate nitrate, sulfate or chloride.

The solutions used for pH adjustment were dilute hydrochloric acid (pH < 1.5), 1.25 M chloroacetic acid/sodium chloroacetate (pH 1.5–3.5), 1.25 M acetic acid/sodium acetate (pH 3.5–7.0), 1.25 M ammonia/ammonium chloride (pH 8.0–10.5) and aqueous ammonia solution (pH 10.5–11.5).

Dioxane was generally used as received, but for the determination of the acidity constants purified in the conventional manner. All the chemicals used were of analytical-reagent grade. Water was redistilled from a hard glass vessel.

A Union-Giken SM-401 spectrophotometer and a Hitachi-Horiba M-5 pH meter were used.

Recommended procedure for the spectrophotometric determination of calcium

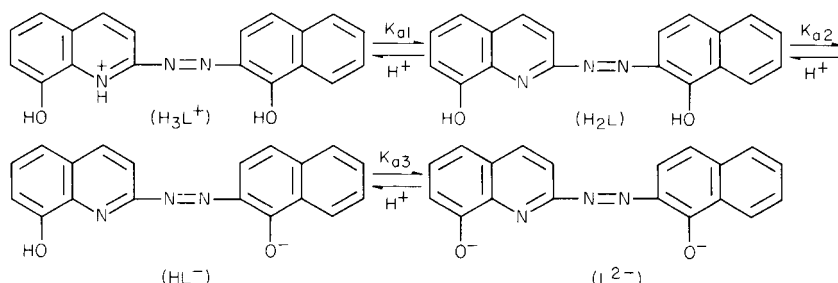
Place 9.0 ml of dioxane in a 25-ml volumetric flask, and add the sample containing 2.5–12.5 μg of calcium, to give a final concentration of 0.1–0.5 mg l^{-1} calcium. Adjust the pH to 8.6–9.0 with an ammonia/ammonium chloride buffer solution. Add 5 ml of 6×10^{-4} M HQAN solution and dilute to the mark with water. Measure the absorbance at 610 nm against a reagent blank.

RESULTS AND DISCUSSION

Acidity constants of HQAN

As HQAN is sparingly soluble in water, the acidity constants were determined in 40% (v/v) dioxane. The spectra of HQAN solutions at different pH

are shown in Fig. 1. The HQAN solution itself is yellow ($\lambda_{\max} = 480 \text{ nm}$) at $\text{pH} < 1.5$, orange ($\lambda_{\max} = 500 \text{ nm}$) in the pH range 2.5–10 and violet ($\lambda_{\max} = 545 \text{ nm}$) at $\text{pH} > 10.5$. The dissociation equilibria of HQAN, therefore, can be written as follows:



The three successive acidity constants are then defined by $K_{a1} = a_{\text{H}^+}[\text{H}_2\text{L}]/[\text{H}_3\text{L}^+]$, $K_{a2} = a_{\text{H}^+}[\text{HL}^-]/[\text{H}_2\text{L}]$ and $K_{a3} = a_{\text{H}^+}[\text{L}^{2-}]/[\text{HL}^-]$, where a_{H^+} is the apparent activity of hydrogen ion measured in 40% dioxane solution by means of a pH meter equipped with a glass electrode which was calibrated with the two standard buffers. Being higher than 12, the $\text{p}K_{a3}$ value was not determined. The $\text{p}K_{a1}$ and $\text{p}K_{a2}$ values, which were evaluated by Hildebrand and Reilly's method [8], are given in Table 1 together with the acidity constants of 8-quinolinol [9] and 2-[2-(4-methylquinolyl)azo]-1-naphthol (4-Me- α -QAN) [10]. The basicity of the heterocyclic nitrogen is reduced by the introduction of the azo group in the adjacent position. When the hydroxyl group of 1-naphthol in 4-Me- α -QAN dissociates, the color of the reagent solution changes from orange to violet. Therefore, the dissociation of HQAN corresponding to K_{a2} is probably that of hydroxyl group of 1-naphthol.

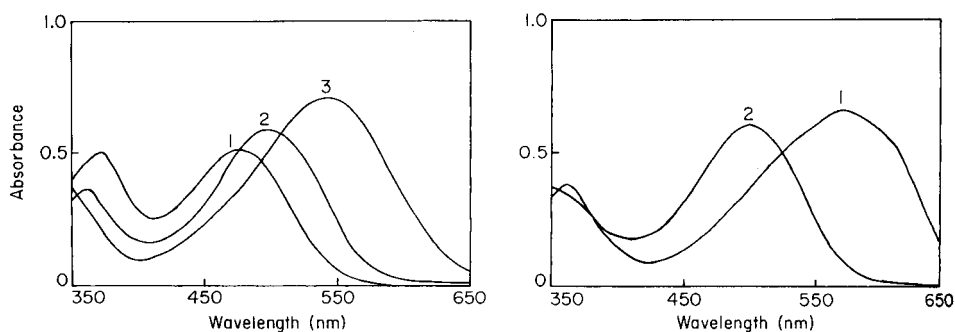


Fig. 1. Absorption spectra of HQAN at different pH: (1) 0.5 M HCl; (2) pH 8.8; (3) pH 12. Conditions: $2.84 \times 10^{-5} \text{ M}$ HQAN, 0.1 M KNO_3 , 40% dioxane.

Fig. 2. Absorption spectra of Ca/HQAN and reagent: (1) calcium chelate; (2) reagent. Conditions: $2.84 \times 10^{-5} \text{ M}$ HQAN, $1.84 \times 10^{-4} \text{ M}$ calcium, pH 8.8, 40% dioxane.

TABLE 1

Acidity constants at ionic strength 0.1 M (KNO₃) in 40% (v/v) dioxane at 25°C

	HQAN	8-Quinolinol ^a	4-Me- α -QAN ^b
pK _{a1}	1.74	3.97	2.55
pK _{a2}	10.41	11.54	10.63
pK _{a3}	>12		

^aRef. [9] (50% dioxane). ^bRef. [10] 50% dioxane.

The HQAN solution in 70% dioxane is stable at least one month at room temperature.

Reaction with metal ions

As shown in Table 2, HQAN reacts with many metal ions to form violet or blue chelates. The copper(II), nickel(II) and cobalt(III) chelates precipitated. In the presence of ascorbic acid, the reagent and the iron chelate decomposed. No color reaction was observed between aluminum and HQAN. Usually the 1:1 chelates were formed with the various metal ions. Calcium, magnesium, strontium and barium formed violet chelates with HQAN, whereas with 4-Me- α -QAN no color reactions were observed with these ions. From these results, HQAN seems to behave as a quadridentate ligand. As the calcium formed a stable chelate with HQAN at a lower pH range than magnesium did, the determination of calcium with HQAN was studied.

Experimental conditions for the determination of calcium

The absorption spectra of HQAN and the calcium chelate at pH 8.82 are shown in Fig. 2. At 570 nm the reagent blank was relatively large, thus the absorbance was measured at 610 nm. When the concentration of dioxane was changed from 40 to 64%, the absorbance of the calcium chelate increased by 7%. Thus an aqueous 50% dioxane solution was preferred.

Constant absorbance was obtained with a ≥ 10 -fold excess of the reagent, although the reagent blank increased with increasing reagent concentrations. As shown in Fig. 3, the absorbance of calcium chelate is constant at pH values higher than 8.5, but the reagent blank increased again with increasing pH. Therefore, the optimum pH range for calcium was from 8.5 to 9.0.

Under the recommended conditions, the calibration graph for calcium was linear for the range 3.0×10^{-6} – 1.2×10^{-5} M (0.12–0.48 mg l⁻¹), and the Sandell sensitivity was 1.8 ng cm⁻².

Interferences. Magnesium reacts with HQAN at a higher pH range than calcium. At pH 8.6, the absorbance produced by magnesium was negligible at 610 nm when the magnesium concentration was less than 1.4×10^{-5} M (0.34 mg l⁻¹). Increase in pH reduced the tolerable amount of magnesium. As the concentration of magnesium is generally much smaller than that of calcium in potable waters (usually around 1:5), magnesium will not interfere.

TABLE 2

Reactions of some metal ions ($C_L = 2.8 \times 10^{-5}$ M, $C_M = 4.0 \times 10^{-4}$ M)

Metal ion added	pH ^a	Color	$\lambda_{\max}$ (nm)	$\epsilon \times 10^{-4}$ (l mol ⁻¹ cm ⁻¹)
Ca(II)	8	Violet	570	2.4
Mg(II)	9	Violet	570	2.3
Sr(II)	9	Violet	568	2.4
Ba(II)	9.5	Violet	572	2.4
Mn(II)	5.5	Blue-violet	578	2.0
Fe(III)	1	Dark violet	530, 670	1.6 (530 nm)
Zn(II)	5.5	Blue-violet	574, 618	2.4 (618 nm)
Cd(II)	4	Blue-violet	578, 611	3.0 (611 nm)
Hg(II)	2	Blue	586, 622	2.9 (622 nm)
Pb(II)	3.5	Blue-green	622	2.4

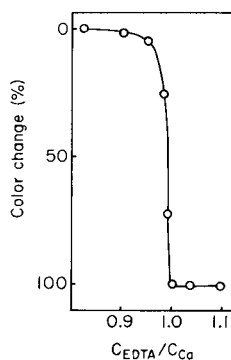
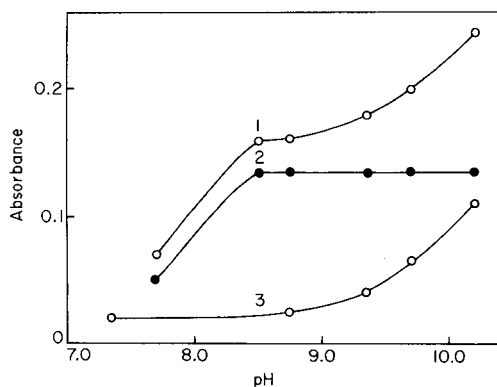
^apH at ca. 50% reaction.

Fig. 3. pH dependence of the Ca/HQAN chelate formation: (1) Ca/HQAN against water; (2) Ca/HQAN against reagent; (3) reagent blank. Conditions: 1.17×10^{-4} M HQAN, 6.02×10^{-6} M calcium, 50% dioxane.

Fig. 4. Photometric titration curve of 1.51×10^{-3} M calcium with 3×10^{-5} M HQAN as indicator at pH 9; absorbance measured at 570 nm.

For the other metal ions likely to be present in potable waters, various masking agents were examined. The results are shown in Table 3. Iron (0.4 mg l^{-1}) could be masked by adding 1 ml of 1% ethylenediamine solution, and nickel, copper, zinc and lead with 2,3-dimercapto-1-propanol. A suitable masking agent could not be found for manganese. A ten-fold molar amount of orthophosphate decreased the absorbance a little.

Determination of calcium in water samples. The method was applied to the determination of calcium in four tap-water samples and two well-water samples. Three portions (0.5–1.5 ml) of a water sample were taken, and the

TABLE 3

Absorbances at 610 nm of metal/HQAN chelates in the presence of masking agents (pH 8.6)^a

Masking agent ^b (1%, 1 ml)	Mn(II) (M) 5.9×10^{-6}	Fe(II) ^c (M) 7.3×10^{-6}	Ni(II) (M) 6.0×10^{-6}	Cu(II) (M) 5.8×10^{-6}	Zn(II) (M) 8.8×10^{-6}	Pb(II) (M) 6.5×10^{-6}
none	0.165	0.087	0.195	0.022	0.250	0.143
<i>o</i> -Phen	0.135	0.160	0.000	0.022	0.055	0.128
En	0.143	0.000	0.025	—	0.195	0.133
AA	0.180	0.160	0.132	0.038	0.200	0.150
TEA	0.155	0.138	—	—	—	—
DTCS	0.165	0.170	0.000	0.010	0.058	0.010
Trien	0.130	0.146	0.033	0.000	0.000	0.116
Thiourea	0.157	0.007	0.170	0.019	0.187	0.127
MPA	0.167	0.111	0.207	0.000	0.077	0.132
DMP	0.026	0.016	0.000	0.000	0.000	0.000

^aAbbreviations: *o*-Phen, 1,10-phenanthroline; En, ethylenediamine; AA, acetylacetone; TEA, triethanolamine; DTCS, *N*-(dithiocarboxy)sarcosine; Trien, triethylenetetramine; MPA, 2-mercaptopropionic acid; DMP, 2,3-dimercapto-1-propanol. ^bIn the presence of each masking agent at the concentration examined, calcium (9.04×10^{-6} M) formed the HQAN chelate completely. ^cWhen Fe(III) was added, no interference was observed up to 2.1×10^{-6} M. At higher concentration, hydrated iron(III) oxide precipitated.

TABLE 4

Determination of calcium in potable waters^a

Sample	Calcium (this method)	(A.a.s.)	Magnesium (EDTA titn.)
Tap water 1	6.01	6.01	1.0
2	4.80	4.85	0.8
3	5.55	5.41	0.9
4	5.04	—	0.7
5	7.42	—	1.1
Well water 1	6.09	6.11	1.5
2	24.65	25.05	2.3

^aAll results are given as mg l⁻¹.

results were averaged (Table 4). When the concentration of calcium was relatively high (well water 2), the water was diluted.

The results by the proposed method are in good agreement with those obtained by atomic absorption spectrometry. The proposed method is very simple for the determination of calcium in potable waters.

Use of HQAN as a metallochromic indicator. The HQAN reagent was also applied to the compleximetric titration of calcium with EDTA at pH 9. A sharp color change from violet to orange was obtained by manual titration. The photometric titration curve is shown in Fig. 4.

The authors express their thanks to Mr. Tadao Ozawa for the atomic absorption spectrometric measurements.

REFERENCES

- 1 H. G. C. King and G. Pruden, *Analyst* (London), 94 (1969) 39.
- 2 I. Iwasaki, M. Yoshida and S. Yoshida, *Bunseki Kagaku*, 20 (1971) 302.
- 3 J. W. Ferguson, J. J. Richard, J. W. O'Laughlin and C. V. Banks, *Anal. Chem.*, 36 (1964) 796.
- 4 B. C. R. Sarkar and U. P. S. Chauhan, *Anal. Biochem.*, 20 (1967) 155.
- 5 K. Oguma, S. Ishino and R. Kuroda, *Bunseki Kagaku*, 33 (1984) 280.
- 6 H. Wada, G. Nakagawa and K. Ohshita, *Anal. Chim. Acta*, 159 (1984) 289.
- 7 T. Ohta, Y. Mori and S. Takagi, *J. Pharm. Soc. Jpn.*, 78 (1958) 697.
- 8 G. H. Hildebrand and C. N. Reilley, *Anal. Chem.*, 29 (1957) 258.
- 9 W. D. Johnston and H. Freiser, *J. Am. Chem. Soc.*, 74 (1952) 5239.
- 10 A. Kawase, *Bunseki Kagaku*, 16 (1967) 569.

DIFFERENCE SPECTROPHOTOMETRIC ASSAY OF MIXTURES OF PHENYLPROPANOLAMINE HYDROCHLORIDE WITH GUAIFENESIN OR DEXTROMETHORPHAN HYDROBROMIDE IN SOLID COUGH FORMULATIONS

HENRY S. I. TAN* and GERALDINE C. SALVADOR

College of Pharmacy, University of Cincinnati Medical Center, Cincinnati, OH 45267 (U.S.A.)

(Received 28th May 1985)

SUMMARY

A difference spectrophotometric method is described for the selective assay of phenylpropanolamine hydrochloride(I) in the presence of guaifenesin(II) or dextromethorphan hydrobromide(III) without prior separation. The method is based on the spectral change upon oxidation of phenylpropanolamine to benzaldehyde by sodium metaperiodate. The difference absorption spectrum is obtained by measuring oxidized against unoxidized phenylpropanolamine. This spectrum exhibits a maximum of 251.5 nm, a minimum at 275 nm and an isosbestic point at 272.5 nm. Absorbance is linear with concentration for 25–100 $\mu\text{g ml}^{-1}$ phenylpropanolamine at 251.5 nm. No changes in the spectra of compounds II and III were observed when these compounds were treated with metaperiodate. Guaifenesin and dextromethorphan are assayed by measuring an aliquot of the sample solution against methanol at 281.5 nm and 286 nm, respectively. Phenylpropanolamine does not interfere at these wavelengths. Calibrations are linear over the range 25–125 $\mu\text{g ml}^{-1}$ for II and III. Overall recoveries ($\pm\text{SD}$, $n = 5$) from simulated tablets were $99.8 \pm 2.6\%$ for I and $100.5 \pm 0.5\%$ for II; from simulated capsules, the recoveries were $99.2 \pm 0.4\%$ for I and $99.6 \pm 0.2\%$ for III. The assay was successfully applied to commercial tablets and capsules containing these compounds.

Phenylpropanolamine hydrochloride is one of the most widely used over-the-counter drugs because of its actions as an appetite suppressant and nasal decongestant. This drug was the focus of a conference held at the New York Academy of Medicine in 1984. The reported adverse effects attributed to the drug necessitated the development of a simple, yet reliable assay method to aid in the quality control of formulations containing phenylpropanolamine.

Most of assay methods involving mixtures of phenylpropanolamine with dextromethorphan or guaifenesin were based on chromatography. Mario and Meehan [1] developed a gas chromatographic method for the simultaneous determination of nonderivatized phenylpropanolamine, dextromethorphan, and guaifenesin in the presence of chlorpheniramine. An automated simultaneous determination of dextromethorphan, guaifenesin and phenylpropanolamine involved spectrophotometric measurements of dextromethorphan via ion-pair formation with bromocresol green, phenylpropanolamine via

the ninhydrin reaction, and guaifenesin via reaction with formaldehyde in methanolic sulfuric acid [2]. Mixtures of phenylpropanolamine with guaifenesin have been analyzed by using a bonded-phase cation-exchange column [3], and by reversed-phase ion-pair h.p.l.c. on a nitrile column [4], phenyl column [5, 6] or octadecylsilane column [7].

Difference spectrophotometry provides an alternative to chromatography. This method provides for automatic cancellation of interferences by other active and/or inactive components in the sample while retaining the inherent convenience of direct reading. Preliminary separation may thus not be necessary. The technique is applicable to all compounds that can be made to undergo reproducible spectral changes [8]. This report describes a spectrophotometric method based on an application of the difference spectrophotometric principle for the assay of phenylpropanolamine in the presence of guaifenesin or dextromethorphan. The spectrum of phenylpropanolamine was changed by oxidation of the compound in alkaline medium to benzaldehyde by means of metaperiodate [9, 10]. This treatment did not change the spectra of guaifenesin and dextromethorphan. The latter compounds were assayed by direct ultraviolet (u.v.) spectrophotometry at a wavelength where phenylpropanolamine did not interfere.

EXPERIMENTAL

Materials, reagents and equipment

The following drugs were used: phenylpropanolamine hydrochloride (Sigma Chemical Co.), guaifenesin (NF grade; International Chemical Labs., Eastchester, NY), dextromethorphan hydrobromide (USP grade; City Chemical Corp., New York). Solutions were aqueous 2.0% (w/v) sodium metaperiodate (Fisher Scientific), aqueous 10.0% (w/v) sodium metabisulfite (Matheson, Coleman & Bell), and aqueous 1.5% (w/v) disodium hydrogen phosphate. All other chemicals used were of analytical-reagent grade.

Instruments used were a DMS-100 u.v.-visible spectrophotometer (Varian Instruments), with bandwidth 1.0 nm, time constant 0.3 s and scan speed 50 nm min⁻¹, or an Acta-V spectrophotometer (Beckman) with slit width 7 mm. All spectrophotometric measurements were made with 1-cm cells.

Procedures

Standard solutions. About 25 mg of phenylpropanolamine hydrochloride and 200 mg of guaifenesin or 30 mg of dextromethorphan hydrobromide were accurately weighed and transferred to a 100-ml volumetric flask. The mixture was dissolved and diluted to volume with methanol.

Phenylpropanolamine hydrochloride. An aliquot (3 ml) of the standard solution was transferred to a 10-ml volumetric flask. To this solution were added 4.0 ml of the phosphate solution and 0.5 ml of the metaperiodate solution. After standing for 5 min, 0.5 ml of the metabisulfite reagent was added (Solution A). To another 10-ml volumetric flask, 0.5 ml of the

metaperiodate reagent, 4.0 ml of the phosphate reagent and 0.5 ml of the metabisulfite reagent were added. After 5 min, 3.0 ml of the standard solution was added (Solution B). Finally, Solutions A and B were diluted to volume with water. The absorbance of Solution A was measured at 251.5 nm against Solution B after raising the zero point (baseline).

Guaifenesin. Exactly 10.0 ml of the standard solution was transferred to a 100-ml volumetric flask and diluted to volume with methanol. From the resulting solution, 3.0 ml was pipetted into a 10-ml volumetric flask, diluted with methanol and mixed. The absorbance of the latter solution was measured against methanol at 281.5 nm.

Dextromethorphan hydrobromide. Exactly 3.0 ml of the standard solution was transferred to a 10-ml volumetric flask, diluted to volume with methanol and mixed. The absorbance of this solution was measured at 286 nm against methanol.

Analysis of tablets or capsules. Twenty tablets were weighed accurately and powdered. For capsules, the contents of twenty capsules were quantitatively removed and weighed accurately. Powder equivalent to 25 mg of phenylpropanolamine hydrochloride was weighed accurately and transferred to a 100-ml volumetric flask. After the addition of about 20 ml of methanol, the contents were sonicated for 10 s, diluted to volume with methanol, mixed and filtered; the first 5 ml of filtrate was discarded.

Two 3.0-ml aliquots of the filtrate were taken to prepare Solutions A and B as described above for the assay of standard phenylpropanolamine hydrochloride. For the assay of guaifenesin or dextromethorphan, an aliquot of the filtrate was subjected to the same procedure as described for the standard solutions of these compounds.

RESULTS AND DISCUSSION

The absorption spectrum of phenylpropanolamine in methanol shows three major peaks (Fig. 1). As can be expected from its chemical structure, no spectral change was observed when this compound was dissolved in acidic, neutral and basic media. Furthermore, as is common with the ephedrine, its molar absorptivity is low. The molar absorptivity of phenylpropanolamine is similar to that of norpseudoephedrine and is approximately 150 and 190 $\text{l mol}^{-1} \text{cm}^{-1}$ in methanol at 252 and 258 nm, respectively [11]. Oxidation of phenylpropanolamine by means of metaperiodate in alkaline medium has been studied [9, 10]. The reaction produces benzaldehyde which has a molar absorptivity of about $1.44 \times 10^4 \text{ l mol}^{-1} \text{cm}^{-1}$ at 241 nm in hydrocarbon solvents [12]. Metaperiodate oxidation did not change the absorption spectra of guaifenesin and dextromethorphan. The spectral change occurring with the oxidation of phenylpropanolamine forms the basis for the determination of this compound by difference spectrophotometry. In this technique, the reference cell contains phenylpropanolamine and the sample cell contains the oxidized phenylpropanolamine (as benzaldehyde) in the same concentration.

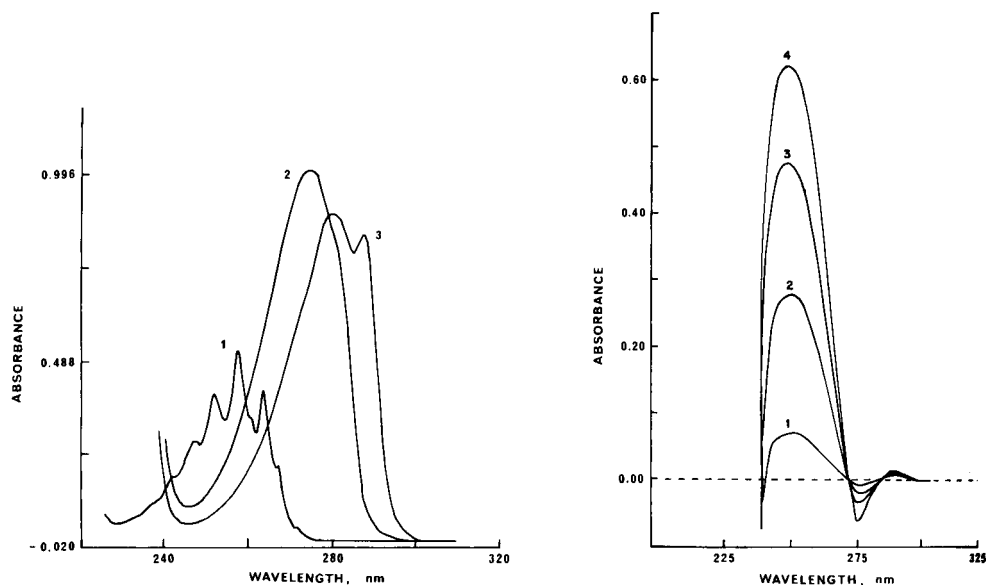


Fig. 1. Absorption spectra in methanol: (1) phenylpropanolamine hydrochloride; (2) guaifenesin; (3) dextromethorphan hydrobromide.

Fig. 2. Difference absorption spectra for different concentrations of phenylpropanolamine hydrochloride: ca. (1) 9.9; (2) 39.4; (3) 59.1; (4) 78.9 mg l⁻¹.

The recorded spectrum is the difference between the spectra of these two species. This difference spectrum exhibited a maximum at 251.5 nm corresponding to the net absorption by the sample, and a minimum at 275.0 nm corresponding to net absorption by the reference solution. An isosbestic point was observed at 272.5 nm (Fig. 2). Because guaifenesin, dextromethorphan and other ingredients are not affected by metaperiodate oxidation and these substances are present in equal concentrations in both the sample and reference cells, the net absorption of these substances is zero. Figure 2 shows the difference spectrum of phenylpropanolamine from simulated guaifenesin or dextromethorphan formulations. This spectrum intersects the baseline at the isosbestic point for the different concentrations of phenylpropanolamine. It confirms that interferences, if present, have cancelled at these points, and strongly suggests that this is true throughout the spectrum [8]. The strong u.v. absorption of excess of metaperiodate was eliminated by the addition of metabisulfite [13].

Guaifenesin or dextromethorphan hydrobromide was assayed by direct absorbance measurement in an area where phenylpropanolamine hydrochloride does not show u.v. absorption. Guaifenesin has an absorption maximum at 272.5 nm in methanol. However, at this wavelength phenylpropanolamine hydrochloride has some residual absorption. The wavelength

selected for guaifenesin was 281.5 nm; the absorbance of phenylpropanolamine is then zero while that of guaifenesin is still high (Fig. 1). Dextromethorphan hydrobromide shows two absorption maxima in methanol, at 278 and 286 nm; the latter wavelength was selected.

In order to show the complete individual absorption spectra in one figure (Fig. 1), the concentrations of the phenylpropanolamine hydrochloride, guaifenesin and dextromethorphan hydrobromide used were not in the ratios of the concentrations found in commercial preparations. When the concentration ratio of phenylpropanolamine hydrochloride to guaifenesin or dextromethorphan hydrobromide is similar to that in commercial preparations, the guaifenesin or dextromethorphan hydrobromide spectrum overlaps the spectrum for phenylpropanolamine hydrochloride severely. In this case, at the wavelength of maximum absorption of phenylpropanolamine (257 nm), the absorbance of this compound is about the same as that of dextromethorphan but is less than 1/15 of that of guaifenesin. This is a result of the low concentrations of phenylpropanolamine hydrochloride, particularly in relation to guaifenesin, coupled with the relatively low molar absorptivity of phenylpropanolamine. Simultaneous multicomponent determination of these combinations is thus not feasible for phenylpropanolamine hydrochloride.

The relationship between absorbance, A , and concentration C (mg ml^{-1}) in the final standard test solution was established for each compound. All data presented for phenylpropanolamine hydrochloride were obtained in the presence of guaifenesin or dextromethorphan hydrobromide whereas all data reported for any of the two latter compounds were obtained in the presence of phenylpropanolamine hydrochloride. Linear calibration was obtained for 25–100 $\mu\text{g ml}^{-1}$ phenylpropanolamine hydrochloride and for 25–125 $\mu\text{g ml}^{-1}$ guaifenesin or dextromethorphan hydrobromide. Typical regression equations were $A = 4.844 C - 0.114$ ($r = 0.998$) for phenylpropanolamine hydrochloride, $A = 9.384 C - 0.015$ ($r = 1.000$) for guaifenesin, and $A = 6.300 C + 0.004$ ($r = 0.999$) for dextromethorphan hydrobromide. Under the experimental conditions, the following $\log \epsilon$ ($\pm \text{SD}$) values were obtained ($n = 5$); phenylpropanolamine hydrochloride (measured as benzaldehyde from difference absorbance) 3.09 ± 0.12 ; guaifenesin 3.25 ± 0.05 and dextromethorphan hydrobromide 3.33 ± 0.02 .

The method was validated by assaying spiked tablet or capsule placebos. Tablet excipients consisted of mannitol, starch, magnesium stearate and hydro-soy oil. Excipients used for the capsule placebo were lactose, starch, calcium sulfate, magnesium stearate and a coloring agent. Five simulated tablets were prepared to contain 8.5–22.6 mg of phenylpropanolamine hydrochloride and 90.9–202.5 mg of guaifenesin in each tablet. After assay by the described procedure, 97.0–103.6% of phenylpropanolamine hydrochloride and 99.7–101.0% of guaifenesin were recovered. This corresponds to an overall percent recovery ($\pm \text{SD}$) ($n = 5$) of $99.8 \pm 2.6\%$ for phenylpropanolamine hydrochloride and $100.5 \pm 0.5\%$ for guaifenesin. The recovery in five simulated capsules containing 24.0–26.4 mg of phenylpropanolamine

hydrochloride and 28.9–29.7 mg of dextromethorphan hydrobromide in each capsule were 98.8–99.6% and 99.3–99.7%, respectively, resulting in an overall percent recovery ($\pm$ SD) ($n = 5$) of $99.2 \pm 0.4\%$ and $99.6 \pm 0.2\%$, respectively. The greater variation in the results of phenylpropanolamine hydrochloride from the mixture with guaifenesin is due to the large variation in the amounts of the compound taken whereas in the mixture with dextromethorphan hydrobromide the amount of phenylpropanolamine hydrochloride was about the same.

The method was successfully applied to the assay of mixtures of the compounds in commercial preparations. The recoveries of phenylpropanolamine hydrochloride and guaifenesin in various tablets with a label claim of 25.0 mg of phenylpropanolamine hydrochloride and 200.0 mg of guaifenesin per tablet, were 94.4–100.4% and 97.8–100.6% of label claim, respectively, with an overall percent label claim ($\pm$ SD) ($n = 5$) of $98.2 \pm 2.3\%$ and $99.4 \pm 1.2\%$, respectively. Assay of commercial capsules with a label claim per capsule of 25.0 mg of phenylpropanolamine hydrochloride and 30.0 mg of dextromethorphan hydrobromide gave recoveries of 97.6–100.0% and 100.3–101.7% of label claim, respectively, corresponding to an overall percent label claim ($\pm$ SD) ($n = 5$) of $98.6 \pm 1.1\%$ and $101.1 \pm 0.6\%$, respectively.

The proposed assay procedure was designed to allow individual dosage unit assay for the usual dosages used in tablet and capsule forms of phenylpropanolamine hydrochloride with guaifenesin or dextromethorphan hydrobromide.

This paper was presented in part at the 131st Annual Meeting of the APhA Academy of Pharmaceutical Sciences, Pharmaceutical Analysis and Control Section, Montreal, Quebec, Canada, May 1984.

REFERENCES

- 1 E. Mario and L. G. Meehan, *J. Pharm. Sci.*, **59** (1970) 538.
- 2 O. W. A. Weber and J. E. Heveran, *J. Pharm. Sci.*, **62** (1973) 1174.
- 3 G. B. Cox, C. R. Loscombe and K. Sugden, *Anal. Chim. Acta*, **92** (1977) 345.
- 4 A. G. Ghanekar and V. Das Gupta, *J. Pharm. Sci.*, **67** (1978) 873.
- 5 N. Muhammad and J. A. Bodnar, *J. Liq. Chromatogr.*, **3** (1980) 113.
- 6 A. G. Ghanekar and V. Das Gupta, *J. Pharm. Sci.*, **67** (1978) 1247.
- 7 G. W. Schieffer and D. E. Hughes, *J. Pharm. Sci.*, **72** (1983) 55.
- 8 T. D. Doyle and F. R. Fazzari, *J. Pharm. Sci.*, **63** (1974) 1921.
- 9 L. Chafetz, *J. Pharm. Sci.*, **60** (1971) 291.
- 10 H. S. I. Tan and G. C. Salvador, *J. Chromatogr.*, **261** (1983) 111.
- 11 J. Holubek (Ed.), *Spectral Data and Physical Constants of Alkaloids*, Vol. VII, Academia Publishing House of the Czechoslovak Academy of Sciences, Prague, 1972, No. 873.
- 12 L. Chafetz, *J. Pharm. Sci.*, **60** (1971) 291.
- 13 J. J. Pisano, *Clin. Chim. Acta*, **5** (1960) 406.

OPTIMAL PEAK AREA DETERMINATION IN THE PRESENCE OF NOISE

J. M. LAEVEN and H. C. SMIT*

*Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht
166, 1018 WV Amsterdam (The Netherlands)*

(Received 7th August 1984)

SUMMARY

A method is presented for determining optimal peak integration intervals on the basis of known peak shapes and noise characteristics. General theoretical considerations lead to conditions yielding optimal integration intervals. Examples of frequently occurring peak shapes and noise types are given, such as gaussian or skewed peak shapes, with band-limited first-order noise or flicker noise superimposed. The optimal integration intervals are approximately independent of the signal-to-noise ratio, and they are considerably smaller than the integration intervals normally used. The resulting expected peak area estimation errors are compared with the estimation error resulting from peak maximum amplitude measurement. On the basis of this comparison, rules of thumb are proposed to determine whether peak maximum measurement or peak integration yields the best results. Simulation of different peak shapes with noise superimposed confirms the results obtained. A flexible method is presented for the optimal measurement of the area of peaks with an unknown shape. This on-line method is simple, and could be used as a simple peak-finding procedure. The method requires almost no computer memory, and can be implemented on a microcomputer. A simulated example of this procedure is given.

In many analytical methods, the information concerning a physical or chemical quantity has to be extracted from a peak-shaped signal superimposed on a background contaminated with noise. The statistical properties of the noise always depend on the detector and system. The peak shape depends on the physicochemical processes involved. The problems involved in extracting the information reliably depend on the complexity of the signals, on prior knowledge about the system, and, of course, on the information required. Many, mostly semi-empirical, procedures have been developed for the information extraction, with varying results.

If the shape of the analytical signal is known to a certain extent, as it usually is, the first step is to apply a filter in order to reduce the noise without affecting the signal. The second step is the extraction of any qualitative information, if required, embedded in the signal. The third step concerns evaluation of the quantitative information required, in many cases represented by some parameters describing the peak, e.g., the peak top amplitude, the peak area or both. Obvious examples of methods where these two parameters provide important information are chromatography and spectrometry.

Depending on the requirements, the peak maximum or peak area can be evaluated by methods of varying complexity and accuracy. If there is no need for high precision and if the signal-to-noise ratio is large, the amplitude and peak area are often easily measured. If high precision is required and if the involved signal is complex, however, it takes much effort to retrieve the analytical signal and subsequently to estimate the required peak parameters in an optimum way. These procedures can be done either on-line or off-line [1-3].

In many analytical methods, peaks are more or less gaussian in shape and the peak area is usually measured by integration over an approximately $4\sigma_p$ interval which is symmetrical with respect to the peak maximum. It is useful to consider this procedure more precisely. From one point of view one can say, when choosing the $4\sigma_p$ interval, that the resulting contribution of bias will be sufficiently small. Larger intervals could cause serious problems related to resolution and smaller intervals yield a significant underestimate of peak area. Therefore the $4\sigma_p$ interval is an acceptable compromise in most practical situations. From another point of view, as noise is also integrated over the same interval, the uncertainty of the resulting area will increase with integration length, in a way dependent on noise type [4]. At first sight, these two points of view do not seem to lead to an optimum in integration length, because the bias cannot be related to the random error caused by noise integration. However, if certain assumptions are made, a procedure can be developed to produce an optimum integration interval, i.e., a minimum error variance in peak area estimation.

The aim of this study is to construct a procedure based on theoretical considerations, which yields some rules of thumb for deciding, in the case of the most frequently occurring peak shapes and noise types, whether and in which circumstances peak integration or peak maximum measurement will give better results. Certain strict assumptions are required to arrive at this procedure. However, for integration of peaks of unknown shape, it is possible to develop a procedure, for which almost no assumptions are necessary, that seems very suitable for simple data-processing in chromatography and related techniques.

PEAK INTEGRATION MODEL

The functions $s(t)$ and $n(t)$, respectively describing the peak shape and the noise, and the measured signal $x(t)$, being the sum of $s(t)$ and $n(t)$, are defined. For convenience it is assumed that the independent variable is given in time units but of course any other unit can be substituted.

$$x(t) = s(t) + n(t) \quad (1)$$

(Table 1 lists the symbols and definitions.)

In addition, a function $I_x(T)$ is defined to describe the integrated measured signal $x(t)$ as a function of the integration length T :

$$I_x(T) = \int_{u-1/2T}^{u+1/2T} x(t)dt \quad (2)$$

provided that the peak shape is symmetric. For asymmetric peaks, the integrated area is divided into left and right parts (with respect to the peak maximum):

$$I_x(T_L) = \int_{u-T'_L}^u x(t)dt \quad (3)$$

$$I_x(T_R) = \int_u^{u+T'_R} x(t)dt \quad (4)$$

where u is the time at the peak maximum. Careful inspection of the integrals can help to formulate the problem of how an optimal estimate of the peak area is to be obtained. Integration over an infinite interval would yield the unbiased peak area, but with a random error of infinite variance because noise is also integrated. If the peak shape is exactly known, the relation between the peak maximum amplitude and the peak area can be found easily, and one could confine oneself to amplitude measurement. For this case, an indirect unbiased peak area estimate is also obtained, but with a random error which is a function of the noise variance and the conversion factor relating the peak top amplitude to the peak area. Alternatively integration over a limited interval can be considered. In this case, the resulting area will always be biased whereas the random error is governed by the integration interval and the noise parameters. In practice, common sense helps to develop the integration rules. The integration interval should be chosen so that the peak has no significant area outside that interval. In addition, some information concerning the peak shape should be available to obtain an estimate of the remaining bias. Furthermore, the noise characteristics have to be known to some extent in order to present the integration result with the appropriate confidence interval. Because the noise within the integration interval is unknown, these characteristics have to be evaluated from large noise records.

From these considerations, it is possible to quantify the peak area and its confidence interval. However, depending on the available prior knowledge, rather complex mathematical procedures may still be necessary, e.g., Kalman filtering (on-line) or curve-fitting procedures (non-linear regression) [5-7]. To arrive at simpler procedures, some prerequisites are necessary; a reliable estimate of the baseline is required and the peak shape and peak maximum position must be known. In this paper, these prerequisites will be assumed. Whether this is realistic is not discussed here. It is shown that these assumptions allow the development of an optimum integration procedure under different conditions. It is possible to analyze the consequence of the assump-

TABLE 1

List of symbols and definitions^a

Symbol	Definition
t	time
$s(t)$	peak shape
u	peak maximum position
$s(u)$	peak maximum amplitude
T	integration interval length (symm. peak)
T'_R	right integration interval length (asymm. peak)
T'_L	left integration interval length (asymm. peak)
$T^1 \equiv u + 1/2T$	right integration limit (symm. peak)
$T^2 \equiv u - 1/2T$	left integration limit (symm. peak)
$T_R \equiv u + T'_R$	right integration limit (asymm. peak)
$T_L \equiv u - T'_L$	left integration limit (asymm. peak)
$I_\infty \equiv \int_{-\infty}^{\infty} s(t)dt$	total peak area (symm. peak)
$I_{R,\infty} \equiv \int_u^{\infty} s(t)dt$	total right peak area (asymm. peak)
$I_{L,\infty} \equiv \int_{-\infty}^u s(t)dt$	total left peak area (asymm. peak)
$I(T) \equiv \int_{T_2}^{T_1} s(t)dt$	peak area in interval T (symm. peak)
$I_R(T_R) \equiv \int_u^{T_R} s(t)dt$	right peak area in interval T'_R (asymm. peak)
$I_L(T_L) \equiv \int_{T_L}^u s(t)dt$	left peak area in interval T'_L (asymm. peak)
$I^1(T) \equiv I(T)/I_\infty$	integrated peak fraction (symm. peak)
$I_R(T_R) \equiv I_R(T_R)/I_\infty$	integrated right peak fraction (asymm. peak)
$I_L(T_L) \equiv I_L(T_L)/I_\infty$	integrated left peak fraction (asymm. peak)
A	asymmetry factor
$n(t)$	(reduced) baseline noise
$E[n(t)] = 0$	baseline noise mean
$E[n^2(t)] \equiv \sigma_n^2$	baseline noise variance

TABLE 1 (continued)

$I_n(\theta) \equiv \int_{T_B}^{T_A} n(t)dt$	integrated noise in interval $T_A - T_B = \theta$
$\sigma_{I_n}^2(\theta) \equiv E[I_n^2(\theta)]$	variance of integrated noise
$R_{nn}(\tau) \equiv \lim_{T \rightarrow \infty} 1/T \int_{-1/2T}^{1/2T} n(t)n(t+\tau)dt$	autocovariance function of baseline noise
$R_{nn}(0) = \sigma_n^2$	baseline noise variance
$x(t) \equiv s(t) + n(t)$	measured signal (peak + noise)
$I_x(T_*) \equiv I_*(T_*) + I_n(T_*)$	integrated measured signal
$\hat{I}_\infty \equiv I_x(T_*)/I_*(T_*)$	peak area estimator
$\mu_2(T_*) \equiv E[I_\infty - \hat{I}_\infty]^2$	error variance in peak area estimation
$\mu'_2(\theta) = d\mu_2(\theta)/d\theta$	first derivative of $\mu_2(\theta)$

^aA subscript asterisk of the integration interval T in the equations is used to abbreviate the notation. It can be replaced, as required, by R if the equation refers to the right part of an asymmetric peak, L if the equation refers to the left part of an asymmetric peak, or nothing at all if the equation refers to a symmetric peak.

tions on the results afterwards. Restrictions may be eased to obtain a less restricted optimum integration procedure. If the mentioned prerequisites are satisfied, the only unknown parameter describing the peak is the area (or the peak maximum amplitude). For an estimate of the true area, one has a measured area, obtained from a freely chosen interval, the normalized peak (with unit area), and the predetermined statistical properties of the noise, i.e., its mean value, variance and autocovariance function. In the following, it is assumed that the baseline is subtracted from the measured signal, and that the mean of the noise is zero.

The normalized peak area in an interval of length T is defined as

$$I^1(T) = (1/I_\infty) \int_{u-1/2T}^{u+1/2T} s(t)dt \quad (5)$$

where I_∞ is the true peak area (integrated over an infinite interval). The estimator for the peak area (Fig. 1) is

$$\hat{I}_\infty \equiv I_x(T)/I^1(T) \quad (6)$$

Substitution of Eqns. 1, 2 and 5 in Eqn. 6 shows the relationship between the estimated and the real peak area:

$$\hat{I}_\infty = I_\infty \left\{ 1 + \left[\int_{u-1/2T}^{u+1/2T} n(t)dt \right] / \left[\int_{u-1/2T}^{u+1/2T} s(t)dt \right] \right\} \quad (7)$$

For a fixed integration interval T , the denominator has a fixed positive value

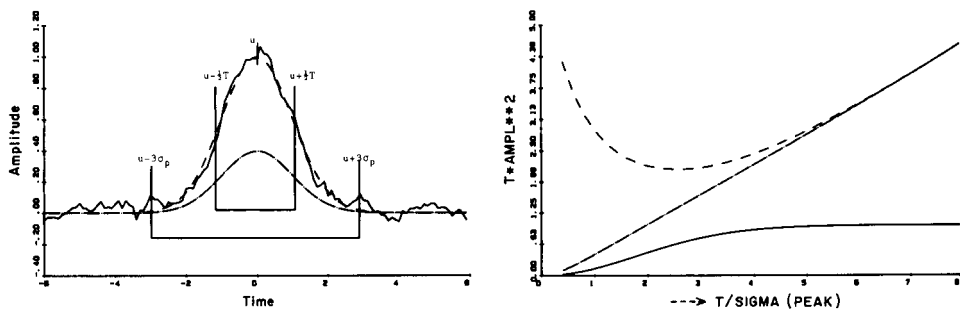


Fig. 1. Area estimator for a symmetric peak (amplitude vs. time): (---) $s(t)$ for a symmetric peak with area I_∞ ; (-.-) $s(t)$ for a symmetric peak with unit area, $I^1(\infty) = 1$; (—) $x(t)$ for a symmetric peak with noise added. First the measured peak $x(t)$ is integrated within the indicated interval (small U-shape), then the result $I_x(T)$ is divided by the predetermined area $I^1(T)$ within the corresponding interval of the peak with unit area. The big U-shape encloses 99.74% ($6\sigma_p$ interval) of the peak area.

Fig. 2. The variance of the peak area estimation error as a function of the integration interval: (—) $I^1(T)^2$, squared area of a peak with unit area (if $T \rightarrow \infty$); (-.-) $\sigma_{I_n}^2(T)$, variance of the integrated baseline noise; (-.-) $\mu_2(T) = \sigma_{I_n}^2(T)/I^1(T)^2$, peak area estimation error variance. The units of the y-axis are arbitrary.

and the numerator is a random variable, thus the expected value of the estimated peak area is

$$E[\hat{I}_\infty] = I_\infty \quad (8)$$

Thus, $\hat{I}_\infty$ is an unbiased estimator for I_∞ . To establish the optimum integration interval, the best procedure is to minimize the error variance for the peak area estimate; this variance is defined as

$$\mu_2(T) \equiv E[I_\infty - \hat{I}_\infty]^2 \quad (9)$$

Substitution of Eqns. 1 and 5 in Eqn. 9 (see Appendix) yields

$$\mu_2(T) = E\{I_n(T)/I^1(T)\}^2 = \sigma_{I_n}^2(T)/I^1(T)^2 \quad (10)$$

where $I_n(T)$ is the integrated noise, and $\sigma_{I_n}^2(T)$ is the variance of the integrated noise as a function of the length of the integration interval T [8]. A close look at Eqn. 10 reveals its characteristics: generally, $\sigma_{I_n}^2(T)$ and $I^1(T)$ are monotonic increasing functions of T (e.g., $I^1(T)$ is the error function in case of a gaussian peak). Figure 2 shows an example of the error variance of the peak area estimate and its constituents. Generally the function of Eqn. 10 has a minimum for some value of T , which will be the optimum integration interval. Minimization leads to

$$I^1(T)d\sigma_{I_n}^2(T)/dT = 2\sigma_{I_n}^2(T)dI^1(T)/dT \quad (11)$$

Depending on the properties of the constituent parts of Eqn. 11, the opti-

imum integration interval can be found. Because $\sigma_n^2(T)$ can be evaluated [4], Eqn. 11 can be refined (Appendix):

$$\int_{u-1/2T}^{u+1/2T} s(t)dt \cdot \int_0^T R_{nn}(\tau)d\tau = 2 \int_0^T (T-\tau)R_{nn}(\tau)d\tau [s(u+1/2T)] \quad (12)$$

For symmetrical peaks, where $R_{nn}(\tau)$ is the autocovariance function (ACVF) of the noise,

$$R_{nn}(\tau) \equiv \lim_{T \rightarrow \infty} (1/T) \int_{-1/2T}^{1/2T} n(t)n(t+\tau)dt \quad (13)$$

$R_{nn}(\tau)$ has to be determined from large noise records to obtain a reliable estimate. For asymmetric peaks the following equations are obtained for the left and right part respectively (Appendix):

$$\int_{u-T'_L}^u s(t)dt \cdot \int_0^{T'_L} R_{nn}(\tau)d\tau = 2 \int_0^{T'_L} (T'_L - \tau)R_{nn}(\tau)d\tau [s(u - T'_L)] \quad (14)$$

$$\int_u^{u+T'_R} s(t)dt \cdot \int_0^{T'_R} R_{nn}(\tau)d\tau = 2 \int_0^{T'_R} (T'_R - \tau)R_{nn}(\tau)d\tau [s(u + T'_R)] \quad (15)$$

Thus, using Eqns. 12, 14 and 15, optimal integration intervals (if any) can be obtained for any peak shape and any (stationary, ergodic) type of noise.

As mentioned before, the information obtained by integration of a peak is related in some way with the peak maximum amplitude:

$$I_\infty = ks(u) \quad (16)$$

where $s(u)$ is measured

$$x(u) = s(u) + n(u) \quad (17)$$

The estimator for $\hat{I}_\infty$ obtained from peak maximum measurement will be

$$\hat{I}_\infty = kx(u) \quad (18)$$

from which the error variance in the peak area estimate can be computed:

$$\mu_2 = E\{\hat{I}_\infty - I_\infty\}^2 = k^2 \sigma_n^2 \quad (19)$$

where σ_n^2 is the variance of the baseline noise. Thus the variances obtained from Eqns. 10 and 19 have to be compared, to decide whether peak-height or peak-area measurement is better.

Specific examples

If a gaussian peak is defined as

$$s(t) = I_\infty/\sigma_p\sqrt{2\pi} \exp[-(t-u)^2/2\sigma_p^2] \quad (20)$$

then the area in an interval T symmetrical with respect to u under the normalized peak (area = 1 for infinite integration) will be

$$I^1(T) = \text{erf}(T/2\sigma_p\sqrt{2}) \quad (21)$$

where the error function is defined as:

$$\text{erf}(z) = (2/\sqrt{\pi}) \int_0^z \exp(-t^2) dt \quad (22)$$

The peak amplitude at the integration boundaries is

$$s(u + 1/2T) = (1/\sigma_p\sqrt{2\pi}) \exp(-T^2/8\sigma_p^2) \quad (23)$$

For asymmetric gaussian peaks, the approximation by Fraser and Suzuki [9] is chosen:

$$s(t) = \{I_\infty / [\sigma_p\sqrt{2\pi} \exp(A^2/4\ln 2)]\} \exp\{-(\ln 2/A^2)\ln^2[1 + A(t-u)/\sigma_p\sqrt{2\ln 2}]\} \quad (24)$$

where A is the asymmetry factor. If the integrated area is divided into left and right parts with respect to the peak maximum, the respective areas of the normalized peak can be evaluated as a function of the integration length T (appendix):

$$I_L^1(T'_L) = 1/2\{\text{erf}[p - 1/2p\ln(1 - p\sqrt{2}T'_L/\sigma_p)] - \text{erf}(p)\} \quad (25)$$

$$I_R^1(T'_R) = 1/2\{\text{erf}(p) - \text{erf}[p - 1/2p\ln(1 + p\sqrt{2}T'_R/\sigma_p)]\} \quad (26)$$

where

$$p \equiv A/2\sqrt{\ln 2} \quad (27)$$

and $I^1(T'_L)$ and $I^1(T'_R)$ are the left and right areas of the normalized asymmetric peak, respectively. The peak amplitudes at the integration boundaries are

$$s(u - T'_L) = (1/\sqrt{2\pi})I_\infty [\sigma_p(1 - p\sqrt{2}T'_L/\sigma_p)] \exp\{-[p - 1/2p\ln(1 - p\sqrt{2}T'_L/\sigma_p)]^2\} \quad (28)$$

$$s(u + T'_R) = (1/\sqrt{2\pi})I_\infty [\sigma_p(1 + p\sqrt{2}T'_R/\sigma_p)] \exp\{-[p - 1/2p\ln(1 + p\sqrt{2}T'_R/\sigma_p)]^2\} \quad (29)$$

The peak-describing part of Eqns. 12, 14 and 15 can now be evaluated. Of course, it is also possible to substitute any other peak-shape model.

Some remarks concerning integration of noise over some interval T are needed. As mentioned above, noise has a random character, thus the result of integrating noise can never be estimated exactly if some analytical signal is superimposed. Applying an (optimal) filter is a first step to reduce the influence of the noise on the parameter of interest. Hereafter, an estimate of the variance of the integrated (reduced) noise over the interval of interest

is made. Because the noise in that interval is unknown, the predetermined statistical parameters of the (stationary, ergodic) noise are used, i.e., the ensemble mean of many intervals of equal length is used. Because the integration interval T is usually small (about 10–20 data points) it should be noted that the statistical parameters used have a considerable standard deviation in that interval.

For band-limited first-order noise the ACVF is

$$R_{nn}(\tau) = \sigma_n^2 \exp(-|\tau|/T_x) \quad (30)$$

which leads to a variance of the integrated noise in the interval T [4] (Appendix) of

$$\sigma_{I_n}^2(T) \approx 2\sigma_n^2 T_x T \quad (31)$$

and

$$d\sigma_{I_n}^2(T)/dT \approx 2\sigma_n^2 T_x \quad (32)$$

For $1/f$ noise the approximated ACVF [10] is

$$R_{nn}(\tau) = [\sigma_n^2/\ln(t_n/t_m)] [E_1(|\tau|/t_n) - E_1(|\tau|/t_m)] \quad (33)$$

where E_1 is an exponential integral [11]

$$E_1(x) = \int_x^\infty \frac{e^{-t}}{t} dt \quad (34)$$

Computation of $\sigma_{I_n}^2(T)$ and its derivative for this type of noise leads to (Appendix)

$$\begin{aligned} \sigma_{I_n}^2(T) = & [2\sigma_n^2/\ln(t_n/t_m)] [T(t_n - t_m) - 1/2(t_n^2 - t_m^2) + t_n^2 E_3(T/t_n) \\ & - t_m^2 E_3(T/t_m)] \end{aligned} \quad (35)$$

where

$$E_n(z) = \int_1^\infty [\exp(-zt)/t^n] dt \quad (36)$$

and

$$d\sigma_{I_n}^2(T)/dT = [2\sigma_n^2/\ln(t_n/t_m)] [t_n - t_m - t_n E_2(T/t_n) + t_m E_2(T/t_m)] \quad (37)$$

Once the necessary expressions for the peak shape and the noise type are derived, they can be substituted in Eqns. 12, 14 and 15 to obtain the relations required to compute the optimum integration intervals.

RESULTS

The optimum integration intervals for some peak shapes with noise superimposed were evaluated. Symmetric gaussian peaks with band-limited, first-order noise or $1/f$ (flicker) noise [12] superimposed, and asymmetric peaks with the same two noise types superimposed were tested.

Gaussian peak with first-order noise

Substitution of Eqns. 21, 23, 30 and 32 in Eqns. 11 and 12 yields

$$\operatorname{erf}(T/2\sigma_p\sqrt{2}) = \sqrt{2/\pi}(T/\sigma_p)\exp(-T^2/8\sigma_p^2) \quad (38)$$

This relation is satisfied for:

$$T_{\text{opt}} \approx 2.8\sigma_p \quad (39)$$

The corresponding error variance in the peak area estimate in this interval is

$$\mu_2(T_{\text{opt}}) = [5.6\sigma_n^2 T_x \sigma_p / \operatorname{erf}^2(0.99)] \approx 8\sigma_n^2 T_x \sigma_p \quad (40)$$

It can be seen that the optimum integration interval for this combination is independent of the time constant and the variance of the noise. The resulting estimation error, however, depends on these two parameters. The result offers the opportunity to formulate a very simple rule of thumb for peak integration for this peak shape and noise type: integrate the peak from peak maximum minus $1.4\sigma_p$ to peak maximum plus $1.4\sigma_p$. Although neither the peak maximum position nor σ_p is exactly known, the two parameter values can be estimated with sufficient accuracy to be used in this approximation by using the estimator of Eqn. 6. It must be mentioned that the μ_2 vs. T curve is rather flat in the minimum region. Consequently, if the integration interval is made somewhat different, the error variance in the peak area estimate will not increase dramatically. Even if the estimated peak maximum position differs by $0.2\sigma_p$ from the true position, the estimated integrated part under the gaussian curve deviates only 1% from the best estimation.

If integration were carried out over a $6\sigma_p$ interval (symmetric with respect to the peak maximum), the error variance in the peak area estimate is only 1.5 times larger than the variance resulting from the optimum integration interval. Consequently, the gain is not very great, but if peaks overlap, the gain can be much greater. Of course, the peak area estimator has to be adopted if the overlap between the peaks is large.

Symmetric peak with flicker noise

Substitution of Eqns. 21, 23, 35 and 37 in Eqns. 11 and 12 yields the following relation:

$$\begin{aligned} \operatorname{erf}(T/2\sigma_p\sqrt{2})[t_n - t_m - t_n E_2(T/t_n) + t_m E_2(T/t_m)] = \\ = \sqrt{2/\pi}(1/\sigma_p) \exp(-T^2/8\sigma_p^2)[T(t_n - t_m) - 1/2(t_n^2 - t_m^2) + t_n^2 E_3(T/t_n) \\ - t_m^2 E_3(T/t_m)] \end{aligned} \quad (41)$$

If $t_n \gg T \approx t_m$, Eqn. 41 can be simplified to

$$\operatorname{erf}(T/2\sigma_p\sqrt{2}) = (1/\sqrt{2\pi})(T/\sigma_p) \exp(-T^2/8\sigma_p^2) \quad (42)$$

If this equation is compared with Eqn. 38 for a gaussian peak contaminated with first-order noise, the difference is only a factor of two in the right part of the equation. Yet Eqn. 42 cannot be satisfied except for $T = 0$, meaning

no integration at all. The general solution of Eqn. 41 is too complicated for the present purpose. Later, a simulated example is given, where an optimum integration interval is obtained. Generally, it should be noted that flicker noise always presents more problems than band-limited first-order noise, because the power of flicker noise in the power spectrum is considerable in the same frequency region where analytical signals are present.

Asymmetric peak with first-order noise

Substitution of Eqns. 25, 26, 28 and 29 in Eqns. 14 and 15 leads to the following expressions for the left and right integration intervals:

$$\operatorname{erf}(p) - \operatorname{erf}(Q) = (8/\pi)^{1/2} (T'_R/\sigma_p) [1/(1 + p\sqrt{2}T'_R/\sigma_p)] \exp(-Q^2) \quad (43)$$

$$\operatorname{erf}(S) - \operatorname{erf}(p) = (8/\pi)^{1/2} (T'_L/\sigma_p) [1/(1 - p\sqrt{2}T'_L/\sigma_p)] \exp(-S^2) \quad (44)$$

where

$$Q \equiv p - \frac{1}{2p} \ln(1 + p\sqrt{2}T'_R/\sigma_p) \quad (45)$$

$$S \equiv p - \frac{1}{2p} \ln(1 - p\sqrt{2}T'_L/\sigma_p) \quad (46)$$

The equations 43 and 44 can be solved for particular values of the parameter p (related to the asymmetry factor of the peak, see Eqns. 24 and 27). In this study the optimum left and right integration intervals were calculated numerically. The resulting optimum intervals are approximated by the equations:

$$T'_L \approx 1.4\sigma_p \exp(-0.57A) \quad (47)$$

$$T'_R \approx 1.4\sigma_p \exp(0.77A) \quad (48)$$

These approximations are not obtained by means of any (expected) model. A simple curve-fitting procedure with an exponential function was used, because the aim was to obtain rules of thumb for practical application in which, if possible, complex relations should be avoided, and because in the limit case ($A = 0$) the curves for asymmetric and symmetric gaussian peaks are identical so that we expect from the first results that the slope of the μ_2 curve vs. the integration interval is flat in the minimum region. Because of the flat shape of this curve small changes of the integration length have no significant influence on the ultimate error variance in the peak area estimate. This variance is obtained from Eqns. 9 and 10 by substituting Eqns. 28, 29 and 32:

$$\mu_2(T_{L,\text{opt}}) = 8\sigma_n^2 T'_L T_x / \{\operatorname{erf}[p - 1/2p \ln(1 - p\sqrt{2}T'_L/\sigma_p)] - \operatorname{erf}(p)\}^2 \quad (49)$$

$$\mu_2(T_{R,\text{opt}}) = 8\sigma_n^2 T'_R T_x / \{\operatorname{erf}(p) - \operatorname{erf}[p - 1/2p \ln(1 + p\sqrt{2}T'_R/\sigma_p)]\}^2 \quad (50)$$

If the approximated integration intervals in Eqns. 47 and 48 were to be

substituted in Eqns. 49 and 50, it would yield an unmanageable result. However, a useful approximation of the result is

$$\mu_2(T_{L,\text{opt}}) \approx 8\sigma_n^2 T_x \sigma_p [\exp(-1.10A) + \exp(1.69A)] \quad (51)$$

$$\mu_2(T_{R,\text{opt}}) \approx 8\sigma_n^2 T_x \sigma_p [\exp(-1.91A) + \exp(0.85A)] \quad (52)$$

Because a zero asymmetry factor should yield the same result as in the case of a symmetric gaussian peak, the respective results have to be compared. Because the integration interval is divided in a right and left part, the total peak area estimate is obtained by a linear combination of the left and right area estimators:

$$\hat{I}_\infty(T_{\text{opt},L} + T_{\text{opt},R}) = \lambda_1 \hat{I}_\infty(T_{\text{opt},L}) + \lambda_2 \hat{I}_\infty(T_{\text{opt},R}) \quad (53)$$

The two estimators obtained are not entirely independent: as the noise is assumed to be correlated, the integrated noise of the left and right integration intervals will also be correlated. However, it can be shown that in practice this effect can be neglected, so that Eqn. 53 can be used. For this case, the weighting coefficients λ_1 and λ_2 are well known:

$$\begin{aligned} \hat{I}_\infty(T_{\text{opt},L} + T_{\text{opt},R}) &= [\mu_2(T_{\text{opt},L}) \hat{I}_\infty(T_{\text{opt},R}) \\ &+ \mu_2(T_{\text{opt},R}) \hat{I}_\infty(T_{\text{opt},L})] / [\mu_2(T_{\text{opt},L}) + \mu_2(T_{\text{opt},R})] \end{aligned} \quad (54)$$

and the ultimate error variance in the peak area estimate is

$$\mu_2(T_{\text{opt},L} + T_{\text{opt},R}) = [\mu_2(T_{\text{opt},L}) \mu_2(T_{\text{opt},R})] / [\mu_2(T_{\text{opt},L}) + \mu_2(T_{\text{opt},R})] \quad (55)$$

When the asymmetry factor decreases to zero, $\mu_2(T_{\text{opt},L})$ and $\mu_2(T_{\text{opt},R})$ are equal because of reasons of symmetry. Thus Eqns. 54 and 55 become

$$\hat{I}_\infty(T_{\text{opt},L} + T_{\text{opt},R}) = 1/2 \hat{I}_\infty(T_{\text{opt},L}) + 1/2 \hat{I}_\infty(T_{\text{opt},R}) \quad (56)$$

$$\mu_2(T_{\text{opt},L} + T_{\text{opt},R}) = 1/2 \mu_2(T_{\text{opt},L}) = 1/2 \mu_2(T_{\text{opt},R}) \quad (57)$$

Substitution of Eqn. 51 or 52 in Eqn. 57, and $A = 0$ in Eqns. 47 and 48 yields

$$T'_L = 1.4\sigma_p \quad \text{and} \quad T'_R = 1.4\sigma_p \quad (58)$$

$$\mu_2(T_{\text{opt},L} + T_{\text{opt},R}) = 8\sigma_n^2 T_x \sigma_p \quad (59)$$

which is the same result as obtained in Eqn. 40.

In Figs. 3 and 4 are shown the optimal integration intervals for symmetric and asymmetric gaussian peaks with band-limited first order noise superimposed. It is seen from Eqns. 47 and 48, that, with increasing asymmetry, the left optimum integration interval decreases, but that the error variance in the peak area estimate increases. The right integration interval increases with the asymmetry and accordingly the error variance in the peak area estimate also increases.

In Fig. 5 the error variances in the peak area estimates are depicted for the left and right integration intervals. Also shown is the ultimate variance,

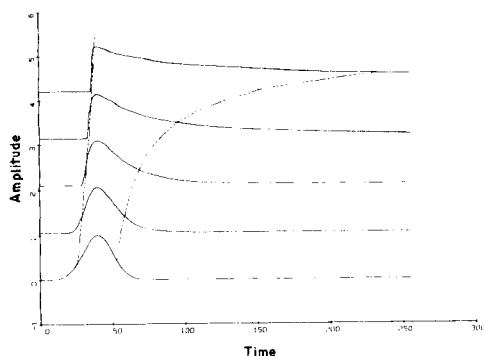
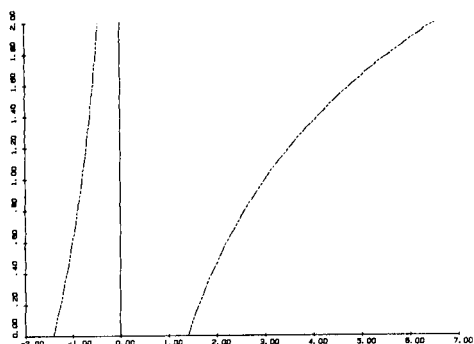


Fig. 3. Left and right optimum integration interval as a function of the asymmetry factor for (skewed) gaussian peaks with band-limited first-order noise superimposed. The asymmetry factor is given on the y-axis and the optimum integration interval in units of the peak standard deviation on the x-axis.

Fig. 4. Left and right optimum integration intervals for (skewed) gaussian peaks (—) with asymmetry factors of 0.0, 0.5, . . . , 2.0, respectively, with band-limited first-order noise superimposed. The limits of optimum integration are indicated by the dashed lines.

resulting from a linear combination of the left and right part estimators according to Eqn. 55. Although the distinct variances are rather sensitive to a change in the asymmetry factor, it can be seen that the ultimate (combined) variance remains approximately constant if the asymmetry factor varies from 0 to 1.

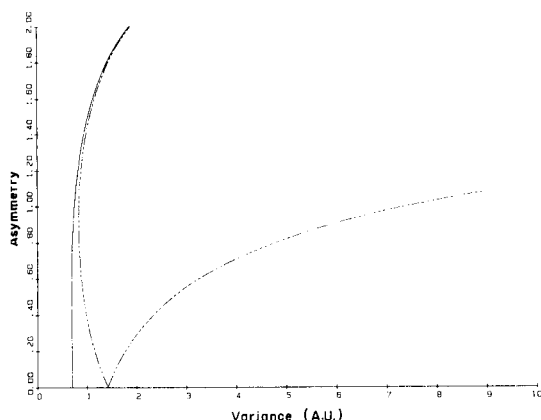


Fig. 5. Peak area estimation error variance (arbitrary units) of (skewed) gaussian peaks with band-limited first-order noise superimposed, as a function of the asymmetry factor. (---) Left curve shows the variance resulting from integration of the left part of the peak; right curve shows the variance resulting from integration of the right part of the peak. (—) Combined variance, resulting from the combined, weighted left and right part variances (Eqn. 55).

Asymmetric peak with flicker noise

Evaluation of the appropriate equations is very complicated for this case and is not done here. As stated for the corresponding case with a symmetric peak, flicker noise is difficult to handle. Depending on the asymmetry factor and the noise time constants t_n and t_m , it is expected that, in some cases, optimum integration intervals will be obtained, but it seems impossible to give simple rules of thumb for such cases.

PEAK MAXIMUM MEASUREMENT

In many analytical problems, measurements of peak height (maximum), and peak area are related. The results obtained for the determination of peak area by integration should therefore be compared with "simple" peak maximum measurement to decide which method is better.

Peak maximum measurement can be represented by

$$x(u) = s(u) + n(u) \quad (60)$$

where $x(u)$ is the measured peak maximum amplitude, $s(u)$ the real peak maximum amplitude, and $n(u)$ the noise contribution. Substitution of $s(u)$ by Eqn. 20 or 24 for symmetric and asymmetric peaks yields:

$$x(u) = (I_\infty / \sigma_p \sqrt{2\pi}) + n(u) \quad (61)$$

$$x(u) = I_\infty / [\sigma_p \sqrt{2\pi} \exp(A^2/4\ln 2)] + n(u) \quad (62)$$

From these equations, the estimators for the peak area are obtained:

$$\hat{I}_\infty = \sigma_p \sqrt{2\pi} x(u) \quad (63)$$

for the symmetric peak, and

$$\hat{I}_\infty = \sigma_p \sqrt{2\pi} \exp(A^2/4\ln 2) x(u) \quad (64)$$

for the asymmetric peak. The corresponding error variances in the peak area estimates are easily computed:

$$\mu_2 = E[\hat{I}_\infty - I_\infty]^2 = 2\pi\sigma_p^2\sigma_n^2 \quad (65)$$

and

$$\mu_2 = 2\pi\sigma_p^2\sigma_n^2 \exp(A^2/4\ln 2) \quad (66)$$

Comparison of peak area integration and peak maximum amplitude

Now that the optimum integration intervals and the corresponding peak area estimation error variances for different noise types and peak shapes have been established, the resulting variances can be compared with the variance resulting from peak maximum amplitude measurement.

For flicker (1/f) noise superimposed on symmetric or asymmetric gaussian peaks, quantitative comparison is not possible, because of the very complicated nature of the relations involved. Yet some observations are possible

The fact that flicker noise exhibits much power down to very low frequencies in the power spectrum, indicates that integration then is much less favourable than in the case of first-order noise. To be more specific, a simple example will show the difficult nature of flicker noise. If the mean value of a signal, with first-order noise or flicker noise superimposed, has to be determined, then the noisy signal is integrated over some interval T . The variance of the integrated noise for both noise types can be roughly approximated: for first-order noise this variance is approximately proportional with the integration interval T , whereas for flicker noise the variance is about proportional with T^2 [4]. Thus, the variance of the error in the determination of the mean is inversely proportional to the integration length for first-order noise, and is independent of the integration length for flicker noise, i.e., increasing the integration length yields a better estimate of the mean if first-order noise is present. But if flicker noise is present, an increase of the integration interval is of no use. A general conclusion for flicker noise is therefore, that peak maximum amplitude measurement should be preferred to avoid a complicated analysis of the flicker noise and peak parameters.

For gaussian and skewed "gaussian" peaks with first-order noise superimposed, quantitative comparison is possible by means of the error variances in peak area estimates. Optimal peak integration will yield better results compared to peak amplitude measurement provided that, for gaussian peaks (Eqns. 40 and 65),

$$8\sigma_n^2 T_x \sigma_p < 2\pi\sigma_p^2 \sigma_n^2 \quad (67)$$

or

$$T_x < 0.785\sigma_p \quad (68)$$

In practice the time constant will usually satisfy the condition, implying integration. But, if the time constant is evaluated or already known, and if the expected standard deviation of the peak is known, condition 68 can be tested and the appropriate method chosen.

For skewed gaussian peaks the same procedure is followed, leading to the following condition using Eqns. 51, 52, 55 and 66:

$$\begin{aligned} 8\sigma_n^2 T_x \sigma_p \{ [\exp(1.69A) + \exp(-1.10A)] [\exp(0.85A) \\ + \exp(-1.91A)] / [\exp(1.69A) + \exp(-1.10A) + \exp(0.85A) \\ + \exp(-1.91A)] \} > 2\pi\sigma_n^2 \sigma_p^2 \exp(A^2/4\ln 2) \end{aligned} \quad (69)$$

or

$$T_x > 0.785\sigma_p \exp(A^2/4\ln 2) \{Z\} \quad (70)$$

where Z is the term within curly brackets in Eqn. 69. If this condition is satisfied, peak maximum amplitude measurement is better than peak integration. The quotient, constituted by the exponentials, decreases from one to

zero if the asymmetry factor increases from zero (gaussian peak) to infinity (in which case the peak resembles a step function). In other words, if asymmetry increases, peak maximum amplitude measurement becomes more favourable than peak area determination, as is well known.

OPTIMAL INTEGRATION OF PEAKS WITH UNKNOWN SHAPE

In the previous sections, strict conditions were imposed regarding the application of an optimum integration procedure, especially concerning the baseline and the peak shape and peak position. In this section, an attempt is made to ease these conditions in order to derive an integration procedure for other cases.

Let $s(t)$ be an integratable function bounded on the interval $[a, b]$, such that

$$I_{a,b} = \int_a^b s(t)dt \quad (71)$$

is finite and

$$s(a) = s(b) = 0 \quad (72)$$

$x(t)$ is a function describing the signal obtained from measurement of $s(t)$:

$$x(t) = s(t) + n(t) \quad (73)$$

with $n(t)$ as superimposed noise. It is assumed that $E[n(t)] = 0$ (the baseline is subtracted). The maximum integration interval is divided in left and right parts:

$$I_{L,a} = \int_a^c s(t)dt \quad (74)$$

$$I_{R,b} = \int_c^b s(t)dt \quad (75)$$

where c is near the peak maximum position.

The (biased) estimators for the left and right peak areas are, respectively,

$$\hat{I}_L(c - T_L) = \int_{T_L}^c x(t)dt = \int_{T_L}^c s(t)dt + \int_{T_L}^c n(t)dt \quad (76)$$

$$\hat{I}_R(T_R - c) = \int_c^{T_R} x(t)dt = \int_c^{T_R} s(t)dt + \int_c^{T_R} n(t)dt \quad (77)$$

The corresponding estimation errors are

$$I_{L,a} - \hat{I}_L(c - T_L) = \int_a^{T_L} s(t)dt - \int_{T_L}^c n(t)dt = \int_a^{T_L} x(t)dt - \int_a^c n(t)dt \quad (78)$$

$$I_{R,b} - \hat{I}_R(T_R - c) = \int_{T_R}^b s(t)dt - \int_c^{T_R} n(t)dt = \int_{T_R}^b x(t)dt - \int_c^b n(t)dt \quad (79)$$

The integration error consists of a biased and a random part. Next the estimation error must be minimized. If the estimation were unbiased, the integration interval is $[a, b]$, but the expected random error would then be relatively large, because it increases with the length of the integration interval. However, by minimizing the error variance in the peak area estimate, an optimum integration interval is obtained. The left and right peak area estimation error variances are, respectively,

$$\begin{aligned} \mu_2(c - T_L) &= E\{I_{L,a} - \hat{I}_{L,c-T_L}\}^2 = \left(\int_a^{T_L} s(t)dt\right)^2 + \sigma_{I_n}^2(c - T_L) \\ &\approx \left(\int_a^{T_L} x(t)dt\right)^2 - 2\sigma_{I_n}^2(T_L - a) + \sigma_{I_n}^2(c - a) \end{aligned} \quad (80)$$

$$\begin{aligned} \mu_2(T_R - c) &= E\{I_{R,b} - \hat{I}_{R,T_R-c}\}^2 = \left(\int_{T_R}^b s(t)dt\right)^2 + \sigma_{I_n}^2(T_R - c) \\ &\approx \left(\int_{T_R}^b x(t)dt\right)^2 - 2\sigma_{I_n}^2(b - T_R) + \sigma_{I_n}^2(b - c) \end{aligned} \quad (81)$$

(the cross terms $E\int s(t)dt \cdot \int n(t)dt$ are zero because $E[n(t)] = 0$, and because $s(t)$ and $n(t)$ are not correlated).

Minimization (see also Eqns. B5, B11, B12) yields

$$|x(T_L)| \int_a^{T_L} x(t)dt = 2 \int_0^{T_L-a} R_{nn}(\tau)d\tau \quad (82)$$

$$|x(T_R)| \int_{T_R}^b x(t)dt = 2 \int_0^{b-T_R} R_{nn}(\tau)d\tau \quad (83)$$

When these equations are satisfied, the resulting integration intervals T'_L and T'_R yield a minimum error in peak area estimate. The absolute signs in the equations are necessary because the bias is one-sided (it is assumed here that only "positive" peaks are of interest). It should be noted that the equations yield only estimated optimum integration intervals, because $x(t)$ has a random component. It can be seen that the equations are satisfied too if $T'_L = a$ and $T'_R = b$. In fact, these values yield maximum error variances in peak area estimation.

The procedure to be followed in practice is as follows. Before the measurements are started, compute the right part of Eqns. 82 and 83 from the (known) noise characteristics. Secondly, start the integration procedure from the (estimated) position a and evaluate for each new measurement the left side of Eqn. 82. The numerical value of the left side will steadily increase (if

a significant peak is present), until Eqn. 82 is satisfied. Because the left side of Eqn. 82 consists of random variables, the equation may be satisfied by coincidence. To prevent this effect, a smoothing technique may be used (e.g., exponential smoothing). Another method of avoiding this effect is to require that a few times in succession the left side exceeds the right side. Especially when the S/N ratio ($s(u)/\sigma_n$) is low, these techniques are very helpful. Thirdly, to find the integration endpoint, another procedure has to be followed, as the integrated part of the signal after the current position is unknown. Because $s(t)$ is a monotonic decreasing function starting from the peak maximum and $s(b) = 0$ (Eqn. 72), the remaining peak area can be roughly estimated. The triangle, determined by the current measured amplitude $x(T_R)$, the current baseline position T_R , and $x(b)$ could be considered as a realistic estimate of the area: $1/2x(T_R)(b - T_R)$. Thus, the following equation is used to determine the integration endpoint:

$$1/2|x(T_R)| x(T_R)(b - T_R) = 2 \int_0^{b-T_R} R_{nn}(\tau) d\tau \quad (84)$$

To prevent premature coincidental termination of the integration the same procedure as applied in the determination of the integration starting point can be used. Finally, the resulting value of the integration from position T_L to T_R is the estimate for the peak area.

Concerning the resulting error variance in the peak area estimate, a few remarks have to be made. The ultimate variance is obtained from Eqns. 80 and 81 in the same way as in Eqn. 55, using a linear combination. It should be noted that the variances in Eqns. 80 and 81 are not the theoretical variances as in the cases of known peak shapes. They consist of terms governed by a combination of measured data and theoretical considerations. Thus, the obtained variance must be considered as an estimate. The use of merely theoretical terms is impossible, because the function describing the peak is unknown. Another point is that the approximation used in Eqns. 80 and 81 is based on the assumption (realistic in practice) that the variance of the integrated noise is roughly proportional to the integration time.

SIMULATION

In order to test the above theoretical results, for peaks with known shape, peaks with noise superimposed were simulated, according to the models described by Eqns. 20 and 24. Noise was simulated using the SAMSON software package [10]. For both first-order noise and flicker noise, 81000 numbers were generated. The baseline noise variance was 1.0. The time constant of the first-order noise was $T_x = 0.3$, and the time constants of the flicker noise were t_m and $t_n = 0.1$ and 3.0, respectively. The results were tested for asymmetry factors $A = 0.0, 0.3$ and 1.0.

The simulation procedure was as follows. The peak involved consists of 81 data points. The optimum left and right integration intervals are found

according to Eqns. 47 and 48. The shortest simulation integration interval is established by subtracting as many times as is necessary one data point from both the (theoretically evaluated) left and the right optimum integration intervals until five data points are left. Five noise data are added to the peak values in that interval, and the area is estimated according to Eqn. 6. Subsequently, the estimated error variance is calculated from Eqn. 9. This procedure is repeated as many times as there are noise numbers available (thus 16200 times in this case), and the mean value of the resulting variances is computed and compared with the theoretical variance by Eqns. 49, 50 and 55. Each consecutive simulated integration interval is made up by adding two data points to the previous interval, expanding both left and right integration limits with one data point, after which the simulation procedure is repeated. Thus, the integration intervals have subsequently a length of 5, 7, 9...81. By using this procedure, the predicted optimum integration interval will be part of the series. To compute the theoretical variance the exact (first-order) noise variance (Eqn. B33) is used and not the approximated variance (Eqn. B34).

In Fig. 6 the resulting error variances in peak area estimates for first-order noise (Fig. 6a–c) and flicker noise (Fig. 6d–f) are shown as a function of the integration interval. For first-order noise the observed and theoretical variances are identical as expected. In Fig. 6(a) the dashed curve represents the curve obtained when the approximated noise variance (Eqn. B34) is used. This curve is different from the exact theoretical curve, especially if the integration interval is small. The position of the minimum, however, is not affected significantly by the approximation. Although this approximated curve is not depicted in Fig. 6(b, c) for asymmetric peak, the result is the same. It should be noted here that the estimated error variance is minimal for the symmetric peak, and increases with asymmetry. The minimal variance can be found by substitution of the simulation parameter values in Eqn. 40.

Although numerical evaluation of the equations for gaussian peaks with flicker noise superimposed was not generally possible in the theoretical part, simulation yields minimum integration intervals, as seen in Fig. 6(d–f). The minima are even weaker than in case of first-order noise. The observed optimal integration intervals seem to be somewhat smaller compared with the first-order case. But as stated before, general rules cannot be derived for this type of noise.

To check the results obtained for unknown peak shapes, a spectrum was generated, using the peak shape model of Eqns. 20 and 24. In addition, first-order noise was generated from the SAMSON software package, and superimposed on the spectrum. The algorithm represented by Eqns. 82 and 84 was used. Peak integration start was detected when the left side of condition 82 exceeded the right side, and peak integration stop when the reverse was true. Figure 7 shows the simulated spectrum, Fig. 8 the curves representing the left and right sides of conditions 82 and 84 and Fig. 9 the curve representing

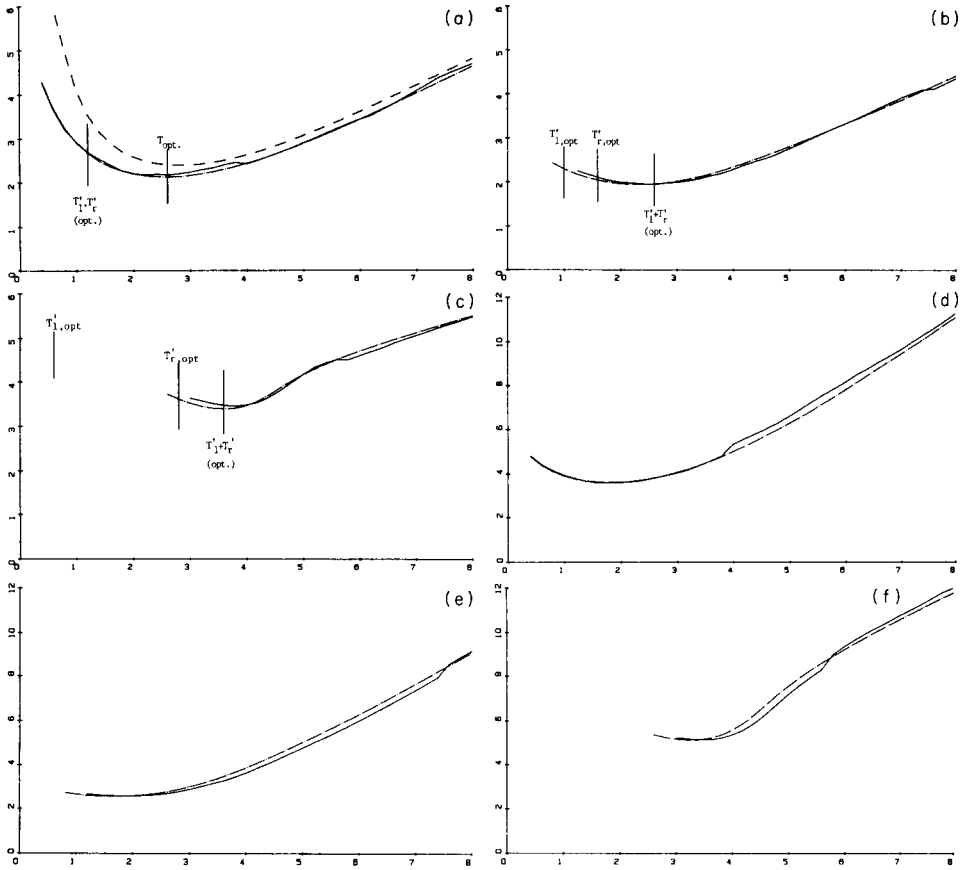


Fig. 6. Theoretical (---) and "simulated" (—) peak area estimation error variance (y-axes) as a function of the integration interval (x-axes in units of the peak standard deviation). The baseline noise variance and the peak standard deviation are 1.0. The variance curves resulting from simulated peaks and noise are the ensemble mean of at least 1000 observations. Curves: (a–c) first order noise, $T_x = 0.3$, asymmetry factor is 0.0, 0.3 and 1.0, respectively; (d–f) flicker (1/f) noise, $t_n = 0.1$, $t_m = 3.0$, asymmetry factor is 0.0, 0.3 and 1.0, respectively.

the measured peak area. The procedure appears to perform well. Even when the signal-to-noise ratio is small, the criterion is able to detect the peak integration start and stop. Of course, the observed peak areas are underestimated, because the integration takes place in a limited interval. For unknown peak shapes, no correction is possible.

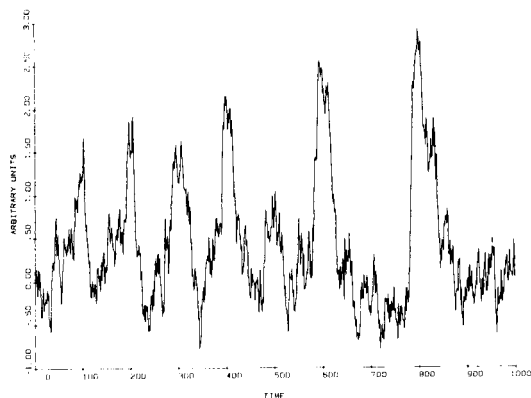


Fig. 7. Simulated chromatogram with first-order noise superimposed ($\sigma_n^2 = 0.09$, $T_x = 7.0$). Peak parameters as below:

u	$s(u)$	σ_p	A	I_∞	u	$s(u)$	σ_p	A	I_∞
100	1.0	10	0.0	25.1	400	2.2	13	0.4	75.9
200	1.4	11	0.2	39.2	600	2.6	14	0.3	94.3
300	1.8	12	0.0	54.1	800	3.0	15	0.7	134.6

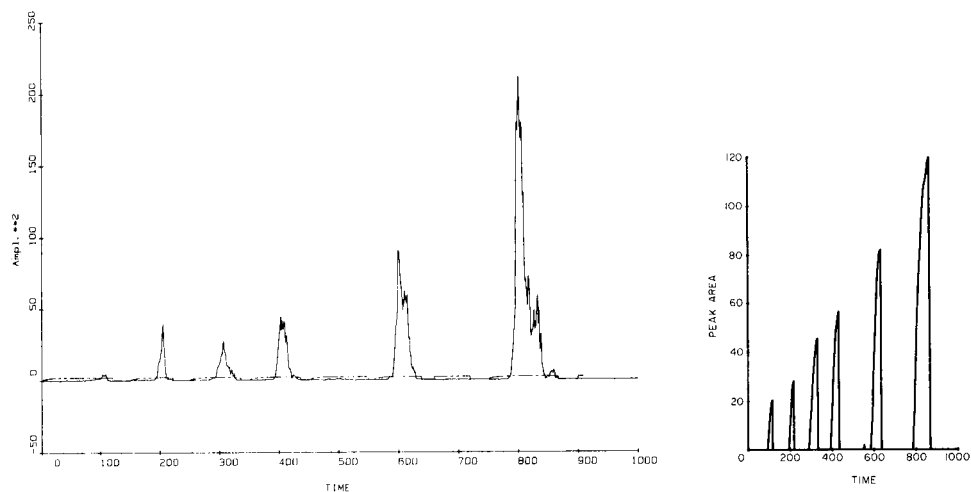


Fig. 8. Integration start/stop criterion for peaks with unknown shape: (---) right side of Eqns. 82 (start) and 84 (stop); (—) left side of Eqns. 82 and 84.

Fig. 9. Observed peak areas using the integration start/stop criterion for unknown peak shapes. Integration is performed within the detected start/stop interval. Area is depicted as a function of time.

CONCLUSIONS

The aim of this study was to derive some rules of thumb for the determination of an analytical quantity, represented by a peak-shaped signal as in chromatography and spectroscopy, for example. Although tedious and rather complex mathematical calculations are necessary to achieve some of the results, they can be described as simple rules of thumb. Of course, the results obtained for known peak shapes are valid only when the peak shape and position are known with sufficient accuracy, and the noise and baseline parameters are known. The described method could be useful for routine analysis, if the peak and noise parameters are known.

The conditions for obtaining an integration procedure for unknown peak shapes are much less strict; almost no prerequisites are necessary. For this case a simple peak-evaluating procedure is obtained. The procedure can be implemented easily on a microcomputer; the computations are simple and no large signal records are required. The method can be refined, for example, by means of smoothing techniques. Further efforts are necessary to derive a useful procedure for peak finding and evaluating from the expressions for the unknown peak-shape procedure presented in this paper. Such a procedure is not preferable if very powerful computing facilities are available. In this case, more sophisticated methods as Kalman filtering or non-linear regression provide better results, because these methods allow simultaneous utilization of all the analytical information available. The drawback of these methods is, however, that depending on the complexity of the model involved, they may be very time-consuming. For most microcomputers, on-line processing will be impossible by these methods.

Comparison of the error variances in peak area estimation resulting from the unknown peak-shape integration procedure and from the known peak-shape integration procedure, shows that the latter yields better results, because the former is biased. Thus, depending on the accuracy of the peak and noise parameters, a choice between the two procedures has to be made.

An important result of this study is that the old controversy around peak integration and peak maximum amplitude measurement can be resolved in certain cases. As expected, the method which will be used depends on the relations between the time constant(s) of the noise and the peak width.

To obtain an ultimate, general method for peak parameter estimation, further study is necessary. Methods must be included to estimate the baseline optimally and to deconvolute overlapping peaks. Integration of all these methods will allow the development of software packages for automatic processing of chromatographic and spectroscopic data.

In conclusion, it is useful to summarize the results of this study as rules of thumb. If the baseline and peak shape are known, and no resolution problems are present Table 2 gives the peak parameter estimation procedure. For peaks with unknown shape, the scheme outlined in Table 3 can be used.

TABLE 2

Rules of thumb for the optimum estimation of the peak area or the peak maximum amplitude for peaks with known shape^a

Peak Sym.	Noise		Integrate if	Left integr. limit	Right integr. limit	Estim. error variance	Measure peak max. if	Estim. error variance
	Asym.	First order						
X		X	$T_x < 0.785\sigma_p$	$u - 1.4\sigma_p$	$u + 1.4\sigma_p$	$8\sigma_n^2 T_x \sigma_p$	$T_x > 0.785\sigma_p$	$2\pi\sigma_p^2\sigma_n^2$
X		X					preferable	$2\pi\sigma_p^2\sigma_n^2$
	X	X	$T_x < 0.785\sigma_p ZC$	$u - 1.4\sigma_p F$	$u + 1.4\sigma_p G$	$8\sigma_n^2 T_x \sigma_p / Z$	$T_x > 0.785\sigma_p ZC$	$2\pi\sigma_p^2\sigma_n^2 C$
	X	X					preferable	$2\pi\sigma_p^2\sigma_n^2 C$

^a $F \equiv \exp(-0.57A)$; $G \equiv \exp(0.77A)$; Z (see Eqn. 70); $C \equiv \exp(A^2/4\ln 2)$.

TABLE 3

Procedure for the optimum estimation of the area of peaks with unknown shape^a

Preparation phase

- (1) Determine the noise type, and the value of the noise parameters.

Location of the peak boundaries

- (2) Choose a sufficient large observation interval a, b , enclosing the peak, so that the peak amplitudes at time $t = a, t = b$ are negligible.

Detection of the integration starting point

- (3) Start integration from $t = a$, and multiply the resulting value at each sample time by the absolute value of the peak amplitude.

- (4) Integrate the ACVF of the noise type over the interval $t - a$, and multiply the result by 2.

For first-order noise (see Eqns. 82, B12, B32, B33) this yields

$$2\sigma_n^2 T_x (1 - e^{-(t-a)/T_x})$$

For flicker noise (see Eqns. 82, B12, B42, B43) this yields

$$[2\sigma_n^2 / \ln(t_n/t_m)](t_n - t_m - t_n E_2[(t-a)/t_n] + t_m E_2[(t-a)/t_m])$$

- (5) If the result of step 3 exceeds the result of step 4, the integration starting point T_L is detected.

Integration phase + detection of integration end point

- (6) Start integration.

- (7) Multiply at each sample time the observed peak amplitude by its absolute value, and by $1/2(b-t)$.

- (8) Integrate the ACVF of the noise type over the interval $b-t$, and multiply the result by 2. For first-order noise and flicker noise, the results can be found by replacement of the factor $t-a$ by $b-t$ in the equations in step 4.

- (9) If the result of step 8 exceeds the result of step 7, the integration end-point T_R is detected.

- (10) Terminate integration. The result of integration between the detected peak integration start and peak integration stop will be biased.

Restart phase

- (11) Return to step 2 to estimate the area of the next peak.

^aThe use of a smoothing technique is recommended to prevent accidental detection of integration start or stop.

APPENDIX. GENERAL CALCULATIONS

$$\mu_2(T_*) = E \{ I_\infty - [(I_*(T_*) + I_n(T_*))/I_*(T_*)] I_\infty \}^2 = E [I_n(T_*)/I_*(T_*)]^2 \quad \text{B1}$$

$$\mu'_2(T_*) = 0 \quad (\text{minimization condition}) \quad \text{B2}$$

$$\frac{d}{dT_*} \frac{\sigma_n^2(T_*)}{[I_*(T_*)]^2} = \left(\frac{[I_*(T_*)]^2 \frac{d}{dT_*} [\sigma_n^2(T_*)] - 2I_*(T_*) \sigma_n^2(T_*) \frac{d}{dT_*} [I_*(T_*)]}{[I_*(T_*)]^4} \right) = 0 \quad \text{B3}$$

Thus, since $I_*(T_*) \neq 0$ if $T_* \neq 0$, the optimal integration condition is

$$I_*(T_*) \frac{d}{dT_*} \sigma_n^2(T_*) = 2\sigma_n^2(T_*) \frac{d}{dT_*} I_*(T_*) \quad \text{B4}$$

The terms of this equation have to be evaluated.

Peak

$$I^1(T) = I(T)/I_\infty = (1/I_\infty) \int_{u-1/2T}^{u+1/2T} s(t)dt \quad (\text{Symmetric}) \quad \text{B5}$$

$$I_R^1(T_R) = I_R(T_R)/I_\infty = (1/I_\infty) \int_u^{u+T'_R} s(t)dt \quad (\text{Asymmetric, right part}) \quad \text{B6}$$

$$I_L^1(T_L) = I_L(T_L)/I_\infty = (1/I_\infty) \int_{u-T'_L}^u s(t)dt \quad (\text{Asymmetric, left part}) \quad \text{B7}$$

$$dI^1(T)/dT = (1/2I_\infty) \{s(u + 1/2T) + s(u - 1/2T)\} \stackrel{\text{symmetry}}{=} s(u + 1/2T)/I_\infty \quad \text{B8}$$

$$dI_R^1(T_R)/dT_R = s(u + T'_R)/I_\infty \quad \text{B9}$$

$$dI_L^1(T_L)/dT_L = s(u - T'_L)/I_\infty \quad \text{B10}$$

Noise

$$\sigma_n^2(T_*) = 2 \int_0^{T_*} (T_* - \tau) R_{nn}(\tau) d\tau \quad \text{B11}$$

$$\begin{aligned} d\sigma_n^2(T_*)/dT_* &= 2d\left[\int_0^{T_*} T_* R_{nn}(\tau) d\tau - \int_0^{T_*} \tau R_{nn}(\tau) d\tau\right]/dT_* \\ &= 2d\left[T_* \Gamma_{nn}(T_*) - T_* \Gamma_{nn}(0) - \tau \Gamma_{nn}(\tau) \Big|_0^{T_*} + \int_0^{T_*} \Gamma_{nn}(\tau) d\tau\right]/dT_* \\ &= 2d\left[\int_0^{T_*} \Gamma_{nn}(\tau) d\tau - T_* \Gamma_{nn}(0)\right]/dT_* = 2\Gamma_{nn}(T_*) - 2\Gamma_{nn}(0) = 2 \int_0^{T_*} R_{nn}(\tau) d\tau \end{aligned} \quad \text{B12}$$

$$\text{where } \Gamma_{nn}(\tau) = \int R_{nn}(\tau) d\tau \quad \text{B13}$$

The results obtained for peak and noise can be substituted in Eqn. B4, leading to optimal peak integration conditions:

$$\int_{u-1/2T}^{u+1/2T} s(t)dt \int_0^T R_{nn}(\tau) d\tau = 2 \int_0^T (T - \tau) R_{nn}(\tau) d\tau [s(u + 1/2T)] \quad \text{B14}$$

$$\int_u^{u+T'_R} s(t)dt \int_0^{T'_R} R_{nn}(\tau) d\tau = 2 \int_0^{T'_R} (T'_R - \tau) R_{nn}(\tau) d\tau [s(u + T'_R)] \quad \text{B15}$$

$$\int_{u-T'_L}^u s(t)dt \int_0^{T'_L} R_{nn}(\tau) d\tau = 2 \int_0^{T'_L} (T'_L - \tau) R_{nn}(\tau) d\tau [s(u - T'_L)] \quad \text{B16}$$

Calculations for specific noise types and peak shapes:

$$s(t) = (I_\infty/\sigma_p\sqrt{2\pi}) \exp[-(t - u)^2/2\sigma_p^2] \quad (\text{gaussian peak}) \quad \text{B17}$$

$$I^1(T) = \text{erf}(T/2\sigma_p\sqrt{2}) \quad \text{B18}$$

$$\text{erf}(z) = 2/\sqrt{\pi} \int_0^z \exp(-t^2) dt \quad \text{B19}$$

$$d \text{erf}(z)/dz = 2\pi^{-1/2} \exp(-z^2) \quad \text{B20}$$

$$dI^1(T)/dT = (1/\sigma_p\sqrt{2\pi}) \exp(-T^2/8\sigma_p^2) \quad \text{B21}$$

$$s(t) = [I_\infty/\sigma_p\sqrt{2\pi} \exp(A^2/4\ln 2)] \exp[-(\ln 2/A^2)\ln^2(1 + A(t-u)/\sigma_p\sqrt{2\ln 2})]$$

(asymmetric gaussian peak) B22

$$I_L^1(T_L) = (1/I_\infty) \int_{u-T_L}^u s(t)dt \quad \text{B23}$$

$$\alpha = [I_\infty/\sigma_p\sqrt{2\pi} \exp(A^2/4\ln 2)] \quad \beta = \ln 2/A^2 \quad \delta = \sigma_p\sqrt{2\ln 2}/A$$

$$1/x = A(t-u)/\sigma_p\sqrt{2\ln 2} \quad \gamma = -AT_L'/\sigma_p\sqrt{2\ln 2} \quad \frac{dx}{dt} = A/\sigma_p\sqrt{2\ln 2} = 1/\delta$$

$$I_L(T_L) = \alpha\delta \int_{\gamma}^0 \exp[-\beta \ln^2(1+x)]dx. \text{ Substitute } y = \ln(1+x)$$

$$I_L(T_L) = \alpha\delta \int_{\ln(1-T_L'/\delta)}^0 \exp(-\beta y^2 - y)dy = 1/2\alpha\delta\sqrt{\pi/\beta} \exp(1/4\beta) \{ \operatorname{erf}[1/2\sqrt{\beta} - \sqrt{\beta}\ln(1-T_L'/\delta)] - \operatorname{erf}(1/2\sqrt{\beta}) \}$$

$$= 1/2I_\infty \left\{ \operatorname{erf} \left[\frac{A}{2\sqrt{\ln 2}} - \frac{\sqrt{\ln 2}}{A} \ln \left(1 - \frac{A}{\sqrt{2\ln 2}} \frac{T_L'}{\sigma_p} \right) \right] - \operatorname{erf} \left(\frac{A}{2\sqrt{\ln 2}} \right) \right\} \quad \text{B24}$$

$$\text{Substitute } p = A/2\sqrt{\ln 2} \quad \text{B25}$$

$$I_L^1(T_L) = 1/2 \{ \operatorname{erf}[p - 1/2p\ln(1 - p\sqrt{2}T_L'/\sigma_p)] - \operatorname{erf}(p) \} \quad \text{B26}$$

$$I_R^1(T_R) = 1/2 \{ \operatorname{erf}(p) - \operatorname{erf}[p - 1/2p\ln(1 + p\sqrt{2}T_R'/\sigma_p)] \} \quad \text{B27}$$

$$I_{L,\infty}^1 = 1/2[1 - \operatorname{erf}(p)] \quad \text{B28}$$

$$I_{R,\infty}^1 = 1/2[1 + \operatorname{erf}(p)] \quad \text{B29}$$

$$\frac{d}{dT_L} I^1(T_L) = (1/\sqrt{2\pi})[1/\sigma_p(1 - p\sqrt{2}T_L'/\sigma_p) \exp\{-[p - 1/2p\ln(1 - p\sqrt{2}T_L'/\sigma_p)]^2\}] \quad \text{B30}$$

$$\frac{d}{dT_R} I^1(T_R) = (1/\sqrt{2\pi})[1/\sigma_p(1 + p\sqrt{2}T_R'/\sigma_p) \exp\{-[p - 1/2p\ln(1 + p\sqrt{2}T_R'/\sigma_p)]^2\}] \quad \text{B31}$$

Noise. For first-order noise:

$$R_{nn}(\tau) = \sigma_n^2 \exp(-|\tau|/T_x) \quad \text{B32}$$

$$\sigma_{I_n}^2(T) = 2\sigma_n^2 T_x \{T - T_x[1 - \exp(-T/T_x)]\} \quad \text{B33}$$

$$\text{Usually } T_x \ll T, \text{ so } \sigma_{I_n}^2(T) \approx 2\sigma_n^2 T_x [T - T_x] \approx 2\sigma_n^2 T_x T \quad \text{B34}$$

$$d\sigma_{I_n}^2(T)/dT = 2\sigma_n^2 T_x \quad \text{B35}$$

For flicker (1/f) noise

$$R_{nn}(\tau) = [\sigma_n^2/\ln(t_n/t_m)] [E_1(|\tau|/t_n) - E_1(|\tau|/t_m)] \quad \text{B36}$$

$$Q \equiv 2\sigma_n^2/\ln(t_n/t_m) \quad \text{B37}$$

$$E_n(z) = \int_1^\infty \{[\exp(-zt)]/t^n\}dt \quad \text{B38}$$

$$E_n(0) = 1/(n-1) \quad (n > 1) \quad \text{B39}$$

$$\frac{d}{dz} E_n(z) = -E_{n-1}(z) \quad (n = 1, 2, 3 \dots) \quad \text{B40}$$

$$E_{n+1}(z) = \frac{1}{n} [e^{-z} - zE_n(z)]$$

B41

$$\sigma_{I_n}^2(T) = QT \int_0^T (E_1(\tau/t_n) - E_1(\tau/t_m)) d\tau \quad (\text{part A})$$

$$- Q \int_0^T \tau \{E_1(\tau/t_n) - E_1(\tau/t_m)\} d\tau \quad (\text{part B})$$

Evaluate part A:

$$A = -QT \int_0^T \{[dE_2(\tau/t_n)/d(\tau/t_n)] - [dE_2(\tau/t_m)/d(\tau/t_m)]\} d\tau$$

$$= -QT[t_n E_2(T/t_n) + t_m - t_n - t_m E_2(T/t_m)]$$

$$B = -Q \int_0^T \tau [E_1(\tau/t_n) - E_1(\tau/t_m)] d\tau$$

Partial integration yields:

$$B = QT[t_n E_2(T/t_n) - t_m E_2(T/t_m)] - Q \int_0^T [t_n E_2(\tau/t_n) - t_m E_2(\tau/t_m)] d\tau$$

$$= QT[t_n E_2(T/t_n) - t_m E_2(T/t_m)] + Q \int_0^T \{t_n^2 [dE_2(\tau/t_n)/d\tau - t_m^2 [dE_2(\tau/t_m)/d\tau]\} d\tau$$

$$= QT[t_n E_2(T/t_n) - t_m E_2(T/t_m)] + Qt_n^2 E_3(T/t_n) - 1/2 Qt_n^2 - Qt_m^2 E_3(T/t_m) + 1/2 Qt_m^2$$

$$\sigma_{I_n}^2(T) = A + B = [2\sigma_n^2/\ln(t_n/t_m)] [T(t_n - t_m) - 1/2(t_n^2 - t_m^2) + t_n^2 E_3(T/t_n) - t_m^2 E_3(T/t_m)]$$

B42

$$d\sigma_{I_n}^2(T)/dT = [2\sigma_n^2/\ln(t_n/t_m)] [t_n - t_m - t_n E_2(T/t_n) + t_m E_2(T/t_m)]$$

B43

Optimum integration intervals for some peak shapes and noise types

Gaussian peak with first order noise. Substitution yields:

$$\text{erf}(T/2\sigma_p\sqrt{2}) = \sqrt{2/\pi}(T/\sigma_p) \exp(-T^2/8\sigma_p^2)$$

B44

$$\text{resulting in: } T_{\text{opt}} \approx 2.8\sigma_p$$

B45

$$\text{and } \mu_2(T_{\text{opt}}) = 5.6\sigma_n^2 T_{\text{opt}} \sigma_p / \text{erf}^2(0.99) \approx 8\sigma_n^2 T_{\text{opt}} \sigma_p$$

B46

Gaussian peak with flicker noise. Substitution yields:

$$\text{erf}(T/2\sigma_p\sqrt{2})[t_n - t_m - t_n E_2(T/t_n) + t_m E_2(T/t_m)]$$

$$= \sqrt{2/\pi}(1/\sigma_p) \exp(-T^2/8\sigma_p^2) [T(t_n - t_m) - 1/2(t_n^2 - t_m^2) + t_n^2 E_3(T/t_n) - t_m^2 E_3(T/t_m)]$$

B47

Suppose $t_n \gg T \approx t_m$

$$\text{erf}(T/2\sigma_p\sqrt{2})\{t_n[1 - E_2(T/t_n)]\} = \sqrt{2/\pi}\{Tt_n - 1/2t_n^2 + 1/2t_n^2[e^{-T/t_n} - T/t_n E_2(T/t_n)]\}$$

$$= \sqrt{2/\pi}(1/\sigma_p) \exp(-T^2/8\sigma_p^2) [Tt_n - 1/2t_n^2 + 1/2t_n^2 - 1/2Tt_n - 1/2Tt_n E_2(T/t_n)]$$

$$= 1/2\sqrt{2/\pi}(1/\sigma_p) \exp(-T^2/8\sigma_p^2) \{T[t_n(1 - E_2(T/t_n))]\}$$

$$\text{which results in } \text{erf}(T/2\sigma_p\sqrt{2}) = (1/\sqrt{2\pi})(T/\sigma_p) \exp(-T^2/8\sigma_p^2)$$

B48

This equation can only be satisfied for $T = 0$, meaning no integration at all.

Asymmetric peak with first-order noise.

$$Q \equiv p - (1/2p)\ln(1 + p\sqrt{2}T'_R/\sigma_p) \quad S \equiv p - (1/2p)\ln(1 - p\sqrt{2}T'_L/\sigma_p)$$

$$\text{Substitution yields: } \operatorname{erf}(p) - \operatorname{erf}(Q) = (\sqrt{8/\pi})(T'_R/\sigma_p)[1/(1 + p\sqrt{2}T'_R/\sigma_p)] \exp(-Q^2) \quad \text{B49}$$

$$\text{and } \operatorname{erf}(S) - \operatorname{erf}(p) = (\sqrt{8/\pi})(T'_L/\sigma_p)[1/(1 - p\sqrt{2}T'_L/\sigma_p)] \exp(-S^2) \quad \text{B50}$$

These equations give different solutions for different asymmetry factors. Numerical approximations yield the following results:

$$T'_L \approx 1.4\sigma_p \exp(-0.57A) \quad \text{B51}$$

$$T'_R \approx 1.4\sigma_p \exp(0.77A) \quad \text{B52}$$

$$\mu_2(T_{L,\text{opt}}) = 8\sigma_n^2 T'_L T_x / \{\operatorname{erf}[p - 1/2p\ln(1 - p\sqrt{2}T'_L/\sigma_p)] - \operatorname{erf}(p)\}^2 \quad \text{B53}$$

$$\mu_2(T_{R,\text{opt}}) = 8\sigma_n^2 T'_R T_x / \{\operatorname{erf}(p) - \operatorname{erf}[p - 1/2p\ln(1 + p\sqrt{2}T'_R/\sigma_p)]\}^2 \quad \text{B54}$$

Substitution of B51 and B52 yields:

$$\mu_2(T_{L,\text{opt}}) \approx 8\sigma_n^2 T_x \sigma_p [\exp(-1.10A) + \exp(1.69A)] \quad \text{B55}$$

$$\mu_2(T_{R,\text{opt}}) \approx 8\sigma_n^2 T_x \sigma_p [\exp(-1.91A) + \exp(0.85A)] \quad \text{B56}$$

*Peak top amplitude measurement**Symmetrical peak.*

$$x(u) = s(u) + n(u) = (I_\infty/\sigma_p\sqrt{2\pi}) + n(u) \quad \text{B57}$$

$$\text{Estimator: } \hat{I}_\infty = \sigma_p\sqrt{2\pi}x(u) \quad \text{B58}$$

$$\mu_2(\hat{I}_\infty - I_\infty) = E[I_\infty + \sigma_p\sqrt{2\pi}n(u) - I_\infty]^2 = 2\pi\sigma_n^2\sigma_p^2 \quad \text{B59}$$

Asymmetrical peak.

$$x(u) = s(u) + n(u) = I_\infty/\sigma_p\sqrt{2\pi} \exp(A^2/4\ln 2) + n(u) \quad \text{B60}$$

$$\text{Estimator: } \hat{I}_\infty = \sigma_p\sqrt{2\pi} \exp(A^2/4\ln 2)x(u) \quad \text{B61}$$

$$\mu_2(\hat{I}_\infty - I_\infty) = 2\pi\sigma_n^2\sigma_p^2 \exp(-A^2/4\ln 2) \quad \text{B62}$$

By comparison of the respective variances, it is possible to establish when peak maximum measurement is preferable: for a symmetric peak, $T_x > 0.785\sigma_p$ (first-order noise). For an asymmetric peak, combining the variances for the left and right integration interval leads to Eqn. 70.

REFERENCES

- 1 G. F. Moler, R. R. Delongchamp and R. K. Mitchum, *Anal. Chem.*, 55 (1983) 842.
- 2 M. A. Mariscotti, *Nucl. Instrum. Methods*, 50 (1967) 309.
- 3 F. Hock, *Chromatographia*, 2 (1969) 334.
- 4 H. C. Smit and H. L. Walg, *Chromatographia*, 9 (1976) 483.
- 5 S. M. Roberts, D. H. Wilkinson and L. R. Walker, *Anal. Chem.*, 42 (1970) 886.
- 6 G. C. Allen and R. F. McMeeking, *Anal. Chim. Acta*, 103 (1978) 73.
- 7 A. H. Anderson, T. C. Gibbs and A. B. Littlewood, *J. Chromatogr. Sci.*, 8 (1970) 640.
- 8 R. P. J. Duursma and H. C. Smit, *Anal. Chim. Acta*, 133 (1981) 67.
- 9 R. D. B. Fraser and E. Suzuki, *Anal. Chem.*, 41 (1979) 37.
- 10 J. M. Laeven, H. C. Smit and J. V. Lankelma, *Anal. Chim. Acta*, 157 (1984) 273.
- 11 M. Abramowitz, *Handbook of Mathematical Functions*, Dover, New York, 1970, pp. 228, 231.
- 12 H. C. Smit and H. L. Walg, *Chromatographia*, 8 (1975) 311.

VOLTAMMETRIC DETERMINATION OF DOXORUBICIN IN URINE BY ADSORPTIVE PRECONCENTRATION AND FLOW INJECTION ANALYSIS

EDWARD N. CHANEY, Jr., and RICHARD P. BALDWIN*

Department of Chemistry, University of Louisville, Louisville, KY 40292 (U.S.A.)

(Received 2nd May 1985)

SUMMARY

Preconcentration and quantitation of the cancer chemotherapy drug, doxorubicin, are achieved via a flow-injection approach utilizing adsorption of the drug onto a carbon paste electrode, medium exchange, and differential pulse voltammetry on the adsorbing surface. The oxidation peak at +0.57 V vs. Ag/AgCl is used for quantitation. Linear response was obtained for concentrations from 10^{-6} M to the detection limit of 10^{-9} M, and it was possible to determine the drug in urine by direct injection of the sample with no preliminary steps required. The sensitivity, linearity, and selectivity of the flow injection approach were markedly improved relative to the earlier batch method in which the adsorption step was done in a stationary sample solution.

Doxorubicin is the most important of a family of anthracycline antibiotics which have gained extensive clinical application in anticancer chemotherapy [1]. Previously, fluorescence [2–4], radio-immunoassay [4, 5], and mass spectrometric [5] procedures suitable for the quantitation of total anthracycline content have been described. In addition, approaches utilizing thin-layer chromatography and high-performance liquid chromatography (h.p.l.c.) with spectrophotometric or fluorescence detection have also been developed [6–12]. These latter approaches, which permit differentiation and quantitation of doxorubicin and its principal metabolites, have rapidly become the method of choice for determination of the drug in complex samples.

Alternatively, several electrochemically-based detection procedures have been suggested for doxorubicin. These range from the use of amperometric detection for monitoring and quantitation following h.p.l.c. [13] to various attempts at direct voltammetric quantitation [14–18]. The former approach has been shown to provide impressive sensitivity while requiring only modest sample pretreatment. Examples of the latter approach include the differential-pulse polarographic procedure of Sternson and Thomas [16] who used reduction of the quinone at the dropping mercury electrode to achieve detection limits of 8×10^{-6} M for total anthracycline content in plasma. Subsequently, the tenacious adsorption of the compound onto carbon paste was utilized to preconcentrate it selectively from urine samples and thus extend

detection down to the 10^{-8} M level [18]. In the latter study, which used oxidation of the drug at low positive potentials, the improved sensitivity was achieved because of the adsorption/preconcentration step while transfer of the adsorbed electrode from the sample solution to a pristine, low-background buffer permitted numerous nonadsorbing interferents to be avoided.

Wang and Freiha [19] have recently demonstrated that the adsorption/preconcentration approach can be effectively adapted for use in flow injection analysis (f.i.a.), thereby combining the improved sensitivity and selectivity described above with the ease of operation inherent in the flow injection method. The model compound used in that work was the drug chlorpromazine which, like doxorubicin, adsorbs relatively rapidly onto various carbon electrode materials and undergoes oxidation at modest positive potentials. In this paper, an analogous coupling of the flow-injection and adsorption/preconcentration approaches is described for the determination of doxorubicin. The improvement in detectability obtained in this manner was an order of magnitude over that which had been obtained with batch preconcentration used earlier. In addition, even at this concentration, direct quantitation of the chemotherapy agent in urine was possible with no sample treatment required.

EXPERIMENTAL

Reagents and apparatus

Doxorubicin was obtained from Adria Laboratories (Columbus, OH) and from Sigma Chemical Co., both in reagent form and in a 1:5 mixture with lactose. Both sample forms were used as received and behaved identically. Other chemicals were reagent or chromatographic grade and were used without further purification. Voltammetric and flow-injection experiments were done in a pH 4.5 buffer solution containing 0.20 M sodium acetate/acetic acid.

The equipment for the flow-injection studies included a Rheodyne Model 7125 sample-loop injector or a Waters Model U6K universal injector, a Perkin-Elmer Series 10 pump, and a thin-layer carbon paste electrode assembly (Bioanalytical Systems, West Lafayette, IN; Model TL-5A). All interconnections were made with 0.010-in. i.d. stainless steel tubing. The working electrode had a geometric surface area of 0.071 cm^2 , and the reference was an Ag/AgCl electrode. For the differential-pulse voltammetric (d.p.v.) detection, a Princeton Applied Research Model 364 polarographic analyzer was used with a Hewlett-Packard Model 7035B X-Y recorder. The carbon paste was prepared in conventional fashion by manually mixing 5 g of graphite powder (Spectropure grade; Fluka Chemical Corp.) with 3 ml of Nujol oil (McCarthy Scientific Corp., Fullerton, CA).

Procedure

Flow injection/preconcentration determinations were done by injecting the desired sample volume into the flowing buffer stream and allowing it to

pass over the carbon paste electrode. The flow rate was usually 0.1 ml min^{-1} , and the electrode was maintained at $+0.30 \text{ V}$ during the adsorption. After the response of the working electrode stabilized (usually after 2 min for a flow rate of 0.1 ml min^{-1}), a d.p.v. scan was recorded from an initial potential of $+0.30 \text{ V}$ (pulse amplitude, 50 mV ; potential scan rate, 2 or 5 mV s^{-1}). A fresh electrode surface was prepared for each preconcentration experiment.

Urine samples containing doxorubicin were prepared by dissolving the desired quantity of the drug in urine specimens obtained from healthy volunteers. No sample treatment steps were used for these experiments.

RESULTS AND DISCUSSION

Flow-injection optimization

In a previous report describing the application of a batch adsorption/preconcentration approach for doxorubicin [18], the preconcentration was accomplished simply by immersing a fresh carbon paste surface into the analyte solution for a brief period and allowing adsorption to occur spontaneously. When attempts involving variation of the adsorption time or the convection rate failed to improve the doxorubicin detection limit significantly, it became apparent that the adsorption was extremely rapid, reaching a saturation level within 30 s of the initial immersion. Consequently, f.i.a. was investigated as a potentially more effective method of presenting the doxorubicin sample for adsorption onto the carbon paste surface.

The feasibility of this approach was initially tested by injecting identical volumes of standard doxorubicin solutions across carbon paste surfaces and recording d.p.v. scans. A fresh surface was prepared before each injection, and the sample plug was allowed to pass completely out of the detector cell before the d.p.v. scan was initiated in the still flowing buffer solution. The resulting voltammograms all exhibited the expected hydroquinone oxidation peak at $+0.57 \text{ V}$ vs. Ag/AgCl [17, 18]. As before, when the preconcentration had been done via the immersion method, the observed current levels were dependent on the drug concentration, with a linear relationship applying only at lower concentrations. In this case, the linear range was from about $1 \times 10^{-9} \text{ M}$ to $1 \times 10^{-6} \text{ M}$. Least-squares fits for five concentrations over this range yielded a calibration line with a slope of $93.1 (\pm 9.9) \text{ nA } \mu\text{M}^{-1}$ and an intercept of $34.1 (\pm 5.5) \text{ nA}$; the standard error of the estimate (S_{yx}) was 7.2 nA with a correlation coefficient of 0.98. Upon exposure to concentrations of the drug higher than 10^{-6} M , the electrode surface began to exhibit saturation effects. It is noteworthy, however, that the doxorubicin concentration causing saturation in the flow-injection procedure occurred at the micromolar level, a range which corresponds to the limit of detection for many direct voltammetric methods.

It was expected that the extent of the doxorubicin adsorption seen in any specific instance would be a function of a number of experimental parameters such as electrode composition and surface area, temperature,

thin-layer cell volume, and exposure time (which in turn is a function of sample volume and flow rate). In subsequent studies, therefore, most of these variables were held constant, and only sample volume and sample flow rate were systematically varied for optimization purposes. Further, upon examination of the adsorption taking place from real sample matrices such as urine, it was evident that the specific sample background also significantly affected the observed currents. Thus, urine samples doped with the same doxorubicin concentration but obtained from different donors (or on different occasions from the same donor) did not normally yield the same adsorption currents. This could be due, for example, to the presence of co-adsorbing but non-electroactive sample constituents which compete for the carbon paste surface but produce no current with d.p.v. In any case, because of the importance of the sample matrix for the adsorption, all subsequent work was done with doxorubicin-containing urine samples. This implies that, even though experimental results obtained for one sample might not be quantitatively identical to those for a second sample, the analytical performance given by the results would realistically reflect that obtainable in actual applications.

The effect of the sample flow rate used during the adsorption period is shown in Fig. 1. The use of rapid analyte transport into and out of the electrode compartment would be advantageous for fast analysis; however, it was also essential that the flow of the sample stream not be so rapid as to com-

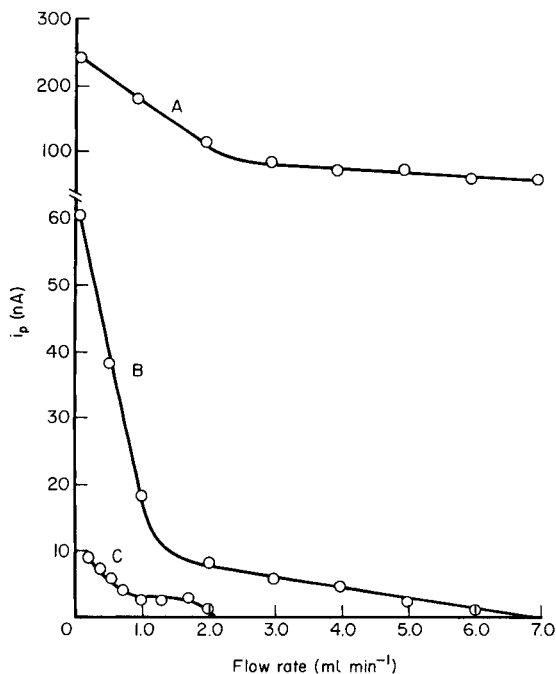


Fig. 1. Effect of sample flow rate on peak current for 6- μ l injection of doped urine containing the following concentrations (μ M) of doxorubicin: (A) 5; (B) 1; (C) 0.5.

promise the extent of analyte adsorption. Typical results obtained for three different concentrations are shown. In all three cases, the sample consisted of a 6- μ l injection from urine specimens doped with the indicated doxorubicin concentration. (Note, however, that different urine samples were used for curves A, B, and C and the exact current levels shown for each concentration cannot be directly compared.) In all cases, initial increases in flow rate up to approximately 2 ml min⁻¹ resulted in a reduction in the observed current; this decrease was most dramatic for the sample with a concentration of 1×10^{-6} M, the upper limit of the 0.10 ml min⁻¹ linear range. Further increases in flow rate resulted in only gradual declines in the adsorption current. This inverse dependence on sample flow rate or, conversely, a direct dependence on sample adsorption time, indicated that maximum sensitivity should be realized under conditions of slow flow rate. Samples possessing higher doxorubicin concentrations (i.e., above the linear range) all exhibited adsorption currents similar in magnitude to those in curve A.

It is interesting to note that essentially the same saturation effect observed under the conditions of Fig. 1 for concentrations greater than micromolar could in principle be produced for lower drug concentrations simply by using appropriately larger injection volumes. In fact, this was precisely the case when standard solutions of doxorubicin prepared in deionized water were evaluated; similar adsorption currents could be generated predictably by utilizing appropriate combinations of analyte concentration, flow rate, and injection volume. However, whenever real sample matrices were involved, the variable nature of the background contribution made it advisable to use as small a sample volume as possible, in this case, 6–20 μ l. This precaution enabled the rapid adsorption of the drug molecule to be favored as much as possible over the comparatively slower adsorption of most background constituents.

Quantitative characteristics

Calibration curves obtained for urine samples under optimum conditions of slow flow rate and small injection volume were found to exhibit linearity from 1×10^{-9} M to 1×10^{-6} M, roughly the same concentration range as for standard solutions in deionized water; the correlation coefficients were typically 0.99 or higher. But, as indicated above, some differences were expected and observed for samples prepared from different urine specimens. In particular, such samples routinely yielded somewhat different least-squares calibration equations and slightly different practical detection limits. Consequently, doxorubicin quantitation was most conveniently done by the method of standard additions. Although this required that at least two adsorption experiments be done for each sample, results obtained in this manner for doped urine specimens were ordinarily within $\pm 5\%$ of the known value as long as the standard addition did not raise the analyte concentration to a value above the linear range of the flow-injection/adsorption method.

The imprecision obtained by this approach was evaluated by comparing a

series of d.p.v. scans after preconcentration of identical aliquots from the same urine solution at several freshly prepared carbon paste surfaces and was generally in the 5–10% range. For example, the mean peak current obtained in this manner for six sequential measurements made on a 5×10^{-7} M sample was 65 nA with a relative standard deviation of 8.7%. This degree of variation is comparable to that expected for the removal and generation of new carbon paste surfaces [20].

In practice, the principal strengths of the flow-injection/adsorption approach for doxorubicin determination are its sensitivity and its simplicity. In both respects, the currently described approach which permits detection in real sample matrices in the nanomolar range with no sample treatment at all is clearly superior to the previously described batch preconcentration approach [18]. Even in the most unfavorable cases for the flow-injection method, the detection limit (i.e., signal/noise = 2) was always lower than the 1×10^{-8} M level found for the batch procedure. Further, the manual sample transfer operations required for the latter are avoided, producing an additional improvement in time and operator involvement. Although the selectivity of both adsorption-based methods towards nonadsorbing interferents is similar, the flow-injection technique appears somewhat more effective in discriminating against adsorbable sample constituents such as uric acid. This is clearly seen in Fig. 2 which shows the d.p.v. scans resulting when both methods were applied to the same urine sample. In curve A, obtained after a 3-min batch preconcentration, the doxorubicin oxidation appeared only as a barely discernible shoulder superimposed on the tail of the much larger wave ascribed to the oxidation of coadsorbed uric acid. Although the adsorption tendency of the latter compound is considerably less than that of doxorubicin, its substantially higher concentration in urine and its slightly lower oxidation potential present a particular problem for the target analysis.

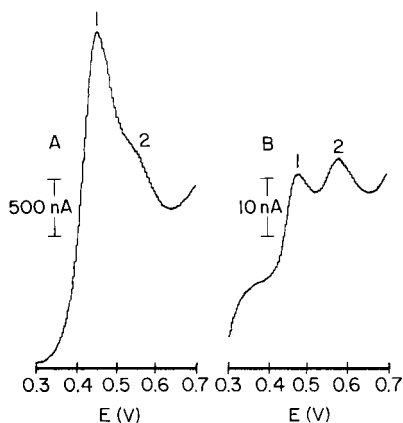


Fig. 2. Differential pulse voltammograms obtained by (A) batch preconcentration and (B) flow-injection preconcentration from a urine specimen with 5×10^{-7} M doxorubicin. (6- μ l injection at 0.1 ml min⁻¹ flow rate.)

Comparative results shown in curve B for the flow-injection mode yielded a pronounced analyte wave with the most dramatic difference being a more than 100-fold decrease in the uric acid current. The net effect was that the doxorubicin signal, the magnitude of which was reduced only slightly in the process, was much better resolved. Although, for the sampling conditions used, the adsorption of the doxorubicin was practically unaltered by the flow mode of sample presentation, the weaker uric acid adsorption was much more drastically affected. Of course, both adsorption approaches offer a remarkable selectivity improvement over direct d.p.v. which provides no real discrimination against uric acid (and other electroactive sample components) and hence is of little or no practical utility without extensive sample treatment.

Direct comparison of the performance of the flow-injection/adsorption approach with h.p.l.c. methods developed for doxorubicin determination is inherently more difficult. Literally dozens of h.p.l.c.-based procedures have been suggested [21]; they entail different times and complexity, have different detection limits, and are compatible with different sample matrices. In terms of real sample detection limits, the best figures reported for h.p.l.c. are in the 2–10 ng ml⁻¹ range; the detection limit of 1×10^{-9} M, or less than 1 ng ml⁻¹, for f.i.a. compares well in this regard. Nearly all reported h.p.l.c. methods require some form of sample treatment, ranging from column enrichment to one or more extraction/evaporation preconcentration steps. By contrast, the f.i.a. performance is achieved with no sample pretreatment; and any external preconcentration procedures suitable for h.p.l.c. usage could, of course, also be applied advantageously to f.i.a. In addition, the freedom of the latter approach from the need to use a chromatographic column in the flow system means that far simpler instrumentation can be utilized.

The most obvious advantage of any h.p.l.c. procedure is its potential for resolving and quantifying separately both the drug itself and its principal metabolites. In the f.i.a. approach, all adsorbing doxorubicin species contribute cumulatively to the single d.p.v. peak. Further, because of the necessity of evaluating the data by standard addition, the minimum of two adsorptions which are required for a single determination brings the total time required to at least 10 min. Also, because no simple chemical or electrochemical procedure was found to be effective for removal of adsorbed doxorubicin, a fresh electrode surface needs to be generated prior to each injection.

In general, this work confirms the suggestion of Wang and Freiha [19] that adsorptive preconcentration and medium exchange done in a flow system can provide a sensitive and selective analytical technique. Doxorubicin seems particularly well suited for such applications because of its extremely vigorous adsorption tendencies. Compared to the chlorpromazine system characterized by Wang and Freiha, doxorubicin adsorption yields considerably larger currents and thus provides linear response to a 100-fold lower detection limit. Clearly, the determination of other strongly adsorbing species by an analogous approach may offer similar advantages.

This work was supported by the College of Arts and Sciences of the University of Louisville.

REFERENCES

- 1 H. L. Davis and T. E. Davis, *Cancer Treat. Rep.*, 63 (1979) 809.
- 2 H. S. Schwartz, *Biochem. Med.*, 7 (1973) 396.
- 3 K. K. Chan and P. A. Harris, *Res. Commun. Chem. Pathol. Pharmacol.*, 6 (1973) 447.
- 4 N. R. Bachur, *Clin. Pharmacol. Ther.*, 21 (1977) 70.
- 5 H. van Vunakis, J. J. Langone, L. J. Riceberg and L. Levine, *Cancer Res.*, 34 (1974) 2546.
- 6 E. Watson and K. K. Chan, *Cancer Treat. Rep.*, 60 (1976) 1611.
- 7 H. G. Barth and A. Z. Canner, *J. Chromatogr.*, 131 (1977) 375.
- 8 R. Baurain, D. Deprez-DeCampeneere and A. Trovet, *Anal. Biochem.*, 94 (1974) 112.
- 9 J. H. Peters and J. F. Murray, Jr., *J. Liq. Chromatogr.*, 2 (1979) 45.
- 10 M. J. Sepaniak and E. S. Yeung, *J. Chromatogr.*, 190 (1980) 377.
- 11 S. Shinozawa, Y. Mimaki, H. Tamano, Y. Araki and T. Oda, *J. Chromatogr.*, 190 (1970) 489.
- 12 S. Shinozawa and T. Oda, *J. Chromatogr.*, 212 (1981) 323.
- 13 C. Akpofure, C. A. Riley, J. A. Sinkule and W. E. Evans, *J. Chromatogr.*, 232 (1982) 377.
- 14 G. M. Rao, J. W. Lown and J. A. Plambeck, *J. Electrochem. Soc.*, 125 (1978) 534.
- 15 C. Molinier-Jumel, B. Malfoy, J. A. Reynaud and G. Aubel-Sadron, *Biochem. Biophys. Res. Commun.*, 84 (1978) 441.
- 16 L. A. Sternson and G. Thomas, *Anal. Lett.*, 10 (1977) 99.
- 17 R. P. Baldwin, D. Packett and T. M. Woodcock, *Anal. Chem.*, 53 (1981) 540.
- 18 E. N. Chaney, Jr. and R. P. Baldwin, *Anal. Chem.*, 54 (1982) 2556.
- 19 J. Wang and B. A. Freiha, *Anal. Chem.*, 55 (1983) 1285.
- 20 R. N. Adams, *Electrochemistry at Solid Electrodes*, M. Dekker, New York, 1969, p. 283.
- 21 A. Aszalos, *J. Liq. Chromatogr.*, 7(S-1), (1984) 69 (and references therein).

CATHODIC STRIPPING VOLTAMMETRY OF 2-MERCAPTOETHANOL

E. CASASSAS*, C. ARIÑO and M. ESTEBAN

*Department of Analytical Chemistry, Faculty of Chemistry, University of Barcelona,
Av. Diagonal 647, 08028-Barcelona (Spain)*

(Received 19th March 1985)

SUMMARY

The behaviour of 2-mercaptoethanol at a hanging mercury drop electrode by cathodic stripping voltammetry (c.s.v.) is studied. The stripping curves are recorded by three scanning modes: rapid-scan direct-current, differential-pulse and fundamental harmonic alternating-current polarography. Under the recommended conditions, pre-electrolysis is done at a potential of 0.0 V vs. Ag/AgCl for 3 min in a medium of pH 6.7 or 8 (Britton-Robinson buffer). Then after 1 min, stripping is done at a scan rate of 6.6 mV s⁻¹ preferably in the differential-pulse mode. The stripping peak at about -0.4 V is used to determine 2-mercaptoethanol within the concentration range 3×10^{-8} – 8×10^{-7} mol l⁻¹. Calibration functions are reported; the standard additions method is preferred near the limit of detection. The interferences of several organic compounds are described.

The importance of thiols in biochemistry and pharmacology has resulted in a plethora of methods for the determination of such compounds. Among these methods, cathodic stripping voltammetry (c.s.v.) is important, as it is useful at submicromolar levels. In the pre-electrolysis step, the thiol is accumulated on the electrode as a sparingly soluble mercury thiolate. Thiol determinations by c.s.v. at a hanging mercury drop electrode (HMDE) have been studied by Bond et al. [1], Forsman [2] and Florence [3]. Cysteine is the compound that has received the greatest attention. Determination of 2-mercaptoethanol by this technique has not been studied previously, and the parameters that influence the determination have not yet been explored. Only Florence [3] described the presence of a stripping peak at -0.51 V, occurring after 3 min of electrodeposition from a 1×10^{-7} mol l⁻¹ solution of 2-mercaptoethanol in a 0.1 mol l⁻¹ sodium borate/0.02 mol l⁻¹ sodium chloride medium, whereas Davidson and Smyth [4] observed the presence of two peaks, at -0.27 V and -0.31 V (but not at -0.51 V) in a 5×10^{-5} mol l⁻¹ solution in a Britton-Robinson buffer.

In work reported here, the cathodic stripping voltammetric behaviour of 2-mercaptoethanol at a HMDE was studied in detail. For the stripping step, direct current polarography (d.c.p.), differential pulse polarography (d.p.p.) and fundamental harmonic alternating-current polarography (a.c.p.) were applied.

EXPERIMENTAL

Chemicals and instrumentation

Aqueous solutions of 2-mercaptoethanol (Merck; analytical-reagent grade) were prepared daily, in air-free twice-distilled water. These solutions were standardized by titration with iodate [5].

Buffer solutions were prepared from Merck (Suprapur-grade) reagents as described by Mongay and Cerdà [6] who modified the B.R. buffer solutions, for the desired pH range at a constant ionic strength of 0.1 mol l^{-1} . For the interference study, the following compounds were used: 2,2'-thiodiacetic acid (Fluka purum), 2,2'-[1,1-methandiylbisthio]diacetic acid (Fluka purum), cystine and cysteine (Carlo Erba), *N*-(2-mercaptopropionyl)glycine (Sigma), glycine (Scharlau) and mono-, di- and tri-ethanolamine (Scharlau).

Voltammograms were obtained with a Metrohm Polarecord E506 attached to an E505 stand. An Ag/AgCl reference electrode (Metrohm EA 441/5) was used in conjunction with a KNO_3 salt bridge. The auxiliary and working electrodes were platinum (Metrohm 285) and a hanging mercury drop electrode (Metrohm EA290), respectively. The polarographic cell was thermostated to $25 \pm 0.1^\circ\text{C}$ and stirred with a constant-speed magnetic stirrer (Metrohm E504).

Procedures

Preliminary procedure. An accurately measured volume of the test 2-mercaptoethanol solution was added to a measured excess of buffer solution of the desired pH and ionic strength in the polarographic cell. The mixed solution was deaerated by bubbling high-purity nitrogen for at least 15 min (for the rest of the experiment, nitrogen was passed above the solution surface). Then, with the stirrer operating, a pre-electrolysis potential was applied for a pre-determined suitable time. After the deposition period and a rest period of 1 min without stirring, a potential scan was applied to the HMDE and the current-potential curve was recorded. The potential scan was made by the three techniques referred to above: rapid-scan d.c., the differential pulse mode, and the fundamental harmonic a.c. mode.

Recommended procedure. A drop of 0.84-mm diameter (4 micrometer divisions) is extruded and a pre-electrolysis potential of 0.0 V is applied to the 2-mercaptoethanol solution (0.1 mol l^{-1} ionic strength, pH 6.0–8.0 with Britton-Robinson buffer) for a pre-electrolysis time of 3.0 min. After a rest time of 1.0 min, the potential scan is run at a scan rate of 6.6 mV s^{-1} . From the height (i_p) of the peak that appears at about -0.4 V , 2-mercaptoethanol concentration is evaluated. The calibration functions are discussed later. The d.p. mode is preferred for the stripping step (with a -40-mV pulse amplitude). At the lower concentration levels, 2-mercaptoethanol is better quantified by means of a standard addition method.

RESULTS AND DISCUSSION

Characteristics of the voltammograms

Within the pH range 4.0–8.6, the stripping voltammograms showed a well defined peak. Peak potential, E_p , was almost independent of the scanning mode used (e.g., at pH 6.0 it was -0.31 V from the d.p. mode, -0.35 V from the rapid-scan d.c. mode and -0.36 V with the a.c.₁ mode), and varied linearly with pH. The slope of the E_p vs. pH plot approached the theoretical value corresponding to an electrode process in which equal numbers of protons and electrons are involved.

In some experiments, an additional peak also appeared, at a potential about 200 mV more negative than the first. The appearance of this second peak did not seem to depend on any particular experimental condition and it did not affect the characteristics of the first peak. Florence, in his exhaustive study on the cystine/cysteine system [3], described the presence of a second peak that appeared in about 20% of the voltammograms (for cysteine) or about 60% of them (for cystine). Florence considered that this peak was due to a change in the morphology of the mercury(II) compound deposited on the electrode surface, probably caused by some impurity in solution, such as a surface-active compound. Miller and Teva [7] attributed the double peak to the presence of both a mercury(I) and a mercury(II) compound in the film, with the more positive peak being due to the mercury(I) compound. Stankovich and Bard [8] suggested that the irregularity observed could be due to a breakdown of the mercury(II) cysteinate film. An extensive study on the behaviour of 2-mercaptoethanol in this laboratory, which will be reported elsewhere, indicated that the almost random formation of the second peak could be due to the presence of a disulfide in the surface film, coming from the anodic oxidation of 2-mercaptoethanol during the pre-electrolysis.

It is, of course, the peak that appears at the more positive potential, designated here as the first peak, which is suitable for analytical use. In order to establish a c.s.v. determination for 2-mercaptoethanol based on this peak, the main operational and instrumental parameters affecting it were optimized.

Evaluation of the experimental and instrumental parameters

Pre-electrolysis potential. It is generally considered that the pre-electrolysis potential (E_{pe}) applied should be more positive than the polarographic half-wave potential of the compound to be anodically oxidized, although in certain instances Davidson and Smyth [4] preferred to use an E_{pe} value equal to that of $E_{1/2}$. For the accumulation of 2-mercaptoethanol ($E_{1/2}$ about -0.50 V, at pH 6.4) on the electrode, the general usage was followed, and several more positive E_{pe} values were tested, ranging from -0.40 V to $+0.50$ V. Figure 1 shows stripping peak heights (maximum peak current, i_p) as a function of E_{pe} , for a 3-min pre-electrolysis time in a 2.12×10^{-6} mol l⁻¹ 2-mercaptoethanol solution at pH 6.4. Maximum peak height was obtained,

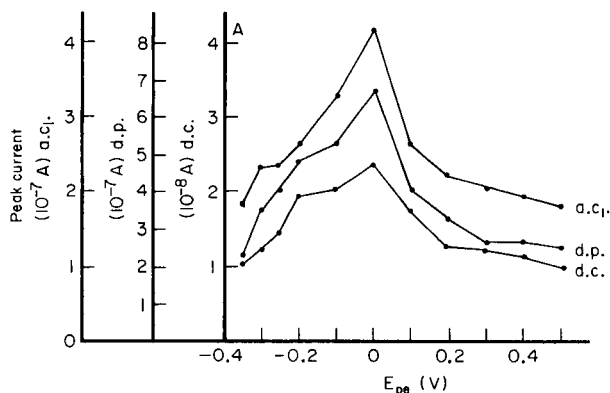


Fig. 1. Dependence of peak current on pre-electrolysis potential, by the three stripping modes, for a 2.12×10^{-6} mol l^{-1} 2-mercaptoethanol solution at pH 6.4 (pre-electrolysis time, 3 min).

whichever scanning mode was used, when the pre-electrolysis potential was 0.0 V. This pre-electrolysis potential was therefore adopted for the analytical procedure. At E_{pe} values lower than 0.0 V, the anodic current responsible for the accumulation of the electroactive species will obviously be lower, and the stripping signal decreases. At more positive potentials, the observed decrease in signal may be due to a decrease in the current yield, the formation of the electroactive species being accompanied by some other anodic reaction, e.g., oxidation of the thiol to disulfide. This sort of variation in signal height with E_{pe} has been observed by other authors [9–11]. Kemula et al. [9] and Bewick et al. [11] explained it on kinetic grounds: at small overpotentials the rate of formation of a crystal in the surface film on the electrode is governed by the slow incorporation of mercury(I) ions into the crystal lattice, but, at more positive potentials these ions are formed so quickly that they cannot be incorporated and are desorbed, forming the mercury(I) compound in solution instead of on the electrode surface.

It must be noted that the above-described second peak appears only at the most positive values of the pre-electrolysis potentials.

Pre-electrolysis time. The influence of the pre-electrolysis time, t_{pe} , (at a potential of 0.0 V) on the peak height obtained in the stripping voltammograms was studied in the range 1–10 min. Peak height, for the first peak, changed with t_{pe} in an unusual way: it decreased with increasing times, as shown in Fig. 2. A pre-electrolysis time of 3.0 min was arbitrarily adopted for the analytical procedure: a good voltammetric signal with excellent reproducibility was obtained after this time interval, which can easily be measured.

This unusual behaviour cannot be explained only by a change in the electrode reaction with time, which could pass from a mercury thiolate-forming reaction to an oxidation reaction yielding disulfide. Some kinetic control of

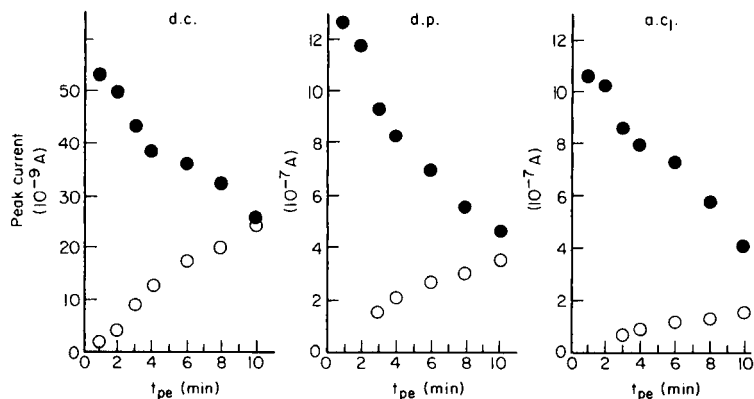


Fig. 2. Dependence of peak current on pre-electrolysis time, by the three stripping modes, for a 9.797×10^{-7} mol l^{-1} 2-mercaptoethanol solution at pH 6.7 (pre-electrolysis potential, 0.0 V): (●) first peak; (○) second peak.

the kind mentioned above must play a role. The mercury thiolate film must be formed initially by very small crystal nuclei, which would be very reactive, but the crystal growth must be fast and the well-formed crystals must be compact enough to resist the potential scan without being fully reduced. In fact, the film material is not wholly stripped in the first potential scan, for a reduction peak, very much decreased in height, appears in a second scan, and persists, slowly decreasing, in successive potential scans.

The second peak, when it appears, shows a normal variation with the length of the pre-electrolysis period, as is also shown in Fig. 2.

Scan rate. The variation of peak height, i_p , vs. the square root of the scan rate, $v^{1/2}$, in the range 3–15 $mV s^{-1}$, was studied for a 1×10^{-6} mol l^{-1} 2-mercaptoethanol solution, at pH 6.7, after a 3-min pre-electrolysis at 0.0 V. For the first peak, the relationship was linear, regardless of the scan mode used, showing that the electrode process is controlled by diffusion of 2-mercaptoethanol from the electrode surface layer to the bulk of solution [12]. A stripping scan rate of 6.6 $mV s^{-1}$ was chosen for the analytical procedure.

Electrode area. A linear dependence was also obtained between peak height and electrode area, indicating that there was no diffusion of the species deposited on the electrode surface to the interior [12]. A 2.20 ± 0.05 -mm² electrode area (corresponding to 4 micrometer divisions) was adopted for further work.

Influence of pH. The influence of pH was studied in the range 4–10 by the three stripping modes. Within the pH range studied, two stripping peaks were observed. Their peak potentials (E_p) were linearly related to pH in the range 4–8.5, as Fig. 3 shows for a solution 7.6×10^{-7} mol l^{-1} in 2-mercaptoethanol. At pH 8.5, the E_p values were more negative than expected from the linearity. Peak height was pH-independent within the pH range 6–8. This range was chosen for analytical application.

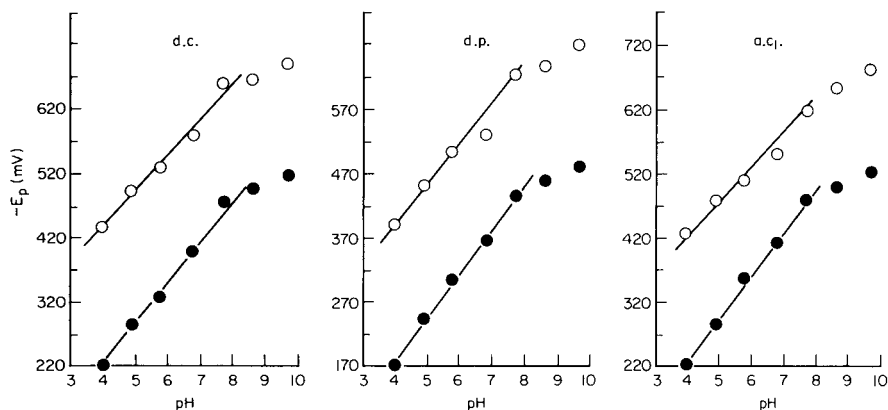


Fig. 3. Dependence of peak potentials on pH values, by the three stripping modes, for $7.6 \times 10^{-7} \text{ mol l}^{-1}$ 2-mercaptoethanol ($t_{pe} = 3 \text{ min}$, $E_{pe} = 0 \text{ V}$): (●) first peak; (○) second peak.

Calibration

When the prescribed pre-electrolysis and rest times and the above-mentioned instrumental parameters were used, a linear dependence was found, regardless of which mode was used for the potential scan, between peak height and 2-mercaptoethanol concentration within the range from 3×10^{-8} to $8 \times 10^{-7} \text{ mol l}^{-1}$. At pH 6.7, this relationship was given by the following calibration functions: $i_p = 7.58 \times 10^{-2}[\text{RSH}] - 6.23 \times 10^{-5}$ ($r^2 = 0.9966$), for experiments made with the rapid-scan d.c. mode; $i_p = 1.722[\text{RSH}] - 1.53 \times 10^{-2}$ ($r^2 = 0.9987$) for experiments with the d.p. mode; and $i_p = 1.506[\text{RSH}] - 3.57 \times 10^{-2}$ ($r^2 = 0.9993$), for experiments with the a.c1. potential scan (where i_p is peak current in μA and $[\text{RSH}]$ is the 2-mercaptoethanol concentration in $\mu\text{mol l}^{-1}$).

From the calibration plots, the detection limits for 2-mercaptoethanol determination were evaluated in the usual way [13]. The limits at pH 6.7 were: $7.35 \times 10^{-8} \text{ mol l}^{-1}$ (or $5.74 \mu\text{g kg}^{-1}$) by the d.c. mode, $4.86 \times 10^{-8} \text{ mol l}^{-1}$ (or $3.80 \mu\text{g kg}^{-1}$) by the d.p. mode and $8.82 \times 10^{-8} \text{ mol l}^{-1}$ (or $6.89 \mu\text{g kg}^{-1}$) by the a.c1. mode. The limits at pH 8 were: $5.14 \times 10^{-8} \text{ mol l}^{-1}$ (or $4.01 \mu\text{g kg}^{-1}$) by the d.c. mode, $3.14 \times 10^{-8} \text{ mol l}^{-1}$ (or $2.45 \mu\text{g kg}^{-1}$) by the d.p. mode and $5.81 \times 10^{-8} \text{ mol l}^{-1}$ (or $45.4 \mu\text{g kg}^{-1}$) by the a.c1. mode.

Main figures of merit of the recommended procedure for 2-mercaptoethanol

As far as the sensitivity, $di_p/d[\text{RSH}]$, is concerned, it can be seen from the above calibration functions that the highest sensitivity of the procedure, $1.722 \mu\text{A } \mu\text{mol}^{-1} \text{ l}$, is obtained when the potential scan is run in the d.p. mode; the a.c1. technique is almost as sensitive, but the rapid-scan d.c. technique is much lower in sensitivity.

As far as the limit of determination of c.s.v. is concerned, the background

signal or instrumental noise in the a.c.₁ mode is more intense than that found for the other techniques, and consequently the reproducibility at the lowest concentration levels is much poorer. Potential scanning by the d.p. technique is, therefore, to be recommended. This mode provided a limit of determination of 3.1×10^{-8} mol l⁻¹; with the other techniques, the limit was higher (about 7×10^{-8} mol l⁻¹) and the precision was worse. At 2-mercaptoethanol concentration lower than the given limits, a voltammetric signal can be obtained but is useless for quantitative purposes.

Interferences

Several compounds potentially capable of interfering in 2-mercaptoethanol determinations were investigated. The compounds chosen represent three different groups: (i) compounds which contain the mercapto group, like cysteine and *N*-(2-mercaptopropionyl)glycine, (ii) compounds which contain a thioether group, like 2,2'-thiodiacetic acid, and 2,2'-[1,1-methandiylbis-thio]diacetic acid, and (iii) compounds without sulphur groups which are of biological interest and can form mercury complexes, like glycine and mono-, di- and tri-ethanolamine. The mercapto compounds interfered strongly; they produced an increase in the voltammetric signal and their tolerance ratio was about 1:1. As was expected from a separate study of thioethers, which will be reported elsewhere [14], among the compounds in this group only the homologues of 2,2'-thiodiacetic acid interfered at concentration ratios higher than 25:1 (for a 2-mercaptoethanol concentration of 10^{-7} mol l⁻¹, the interference being greater at lower 2-mercaptoethanol concentrations). Other thioethers and the compounds in the third group studied did not interfere.

REFERENCES

- 1 A. M. Bond, S. B. Thomsom, D. J. Tucker and M. H. Briggs, *Anal. Chim. Acta*, 156 (1984) 33.
- 2 U. Forsman, *Anal. Chim. Acta*, 146 (1983) 71; 156 (1984) 43.
- 3 T. M. Florence, *J. Electroanal. Chem.*, 97 (1979) 219.
- 4 I. E. Davidson and W. Franklin Smyth, *Anal. Chem.*, 49 (1977) 1195; *Anal. Chim. Acta*, 147 (1983) 53.
- 5 S. Siggia, *Quantitative Organic Analysis via Functional Groups*, 3rd edn., Wiley, New York, 1963.
- 6 C. Mongay and V. Cerdà, *An. Quim. B*, 74 (1978) 445.
- 7 I. R. Miller and J. Teva, *J. Electroanal. Chem.*, 36 (1972) 157.
- 8 M. T. Stankovich and A. J. Bard, *J. Electroanal. Chem.*, 75 (1977) 487.
- 9 W. Kemula, Z. Kublik and J. Taraszewska, *Microchem. J. Symp.*, 1 (1962) 865.
- 10 Z. Galus, in W. Fresenius (Ed.), *Reviews on Analytical Chemistry (Euroanalysis Conference II)*, Masson, Paris, 1977, p. 65.
- 11 A. Bewick, M. Fleischmann and H. R. Thirsk, *Trans. Faraday Soc.*, 58 (1962) 2200.
- 12 J. P. Perchard, M. Buvet and R. Molina, *J. Electroanal. Chem.*, 14 (1967) 761.
- 13 R. Caulcutt and R. Boddy, *Statistics for Analytical Chemists*, Chapman and Hall, London, 1983, p. 201.
- 14 E. Casassas, M. Esteban and C. Ariño, submitted for publication.

ELECTROANALYTICAL STUDIES OF THE PESTICIDE MENAZON AND ITS HYDROLYSIS PRODUCTS

J. HERNÁNDEZ MÉNDEZ*, R. CARABIAS MARTÍNEZ, M. E. GONZÁLEZ LÓPEZ
and M. CUESTA GONZÁLEZ

*Department of Analytical Chemistry, Faculty of Chemistry, University of Salamanca,
Salamanca (Spain)*

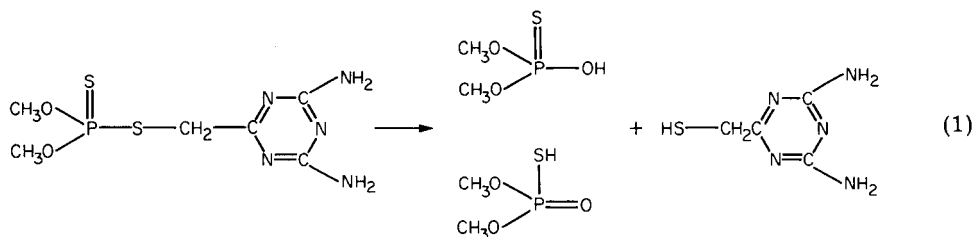
(Received 15th March 1985)

SUMMARY

An electroanalytical study based on d.c. polarography, differential pulse polarography (d.p.p.) and coulometry is described for Menazon, *S*-[4, 6-diamino-1,3,5-triazin-2-yl)-methyl]-*O,O*-dimethylphosphorodithioate, and its alkaline hydrolysis products in a (1 + 4) methanol/water medium. The responses of the different species are studied for analytical utility. Menazon itself produces two diffusion-controlled cathodic waves or peaks at pH 4.78; the determination limit is 5.5×10^{-7} M for the d.p. peak at about -0.8 V vs. Ag/AgCl. The hydrolysis products obtained in 0.1 M sodium hydroxide give rise to four anodic waves. Acidification of the alkaline medium to pH 4.3 produces three well-defined waves, two cathodic and one anodic. The responses for the cathodic processes are linear with concentration over 2–2.5 orders of magnitude; the anodic wave is adsorption-controlled and provides linear response only at low concentrations. The detection limit for the hydrolysis products is 0.17×10^{-6} M. Reaction mechanisms are proposed.

Methods for the determination of pesticides and/or their degradation products are of evident interest. Most methods are spectrophotometric or chromatographic; electroanalytical techniques have been applied mainly to the determination of pesticides containing the electroactive nitro group [1–4]. Of special interest is the early work on pesticide residues reported by Martens and Nangniot [5] and Nangniot [6], and the reviews of applications of electroanalytical techniques by Gajan [7].

The present work is concerned with an electroanalytical study of the pesticide Menazon, *S*-[(4,6-diamino-1,3,5-triazin-2-yl)methyl]-*O,O'*-dimethylphosphorodithioate, and its hydrolysis products. Direct current polarography (d.c.p.), differential pulse polarography (d.p.p.) and coulometric techniques were applied. Menazon is stable at temperatures below 50°C and in media which are neither too acid nor too alkaline. In alkaline media, the compound is hydrolyzed quite rapidly, producing 4,6-diamino-1,3,5-triazin-2-methylmercaptan and the corresponding thiophosphate which may be found either in the thione (alcoholic media) or thiol (aqueous media) form:



EXPERIMENTAL

Apparatus and reagents

A Metrohm E-505 polarograph was used with a mercury forced-drop electrode (Metrohm EA-1019-1), a platinum auxiliary electrode (Metrohm EA-285) and a Ag/AgCl reference electrode (Metrohm EA-441-5). For the coulometric study, an Amel model 563 electroanalytical apparatus was used with a mercury pool working electrode.

Solutions of Menazon were prepared by dissolving the solid product (99.5% purity; Zeltia-Agraria) in 1:1 methanol/water. Other solutions were 0.2 M acetic acid, 0.2 M sodium acetate, 2.5 M sodium hydroxide and 0.6 M Britton-Robinson buffer. All solutions were prepared from analytical-grade chemicals with twice-distilled water.

Procedures

The polarographic study of Menazon was done in a 0.06 M acetic acid/0.04 M sodium acetate solution in (1 + 4) methanol/water. After nitrogen had bubbled through the solution for 10 min to deaerate it, the corresponding d.c. or d.p. polarograms were recorded; in the latter case the pulse amplitude was -50 mV and the scan rate was 4 mV s $^{-1}$. In both techniques, the drop time was 2 s.

In the studies of the alkaline hydrolysis products of Menazon, the solution of Menazon in (1 + 4) methanol/water was deaerated for 10 min after which 2.0 ml of 2.5 M sodium hydroxide was added and the pesticide was left to hydrolyze for 40 min under nitrogen. Once hydrolysis was complete, the d.c. or d.p. polarograms were recorded. To study the hydrolysis products in an acidic medium, the procedure was the same as above except that after the 40 min of hydrolysis, Britton-Robinson buffer was added to give the desired pH.

RESULTS AND DISCUSSION

Polarographic behaviour of Menazon and its hydrolysis products

The electroanalytical behaviour of Menazon is largely influenced by the pH of the medium. In mildly acidic media (pH 4.8) it exhibits two cathodic waves (C-1 and C-2) at peak potentials close to -0.84 and -1.30 V (Fig. 1a);

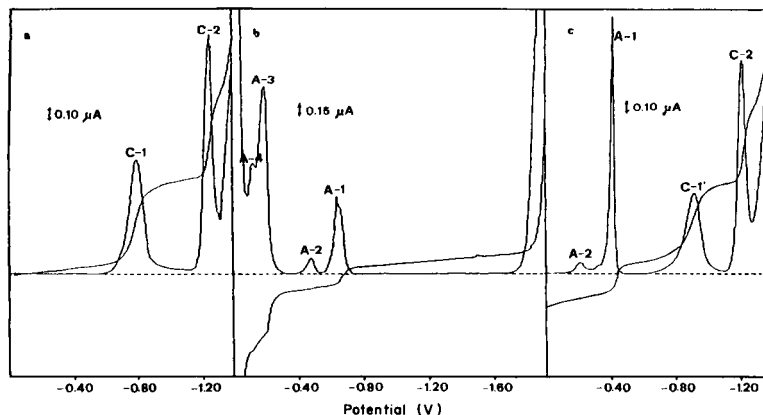


Fig. 1. Polarograms (d.c. and d.p.) produced by Menazon and its alkaline hydrolysis products at different pH values in (1 + 4) methanol/water. (a) 2.0×10^{-4} M Menazon in the acetate buffer of pH 4.78; (b) hydrolysis products of 2.0×10^{-4} M Menazon in 0.1 M NaOH; (c) hydrolysis products of 1.7×10^{-4} M Menazon in 0.1 M NaOH/0.07 M Britton-Robinson buffer at pH 4.3.

these waves are shifted towards more negative potentials as the pH is increased. At pH 8, only wave C-1 is observed. In more strongly alkaline medium (Fig. 1b), the reduction waves disappear and four anodic waves, or d.p.p. peaks, appear; these peaks (A-1 to A-4) are related to the hydrolysis products of the pesticide. Because hydrolysis is not immediate, the magnitudes of these waves or peaks increase with time (Fig. 2), reaching constant values at 40 min; wave A-3 behaves differently because it is not diffusion-controlled (see below). The results in alkaline medium are only obtained if hydrolysis and measurements are done in the absence of oxygen. In its presence, there

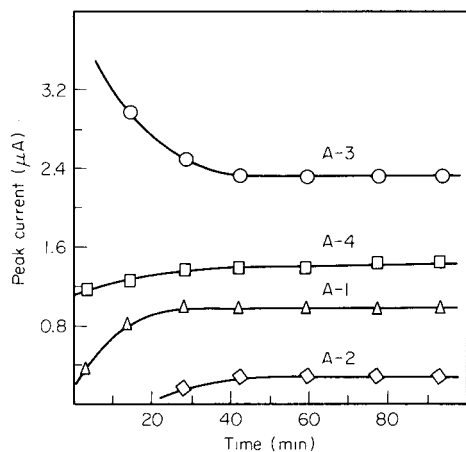


Fig. 2. Variation of the peak currents produced by the hydrolysis products as a function of time. Conditions as for Fig. 1b.

is a poorly controlled oxidation of the hydrolysis products and of the mercury electrode; mercury is easily oxidized in the presence of thiol groups [8]. These effects produce a series of waves which are not reproducible, difficult to study, and even harder to interpret.

If, once alkaline hydrolysis has taken place in the absence of oxygen, the solution is acidified (pH 4.3), the polarogram obtained exhibits two anodic waves (A-1 and A-2) at potentials of -0.44 and -0.24 V, respectively, and three cathodic waves (C-1', C-2 and C-3) at potentials of -0.93 , -1.22 and -1.68 V, respectively (Fig. 1c). The C-3 wave, which does not appear in the polarograms of unhydrolyzed Menazon, is not shown in Fig. 1(c) because it is poorly defined and is very close to the electrolyte reduction wave. The other two waves are reproducible and are unaffected by the presence of oxygen, showing that in acidic media the hydrolysis products of Menazon are not oxidized and mercury/thiol reactions do not occur. The C-1' wave related to the hydrolysis products of Menazon in acidic medium has a potential similar to that of C-1 caused by the unhydrolyzed Menazon.

In order to determine the number of protons involved in the various electrode processes, the variation of $E_{1/2}$ with pH was studied for the best-defined waves, i.e., C-1, C-2 (Menazon) and C-1', C-2 and A-1 (hydrolysis products). Waves A-3 and A-4 were not studied because they appear only in media with pH > 13 ; wave C-3 was ignored for the reasons outlined above; wave A-2 appeared only at Menazon concentrations exceeding 2.5×10^{-5} M. The changes of $E_{1/2}$ with pH are shown in Fig. 3; the coefficients $\Delta E_{1/2}/\Delta \text{pH}$ are shown in Table 1. For waves C-1, C-1' and C-2, the slopes of the plots change at pH values close to 3.8, a value which is similar to the $\text{p}K_a$ value of Menazon [9]. The A-1 half-wave potential varies with pH up to pH 6.0 but remains almost constant at higher pH.

The logarithmic data for the C-1 and C-2 waves of unhydrolyzed Menazon

TABLE 1

Determination of the number of protons involved (p) in the C-1 and C-2 waves of Menazon and the A-1, C-1' and C-2 waves of the hydrolysis products of the pesticide

Wave	pH	$\Delta E_{1/2}/\Delta \text{pH}$	$\Delta E/\Delta \log [(i_d - i)/i]$	p
C-1	< 3.8	0.056	0.074	0.8
	> 3.8	0.082	0.064	1.3
C-2	< 3.8	0.059	0.046	1.3
	> 3.8	0.088	0.039	2.2
A-1	< 6	0.058	0.026 ^a	2.2
C-1'	< 3.8	0.069	0.061	1.1
	> 3.8	0.112	0.084	1.3
C-2	< 3.8	0.048	0.040	1.2
	> 3.8	0.090	0.040	2.3

^a $\Delta E/\Delta \log [(i - i_c)/(i_a - i)^2]$.

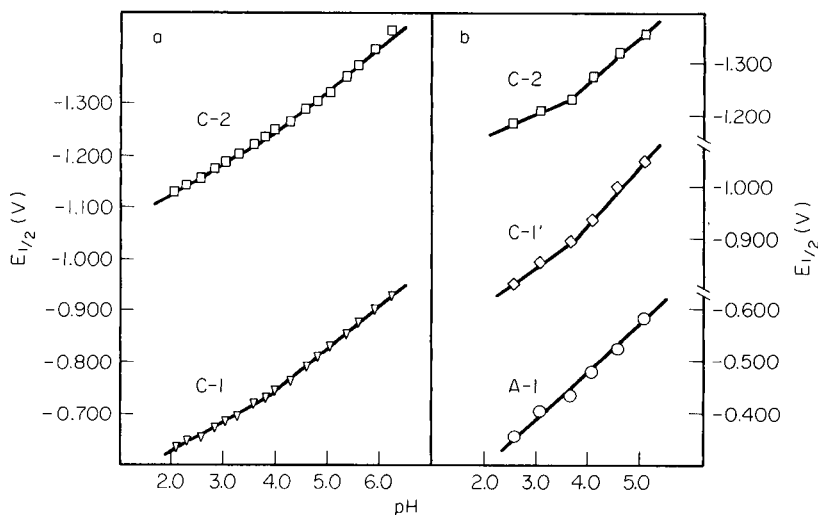


Fig. 3. Variation of $E_{1/2}$ with pH: (a) Menazon; (b) hydrolysis products. For details, see text.

and the C-1' and C-2 waves of hydrolyzed Menazon suggest that these processes are irreversible (Table 1). The A-1 anodic wave has a cathodic component which together with other data indicates that this wave is caused by the oxidation of mercury in the presence of thiol. For this wave, a plot of $\log [(i - i_c)/(i_a - i)^2]$ vs. potential had a slope of 0.026, showing that the reaction is reversible if two electrons are exchanged in the electrode process.

The number of protons exchanged in the different reactions was evaluated from the measured value of $E_{1/2}$ and the values calculated for αn [10] (Table 1). For the C-1 process, only one proton was involved in the electrode reaction, whereas for the C-2 wave, one proton was involved at pH < 3.8 and two protons at higher pH values. A similar study of the hydrolysis products of Menazon showed that one proton was involved in the C-1' process; for the C-2 process, the results obtained were the same as those for the unhydrolyzed pesticide. For the A-1 wave, two protons were involved at pH < 6.0 but no protons were exchanged at higher pH (Table 1).

Coulometric study

The coulometric study was conducted with unhydrolyzed Menazon at pH 4.8. With a mercury pool electrode and an applied potential of -1.1 V, the reduction products related to the C-1 wave must be species which give rise to oxidation waves. Figure 4(a) shows the polarograms before and after coulometry; curve II shows the disappearance of the C-1 wave and the appearance of an anodic wave. It is also possible to observe the formation of a dark film on the mercury pool. However, it was not possible to obtain reproducible results from this coulometric analysis with respect to the number of electrons involved.

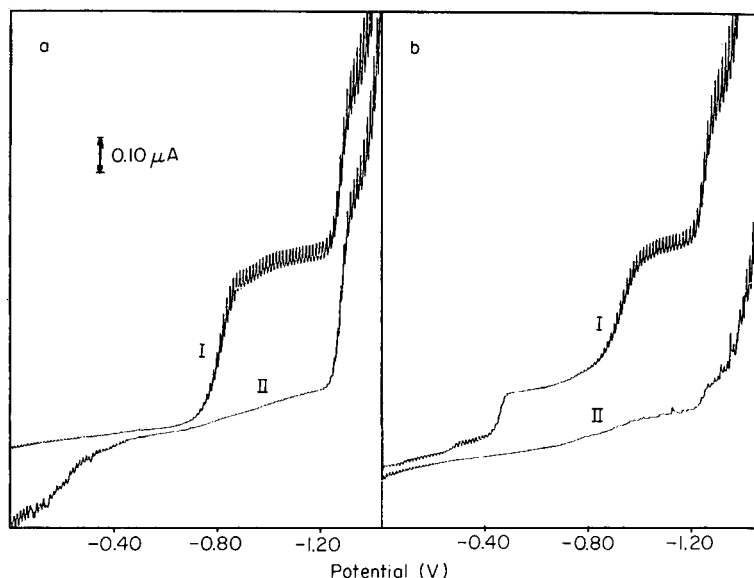


Fig. 4. Coulometric study in (1 + 4) methanol/water. (a) 2.0×10^{-4} M Menazon in acetate buffer pH 4.8: (I) before coulometry; (II) after coulometry at -1.1 V. (b) Hydrolysis products (1.7×10^{-4} M) from 0.1 M NaOH with adjustment to pH 4.3: (I) before coulometry; (II) after coulometry at -0.15 V.

When coulometry at pH 4.3 was used for the hydrolysis products of Menazon, at a potential of -0.15 V it was possible to evaluate accurately that one electron was involved in the A-1 process per mole of oxidized substance. In the polarogram recorded after coulometry (Fig. 4b, curve II), no waves are seen because during the electrode reaction an insoluble product is formed by reaction of the electroactive substance with mercury. Comparison of the limiting current of the A-1 d.c. wave with those of the C-1' and C-2 waves suggests that two electrons are transferred per mole of substance in the latter cases.

Influence of temperature

The influence of temperature on the limiting currents in d.c.p. and on the peak currents in d.p.p. was studied for the C-1, C-2 (unhydrolyzed Menazon) and C-1', C-2, C-3, A-1 and A-2 (hydrolysis products) waves. The temperature coefficients were also evaluated; they were found to be 1.79 and 1.27 for the C-1 and C-2 waves of Menazon, and 1.30, 1.70 and 1.30 for the A-2, C-1' and C-2 processes of the hydrolysis products, respectively. The processes C-1, C-2 and C-1' are diffusion-controlled. The A-1 wave shows a zero temperature coefficient in both d.c.p. and d.p.p. so that the process cannot be diffusion-controlled; nor can the wave be kinetic in nature so that adsorption processes must be assumed; as the temperature increases, the amount of substance adsorbed decreases and oxidation takes place at more anodic potentials

than those corresponding to the A-1 wave. The A-2 wave shows a positive temperature coefficient; this wave cannot be observed at temperatures lower than 20°C but is well-defined at temperatures greater than 30°C (Fig. 5).

The C-3 wave can only be measured by d.p.p.; it shows a negative temperature coefficient, and it shifts towards more negative potentials with increasing temperature. The temperature coefficient and the peak currents obtained suggest that it is a hydrogen catalytic wave with adsorption. Vlček [11] reported that the presence of thiol groups favours proton reduction. As the C-3 wave comes from the hydrolysis products of Menazon, the thiol group could be responsible.

Influence of pulse amplitude and drop time

The effect of variable pulse amplitude is shown in Fig. 6; the results fit theoretical predictions [12] for peaks A-1, C-2 and C-3, but not for the C-1' and C-1 peaks. The plots for the A-2 peak are not shown because it is poorly defined (under these pH and temperature conditions) for pulse amplitudes less than 50 mV.

The results obtained for the effect of drop time (τ) on the waves from Menazon and its hydrolysis products showed that for the C-1, C-2 and C-1' waves, there was a linear relationship between i_p and $\tau^{2/3}$. For the C-3 and

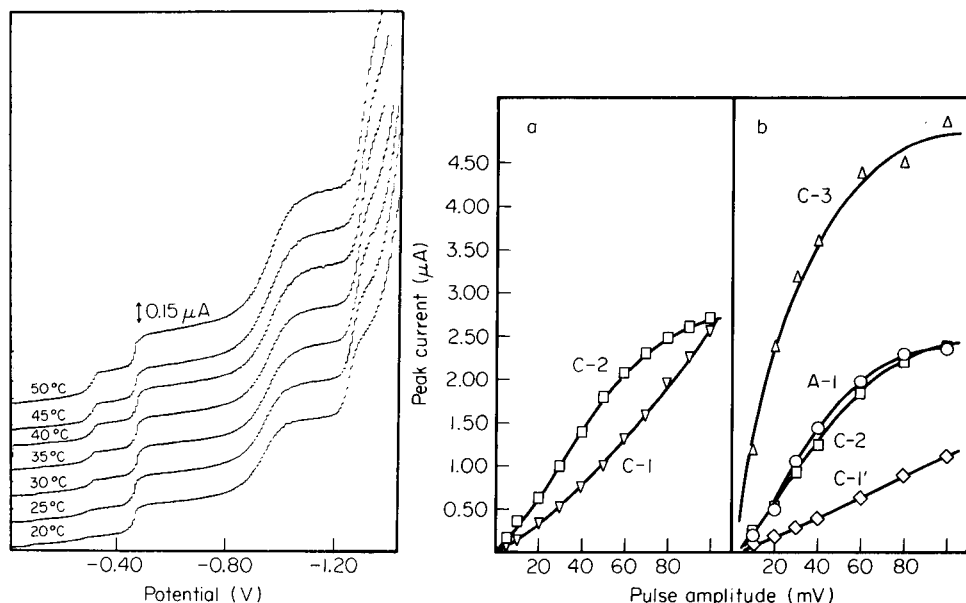


Fig. 5. Polarograms (d.c.) of the alkaline hydrolysis products of Menazon (1.7×10^{-4} M) at different temperatures (pH 4.3).

Fig. 6. Variation in peak currents with pulse amplitude: (a) Menazon (2.0×10^{-4} M) in (1 + 4) methanol/water at pH 4.8; (b) hydrolysis products (1.7×10^{-4} M) from 0.1 M NaOH with adjustment to pH 4.3. Scan rate 4 mV s^{-1} .

A-1 waves, the relationship was not linear; thus these waves are not diffusion-controlled. The A-2 peak was badly defined for drop times of <3 s.

Calibration

Calibration plots were obtained for both conventional and differential pulse polarography in different media when limiting current and peak current were used, respectively.

Unhydrolyzed pesticide. The results obtained for the C-1 and C-2 waves in the acetate buffered media are shown in Table 2. Good linear ranges were obtained in both cases over 2–2.5 orders of magnitude above the determination limits. The determination limits were calculated according to the criteria of Winefordner and Long [13].

Hydrolyzed pesticide. When the alkaline medium was used, the A-1 wave in d.c. polarography provided linear calibration over two orders of magnitude. The A-3 wave gave a linear response only up to about 9.0×10^{-5} M. With d.p.p., the A-1 peak provided a linear relationship for concentrations lower than 1.4×10^{-4} M and over the whole range studied (up to 10^{-3} M) if the

TABLE 2

Calibration data for d.c. and d.p. polarography

Sample	Medium ^a	Wave	Slope ($\mu\text{A mM}^{-1}$)	Intercept (μA)	<i>r</i>	s.d. (μA)	Determination li (10^{-6} M)		
							I	II	III
<i>D.c. polarography (2-s drop time, 25°C)</i>									
Menazon	Ac	C-1	2.84 ± 0.05	0.008 ± 0.005	0.998	0.004	4.23	4.48	6
		C-2	3.18 ± 0.14	0.036 ± 0.013	0.992	0.004	3.78	4.40	12
Hydrolysis products	Na	A-3 ^c	4.77 ± 0.07	0.017 ± 0.004	0.999	0.004	2.51	2.76	3
		A-1	1.35 ± 0.04	0.006 ± 0.004	0.997	0.007	15.61	17.38	17
	BR	A-1 ^d	1.43 ± 0.08	−0.002 ± 0.004	0.992	0.005	10.45	13.68	13
		C-1'	3.28 ± 0.02	−0.015 ± 0.008	0.997	0.006	5.48	6.08	9
		C-2	4.05 ± 0.10	−0.000 ± 0.009	0.999	0.004	2.96	3.27	7
<i>D.p. polarography^e</i>									
Menazon	Ac	C-1	4.40 ± 0.03	−0.001 ± 0.003	0.999	0.0008	0.55	0.56	1
		C-2	8.92 ± 0.05	0.012 ± 0.005	0.999	0.0019	0.64	0.65	1
Hydrolysis products	Na	A-3 ^f	35.48 ± 0.55	−0.033 ± 0.022	0.999	0.0020	0.17	0.18	1
		A-1 ^g	6.90 ± 0.13	−0.024 ± 0.008	0.996	0.0004	0.17	0.18	5
	BR	A-1 ^h	4.21 ± 0.15	−0.008 ± 0.002	0.997	0.0005	0.36	0.42	1
		C-1'	3.68 ± 0.03	−0.004 ± 0.002	0.999	0.0009	0.73	0.75	1
		C-2	8.92 ± 0.03	−0.006 ± 0.002	0.999	0.0006	0.20	0.20	0

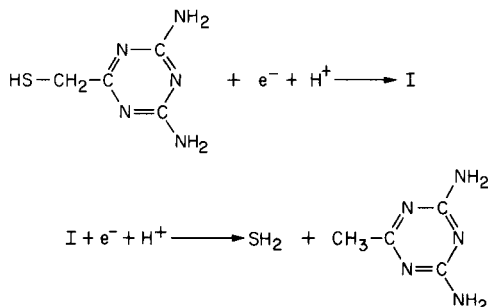
^a Ac, 0.06 M acetic acid/0.04 M sodium acetate. Na, NaOH 0.1 M. BR, Britton-Robinson buffer at pH 4.3. ^b Determination limit calculated in 3 ways: (I) $3 S_B/m$; (II) $3 S_B/(m - t_\alpha S_m)$; (III) $3 [S_B^2 + S_i^2 + (i/m)^2 S_m^2]^{1/2}/m$. ^{c,d} Only at concentrations lower than 8.65×10^{-5} M (c) and 8.47×10^{-5} M (d). ^e Conditions: ΔE , -50 mV; s-2 drop time; scan rate 4 mV s⁻¹; 25°C. ^{f-h} Only at concentrations lower than 8.25×10^{-5} M (f) 1.44×10^{-4} M (g) and 2.54×10^{-5} M (h).

sum of the peak heights for the A-1 and A-2 processes was plotted. It should be noted that the A-2 peak appeared for concentrations exceeding 1.4×10^{-4} M. This behaviour is in agreement with expectation if it is recalled that the A-1 process is an adsorption pre-wave and A-2 is caused by uninhibited oxidation. The A-3 process provided a linear response for concentrations below 8.0×10^{-5} M.

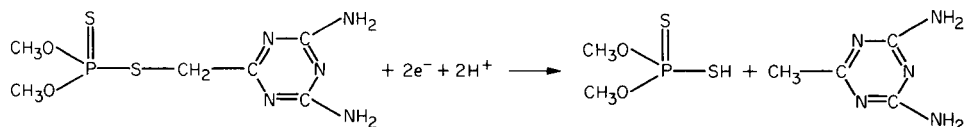
When the solutions of hydrolyzed Menazon were acidified to pH 4.3 by the addition of Britton-Robinson buffer, only the A-1, C-1' and C-2 processes were well-defined. The A-2 wave was also well-defined for high concentrations of the pesticide. The results obtained for these waves are also shown in Table 2. The determination limits are improved in d.p.p. The best waves for analytical purposes are C-1, C-1' and C-2 in view of their better definition and their wider interval of linearity. However, the analytical use of one or the other wave would be influenced by the presence of other species in the solution.

Proposed mechanisms

The C-1' cathodic wave could be caused by breakage of the C-S bond [14] with an exchange of two electrons and 2 protons in the overall reaction and of 1 electron and 1 proton in the rate-determining electron-transfer step:



where I is an unspecified intermediate. The C-1 process produced by unhydrolyzed Menazon has a potential similar to that of the C-1' wave, but the groups bound to the sulphur make it more electropositive, thereby facilitating breakage of the C-S bond, and the half-wave or peak potential appears at slightly more positive potentials than those of the C-1' wave. The overall electrode reaction could be

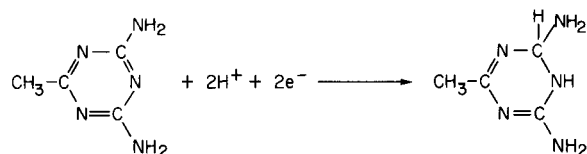


progressing in two steps again.

In order to examine this mechanism, nuclear magnetic resonance was applied to the electrode reaction products after coulometry of Menazon at -1.1 V; the methyl group of the 2-methyl-4,6-diamino-1,3,5-triazine was

identified. This mechanism is in agreement with the polarographic data; after coulometry at -1.1 V, the C-1 wave disappears and an anodic wave related to oxidation of mercury in the presence of the dimethylphosphorodithioate formed is observed.

The process denominated C-2, shown by both hydrolyzed and unhydrolyzed pesticide, may be due to the reduction of a double bond of the triazine, involving 2 electrons and 2 protons in the rate-determining step when the solution pH is greater than 3.8:



At $\text{pH} < 3.8$, the pK_a value for Menazon, the triazine would be protonated and only 1 proton would be exchanged, giving the same reaction product as immediately above.

The A-1 wave is anodic, and an insoluble product is formed during coulometry conducted at potentials on the limiting current plateau. It seems probable that this insoluble species is $(\text{RS})_2\text{Hg}$, formed during the oxidation of mercury in the presence of the mercaptan in reaction (8). This would be the electrode reaction at $\text{pH} < 6.0$; if the solution pH were higher, the mercaptan would not be protonated and protons would not be involved in the oxidation. The absence of proton involvement in the electrode reaction at $\text{pH} > 6$ explains why the half-wave potential of this process remains practically constant for these pH values. This mechanism also explains why all the waves disappear, once coulometry has been done at -0.15 V; the relevant electroactive species vanishes from solution on forming an insoluble compound with mercury. The process designated as A-2, as has been detailed above, is due to the same oxidation of mercury in the presence of the mercaptan, but without the insoluble salt being adsorbed onto the electrode.

REFERENCES

- 1 M. R. Smyth and J. G. Osteryoung, *Anal. Chim. Acta*, 96 (1978) 335.
- 2 H. Benadikova, M. Popl and V. Jakubickova, *Collect. Czech. Chem. Commun.*, 48 (1983) 2636.
- 3 C. V. Bowen and F. I. Edwards, *Anal. Chem.*, 22 (1950) 706.
- 4 M. Zietek, *Mikrochim. Acta*, (1976) 549.
- 5 P. H. Martens and P. Nangniot, *Residue Rev.*, 2 (1963) 26.
- 6 P. Nangniot, *Anal. Chim. Acta*, 31 (1964) 166; *Bull. Rech. Agron. Gembloux*, 3 (1968) 182.
- 7 R. J. Gajan, *Residue Rev.*, 5 (1964) 80; 6 (1964) 75.
- 8 F. Peter and R. Rosset, *Anal. Chim. Acta*, 79 (1975) 47.
- 9 A. Calderbank and J. B. Turner, *Analyst*, 87 (1962) 273.
- 10 L. Meites, *Polarographic Techniques*, 2nd edn., Wiley, New York, 1965, Chap. 5.
- 11 A. A. Vlček, *Z. Elektrochem.*, 61 (1957) 1014.

- 12 A. M. Bond, *Modern Polarographic Methods in Analytical Chemistry*, M. Dekker, New York, 1980, Chap. 6.
- 13 J. D. Winefordner and G. L. Long, *Anal. Chem.*, 55 (1983) 712A.
- 14 P. Zuman, *Polarography 1964. Proceedings of the third International Conference*. Southampton, Macmillan, London, 1966, p. 699.

COMPARISON OF THE UTILITY OF SEVERAL TENSAMMETRIC TECHNIQUES FOR THE DETERMINATION OF TRACES OF SURFACTANTS AFTER PRECONCENTRATION AT THE HANGING MERCURY DROP ELECTRODE

EWA BEDNARKIEWICZ and ZENON KUBLIK*

Department of Chemistry, University of Warsaw, Pasteura 1, Warsaw 02093 (Poland)

(Received 13th March 1985)

SUMMARY

After preconcentration of surfactants at the HMDE, four tensammetric signals were examined to establish the optimum conditions for the determination of ultratraces of surfactants in alkaline, neutral and acidic media. The signals studied were: (1) the depth of the depression occurring on the curves obtained in a.c. fundamental tensammetry; and (2–4) the height of the peaks on the curves obtained in a.c. fundamental tensammetry, a.c. second-harmonic tensammetry and differential pulse tensammetry, respectively. The lowest detection limits found for Triton X-100 and a polyethylene glycol (PEG-4000) were 10 and $2 \mu\text{g dm}^{-3}$, respectively. Traces of surfactants found in supporting electrolytes (ca. $25 \mu\text{g dm}^{-3}$) gave desorption peaks at potentials around -1 V . These contaminants had no significant effect on the peaks of Triton X-100 and PEG-4000 and they affected the SDS peak markedly. The reproducibility of the results obtained for SDS was poorer than for Triton or PEG.

The presence of surfactants in solution modifies the tensammetric curve essentially. In the main portion of the curve, the base current is depressed and, in most cases, adsorption/desorption peaks appear at the edges of the potential range of adsorption [1]. Variations in surfactant concentration affect the depth of the depression as well as the height of the peak. Both effects may be exploited as analytical signals for the determination of surfactants in solution [2]. The possibilities of the method based on the depression for the determination of ultratraces of surfactants were studied previously [3]. The aim of the present work was to compare the potentialities of three tensammetric techniques in conjunction with the preconcentration of surfactants on the HMDE, in order to extend prior work on exploitation of the tensammetric signals obtained after preconcentration of surfactants at mercury electrodes [2–8]. The following techniques were chosen for comparison: alternating-current fundamental tensammetry (a.c.f.t.), alternating-current second-harmonic tensammetry (a.c.s.h.t.) and differential-pulse tensammetry (d.p.t.). In d.p.t., the main portion of the capacitive contribution to the current is removed but, according to Myers and Osteryoung [9], the d.p. voltammogram in the absence of the faradaic reaction is essentially a

differential capacity curve. The a.c.f.t. technique gives two analytical signals, the depression of the base current and desorption peaks. In the remaining techniques, the desorption peaks served as the analytical signals.

EXPERIMENTAL

The tensammetric curves obtained in a.c.f.t. and a.c.s.h.t. were recorded with a Radelkis OH-105 polarograph and a three-electrode arrangement with an applied alternating voltage of 15 and 30 mV, respectively. The d.p.t. curves were recorded with a Unitra PPO4 pulse polarograph connected with a Riken-Denshi F-3C XY recorder; the pulse amplitude was 20 mV. Platinum foil with a surface area of 1 cm^2 was used as the counter electrode with a saturated calomel reference electrode. The working electrode was a hanging mercury drop electrode (HMDE) of the type described by Kemula and Kublik [10]; the surface area of the electrode was always the same (3.8 mm^2).

Solutions were prepared from reagent-grade chemicals and water purified as described by Bednarkiewicz et al. [3]. Some portions of the supporting electrolytes were further purified with active charcoal as described by Bednarkiewicz and Kublik [11]. The following surfactants were used: Triton X-100 (Loba, Austria), polyethylene glycol 4000 (PEG-4000; Carl Roth OHG) and sodium dodecyl sulphate (SDS; Loba, Austria). Stock solutions of Triton X-100 (40 mg dm^{-3}), PEG-4000 (30 mg dm^{-3}) and SDS (100 mg dm^{-3}) were prepared by dissolving the commercial products in distilled water. The SDS stock solution was prepared immediately before use. Small aliquots of the surfactant stock solution were added by microsyringe to a known volume of solution in the cell. The concentrations of unknown surfactants present in the supporting electrolytes and determined in the blank tests were expressed in terms of concentrations of Triton X-100 taken as the standard substance. Solutions were deaerated with argon. Experiments were done at $20 \pm 1^\circ \text{C}$.

RESULTS

Blank tests

In determinations of trace levels of substances, the establishment of proper blank curves is very important. In preliminary experiments, optimum conditions for obtaining the best blank curves were studied. Supporting electrolyte solutions of "normal" purity were prepared as usual from analytical-grade reagents and distilled water; solutions of greater purity were obtained by further purification of the "normal" solutions with activated charcoal. Some results obtained for acidic, neutral and alkaline solutions are presented in Fig. 1. Without preconcentration, the solutions with normal and improved purity gave identical curves, indicating that such curves are unaffected by traces of surfactants present in the solutions studied. These curves were used in further experiments as convenient reference curves. After preconcentration, at -0.6 V with stirring, the tensammetric curves were recorded towards

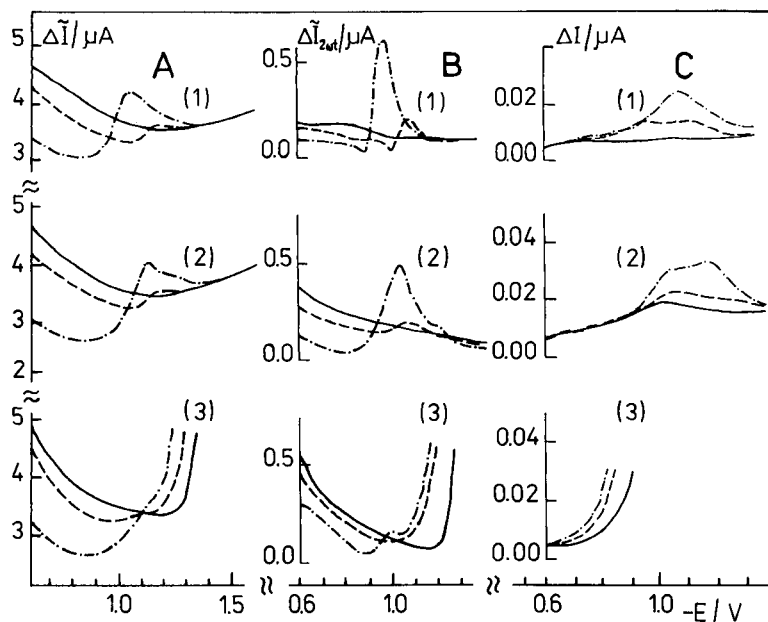


Fig. 1. Blank tests obtained for 0.1 mol dm^{-3} solutions of: (1) lithium hydroxide; (2) lithium perchlorate; (3) perchloric acid. Technique used: (A) a.c.f.t.; (B) a.c.s.h.t.; (C) d.p.t. Curves: (—) solutions of normal purity without preconcentration; (---) solutions of normal purity after preconcentration for 100 s at -0.6 V with stirring; (····) solutions purified with active charcoal, after preconcentration as before.

negative or positive potentials. The positive branches of the tensammetric curves either did not show desorption peaks or the peaks were irreproducible. Therefore, in Fig. 1 only the negative branches of the tensammetric curves are presented.

It is evident that surface-active contaminants present in neutral and alkaline solutions with normal purity give both the depression and the desorption peaks. The peaks obtained by a.c.f.t. and a.c.s.h.t. are quite well defined, whereas the peaks obtained by d.p.t. are drawn out. For the purified alkaline and neutral solutions, desorption peaks either do not occur or are very small, whereas the depression effect remains well defined though its depth is small. In acidic solutions, the desorption peaks of contaminants are significantly overlapped by the hydrogen evolution current, but the depression effect observed in a.c.f.t. remains well developed.

The size of the signals increased for longer preconcentration times but the precision of the enhanced signals thus obtained was poor. A preconcentration time of 100 s was the optimum. The concentrations of surfactants, expressed in terms of Triton X-100 concentration, found in solutions with normal and enhanced purity on the basis of the depression effect are presented in Table 1.

TABLE 1

Determination of unknown surface-active contaminants in the supporting electrolytes used

(The concentrations, expressed in terms of Triton X-100, were found from the depression depth after 100-s preconcentration in stirred solutions)

Solution (0.1 mol dm ⁻³)	Depression depth (μ A)	Conc. of surfactant ^a (μ g dm ⁻³)	SD (μ A)	RSD (%)
LiOH	0.58	41	0.083	14.3
LiOH ^b	0.31	22	0.042	13.5
LiClO ₄	1.30	93	0.061	11.5
LiClO ₄ ^b	0.39	28	0.042	10.7
HClO ₄	1.38	100	0.074	5.4
HClO ₄ ^b	0.28	20	0.014	5.0

^aMean of 3–5 determinations with standard deviation (SD) and relative standard deviation (RSD). ^bSolutions purified with active charcoal.

The behaviour of Triton X-100

Figure 2 shows a comparison of the shapes of the tensammetric curves obtained for increasing concentrations of Triton X-100 in neutral solutions. The desorption peak of Triton X-100 occurs at -1.65 V, i.e., there is a sufficient difference between the potentials of this peak and the peaks of contaminants shown in Fig. 1. Preconcentration at -1.3 V eliminates the action of contaminants easily. Under these conditions, both the depression and the desorption peaks are well defined except for the d.p.t. technique when the peak becomes very close to the hydrogen evolution wave. Figure 2 shows that a preconcentration time of 100 s is sufficient to detect easily as little as $50 \mu\text{g dm}^{-3}$ Triton X-100 by a.c.f.t. or $10 \mu\text{g dm}^{-3}$ by a.c.s.h.t.

In acidic solutions, the tensammetric desorption peak of Triton X-100 was masked by the hydrogen evolution current. Triton X-100 could still be determined on the basis of the depression effect, but the influence of contaminants had then to be taken into account. In alkaline solutions, the peaks obtained from a.c.f.t. and a.c.s.h.t. were slightly lower and broader than those obtained in neutral medium but the peak obtained by d.p.t. was better separated from the hydrogen evolution current.

Figure 3 shows the dependence of the heights of the analytical signals obtained by a.c.f.t. and a.c.s.h.t. techniques on the concentration of Triton X-100 in solution. With preconcentration for 100 s, saturation of the mercury surface was attained at Triton X-100 concentrations of about $250 \mu\text{g dm}^{-3}$ for the peaks, or 150 – $200 \mu\text{g dm}^{-3}$ for the depression. As expected, plots obtained for longer or shorter preconcentration times showed the saturation limits at lower or higher Triton X-100 concentrations, respectively. For small Triton X-100 concentrations, the plots are linear though the line representing the a.c.f.t. peaks has a positive intercept.

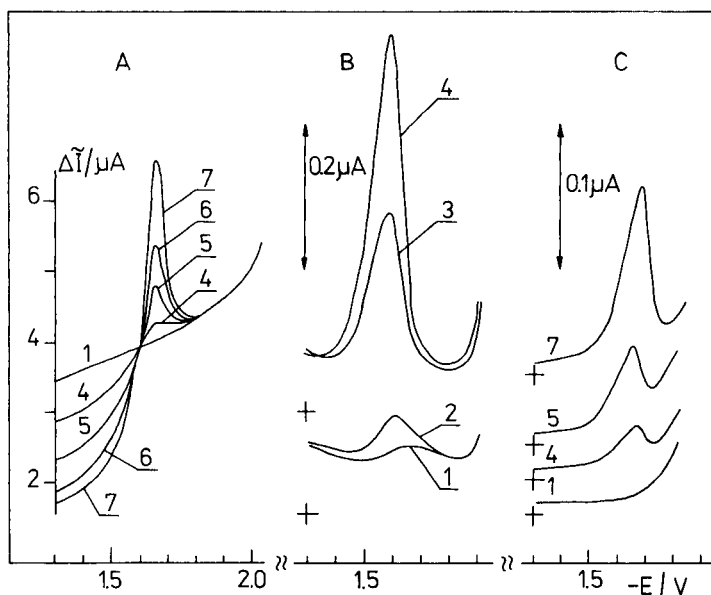


Fig. 2. Tensammetric curves obtained after preconcentration for 100 s at -1.3 V with stirring. Technique used: (A) a.c.f.t.; (B) a.c.s.h.t.; (C) d.p.t. Curves for 0.1 mol dm^{-3} LiClO_4 with increasing concentration of Triton X-100: (1) 0; (2) 10; (3) 20; (4) 50; (5) 100; (6) 150; (7) $250 \mu\text{g dm}^{-3}$.

The peaks obtained for Triton X-100 in alkaline solutions by d.p.t. were smaller than those obtained with the other techniques, but the background currents were small and smooth. The calibration plot obtained, with seven points ranging from 10 to $200 \mu\text{g dm}^{-3}$ Triton X-100, can be described by $i_p = 2.38 + 0.57 C$ (correlation coefficient 0.994), where current is expressed in nA and concentration in $\mu\text{g dm}^{-3}$. The detection limit found in neutral medium on the basis of the depression effect was $10 \mu\text{g dm}^{-3}$. The detection limits found on the basis of the desorption peaks obtained by a.c.f.t., a.c.s.h.t. and d.p.t. were 30, 10 and $30 \mu\text{g dm}^{-3}$, respectively. The detection limit found by d.p.t. in alkaline solution decreased to $10 \mu\text{g dm}^{-3}$. The standard deviations found for determinations of Triton X-100 are given in Table 2.

The behaviour of polyethylene glycol

The preconcentration of small amounts of various polyethylene glycols on the HMDE from stirred solutions was studied by Batycka and Łukaszewski [7] with the a.c.f.t. technique. Because PEG-4000 is strongly adsorbed on the mercury surface and its desorption occurs at very negative potentials, PEG's cannot be determined in acidic solutions on the basis of its desorption peak. Exploitation of the depression effect enables PEG-4000 to be determined in acidic solution.

The curves obtained in neutral medium were well defined for higher concentration of PEG-4000, but for low concentrations the desorption peak and

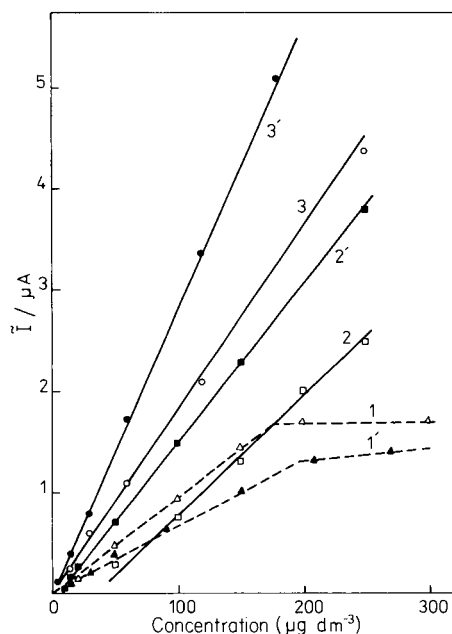


Fig. 3. Calibration plots: (1–3) Triton X-100 in $0.1 \text{ mol dm}^{-3} \text{ LiClO}_4$; (1'–3') PEG-4000 in $0.1 \text{ mol dm}^{-3} \text{ LiOH}$. Measured signals: (1,1') capacity depression; (2,2') a.c.f.t. desorption peaks; (3,3') a.c.s.h.t. desorption peaks. Preconcentration for 100 s at -1.3 V with stirring.

TABLE 2

Standard deviations (all based on 5 measurements) calculated for the surfactants tested

Solution	Conc. ($\mu\text{g dm}^{-3}$)	A.c.f.t.				A.c.s.h.t.		D.p.t.	
		Depression		Peak		Peak		Peak	
		SD (μA)	RSD (%)	SD (μA)	RSD (%)	SD (μA)	RSD (%)	SD (μA)	RSD (%)
Triton X-100/ 0.1 M LiClO_4	50	0.054	13.2	0.056	11.0	0.119	17.0	0.0076	20
PEG-4000/ 0.1 M LiOH	30	0.027	11.7	0.057	11.9	0.117	16.5	0.007	6.2

the cathodic limiting current were not well separated. This separation improved in lithium hydroxide solutions. Some curves obtained for PEG-4000 in this medium are given in Fig. 4. The peaks are narrower than those obtained for Triton X-100 in alkaline solution. The potential of the PEG-4000 desorption peak is -1.83 V , so that adsorption of contaminants in the solutions can usually be eliminated by proper choice of the preconcentration potential.

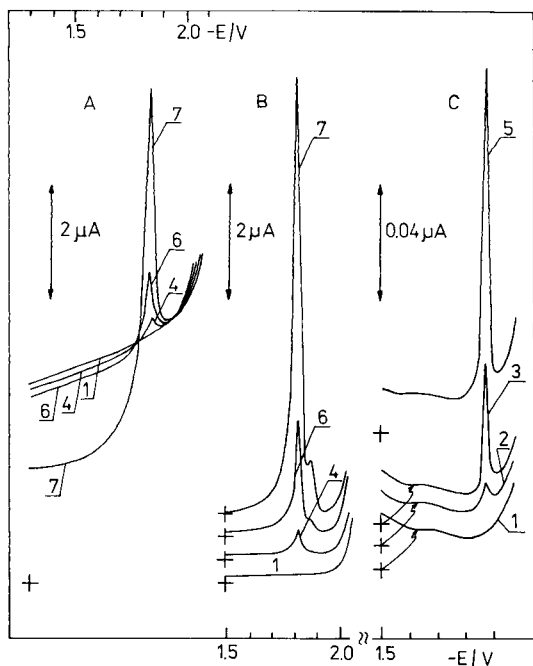


Fig. 4. Tensammetric curves obtained for PEG-4000 in 0.1 mol dm^{-3} LiOH after preconcentration for 100 s at -1.3 V with stirring. Technique: (A) a.c.f.t.; (B) a.c.s.h.t.; (C) d.p.t. Concentration of PEG: (1) 0; (2) 2; (3) 10; (4) 15; (5) 30; (6) 60; (7) $250 \text{ } \mu\text{g dm}^{-3}$.

Here again, the background current shown in a.c.f.t. is significant and is also potential-dependent, so that it is impossible to achieve reproducible curves at enhanced recorder sensitivities. The background currents obtained in a.c.s.h.t. and d.p.t. are smaller and these techniques provide better conditions for the determination of very small concentrations of PEG-4000.

Calibration plots for PEG-4000 based on the depth of the depression and the height of the peaks by a.c.f.t. and a.c.s.h.t. are shown in Fig. 3. The depression for PEG-4000 is smaller than that found for the same concentration of Triton X-100. In contrast, the desorption peaks for PEG are markedly higher than those for Triton X-100, largely because of their decreased width. The dependence of the peak height (in μA) obtained by d.p.t. on the PEG-4000 concentration (in $\mu\text{g dm}^{-3}$) can be described by the equation $i_p = 1.72 + 3.71 C$, which is valid, with a correlation coefficient of 0.997, in the concentration range $2\text{--}150 \text{ } \mu\text{g dm}^{-3}$. The slope of this line, i.e., the sensitivity of the method, is markedly higher than that for Triton X-100 (see above). Although the calibration plot for PEG-4000 covers a smaller range of concentrations than that for Triton X-100, the equation has a better correlation coefficient and smaller intercept. Thus PEG-4000 can be determined more precisely than Triton X-100.

The detection limit found in lithium hydroxide solutions for the PEG-4000, after a 100-s preconcentration time, on the basis of the depression effect was $15 \mu\text{g dm}^{-3}$. The detection limits found on the basis of desorption peaks in a.c.f.t., a.c.s.h.t. and d.p.t. were 15, 5 and $2 \mu\text{g dm}^{-3}$, respectively. The standard deviations found for $30 \mu\text{g dm}^{-3}$ of PEG-4000 (Table 2) are comparable with those found for $50 \mu\text{g dm}^{-3}$ Triton X-100.

The behaviour of dodecylsulphate

Sodium dodecylsulphate (SDS) is less strongly adsorbed on the mercury surface than Triton X-100 or PEG-4000. The potential of the negative desorption peak is close to -1 V , i.e., in the potential range where the unknown surface-active contaminants of the supporting electrolytes are also desorbed. Accordingly, it is impossible to determine, by the techniques used, markedly lower concentration of SDS than the values obtained in blank tests (see Table 1). Experiments done with SDS concentrations in the range $40\text{--}400 \mu\text{g dm}^{-3}$ were not promising. The reproducibility of the shape and height of the peaks was poor. In some concentration ranges, the peak split. The signal heights were not strictly proportional either to the deposition time or to the concentration of SDS. In strongly acidic solutions, the desorption peak was severely overlapped by the hydrogen evolution current. The best results were obtained for the depression effect in acidic solution. The calibration plot obtained for these conditions is presented in Fig. 5, which also illustrates the dramatic change in the properties of SDS in acidic and alkaline solutions. For the same concentrations of SDS, the signal obtained in acidic solution is 7 times higher than that obtained in alkaline medium.

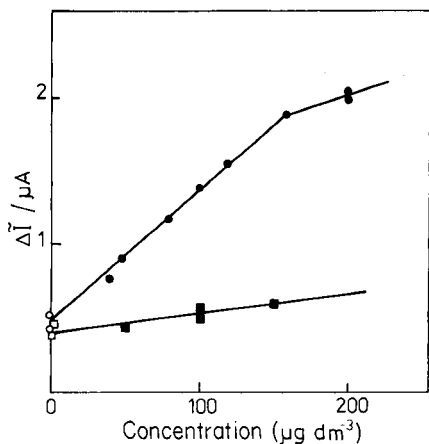


Fig. 5. Calibration plots for SDS in 0.1 mol dm^{-3} solutions of: (●) HClO_4 ; (■) LiOH . The capacity depression obtained after 100-s preconcentration at -0.6 V with stirring is measured. (○, □) Blank tests.

DISCUSSION

The results presented show that these tensammetric techniques can be used in conjunction with preliminary preconcentration of surfactants on the HMDE in stirred solution to detect as little as a few $\mu\text{g dm}^{-3}$ of surfactants. Work at this concentration level requires thorough testing of the purity of the supporting electrolytes used. The concentrations of surface-active contaminants present in supporting electrolytes prepared from twice-distilled water and analytical-grade reagents were found to lie in the range 40–100 $\mu\text{g dm}^{-3}$. In solutions purified by passage through active charcoal, the concentration of surfactants decreased to about 25 $\mu\text{g dm}^{-3}$. Previously, this problem seems to have been noticed only by Jehring and Stolle [2] who detected surface-active contaminants in the supporting electrolyte used but did not determine their concentrations.

The tensammetric determination of traces of Triton X-100 and PEG-4000 were quite easy, particularly in neutral or alkaline solutions, where the obstacles created by contaminants can easily be eliminated by an appropriate choice of the preconcentration potential. The detection limits found for Triton X-100 and PEG-4000 in this work are smaller than those given in the literature [2, 4, 7].

In contrast, the determination of SDS encounters serious difficulties caused partly by overlap of the SDS desorption peak and the peak of the contaminants, and partly by the complex character of the SDS adsorption/desorption process. Some authors [12–14] studying the behaviour of SDS by a.c.f.t. in neutral solutions, observed complex curves with two positive and two negative peaks. According to Takahashi [15], the rate of the adsorption process of alkyl sulphates on the mercury surface may be controlled by an activation process.

Comparisons based on all the data described in this work do not allow a choice of a "best technique" or "best signal" fitting all the substances studied. Each technique has its advantages and disadvantages. For example, d.p.t. enabled the lowest concentration of PEG-4000 in this work to be determined (2 $\mu\text{g dm}^{-3}$), but it was inefficient in determining traces of SDS. Exploitation of the a.c.s.h.t. and d.p.t. techniques, which eliminate a significant portion of the capacitative current, usually led to increased sensitivity (see, e.g., Fig. 3) but these more sophisticated techniques did not offer improved precision (see Table 2). The depression of the capacity current is moderately sensitive to all the surfactants studied but it is not selective and therefore may be severely affected by traces of surfactants present as contaminants.

This work was done as part of the Project MR-I-32.

REFERENCES

- 1 B. Breyer and H. H. Bauer, *Alternating Current Polarography and Tensammetry*, Interscience, New York, 1963.

- 2 H. Jehring and W. Stolle, *Collect. Czech. Chem. Commun.*, 33 (1968) 1670.
- 3 E. Bednarkiewicz, M. Donten and Z. Kublik, *J. Electroanal. Chem.*, 127 (1981) 241.
- 4 D. R. Canterford and R. J. Taylor, *J. Electroanal. Chem.*, 98 (1979) 25.
- 5 B. Čosović and V. Vojvodić, *Limnol. Oceanogr.*, 27 (1982) 361.
- 6 R. Kalvoda, *Anal. Chim. Acta*, 138 (1982) 11.
- 7 H. Batycka and Z. Łukaszewski, *Anal. Chim. Acta*, 162 (1984) 207, 215.
- 8 N. K. Lam and M. Kopanica, *Anal. Chim. Acta*, 161 (1984) 315.
- 9 D. J. Myers and J. Osteryoung, *Anal. Chem.*, 46 (1974) 356.
- 10 W. Kemula and Z. Kublik, *Anal. Chim. Acta*, 18 (1958) 104.
- 11 E. Bednarkiewicz and Z. Kublik, *J. Electroanal. Chem.*, 165 (1984) 195.
- 12 H. Jehring, *Electrosorptionsanalyse mit der Wechselstrompolarographie*, Akademie Verlag, Berlin, 1974.
- 13 D. Vollhard, *Colloid Polym. Sci.*, 254 (1976) 64.
- 14 D. Britz and J. Mortensen, *J. Electroanal. Chem.*, 127 (1981) 231.
- 15 K. Takahashi, *Electrochim. Acta*, 13 (1968) 1609.

THE ELECTROLYTIC EFFICIENCY OF AMPEROMETRIC DETECTION IN NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

H. GUNASINGHAM, B. T. TAY and K. P. ANG

Department of Chemistry, National University of Singapore, Kent Ridge, 0511 (Singapore)

(Received 16th July 1984)

SUMMARY

The electrolytic efficiency of the wall-jet detector in normal-phase high-performance liquid chromatography (between 0.1 and 5%) is significantly less than its efficiency in reversed-phase separations. A large-volume wall-jet cell with a Ag/Ag^+ reference serves as the detector system in evaluating the effects of various chromatographic conditions on efficiency. Anthraquinone, phenanthrenequinone (both at 10^{-3} – 10^{-4} M) and three estrogen steroids (at 10^{-2} – 10^{-3} M) are used as samples with ethanol/hexane eluents. The effect of pH depends on the sample; increased ethanol concentrations and lower flow rates improve the efficiency.

Electrochemical detectors in high-performance liquid chromatography (h.p.l.c.) have gained increasing popularity because of their high sensitivity and selectivity [1]; they can be particularly useful for compounds of bio-medical importance. Such detectors are generally based on solid electrodes; various configurations have been used, including the thin-layer cell [2, 3], reticulated vitreous carbon cell [4] and wall-jet cell [5, 6]. Usually, the sensitivities of different electrochemical detectors are compared on the basis of the peak currents obtained for a particular compound or class of compounds. It is, however, more meaningful if detectors are compared on the basis of their electrolysis efficiency; that is, the percentage of solute that is electrolysed as it is eluted through the electrochemical cell.

Differences in efficiencies between different detectors arise from their intrinsic characteristics (hydrodynamic performance) as well as extrinsic factors related to the chromatographic conditions. Hanekamp and Van Nieuwkerk [7] suggested the dimensionless Reynold's number, Re , as a measure of detector performance. $Re = ul/\nu$, where u is the average linear velocity of the fluid, l the length of the electrode and ν the kinematic viscosity. The signal/noise ratio of the wall-jet detector is proportional to $Re^{3/4}$, whereas for thin-layer detectors it is proportional to $Re^{1/2}$ [7]. Another factor affecting efficiency is the electrode area. As electrolysis takes place at the working electrode, increasing the electrode area should enhance electrolytic efficiency.

For the wall-jet electrode, the current is proportional to the radius of the electrode to the power $3/4$. In practice, however, as the electrode area increases so does the background noise. Another factor which limits the use of a large electrode area is the resulting increase in band spreading. Because of its large working electrode area, efficiencies of up to 46% are possible for the reticulated vitreous carbon detector [4]; for the thin-layer and wall-jet detectors, which have much smaller working electrode areas, electrolytic efficiencies of between 0.1 and 10% are typical.

The efficiencies of electrochemical detectors that have so far been obtained are for analyses in the aqueous eluents used in reversed-phase or ion-exchange chromatography. No comparable work has been reported which has evaluated efficiencies for amperometric detection in normal-phase h.p.l.c. Recently, the practical aspects of applying electrochemical detectors in normal-phase h.p.l.c. were described [8, 9]. Among the benefits of using non-aqueous eluents are the increase in working potential range and reduced adsorption problems. In this paper, it is shown that the electrolytic efficiency of amperometric detection in normal-phase h.p.l.c. is comparable to that obtained when aqueous eluents are monitored, for both reductive and oxidative modes. The electrolytic efficiency is discussed in relation to the influence of applied potential, pH, flow rate, eluent composition and solute concentration. The test compounds are polar organic species which show reasonably high electrochemical activity.

EXPERIMENTAL

Instrumentation

The electrochemical system. The large volume wall-jet cell used was identical to the glass/teflon design described earlier [9, 10]. The cell volume is 35 ml which is much larger than that of the original cell (about 100 μ l) designed by Fleet and Little [5]. The cell was positioned below a PARC model 303 electrode system (Princeton Applied Research Corporation) and the model 303 platinum counter electrode was used with a glassy carbon working electrode (2.5 mm diameter; Tokai Mfg., Tokyo). The Ag/Ag⁺ reference electrode consisted of a silver wire dipped into a saturated solution of silver nitrate in ethanol which was 12 mM in tetrabutylammonium fluoroborate [9]. All potentials quoted in this paper are with respect to this Ag/Ag⁺ reference system. The nozzle of the jet inlet was kept 4 mm away from the working electrode to avoid interfering with the hydrodynamic boundary layer, thereby ensuring maximum sensitivity [9].

The model 303 electrode system was controlled by a PARC model 174A polarographic analyser in the d.c. mode. The current responses were recorded on a Perkin-Elmer R-100 recorder. Peak areas were measured with a Hewlett-Packard model 3390 A reporting integrator.

The h.p.l.c. system. A Perkin-Elmer series 4 microprocessor-controlled solvent delivery system was used. Eluents were deaerated by purging with helium

in the solvent chamber and were kept under pressure (50–60 kPa) throughout the chromatographic separations. This feature of the h.p.l.c. is especially useful for reductive electrochemical detection [11]. The chromatographic column used was a LiChrosorb Si-60 (10 μ m) normal-phase column (250 $\times$ 4.6 mm). Samples were injected through a Rheodyne 7125-075 injector, with a 6- μ l loop. The wall-jet detector was connected to the column with 20 cm of 0.55-mm i.d. stainless steel followed by 5 cm of teflon tubing of the same diameter.

Reagents

All chemicals and eluents were of analytical-reagent grade and were used as received. The eluents, hexane and ethanol, and silver nitrate were obtained from Merck. Tetrabutylammonium tetrafluoroborate, and the test compounds, anthraquinone and phenanthrenequinone, were from Tokyo Kasei Organic Chemicals. The estrogen steroids, estrone, β -estradiol and estriol, were obtained from Sigma Chemical Co. Solutions of test compounds were prepared in ethanol; unless specified otherwise, the solutions injected were 10^{-3} – 10^{-4} M in a quinone or 10^{-2} – 10^{-3} M in a steroid.

The eluents used for the separation of the quinones were prepared as follows: (i) "neutral", 40:60 hexane/ethanol; (ii) "acidic", 40:60 hexane/ethanol with 80 mM acetic acid (Merck); (iii) "alkaline", 40:60 hexane/ethanol with 80 mM ammonia (Ajax Chemicals, Sydney, Australia). For the estrogen steroids, 80:20 hexane/ethanol with 80 mM ammonia served as eluent. Tetrabutylammonium tetrafluoroborate was added to all the eluents to give 12 mM solutions.

Method

The amount of solute electrolysed was estimated by integrating the area under the amperometric current/time trace obtained as the solute was eluted through the wall-jet cell. The percentage electrolysed (or degree of conversion) was then calculated as a percentage of the theoretical charge that would be obtained for electrolysis of the entire solute injected into the column. For the quinones, the theoretical charge was estimated for a two-electron process; for the estrogen steroids, a one-electron process involving oxidation of the phenolic moiety was assumed. This approach to estimating the degree of conversion differs from that of Wang and Dewald [4] who calculated as a percentage of the conversion of potassium hexacyanoferrate under steady-state conditions.

RESULTS AND DISCUSSION

Effect of applied potential and pH

In general, the percentage of electroactive species electrolysed increases with applied potential, reaching a limiting value at a characteristic potential, similarly to plots of applied potential against current response obtained in

potential step techniques [12]. Figure 1 shows the effect of applied potential on the percentage electrolysed for the two quinones in neutral media. Figure 2 is the analogous plot for the estrogen steroids. As can be seen, the relationships are similar, although a lower degree of conversion is noted for the steroids. Figures 1 and 2 also show that the optimum applied potential can depend on the individual compound.

In aprotic non-aqueous solvents, quinones are reduced in two successive one-electron steps. This straightforward behaviour can be altered by changes in the diffusion layer concentrations of the quinone, semiquinone or the dianion caused by variations in pH which may result in shifts from kinetic to diffusion control of the electrolysis [13]. Figure 3 shows plots of efficiency against potential for anthraquinone in the above-mentioned neutral, alkaline and acidic eluents. The greatest efficiency for anthraquinone is in the neutral medium; the same trend was observed for phenanthrenequinone. The plots also show that the optimum applied potential is dependent on pH conditions. The difference in behaviour in acidic medium may be related to the shift in the two-electron reduction from a two-step to a one-step process.

Effect of flow rate

Figure 4 shows plots of the percentage of quinones electrolysed against flow rate in the neutral media with the working electrode held at the optimum

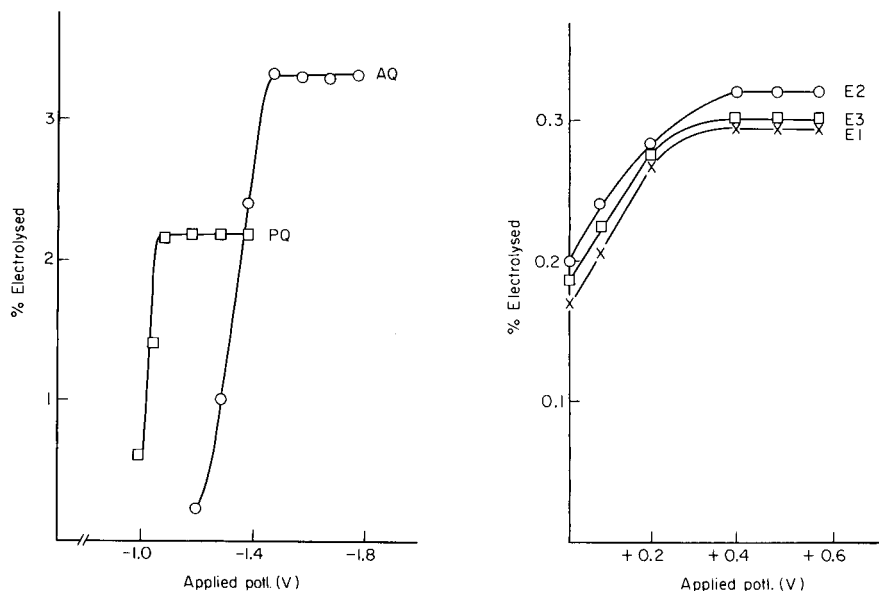


Fig. 1. Effect of applied potential on percentage of quinone electrolysed. AQ, anthraquinone (1.2 μg); PQ, phenanthrenequinone (1.2 μg); eluent composition, 40:60 hexane/ethanol; flow rate, 1.0 ml min⁻¹.

Fig. 2. Effect of applied potential on percentage of estrogen steroid electrolysed. E1, estrone (2.3 μg); E2, β -estradiol (2.3 μg); E3, estriol (2.5 μg); eluent composition, 80:20 hexane/ethanol with 80 mM ammonia; flow rate, 1.0 ml min⁻¹.

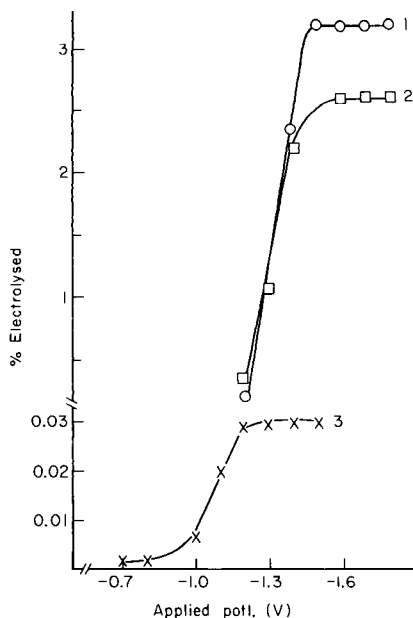


Fig. 3. Effect of applied potential on percentage of anthraquinone (1.25 μg) electrolysed in neutral (1), alkaline (2) and acidic (3) media (see Experimental) at a flow rate of 1.0 ml min^{-1} .

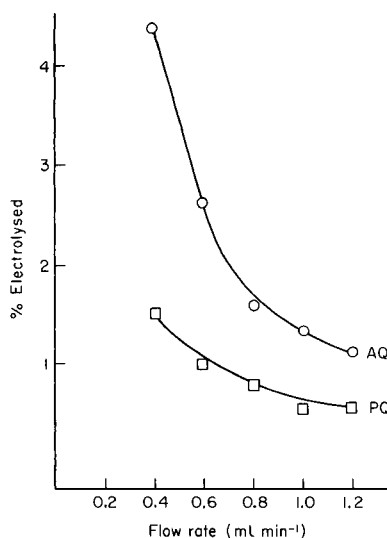


Fig. 4. Influence of flow rate on percentage of the quinone electrolysed. Eluent composition, 40:60 hexane/ethanol; applied potential, AQ at -1.5 V and PQ at -1.3 V ; 1.2 μg of each quinone injected.

potential for each quinone. As expected, the degree of conversion decreases with increasing flow rate for both quinones, a trend which was also observed for alkaline and acidic conditions. Figure 5 shows that the trends are similar for the efficiency for estrogen steroids when the flow rate is increased. The electrolytic efficiency decreases as the flow rate increases, because of the decrease in residence time of the electroactive species at the working electrode [5].

The amperometric signal is related to the instantaneous flow rate and the concentration of electroactive species in the detector. In the case of the wall-jet cell, the peak current is given by

$$I_p = 1.38 nFC_{\max} D^{2/3} \nu^{-5/12} a^{1/2} V^{3/4} R^{3/4} \quad (1)$$

where n , F , and D have their usual meanings; $C_{\max}$ is the concentration at the peak of the solute profile in the cell, ν is the kinematic viscosity, a is the diameter of the inlet electrode, V is the volume flow rate, and R is the radius of the working electrode. Equation 1 predicts that the peak current is related to the volume flow rate to the power of 3/4. Plots of log peak current against log flow rate gave straight lines with an average gradient of 0.72 (r.s.d. 2%)

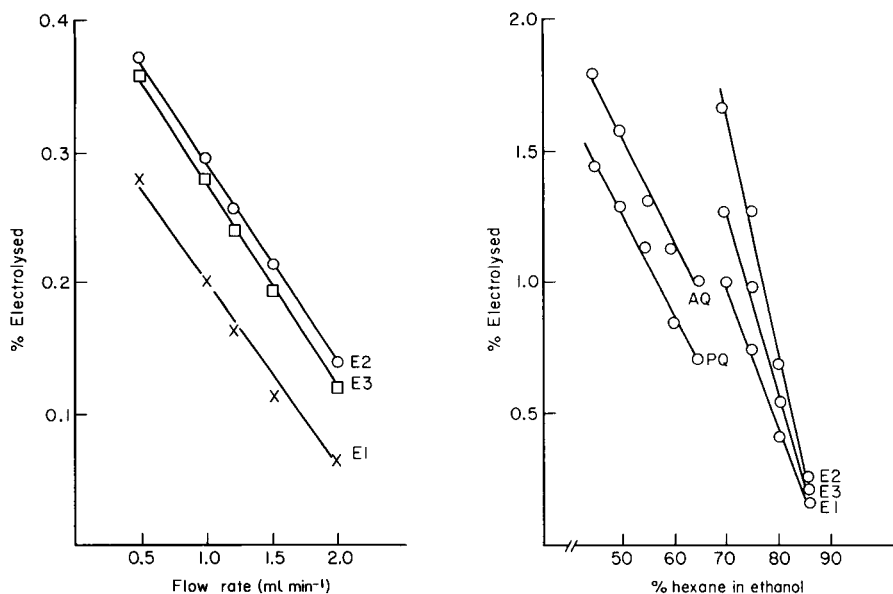


Fig. 5. Influence of flow rate on percentage of estrogen steroid electrolysed. Eluent composition, 80:20 hexane/ethanol with 80 mM ammonia; applied potential, +0.4 V; 2.3 μg each of E1 and E2; 2.5 μg of E3.

Fig. 6. Effect of eluent composition on percentage electrolysed. Applied potential, AQ and PQ at -1.3 V and E1, E2 and E3 at +0.4 V; 0.91 μg AQ, 0.78 μg PQ, 1.4 μg E1 and E2, 1.5 μg E3; flow rate, 1.0 ml min^{-1} .

for the quinones (in the various pH eluents) as well as for the estrogen steroids. This is in close agreement with the theoretical value. The agreement of flow rate dependency with the theoretical value contrasts with most other reported works with aqueous eluents, where the value is closer to the power of $1/2$, a value that is characteristic of thin-layer cells. The difference may be attributed to three features: (1) the inlet and working electrode not being aligned correctly; (2) interference of the inlet nozzle in the hydrodynamic boundary layer; and (3) the inlet diameter being too large.

In order to have a true wall-jet configuration, the free jet emerging from the inlet must be very small compared to the working electrode diameter. Further, the radial flow of the jet must not be disturbed, which means that the inlet nozzle has to be well clear of the hydrodynamic boundary layer formed at the working electrode. It is also necessary to use a large-volume cell; constricting the cell volume leads to eddy formation within the boundary layer caused by the backflow of solution from outside the jet.

Effect of concentration of solute and eluent composition

Separate mixtures of quinones and estrogen steroids of varying concentrations were analysed chromatographically. In the case of the quinones, the

concentrations were in the range 10^{-3} – 10^{-4} M and the eluent composition was 55:45 hexane/ethanol; for the estrogen steroids, the concentrations were in the range 10^{-2} – 10^{-3} M and the eluent was 80:20 hexane/ethanol. Plots of percentage electrolysed against concentration showed no apparent dependence for the two mixtures, even at the lower concentrations. The independence of the efficiency shows that background noise is tolerable even when the detector is operated at high sensitivity.

An increase in the proportion of ethanol in the eluent led to an increase in the electrolytic efficiency for both the quinones as well as the estrogen steroids. This is shown in Fig. 6 which gives plots of percentage electrolysed against the percentage of hexane in the eluent composition. The increase in electrolytic efficiency is presumably due to the greater solubility of the polar organic compounds in ethanol.

Figure 7 shows the chromatographic traces obtained for the quinone and estrogen steroid mixtures near the limits of detection of the wall-jet cell. At such sensitivities, subnanogram detection levels can be achieved with a signal:noise ratio of slightly over 2. The traces are typical for polar polyaromatic compounds with 2–4 benzene and heterocyclic rings. Better sensitivity may be achieved with simpler aromatics, especially catechol derivatives.

In aqueous eluents, the wall-jet detector shows almost a tenfold increase in electrolytic efficiency. For acetonitrile/water eluents and a neutral electrolyte (e.g., KNO_3), the efficiency for the quinones may be as high as 22%; for the estrogen steroids, efficiency is close to 4%.

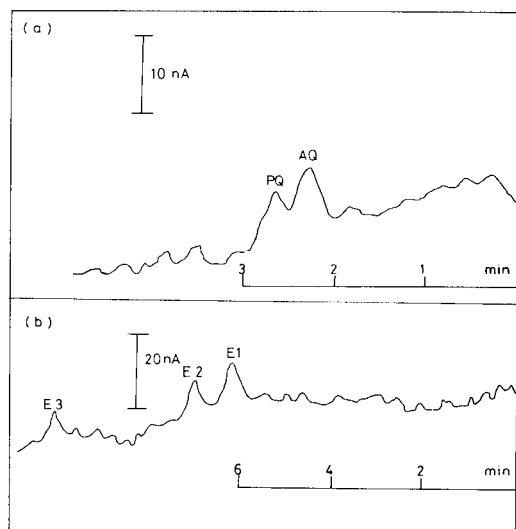


Fig. 7. Wall-jet detector traces. (a) 0.68 ng each of AQ and PQ; eluent composition, 55:45 hexane/ethanol with 80 mM ammonia; applied potential, -1.3 V; flow rate, 1.0 ml min^{-1} ; current range, 100 nA. (b) 0.60 ng each of E1, E2 and E3; eluent composition, 80:20 hexane/ethanol with 80 mM ammonia; applied potential, $+0.4$ V; flow rate, 1.0 ml min^{-1} ; current range, 200 nA.

Thus, eluent composition plays a major role in governing the electrolytic efficiency of amperometric detectors. Although the efficiency for the wall-jet detector is lower for normal-phase h.p.l.c. than for reversed-phase separations, other factors set out in earlier papers [8, 9] may outweigh this consideration, particularly if high sensitivity is not required.

The award of a University Research Scholarship to B. T. Tay as well as a research grant to K. P. Ang and H. Gunasingham from the University is gratefully acknowledged.

REFERENCES

- 1 D. A. Roston, R. E. Shoup and P. T. Kissinger, *Anal. Chem.*, 54 (1982) 1471A.
- 2 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.
- 3 K. Brunt and C. H. P. Bruins, *J. Chromatogr.*, 172 (1979) 3.
- 4 J. Wang and H. D. Dewald, *J. Chromatogr.*, 285 (1984) 281.
- 5 B. Fleet and C. J. Little, *J. Chromatogr. Sci.*, 12 (1974) 747.
- 6 M. Varadi, Z. Feher and E. Pungor, *J. Chromatogr.*, 90 (1974) 259.
- 7 H. B. Hanekamp and H. J. Van Nieuwkerk, *Anal. Chim. Acta*, 121 (1980) 13.
- 8 H. Gunasingham and B. Fleet, *J. Chromatogr.*, 261 (1983) 43.
- 9 H. Gunasingham, B. T. Tay and K. P. Ang, *Anal. Chem.*, 56 (1984) 2422.
- 10 H. Gunasingham, B. T. Tay, K. P. Ang and L. L. Koh, *J. Chromatogr.*, 285 (1984) 103.
- 11 F. Senftleber, D. Bowling and M. S. Stahr, *Anal. Chem.*, 55 (1983) 810.
- 12 C. E. Lunte and P. T. Kissinger, *Anal. Chem.*, 55 (1983) 1458.
- 13 J. Q. Chambers, in S. Patai (Ed.), *The Chemistry of Quinonoid Compounds*, Wiley, New York, 1974, Chap. 14.

SEPARATION OF MICROAMOUNTS OF FLUORIDE COEXISTING WITH LARGE AMOUNTS OF ALUMINUM AND SILICA BY IMPROVED TRIMETHYLSILYLATING DISTILLATION

KENTARO TSUCHIYA, TAKASHI IMAGAWA, KAZUHISA YAMAYA and MINORU YOSHIDA*

Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, Meguro-ku, Tokyo (Japan)

(Received 1st April 1985)

SUMMARY

The previously reported distillation method for the separation of fluoride as trimethylfluorosilane is much improved. When hexamethyldisilazane serves as the silylating agent, the formation of trimethylfluorosilane is quantitative in the pH 0–1 region where the interference of silica can be avoided and distillation is rapid at room temperature. Aluminum is effectively masked by pyrophosphoric acid. Almost all ions tested can be tolerated in 1000-fold molar amounts to fluoride. Over 98% recovery is obtained for 49 μg of fluoride in the presence of 80 mg of aluminum plus 200 mg of silica. Concentration of fluoride from very dilute ($\mu\text{g l}^{-1}$) solutions is easily achieved. Satisfactory results were obtained for standard rocks.

A rapid distillation method for the separation of fluoride based on the formation of trimethylfluorosilane (TMFS), which is a development of a microdiffusion method devised by Taves [1], was reported by Yoshida et al. [2]. In that method, the sample solution was treated with hexamethyldisiloxane (HMDSO) and sulfuric acid. The volatile TMFS thus formed was expelled with a nitrogen carrier and collected in an alkaline absorbing solution. Over 97% recovery of fluoride was obtained within 30 min without the serious dilution that accompanies the conventional steam distillation, so that significant preconcentration of fluoride from dilute solutions could be achieved. The interference of aluminum and some other metal ions could be eliminated by the addition of phosphoric acid. Although the method was applicable to various samples, HMDSO needs strong acidic medium (over 7 mol l^{-1} sulfuric acid) to react quantitatively with fluoride. In such conditions, silicic acid polymerizes rapidly and interferes seriously with the volatilization of fluoride, because of occlusion of fluorides in the gelatinous hydrated silica.

In this paper, the use of the more reactive silylating reagent, hexamethyldisilazane (HMDS), which reacts at lower acidity, is recommended. Conjoined with the improvement of the distillation apparatus, over 98% recovery can be achieved from reaction media at pH 0–1, and the interference by silicate

is prevented. Pyrophosphoric acid is used as the reaction medium, because it has a stronger masking ability for aluminum than orthophosphoric acid. The proposed method is applicable to the analysis of geochemical materials with high contents of silica and aluminum, such as silicate rocks. Fluoride at a level of $1 \mu\text{g l}^{-1}$ can be determined by distilling from 500-ml sample solutions.

EXPERIMENTAL

Apparatus

The distillation apparatus made of borosilicate glass is shown in Fig. 1. The reaction tube (A) for the ordinary distillation is 25 mm in inner diameter and 230 mm long (capacity 100 ml), and the absorption tube (E) is 14 mm in inner diameter and 150 mm long (capacity 20 ml). Sintered-glass filter balls of 20–30- μm pore size (G) are fitted at the ends of the gas-inlet tubes of both the reaction and the absorption tubes. All parts are connected by ground-glass joints sealed by phosphoric acid and fastened with springs to withstand inner pressures. The reaction tube for the concentrating distillation is of 700-ml capacity (46-mm i.d., 450-mm length). A suitably larger adaptor (C) and a longer gas-inlet tube (B) are prepared.

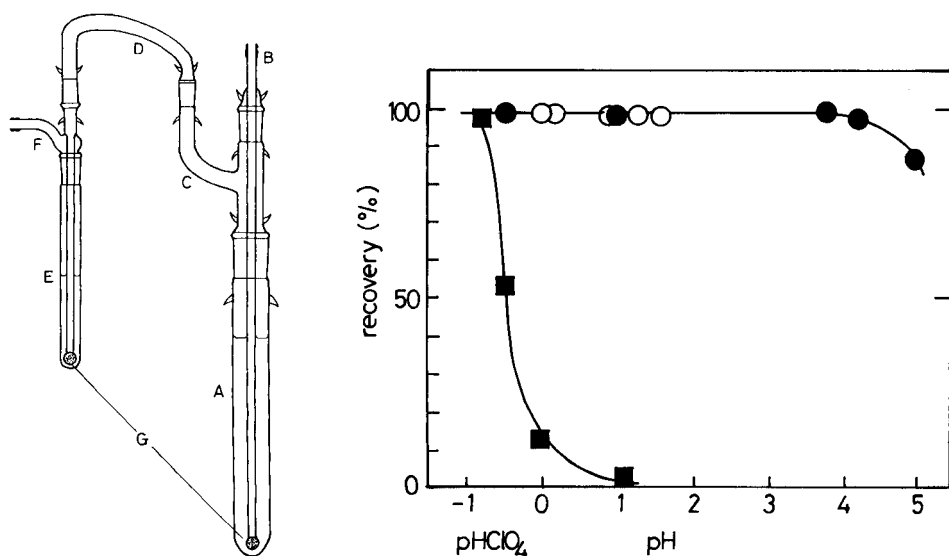


Fig. 1. The distillation apparatus. A, reaction tube; B, gas-inlet tube; C, adaptor; D, connecting tube; E, absorption tube; F, gas-inlet tube for absorption tube; G, sintered-glass filter balls (20–30- μm pore size).

Fig. 2. Effect of acidity on the recovery of fluoride (49 μg) without masking agent (55 μl HMDS; 30 min): (●) without interfering substance; (■) with 27 mg Al^{3+} ; (○) with 100 mg SiO_2 .

Potentiometric determination of fluoride was done with a Toa-Denpa model IM-20E ion meter with an F-125 fluoride-selective electrode and an HS-305DP reference electrode; pH was measured with a Toa-Denpa model HM-5ES pH meter with a GST-155C combined glass electrode.

Reagents

Standard fluoride solution (1000 mg l^{-1}) was prepared from sodium fluoride dried by heating in a platinum crucible at a low red heat; working standards were prepared by suitable dilution. Hexamethyldisilazane was an extra-pure reagent ($>95\%$ by gas chromatography; Tokyo Kasei Kogyo Co.).

Modified total ionic strength adjustment buffers. For TISAB A, 28.5 ml of acetic acid, 51.0 g of sodium nitrate and 58.8 g of trisodium citrate dihydrate were dissolved in 250 ml of deionized-distilled water; the pH was adjusted to 5.5 with 5 mol l^{-1} sodium hydroxide and the solution was diluted to 500 ml. For TISAB B, the preparation was the same as for A, except that 29.7 g of sodium nitrate was used. The difference in the amount of sodium nitrate between A and B corresponds to the amount produced by neutralization of the absorbing solution with nitric acid.

Absorbing solution. About 0.1 mol l^{-1} sodium hydroxide was prepared and the volume (V_a) needed to neutralize 2.0 ml of $0.50 \pm 0.01 \text{ mol l}^{-1}$ nitric acid was determined by titration to a methyl orange end-point. This nitric acid solution was prepared by diluting ca. 1 mol l^{-1} nitric acid which had been standardized in the usual way.

Phosphoric acid solutions. Pyrophosphoric acid (ca. 75% P_2O_5 , chemically pure; Wako Junyaku Kogyo Co.) was diluted with the same volume of water in an ice-bath to avoid rapid hydrolysis; this solution was prepared daily and stored below 10°C . Purified orthophosphoric acid (ca. 10 mol l^{-1}) was prepared by diluting reagent-grade phosphoric acid (500 g) with the same volume of water, adding 5 ml of HMDS, and evaporating to about 450 ml on a boiling water bath with nitrogen bubbling.

All other chemicals were of analytical grade and were used as received. Deionized-distilled water was used throughout.

Recommended procedures

Ordinary distillation. Place V_a ml (defined above; ca. 10 ml) of the absorbing solution in the absorption tube (E). Connect the apparatus as shown in Fig. 1 except for the reaction tube. Transfer up to 50 ml of sample solution to the reaction tube. Dilute to about 50 ml, if necessary, and add 6 ml of the pyrophosphoric acid solution; the concentration of the acid will become about 1 mol l^{-1} in terms of orthophosphoric acid and the pH 0.5–1. Immediately after the addition of $55 \mu\text{l}$ of HMDS by microsyringe, connect the reaction tube to the adapter. Introduce nitrogen at the rate of about 70 ml min^{-1} for 30 min, and then remove the absorption tube. Transfer this solution quantitatively with a 25-ml volumetric flask containing 2.0 ml of modified TISAB B and combine the washings of the absorption tube. Dilute to the mark.

Concentrating distillation. Place up to 500 ml of sample solution in the 700-ml reaction tube. Distil in the same way as described above, adding 30 ml of the purified orthophosphoric acid and 0.55 ml of HMDS.

Potentiometric measurement. Add 2.0 ml of the 0.50 mol l⁻¹ nitric acid to the distillate prepared as above in the 25-ml volumetric flask. Transfer this solution to a 50-ml beaker. Determine the concentration of fluoride with the calibrated ion meter by the single-point method. The solutions used for calibration are prepared by adding 2.0 ml of modified TISAB A to 25 ml of standard fluoride solutions.

RESULTS AND DISCUSSION

Improvement of the silylating reagent and distillation apparatus

As already stated, the use of HMDSO needs over 7 mol l⁻¹ sulfuric acid medium to obtain satisfactory recovery. To avoid the interference of silicate, a silylating reagent reactive in weakly acidic media is required. Thus HMDS and trimethylsilylimidazole were tested preliminarily by using the previously reported distillation apparatus which lacks the sintered-glass filter ball at the gas-inlet tube of the reaction vessel [2]. Without interfering substance, over 97% fluoride recovery was obtained even at pH 2 by using HMDS. Trimethylsilylimidazole is known to be more reactive than HMDS in organic silylation reactions. In acidic aqueous media, however, both reagents are hydrolyzed quickly to generate trimethylsilanol and HMDSO. Therefore, the reactivities with fluoride were almost the same for both reagents. HMDS was selected because of ease of handling and low cost.

The marked effect of applying sintered-glass bubblers on the absorption of TMFS in alkali solution was reported previously [2]. It was expected that a larger interface area between the solution and the carrier gas would also result in rapid sweeping of TMFS from the reaction solution. A sintered-glass filter ball (20–30- μ m pore size) was attached to the gas-inlet tube of the reaction vessel and a tubular vessel was adopted instead of the pear-shaped one used before. Very fine bubbles pervaded the solution uniformly and the distillation was completed in 7 min from pH 1 solution without interfering substances.

Initially, two absorption vessels were connected in series, but subsequent examination showed that TMFS was caught quantitatively in one vessel containing 0.1 mol l⁻¹ sodium hydroxide when the glass frit was used. One tall absorption tube was used therefore to avoid excessive resistance to the nitrogen flow.

Effects of acidity, silica and aluminum

The variation of the recovery of fluoride (49 μ g) with the concentration of acid or pH of the reaction solution by 30-min distillation is shown in Fig. 2. Acidity of the solution below pH 2 was adjusted by perchloric acid and by acetate buffers in the higher pH region. In the absence of any interfering ions,

99% recovery was obtained even at pH 4. Coexistence of aluminum (27 mg) retarded the distillation markedly and 6 mol l⁻¹ perchloric acid was needed for 98% recovery without masking agents. But silicate (100 mg as SiO₂) did not interfere in the pH range 0–2, although it had a fatal effect on the distillation process in the strong acidic region.

A stable foam was formed during the bubbling and overflowed into the absorption tube when a silicate-rich solution was distilled from a strongly acidic medium containing more than 2 mol l⁻¹ perchloric acid. Surface-active agents and higher alcohols were tested for their defoaming ability, but none was sufficiently effective. This foaming also occurred when sample solutions with high silica contents were distilled after long standing at nearly neutral pH conditions. Boiling of such solutions with addition of alkali made it possible to distil at about pH 1. These observations suggest that the reaction between colloidal silica and trimethylsilanol is responsible for the foaming phenomena. When the sample solution contains a large amount of colloidal silica, it must be depolymerized by the pretreatment by alkali. Coexistence of up to 200 mg (as SiO₂) of ionic silicate can be tolerated in the distillation at around pH 1 where the rate of polymerization of silica is slowest [3, 4]. A sodium metasilicate reagent was used in these experiments; this reagent contained a small amount of fluoride (e.g., 0.5 µg F/200 mg SiO₂) and the blank value was subtracted from the values found.

Masking of aluminum. As has been stated, aluminum reduces seriously the recovery of fluoride in the range pH 0–2, where alone the disturbance from silicate can be avoided. The interference of aluminum was eliminated by adding phosphoric acid in the previous method [2]. The recovery of 49 µg of fluoride in the presence of 70 mg of aluminum (molar ratio 1:10³) is plotted against the concentration of orthophosphoric acid in the reaction medium in Fig. 3. As can be seen such a large amount of aluminum is not masked at around pH 1.

The masking ability of pyrophosphoric acid was presumed to be superior to that of orthophosphoric acid from the formation constants of complexes of their anions with trivalent metal ions although those with aluminum were not listed [5]. Addition of 0.6 mol l⁻¹ pyrophosphoric acid made it possible to obtain 99% recovery in the presence of 70 mg of aluminum as is also shown in Fig. 3. So-called pyrophosphoric acid is known to be a mixture of ortho-, pyro-, tripoly- and tetrapoly-phosphoric acid. An acid with 75% P₂O₅ (by weight) was reported to have the composition 66.1% H₃PO₄ + 29.3% H₄P₂O₇ + 4.6% H₅P₃O₁₀ [6]. The concentration of the acid is represented in terms of total phosphate calculated as mol l⁻¹ H₃PO₄ in this paper.

The amounts of aluminum which could be masked by 1 mol l⁻¹ ortho- or pyro-phosphoric acid at pH 1.2 ± 0.1 were determined in the coexistence of 200 mg SiO₂. As shown in Fig. 4, 80 mg of aluminum plus 200 mg of silica were tolerated in the pyrophosphoric acid medium. In contrast, more than 40 mg of aluminum interfered in the orthophosphoric acid medium. The potassium aluminum sulfate used in this study contained a little fluoride (0.5 µg F/140 mg Al), and all the data were corrected for this blank value.

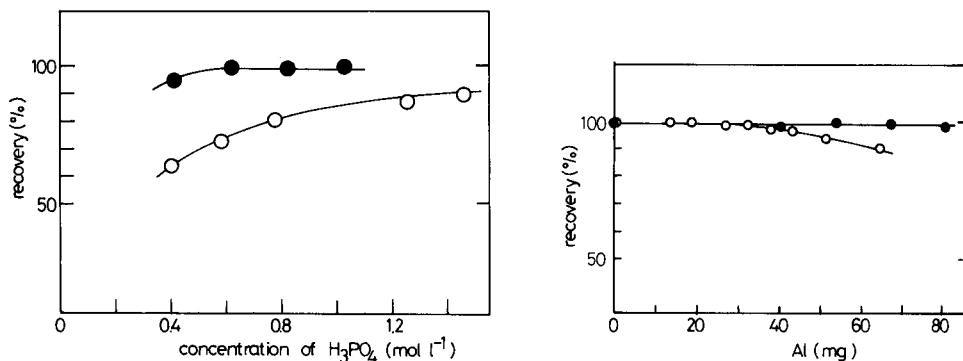


Fig. 3. Masking of aluminum by ortho- and pyro-phosphoric acids ($49 \mu\text{g F}^-$; 70 mg Al^{3+}): (○) orthophosphoric acid; (●) pyrophosphoric acid. Concentration of acids are presented in terms of H_3PO_4 (see text).

Fig. 4. Masking of aluminum in the presence of 200 mg of silicate (as SiO_2) at $\text{pH } 1.2 \pm 0.1$: (●) by 1.0 mol l^{-1} pyrophosphoric acid; (○) by 0.97 mol l^{-1} orthophosphoric acid. (Fluoride $49 \mu\text{g}$.)

Other conditions

The amounts of HMDS necessary for the quantitative recovery of fluoride were examined. Addition of $55 \mu\text{l}$ of HMDS, which corresponds to a concentration of 0.7 mg ml^{-1} in the distillation solution, was sufficient to obtain 99% recovery for at least $250 \mu\text{g}$ of fluoride. The molar ratio of HMDS to fluoride was then about 40.

All the experiments were done at room temperature ($16\text{--}20^\circ\text{C}$). Higher temperatures accelerate the distillation a little, but did not bring about any appreciable reduction in the distillation time.

Potentiometric determination of fluoride was done under the conditions established in earlier work [2, 7].

Effect of diverse ions

The effects of ions which may affect the recovery of fluoride were investigated under the conditions of the ordinary distillation. Very few of the ions tested interfered with the distillation in 1000:1 molar ratios to $49 \mu\text{g}$ of fluoride (Table 1). Zirconium did not interfere in a 100-fold amount, but a 1000-fold amount caused the whole solution to set to a stiff gel composed of zirconyl phosphates which completely prevented distillation. When oxalic acid at a concentration of 0.5 mol l^{-1} was used instead of phosphoric acids, 99% recovery of fluoride was obtained in the presence of 1000-fold amount of zirconium.

Chromium(III) interfered in 100-fold amounts, contrary to expectation from the formation constants of chromium complexes. It was added as dichromate to the sample solution and reduced to Cr(III) by hydrogen peroxide in acidic media. In the first experiment, dichromate was reduced in

TABLE 1

Effect of diverse ions on the recovery of 49 μg of fluoride

Ion	Added as	Amount added (mg)	Molar ratio	Recovery (%)
Mg^{2+}	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	63	1000	99.0
		188	3000	98.0
Ca^{2+}	CaCl_2	103	1000	99.0
		310	3000	98.0
Borate	H_3BO_3	90 ^a	1000	99.5
Ba^{2+}	$\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$	354	1000	99.0
Fe^{3+}	$\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	144	1000	99.5
Ti(IV)	$\text{Ti}(\text{SO}_4)_2 \cdot x\text{H}_2\text{O}$	124	1000	98.0
Ni^{2+}	$\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	151	1000	99.0
Zr(IV)	ZrOCl_2	24	100	98.0
		235	1000	99.0 ^b
Cr^{3+}	$\text{K}_2\text{Cr}_2\text{O}_7 + \text{H}_2\text{O}_2$	13	100	62.2 ^c
		13	100	94.0 ^d
		13	100	99.0 ^e
Cr(VI)	$\text{K}_2\text{Cr}_2\text{O}_7$	258 ^f	1000	98.0
La^{3+}	$\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$	358	1000	100
Hg^{2+}	HgSO_4	516	1000	98.5

^aCalculated as B_2O_3 . ^b0.5 mol l^{-1} oxalic acid was used. ^c Cr(VI) was reduced in the presence of fluoride. ^dFluoride was added after the reduction of Cr(VI) . ^e Cr(VI) was reduced in the presence of pyrophosphoric acid and then fluoride was added. ^fCalculated as CrO_3 .

the presence of fluoride and pyrophosphoric acid was added later; recovery was very low. The recovery was fairly high when fluoride was added after the reduction. When dichromate was reduced in the presence of pyrophosphoric acid and fluoride was added afterward, 99% recovery was obtained. These results may be explained from the well known fact that the rates of ligand-exchange reactions of chromium(III) complexes are extremely slow. The effect of chromium in actual samples may, therefore, differ greatly, according to their individual histories. Some counterplan other than usual masking, e.g., oxidation to Cr(VI) , is required.

Large amounts of carbonate affected the result. The pH of the absorbing solution was lowered by carbon dioxide generated by acidification of the sample solution and TMFS was not absorbed quantitatively. To avoid this effect, such sample solutions were acidified by perchloric acid and carbon dioxide was expelled by nitrogen bubbling before the addition of HMDS.

Concentrating distillation

The lower limit of determination for the fluoride-selective electrode is usually described as 0.02 $\mu\text{g ml}^{-1}$, but the response of the electrode becomes very slow when the fluoride concentration is less than 0.1 $\mu\text{g ml}^{-1}$ [8]. In

addition, most separation methods are accompanied by some dilution of the sample. The determination of less than 0.1 mg l^{-1} fluoride in actual samples is, therefore, very difficult.

In the proposed method, the fluoride concentration is doubled simultaneously with the separation when 50-ml samples are distilled. To obtain a better concentration ratio, the volume of the distillation solution was increased to 500 ml with larger vessels, the volume of the absorbing solution being unchanged. Then, a 20-fold concentration could be achieved. Fluoride at the $\mu\text{g l}^{-1}$ level can be determined after the distillation with addition of 0.3 mol of orthophosphoric acid and 0.55 ml of HMDS.

Commercially available phosphoric acids often contain small amounts of fluoride (e.g., 40 ng ml^{-1} in an analytical-grade phosphoric acid) and this cannot be neglected in the determination of such low concentrations of fluoride. Orthophosphoric acid was purified by heating diluted (1 + 1) acid with addition of HMDS. Pyrophosphoric acid is hydrolyzed by this treatment. Aluminum in a 1000-fold (molar) ratio to $4.9 \mu\text{g}$ of fluoride could be masked by orthophosphoric acid under the condition of the concentration distillation. Samples which need concentrating distillation would seldom contain such a high concentration of aluminum and the purified orthophosphoric acid was used for the concentrating distillation.

Storage of the distillate

Excess of trimethylsilanol is volatilized and partially caught in the absorbing solution in the distillation process. It follows from the principle of the method that fluoride may be lost from the distillates on standing in weakly acidic conditions. To test this, the distillates obtained by the ordinary distillation procedure were allowed to stand for 24 h after neutralization by nitric acid (pH 5.6). The loss of fluoride was less than 0.5% (relative) at the $1 \mu\text{g ml}^{-1}$ level and 3–5% at the $0.1 \mu\text{g ml}^{-1}$ level. If the potentiometric measurements cannot be made on the same day as the distillation, the solution should be stored without prior neutralization, especially in the case of concentrating distillation.

Application

The standard rock samples JG-1 and JB-1 from the Geological Survey of Japan were analyzed for fluorine by the proposed method. In a nickel crucible, 100–300 mg of powdered sample was weighed and fused with a 8–10-fold amount of sodium peroxide. The cake was dissolved with about 30 ml of water and centrifuged in a 50-ml tube. The supernatant liquid was transferred to a 100-ml volumetric flask. The residue was washed with a small amount of water and re-centrifuged. The washings were added to the flask and the solution was diluted to the mark. A 50-ml aliquot of the solution was transferred to the reaction tube of the distillation apparatus. The pH was adjusted to approximately 1 by the addition of perchloric acid. Carbon dioxide was expelled by passing nitrogen through the solution.

TABLE 2

Determination of fluorine in some standard rock samples. (Number of determinations, 3)

Sample	F content ($\mu\text{g g}^{-1}$)	
	This work ^a	Reported values ^b
JG-1 Granodiorite	502 (1.52)	470–521 (mean 498)
JB-1 Basalt	403 (1.11)	360–433 (mean 390)

^aMean of 3 determinations with relative standard deviation (%) in parentheses. ^bFrom [9]. One value for JG-1 which deviated largely from other values is omitted.

Fluoride was separated and determined as stated in the ordinary distillation procedure. The results were highly reproducible and agreed well with the recommended values [9] (Table 2).

REFERENCES

- 1 D. R. Taves, *Anal. Chem.*, 40 (1968) 204; *Talanta*, 15 (1968) 969, 1015.
- 2 M. Yoshida, M. Kitami, N. Murakami and T. Katsura, *Anal. Chim. Acta*, 106 (1979) 95.
- 3 I. Iwasaki, T. Tarutani, T. Katsura and H. Arino, *Nippon Kagaku Zasshi*, 75 (1954) 857.
- 4 K. Goto, *Nippon Kagaku Zasshi*, 76 (1955) 726.
- 5 IUPAC, *Stability Constants of Metal-Ion Complexes, Part A Inorganic Ligands*, Pergamon, Oxford, 1982, p. 133, 138.
- 6 S. Ohashi and H. Sugatani, *Bull. Chem. Soc. Jpn.*, 30 (1957) 864.
- 7 M. Yoshida, Y. Makiyama and T. Katsura, *Nippon Kagaku Kaishi*, (1978) 1375.
- 8 R. C. Hawkins, L. P. V. Corriveau, S. A. Kushneriuk and P. V. Wong, *Anal. Chim. Acta*, 102 (1978) 61.
- 9 A. Ando, H. Kurasawa, T. Ohmori and E. Takeda, *Geochem. J.*, 8 (1974) 175.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH POST-COLUMN REACTION DETECTION FOR THE DETERMINATION OF RARE-EARTH ELEMENTS IN PHOSPHORIC ACIDS PRODUCED FOR THE MANUFACTURE OF PHOSPHATE FERTILIZERS

J. A. TIELROOY, J. C. KRAAK and F. J. M. J. MAESSEN*

Laboratorium voor Analytische Scheikunde, Universiteit van Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)

(Received 20th May 1985)

SUMMARY

The isolation of rare earth elements (REE) from technical phosphoric acids and their determination by h.p.l.c. with post-column reaction detection are examined with different liquid chromatographic techniques. A combination of procedures provides optimum analytical conditions. Twelve REE can be separated within 20 min by applying the following procedures: (i) separation of REE from calcium by addition of ammonium pyrrolidinedithiocarbamate to the sample solution, passage through a disposable column (SepPak C18), and use of dilute perchloric acid for desorption; (ii) separation of REE by using a chemically bonded strong cation-exchanger (Servachrom Si-100) and gradient elution with α -hydroxyisobutyric acid. The performance of this combination is discussed in relation to the restrictions imposed by the post-column reaction detector based on complexation with 4-(2-pyridylazo)resorcinol. Results obtained for two types of technical phosphoric acid are presented.

In the fertilizer industry, millions of tons of natural phosphates are processed annually. Depending on their nature and the location of the deposits, the contents of rare-earth elements (REE) range between 0.01 and 1% [1]. So far, the distribution of the REE over the various intermediate products, by-products, waste products and end products that are generated in industrial processes is completely unknown. It should be realised, on the one hand, that even if only a minor part of the REE contents of the raw material eventually reaches the end product (i.e., the fertilizer), this would lead inevitably to a continuous increase of the REE loading of arable land. On the other hand, if the REE were concentrated in a specific by-product or waste product, their recovery might be economically feasible. An impression of the proportions of this problem was given by Altschuler [1] who reported that the quantity of potentially recoverable REE amounted to over ten thousand tons in 1978 in the U.S.A. In that year, eight million tons of wet-process phosphoric acid were produced from twenty-two million tons of natural phosphates. Much research is being done to recover REE in one of the fertilizer process stages. So far, however, only a few companies have successfully recovered REE from natural phosphates on a commercial basis [2].

Considering the possible environmental and economic aspects associated with the production of fertilizers from natural phosphates on the one hand, and the strongly varying REE contents of the raw material on the other hand, there is an urgent need for rapid and reliable analytical methods which are suitable for routine determination of the REE in the products mentioned.

For the determination of REE in geological and related materials, a wide variety of analytical techniques has been used. Successful applications were reported for d.c. arc and plasma arc emission spectrometry [3, 4], instrumental neutron activation analysis [5-7], γ -spectrometry [8, 9], isotope dilution mass spectrometry [10], graphite-furnace and flame atomic absorption spectrometry [11, 12], and inductively-coupled plasma/atomic emission spectrometry (i.c.p./a.e.s.) [13-19]. However, because of interelement interferences or lack of sensitivities, analysis for REE at trace level concentrations still encounters serious problems when the instrumental analytical techniques are used directly without specific sample treatment [19].

Depending on the nature of the technique, but also on the number and concentration ratios of the REE in the sample, the required treatment procedures comprise separation of the analyte from the matrix, analyte preconcentration, and separation of the REE analytes. Thus, Jung and Specker [8] determined REE in nuclear fuels by γ -spectrometry after thin-layer chromatographic enrichment of the analytes. Wyttenbach et al. [6] isolated REE as a group from neutron-activated rock samples by liquid-liquid extraction with thenoyltrifluoroacetone/phenanthroline in chloroform. For establishing REE abundances in chondrites, Masuda et al. [10] separated the analytes from major elements by making use of cation-exchange resins. Walsh et al. [14], Broekaert and Hörmann [16], and Crock and Lichte [17] also used ion-exchange procedures, applying Dowex AG50W-X8 resin for analyte/matrix separation and analyte preconcentration for the determination of REE in a wide variety of geological materials by i.c.p./a.e.s.

Yoshida and Haraguchi [19] applied i.c.p./a.e.s. interfaced with high-performance liquid chromatography (h.p.l.c.) for the separation of REE prior to the analysis of rock standard reference material, REE ores and high-purity REE reagents. A strong cation-exchange resin served as the stationary phase and an aqueous solution of ammonium lactate as the mobile phase. By flow programming, spectral interferences from accompanying REE, as well as other concomitant elements, could be reduced sufficiently.

Regarding the practice of analysis for REE, it should be realized that rare-earth elements are not as "rare" as their name implies [11, 20]. Considering the progress made in the field of cation separation in recent years (see, e.g., [21]) and because of its instrumental simplicity, it is of importance to examine how far the determination of REE by h.p.l.c., without element-specific detection, can meet the present demands. Previous achievements in this respect may be summarized as follows.

Elchuk and Cassidy [22] described the separation of REE on the strong-acid ion-exchangers, Partisil 10-SCX, Nucleosil 10-SA, and Aminex, using a

solution with α -hydroxyisobutyric acid (HIBA) as the eluent and various complexing reagents for post-column reaction detection. With the column materials used, good separations and attractive sensitivities were obtained. Weuster and Specker [23] determined the REE contents of REE ores and technical products by h.p.l.c. after separation on a silica column packing with an eluent system containing a mixture of di-isopropyl ether, nitric acid, and tetrahydrofuran (THF). Fluorescence quenching of THF, caused by the individual elements, served for detection. The procedure satisfied the aims intended, but relatively poor resolution and selectivities were obtained for the heavier REE. Using h.p.l.c. for the determination of REE in monazite sand and high-purity rare-earth oxides, Jon-Mau Hwang et al. [24] separated the fourteen REE on a chemically bonded cation-exchanger column with HIBA as the eluent. After complexation with arsenazo-III, the eluted elements were detected with a variable-wavelength spectrophotometer. Good sensitivity was obtained but the separation of the REE was not complete. Wang-nang Wang et al. [25] also used chemically bonded cation-exchange columns in combination with different types of chelating eluents, such as citric acid, tartaric acid, 2-methylactic acid, and lactic acid, for the determination of transition and rare-earth elements in aqueous test solutions, with post-column reaction detection. 4-(2-Pyridylazo)resorcinol and Arsenazo I proved to be very sensitive reagents in the spectrophotometric determination of the REE. Without pre-concentration on the analytical column itself, detection limits at the $0.1 \mu\text{g ml}^{-1}$ level were attained for most of the REE studied.

These results indicate that h.p.l.c. with post-column reaction detection is an attractive possibility for determining REE in products such as technical phosphoric acids. The aim of the present work was to investigate the potential of this general method for the determination of REE in phosphoric acids derived from natural phosphates.

EXPERIMENTAL

Apparatus

The h.p.l.c. system consisted of a gradient pump (Perkin Elmer Series 3) and an injection valve (Rheodyne 7125) fitted with sample loops of different volume. All components and tubing in contact with the mobile phase consisted of S316 stainless steel. The post-column reactor consisted of a home-made T-piece constructed from 0.5 mm stainless steel capillaries and a single-bead-string reactor (20 cm length, 0.5 mm i.d., filled with glass beads of 0.3-mm diameter). The reagent was delivered by a peristaltic pump (Ismatec). The eluate was monitored by a spectrophotometer (Siemens PM2000 variable-wavelength detector) set at 520 nm. For matrix separation, disposable columns (SepPak-C18, Waters Ass.) were used. The sample liquids were pumped through these columns by a peristaltic pump (Ismatec) at a flow rate of 1.5 ml min^{-1} .

Reagents and materials

The REE stock solutions were obtained (Hicol, Alpha Products) or were prepared from rare-earth oxides (Merck or Fluka). The oxides were successively heated for 2 h at 700°C and dissolved in the recommended acid, and the solutions were finally diluted with subboiled water to the required volume. Monazite sand was kindly donated by Dr Pham Luan (Vietnam). The reagent used for post-column reaction was 4-(2-pyridylazo)resorcinol (PAR; Aldrich Chemicals). α -Hydroxyisobutyric acid (HIBA; Aldrich Chemicals) was obtained in different degrees of purity (95% and 99%). Both products required purification, which was done by vacuum sublimation and by crystallization from tetrachloromethane, respectively. Glass beads with immobilized 8-quinolinol (particle size 100 μ m; Pierce Chemicals) were used. The complexing reagents, ammonium pyrrolidinedithiocarbamate (APDC) and 8-quinolinol (both from Merck) were used as received. The chemically bonded cation-exchangers used were Nucleosil SA (particle size 5 μ m; Macherey-Nagel) and Servachrom Si-100 (particle size 5 μ m; Serva). The h.p.l.c. columns (150 $\times$ 4.6 mm) were packed by a slurry technique with methanol/isopropanol (1:1) as slurry and methanol as the displacing liquid, up to a maximum pressure of 40 MPa.

Procedures

Sample preparation. The phosphoric acid samples (20–50 μ l) were dissolved in 25 ml of an acetate (pH 4.5) or borax (pH 8) buffer solution. Monazite samples were dissolved in a small amount of nitric acid and then evaporated nearly to dryness. The residue was dissolved in concentrated hydrochloric acid and subsequently diluted with subboiled water.

Post-column reaction detection. Excellent sensitivity for detection of individual REE was obtained with a PAR solution of the following composition: 2×10^{-4} M PAR, 2 M ammonia, and 1 M ammonium acetate [22] mixed with the eluent in a ratio of 1:1.5. Considerable reduction of the noise level was achieved by using a single-bead-string reactor placed at a short distance from the reagent introduction plug. In this way, mixing of eluate and reagent was improved without causing noticeable extra broadening of peaks.

Matrix separation. Because the phosphoric acids to be analysed are prepared from natural calcium phosphates as the major raw material, the concentration of matrix elements, and in particular of calcium, is high (see Table 1). Because of the excess of calcium, the chromatographic column can be overloaded locally, which may have detrimental effects on the separation. Another complication associated with the high calcium content of the samples stems from the ability of PAR to form calcium complexes. Consequently, if an analyte is eluted together with more or less significant amounts of calcium, the detection is affected proportionally. Given these considerations, it is clear that REE/matrix separation is a prerequisite for achieving reliable REE determinations when h.p.l.c. procedures are used with post-column reaction detection.

TABLE 1

Rough concentrations ($\mu\text{g ml}^{-1}$) of matrix and rare-earth elements in two types of technical phosphoric acids

Element	Conc. ($\mu\text{g ml}^{-1}$)		Element	Conc. ($\mu\text{g ml}^{-1}$)	
	Type 1	Type 2		Type 1	Type 2
Ca	5000	80000	REE (total)	60	120
Si	1500	200	La	15	30
Fe	3000	1000	Ce	10	20
Al	3000	1000	Nd	10	20
K	500	200	Pr	—	10
Na	1000	2000			
Mg	4000	1000			
Cr	300	40			
Ni	60	10			
Zn	500	60			

Analyte/matrix separation and separation of the REE were examined by using single as well as off-line dual column systems. Figure 1 schematically shows the approach followed. When a single column was used (route a), the test or sample solutions were introduced directly into the analytical column A. Using a test solution which contained salts of 12 REE but no concomitant matrix metal ions, the following single column procedures were examined first:

- (A1) a reversed-phase column combined with a buffered aqueous mobile phase containing hydrogen diethylhexyl phosphate (HDEHP);
- (A2) a chemically bonded strong cation-exchanger (Nucleosil SA) and gradient elution with α -hydroxyisobutyric acid (HIBA);
- (A3) the same system as described under (A2) but with Servachrom Si-100 as the ion-exchanger.

The study was continued with an attempt to achieve analyte/matrix separation on the analytical column described (procedure A3), with preservation of the REE separation (see Fig. 2). Finally, a method aimed at complete analyte/matrix separation prior to the REE separation was considered. For that purpose, an additional column was used, which served exclusively to separate the matrix from the group of REE (see Fig. 1, route b, column B and Fig. 3). For that approach, the following procedures were tested:

- (B1) the pH-adjusted sample solution was passed through a bed of glass beads with immobilized 8-quinolinol (8-Q), and perchloric acid was used to desorb the REE from the column;
- (B2) the sample solution was mixed with a chelating agent (8-Q) prior to its passage through a disposable column containing reversed-phase material (Sep-Pak C-18) and methanol or perchloric acid was used to desorb the REE;

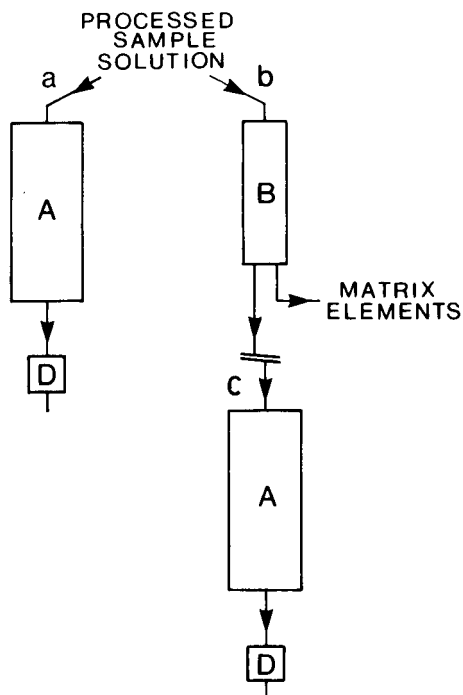


Fig. 1. Schematic diagram of the chromatographic routes examined. (A) Analytical column; (B) column for matrix separation; (D) spectrophotometric detector. (a) Single column procedure; (b) dual column procedure; (c) matrix-free, mutually unseparated REE.

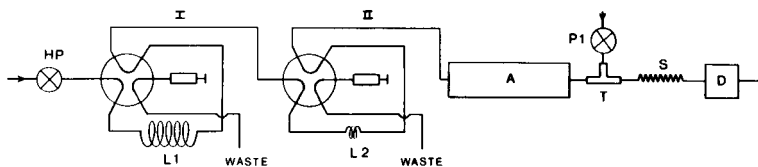


Fig. 2. Scheme of the instrumentation for the single column procedure. (HP) High-pressure gradient pump; (I) injection valve 1; (II) injection valve 2; (L1) loop 1, 3.6 ml; (L2) loop 2, 20 µl; (A) analytical column; (T) mixing tee; (P1) peristaltic pump; (S) single-bead-string reactor; (D) spectrophotometric detector.

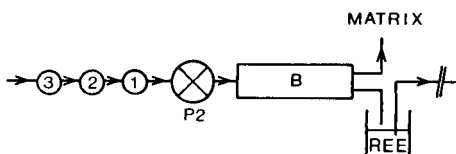


Fig. 3. Scheme of the instrumentation for the matrix separator column in the dual column procedure. (B) Column for REE/matrix separation; (P2) peristaltic pump; (1) sample with or without complexing agent; (2) buffer; (3) eluent, 0.1 M perchloric acid or methanol.

(B3) the procedure described under (B2) was used but with ammonium pyrrolidinedithiocarbamate (APDC) as the complexing agent and with perchloric acid only for desorption of the REE.

RESULTS AND DISCUSSION

Separation of REE

The reversed-phase system with HDEHP in the mobile phase can be considered as a solvent-generated liquid-liquid system; HDEHP adsorbs strongly on the hydrophobic support and forms an immobilized layer of HDEHP on the surface of the particles. This system provided good selectivity for the REE, but for the elution at least 1 M perchloric acid had to be used. As a result of such a high acid concentration, a significant amount of metals from the pump, column, and tubing, dissolved in the mobile phase. This seriously affected the detection of the REE. Therefore, a phase system involving a less aggressive mobile phase was investigated.

For that purpose, a chemically bonded strong cation-exchange packing was used (Nucleosil SA; procedure A2). With this material, good selectivity was found, but its ion-exchange capacity appeared to be very small. Only samples with a very low REE concentration could be separated (20 μ l maximum with a total REE concentration of 5 μ g ml⁻¹). Moreover, it was found that another batch of Nucleosil SA, for unknown reasons, provided no retention of REE at all.

Better results were obtained with the ion-exchange material Servachrom Si-100, which had a capacity of 0.6 mmol g⁻¹ (procedure A3). Aqueous solutions of HIBA adjusted with ammonia in the pH range 3.5–6 were used. Optimum separation conditions were obtained at pH 4.7. The separation was optimized by variation of the gradient in curve and slope. The eventual choice was a 2-step linear gradient with a HIBA concentration of 0.02–0.2 M in 20 min (Fig. 4A). In all experiments, the background was increased at the positions of the peaks corresponding to Gd, Eu, Sm, Nd, Ce and La. This background increase appeared even when blanks were injected (Fig. 4B). In order to explain this phenomenon, the effluent was collected in fractions; i.c.p./a.e.s. analysis of these fractions showed the presence of iron, chromium and nickel at the μ g ml⁻¹ level, and in ratios nearly corresponding to the ratio of these metals in S316 steel alloy.

When gradient elution is used with a very low starting concentration of HIBA, the REE are strongly retained. This behaviour can be used to achieve on-column preconcentration. No significant change in peak width was observed when a fixed amount of REE was introduced on the analytical column, whether in 20 μ l or in 4.0 ml of 0.1 M perchloric acid. The relative large capacity of the ion-exchanger permits analysis of samples differing widely in REE concentrations, as is demonstrated in Fig. 5 and Table 2, for two monazite samples with 100 and 1000 μ g ml⁻¹ REE.

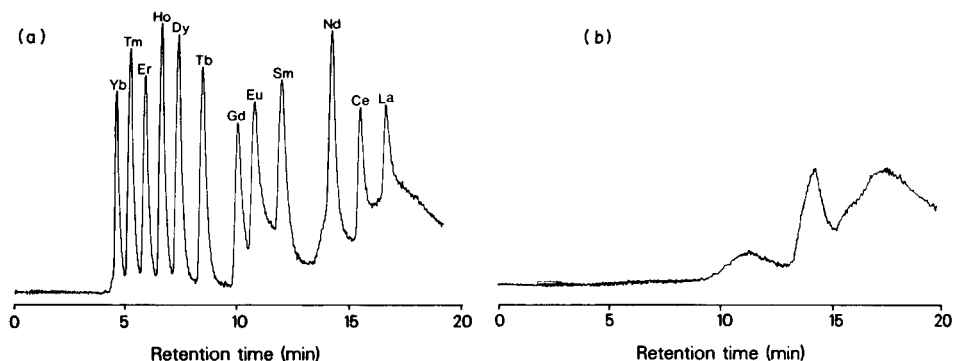


Fig. 4. (A) Separation of twelve REE after injection of 20 μl containing 10 $\mu\text{g ml}^{-1}$ each of the REE. (B) Baseline after injection of 20 μl of water. Experimental conditions: column, Servachrom Si-100, 4.6 $\times$ 150 mm; gradient 0.02–0.2 M HIBA in 24 min; flow rate 1.5 ml min^{-1} ; detection after post-column reaction with PAR (2×10^{-4} M); reagent flow 1 ml min^{-1} ; $\lambda = 520$ nm.

TABLE 2

Results obtained for monazite sand (cf. Fig. 5A)

Element	Concentration (%) ^a		Element	Concentration (%) ^a	
	Indicative ^b	Found ^c		Indicative ^b	Found ^c
Dy	—	} 0.04	Nd	13—14	17.8
Y	} 4		Pr	5—6	4.2
Gd			Ce	44	41.3
Eu		0.2	La	28	33
Sm	2—3	2.5			

^aExpressed as a percentage of the rare-earth oxides in the solid sample. ^bD.c. arc method.

^cH.p.l.c. with post-column detector.

Separation of REE and matrix

Single column procedure (Fig. 1, route a, column A). For the single column procedure, used for the analyte/matrix separation and separation of the REE, the analytical column, sample injection valve and detection system were the same as for the dual column procedures (Fig. 2). After introduction of the sample on analytical column A by means of injection valve II, analyte and most of the matrix are concentrated on top of the analytical column. Subsequently, by switching injection valve I, 3.6 ml of 0.1 M or 0.2 M perchloric acid, present in loop I, is pumped through the column. Most of the matrix elements are eluted by the acid, but the REE are not (Table 3).

The REE, which are then matrix-free, can be separated mutually by starting the gradient. Because it is less corrosive than other mineral acids, perchloric acid was chosen for removal of the matrix. Although calcium can be

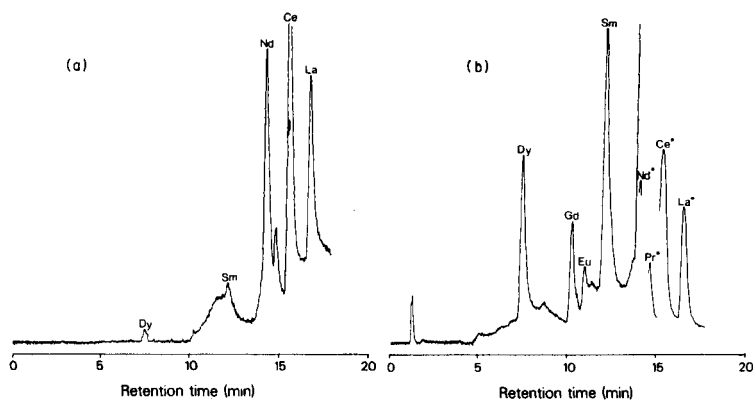


Fig. 5. Separation of REE in monazite sand. Total concentration of REE: (A) $100 \mu\text{g ml}^{-1}$; (B) $1000 \mu\text{g ml}^{-1}$. Experimental conditions: injection volume $20 \mu\text{l}$; other conditions as in Fig. 4; asterisked peaks were from 10 times attenuated signals.

TABLE 3

Contents of ytterbium and calcium ($\mu\text{g ml}^{-1}$) in collected eluent fractions for the single column procedure

(Elution with 0.1 M HIBA; injection volume $20 \mu\text{l}$ containing $500 \mu\text{g ml}^{-1}$ Ca and $50 \mu\text{g ml}^{-1}$ Yb, followed by injection of 3.6 ml of perchloric acid)

HClO_4 (M)	Test element	Fractions (ml)				
		0–1.2	1.2–3	3–6	6–8	8–14
0.2	Yb	1	3	—	100	45
	Ca	—	5700	2	—	—
0.1	Yb	—	—	—	175	10
	Ca	—	5000	50	—	—

removed satisfactorily, the method described has the following disadvantages. First, of the matrix elements, calcium is the only one which is removed effectively; other matrix metals are not completely washed out and may interfere in the detection. Secondly, even the relatively mild perchloric acid, used for matrix elution, causes some dissolution of metals from the h.p.l.c. system. Finally, overloading the column with matrix elements or the subsequent removal of the matrix may cause displacement of REE by matrix elements.

Dual column procedure (Fig. 1, route b, column B). In order to achieve an analyte/matrix separation that does not suffer from the disadvantages of the single column procedure, an off-line dual column system was tested. An advantage of such a system is the shorter time for analysis, because the matrix separation can be done during the separation of the REE from a previous sample. Three different systems were investigated for analyte/matrix separation by means of a separate column for the removal of the matrix (see

procedures B1, B2, B3 and Fig. 3). The procedures are based on the property of complexing reagents to form complexes with the REE but not with major matrix elements like calcium.

8-Quinolinol immobilized on glass beads was used at pH 8 (procedure B1). The buffered sample solution was pumped through the column at a flow rate of 1.5 ml min^{-1} . The adsorbed REE could be desorbed from the column with a few millilitres of an acidic solution. However, the capacity of the column material was rather low, and it appeared to decrease notably after the material had been used several times. When an ytterbium solution (1 mg l^{-1}) was pumped through the column at a flow rate of 1.5 ml min^{-1} , ytterbium was present to a significant extent even in the first 1-ml fraction of the effluent. The ytterbium concentration in this fraction increased with increasing loading of the column and with increasing flow rate. This phenomenon points to slow complex formation. However, if very low flow rates are used, acceptable results can be obtained with this material. Quite apart from the time-consuming aspect, a drawback of this method is the poor capacity and stability of this packing.

Therefore, the REE were complexed in the sample solution with 8-quinolinol. Then, the solution was pumped through a SepPak C-18 reversed-phase disposable column. The complexes were adsorbed, while the uncomplexed elements, e.g., alkali and alkaline earth metals, remained in solution and left the column unretained. To remove all the uncomplexed metals, a buffer solution was pumped through the column after completion of sample introduction. Subsequently, the REE were eluted from the column with 5 ml of acid or methanol. The complexing agents tested were 8-quinolinol at pH 8 (procedure B2 and Table 4) and APDC at pH 4.3 (procedure B3 and Table 5). However, under the conditions needed for formation of the REE complexes, both reagents also formed strong complexes with iron and aluminium. When the column was eluted with methanol, the intact complexes, of the REE as well as Fe and Al, were eluted. If, however, the elution was done with perchloric acid, only the REE cations were eluted, the complexes of Fe and Al remaining intact and adsorbed. Because this procedure enables elements like Fe and Al to be separated at least partly from the REE, acidic elution was preferred.

TABLE 4

Efficiency of REE/calcium separation

(Concentration and corresponding recovery of calcium and some test REE in the hydrochloric acid fraction for procedure B2. Elution with 3.5 ml of 1 M HCl; sample volume $40 \mu\text{l}$ containing $40 \mu\text{g ml}^{-1}$ each of the REE and $1000 \mu\text{g ml}^{-1}$ Ca.)

Element	Ca	Yb	Sm	Ce
Conc. (ng ml^{-1})	121	465	437	441
Recovery (%)	1.05	101.7	95.6	96.5

TABLE 5

Efficiency of analyte/matrix separation for artificial technical phosphoric acid (Concentration and corresponding recovery of matrix elements and REE for procedure B3. Elution with 5 ml of 0.1 M perchloric acid; sample volume 200 μ l containing 20 μ g ml⁻¹ each of the REE, 1000 μ g ml⁻¹ Fe, and 80 g l⁻¹ Ca.)

Element	Yb	La	Ce	Nd	Sm	Ca	Fe
Conc. (ng ml ⁻¹)	740	790	800	750	740	1300	900
Recovery (%)	92.5	98.5	100	94	92.5	0.04	4

For the matrix/analyte separation in natural phosphates and their corresponding phosphoric acids, problems arose during adjustment of the sample solution at pH 8. Even in very diluted solutions, a precipitate of calcium carbonate developed. Because of possible co-precipitation of the REE, the precipitate cannot be removed. Consequently, under these circumstances a good analyte/matrix separation is not possible. Therefore, 8-quinolinol cannot be used for samples with a calcium content of the order of a few percent.

Then APDC was tested as the complexing agent at pH 4.3. Because diluted sample solutions can be adjusted to this pH without precipitation of calcium carbonate, the procedure involving this complexing reagent (procedure B3) is suitable for samples with a high calcium content. This analyte/matrix separation procedure was used to analyse two types of phosphoric acids with h.p.l.c. and the post-column reaction detector (Fig. 6). Results are summarized in Table 6. With regard to the detection limits, it should be realised that their numerical values are inversely proportional to the volume of the sample loop used.

Assessment of the procedures examined

The procedures A1–3 and B1–3 were outlined in the Experimental section. Here, their general advantages and disadvantages are assessed. With procedure A1, good separation of REE is possible, but detection is strongly affected by metals dissolved from components of the instrumentation. For this reason, satisfactory results can be obtained only if the h.p.l.c. system is highly resistant to corrosion.

Although twelve REE could be separated rapidly on a column packed with a specific batch of Nucleosil SA (procedure A2), the capacity of this batch was still too small to permit determination of REE in samples with widely varying analyte concentrations. With the chromatographic system used in procedure A3 attractive results were obtained. Separation of twelve REE was possible within 20 min. On-line analyte/matrix separation on the analytical column yielded good results for samples which did not have extremely low REE contents.

Because of the low flow rates required in procedure B1, analyte/matrix separation with immobilized 8-quinolinol was very time-consuming. Additional disadvantages associated with the use of 8-quinolinol were the decreasing

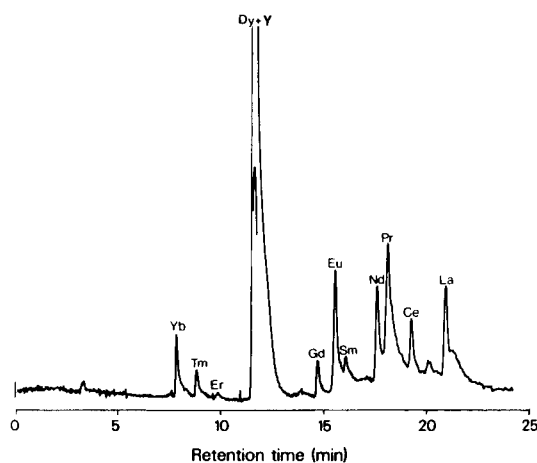


Fig. 6. Determination of REE in technical phosphoric acid after matrix separation. Injection volume 3.6 ml; for other experimental conditions, see Fig. 4.

TABLE 6

Results obtained with a combination of procedures A3 and B3 (with a 1000- μ l loop) for the analysis of two types of phosphoric acids

Element	Yb	Tm	Er	Dy+Y	Gd	Eu	Sm	Nd	Pr	Ce	La
<i>Type 1</i>											
Conc. ($\mu\text{g ml}^{-1}$)	4	2	0.5	115	3	15	2	8	11	7	13
RSD (%) ^a	7	8	22	5	7	5	10	6	7	7	8
<i>Type 2</i>											
Conc. ($\mu\text{g ml}^{-1}$)	7	3	1	95	7	22.6	4	14	22	12	24
RSD (%) ^a	6	7	15	5	7	5	8	6	6	8	8
DL ^b (ng ml ⁻¹)	40	40	60	60	80	80	80	70	80	100	100

^aRelative standard deviation (RSD) based on quadruplicate measurements. ^bDetection limit (3σ).

capacity of this material upon repetitive use and the relatively high pH required for optimum operation. At pH 8, calcium carbonate precipitates easily if there are high calcium contents in the sample solutions. Good results were obtained with sample solutions that could be adjusted with a buffer solution to pH 8 without causing precipitation (procedure B2). However, the phosphoric acid samples required considerably lower pH values. In procedure B3, involving the use of APDC at pH 4.3, excellent analyte/matrix separation for phosphoric acid samples could be achieved without the occurrence of precipitate formation.

From the discussion of the performance of the procedures examined, it is obvious that procedure B3 for matrix removal followed by procedure A3 for

mutual REE separation provides the optimum conditions for the analysis of phosphoric acids of the type under consideration. With that combination, good reproducibility and accuracy were obtained for most of the analytes. However, in some cases, the sample may still contain interfering cations which are not, or not completely, removed in the analyte/matrix separation step. The extent to which their peaks overlap with the REE peaks decides the accuracy of the analysis; the accuracy depends on both the nature and the concentration of metals accompanying the REE after the analyte/matrix separation. Another possible complication originates from the observation that the retention times are not governed exclusively by the HIBA concentration but also, to some extent, by the nature and concentration of the cations. This means that the retention times are slightly dependent on the composition of the sample. Consequently, problems can arise with the identification of eluted elements, especially if some concomitant interfering elements are still present.

Simplification of the analytical procedure, as well as improvement of the reliability, could be achieved by using an element-specific detector. Therefore, further work is in progress to apply inductively-coupled plasma/atomic emission spectrometry for REE detection.

The authors are greatly indebted to Drs. A. J. B. Beeren and Dr. R. S. Deelder (D.S.M. Research Department, Geleen, The Netherlands) for useful discussions and for providing the phosphoric acid samples.

REFERENCES

- 1 Z. S. Altschuler, Proceedings of the 2nd International Congress on Phosphorus Compounds, Boston, April, 1980, IMPHOS Pub., Boston, 1980, p. 605.
- 2 N. Lounamaa, T. Matila, V. P. Judin and H. E. Sund, Proceedings of the 2nd International Congress on Phosphorus Compounds, Boston, April, 1980, IMPHOS Pub. Boston 1980, p. 759.
- 3 K. Dittrich, M. Gajek and P. Luan, *Chem. Anal.*, 23 (1978) 41.
- 4 M. A. Eid, K. M. El Behery, A. A. Fakhry and F. A. El Anany, *Spectrochim. Acta*, Part B, 38 (1983) 495.
- 5 P. J. Potts, O. W. Thorpe and J. S. Watson, *Chem. Geol.*, 34 (1981) 331.
- 6 A. Wytttenbach, S. Bajo and L. Tobler, *J. Radioanal. Chem.*, 78 (1983) 283.
- 7 P. Vukotic, *J. Radioanal. Chem.*, 78 (1983) 105.
- 8 K. Jung and H. Specker, *Fresenius Z. Anal. Chem.*, 300 (1980) 15.
- 9 G. Steidl, K. Bachmann and F. Dienstbach, *Fresenius Z. Anal. Chem.*, 314 (1983) 146.
- 10 A. Masuda, N. Nakamara and T. Tanaka, *Geochim. Cosmochim. Acta*, 37 (1973) 239.
- 11 S. J. Horsky, *At. Spectrosc.*, 1 (1980) 129.
- 12 R. Keil, *Fresenius Z. Anal. Chem.*, 303 (1980) 188.
- 13 J. A. C. Broekaert, F. Leis and K. Laqua, *Spectrochim. Acta*, Part B, 34 (1979) 73.
- 14 J. N. Walsh, F. Buckley and J. Barker, *Chem. Geol.*, (1981) 141.
- 15 I. B. Brenner, A. E. Watson, T. W. Steele, E. A. Jones and M. Gonçalves, *Spectrochim. Acta*, Part B, 36 (1981) 785.
- 16 J. A. C. Broekaert and P. K. Hormann, *Anal. Chim. Acta*, 124 (1981) 421.
- 17 J. G. Crock and F. E. Lichte, *Anal. Chem.*, 54 (1982) 1329.
- 18 A. Bolton, J. Hwang and A. Van der Voet, *Spectrochim. Acta*, Part B: 38 (1983) 165.

- 19 K. Yoshida and H. Haraguchi, *Anal. Chem.*, 56 (1984) 2580.
- 20 A. A. Levinson, *Introduction to Exploration Geochemistry*, Applied Publishing Ltd., Calgary, Canada, 1974.
- 21 Zhang Tao, *Talanta*, 30 (1983) 864.
- 22 S. Elchuk and R. M. Cassidy, *Anal. Chem.*, 51 (1979) 1434.
- 23 W. Weuster and H. Specker, *Fresenius Z. Anal. Chem.*, 304 (1980) 6.
- 24 Jon-Mau Hwang, Jeng-Shang Shih, Ju-Shih, Ju-Chai Yeh and Shaw-Chii Wu, *Analyst*, 106 (1981) 869.
- 25 Wang-nang Wang, Yeong-jgi Chen and Mou-tai Wu, *Analyst (London)*, 109 (1984) 281.

A SELENIUM-SELECTIVE CHROMATOGRAPHIC DETECTOR BASED ON ISOTOPE CLUSTERS

ROBERT J. ANDEREGG

Department of Chemistry, University of Maine, Orono, ME 04469 (U.S.A.)

(Received 29th April 1985)

SUMMARY

A selenium-selective chromatographic detector based on mass spectrometry is described. Full mass spectra are collected continuously from the gas chromatographic effluent. A computer program then searches the spectra for the naturally-occurring selenium isotope clusters, indicating which spectra in the data set are most likely to contain those clusters. The device involves a selective data-reduction technique, as opposed to a selective data-collection process; it therefore possesses some useful advantages. The method was tested on the EPA/NIH Mass Spectral Data Base and on the analysis of trimethylsilylated amino-acid mixtures including selenomethionine.

Selenium is an interesting element in biochemistry both because of its role as an essential nutrient and because of its toxicity [1]. Despite this importance, surprisingly little is known about the actual chemical form in which selenium occurs in organisms. The element has been associated with vitamin E metabolism [2], is incorporated into several proteins [3–7] and t-RNA's [8], and seems to have anti-carcinogenic and anti-mutagenic properties [9]. Only in a few proteins has the precise chemical form been defined; and in these, not unexpectedly, the form is as the seleno analog of cysteine [10, 11]. Most of the methods proposed for quantifying selenium in biological samples by neutron activation analysis [12], fluorescence [13], gas chromatography [14], mass spectrometry [15], or atomic emission spectrometry [16] measure total selenium, rather than identify the actual chemical species involved. Chromatographic methods capable of resolving selenium-containing species have appeared in the literature [14, 17, 18], relying on chromatographic retention times and/or incorporation of radioactive ^{75}Se for verification of the presence of selenium. Element-selective chromatographic detectors based on microwave [19] or inductively-coupled [20] plasma emission or atomic absorption [21] could, in principle, be used for selenium detection; but so far, few reports of selenium-selective detectors have appeared [21, 22]. Unfortunately, these devices provide little structural information other than the presence or absence of certain elements. They can be classified as selective data collectors.

Gas chromatography/mass spectrometry (g.c./m.s.) and liquid chromatography/mass spectrometry (l.c./m.s.) can provide a wealth of structural information about compounds being analyzed, but the methods are relatively non-selective; all reasonably volatile compounds leaving the chromatograph are ionized and detected. This lack of specificity is a disadvantage in analysis for selenium compounds in biological samples because the selenium species are present at low levels in a complex matrix of compounds which do not contain selenium. Computer methods have been developed to improve the apparent selectivity of g.c./m.s. and l.c./m.s. data, taking advantage of the fact that many elements, including selenium, have multiple naturally-occurring stable isotopes and thus show characteristic patterns of ions in their mass spectra. Chlorine and bromine are the most familiar examples, each having two isotopes. Selenium has six naturally-occurring isotopes. The computer technique, isotope cluster chromatography [23], searches a g.c./m.s. or l.c./m.s. data set and locates mass spectra which display an isotope pattern as requested by the analyst. The method has been used for the detection of chlorine-containing [24, 25] and stable isotope-labeled species [26]. This paper reports the extension of the isotope cluster chromatographic technique to search for selenium-containing species in a mass spectral data set.

EXPERIMENTAL

Computer program

A Hewlett-Packard 5985-B g.c./m.s. system was used in all the experiments. Computer programs were written in BASIC with FORTRAN subroutines and are available from the author. The computer used was an H-P 1000 E, the data system for the g.c./m.s. instrument.

The isotope cluster-searching algorithm is based on previous work [23]. Briefly, the known isotope pattern (of selenium, in this case) is considered to be analogous to a "library" spectrum. An equal-sized part of an unknown mass spectrum is considered to be analogous to an "unknown". After a pre-search has tested the unknown to see if key ions are present, a similarity index [27] is calculated. This measures the similarity of the known cluster to the spectral fragment, and has a value ranging from 0.0 for a very bad match, to 1.0, for a perfect match. If this similarity index is greater than a threshold value (input by the user), the index is weighted for the intensity of the potential isotope cluster and added to a running score. The mass window is then incremented and a new part of the unknown spectrum is compared to the known cluster, and the process is repeated. In this fashion, each mass spectrum is assigned a total score based on the likelihood that it contains the isotope pattern of interest and on the intensity of that cluster. A relatively high score indicates a high probability of an intense isotope pattern of the correct type being present; a score of zero or a relatively low score indicates either that no cluster is present or that clusters which are present are of very low abundance. Because the threshold and weighting

factors in the program are variable, the scores only have meaning in a relative sense. Scores calculated on one g.c./m.s. data set and based on one set of threshold and weighting factors must not be compared with scores calculated by using a different set of values for threshold and weighting factors.

To test the program on a large data set with many different compound types, the EPA/NIH Mass Spectral Data Base [28] was used. This library contains 31,579 reference mass spectra, including 168 spectra of compounds containing selenium. Of these 168, 22 were found to be unsuitable because of spectral abbreviation or errors; and these 22 were not considered in subsequent analyses. Full spectra (not abbreviated) were used in all searches. The time required for spectral searching varied with the complexity of the spectrum, but the first 10,000 library spectra were searched in about 35 min, or at a rate of just under 300 spectra per minute.

Samples and equipment

Amino acids (including seleno-amino acids) and mixtures of amino acids were purchased from commercial sources and used without further purification. An amino acid standard mixture containing 250 nmol of each amino acid was dissolved in 0.25 ml of pyridine and 0.50 ml of bis(trimethylsilyl)-trifluoroacetamide (BSTFA), and heated at 60°C for 30 min. A portion (1–2 μ l) was injected onto the g.c. column. The g.c. column was a 25 m (0.25 mm i.d.) fused silica capillary column coated with SE-30 (J & W Scientific, Rancho Cordova, CA). Splitless injection at 250°C was used. The g.c. oven was temperature-programmed from 70 to 300°C at 8°C min⁻¹. The entire effluent (ca. 1 ml min⁻¹ He) was introduced into the m.s. ion source via a transfer line maintained at 275°C. The ion source was kept at 200°C. Ionization with 70-eV electrons was used.

RESULTS

The isotope pattern of selenium is very distinctive. Six naturally-occurring stable isotopes are spread over a range of nine daltons, with ⁸⁰Se being the most abundant [⁷⁴Se (0.9%), ⁷⁶Se (9.0%), ⁷⁷Se (7.6%), ⁷⁸Se (23.5%), ⁸⁰Se (49.8%), ⁸²Se (9.2%)]. The mass spectrum of any compound which contains selenium should show a pattern of ions of the same relative intensities. The mass spectrum of 2-(methylseleno)furan [28] clearly exhibits selenium isotope clusters beginning at m/z 87 (CHSe), m/z 113 (C₃H₃Se), m/z 127 (C₄H₅Se), m/z 141 (C₄H₃OSe), and m/z 156 (C₅H₆OSe). The computer assigned these clusters similarity indices of 0.81, 0.89, 0.81, 0.94, and 0.96, respectively, indicating good matches. When the weighting and intensity factors were included, this mass spectrum received a score of 36 100, a very high value. In contrast, the mass spectrum of cholesterol, a molecule which does not contain selenium, received a score of zero, because no part of the spectrum had a similarity index (relative to the known selenium pattern) of greater than 0.1.

An adjustable threshold value for the similarity indices, set by the user, controls the selectivity of the algorithm. Spectral fragments which have a similarity index less than this threshold are rejected as being unlikely to contain selenium. A high threshold value, 0.9 for example, results in the rejection of nearly all non-selenium-containing compounds, but also rejects many selenium compounds with mass spectral patterns that are weak or slightly distorted. A lower threshold, 0.7, locates most selenium compounds, but also finds many "false positive" results. Table 1 shows the effect of varying the threshold value on the number of compounds "found", i.e., those compounds assigned a non-zero total score. In practice, it was found empirically that a threshold of 0.85 is a useful value. The Mass Spectral Data Base includes mass spectra of variable quality from a wide range of instruments and conditions. When the isotope cluster-locating program was used on data generated on the instrument used here, it was found, as expected, that its performance was substantially better, both in terms of higher similarity indices for selenium clusters, and in rejection of non-selenium compounds.

Even among the non-zero scores, the magnitude of the score gives some indication of the likelihood of a selenium cluster pattern being present. At a threshold level of 0.8, selenium-containing compounds with mass spectra that received non-zero scores had an average score of 19,000. Non-selenium-containing compounds with mass spectra that received non-zero scores had an average score of 1800 (Table 1). The reasons for both positive interferences (non-selenium compounds receiving non-zero scores) and negative interferences (selenium compounds receiving low or zero scores) are discussed below.

In the earlier work with chlorine-selective detection, it was frequently found necessary to search for the isotope patterns caused by multiple chlorine atoms in the same molecule or fragment [24]. With selenium, this was not found to be the case. One reason is that multiple selenium atoms are relatively unusual. Only 43 compounds out of the 31 579 mass spectra in the data base have more than one selenium atom; only 4 out of 31 579 have more than two selenium atoms. The vast majority of the compounds containing multiple selenium atoms showed, in their mass spectra, fragment ions containing a single selenium atom, and hence a clear selenium isotope pattern.

TABLE 1

Effect of similarity index threshold on the results of a selenium search of the mass spectral data base [28]

Threshold	Se cpds. with score > 0 ^a	Average score		Non-Se cpds. with score > 0 ^b	Average score	
		All Se cpds.	Se cpds. with scores > 0		All non-Se compounds	Non-Se c with scor
0.9	54 (37%)	7175	19400	3 (0.01%)	10 ⁻²	40
0.8	120 (82%)	16000	19000	546 (2%)	30	1800
0.7	138 (95%)	18000	19000	2906 (9%)	213	2300

^aNumber of compounds (and %) from a total of 146. ^bNumber of compounds (and %) from a total of 31 411.

Consequently, these spectra were located by the computer. While a search for Se_2 patterns could have been conducted, it would have nearly doubled the search time of the program, and so it was not considered necessary at this time.

To demonstrate how the program would perform in the reduction of mass spectral data, a mixture of amino acids, including selenomethionine, was prepared. When the mixture was trimethylsilylated (TMS) and analyzed by g.c./m.s., the total ion chromatogram (Fig. 1b) showed a large number of peaks; over 300 mass spectra were collected in the 17-min experiment. The selenium-selective program was directed to search through the 300 spectra, seeking those which displayed a selenium isotope-cluster pattern. Each mass spectrum was assigned a score (as described under Experimental); a plot of these scores vs. scan number produced the selenium-selective chromatogram (Fig. 1a). Only one peak, at scan 157, is indicated as likely to contain selenium clusters. The mass spectrum of this compound is shown in Fig. 2. It is from the di-TMS derivative of selenomethionine; and it clearly shows the selenium isotope clusters beginning at m/z 218 and m/z 335. Searching for the selenium clusters is quite fast; the 300 spectra were searched in just under 2 min.

DISCUSSION

The availability of computerized instrument systems has led to data-collection rates that far exceed the rate at which data can be interpreted. In an effort to limit the amount of data which must be interpreted in a large data set, computer software is being developed here [23–26] that will sort through the data set, seeking spectral features described by the operator, a process of selective data reduction. The selenium-selective “detector” described herein is intended to be an aid to, not a replacement for, the human

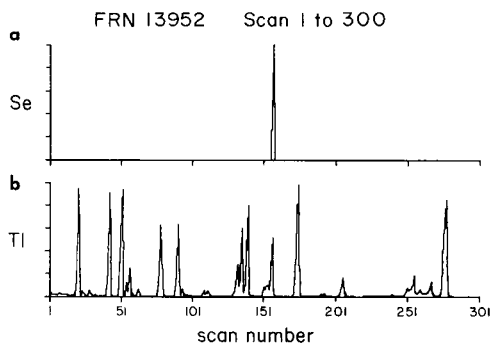


Fig. 1. Selenium-selective chromatogram (a) and total ionization profile (b) from the g.c./m.s. of the mixture of trimethylsilylated amino acids.

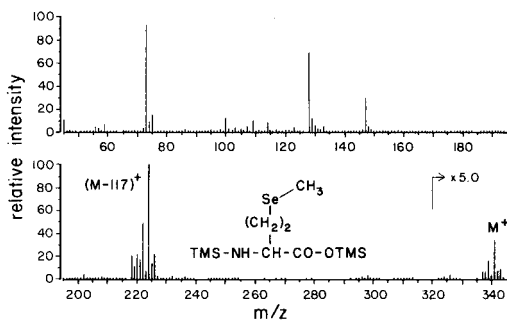


Fig. 2. Mass spectrum of *N,O*-di(trimethylsilyl)selenomethionine.

interpreter. Its function is to sort quickly through large numbers of mass spectra and indicate which of these are the most likely to contain selenium isotope clusters.

The sensitivity and detection limit of this system are, of course, a function of the mass spectrometer used. The entire mass range must be continuously scanned, because one does not know at the start of the experiment where the selenium clusters may occur. This mode of operation is less sensitive than the single-ion monitoring mode, but is more generally useful when dealing with unknowns, because of the large amount of spectral data collected. The sensitivity of the mass spectrometer can often be improved by utilizing chemical-ionization or negative-ion detection. The spectral data from these operation modes can also be analyzed by the isotope-cluster searching program, provided only that enough ions are counted to exhibit a statistical distribution of the selenium isotopes. Finally, because the g.c./m.s. data are unchanged by the search, the same data can be subjected to manual interpretation, library searching, other isotope cluster searches, or any other procedures for data handling.

The selectivity of this selenium detection system must be assessed somewhat differently than other element-selective detectors. For most element-selective detectors, the selectivity can be defined as "the ratio of the detectability of a potentially interfering substance to the detectability of a desired substance" [29]. This definition cannot be used in the present situation. If the similarity indices of all spectral fragments of a potentially interfering substance fall below the threshold value, the mass spectrum is assigned a score of zero. Increasing the concentration of the substance does not change the mass spectrum (within the limits of the instrument), so the similarity indices will not change and the score will remain zero. This would correspond to a selectivity factor, as defined above, which approaches infinity. A more meaningful indicator of selectivity might be to compare the average score assigned to all mass spectra in the data base which arise from compounds that contain selenium to that of mass spectra which do not contain selenium

(Table 1). At the 0.8 threshold level, this would correspond to a selectivity factor of about 500 (16000/30). As the threshold is set higher, the selectivity increases, although many selenium compounds are missed. If the threshold is set lower, the selectivity predictably decreases.

The selectivity of the proposed method serves a somewhat different function than that of conventional element-selective detectors. In those techniques, data are collected or rejected based on the selectivity of the device. In our method, all data are collected, regardless of whether or not selenium is present. The selectivity factor gives some indication of the degree of data reduction. If the analyst uses a set of search parameters that makes the selectivity too low, the search can be repeated with different search parameters on the same data set, without re-analyzing the sample. In any event, the search results are intended to be reviewed and further interpreted by the operator, so that the final decision about the presence or absence of selenium can be made by the investigator, not by the instrument.

Four types of interferences were identified in the work with chlorine-selective detection [24], as well as in the current selenium work. Type I interferences are selenium compounds with mass spectra, under the conditions used to obtain the spectra, that do not show the expected selenium cluster pattern. This can occur either because the fragment ions containing selenium are very weak or because there is a distortion of the cluster pattern by overlapping fragment ions. For example, 2-methylbenzoselenophene readily loses a proton under electron bombardment, giving rise to a $(M - 1)^+$ ion in the mass spectrum [28]. The $(M - 1)^+$ pattern overlaps with the M^+ pattern, resulting in a complex pattern not immediately recognizable as a selenium isotope cluster. In general, these spectra are not located by the program and thus constitute a negative interference. Type II interferences are selenium compounds which also contain other elements that have distinctive isotope patterns, e.g., bromine. It may happen that in the fragmentation of the molecule, the selenium and bromine remain together and so the isotope patterns always overlap. The distorted isotope pattern may be recognized by the computer, but assigned a lower similarity index; or if the pattern is sufficiently distorted, the computer may not recognize it at all. This again is a negative interference. Fortunately, it does not occur often. The isotopes of carbon, hydrogen, oxygen, nitrogen, sulfur, and silicon do not distort the selenium isotope pattern sufficiently to make it unrecognizable to the computer. The mass spectrum in Fig. 2, because it results from a TMS derivative, shows isotope patterns of selenium distorted by the presence of silicon; but the distortion is minor, and the spectrum is still easily located by the computer. Chlorine and bromine cause more serious interference, but frequently, in the fragmentation of these molecules, the selenium is observed in fragments without the halogen; and in those fragments, the selenium isotope cluster is clear and readily located. The Type II interferences are also negative interferences, resulting in the program missing a compound that does, in fact, contain selenium. It is

interesting to note that the Type I interference, because it occurs as a result of fragmentation, could be suppressed by using a softer ionization technique such as chemical ionization, in which fragmentation is suppressed. The Type II interference will be present in both electron-impact and chemical-ionization techniques.

Two other types of interference cause non-selenium-containing compounds to be detected, and so provide positive interferences. Type III interferences result when, because of a particular fragmentation pathway, a cluster of ions is formed which looks similar to the selenium pattern. The likelihood of this happening is a function of the isotope pattern being sought. Obviously, a simple pattern like that from a single chlorine atom (a doublet in a ratio of 3:1) will occur more often by chance than will a relatively complex pattern like that of selenium. Still, the majority of the false positives found by the selenium-selective detector was of this type. Type IV interferences occur when a molecule contains an element or combination of elements with an isotope pattern that resembles that of selenium. This occurrence is rare with a pattern as complex as that of selenium, and no examples were observed in the 31 579 mass spectra in the data base used in this study. It should be noted again that the Type III interferences, which result from fragmentation, would be suppressed by soft ionization techniques, while the Type IV interferences would not.

The technique of selenium-selective detection has great potential for studying selenium metabolism and biochemistry. One can take advantage of the high information content of the mass spectrometer as a chromatographic detector, without having to conduct a laborious scan-by-scan manual search for selenium isotope clusters. Metabolites of selenium compounds and side-products of derivatization reactions can easily be located in the chromatograms without the incorporation of radioactive selenium. In the field of protein structure determination, mass spectrometry is playing an increasingly greater role; a wide range of derivatives is now available for g.c./m.s. and l.c./m.s. of peptides [30]. The methods described here can, in principle, be applied to any mass spectral data set for rapid location of the selenium-containing species.

The author acknowledges Jacques LaBrosse for helpful discussions and the National Science Foundation for financial support (Grant CHE-8209056).

REFERENCES

- 1 National Academy of Sciences Committee, Selenium, National Academy of Sciences Publishing Office, Washington, D.C., 1976.
- 2 T. C. Stadtman, *Science*, 183 (1974) 915.
- 3 M. A. Montsenbocker and A. L. Tappel, *Biochim. Biophys. Acta*, 719 (1982) 147.
- 4 J. E. Cone, R. Martin del Rio, J. N. Davis and T. C. Stadtman, *Proc. Nat. Acad. Sci. U.S.A.*, 73 (1976) 2659.
- 5 K. P. McConnell, R. M. Burton, T. Kute and P. J. Higgins, *Biochim. Biophys. Acta*, 588 (1979) 113.

- 6 M. A. Motsenbocker and A. L. Tappel, *Biochim. Biophys. Acta*, 709 (1982) 160.
- 7 I. Yamamota, T. Saiki, S.-M. Liu and L. G. Ljungdahl, *J. Biol. Chem.*, 258 (1983) 1826.
- 8 A. J. Wittwer, *J. Biol. Chem.*, 258 (1983) 8637.
- 9 J. A. Milner, H. J. Thompson, S. E. Martin, M. Schillaci, H. H. Draper, R. P. Bird, C. K. Chow and G. C. Gaiola, *J. Agric. Food Chem.*, 32 (1984) 421.
- 10 R. A. Condell and A. L. Tappel, *Biochim. Biophys. Acta*, 709 (1982) 304.
- 11 R. J. Kraus, S. J. Foster and H. E. Ganther, *Biochem.*, 22 (1983) 5853.
- 12 H. J. M. Bowen and P. A. Cawse, *Analyst (London)*, 88 (1963) 721.
- 13 R. Hasunuma, T. Ogawa and Y. Kawanishi, *Anal. Biochem.*, 126 (1982) 242.
- 14 S. Dilli and I. Sutikno, *J. Chromatogr.*, 300 (1984) 265.
- 15 D. C. Reamer and C. Veillon, *Anal. Chem.*, 53 (1981) 2166.
- 16 E. de Oliveira, J. W. McLaren and S. S. Berman, *Anal. Chem.*, 55 (1983) 2047.
- 17 C. De Marco, P. Cossu, S. Dernini and A. Rinaldi, *J. Chromatogr.*, 129 (1976) 369.
- 18 A. Rinaldi, P. Cossu and C. De Marco, *J. Chromatogr.*, 120 (1976) 221.
- 19 W. R. McLean, D. L. Stanton and G. E. Penketh, *Analyst (London)*, 98 (1973) 432.
- 20 D. L. Windsor and M. B. Denton, *Anal. Chem.*, 51 (1979) 1116.
- 21 D. Chakraborti, D. C. J. Hillman, K. J. Irgolic and R. A. Zingaro, *J. Chromatogr.*, 249 (1982) 81.
- 22 D. O. Duebelbeis, S. Kapila, S. E. Manahan and C. R. Vogt, *Pittsburgh Conference for Analytical Chemistry, Atlantic City, 1984*, paper 329.
- 23 R. J. Anderegg, *Anal. Chem.*, 53 (1981) 2169.
- 24 J. L. LaBrosse and R. J. Anderegg, *J. Chromatogr.*, 314 (1984) 83.
- 25 J. L. LaBrosse and R. J. Anderegg, *J. Chromatogr.*, 314 (1984) 93.
- 26 R. J. Anderegg, *J. Chromatogr.*, 275 (1983) 154.
- 27 H. S. Hertz, R. A. Hites and K. Biemann, *Anal. Chem.*, 43 (1971) 681.
- 28 S. R. Heller and G. W. A. Milne (Eds.), *EPA/NIH Mass Spectral Data Base*, US Government Printing Office, Washington, D.C., 1978.
- 29 C. H. Hartmann, *Anal. Chem.*, 43 (1971) 113A.
- 30 K. Biemann, in G. R. Waller and O. Derner (Eds.), *Biochemical Applications of Mass Spectrometry*, 1st Supplemental Volume, Wiley-Interscience, New York, 1980, pp. 469–525.

SENSITIVE FLOTATION-SPECTROPHOTOMETRIC DETERMINATION OF GOLD BASED ON THE GOLD(III)/BROMIDE/RHODAMINE 6G SYSTEM

Z. MARCZENKO* and K. JANKOWSKI

Department of Analytical Chemistry, Technical University, 00-664 Warsaw (Poland)

(Received 25th February 1985)

SUMMARY

A very sensitive flotation-spectrophotometric method based on the gold(III)/bromide/tribromide/rhodamine 6G (R6G) system is described. In the presence of bromine, the dibromoderivative (R6G-Br) is formed. The gold compound is floated with di-isopropyl ether, washed with 1 M sulphuric acid and dissolved in methanol, and the absorbance of the solution is measured at 545 nm. The floated compound is considered to be an adduct of the formula $[(R6G-Br^+)(AuBr_4^-)] \cdot 12[(R6G-Br^+)(Br_3^-)]$. The apparent molar absorptivity is $11.4 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$. Important interferences are Pd, Ag, Hg(II) and Tl(III), belong to the most interfering metals; after a preliminary extraction of gold from acidic bromide medium into di-isopropyl ether, only thallium(III) interferes significantly. The proposed method is applied to determining gold traces in a blister copper sample. Results obtained prove the good precision and accuracy of the method.

Sensitive flotation-spectrophotometric methods for the determination of all six platinum metals have been described [1–7]. The methods are based on ion-associates of basic dyes with polyvalent anionic complexes of these metals. Their high sensitivity is due to the fact that the compound floated contains more than one monovalent dye cation per atom of the metal determined. In some cases, the sparingly soluble compounds are not simple ion-associates, but adducts composed of an ion-associate of the polyvalent metal-containing anion and of an ion-associate of the dye with another anion present in the system [4, 5, 7].

The development of similar methods for determining gold was then investigated. It proved to be impossible to find a polyvalent anionic complex of gold that would associate with more than one dye cation. Nevertheless, the participation of monovalent anionic complexes was successful, with AuI_2^- in the iodide system, and $AuBr_4^-$ in the bromide system. The simple (1:1) ion-pair of gold with a suitable basic dye is precipitated and floated as a relatively stable adduct with the ion-pair (salt) of the basic dye with triiodide or tribromide ions. The Au(I)/iodide/triiodide/methylene blue system has been reported [8]. Here, a very sensitive method based on the system Au(III)/bromide/tribromide/rhodamine 6G is described.

EXPERIMENTAL

Reagents and apparatus

Gold standard solution (1 mg Au ml^{-1}) was prepared by dissolving the pure metal in aqua regia, evaporating the solution almost to dryness on a water bath, adding 4 ml of concentrated hydrochloric acid, evaporating to half the volume and diluting with water in a volumetric flask to 100 ml. Dilution with 0.1 M hydrochloric acid was used as required.

Rhodamine 6G (R6G; BDH Chemicals) was used as a $5 \times 10^{-4} \text{ M}$ (ca. 0.02%) solution, potassium bromide as 0.3 M (ca. 3.5%) and 4 M (ca. 50%) solutions, and potassium bromate as a $1 \times 10^{-2} \text{ M}$ (ca. 0.2%) solution.

A Specord u.v.-visible recording spectrophotometer and a VSU2-P spectrophotometer with 1-cm cells were used.

Procedures

Flotation and measurement of gold. To the acidic solution (sulphuric acid), containing not more than $3 \mu\text{g}$ of gold, in a separatory funnel (carefully degreased), add 1 ml of the 0.3 M bromide, 2.5 ml of the bromate solution and 2 ml of the R6G solution, mixing carefully. The final sulphuric acid concentration should be about 1 M in 20 ml of aqueous phase. Shake the solution with 10 ml of di-isopropyl ether for about 1 min. Discard the aqueous phase carefully and wash the ether and the separated compound by shaking with two successive 20-ml portions of 1 M sulphuric acid for about 1 min each. Discard both liquid phases, and dissolve the remaining precipitate with methanol. Transfer the solution to a 25-ml volumetric flask and dilute to the mark with methanol. Measure the absorbance at 545 nm against a reagent blank prepared in the same way.

Determination of gold in copper. For the extractive separation of gold, dissolve 0.5–1 g of the sample in 20 ml of aqua regia and evaporate until the residue is just moist. Repeat the operation after adding 10 ml of concentrated hydrochloric acid. Dissolve the residue in 20 ml of 6 M hydrochloric acid. Transfer the solution to the separatory funnel, add 4 ml of the 4 M bromide solution, and dilute with water to 30 ml. Shake with two successive 5-ml portions of di-isopropyl ether for 2 min each. Wash the combined extracts with 10 ml of a solution 2 M in sulphuric acid and 0.2 M in bromide by shaking for 2 min. Discard the aqueous phase.

To determine the gold in the ether phase, add 10 ml of 2 M sulphuric acid, 1 ml of the 0.3 M bromide, 2 ml of the bromate solution, and 2 ml of the R6G solution. Dilute the aqueous phase to 20 ml with water and shake the liquid phases for 1 min. Then wash and dissolve the precipitate as described above.

RESULTS AND DISCUSSION

Preliminary studies

In the acidic (ca. 1 M sulphuric acid) bromide medium, gold(III) forms AuBr_4^- . In the presence of a suitable basic dye, a precipitate is formed and

floats when shaken with a slightly polar solvent. This precipitate consists of the $[(\text{dye})(\text{AuBr}_4)]$ ion-pair and the dye bromide salt. The separated compounds decompose when the precipitate is washed, and dissolve in the aqueous phase. In the presence of bromine (which forms tribromide with the bromide present), the gold compound which is formed and floated does not decompose when the co-precipitated excess of the dye is washed out.

Benzene, toluene, xylene, cyclohexane and di-isopropyl ether were examined as the solvent, but only the ether was useful. The other solvents extracted ion-pairs or free bromine. The quality of the di-isopropyl ether used was important; it should not contain peroxides. More than ten xanthene, triarylmethane and azine basic dyes were examined. Only rhodamine 6G was fully satisfactory. With some dyes (methylene blue, Nile blue A) the formed compounds decomposed during washing.

The gold(III)/bromide/rhodamine 6G system

From a medium containing dilute sulphuric acid, bromide and bromine, rhodamine 6G produces a precipitate which, on shaking with di-isopropyl ether, accumulates at the phase boundary. The gold (as tetrabromoaurate) present in the aqueous phase is quantitatively separated as a compound with the dye, apart from the tribromide salt of the dye.

Maximum and reproducible results were obtained when the concentration of bromine, produced by the reaction of bromate with bromide, was relatively high. It should be present in a mole ratio of ca. 50:1 compared to the dye. In the presence of a considerable excess of bromine, rhodamine 6G changes its colour; there is a bathochromic shift of ca. 20 nm in the wavelength of maximum absorbance. Under these conditions, the bromination of the dye occurs.

The optimum concentrations of sulphuric acid, bromide, bromate and rhodamine 6G, as well as the shaking time and number of washings, were investigated. Figure 1 shows the influence of the sulphuric acid concentration on the formation and flotation of the gold compound. Maximum absorbance was obtained in the range 0.5–1.5 M sulphuric acid. Below 0.5 M acid the formation of bromine is slow. At higher acidity, the concentration of the monovalent dye cation decreases in favour of the protonated forms. Figure 2 presents the effect of bromide and bromate concentrations. For the range $1.0\text{--}1.5 \times 10^{-3}$ M bromate, the concentration of bromide should be $1.0\text{--}2.5 \times 10^{-2}$ M. Figure 3 shows the dependence of the absorbance on the rhodamine 6G concentration. For the concentration range $4\text{--}6 \times 10^{-5}$ M, results were reproducible, with a suitably small reagent blank. Further increase in the concentration of the dye increased the reagent blank.

Dilute sulphuric acid (ca. 1 M) was most suitable for washing the gold compound. Two portions of this acid ensured that the free dye was largely removed (the blank value was about 0.06), and that the results were reproducible. Further washing partly decomposed the separated gold compound.

In both flotation and washing, equilibrium was reached after shaking for

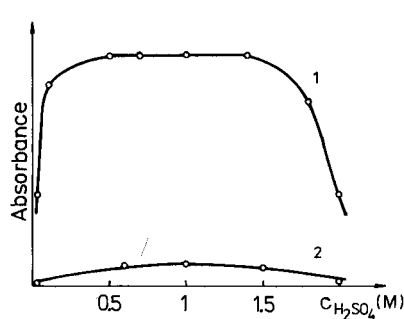


Fig. 1. Effect of the sulphuric acid concentration on the formation and flotation of the gold compound: (1) absorbance of the gold compound solution against the blank; (2) blank against methanol.

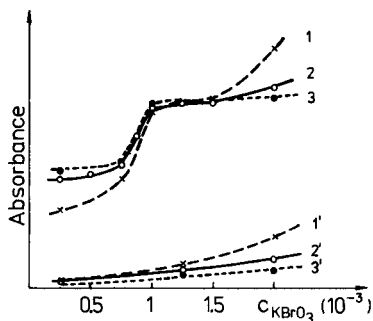


Fig. 2. Dependence of the absorbance of the methanolic solution of the gold compound on the bromide and bromate concentrations. Bromide concentration: (1) 2.5×10^{-3} M, (2) 1.5×10^{-3} M, (3) 1×10^{-3} M (absorbance measurements against the blank). Blank values (1', 2', 3') measured against methanol.

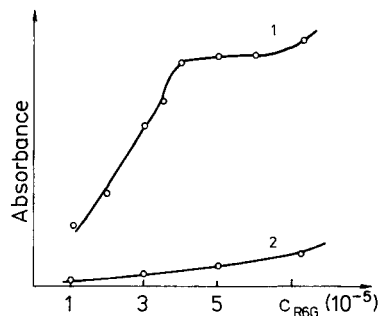


Fig. 3. Effect of the rhodamine 6G concentration on the formation and flotation of the gold compound (1 M H_2SO_4 during flotation): (1) absorbance of the gold compound solution against the blank; (2) blank against methanol.

Fig. 4. Spectrophotometric titration of 1×10^{-6} mol of rhodamine 6G with bromine. Absorbance measurements at 545 nm in water/methanol (90%) solutions against methanol.

about 1 min. The washed precipitate dissolved readily in both methanol and acetone; methanol was preferred. During flotation and washing of the precipitate, the separatory funnel should be kept from direct sunlight, which reduced the absorbances significantly. The sulphuric acid medium seems to be optimal. In the presence of large quantities of chloride, part of the gold may be present as mixed chloride/bromide complexes.

Composition of the floated gold compound

In order to investigate the composition of the floated compound, it was necessary to establish the chemical composition of brominated rhodamine

6G. A solution of rhodamine 6G in 1 M sulphuric acid was treated with bromine; Aqueous solutions with a constant quantity of R6G were mixed with solutions of bromide (in excess) and different amounts of bromate, and the aqueous pseudosolution containing the precipitate of the dye salt was diluted (1 + 9) with methanol. At the wavelength of maximum absorption for brominated R6G (545 nm), the absorbance of the R6G was small. Figure 4 shows that two molecules of bromine react with one molecule of the dye. Accordingly, the product is a dibromo derivative of rhodamine 6G; this was confirmed by elemental analysis (C, H, N, Br).

The molar absorptivity for methanolic solutions of the brominated dye (R6G-Br) was about 9×10^4 at 545 nm. Given the molar absorptivities of the floated complex and of the dye, it can be calculated that the separated gold compound contains about 13 molecules of the dye to one gold atom. At such a high mole ratio of R6G-Br to gold, it is difficult to confirm the composition by other methods (e.g., the Bent-French method, elemental analysis or radiometry). It seems likely that the separated compound is an adduct, with the formula $[(\text{R6G}-\text{Br}^+)(\text{AuBr}_4^-)] \cdot 12[(\text{R6G}-\text{Br}^+)(\text{Br}_3^-)]$.

Determination of gold

Under the optimal conditions as outlined in the Procedures (1 M sulphuric acid, 1.5×10^{-2} M bromide, 1.2×10^{-3} M bromate and 5×10^{-5} M R6G), the system obeyed Beer's law in the range 0.2–3 μg of gold. For higher gold concentrations, a negative deviation was observed. The molar absorptivity was $11.4 \times 10^5 \text{ l mol}^{-1} \text{ cm}^{-1}$ at 545 nm (specific absorptivity $a = \epsilon/\text{atomic mass} \times 1000 = 5.8$). Table 1 shows the precision and accuracy of the method for three quantities of gold.

The effects of some elements on the determination of gold by the proposed method were examined. The results are shown in Table 2. Significant effects were found for Pd, Ag, Hg(II) and Tl(III). Interferences caused by Pt, Os and Bi were less serious. In the presence of larger amounts of bismuth, its sparingly soluble compound is floated and then dissolves during washing.

The preliminary separation of gold by extraction from acidic bromide

TABLE 1

Statistical evaluation of the results of the determination of gold

Gold (μg)		Standard deviation ^a (μg)	Relative standard deviation (%)	Confidence limits (probability level 0.95)
Added	Found			
0.80	0.83	0.04	5.0	0.83 ± 0.04
2.00	2.05	0.07	3.5	2.05 ± 0.07
3.00	2.93	0.09	3.0	2.93 ± 0.09

^aFor seven determinations.

TABLE 2

Effect of some elements on the determination of gold (1 μ g) by the proposed method

Metal	Mass ratio M: Au	Gold recovery (%)	Metal	Mass ratio M: Au	Gold recovery (%)
Pd	0.2	103 105	Tl	0.2	105 105
	1	127 124		1	125 122
	5	211 218		10	186 192
Pt	10	102 102	In	100	108 105
	50	123 128		500	144 138
	100	186 179		2000	192 200
Ir	10	104 102	Fe	100	107 105
	50	110 112		500	119 122
	100	117 119		2000	171 165
Rh	10	105 107	Pb	100	100 102
	50	119 117		500	115 118
	100	129 126		2000	145 141
Os	1	100 102	Cu	100	100 102
	10	117 120		2000	133 130
	100	290 283		10 000	143 148
Ag	1	109 106	Te	100	102 100
	5	123 123		500	108 106
	10	137 140		2000	114 112
Hg	2	104 102	Bi	20	98 100
	10	124 127		100	90 86
	50	208 213		2000	55 48

solution with di-isopropyl ether [9, 10] was found to be very convenient. This method was re-examined and adapted for the separation of trace amounts of gold. The most effective extraction was achieved with a 4 M hydrochloric acid/0.5 M potassium bromide mixture (see Procedures). Washing the extract removed chloride and some interfering metals, e.g., iron.

The preliminary extraction of gold increases significantly the selectivity of the method. Only thallium(III), which undergoes partial extraction and flotation as the ion-associate, still interferes. There was no interference from 200-fold amounts of In, 1000-fold amounts of Ir, Rh, Pt, Pd, Os, Ag, Hg, or Fe, or 10 000-fold amounts of Cu, Pb, Bi, or Te.

The method developed was applied to the determination of gold in blister copper with the results shown in Table 3. The recovery of the gold added was 93–96%. The results were calculated by using a standard curve prepared on the basis of gold solutions with known amounts of Cu, Pb, Ag and Fe similar to those contained in the analyzed copper sample.

Conclusions

The above results show that it is possible to produce very sensitive flotation-spectrophotometric methods based on a simple (1:1) ion-pair of the

TABLE 3

Determination of gold in blister copper

Sample weight (g)	Gold added (μg)	Gold found (μg)	Au content in copper ^a ($\times 10^{-5}\%$)
1.00	—	1.00, 1.08, 1.12	10.67
0.50	—	0.54, 0.52, 0.48	10.23
1.00	1.00	2.02, 1.94, 2.14	10.83
0.50	1.00	1.52, 1.48, 1.46	10.63

^aEach result listed is the mean from the 3 results given in the third column. Statistical data: $\bar{x} = 10.6 \times 10^{-5}\%$, $s = 0.65 \times 10^{-5}\%$, $s_r = 6.1\%$, $\mu_{0.95} = (10.6 \pm 0.43) \times 10^{-5}\%$.

metal determined provided that it is accompanied by the formation of an adduct with the dye salt molecules. A gold adduct having a very advantageous (from the analytical point of view) dye:metal ratio was obtained. This spectrophotometric procedure may be considered as a kind of amplification method.

It is most unusual to obtain such good precision as is shown in Table 1 on the basis of such a complex adduct (M:BD = 1:13). The sensitivity of the proposed method is many times higher than that of any previous spectrophotometric method for determining gold [1, 10].

This work was supported by Research Program MR-I-32.

REFERENCES

- 1 Z. Marczenko, *CRC Crit. Rev. Anal. Chem.*, 11 (1981) 195.
- 2 Z. Marczenko and J. Uścińska, *Microchem. J.*, 26 (1981) 453.
- 3 Z. Marczenko and M. Jarosz, *Analyst*, 106 (1981) 751.
- 4 Z. Marczenko, M. Balcerzak and H. Pasek, *Mikrochim. Acta*, 2 (1982) 371.
- 5 Z. Marczenko and K. Kalinowski, *Anal. Chim. Acta*, 144 (1982) 173.
- 6 Z. Marczenko and K. Kalinowski, *Mikrochim. Acta*, 2 (1983) 169; *Anal. Chim. Acta*, 153 (1983) 219.
- 7 K. Kalinowski and Z. Marczenko, *Mikrochim. Acta*, 1 (1985) 167.
- 8 Z. Marczenko and K. Jankowski, *Talanta*, 32 (1985) 291.
- 9 E. B. Sandell and H. Onishi, *Photometric Determination of Trace Metals, General Aspects*, Interscience, New York, 1978.
- 10 Z. Marczenko, *Spectrophotometric Determination of Elements*, Ellis Horwood, Chichester, 1976.

SHORT-TERM STABILITY OF TRACE METALS IN ESTUARINE WATER SAMPLES

J. A. CAMPBELL*, M. J. GARDNER and A. M. GUNN

WRc Environment, Medmenham Laboratory, Henley Road, Medmenham, P.O. Box 16, Marlow, Bucks. SL7 2HD (Great Britain)

(Received 12th April 1985)

SUMMARY

The short-term (up to 24 h) stability of estuarine water with high (22‰) and low (<1‰) salinity, stored unacidified in polythene containers prior to filtration, preservation and determination of dissolved trace metals, was assessed. For the low-salinity sample a massive (140%) increase in copper concentration was observed, indicating desorption from the particulate phase; a similar, although less pronounced (11%) effect was found for the high-salinity sample. Evidence was also obtained for a significant loss (30%) of lead from a pre-filtered low-salinity sample, suggesting appreciable adsorption to the container walls. For cadmium and nickel, no substantial changes in concentration were observed.

The separation of “dissolved” and “particulate” fractions is fundamental to many studies of trace metals in natural waters. This is often achieved by filtration of a sample through a filter membrane. The desire to manipulate the sample in an environment in which the likelihood of contamination can be adequately controlled makes it preferable to conduct the filtrations in the laboratory. However, the possibility that important changes in dissolved and particulate fractions may occur if filtration is delayed must not be overlooked. This paper describes work undertaken to assess the changes that can occur in samples from one particular estuary over a time period of ca. 24 h. This storage time corresponded to that between sample collection and subsequent filtration in a laboratory for a survey of metals concentrations in the estuary of the river Mersey in Northwest England. The objectives of the tests described here were twofold: (1) to make a direct assessment of any losses of dissolved metal by adsorption to the sample containers used in the survey; and (2) to evaluate (given the information from (1)) changes in the proportions of dissolved and particulate metal during storage.

EXPERIMENTAL

Apparatus

All apparatus which was to come into contact with the samples was acid-washed and thoroughly rinsed in deionised water. The filtration equipment

used was the Sartorius SM-16510 polycarbonate filter unit with Millipore HAWP04700 (47-mm, 0.45- μ m) filter membranes. In order to avoid the need to acid-wash and rinse filter units and membranes in the field, it was decided to prepare a sufficient number of units, each with an acid-leached, rinsed filter, before the test. A different unit was used for each filtration. The alternative approach, of not acid-washing the filter unit and/or filter between samples, did not permit the assumption that losses of dissolved metal by adsorption to the filtration apparatus would be comparable for different aliquots of sample. Such adsorptive losses would, if they were not constant, introduce a spurious change in dissolved metal concentration which could not be distinguished from effects of interest.

Sample collection and treatment

Tests were made on samples of both high (22‰) and low (<1‰) salinity. These were collected from two locations on the Mersey estuary by sampling from the bank with a plastic bucket and line. For each of these sample types, two sub-samples (about 4 l each) were prepared at the sampling location: (1) a sub-sample prefiltered through a 0.45- μ m membrane, homogenised and divided for storage in two 2-l polyethylene bottles (low-density polythene, Kartell Plastics) of the type used routinely in survey work; and (2) a sub-sample homogenised and divided for storage in two 2-l polythene bottles without prior filtration. Four 250-ml aliquots of each sub-sample (two from each of the 2-l bottles) were filtered, immediately after preparation (to establish an initial sample composition at time designated t_0) and subsequently after intervals of about 1 h, 4 h and 24 h (times t_1 , t_2 and t_3 , respectively). The filtrate of each 250-ml aliquot was collected in a vessel containing nitric acid as a preservative to give a final concentration of 0.5% (v/v), i.e., pH $\approx$ 1.5. Preserved filtered samples were transferred to 250-ml polythene bottles. Eight field blank samples of deionised water were filtered during the course of the experiment.

The 16 samples, comprising preserved 250-ml aliquots from times t_0 to t_3 for a given sub-sample (e.g., high salinity, unfiltered, plus a quarter of the field blanks) were analysed in a single batch by using the method described by Danielsson et al. [1]. Five trace metals, cadmium, copper, lead, nickel and zinc, were determined. Unfortunately, the zinc results were subject to serious contamination, possibly as a result of the filtration operation in the field. No useful conclusions could be drawn concerning the stability of that element.

The second filtration of the previously filtered sub-samples was an attempt to achieve comparability with respect to adsorptive losses during filtration between the two types (filtered and unfiltered) of sub-sample prepared at each sampling location. It is recognised, however, that any coagulation of sub-0.45 μ m particles occurring in the pre-filtered sub-samples, to produce particles retained in the second filtration, would be indistinguishable from adsorptive losses from the dissolved phase. The design of test was based on

a relative standard deviation of approximately 5% for the results. It was calculated, given this precision, that four replicate determinations on each sub-sample for each storage time would give an expectation that a true change in metal concentration of approximately 15% would be detected as statistically significant at the 0.05 probability level. The introduction of additional variability, caused by sampling or contamination in the field would of course degrade the power of the test as would relative standard deviations greater than 5% encountered for metal concentrations near the limit of detection of the method used. Such degradation of the power of test was observed in some cases (see confidence limits in Tables 1 and 2).

RESULTS

Stability test data for the samples of high and low salinity are summarised in Tables 1 and 2, respectively. Estimates of the variances of the differences between the mean concentrations at t_0 and at t_1 , t_2 and t_3 were calculated. Values for maximum possible change in concentration were obtained under the assumption that all four results for each sub-sample were independent. Concentrations of the elements under study in the field blank samples were found to lie in the ranges: 0.005–0.020 $\mu\text{g l}^{-1}$ cadmium, 0.16–0.41 $\mu\text{g l}^{-1}$ copper, 0.13–0.57 $\mu\text{g l}^{-1}$ lead and –0.01–0.30 $\mu\text{g l}^{-1}$ nickel. Detection limits (4.65 standard deviations) for the extraction/atomic absorption spectrometric procedure [1], estimated from three blank determinations made in each of the five batches analysed for the test, were 0.035 $\mu\text{g l}^{-1}$ for Cd, 0.65 $\mu\text{g l}^{-1}$ for Cu, 0.75 $\mu\text{g l}^{-1}$ for Pb and 0.50 $\mu\text{g l}^{-1}$ for Ni. Thus, it would not appear that contamination of samples during filtration in the field presented a major problem.

DISCUSSION

Copper

It is appropriate to discuss the data for each element separately. Of the trace elements determined in this investigation, copper showed the most pronounced and, perhaps, the most surprising behaviour. The copper data are summarised in Fig. 1. There was no evidence of a change in the dissolved copper concentrations in the filtered, high-salinity sample (maximum possible change for $p = 0.05$ at 27 h is –13.6%) over the course of the experiment. In the unfiltered sample, a gradual increase of dissolved copper was observed and was significant at t_3 (the mean change with respect to t_0 is 11%). A similar effect was found for the low-salinity sample; there was no significant change in the dissolved copper content of the filtered sample (maximum possible change for $p = 0.05$ at 24 h is –8.5%). A dramatic increase was observed for the unfiltered sample. After one hour, the mean concentration had increased by 21% and at t_3 (24 h) the increase was 142%. The changes at times t_1 , t_2 and t_3 are all highly significant. This enhancement

TABLE 1

Summary of stability test data for the high-salinity sample

Metal/concn. ($\mu\text{g l}^{-1}$)	Change in metal concn. ($\mu\text{g l}^{-1}$) ^a					
	Mean	Max. possible ^b	Mean	Max. possible ^b	Mean	Max. possible ^b
<i>A. High-salinity, filtered sample</i>						
	Time (t_1) = 1.2 h		Time (t_2) = 4.2 h		Time (t_3) = 27 h	
Cd/0.250	-0.012 (-4.8)	-0.035 (-14.1)	0.024 (9.6) ^c	0.047 (18.8)	-0.007 (-2.8)	-0.025 (-10.0)
Cu/4.58	-0.42 (-9.2)	-0.85 (-18.5)	-0.02 (-0.04)	-0.61 (-13.4)	-0.18 (-3.9)	-0.62 (-13.6)
Pb/3.51	-0.27 (-7.7)	-0.81 (-23.1)	-0.39 (-11.1)	-0.96 (-27.3)	-0.30 (-8.5)	-0.68 (-19.4)
Ni/5.95	-0.13 (-2.2)	-0.32 (-5.4)	0.00 (0.0)	0.40 (6.7)	-0.09 (-1.5)	-0.47 (-7.9)
<i>B. High-salinity, unfiltered sample</i>						
	Time (t_1) = 1.1 h		Time (t_2) = 4.7 h		Time (t_3) = 27 h	
Cd/0.233	-0.003 (-1.2)	-0.03 (-12.2)	0.004 (1.7)	0.15 (6.4)	0.011 (4.8)	0.033 (14.3)
Cu/4.41	0.027 (0.6)	0.23 (5.1)	0.15 (3.4)	0.31 (7.1)	0.48 (11.0) ^c	0.64 (14.5)
Pb/2.63	0.15 (5.9)	0.58 (22.0)	0.25 (9.7)	0.70 (26.7)	0.23 (8.6)	0.58 (22.0)
Ni/5.36	0.10 (1.8)	0.23 (4.3)	0.11 (2.0)	0.26 (4.8)	0.08 (1.4)	0.30 (5.6)

^a% changes are given in parentheses. ^bMaximum possible change in concentration ($p = 0.05$). ^cStatistically significant change in concentration.

TABLE 2

Summary of stability test data for the low-salinity sample

Metal/concn. ($\mu\text{g l}^{-1}$)	Change in metal concn. ($\mu\text{g l}^{-1}$) ^a			Mean	Max. possible ^b	Mean	Max. possible ^b	Time (t_3) = 24 h	Mean	Max. possible ^b
	Mean	Max. possible ^b								
A. Low-salinity, filtered sample										
	Time (t_1) = 1.5 h			Time (t_2) = 3.5 h			Time (t_3) = 24 h			
Cd/0.047	-0.007 (-14.4)	-0.019 (-39.8)		-0.001 (-2.7)	-0.012 (-26.5)	-0.008 (-18.1)	-0.019 (-41.3)			
Cu/2.40	-0.05 (-2.3)	-0.19 (-7.8)		0.03 (1.3)	0.21 (8.6)	-0.06 (-2.6)	-0.20 (-8.5)			
Pb/2.11	-0.58 (-27.7) ^c	-1.04 (-49.2)		-0.65 (-30.9) ^c	-1.10 (-52.1)	-0.69 (-32.7) ^c	-1.10 (-52.1)			
Ni/7.63	-0.13 (-1.8)	-0.73 (-9.6)		-0.37 (-4.8)	-1.01 (-13.2)	-0.41 (-5.4)	-1.09 (-14.3)			
B. Low-salinity, unfiltered sample										
	Time (t_1) = 1 h			Time (t_2) = 17 h			Time (t_3) = 24 h			
Cd/0.021	-0.015 (-73.5) ^c	-0.030 (-144)		0.017 (80.7)	0.037 (180)	-0.007 (-32.5)	-0.023 (-110)			
Cu/2.46	0.52 (21.0) ^c	0.74 (30.2)		2.92 (119) ^c	3.16 (129)	3.48 (142) ^c	3.70 (151)			
Pb/1.49	0.10 (6.5)	0.32 (21.8)		0.24 (16.3)	0.86 (58.3)	-0.05 (-3.5)	-0.37 (-25.2)			
Ni/7.62	-0.70 (-9.2)	-1.92 (-25.2)		-0.43 (-5.6)	-1.76 (-23.1)	-0.73 (-9.5)	-2.05 (-27.0)			

^a% changes are given in parentheses. ^bMaximum possible change in concentration ($p = 0.05$). ^cStatistically significant change in concentration.

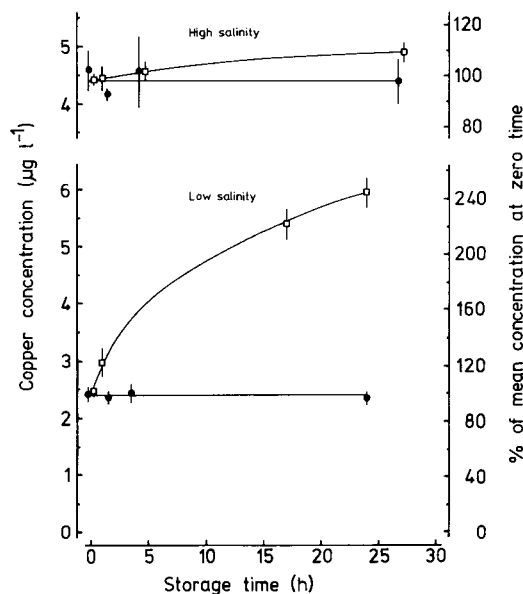


Fig. 1. Dissolved copper versus storage time for filtered and unfiltered samples with high salinity (top) and low salinity (bottom): (○) unfiltered; (●) filtered.

of dissolved copper concentration represents a massive desorption of particulate-bound copper. Such a desorption could reflect the equilibration of particles of riverine origin with brackish water, resulting in release of copper and producing minor changes in pH which could accelerate the desorption over the longer storage periods.

Lead

The susceptibility of dissolved lead in fresh-water samples to adsorptive losses on to container walls and during filtration has been recognised [2]. Moreover, in the marine environment, the chemical behaviour of lead is largely controlled by scavenging by particulate matter [3, 4]. It is therefore not surprising that significant losses of lead were recorded at t_1 , t_2 and t_3 for the low-salinity, filtered sample. At the end of the experiment, the mean percentage loss was 32.7%. It is worth noting that at t_1 , 1.5 hours after collection, a 27% mean loss was recorded. This indicates extremely rapid initial removal of lead from solution. No significant changes were detected for the unfiltered sample, though the power of the test for time t_2 was particularly poor. The insignificant change in lead concentration in the unfiltered sample is not necessarily inconsistent with the losses observed with the filtered sample. Adsorption losses in the unfiltered sample could be compensated by release of lead to solution from the particulate matter. This interpretation for the lead data is, however, very speculative. The mean dissolved lead concentration of the filtered sample at t_0 is $2.11 \mu\text{g l}^{-1}$ compared to $1.49 \mu\text{g l}^{-1}$

in the unfiltered sample. If this difference represents contamination during the initial filtration, then it is possible that the significant losses observed at t_1 , t_2 and t_3 merely represent adsorptive loss of the contaminant lead whilst the equilibrium of the natural lead content is maintained. (At t_3 , the lead concentrations in the filtered sample ($1.43 \mu\text{g l}^{-1}$) and the unfiltered sample ($1.44 \mu\text{g l}^{-1}$) are virtually identical). There were no significant changes in the dissolved lead concentrations of either the filtered or the unfiltered high-salinity sample. The maximum possible change ($p = 0.05$) was ca. -27% (filtered sample) and $+27\%$ (unfiltered sample), in both cases at time t_2 . Adsorptive losses are likely to be inhibited by competition for the active sites by major ions. Again, there is some evidence for possible contamination during the initial filtration of the bulk sample. However, no loss of lead was recorded.

Nickel

No statistically significant changes in dissolved nickel concentration were observed for either the high- or low-salinity samples. In the case of the high-salinity, unfiltered sample, the maximum possible change ($p = 0.05$) in dissolved nickel concentration was $+5.6\%$ at time t_3 . However, the power of the test for the low-salinity, unfiltered sample was poorer, the maximum possible change ($p = 0.05$) in dissolved nickel concentration being -27% at t_3 .

Cadmium

For the high-salinity estuarine water, one significant change was recorded at t_2 (4.2 h) in the filtered aliquot. Over the complete series, however, there was no consistent loss or gain trend. The unfiltered high-salinity aliquot showed no significant deviation from the mean concentration at t_0 . Unfortunately, the mean cadmium concentration of the low-salinity sample was close to the limit of detection for the analytical procedure ($0.035 \mu\text{g l}^{-1}$). The power of the test was therefore much poorer than expected. Clearly, although at t_1 the unfiltered aliquot gave a significant loss, this must be treated with caution, especially as the trend for both filtered and unfiltered samples was extremely erratic through the experiment.

CONCLUSIONS

While the short-term instability of samples of natural waters, with respect to their trace metal content, is recognised by most workers, logistical difficulties in the field often lead to delays between sample collection and filtration and preservation. The above results, however, show conclusively that there is enhancement, during storage, of dissolved copper concentrations in samples of unfiltered estuarine waters whether of high salinity or low salinity. These increases amounted to 10% for the high-salinity sample and 142% for the low-salinity sample. No statistically significant losses of dissolved copper to the container walls were found for either the low- or high-salinity sample.

There was, however, evidence of lead adsorption from filtered, unpreserved low-salinity samples. In the cases of nickel and cadmium, no consistent, significant changes of the dissolved concentrations were discerned in either the unfiltered or filtered sample, at either salinity.

The effects measured in this study may, in principle, be unique for the Mersey estuary (e.g., as a result of specific pollutant loadings on particulate matter). However, the apparent instability of copper in estuarine water samples should be of concern when sampling exercises are planned. Obviously, for reasons of contamination and safety, handling of samples and acidic preservative agents in controlled laboratory conditions is preferable. Nevertheless, if an accurate estimate of, say, dissolved copper concentrations is required (particularly in low-salinity waters) then an appropriate protocol for sample collection and processing should be designed to guard against the generation of seriously biased data. Although, in these experiments, only copper showed clear-cut changes in unfiltered samples, the power of test was not always as high as had been expected. There is clearly a need for further investigations of the stability of unfiltered samples, to cover a wider range of sample types with better statistical discrimination. The results obtained in this study will, it is hoped, provide a stimulus for such further work both in terms of accurately determining the trace metal content of natural waters and also in evaluating the processes which control the equilibria maintaining the partitioning of metals between the "dissolved" and "particulate" phases in the aquatic environment.

We are grateful to Dr. S. C. Warren, Director, WRc Environment for permission to publish this paper.

REFERENCES

- 1 L.-G. Danielsson, B. Magnusson, S. Westerlund and K. Zhang, *Anal. Chim. Acta*, 131 (1982) 63.
- 2 M. J. Gardner and D. T. E. Hunt, *Analyst* (London), 106 (1981) 471.
- 3 B. K. Schaule and C. C. Patterson, *Earth Planet. Sci. Lett.*, 54 (1981) 97.
- 4 F. Elbaz-Poulichet, P. Holliger, W. W. Huang and J.-M. Martin, *Nature* (London), 308 (1984) 409.

SIMPLE AND MIXED LIGAND COMPLEXES OF COPPER(II) WITH POLYFUNCTIONAL PHENOLIC COMPOUNDS AS MODELS OF NATURAL SUBSTANCES

ISABELLE LAMY**, MICHEL SEYWERT, MONIQUE CROMER and
JEAN-PIERRE SCHARFF*

Laboratoire de Chimie Minérale (Chimie Bioinorganique), Formation PIREN (Programme Interdisciplinaire de Recherche sur l'Environnement du CNRS), Université Claude Bernard LYON I, 43 boulevard du 11 Novembre 1918, 69622 Villeurbanne, Cedex (France)

(Received 16th April 1985)

SUMMARY

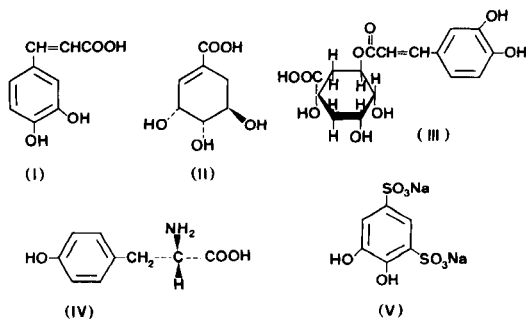
In order to analyse metal complexation with polyfunctional phenolic compounds as ligand models of natural substances, a detailed examination is described for five simple binary complexes and three ternary mixed ligand complexes at 25°C ($\mu = 0.1$ M NaClO₄). The ligands are tyrosine, 4,5-dihydroxy-1,3-benzene disulfonic acid, disodium salt (tiron), 3,4-dihydroxycinnamic acid (caffeic acid), 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid (shikimic acid) and 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate) (chlorogenic acid). The ternary systems are Cu(II)/H_qA/tiron, where H_qA is tyrosine, caffeic or chlorogenic acids. Potentiometric data were used successively to evaluate the protonation of each individual ligand, to detect simple and mixed complexes (including protonated species) and to determine their stability constants (a set of 33 values of constants with several original data is provided). The calculated distribution (speciation) of each species as a function of pH is indicated. Mixed coordination enhances the stability of complexes and the stabilization is expressed in terms of various parameters. The results emphasize that mixed ligand complex formation is essential to studies of multiple equilibria.

In the field of environmental research, the determination of the different individual physicochemical species formed by an element is needed to evaluate most of its properties, and to understand various factors such as the mobilization, the transport mode and the bioavailability of trace metal cations. In contrast to organic pollutants, metals are not biodegradable and once they have entered the geochemical cycle, their reactivity is controlled to a large extent by their speciation [1, 2]. Thus, many studies have shown that the free metal ion (fully hydrated) is more toxic than complexes and species associated with colloidal particles. Humic substances from sediments, soils and natural waters play an important role in nature because of their

**Permanent address: Institut National de la Recherche Agronomique, Station de Science du Sol, route de Saint-Cyr, 78000 Versailles, France.

cation-exchange properties and metal-binding ability [3]. The behaviour of trace metals towards these ill-defined dissolved and particulate natural compounds is very complicated because of the large number of possible interactions [4]. Thus it can be useful to analyse complexation phenomena in complicated natural systems by initially considering simple ligands as models [5]. Many computer programs describing aquatic models are now available for calculating speciation in multimetal/multiligand systems at equilibrium [6–10]. Geochemical modelling is extremely valuable in predicting physico-chemical trends under changing conditions. But, of course, any model is only as good as the equilibrium constants used, as well as the preconceived ideas of the modellers in their particular description of a system. Extensive compilations of thermodynamic data for complexes have been published [11, 12], but most of these data relate to simple homogeneous ligand complexes. However, mixed ligand complexation is expected to be favoured statistically and will likely dominate in multiligand systems [13, 14]. Buffle [15] showed that taking into account the possible formation of such mixed species, leads to better agreement between the results of various authors concerning the association of copper(II) with fulvic substances. New concepts (such as the stability function concept [14]) describing the binding of a metal ion in a ligand mixture need reliable data on the formation of mixed ligand complexes. Recently Gamble et al. [16] pointed out that studies of metal complexation by humic compounds must include metal/multiligand equilibria. All these statements emphasize the importance of producing more data on the stability constants of mixed ligand complexes.

This paper is concerned with a study of simple and mixed complexation of copper(II) with five polyfunctional ligands as models of natural compounds. In a previous investigation [5], caffeic acid (I), a constituent of plants (probably only in conjugated forms such as chlorogenic acid), was chosen, because its carboxyl and phenolic hydroxyl groups mimic several naturally occurring organic substances. In the same way, shikimic acid (II) is a major biosynthetic precursor; metabolism via the shikimate pathway [17] gives rise to a large number of aromatic compounds related to aromatic amino acids such as L-tyrosine (IV). Chlorogenic acid (III) is also an important factor in plant metabolism, forming caffeic acid on hydrolysis. Complementarily, tiron (V) was used as a model of a soluble *o*-diphenolic acid.



Complexation experiments were done with these five ligands and copper(II), an essential element in soil and aquatic environments.

EXPERIMENTAL

Copper(II) perchlorate solutions (p.a.; Fluka) were standardized against EDTA by using 4-(2-thiazolylazo)-resorcinol (TAR) as indicator [18]. The ligands tested were 4,5-dihydroxy-1,3-benzene disulphonic acid, disodium salt (tiron; Merck), 3,4-dihydroxycinnamic acid (caffeic acid; Aldrich), 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid (shikimic acid; Sigma), 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate (chlorogenic acid; Sigma), all used as received. The L-tyrosine (p.a.; Fluka) was recrystallized; its further solubilization was thus facilitated. Solutions containing weighed amounts of ligands, with or without perchloric acid, were titrated against carbonate-free 0.1 M sodium hydroxide, both in the absence and presence of metal ions for different ratios, $R = C_A/C_M$, of total ligand to total metal concentrations. Potentiometric measurements were made at $25 \pm 0.1^\circ\text{C}$ in 0.1 M sodium perchlorate medium, with a stream of nitrogen over the solution to limit oxidation phenomena. An automatic titrator (Tacussel TT Processor) was used as previously described [19]; it was fitted with extension glass and calomel electrodes for recording direct and differential titration curves. In some experiments, pH readings were combined with direct determinations of the free metal ion concentration by means of a copper(II)-ion selective electrode (Tacussel). A general computer program [20] was used for interpretation of pH titration data.

RESULTS AND DISCUSSION

Ligand protonation constants

Titration data (volume and pH) obtained for various concentrations of each ligand (usually in the C_A range 7×10^{-4} – 2×10^{-3} M) were converted to $(\bar{q}_0; \text{pH})$ curves, $\bar{q}_0$ being the average number of protons bound per mole of ligand. The ligand protonation constants shown in Table 1 were evaluated in

TABLE 1

Ligand protonation constants (25°C , 0.1 M NaClO_4)

Compound	$\text{p}K_{a1}^a$	$\text{p}K_{a2}$	$\text{p}K_{a3}$
Caffeic acid ^b	11.8	8.66	4.45
Chlorogenic acid	11.5	8.27	3.37
Shikimic acid	12.8	11.5	4.01
Tyrosine	9.98	9.03	2.00
Tiron ^b	11.7	7.61	—

^a K_{aq} refers to $[\text{H}_{q-1}\text{A}]/[\text{H}_q\text{A}]$. ^b From [5].

the conventional way (those of caffeic acid and tiron had already been calculated under the same experimental conditions [5]). The accuracy of the pK_{a1} values is poor because of inevitable oxidation of the *o*-diphenolic ligands in strong alkaline media.

Except for shikimic acid, for which no published data were found, these values are in good agreement with those previously reported for tyrosine [11, 12, 21] and chlorogenic acid [22].

Binary systems: stability constants of simple Cu(II)/ligand complexes

Copper(II)/tiron (H_2A^{2-}) and Cu(II)/caffeic acid (H_3A). These complexes were studied previously [5]. The equilibrium constants found are included in Table 2.

Copper(II)/chlorogenic acid (H_3A). The ligand/metal ratios examined were in the range $R = 2-10$ with $C_A = 10^{-3}$ M. Under these conditions, the appearance of a precipitate in the vicinity of pH 5 was not verified; such a precipitate was attributed by Timberlake [22] to the formation of the very slightly soluble neutral $CuHA$ complex in 1:1 mixtures of chlorogenic acid and copper(II) chloride (2.5×10^{-3} M).

The formation curves $\bar{r} = f(-\log a)$ ($\bar{r}$ is the average number of ligands bound per mole of metal ion and a is the free concentration of the anionic deprotonated form of the ligand) obtained for six different values of R were not exactly superimposable, especially in acidic medium (Fig. 1). As was found previously in the case of the complexation of Cu(II) by caffeic acid [5], a slight dispersion was observed, which suggests the presence of at least one protonated species having the general formula $M_pH_qA_r$. The presence of one MHA species was confirmed by the mean of measurements with the ion-selective electrode (i.s.e.) in acidic medium and a first estimation of its stability constant β_{111} was available by plotting the Leden function reduced to $(C_M - m)/ma = \beta_{101} + \beta_{111}h$, where m is the free metal ion concentration.

All the stability constants β_{pqr} were fitted to the experimental data by using the ACREF 3AM least-squares computer program [20] applied to the curves $\bar{q} = f(pH)$ with the pit-mapping procedure. The set of overall stability constants which minimized the sum $U = \Sigma(\bar{q}_{exp} - \bar{q}_{calc})^2$ is reported in

TABLE 2

Overall stability constants for the simple Cu(II)/ligand systems (25°C; 0.1 M NaClO₄)

System	$\log \beta_{101}^a$	$\log \beta_{102}$	$\log \beta_{111}$	$\log \beta_{121}$	$\log \beta_{122}$
Cu(II)/caffeic acid ^b	12.85	22.74	17.83	—	—
Cu(II)/chlorogenic acid	12.39	22.12	16.65	—	—
Cu(II)/tiron ^b	13.41	23.38	—	—	—
Cu(II)/tyrosine	—	15.48	17.75	25.39	34.70
Cu(II)/shikimic acid	13.06	—	19.46	—	—

^a β_{pqr} refers to $[M_pH_qA_r]/m^p h^q a^r$. ^bFrom [5].

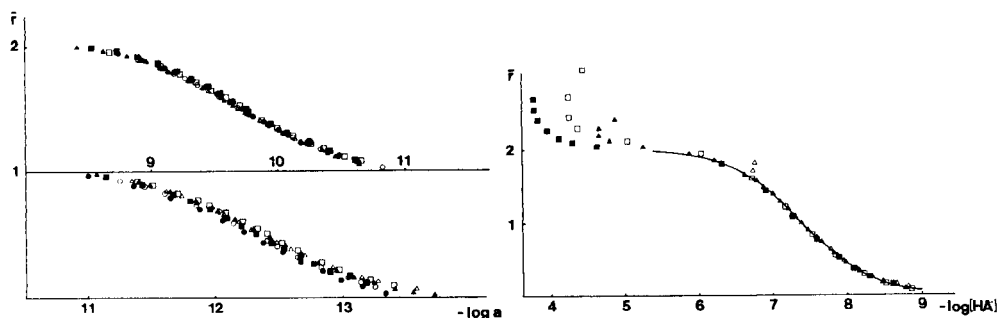


Fig. 1. Formation curves for the Cu(II)/chlorogenic acid system for different values of R : ($\bullet$) 10; ($\blacktriangle$) 8.3; ($\blacksquare$) 7.3; ($\circ$) 5; ($\square$) 3.5; ($\triangle$) 2.

Fig. 2. Formation curves for the Cu(II)/tyrosine system for different values of R : (Δ) 1.76; ($\square$) 3.52; ($\blacktriangle$) 2.51; ($\blacksquare$) 5.03. (—) Calculated curve.

Table 2. In contrast to the work of Timberlake [22], the formation of CuH_2A was not detected; furthermore, he failed in attempts to evaluate his experimental data by use of equations based on the presence of Cu^{2+} , CuH_2A^+ , CuHA and CuA^- . In fact, the situation appears to be identical to that observed for the case of caffeic acid [5]. It was previously reported that, in the Cu(II)/tiron system, it was not possible (in conformity with the results of Sigel et al. [23]) to prove the doubtful existence of a protonated complex CuHA . So, it seems likely that in the MHA species, the protonation of the HA^{2-} ligand affects the carboxyl group (see below for comparison with other ligands).

Copper(II)/tyrosine (H_2A). Four ratios of concentrations were studied, varying from 1.8 to 5 ($C_A = 7 \times 10^{-4}$ M and 10^{-3} M). In the range pH 3–7, the nature of the complexing species is expected to be HA^- , as the ligand behaves in the same way as α -amino acids with an aliphatic side-chain, coordination occurring through the amino and carboxyl groups while the phenolic hydroxyl remains protonated. The formation curves $\bar{r} = f(-\log [\text{HA}^-])$ were perfectly superimposable for different values of R with $\bar{r}$ in the range 0–2, indicating that 1:1 (CuHA) and 1:2 ($\text{Cu}(\text{HA})_2$ or CuH_2A_2) complexes are formed (Fig. 2). The dispersion observed for $\bar{r} > 2$ corresponds to species not involved in the classical Bjerrum method and resulting from successive deprotonation of the CuH_2A_2 complex leading to CuHA_2 (pH > 7) and then CuA_2 (pH > 9) species. The stability constants were computed from the $\bar{q} = f(\text{pH})$ curves (Fig. 3 and Table 2).

Copper(II)/shikimic acid (H_3A). For various ligand/metal ratios ($R = 4.24$, 5.65 and 8.48, with $C_A = 1.683 \times 10^{-3}$ M), complexation took place above pH 6.5 and released a maximum of two protons per mole of copper(II) ion. Formation curves, $\bar{r} = f(-\log a)$, calculated for the three ratios were not superimposable (Fig. 4) (with $\bar{r}$ in the range 0–1), indicating the simultaneous presence of several complexes of type MH_qA . Particularly, the existence of the CuHA and CuA species was revealed by plotting the Leden function

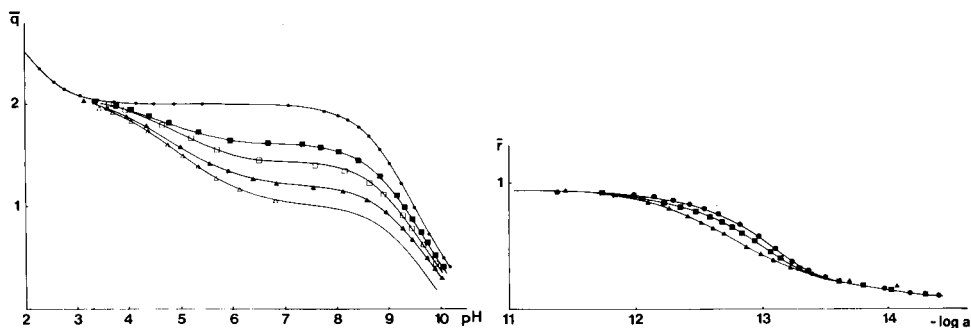
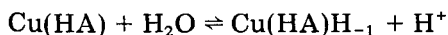


Fig. 3. $\bar{q} = f(-\log h)$ curves for the Cu(II)/tyrosine system for different values of R : (Δ) 1.76; ($\square$) 3.52; ($\blacktriangle$) 2.51; ($\blacksquare$) 5.03. ($\bullet$) $\bar{q}_0 = f(-\log h)$; (—) calculated curves.

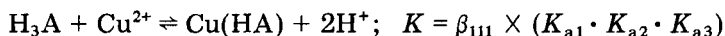
Fig. 4. Formation curves for the Cu(II)/shikimic acid system for different values of R : ($\bullet$) 4.24; ($\blacksquare$) 5.65; ($\blacktriangle$) 8.48.

$(C_M - m)/ma$ vs. h ; this gives an estimate of the stability constants β_{111} and β_{101} . However, the shape of the plots of $\bar{r}$ vs. $-\log a$ suggests the formation of at least one other complex and seems typical of hydroxide binding according to the reaction:



with $K_a = [\text{Cu}(\text{HA})\text{H}_{-1}]h/[\text{CuHA}]$, K_a being related to the first hydrolysis constant of Cu(II): $\log \beta_{1-10} < -8$ in 0.1 M NaClO_4 medium [24]. Then, the overall constant of the species $\text{Cu}(\text{HA})\text{H}_{-1}$ should be: $\beta'_{101} = \beta_{1-10} \times \beta_{111}$. It was observed that the values of the constants β'_{101} and β_{101} are very close ($\log \beta'_{101} = 12.81$, $\log \beta_{101} = 13.06$) but it was not possible to distinguish between these species. The following sequence of complexes is proposed tentatively for typifying the formation curves: $\text{Cu}(\text{HA})$, $\text{Cu}(\text{HA})\text{H}_{-1}$, CuA . Computation of the data ($\bar{q}$, pH) gave the results reported in Table 2.

Given these evaluations of stability constants for all the complexes formed, it was of interest to calculate the related speciation curves. For each system, the same ratio of concentrations ($C_A = 2 \times 10^{-3}$ M and $R = 10$) was chosen for visualizing the importance and the pH range of complex formation. For clarity, only the results obtained for the species CuHA and CuA are shown in Fig. 5. In the two systems Cu(II)/chlorogenic acid and Cu(II)/caffeic acid, the concentration of CuHA complex is maximal at about pH 5 where carboxyl groups can be still protonated. The constant K relative to the equilibria



were calculated as $\log K = -6.49$ (chlorogenic acid) and -7.08 (caffeic acid). These values are comparable with that obtained in the case of tiron ($\log K = -5.9$) for the equilibria $\text{H}_2\text{A} + \text{Cu}^{2+} \rightleftharpoons \text{CuA} + 2\text{H}^+$ where the two phenolic groups were implicated during the complexation [5]. Thus it is suggested

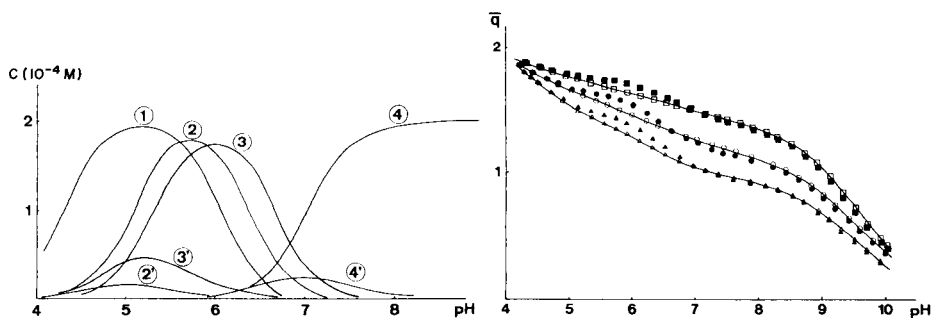


Fig. 5. Speciation curves for binary systems of Cu(II) with different ligands ($R = 10$): (1) tiron; (2) chlorogenic acid; (3) caffeic acid; (4) shikimic acid. (1–4) MA species; (2'–4') MHA species.

Fig. 6. $\bar{q} = f(\text{pH})$ curves for the ternary Cu(II)/tyrosine (A)/tiron (B) system. $R = (C_M : C_A : C_B)$: (●) (1:2.5:2.5), $C_A = 1.0 \times 10^{-3}$ M; (▲) (1:1.75:1.75), $C_A = 7.0 \times 10^{-4}$ M; (■) (1:5:2.33), $C_A = 1.4 \times 10^{-3}$ M. (—) Experimental curves. Filled symbols represent calculated points without considering the mixed species; open symbols represent calculated points with refined β_{pqr} constants.

that CuHA is protonated through a carboxylate group. For shikimic acid, this hypothesis fails because of the major presence of CuHA at about pH 7 (and the $\log K = -8.85$ found differs markedly from the previous values). In this case, protonation of the ligand probably involves a hydroxyl group.

Ternary systems: stability constants of Cu(II)/mixed ligand complexes

Copper(II)/H_qA/tiron (H_qA = caffeic acid, chlorogenic acid or tyrosine). Mixed complexes in which the metal ion is bonded simultaneously to two or more different complexing species are probably formed in most solutions which contain metal ions and more than one kind of ligand. Such mixed complexes are likely to be important because under biological or environmental conditions multiligand/multimetal ion equilibria predominate. The simplest of these species are ternary complexes $M_pH_qA_rB_s$ (i.e., one metal ion and two different ligands A and B).

For such complicated systems, various computational methods are available. Here, the earlier computer program [20] is used first to refine the corresponding stability constants $\beta_{pqr} = [M_pH_qA_rB_s] / m^p h^q a^r b^s$, and then to calculate the speciation of all the species existing at equilibrium. To refine the stability constants, the input data required are the initial approximate β_{pqr} values and information on the experimental conditions such as pH, stoichiometric total concentrations C_M , C_A , C_B , etc.

According to the rule outlined earlier [25], statistically, a metal ion prefers to combine with two different ligands rather than with two molecules of the same ligand. Sharma and Schubert [26] generalized this idea for a complex MA_rB_s and expressed the statistical factor as $S = (r + s)! / r!s!$. If the stability constants of parent complexes are known, the corresponding β_{10rs} constant

can be estimated from the relationship:

$$\log \beta_{10rs}(\text{calc.}) = \log S + [r/(r+s)] \log \beta_{10(r+s)0} + [s/(r+s)] \log \beta_{100(r+s)}$$

These calculated values were used in preliminary computations to find the conditions of pH, component ratios, and concentrations corresponding to the maximum concentration of the mixed species.

Data treatment in the detection of the mixed complexes and the evaluation of their stability constants were identical in both systems. The experimental titration curves ($\bar{q}_{\text{exp}}$ vs. pH) were first compared to the family of curves ($\bar{q}_{\text{calc}}$ vs. pH) computed with the known stability constants β_{pqro} and β_{pqos} of the parent binary complexes (the constants of the possible mixed complexes were set equal to zero). These curves overlapped within their limit of error in some ranges (Fig. 6) but disagreed elsewhere; the differences between $\bar{q}_{\text{calc}}$ and $\bar{q}_{\text{exp}}$ were attributed to the formation of the mixed species. The introduction into the calculation of the approximate values of β_{10rs} provided a significant decrease in this difference and an iterative process minimized the sum of squares $\Sigma(\bar{q}_{\text{calc}} - \bar{q}_{\text{exp}})^2$.

For the Cu(II)/caffeic acid/tiron system (Fig. 7), the values $\bar{q}_{\text{calc}}$ and $\bar{q}_{\text{exp}}$ do not agree below pH 6, in spite of the fact that the MAB species was taken into account; so the existence of a further protonated species M(HA)B seems probable. With that assumption, better agreement is obtained between the two curves. The situation is identical for the Cu(II)/tyrosine/tiron system (Fig. 8), where a M(HA)B species is first formed and is by far the predominant mixed species until it changes to a MAB complex when tyrosine is deprotonated in alkaline medium (pH > 8). However, the analogous protonated species could not be found in the Cu(II)/chlorogenic acid/tiron system because, as clearly shown in Fig. 9, the formation of MAB begins when the MHA species has already disappeared.

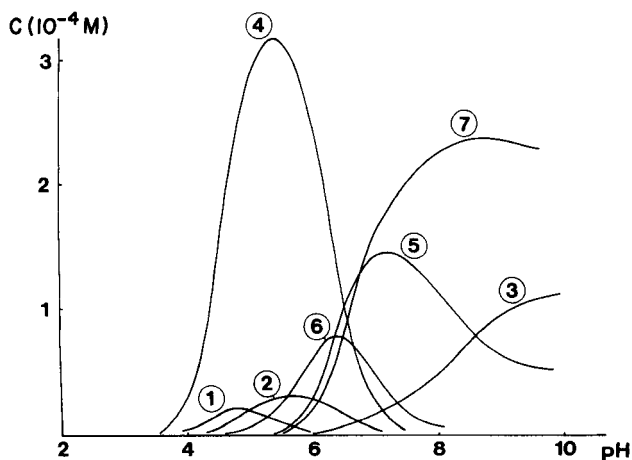


Fig. 7. Speciation curves for the Cu(II)/caffeic acid (A)/tiron (B) system; $C_A = 1.962 \times 10^{-3}$ M; $C_B = 7.0 \times 10^{-4}$ M; $C_M = 3.967 \times 10^{-4}$ M. Curves: (1) MHA; (2) MA; (3) MA_2 ; (4) MB; (5) MB_2 ; (6) MHAB; (7) MAB.

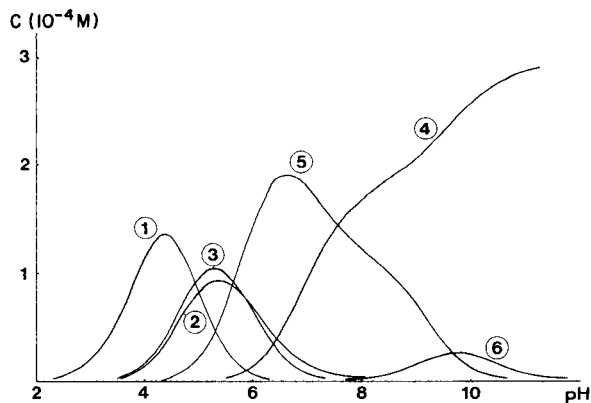


Fig. 8. Speciation curves for the Cu(II)/tyrosine (A)/tiron (B) system; $C_A = 1.4 \times 10^{-3}$ M; $C_B = 7.0 \times 10^{-4}$ M; $C_M = 3.967 \times 10^{-4}$ M. Curves: (1) MHA; (2) MH_2A_2 ; (3) MB; (4) MB_2 ; (5) MHAB; (6) MAB. ($[MA_2]$ and $[MHA_2] < 10^{-5}$ M).

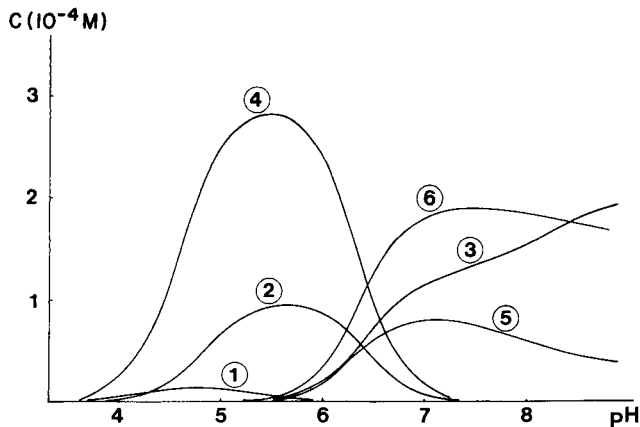


Fig. 9. Speciation curves for the Cu(II)/chlorogenic acid (A)/tiron (B) system; $C_A = 2 \times 10^{-3}$ M; $C_B = 5 \times 10^{-4}$ M; $C_M = 4 \times 10^{-4}$ M. Curves: (1) MHA; (2) MA; (3) MA_2 ; (4) MB; (5) MB_2 ; (6) MAB.

All the corresponding original values of the refined stability constants are listed in Table 3.

There are three common ways of quantifying the stability of mixed ligand complexes [13, 25]: (i) the difference $\Delta_{10rs} = \log \beta_{10rs(\text{exp})} - \log \beta_{10rs(\text{calc})}$ is usually found to be positive; (ii) for the reaction $MA_2 + MB_2 \rightleftharpoons 2 \text{ MAB}$ (formation constant = χ_{11}), the position of this equilibrium, and consequently the tendency to mixed complex formation is determined by the sign and the value of χ_{11} (for statistical reasons the value expected for $\log \chi_{11}$ is 0.6) where $\log \chi_{11} = 2 \log \beta_{1011} - (\log \beta_{1020} + \log \beta_{1002})$; and (iii) the use of an alternative expression [13] for mixed complex formation by considering the equilibria: $M + B \rightleftharpoons MB$; $K_{MB}^M = [MB]/mb$, $MA + B \rightleftharpoons MAB$; $K_{MAB}^{MA} = [MAB]/[MA]b$.

TABLE 3

Overall stability constants and characteristic stabilization parameters for Cu(II) mixed ligand complexes (25°C, 0.1 M NaClO₄)

System	$\log \beta_{1011}$	$\log \beta_{1111}$	$\log \chi_{11}$	Δ_{11}	$\Delta \log K_{11}$
Cu(II)/caffeic acid/tiron	23.55	30.04	0.98 ^a — ^b	0.19 —	—2.71 —1.2
Cu(II)/chlorogenic acid/tiron	23.05	—	0.60	0.00	—2.75
Cu(II)/tyrosine/tiron	20.02	29.71	1.18 ^a 1.34 ^b	0.29 0.37	— —1.45

^aMAB formation. ^bM(HA)B formation. The absence of values indicates that the calculation is impossible because of the lack of data for the parent complex.

Sigel [13] used the difference between the stabilities of the binary and of the ternary complexes to characterize the tendency to form ternary complexes:

$$\Delta \log K = \log K_{\text{MAB}}^{\text{MA}} - \log K_{\text{MB}}^{\text{M}} = \log K_{\text{MAB}}^{\text{MB}} - \log K_{\text{MA}}^{\text{M}}$$

This relationship may be generalized to

$$\Delta \log K_{rs} = \log \beta_{10rs} - (\log \beta_{10r0} + \log \beta_{100s})$$

An experimental value of $\Delta \log K < 0$ is expected and an increase in this value indicates that mixed ligand complex formation is favoured.

The two systems involving caffeic acid and tyrosine show that other factors than purely statistical ones influence the stability of the mixed ligand complexes ($\log \chi > 0.6$ and $\Delta > 0$), whereas the stabilization of the mixed complex involving chlorogenic acid can be accounted for by statistical factors alone ($\log \chi = 0.6$ and $\Delta \approx 0$).

Conclusion

In order to assess the real behaviour of a metal ion in a natural system, reliable and accurate data about its physicochemical speciation are needed, because complexation can play an important role in phenomena such as mobilization, transport, adsorption, weathering, bioavailability and toxicity in soil and water environments. The classical approach consists of computer calculations based on a modelling process that assumes knowledge of all the various species involved for which chemical thermodynamic data are required.

The present results confirm that in complicated systems the formation of only simple binary complexes may be regarded as more the exception than the rule; models and computed calculations must take into account the formation of mixed complexes. As reported by various authors, the data available on the formation of such species are very sparse [27]. Nevertheless, it is shown here that a good mathematical estimation of the effect of mixed ligand complexation can easily be obtained by considering the characteristic stabilization parameters of ternary complexes (Table 3). It must be noted that

mixed complex formation is also implicated in the coordination chemistry of the oxide/water interface [28] and that further investigations in natural systems should take into account not only mixed complexes with more than two different ligands but also competitive reactions between different metal ions for the organic matter [29].

Support of this work by the French Scientific Research National Centre (CNRS), the National Institute for Agronomic Research (INRA; for I.L.) and the Environment Ministry is gratefully acknowledged.

REFERENCES

- 1 T. M. Florence, *Talanta*, 29 (1982) 345.
- 2 W. Salomons and U. Förstner, *Metals in the Hydrocycle*, Springer Verlag, Berlin, 1984.
- 3 R. F. Christman and E. T. Gjessing, *Aquatic and Terrestrial Humic Materials*, Ann Arbor Science, Ann Arbor, MI, 1983.
- 4 F. J. Stevenson, *Humus Chemistry*, Wiley, New York, 1982.
- 5 Y. Bizri, M. Cromer, I. Lamy and J. P. Scharff, *Analysis*, 13 (1985) 128.
- 6 D. D. Perrin and I. G. Sayce, *Talanta*, 14 (1967) 833.
- 7 N. Ingri, W. Kakolowicz, L. G. Sillén and B. Warnquist, *Talanta*, 14 (1967) 1261.
- 8 Ting-po I and G. H. Nancollas, *Anal. Chem.*, 44 (1972) 1940.
- 9 G. Sposito and F. T. Bingham, *J. Plant Nutr.*, 3 (1981) 35.
- 10 D. K. Nordstrom, L. N. Plummer, T. M. L. Wigley, T. J. Wolery, J. W. Ball, E. A. Jenne, R. L. Bassett, D. A. Crerar, T. M. Florence, B. Fritz, M. Hoffman, G. R. Holdren, Jr., G. M. Lafon, S. V. Mattigod, R. E. McDuff, F. Morel, M. M. Reddy, G. Sposito and J. Thraikill, in E. A. Jenne (Ed.), *Chemical Modeling in Aqueous Systems*, ACS Symp. Ser. No. 93, 1979, Chap. 38.
- 11 L. G. Sillen and A. E. Martell, *Stability Constants of Metal Ion Complexes*, The Chemical Society, London, 1964, 1971.
- 12 R. M. Smith and A. E. Martell, *Critical Stability Constants*, Plenum, New York, 1974, 1976, 1977.
- 13 H. Sigel, *Angew. Chem. Int. Ed. Engl.*, 14 (1975) 394.
- 14 P. MacCarthy and G. C. Smith, in E. A. Jenne (Ed.), *Chemical Modeling in Aqueous Systems*, ACS Symp. Ser. No. 93, 1979, Chap. 10.
- 15 J. Buffle, *Anal. Chim. Acta*, 118 (1980) 29.
- 16 D. S. Gamble, P. MacCarthy, E. M. Perdue and G. C. Smith, in *Proceedings of 2nd IHSS meeting*, Birmingham (U.K.), 1984.
- 17 J. Mann, *Secondary Metabolism*, Oxford University Press, Oxford, 1978.
- 18 B. S. Jensen, *Acta Chem. Scand.*, 14 (1960) 927.
- 19 Y. Bizri, M. Cromer, J. P. Scharff, B. Guillet and J. Rouiller, *Geochim. Cosmochim. Acta*, 48 (1984) 227.
- 20 M. Cromer-Morin, J. P. Scharff and R. P. Martin, *Analysis*, 10 (1982) 92.
- 21 D. D. Perrin, *Stability Constants of Metal Ion Complexes*, Part B, IUPAC. Chemical Data Series No. 22, Pergamon, Oxford, 1979.
- 22 C. F. Timberlake, *J. Chem. Soc.*, (1959) 2795.
- 23 H. Sigel, P. R. Huber, R. Griesser and B. Prijs, *Inorg. Chem.*, 12 (1973) 1198.
- 24 C. F. Baes and R. E. Mesmer, *The Hydrolysis of Cations*, Wiley, New York, 1976.
- 25 R. P. Martin, M. M. Petit-Ramel and J. P. Scharff, in H. Sigel (Ed.), *Metal Ions in Biological Systems*, M. Dekker, New York, 1973, Chap. 1.
- 26 V. J. Sharma and J. Schubert, *J. Chem. Educ.*, 46 (1969) 506.

- 27 H. Sigel, Metal Ions in Biological Systems. Circulation of Metals in the Environment, M. Dekker, New York, Vol. 18, 1984.
- 28 P. W. Schindler, in H. Sigel (Ed.), Metal Ions in Biological Systems. Circulation of Metals in the Environment, M. Dekker, New York, Vol. 18, 1984, Chap. 4.
- 29 D. S. Gamble, M. Schnitzer, H. Kerndorff and C. H. Langford, *Geochim. Cosmochim. Acta*, 47 (1983) 1311.

EVALUATION OF ANALYTICAL INTERLABORATORY COMPARISONS AND CERTIFICATION OF REFERENCE MATERIALS

L. PSZONICKI*

International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna (Austria)

(Received 16th April 1985)

SUMMARY

A procedure for the evaluation of results of interlaboratory comparisons of trace multi-component determinations is presented. The distribution of interlaboratory comparison data may differ significantly from the normal distribution and, therefore, various types of median and modal values approximate their central values better than the mean. The simple median and its confidence interval, calculated on the basis of a nonparametric distribution after the rejection of outlying results, is shown to be the optimal indicator of the central values. The total procedure consists of three parts: statistical evaluation of the data, classification of the results according to a special set of analytical criteria, and graphic presentation of the data. The application of the results of such comparisons for certification of analytical reference materials is discussed.

Interlaboratory comparisons have been organized by the Analytical Quality Control Service (AQCS) of the International Atomic Energy Agency (IAEA) for close to twenty years. The primary aim of this programme was to support laboratories engaged in the analysis of nuclear materials and in the development of nuclear analytical methods. Laboratories which applied non-nuclear methods were invited to participate also, to allow a comparison between methods. It may be assumed, as a rough estimate, that the contribution of various analytical methods to the results provided for the individual exercises is proportional to the actual frequency of their common application. Neutron activation analysis, the most popular and versatile nuclear method, is used predominantly for multi-element trace determinations, thus the interlaboratory comparison programme expanded quickly in all directions in which trace analysis is applied, e.g., environment protection, food control, biology and geology. The participation in the programme of a large number of laboratories made it possible to consider certification of the materials compared and their distribution as analytical reference materials.

The number of results received in individual interlaboratory comparisons

*Present address: Institute of Nuclear Chemistry and Technology, Dorodna 16, 03-195 Warsaw, Poland.

depends on the type of the material and on the number of components determined in it. Usually several hundred results are received and a special procedure is required for their objective evaluation. An earlier evaluation procedure, proposed by Dybczynski et al. [1, 2] and based on the assumption of a normal distribution of the data, was applied for some years. The large amount of experimental material collected during this time provided new information on specific features of the results of the interlaboratory comparison and this experience, as well as the experience of other organizers of such comparisons [3–5], suggested that the normal distribution is not always the best basis for data evaluation.

The new procedure presented below is valid for any continuously distributed sets of data and it does not require simplifying assumptions. It comprises three parts: (1) computation of statistical characteristics of the set of data; (2) classification of the results of statistical evaluation according to a special set of analytical criteria; and (3) plotting of the data distribution.

PLOTTING OF DATA DISTRIBUTION

Graphic illustration of the experimental data distribution is not essential for their statistical evaluation, but it usually provides condensed and easily understood information on their specific properties. It allows not only a semiquantitative estimate of the significance of the total set and of the individual data points in it, but is also helpful for evaluation of the analytical meaning of the calculated statistical characteristics. In general, it facilitates any consideration concerning the data to be evaluated and, therefore, the plotting procedure applied is described first. Yet, the significance of the plot of data distribution should not be overrated. Computer simulation of a large number of data sets chosen at random from a well defined general population, e.g., a normally distributed one, shows that the distribution of a significant number of these sets is strongly deformed. Therefore, it should be kept in mind that the experimental data are random values and that their plot represents the distribution only of the given experimental set and not the distribution of the general population, in the statistical sense, to which this experimental set belongs. The final form of the plot depends also on the plotting procedure applied and on the discrimination level of local statistical fluctuations of random data points.

Many different graphic methods for illustrating analytical data are described in the literature, e.g., conventional histograms, box plots [6], moving histograms [3], plots of data arranged by their ascending values [4, 7, 8], or DISTRI plots which present the sum of Gaussian curves found for the separate sub-populations [9]. Conventional histograms and moving histograms are the most convenient of these methods for presentation of interlaboratory comparison results. However, as was shown by Abbey et al. [3], the form of conventional histograms is strongly affected by the arbitrarily chosen size of class intervals and by the location of the boundaries between these intervals, so that for the same set of data various significantly different

histograms may be obtained. The moving histogram procedure which was devised as a method of finding the modal value also shows some shortcomings when it is used for plotting data distribution. To avoid these disadvantages a new approach to data plotting was prepared.

The diagrams obtained by this new procedure are similar, to some extent, to the moving histograms, but they are constructed on a different basis. The distribution of data is represented by the relative local densities of data points plotted as a function of values of these points. The local density is defined as the number of data points per one concentration unit in the immediate vicinity of a given point.

Procedure

First, all accepted results are treated as a set of data points and arranged by ascending values from x_1 to x_n . Then, in step 2, for every data point a new corrected value is calculated as the average value of this point and of two neighbouring points. This operation is repeated $0.1 m$ times, where m is the total number of data points rounded to full tens. The values of the extreme points x_1 and x_n are kept constant and equal to the original values.

In step 3, the absolute local density value is calculated for every corrected point as the reciprocal value of the mean distance between this point and two neighbouring points. The function obtained is smoothed by calculation for each point of the mean value of local densities referred to this point and to $0.2 m$ neighbouring points. This operation is repeated $0.1 m$ times. The procedure may be applied also to the end points x_1 to x_m and x_{n-m} to x_n by assuming on both sides of the set the existence of m hypothetical auxiliary points. Experimentally it was found that the deformation of tails of a plot may be considered insignificant when the distance of these auxiliary points from the lower and upper extreme points x_1 or x_n is calculated as equal to $2^k(x_3 + x_2 - 2x_1)$ or $2^k(2x_n - x_{n-1} - x_{n-2})$, respectively, where $k = 0, 1, \dots, (m - 1)$ for the consecutive auxiliary points counted from the centre of the set.

In step 4, the smoothed function of absolute local densities is transformed to the relative local density function $\Phi(x)$ by normalizing its maximum to 100. The latter is plotted against the corrected values of data as a continuous line with small filled circles (see Fig. 1). Finally, for multimodal curves (more than one maximum), the function $\Phi(x)$ is processed further by the same smoothing procedure as in step 3. The smoothing is repeated until a unimodal function is achieved. This function is plotted in Fig. 1B as a continuous line without circles.

Step 2 homogenizes data positions in small intervals of concentration containing only a few data points. It allows the calculation of local densities for clusters of three or more coinciding points and diminishes local irregularities of the density function but does not affect the form of the function. Step 3 reduces the statistical fluctuation of the density of random data without changing significantly the general form of the plotted function. A serious

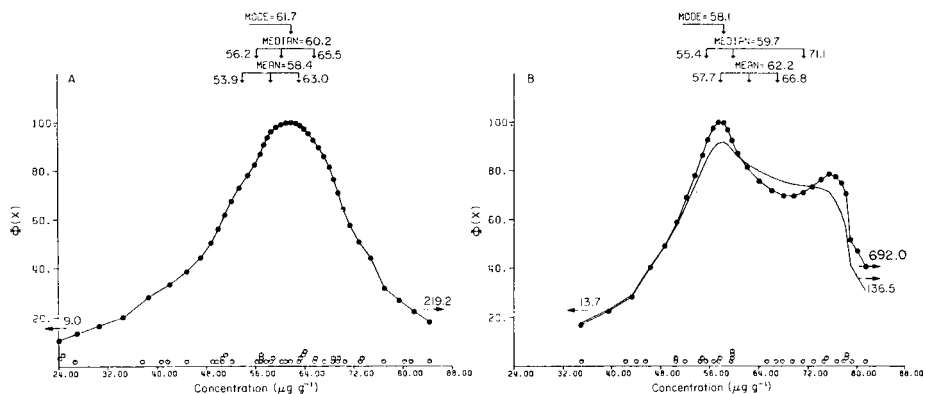


Fig. 1. Distribution of results of the determination of chromium and lead in the Interlaboratory Comparison of IAEA/SOIL-7 (1983): (A) chromium; (B) lead.

deformation is usually caused by transformation of a multimodal curve to a unimodal one (final step). In this operation, some important information may be lost, and therefore the transformed function should always be considered together with its primary form.

Results

Figure 1 shows the distribution of results obtained for chromium and lead determinations in the interlaboratory comparison run on IAEA/SOIL-7 [10]. The numerical values of these results are listed in Table 1. The figure also contains other information. The positions of the original results are indicated by open circles directly above the abscissa; the values of means and medians are shown accompanied by their confidence intervals and the value of the mode is indicated by vertical arrows in the upper part of the plot. The values of outlying results are printed at the horizontal arrows indicating the direction of their position.

The modal values were taken as the values of concentration corresponding to the maximum of the local density function $\Phi(x)$. For multimodal plots the modal values were estimated after the transformation of these plots to the unimodal form. The values of mode (M) determined in this way are simply a byproduct of the plotting procedure applied and their advantages or shortcomings in comparison to the moving histogram mode (MHM) [3] or dominant cluster mode (DCM) [11] will not be discussed here. In fact, they are compromise values usually placed between MHM and DCM, e.g., the various modal values of data presented in Fig. 1A are 58.8 (MHM), 61.7 (M), 61.9 (DCM) while in Fig. 1B the values are 57.1 (MHM), 58.1 (M), and 59.7 (DCM). Taking into account the dispersion of the data and the size of the confidence intervals of the estimated central values, the difference between those three modal values may be considered as insignificant.

TABLE 1

Results of interlaboratory comparison of determinations of chromium and lead in IAEA/ SOIL-7

No.	Method code no. ^a	Result ($\mu\text{g g}^{-1}$)	Notes ^b	No.	Method code no. ^a	Result ($\mu\text{g g}^{-1}$)	Notes ^b	No.	Method code no. ^a	Result ($\mu\text{g g}^{-1}$)	Notes ^b
<i>Chromium</i>											
1	2	9	*	16	2	57		31	2	67	—
2	—	24		17	3	57	+	32	1	69	+
3	2	25	—	18	2	57		33	1	69	+
4	—	27		19	4	58		34	1	69	
5	2	37		20	2	59		35	1	70	+
6	2	41	—	21	1	59	+	36	4	71	
7	2	42		22	1	60		37	3	73	+
8	3	45	—	23	1	61	+	38	1	73	
9	2	49		24	2	62		39	1	77	
10	2	50		25	1	63	+	40	3	80	
11	2	51		26	1	63	—	41	1	81	—
12	2	51		27	1	64	+	42	—	84	
13	2	51		28	1	64	+	43	4	219	*
14	2	52	—	29	2	66	—				
15	2	56	+	30	2	66					
<i>Lead</i>											
1	2	14	*—	13	2	58	—	25	—	75	
2	2	35	—	14	2	58	—	26	4	75	
3	2	42		15	4	60		27	—	77	
4	2	44		16	2	60		28	4	78	
5	3	46	—	17	4	60		29	2	78	
6	2	51	—	18	4	60		30	2	78	+
7	2	51		19	2	65	+	31	2	79	—
8	4	52		20	4	67		32	2	81	—
9	2	55		21	2	68	+	33	2	137	*
10	2	55		22	2	69		34	2	682	*—
11	2	55		23	2	71	—				
12	5	57	—	24	2	73					

^aMethod code no.: (—) Not Reported; (2) atomic absorption spectrometry; (3) atomic emission spectrometry; (4) x-ray fluorescence; (5) spark-source mass spectrometry. ^bNotes: (*) Results rejected as outliers; (+) results provided by "good" laboratories; (—) results provided by "bad" laboratories.

STATISTICAL EVALUATION

The best indicators of the central value

An objective evaluation of interlaboratory comparison data is only possible by means of a statistical procedure. The arithmetic mean and its confidence interval, calculated on the basis of a normal distribution, are not always sufficiently reliable as indicators of the central value of the collected data. Ellis and Steele [5] found that the order of efficiency of various indicators is as follows: DCM, modian, Gastwirth median, median, trimmed mean, mean, and trimean. Some of these indicators were tested on many sets of data obtained for two artificially prepared materials, IAEA/W-3/1 Fresh Water [12] and IAEA/Air-3/1 Air Filter [13], which had been spiked with known amounts of trace components. The results were similar for both materials; those for the air filters are listed in Table 2. The data for barium and copper

TABLE 2

Comparison of input values and various indicators of central values for the results of inter-laboratory comparison IAEA/AIR-3/1

Element	Input value (μg)	Mean		Mode		Median without outlier	
		Value (μg)	Confidence limits (0.05) (μg)	Value (μg)	Confidence limits (0.05) (μg)	Value (μg)	Confidence limits (0.05) (μg)
As	5.60	4.59	3.44–5.74	5.00	2.13–6.63	5.00	4.61–5.74
Au	1.15	1.06	0.83–1.30	1.11	0.69–1.36	1.06	0.69–1.36
Cd	9.96	9.82	9.08–10.6	10.3	8.87–11.4	10.00	8.97–10.8
Co	1.12	1.18	0.94–1.41	1.01	0.59–1.35	1.11	0.85–1.42
Cr	5.06	4.82	4.53–5.11	5.00	4.42–5.52	5.00	4.50–5.09
Fe	208	200	194–208	207	186–215	204	196–210
Hg	1.00	0.78	0.36–1.19	0.74	0.02–1.37	0.73	0.21–1.37
Mn	31.9	30.1	28.4–31.7	33.0	28.8–39.0	30.2	28.0–32.0
Mo	1.14	1.57	0.41–2.72	0.80	0.59–3.50	1.13	0.59–3.50
Ni	8.00	7.38	6.76–7.99	7.30	5.38–8.18	7.33	7.06–8.18
Pb	111	102	95–110	112	95.5–120	105	95–112
Se	1.06	1.01	0.91–1.12	1.07	0.86–1.14	1.00	0.86–1.14
U	1.02	1.00	0.87–1.12	0.99	0.77–1.17	0.97	0.77–1.17
V	8.00	7.24	6.46–8.02	8.00	6.07–9.04	7.84	5.87–8.29
Zn	152	141	135–148	143	131–157	143	132–152

for which erroneously low values were found by most laboratories, are excluded from the table.

The values given for the mean and of the mode were estimated for the data after rejection of outlying results by a statistical test (see next section). The values of the median were calculated for all the data and for the data after rejection of outliers. The values of the Gastwirth median, the calculation of which strongly discriminates against the effects of extreme results, were calculated on the basis of all data. The confidence intervals of the mean were calculated in the usual way by assuming a normal distribution of data at a significance level of 0.05; those of the median were estimated from tables [14, 15] which are valid for any continuous non-parametric distribution. The confidence intervals of the mode were evaluated as "the smallest range containing R data points, where $R = 1.62N^{0.728}$ for 95% confidence and R is rounded to the nearest integer" [11]. The Gastwirth medians were calculated from the formula $GAS = 0.4Md + 0.3(HT + LT)$, where Md is the median, HT the upper tertile, and LT the lower tertile. Their confidence intervals were accepted to be the same as for the medians with outliers, as suggested by Ellis [15].

Table 2 shows that the smallest relative deviations from the input values (spiked concentration values) for the largest number of elements determined

Element	Confidence limits (0.05) (μg)	Gastwirth median (μg)	Relative deviation (%) from the input value				
			Mean	Mode	Median without outliers	Median with outliers	Gastwirth median
As	4.61–6.63	5.15	18	11	11	10	8
Br	0.69–8.00	1.11	8	4	8	7	4
Cd	8.97–10.8	9.89	1	3	0.4	0.4	0.7
Co	0.92–2.07	1.23	5	10	0.9	12	10
Cu	4.43–5.13	4.88	5	1	1	1	4
Fe	185–210	202	4	0.5	2	3	3
Mn	0.21–5.63	0.95	22	26	27	13	5
Ni	27.7–32.8	30.0	6	3	5	6	6
Pb	0.59–3.50	1.13	38	30	0.9	0.9	0.9
Pb	7.06–8.35	7.67	8	9	8	4	4
Pb	95–115	107	8	0.9	5	3	4
Pb	0.88–5.27	1.08	5	0.9	6	4	2
Pb	0.92–2.50	1.05	2	3	5	3	3
Pb	5.87–8.29	7.46	10	0.0	2	2	7
Pb	132–150	141	7	6	6	7	7
Average relative deviation from the value			9.8	7.2	5.9	6.9	4.6

were found for the mode (8 elements) followed by the Gastwirth median (5 elements), median without outliers (5 elements), median with outliers (4 elements), and mean (1 element). These results agree well with those of Ellis and Steele [5], but except for the mean, the differences between the various estimates of the central value seem to be insignificant. If the average relative deviation calculated for all fifteen elements is taken as the indicator of efficiency, another order is obtained: Gastwirth median, median without outliers, median with outliers, mode and mean. It may be assumed, therefore, that the values of the mode and of the three investigated types of median are more or less equivalent as estimates of the central value.

The quality and the size of confidence intervals may be used as another test criterion. Table 2 shows that the confidence intervals of the mean do not contain the input values for four (Mn, Ni, Pb and Zn) of the fifteen elements. These four input values, however, are within the confidence intervals of the other estimates. Consequently, not only is the mean itself a bad estimate but also its confidence interval does not describe correctly the range in which the central value should be placed with a defined probability. Both these facts suggest that normal distribution should be rejected as a basis for evaluation of interlaboratory comparison data and an indicator of the central value other than the mean should be accepted.

Any estimate of the central value results from a set of random data and its value is also random. Therefore, correct evaluation of confidence limits of the estimate is as important as the calculation of the central value itself. Of course, the confidence interval should be as small as possible because its size decides on how precisely the central value is approximated. Table 2 indicates that, except for the mean, the smallest confidence intervals are found for the median without outliers followed by mode and median with outliers. For some elements, the confidence intervals of the two last estimates are very large in relation to the intervals of the median without outliers, e.g., the intervals of the mode calculation for arsenic, mercury and manganese, or the intervals of the median-with-outliers calculation for gold, cobalt, mercury, manganese, uranium and vanadium. Although the median itself is not sensitive to the presence of outlying results in a set of data, its confidence interval may be greatly affected, particularly when the number of outliers is relatively large and most of them are placed on one side of the set. Therefore, the preliminary elimination of outlying data is also important when the median is used as the indicator of central value.

The above considerations explain why, in the proposed method, the median, estimated after rejection of outlying data, and its confidence interval, calculated for a non-parametric distribution, were accepted as the optimal approximation of central value. The mean and the mode, although always calculated, are used only as additional information.

Rejection of outlying data

The criteria commonly used for the rejection of outlying results from a set of analytical data [2] are constructed on the assumption of normal distribution and could not be used in a procedure which is based on nonparametric distribution. Therefore, the test proposed by Veglia [16] was applied; it is based on the Tchebycheff inequality and is valid for any continuous distribution. The calculation involved is

$$h = |x_j - \bar{x}_{n-1}| (S_{n-1})^{-1} [n/(n-1)]^{1/2}$$

where x_j is the value of the point to be tested, $\bar{x}_{n-1}$ the arithmetic mean of the set without point j , S_{n-1} the standard deviation of the set without point j , and n the total number of data in the set. Any extreme result should be rejected as an outlier if the value calculated for h is larger than a critical value. For a significance level of 0.05, the critical value of h is 3.162.

The above test works well, with one exception. Sometimes, when the number of data is small (about ten) and two outliers are placed at approximately the same large distance from the centre of the set, the test is ineffective. This shortcoming was removed by a small empirical modification. When the h value of a successive data point is smaller than 3.162, the testing procedure is not stopped as in the original test, but the point is provisionally excluded from the set of data and the next point is tested. If for this next point the h value is also smaller than 3.162, both points are accepted and the testing is stopped. However, if the h value for the second point is larger than the

critical one, then both points are rejected as outliers and the testing is continued for the next data points of the set.

Statistical procedure

The steps in the statistical evaluation procedure used may be described briefly as follows. First, laboratory means are calculated on the basis of the individual determinations reported by each laboratory. All laboratory means for the component determined are then treated as a set of data and arranged in the order of increasing values (see Table 1). The set of data is tested for outliers by the modified Veglia procedure.

For the set of accepted data the overall median, its confidence limits at a significance level of 0.05, and the relative uncertainties (relative confidence interval) are calculated (the confidence interval of the median may be asymmetric). The overall mean and its confidence limits and the mode are also calculated for additional information. The medians of groups of data points obtained by various analytical methods are then calculated. Finally, the results of the statistical evaluation are listed separately for each component, as shown in Table 3.

QUALIFICATION OF THE STATISTICAL CHARACTERISTICS BY ANALYTICAL CRITERIA

The results of a statistical evaluation of the interlaboratory comparison data cannot be considered as ultimate because their analytical sense is usually ambiguous and requires to be interpreted in the light of additional analytical

TABLE 3

Results of statistical evaluation of the determination of chromium and lead in IAEA/ SOIL-7

Element	No. of accepted results	Outliers (%)	Overall median			Obtained by individual analytical method		
			Value ($\mu\text{g g}^{-1}$)	Confidence limit (0.05) ($\mu\text{g g}^{-1}$)	Relative uncertainty (%)	Code no. ^a	No. of accepted results	Median value ($\mu\text{g g}^{-1}$)
Cr	41	5	60	56–66	–7; +9	1	14	66
						2	18	52
						3	4	65
						4	2	64
Pb	31	9	60	55–71	–7; +19	2	20	59
						3	1	46
						4	7	69
						5	1	57

^aMethod code no.: (1) neutron activation analysis; (2) atomic absorption spectrometry; (3) atomic emission spectrometry; (4) x-ray fluorescence spectrometry; (5) spark-source mass spectrometry.

information such as concentration level, number of different analytical methods used, agreement between these methods, and many other criteria. Sometimes, a concentration value based on the agreement of only two results provided by laboratories from different countries and obtained by different methods, may be considered as more reliable than another value based on fifteen results obtained by the same analytical method, particularly if the accuracy of this method is not well confirmed.

The digital (Tables 1 and 3) and analog (Fig. 1) ways of presentation of data distribution and of its statistical characteristics are complementary and together with all available analytical information they form a proper basis for the final evaluation of interlaboratory comparison results.

A high degree of objectivity in the final evaluation can be assured only by the application of precisely defined criteria. Because there are no commonly accepted criteria of this type, it was necessary to formulate them for the purposes of the IAEA Intercomparison Programme. The criteria accepted primarily [17] were tested and then improved on the basis of many comparison exercises. Their present form is as follows:

- (1) for a given concentration range, the relative uncertainty of the overall median (at a significance level of 0.05) does not exceed the following limits: $\pm 20\%$ for 100–1000 $\mu\text{g g}^{-1}$, $\pm 30\%$ for 10–100 $\mu\text{g g}^{-1}$, -50% to $+60\%$ for 0.1–10 $\mu\text{g g}^{-1}$, and -50% to $+100\%$ for $<0.1 \mu\text{g g}^{-1}$;
- (2) the overall median is based on data obtained by at least two different analytical methods;
- (3) more than half of the medians of all groups of results obtained by different analytical methods and calculated on the basis of at least three results is enclosed inside the confidence limits found for the overall median;
- (4) the relative number of results rejected as outliers does not exceed (a) 0% (no outliers), (b) 30%, and (c) 50%;
- (5) the overall median is calculated on the basis of at least the following number of results provided by different laboratories (a) 5, (b) 3, and (c) 2.

All concentration values (overall medians) obtained by a statistical evaluation are tested by the criteria mentioned above and classified as follows:

Class A: values recommended with a high degree of confidence must fulfil at least criteria 1, 2, 3, 4b and 5a;

Class B: values recommended with an acceptable degree of confidence must fulfil at least criteria 1, 2, 3, 4c and 5a, or 1, 2, 3, 4b and 5b or 1, 4a and 5a (the last set of criteria is applicable for components to be determined usually by only one analytical method which is significantly more effective than all others, e.g., neutron activation analysis for some rare earth elements at a ng g^{-1} level);

Class C: information values must fulfil at least criteria 4a and 5c, or 4c and 5b, and their confidence intervals should be reasonable for the given concentration level;

Class D: meaningless values which do not satisfy even the reliability criteria for Class C; these are regarded as meaningless until additional results are obtained for the components concerned.

All data necessary for final evaluation of the results of chromium and lead in SOIL-7 (Table 1) by the above-defined criteria are listed in Table 3.

CERTIFICATION OF THE REFERENCE MATERIALS

The certification of a batch of material as an analytical reference material requires the determination of all certified values with good accuracy and precision. Of course, when the certification is based on interlaboratory comparison data which are usually widely dispersed (e.g., the data in Table 1), the confidence intervals of the estimated central values are relatively large. To try to solve this problem, some authors have suggested that the certification should be based on the results provided by a small group of highly qualified selected laboratories. The advantages and shortcomings of such a procedure were discussed by Abbey [8] and by Kosta and Byrne [18]. It seems, however, that this way would be effective only for the certification of a small number of selected components. For multi-element trace determinations, it can hardly be achieved in practice. Very few laboratories are highly experienced in the determination of a large number of trace components in a given material and the choice of a group of selected laboratories would be faced with serious difficulties. A likely situation would be typified by the following example.

For the interlaboratory comparison of the IAEA/SOIL-7 material more than 15 results (laboratory means) per element were received for 28 elements. On the basis of these data, the 52 participating laboratories, which provided their results for at least five elements, were classified into one of the following groups: (1) "good" laboratories, from which more than 50% of the results were within the estimated confidence intervals of the central values; (2) "acceptable" laboratories, from which 20–50% of the results were within the confidence intervals; and (3) "bad" laboratories, from which less than 20% of all results were within the confidence intervals. Only 13 of the 52 laboratories could be included in the first group. However, although most of their results were close to the central values, others were dispersed widely and many had to be rejected as outliers (Table 4). If only the results of this selected group of "good" laboratories had been taken into account for the evaluation, then the number of available data would have been greatly reduced and some would still be widely dispersed. In consequence, for some elements there would have been a serious deterioration of the quality of the estimated statistical characteristics instead of the expected improvement.

For chromium and lead (Table 1), the results provided by the "good" laboratories are marked with a plus (+) and those provided by the "bad" laboratories by a minus (−). If only the results of selected "good" laboratories are taken for evaluation, then for chromium the number of data is reduced from 41 to

TABLE 4

Quality of results provided by selected ("good") laboratories for IAEA/Soil-7

Lab. no.	Percentage of results		
	Within conf. limits	Acceptable	Outlying
1	87	6	7
2	80	20	—
3	75	25	—
4	67	16	17
5	65	35	—
6	64	27	9
7	63	37	—
8	62	30	8
9	62	30	8
10	60	40	—
11	60	35	5
12	58	38	4
13	52	39	9

11 and the new median found is $64 \mu\text{g g}^{-1}$ with a confidence interval of $57\text{--}70 \mu\text{g g}^{-1}$. This confidence interval is larger than that shown in Table 3 which was calculated on the basis of all the data. For lead, the number of data is reduced to only 3, all obtained by the same analytical method, and their evaluation is not sensible. A procedure based on the elimination of data provided by the "bad" laboratories is also ineffective. In such a case, the number of available data is significantly larger, 33 for chromium and 22 for lead, but the confidence intervals found are almost the same as, or larger than, those in Table 3, i.e., $57\text{--}66 \mu\text{g g}^{-1}$ for chromium and $55\text{--}75 \mu\text{g g}^{-1}$ for lead.

The above outline suggests that the best basis for the establishment of certified values of trace concentrations is the use of a large number of analytical results with rejection of only obvious outliers. The relatively wide dispersion of data seems to be due rather to the present state of the art of the analytical methods used than to the fact that some participating laboratories produce bad results. If the number of data is sufficiently large, the extremely bad results are rejected as outliers or they are placed at the periphery of the set of data and only slightly affect the values of the median and its confidence limits. Therefore, the values of the overall medians, calculated on the basis of all accepted results, which passed the qualification criteria defined above for Classes A and B are the optimal certified values for trace concentrations.

The empirical qualification criteria, formulated on the basis of large amounts of experimental material, are of a general character; for some special materials or determinations, they may be modified appropriately. Some of these criteria, particularly the first one which accepts relatively large

confidence intervals, may be considered as controversial. In fact, these criteria were formulated as a compromise between the desirable precision and the present analytical possibilities. The accepted confidence intervals, although relatively large, are small in comparison to the dispersion of analytical results presently produced at trace concentrations. In this situation, it seems to be useful to certify the concentrations of a larger number of trace components by accepting a relatively large, but still reasonable, confidence interval than to reduce the number of certified components only to those which can be determined very precisely.

DISCUSSION

The method presented assures the evaluation of interlaboratory comparison data on the basis of objective statistical characteristics with regard to all available analytical information. Moreover, the results obtained at one stage of the procedure are verified by the results of other stages, thus avoiding premature conclusions. The way in which this procedure works is shown in the following examples.

The data distribution plot for lead in SOIL-7 (Fig. 1B) shows a bimodal curve which suggests the existence of two independent sets of data, one displaced with respect to the other, with the central values placed at $58 \mu\text{g g}^{-1}$ and $76 \mu\text{g g}^{-1}$, respectively. The information contained in Table 1 indicates that the results (except four) were obtained by two analytical methods, atomic absorption spectrometry (21 results) and x-ray fluorescence spectrometry (7 results). The groups of results obtained by each of these two methods are dispersed over the total interval of concentration and cannot be correlated with the two maxima of the data distribution plot. This is also confirmed by the fact that both median values calculated for the group of results are placed within the confidence interval of the overall median (Table 3). Also no correlation could be found with the pretreatment methods used by various laboratories for the preparation of the soil samples (data not presented in the table) or with other available information. The existence of two maxima is therefore considered as accidental, without any consequence for the result of evaluation, and the overall median and its confidence interval can be accepted as a correct approximation of the central value. The possibility of accidental formation of a two or even higher modal distribution for a set containing thirty random data is easily confirmed by a computer simulation of many such sets taken from a unimodal population. With an increasing number of data, the bimodal distribution is transformed into a unimodal one with the maximum situated usually between the two primary maxima, not far from the highest one.

A completely opposite situation is found in the case of the chromium determination in SOIL-7. The distribution plot (Fig. 1A) is perfectly unimodal, however, as can be seen in Table 3, the medians of groups of results obtained by the two most frequently used analytical methods, neutron

activation analysis and atomic absorption spectrometry, are situated outside the confidence interval of the overall median. A detailed examination of the data in Table 1 indicates that the groups of results obtained by these two methods are shifted one with respect to the other. Their medians are $52 \mu\text{g g}^{-1}$ and $65 \mu\text{g g}^{-1}$ and their confidence intervals are $61\text{--}74 \mu\text{g g}^{-1}$ and $49\text{--}59 \mu\text{g g}^{-1}$, respectively. Because no additional information is available to prove which of the two methods is the more accurate for the investigated material, and because the few results obtained by other methods are widely dispersed, only the overall median may be considered as the best approximation of the "true" value. Its confidence interval, however, cannot be accepted, because only one of the confidence intervals estimated for the results of individual methods can be correct and it is not known which one. The accepted new confidence interval must therefore contain the intervals of both groups of results and its size should be $49\text{--}74 \mu\text{g g}^{-1}$. The overall median with the new estimated confidence interval passes the qualification criteria and, therefore may be accepted as the certified value of concentration.

The examples discussed above demonstrate that the characteristics resulting from data processing by the proposed procedure enable a relatively simple evaluation even of complex sets of interlaboratory comparison data. Most of the data sets, however, do not show any inconsistencies between the results obtained by different analytical methods or any anomalies in the data distribution. Their evaluation is done automatically and any finer considerations such as those shown above are unnecessary.

CONCLUSIONS

The distribution of results of an interlaboratory comparison of trace component determinations may differ significantly from a normal one. The application of evaluation procedures which assume a normal distribution can cause errors in such cases and, therefore, procedures valid for any type of continuous distribution should be preferred.

A comparison of some types of median and mode values suggests that the differences between these characteristics are usually insignificant in comparison to the size of their confidence intervals resulting from the dispersion of the data. The simple median calculated after rejection of outlying results has been found to be optimal because its confidence interval may be estimated most precisely.

The dispersion of trace component data in interlaboratory comparisons can be quite wide; at low $\mu\text{g g}^{-1}$ and at ng g^{-1} levels, it is often about half an order of magnitude. This situation seems to be due rather to the present state of the analytical methods used and to the lack of suitable reference materials than to improper work by many laboratories. The collected data indicate that at the level of low concentrations even highly qualified laboratories produce a number of outlying results. The wide dispersion of these data causes serious difficulties in the certification of reference materials. The lack

of suitable reference materials, however, is one of the main reasons for the dispersion of the data. This vicious circle may be broken only by finding a reasonable compromise between the desirable precision (the size of confidence intervals) of the values to be accepted for certification and the present analytical possibilities. This compromise, however, can be over the precision and not over the accuracy. All requirements concerning accuracy should be kept very strictly at a high level. It is more useful to have a reference material with certified values less precisely defined than is desirable for some special purposes, than to have no reference material. This seems to be the most effective possibility for gradual improvement of analytical performance and for future improvement of precision in the certification of reference materials for trace components.

The author is grateful to Dr. O. Suschny of the IAEA Laboratory Seibersdorf for helpful discussions and to Ms. K. Lewis-Goettler of the IAEA Computer Section for the preparation of the computer program and the computation of data.

REFERENCES

- 1 R. Dybczynski, A. Tugsavul and O. Suschny, *Analyst* (London), 103 (1978) 734.
- 2 R. Dybczynski, *Anal. Chim. Acta*, 117 (1980) 53.
- 3 S. Abbey, R. A. Meeds and P. G. Belanger, *Geostand. Newsl.*, 3 (1979) 121.
- 4 B. Lister, *Geostand. Newsl.*, 6 (1982) 175.
- 5 P. J. Ellis and T. W. Steele, *Geostand. Newsl.*, 6 (1982) 207.
- 6 R. McGill, J. W. Tukey and W. A. Larsen, *Am. Stat.*, 32 (1978) 12.
- 7 A. Colombo, *Geostand. Newsl.*, 4 (1980) 239.
- 8 S. Abbey, *Geostand. Newsl.*, 5 (1981) 13.
- 9 F. M. Herber and H. J. Pieters, *Fresenius Z. Anal. Chem.*, 311 (1982) 691.
- 10 L. Pszonicki, A. N. Hanna and O. Suschny, International Atomic Energy Agency, Report No. IAEA/RL/112, Vienna, May 1984.
- 11 P. J. Ellis, J. Copelowitz and T. W. Steele, *Geostand. Newsl.*, 1 (1977) 47.
- 12 L. Pszonicki, A. Veglia and O. Suschny, International Atomic Energy Agency, Report No. IAEA/AL/94, Vienna, May 1982.
- 13 L. Pszonicki, A. Veglia and O. Suschny, International Atomic Energy Agency, Report No. IAEA/RL/95, Vienna, June 1982.
- 14 R. D. Remington and M. A. Schork, *Statistic with Application to the Biological and Health Science*, Prentice Hall, Engelwood Cliffs, NJ, 1970.
- 15 P. J. Ellis, *Geostand. Newsl.*, 5 (1981) 161.
- 16 A. Veglia, International Atomic Energy Agency, Report No. IAEA/AL/84, Vienna, August 1981.
- 17 R. Dybczynski, A. Tugsavul and O. Suschny, *Geostand. Newsl.*, 3 (1979) 61.
- 18 L. Kosta and A. R. Byrne, *J. Radioanal. Chem.*, 69 (1982) 117.

THE SUSCEPTIBILITY OF CALIBRATION METHODS TO ERRORS IN THE ANALYTICAL SIGNAL

G. J. KEMP^a

Department of Clinical Chemistry, Northern General Hospital, Sheffield (Great Britain)

(Received 20th February 1985)

SUMMARY

Calibration methods differ as to the number and concentration of calibration standards, and whether these are added to the samples or separate from them. The four main calibration methods (single separate or added standard and multiple separate or added standards) and some modifications are described mathematically and subjected to error-propagation analysis, to examine the likely effects of errors in the analytical signal on the overall accuracy and precision of the concentration estimate. Comparison of the results throws light on the influence of the number, concentration and nature of the calibration standards, the effects of sample and standard replication, and the costs and benefits of blank measurements. It is shown that all standard-addition methods are immune to proportional signal error, but more sensitive to nonlinearity. In separate-standard methods, all bias disappears when the true sample concentration (x_s) is equal to the standard concentration or to the mean standard concentration ($\bar{x}$). Only the multiple separate standard method is unaffected by constant error common to sample and standards, without blanking. In multiple-standard methods, precision is best at $x_s = \bar{x}$. Precision is always improved by increasing the number of sample and standard measurements; standard-addition methods respond best to sample replication. Whichever calibration method is used, recovery correction will eliminate proportional concentration error, at the cost of decreased precision.

There are several kinds of calibration method. Multiple-standard techniques based on simple linear regression are in common use. Their limitations are well-known, and many refinements have been put forward [1–3]. The precision of the method is known to depend on the number and concentration of the calibration standards [4], among other things. The standard-addition variant is often used when matrix interference is a problem [5]. The simplicity of the single-standard calibration method makes it popular in many situations, despite its proneness to systematic error [6, 7].

In this paper, the mathematical basis of the various calibration methods is described, and an error-propagation analysis is used to investigate the way in which errors in the analytical signal can be expected to affect the overall accuracy and precision of the sample concentration estimate. The susceptibility of the different methods to three main types of signal error is

^aPresent address: Department of Human Metabolism and Clinical Biochemistry, University of Sheffield Medical School, Sheffield S10 2RX, Great Britain.

examined, and the effects of changes in the number and concentration of calibration standards, and of the use of blank measurements, are assessed. These theoretical results provide a framework for investigating the suitability of candidate calibration methods, and for examining the errors inherent in existing methods.

The mathematical symbols used are defined in Table 1, and in the text as they occur.

CALIBRATION METHODS

In all quantitative analytical methods, a signal generated from a sample is used to derive an estimate of the unknown analyte concentration. The basic relation between signal, y , and concentration, x , in a method with linear response is

$$y = bx \quad (1a)$$

where b is the slope of the calibration curve, sometimes known as the sensitivity. The estimated value of the sample concentration, $\hat{x}_s$, corresponding to the sample signal, y_s , is therefore given by

$$\hat{x}_s = y_s/b \quad (1b)$$

TABLE 1

Symbols used in this paper

x, y, A	Concentration, signal, absorbance.
A_0, A_1	Blank absorbance, absorbance at time t_1 .
$x_s, \hat{x}_s, \hat{x}'_s$	True, estimated and corrected sample concentration.
$x_c, \bar{x}$	Standard concentration, and mean concentration.
y_s, y_c, y_{s+c}	Signal of sample, standard and spiked sample.
$\bar{y}, \bar{y}_{s+c}$	Mean signal of standards, and spiked samples.
$d\hat{x}_s, s\hat{x}_s$	Bias and standard error of $\hat{x}_s$.
s_y	Standard deviation of signal.
$\bar{U}, V, W$	Coefficients of proportional, constant and
U_y, V_y, W_y	quadratic error in absorbance, signal (y),
U_x, V_x, W_x	and concentration estimate (x).
$a, \hat{a}; b, \hat{b}$	True and estimated calibration intercept and slope.
n, N	Numbers of sample replicates and standards.
x_i, I	Lowest x_i , and the interval between adjacent x 's.

$$D = \left[\sum_1^N (x - \bar{x})(x^2 - \bar{x}^2) \right] / \sum_1^N (x - \bar{x})^2$$

$$E = (x_s - \bar{x})^2 / \sum_1^N (x - \bar{x})^2$$

$$r = (x_s - \bar{x})/\bar{x}$$

$$z = x_s/x_c$$

Sometimes b is known from theory, but more usually an estimate, $\hat{b}$, is made by means of a calibration procedure based on measurements made on standards of known concentration. All signal measurements are liable to error; the subject of this paper is the effect of signal errors on the concentration estimate, and the way in which this is influenced by the choice of calibration method.

An important distinction must be drawn between calibration methods in which the standards are separate from the sample, and those in which they are added to the sample. In separate-standard methods, the calibration curve is drawn by plotting the standard signal, y_i , against the i th standard concentration, x_i . In a standard-addition method, the signal, y_{s+i} , given by the i th spiked sample aliquot is plotted against x_i , the expected concentration increase on spiking. The second important distinction concerns the number of standard concentrations, N , which may be one or many.

There are therefore four main calibration methods. First, in single-standard (or one-point) calibration with a separate standard, which is called here the SSS (single separate standard) method, the calibration graph is the straight line which joins the origin to the point corresponding to the concentration, x_c , and signal, y_c , of the standard. Algebraically [6],

$$\hat{x}_s = y_s x_c / y_c \quad (2a)$$

Secondly, in single-standard calibration with standard addition, called here the SAS (single added standard) method, the calibration plot joins the points corresponding to the spiked and unspiked aliquots of sample, the signals of which are y_{s+c} and y_s , respectively:

$$\hat{x}_s = x_c y_s / (y_{s+c} - y_s) = x_c / [(y_{s+c} / y_s) - 1] \quad (2b)$$

In the absence of error, $y_{s+c} = y_s + y_c$. Thirdly, in multiple-standard calibration with separate standards, called here the MSS (multiple separate standards) method, $\hat{x}_s$ is calculated from the estimated slope and intercept, a , of the calibration plot; $\hat{a}$ and $\hat{b}$ are usually derived from regression analysis of the pairs of values y_i and x_i , and in the absence of error $\hat{a} = a = 0$. It is convenient to write the expression for $\hat{x}_s$ in a form in which $\hat{a}$ is replaced by $\bar{x}$ and $\bar{y}$, the mean concentration and signal of the standards:

$$\hat{x}_s = (y_s - \hat{a}) / \hat{b} = [(y_s - \bar{y}) / \hat{b}] + \bar{x} \quad (3a)$$

Fourthly, in multiple-standard calibration with standard addition, called here the MAS (multiple added standards) method, $\hat{x}_s$ is estimated as the absolute value of the x -axis intercept of the calibration curve [5], which is, in effect, the calibration concentration needed to double y_s :

$$\hat{x}_s = \bar{x} + [(2y_s - \bar{y}_{s+c}) / \hat{b}] \quad (3b)$$

The mean calibration concentration, $\bar{x}$, differs from that in the MSS method in that it necessarily contains $x = 0$, which corresponds to the unspiked sample measurement. (It is possible in principle to use the intercept as an

estimate of y_s ; y_s itself is not then needed; this theoretical possibility is outlined in Appendix 2.) The mean signal, $\bar{y}_{s+c}$, includes y_s itself, and $\bar{y}_{s+c} = y_s + \bar{y}_c = b(x_s + \bar{x})$.

Whichever calibration method is used, it is possible to correct the estimate $\hat{x}_s$ by means of the analytical recovery. An aliquot of sample is spiked to give an expected concentration increment x_c . The calibration method is then used to estimate the concentrations of unspiked and spiked aliquots, $\hat{x}_s$ and $\hat{x}_{s+c}$, respectively, and these are used to estimate the analytical recovery [8]. In a linear method, this estimates the proportional error in $\hat{x}_s$, and so the corrected estimate of the sample concentration, $\hat{x}'_s$, is calculated as

$$\hat{x}'_s = \hat{x}_s / \text{Recovery} = \hat{x}_s x_c / (\hat{x}_{s+c} - \hat{x}_s) = x_c / [(\hat{x}_{s+c} / \hat{x}_s) - 1] \quad (4)$$

There is an obvious analogy with the SAS calibration method shown in Eqn. 2b, where a similar correction is applied at the level of signal rather than concentration estimate.

BIAS IN ESTIMATED SAMPLE CONCENTRATION

From any equation giving $\hat{x}_s$ as a function of y_s and the concentrations and signals of the calibration standards, the theory of error transmission [9], the basic principles of which are outlined in Appendix 1, allows the random and systematic errors in $\hat{x}_s$ to be expressed as a function of those in the sample and standard signals. This section deals with systematic errors, expressed as absolute or relative bias in $\hat{x}_s$, which are symbolised by $d\hat{x}_s$ and $d\hat{x}_s/x_s$, respectively.

For calibration without standards, the relative bias of a quotient is the difference of the relative biases of its components, thus from Eqn. 1b,

$$d\hat{x}_s/x_s = (dy_s/y_s) - (d\hat{b}/b) \quad (5a)$$

For the SSS method, similarly, from Eqn. 2a,

$$d\hat{x}_s/x_s = (dy_s/y_s) - (dy_c/y_c) \quad (5b)$$

and for the SAS method, from the second part of Eqn. 2b,

$$d\hat{x}_s/x_s = -d[(y_{s+c}/y_s) - 1] / [(y_{s+c}/y_s) - 1]$$

Because $d[(y_{s+c}/y_s) - 1] = d(y_{s+c}/y_s)$, and $y_{s+c} - y_s = y_c$,

$$\begin{aligned} d\hat{x}_s/x_s &= -d(y_{s+c}/y_s) y_s / y_c = -[(dy_{s+c}/y_{s+c}) - (dy_s/y_s)] y_{s+c} / y_c \\ &= -[(dy_{s+c}/y_{s+c}) - (dy_s/y_s)] (1 + z) \end{aligned} \quad (5c)$$

where $z = y_s/y_c = x_s/x_c$.

In spectrophotometric procedures, the analytical signal, y , may be an absorbance (A), a blanked absorbance ($A - A_0$), or in kinetic methods [10], a change in absorbance (e.g., $A_2 - A_1$). There are theoretical grounds [6],

for believing that the bias in absorbance can be expressed as

$$dA = (UA) + V + (WA^2) \quad (6a)$$

where U , V , and W are the coefficients of proportional, constant and quadratic error. Photometric errors in absorbance [11] have an exponential dependence on A , but Eqn. 6a should be a reasonable approximation when A is small. It follows from this expression that

$$d(A - A_0) = (UA) + (WA^2) \quad (6b)$$

where A_0 is the absorbance of the (perfect) blank.

In fact, when y is any sort of analytical signal, it should be possible to find a quadratic approximation which will describe a small bias in y , in these general terms:

$$dy = (U_y y) + V_y + (W_y y^2) \quad (6c)$$

Here U_y , V_y and W_y are the proportional, constant and quadratic signal-error coefficients. Sometimes these will simply be a mathematical convenience, but they can often be interpreted in terms related to the analytical process; some examples are given in Table 2. From Eqns. 6a and 6b, in equilibrium spectrophotometric methods, $U_y = U$, $W_y = W$, and $V_y = V$ in unblanked methods and zero with blanking.

Equations 5a, 5b and 5c give $d\hat{x}_s/x_s$ as a function of dy ; by using Eqn. 6c, this can be replaced by the appropriate error coefficients, to show how the three types of signal error contribute to the overall error in $\hat{x}_s$. Table 3 gives the results of this analysis for single-standard calibration methods. It is assumed for simplicity that V and W are the same for both sample and standard measurements. In separate-standard methods this may well not be true (if, for example, samples and standards are made up in different matrices), and the error equations can be modified accordingly. It is an advantage of standard-addition methods that because samples and standards share the same matrix, their error coefficients are likely to be similar; it is assumed here that they are identical.

TABLE 2

Likely analytical causes of the three types of signal error

Constant error	Proportional error	Quadratic error
Sample turbidity	Volumetric error	Decay/dissociation of product ^a
Reagent absorbance	Gravimetric error	Reagent depletion ^a
Nonspecificity	Incomplete separation or derivatisation	Instrumental nonlinearity
Zero error/drift ^a	Loss/decay of analyte	
Carryover ^a	Matrix evaporation	
Contamination	Errors in timed processes	

^aSee [6] for details.

TABLE 3

Bias in sample concentration estimate in the four main calibration methods^a

Method	Expression for bias, $d\hat{x}_s/x_s$
Single separate standard (SSS)	$\Delta U_y + [V_y(y_s^{-1} - y_c^{-1})] + [W_y(y_s - y_c)]$
Single added standard (SAS)	$(V_y/y_s) - (W_y \cdot y_s + c)$
Multiple separate standards (MSS)	$\Delta U_y + (\Delta V_y/y_s) + [W_y b r \{[\bar{x}(r + 2)] - D\}/(1 + r)]$
Multiple added standards (MAS)	$(V_y/y_s) - [W_y b \{[\bar{x}(r^2 + 2r + 2)] - (Dr)\}/(1 + r)]$

^a $\Delta U_y = U_y(\text{sample}) - U_y(\text{standard})$; similarly ΔV_y . When y is a blanked absorbance, V_y and ΔV_y are zero.

Some conclusions can be drawn from Table 3 about the way in which signal errors contribute to bias in the concentration estimate in single-standard calibration methods. Thus blanking eliminates constant absorbance errors in equilibrium procedures (kinetic methods are immune to constant errors anyway [10]). Constant signal errors are most important at low sample and standard concentrations [7]; quadratic errors are more important at higher concentrations. Further, the SAS method is immune to proportional error, because U is identical for samples and standards: it is more susceptible to quadratic error than the SSS method, especially at high standard concentrations. Finally, when sample and standard errors are equal, all errors disappear in the SSS method, provided that the error coefficients are identical for sample and standard (if they are not, net bias in $\hat{x}_s$ may still be zero at some other values of x_s and x_c); sensible choice of calibration concentration can therefore minimise the effects of signal errors. However, constant and quadratic errors affect accuracy in the SAS method at all values of x_s and x_c (except that $d\hat{x}_s/x_s = 0$ in the unlikely event that $V_y/W_y = y_{s+c}y_s$); the optimum value of x_c is less easy to judge.

For multiple-standard calibration, it is convenient to apply the analysis of errors to forms of the equations for $\hat{x}_s$ in which the intercept does not appear. For the MSS method, using the second part of Eqn. 3a and the results in Appendix 1 yields

$$\begin{aligned} d\hat{x}_s &= d[(y_s - \bar{y})/\hat{b}] = [(y_s - \bar{y})/b] \{ [d(y_s - \bar{y})/(y_s - \bar{y})] - (d\hat{b}/b) \} \\ &= [(dy_s - d\bar{y})/b] - [(y_s - \bar{y})d\hat{b}/b^2] \end{aligned} \quad (7)$$

Next, $d\hat{b}$ must be evaluated. From the theory of regression analysis [9],

$$\hat{b} = \sum_1^N [(y - \bar{y})(x - \bar{x})] / \sum_1^N (x - \bar{x})^2 \quad (8a)$$

and so

$$d\hat{b} = \sum_1^N d[(y - \bar{y})(x - \bar{x})] / \sum_1^N (x - \bar{x})^2 = \sum_1^N [(x - \bar{x})(dy - d\bar{y})] / \sum_1^N (x - \bar{x})^2 \quad (8b)$$

Substituting for the signal errors by means of Eqn. 6d,

$$d\hat{b} = \sum_1^N \{ [U_y(y - \bar{y})(x - \bar{x})] + [W_y(y^2 - \bar{y}^2)(x - \bar{x})] \} / \sum_1^N (x - \bar{x})^2 \quad (8c)$$

where U_y and W_y refer to standard measurements. From Eqns. 8a, 8c and 1a,

$$d\hat{b}/b = U_y + \left\{ W_y b \sum_1^N [(x - \bar{x})(x^2 - \bar{x}^2)] / \sum_1^N (x - \bar{x})^2 \right\}$$

which will be abbreviated to

$$d\hat{b}/b = U_y + (W_y b D) \quad (8d)$$

It is shown in Appendix 3 that for N standards, equally spaced at concentration intervals I starting from zero, $D = I(N - 1)$. Substituting for dy and $d\bar{y}$ in Eqn. 7 by means of Eqn. 6c, and for $d\hat{b}$ by means of Eqn. 8d, gives an expression for $d\hat{x}_s/x_s$ in terms of the signal-error coefficients for the MSS method: this is shown in Table 3.

Similarly, for the MAS method, from Eqn. 3b,

$$\begin{aligned} d\hat{x}_s &= d[(2y_s - \bar{y}_{s+c})/b] = [(y_s - \bar{y})/b] \{ [d(2y_s - \bar{y}_{s+c})/(y_s - \bar{y})] - (d\hat{b}/b) \} \\ &= [(2dy_s - d\bar{y}_{s+c})/b] - [(y_s - \bar{y})d\hat{b}/b^2] \end{aligned} \quad (9)$$

bearing in mind that $\bar{y}_{s+c} = y_s + \bar{y}$, and so $2y_s - \bar{y}_{s+c} = y_s - \bar{y}$. In this method, the slope is estimated by

$$\hat{b} = \sum_1^N [(x - \bar{x})(y_{s+c} - \bar{y}_{s+c})] / \sum_1^N (x - \bar{x})^2$$

where $\bar{x}$ includes one zero for each sample measurement. By using an analysis similar to that used above for the MSS method,

$$\begin{aligned} d\hat{b} &= \sum_1^N [(x - \bar{x})(dy_{s+c} - d\bar{y}_{s+c})] / \sum_1^N (x - \bar{x})^2 \\ &= \sum_1^N \{ [U_y(y_{s+c} - \bar{y}_{s+c})(x - \bar{x})] + [W_y(y_{s+c}^2 - \bar{y}_{s+c}^2)(x - \bar{x})] \} / \sum_1^N (x - \bar{x})^2 \end{aligned}$$

Because $y_{s+c} - \bar{y}_{s+c} = y - \bar{y}$, and $y_{s+c}^2 - \bar{y}_{s+c}^2 = y^2 - \bar{y}^2 + [2y_s(y - \bar{y})]$, then from Eqn. 1a,

$$d\hat{b}/b = U_y + W_y b(D + 2x_s) \quad (10)$$

where D is as defined above. As expected, in the MAS method, slope error caused by the quadratic signal error depends on the sample concentration,

which determines the starting signal for the calibration graph. Substitution for $d\hat{b}$ and for the signal errors in Eqn. 9 gives an expression for $d\hat{x}_s/x_s$ as a function of signal error in the MAS method; this is shown in Table 3.

From these results, some conclusions can be drawn about the effects of the three types of absorbance error on the overall bias in $\hat{x}_s$ in multiple-standard calibration methods. First, proportional signals do not affect the MAS method; constant errors do. Secondly, constant errors which are identical for samples and standards do not affect the MSS method. Thirdly, both methods are susceptible to quadratic error; the MAS method is the more sensitive, and suffers more at high sample concentrations; in the MSS method, quadratic error has no effect when $x_s = \bar{x}$; in both methods, its effect increases with the number and concentration of the standards. Finally, blanking removes constant errors.

Clearly, if quadratic error is significant, the assumption of linearity of response (Eqn. 1a), must be abandoned and some more appropriate curve-fitting procedure adopted. A further effect of quadratic error in a regression-based calibration method will be to increase the apparent signal variance about the fitted line.

The results in Table 3 can also be used to express $d\hat{x}_s$ in terms of three concentration-based error coefficients, as have been used elsewhere. These are defined so that

$$d\hat{x}_s = (U_x x_s) + V_x + (W_x x_s^2) \quad (11)$$

The constant concentration-error coefficient, V_x , is the bias in $\hat{x}_s$ at $x_s = 0$. It is interesting to examine the way in which signal errors contribute to these three coefficients. By using Eqn. 1a the expressions in Table 3 can be rewritten to give $d\hat{x}_s$ in terms of x_s and x_s^2 ; by comparison with Eqn. 11, U_x , V_x and W_x can be expressed in terms of U , V and W . As an example, in the SSS method,

$$\begin{aligned} d\hat{x}_s &= (\Delta U_y x_s) + [V_y x_s (y_s^{-1} - y_c^{-1})] + [W_y x_s (y_s - y_c)] \\ &= (\Delta U_y x_s) + [V_y x_s (x_s^{-1} - x_c^{-1})/b] + [W_y b x_s (x_s - x_c)] \\ &= (V_y/b) + \{[\Delta U_y - (V_y/y_c) - (W_y y_c)] x_s\} + (W_y b x_s^2) \end{aligned} \quad (12)$$

and so $V_x = V_y/b$, $W_x = W_y b$, and $U_x = U_y - (V_y/y_c) - (W_y y_c)$. Further examples are given in Table 4, which shows the composition of the three concentration-error coefficients for each of the calibration methods examined in Table 3.

Several points emerge from these results. In single-standard methods, only constant signal error contributes to constant concentration error; in multiple-standard methods quadratic signal error also contributes to V_x , because non-linearity affects estimation of the intercept. Further, only quadratic signal error can give rise to quadratic concentration error (its sign being reversed in standard-addition methods); it also contributes to U_x in all methods. Proportional signal error, however, appears only as proportional concentration error.

TABLE 4

The three coefficients of concentration error expressed in terms of signal-error coefficients and calibration signals^a

Method	Constant error	Proportional error	Quadratic error
SSS	V_y/b	$\Delta U - (V_y/y_c) - (W_y y_c)$	$W_y b$
SAS	V_y/b	$-W_y y_c$	$-W_y b$
MSS	$(\Delta V_y/b) + [W_y \bar{y}(D - \bar{x})]$	$\Delta U_y - (W_y b D)$	$W_y b$
MAS	$(V_y/b) + [W_y \bar{y}(D - \bar{x})]$	$-W_y b D$	$-W_y b$

^aConstant error = V_x ; proportional error = U_x ; quadratic error = W_x : calculated from Eqn. 11 and Table 3. ΔV_y is assumed to be zero in single-standard methods. If y is a blanked absorbance, then $V_y = 0$.

Finally, the standard concentration(s) affect proportional error in all methods, and constant error in multiple-standard methods.

Applying an analysis of errors to Eqn. 4, and substituting for $d\hat{x}_s$ by means of Eqn. 11 yields an expression for $d\hat{x}'_s$ in terms of V_x and W_x :

$$d\hat{x}'_s/x_s = (V_x/x_s) - [W_x(x_s + x_c)] \quad (13)$$

Thus the use of a recovery correction removes proportional concentration error at the cost of increased sensitivity to quadratic error, which becomes more significant as x_c increases, although the net bias is zero if x_c happens to be $(V_x/x_s) - (W_x x_s)$. One component of proportional concentration error is proportional signal error, and this is the main reason for using a recovery correction when there is incomplete extraction or derivatisation of analyte, for example.

In Table 5, the analysis of bias discussed above is applied to a hypothetical example. The table shows the estimated sample concentration compared to its true value over a range of concentrations, for each of the calibration methods applied to an equilibrium spectrophotometric procedure which suffers from significant absorbance errors. Notable features are how net bias is zero at $x_s = x_c = \bar{x}$ in the separate-standard methods, and the large bias that quadratic absorbance error introduces in standard-addition methods, particularly at high sample concentrations.

The use of blank correction

The use of blank corrections for constant absorbance errors was described above, and the effect on precision will be discussed shortly. A few general comments must be made here. From the point of view of bias, the zero-sample, which contains everything but the analyte, is the perfect blank, and its absorbance is equal to V . It is assumed here that this is true of any blank, although in fact sample and reagent blanks of the conventional kind merely reproduce the major components of V .

TABLE 5

Estimated and true sample concentrations for various calibration methods applied to a spectrophotometric procedure with absorbance bias^a

Method	True sample concentration						
	0	1	2	3	4	5	6
SSS	0.40	1.30	2.16	3.00	3.81	4.59	5.33
SSS+B	0.00	1.03	2.03	3.00	3.94	4.85	5.73
SAS	0.40	1.46	2.55	3.67	4.82	6.00	7.21
SAS+B	0.00	1.06	2.15	3.27	4.42	5.60	6.81
MSS	-0.14	0.94	2.02	3.00	3.99	4.94	5.87
MAS	0.27	1.37	2.51	3.60	4.68	6.09	7.35
MAS+B	-0.14	0.97	2.11	3.20	4.28	5.69	6.95
MMAS	0.54	1.64	2.78	3.94	5.14	6.36	7.62
MMAS+B	0.14	1.24	2.38	3.54	4.74	5.96	7.22

^aThe numbers shown are the estimated sample concentrations, calculated as $\hat{x}_s = x_s + d\hat{x}_s$ from expressions in Table 3. MMAS is the modified MAS method, discussed in Appendix 2; B signifies a blanked method. For the single-standard methods, $x_c = 3$; for multiple-standard methods, $x_i = 0$, $I = 1$, and $N = 7$ (so $\bar{x} = 3$); for all methods $b = 0.05$; the absorbance errors are $U = -0.08$, $V = 0.02$ and $W = -0.3$ for samples and standards.

From the point of view of precision, the most important distinction concerns the relation between sample and blank. A sample blank measurement is often made on a separate aliquot from that used for the sample signal measurement. Blanking therefore removes any component of constant error that does not vary between different aliquots. Sometimes, however, the blank and sample measurements are made on the same aliquot, e.g., before and after reagent addition. This resembles a "two-point" kinetic method, and removes any component of constant error which does not vary within a single aliquot. A reagent blank, or a zero-sample taken right through the analytical process, usually serves a complete batch of samples. Only errors that do not vary between samples can be corrected for in this way. Obviously, errors that are not removed by blanking contribute to overall bias and precision. As will be seen, a gain in accuracy may cost precision.

PRECISION OF ESTIMATED SAMPLE CONCENTRATION

The random error in $\hat{x}_s$ is calculated in a similar way to the bias (see Appendix 1), and is expressed as the absolute or relative standard deviation, $s_{\hat{x}_s}$ or $s_{\hat{x}_s}/x_s$, respectively. For calibration without standards, from Eqn. 1b, and because the relative variance of a quotient is the sum of the relative variances of its components,

$$(s_{\hat{x}_s}/x_s)^2 = (s_{y_s}/y_s)^2 + (s_b/b)^2 \quad (14a)$$

Similarly, for the SSS method, from Eqn. 2a,

$$(s_{\hat{x}_s}/x_s)^2 = (s_{y_s}/y_s)^2 + (s_{y_c}/y_c)^2 \quad (14b)$$

and for the SAS method, from Eqn. 2b, by a derivation similar to that of Eqn. 5c,

$$\begin{aligned} (s_{\hat{x}_s}/x_s)^2 &= s_{(y_s+c/y_s)}^2 / [(y_s+c/y_s) - 1]^2 = s_{(y_s+c/y_s)}^2 z^2 \\ &= [(s_{y_s+c/y_s+c})^2 + (s_{y_s}/y_s)^2] (1+z)^2 \end{aligned} \quad (14c)$$

When y is an absorbance, its bias can be expressed in terms of U , V and W (see above). If any variation in W is ignored, and if U and V are the mean values of population distributions with variances s_U^2 and s_V^2 , respectively, then if all errors are small, the variance in absorbance, s_A^2 , is given approximately by

$$s_A^2 = s_V^2 + (s_U A)^2 \quad (15)$$

which is the same as regarding signal variance as the sum of two components: a constant standard deviation, and a constant relative standard deviation [12]. Of course, s_U and s_V can exist even when the mean values are zero.

The variance of the constant absorbance error, s_V^2 , or more generally, of the constant signal error, $s_{V_y}^2$, is equal to the signal variance of the zero-sample. This is important in the theory of detection limits [13, 14], and so the effect of blanking on $s_{V_y}^2$ will be discussed briefly.

The case of $s_{V_y}^2$ measured over a batch of samples (e.g., as the variance in x_s for a batch of different zero-samples) is discussed first. It follows from Eqn. 15 that when $y = A$, $s_{V_y} = s_V$. When $y = A - A_0$, and A_0 is measured only once for a batch of samples, then any component of V which remains constant over the whole batch is eliminated, and the variances of the remaining components are increased by the subtraction of A_0 from each A ; it follows that $s_{V_y}^2 = 2s_V^2$. Now, s_V^2 is the sum of the between-sample (BS) and within-sample (WS) variance in V . When A_0 is measured once for each sample, any component of V which remains constant for each measurement on a single sample is eliminated, and the variances of the others are added: it follows that $s_{V_y}^2 = 2s_{V(WS)}^2$. Now, $s_{V(WS)}^2$ is the sum of between-aliquot (BA) and within-aliquot (WA) variance. When A and A_0 are measured on the same aliquot of each sample, only those components of V which can vary between two measurements on the same aliquot will fail to be eliminated: it follows that $s_{V_y}^2 = 2s_{V(WA)}^2$. This is analogous to the "two-point" kinetic method, where only components of V that vary between t_1 and t_2 fail to be eliminated.

Some conclusions can be drawn about the effect of blanking on precision at $x_s = 0$. First, batch-blanking always increases imprecision; when it is used (e.g., a reagent blank), however, it is assumed that s_V will be fairly small. Secondly, same-sample blanking will increase precision if $(s_{V(WS)}/s_V)^2 \leq 0.5$, i.e., if less than half the total variance in V acts within-sample. Finally, same-aliquot blanking will increase precision if $(s_{V(WA)}/s_V)^2 \leq 0.5$, i.e., if less than half the total variance in V acts within-aliquot.

The case of $s_{V_y}^2$ measured on several aliquots of a single zero-sample is now discussed. Batch-blanking and same-sample blanking are now effectively the same thing, and $s_{V_y}^2 = 2s_{V(WS)}^2$; same-aliquot blanking gives $s_{V_y}^2 = 2s_{V(WA)}^2$, which represents improvement if less than half of the within-sample variance acts within-aliquot.

Various types of constant error can be expected to vary in different ways: for reagent absorbance, s_V should be low; for sample turbidity or analytical nonspecificity, $s_{V(WS)}$ should be low, but $s_{V(BS)}$ may be significant. With instrumental errors such as baseline drift or with carryover, $s_{V(BA)}$ may be a significant fraction of s_V . If the approximate composition of s_V can be assessed in this way, the effects of blanking on precision at $x_s = 0$ can be predicted from the results given above. In a similar way, standard-addition methods are usually (but not necessarily) based on measurements made on several spiked and unspiked aliquots of each sample. Any component of proportional signal error which does not vary within-sample will be eliminated, and only the within-sample variance in U will contribute to overall imprecision.

It is now possible to calculate $s_{\hat{x}_s}^2$ in terms of the variance of the constant and proportional absorbance errors, for single-standard calibration methods (blanked and unblanked) applied to equilibrium spectrophotometric procedures. For the SSS method, from Eqns. 14b and 15,

$$(s_{\hat{x}_s}/x_s)^2 = 2s_U^2 + [s_{V_y}^2(A_s^{-2} + A_c^{-2})] \quad (16a)$$

where $s_{V_y}^2$ is as defined above. For the SAS method, from Eqns. 14c and 15,

$$(s_{\hat{x}_s}/x_s)^2 = (1 + z)^2 \{2s_U^2 + [s_{V_y}^2(A_{s+c}^{-2} + A_s^{-2})]\} \quad (16b)$$

and when A_s and A_{s+c} are measured on separate aliquots, the relevant s_U is $s_{U(WS)}$, the within-sample variance in U .

Multiple-standard methods are more difficult to assess, because the estimates of slope and intercept are correlated. It is necessary to use forms of the equations from which the intercept has been eliminated. For the MSS method, by applying the usual analysis of errors to Eqn. 3a,

$$\begin{aligned} s_{\hat{x}_s}^2 &= s_{[(y_s - \bar{y})/\hat{b}]}^2 = [(y_s - \bar{y})/b]^2 \{ [s_{(y_s - \bar{y})}/(y_s - \bar{y})]^2 + (s_{\hat{b}}/b)^2 \} \\ &= [(s_{y_s}^2 + s_{\bar{y}}^2)/b^2] + [(y_s - \bar{y})^2 s_{\hat{b}}^2/b^4] \end{aligned} \quad (17a)$$

and for the MAS method, from Eqn. 3b, analogously,

$$s_{x_s}^2 = [(s_{2y_s}^2 + s_{2y_{s+c}}^2)/b^2] + [(y_s - \bar{y})^2 s_{\hat{b}}^2/b^4] \quad (17b)$$

From the theory of regression analysis [9], these component variances can be evaluated as follows: if s_y^2 is the signal variance (the residual variance about the regression line), and if n sample replicates and N standards are used, then $s_{y_s}^2 = s_{2y_s}^2 = s_y^2/n$; $s_{\bar{y}}^2 = s_y^2/N$ and $s_{2y_{s+c}}^2 = s_y^2/(N + n)$; in both methods the variance of the slope is given by $s_{\hat{b}}^2 = s_y^2/\Sigma_1^N (x - \bar{x})^2$, bearing in mind that the sum of squares in the MAS method contains one zero for each of the n

sample replicates. Finally, from Eqn. 1a, $\bar{y} = b\bar{x}$ and $\bar{y}_{s+c} = b(x_s + \bar{x})$, where $\bar{x}$ in the MAS method contains one zero for each sample replicate. Substitution for these quantities in Eqns. 17a and 17b gives the expressions shown in Table 6 (see also below). The term $(x_s - \bar{x})^2 / \sum_1^N (x - \bar{x})^2$ has been called the eccentricity term, E [4]; here its value at $x_s = 0$ is called E_0 . If the relative distance of x_s from $\bar{x}$ is defined as $r = (x_s - \bar{x})/\bar{x}$, then the value of the eccentricity term at any sample concentration is $E = E_0 r^2$. The term r is thus related to the eccentricity parameter [4], which is defined as $\rho = (x_s - \bar{x})/(x_N - x_1)$: in fact, $r = \rho(x_N - x_1)/\bar{x}$. From the results shown in Appendix 3, if N standards are equally spaced at concentration intervals I , and the lowest standard, x_1 , is equal to zero, then E_0 is given by $3(N-1)/[N(N+1)]$: some values are shown in Table 7, which shows how E_0 approaches zero as N increases.

For simple linear regression to be valid, several conditions must be met [4]. One of these is the condition of homoscedasticity, which requires that s_y be independent of y over the range of interest; if y is an absorbance, this is equivalent to the condition that s_U be negligible. If this condition is met, the earlier expressions for precision in single-standard methods can be rewritten, to facilitate direct comparison with the multiple-standard methods. It is convenient to assume also that there are n sample and N standard replicates, and amend the equations accordingly: the resulting expressions, from Eqns. 14b and 14c, are given in Table 6.

The expression for $s_{\hat{x}_s}^2$ in the SSS method contains a term independent of x_s , and a term proportional to z^2 , and so to x_s^2 : these correspond to "additive" and "multiplicative" random error [15], and if s_U or errors in calibration concentration cannot be neglected they will contribute to the latter. It is clear from Table 6 that an "additive" component of $s_{\hat{x}_s}^2$ exists in the other methods as well, but the dependence on x_s is (theoretically) more complicated; the non-additive terms are proportional to $(1+z)^2$ in the SAS method, and to r^2 in multiple-standard methods.

It is now possible to compare precision in the various calibration techniques.

TABLE 6

Precision of sample concentration estimate in various calibration methods^a

Method	Expression for precision, $(s_{\hat{x}_s}/x_s)^2$
SSS	$(s_y/b)^2 [(1/n) + (z^2/N)]$
SAS	$(s_y/b)^2 \{[(1+z)^2/n] + (z^2/N)\}$
MSS	$(s_y/b)^2 [(1/n) + (1/N) + (r^2 E_0)]$
MAS	$(s_y/b)^2 [(1/n) + (N+n)^{-1} + (r^2 E_0)]$

^a s_y is assumed to be independent of y .

TABLE 7

Values of E_0 for equally-spaced calibration concentrations starting at $x_1 = 0$, expressed as a function of the number of standards, N

N	3	4	5	7	10	15	∞
E_0	0.50	0.45	0.40	0.32	0.25	0.18	0.00

Single-standard methods

From the results in Table 6, the ratio of s_{x_s} in the SAS method to that in the SSS method can be calculated. If $n = N = 1$, and conditions are identical in both methods, this ratio is equal to $[(1 + z)^2 + z^2]^{1/2}/(1 + z^2)^{1/2}$. Some values are given in Table 8, which shows that precision is always better in the SSS method, and that the difference is greatest when x_s is about half x_c .

Both methods respond identically to standard replication; however, the SAS method shows more improvement in precision with sample replication, because the N term is multiplied by $(1 + z)^2$, which is larger than one.

Multiple-standard methods

If an MSS method with $N + 1$ standards (including zero) is compared with an MAS method with N standards and one sample measurement, then precision is identical in both methods (however, if constant signal error is present or possible, the MAS method will need to be blanked, which may increase s_y , as was shown above). The two methods differ in their response to sample replication. The MSS method behaves like the SSS method: in the MAS method, however, the use of sample replicates has the additional effect of adding extra zeros to $\bar{x}$ and $\Sigma_1^N (x - \bar{x})^2$, and so decreasing E_0 . Precision at $x_s = 0$ is therefore more sensitive to sample replication in the MAS method, because each sample adds one more point to the regression line; the MAS method therefore resembles single-standard methods in that sample replication alone can improve precision at $x_s = 0$ indefinitely. Furthermore, at non-zero x_s (i.e., at $r \neq -1$), sample replication has the additional effect of reducing $\bar{x}$ and thereby changing r : when $x_s < \bar{x}$ (r negative), sample replication reduces r^2 ; when $x_s > \bar{x}$ (r positive), r^2 is increased; if x_s starts smaller than $\bar{x}$, and n is increased, then eventually $\bar{x}$ will fall below x_s , and so r must eventually become positive. The overall effect on precision at nonzero x_s will be the result of these three processes: reduction of sample measurement error, addition of points to the regression line, and reduction in $\bar{x}$. In the MSS method, only the first applies.

Single and multiple-standard methods compared

Precision at $x_s = 0$ is always better in single-standard methods, where $s_{x_s}^2$ at $x_s = 0$ is $(s_y/b)^2/n$, than in multiple-standard methods, where it is given by $[(s_y/b)^2/n] + s_a^2$. The variance, s_a^2 , of the estimated intercept is equal to

TABLE 8

The ratio of the imprecisions of the sample concentration estimates in comparable SSS and SAS methods^a

z	0.00	0.25	0.50	1.00	1.50	2.00	3.00	∞
Ratio	1.00	1.24	1.41	1.50	1.62	1.61	1.58	$2^{1/2} \simeq 1.41$

^aThe ratio shown is that of $s_{\hat{x}_s}$ in the SAS method to $s_{\hat{x}_s}$ in the SSS method, with $n = N = 1$. The maximum value of the ratio, $[2(5^{1/2} + 2)/(5^{1/2} + 1)]^{1/2} \simeq 1.62$, is reached at $z = 1/2(5^{1/2} + 1) \simeq 1.62$.

$s_y^2(N^{-1} + E_0)$, and approaches zero as N increases; in fact, when N is very large, $s_{\hat{x}_s}^2 = (s_y/b)^2/n$ in all methods at all concentrations, which represents the irreducible error of the sample measurement.

Comparing precision at nonzero x_s is more difficult, but, as an example, an SSS method with N standards of concentration x_c , and an MSS method with N standards of mean concentration $\bar{x} = x_c$ may be compared. From Table 6, they will have the same precision at a sample concentration given by $(z^2 - 1) = NR^2E_0 = N(z - 1)^2E_0$. Solution of this quadratic gives $z = 1$ and $z = (NE_0 + 1)/(NE_0 - 1)$. This second solution will be positive if NE_0 exceeds one, which is certainly the case for equally-spaced standards. It follows that the two methods have equal precision at $x_s = x_c$, and at $x_s = x_c(NE_0 + 1)/(NE_0 - 1)$, and that between these two concentrations the MSS method has the better precision.

Lastly, by applying the usual analysis of errors to Eqn. 4, the cost in precision of using a recovery correction can be calculated. The derivation resembles that of Eqn. 14c. If $s_{\hat{x}_s}$ can be treated as constant, then

$$s_{\hat{x}_s'}^2 = s_{\hat{x}_s}^2[z^2 + (1 + z)^2] \quad (18a)$$

where $z = x_s/x_c$, as before. If x_s is approximately equal to x_c , therefore, then $s_{\hat{x}_s}$ is roughly doubled by the use of the correction. If, however, it is truer to say that the relative precision of the calibration method, $s_{\hat{x}_s}/x_s$, is constant, then Eqn. 18a can be rewritten as

$$(s_{\hat{x}_s'}/x_s)^2 = 2(1 + z)^2(s_{\hat{x}_s}/b)^2 \quad (18b)$$

and so if x_s and x_c are approximately equal, then $s_{\hat{x}_s}$ is roughly tripled by the use of the correction. In most cases, the loss of precision will be between those extremes.

Table 9 shows the relative and absolute precision of the various calibration methods applied to a hypothetical equilibrium spectrophotometric procedure. The single-standard methods are shown both with a single standard measurement ($N = 1$), and with seven standard replicates ($N = 7$): this illustrates not only the beneficial effect of standard replication, but also the differing effects of grouping several standards at one concentration, and

TABLE 9

Absolute and relative standard error of the sample concentration estimate for various calibration methods applied to a spectrophotometric procedure^a

Method	Standard errors for sample concentrations 0–6						
	0	1	2	3	4	5	6
SSS ^b	0.060	0.063 (6.3%)	0.072 (3.6%)	0.085 (2.8%)	0.10 (2.5%)	0.12 (2.3%)	0.13 (2.2%)
SSS ^c	0.060	0.061 (6.1%)	0.062 (3.1%)	0.064 (2.1%)	0.067 (1.7%)	0.071 (1.4%)	0.075 (1.3%)
SAS ^b	0.060	0.083 (8.3%)	0.11 (5.4%)	0.13 (4.5%)	0.16 (4.0%)	0.19 (3.8%)	0.22 (3.6%)
SAS ^c	0.060	0.080 (8.0%)	0.10 (5.1%)	0.12 (4.1%)	0.14 (3.6%)	0.16 (3.3%)	0.19 (3.1%)
MSS } MAS }	0.073	0.068 (6.8%)	0.065 (3.3%)	0.064 (2.1%)	0.065 (1.6%)	0.068 (1.4%)	0.073 (1.2%)
MMAS	0.041	0.051 (5.1%)	0.061 (3.1%)	0.072 (2.4%)	0.082 (2.1%)	0.093 (1.9%)	0.11 (1.7%)

^aCalibration methods as in Table 5, with $s_y = 0.003$. The table shows s_{x_s} , with $(100s_{x_s}/x_s)\%$ in parentheses. ^bSingle standard measurement ($N = 1$). ^cReplicate standard measurements ($N = 7$).

distributing the same number about that mean concentration. It can be noted that precision at $x_s = 0$ is better in the single-standard methods, where it is unaffected by standard replication. The SSS method ($N = 7$) continues to have the better precision up to $x_s = 3$, above which the MSS method is the more precise; above $x_s = 7.8$ the SSS method ($N = 7$) is again the better of the two. Also shown in Table 9 is the “modified” MAS method (MMAS), which is discussed in Appendix 2; this has the best precision of all at $x_s = 0$, and the worst when $x_s \geq 3$.

APPENDIX 1: THE THEORY OF ERROR TRANSMISSION

A variable y , which is a function of $x_1 \dots x_n$ is considered: $y = f(x_1 \dots x_n)$. The theory of error transmission describes how errors in $x_1 \dots x_n$ are transmitted to y . It assumes that all errors are small, and that random errors are independent and approximately Gaussian. The general formulae are

$$dy = (\partial f / \partial x_1) dx_1 + \dots (\partial f / \partial x_n) dx_n$$

$$s_y^2 = (\partial f / \partial x_1)^2 s_{x_1}^2 + \dots (\partial f / \partial x_n)^2 s_{x_n}^2$$

Some useful special cases are as follows. If $y = x_1 + x_2$, then $dy = dx_1 + dx_2$, and if $y = x_1 - x_2$, then $dy = dx_1 - dx_2$. In both cases, $s_y^2 = s_{x_1}^2 + s_{x_2}^2$. Further, if $y = x_1/x_2$, then $dy/y = (dx_1/x_1) - (dx_2/x_2)$, and if $y = x_1 x_2$, then $dy/y = (dx_1/x_1) + (dx_2/x_2)$. In both cases, $(s_y/y)^2 = (s_{x_1}/x_1)^2 + (s_{x_2}/x_2)^2$. A further assumption is made here, that errors in the calibration concentrations are negligible, which is obviously not necessarily true.

APPENDIX 2: ANOTHER VERSION OF THE MAS METHOD

In the MAS method, in the absence of error, the intercept, a , is equal to y_s ; x_s could therefore be estimated as $\hat{x}_s = \hat{a}/\hat{b} = \{(\bar{y}_{s+c}/\bar{b})\} - \bar{x}$. In principle, the sample signal itself is not necessary. The bias in $\hat{x}_s$ is given by

$$d\hat{x}_s = (\bar{y}_{s+c}/b)[(d\bar{y}_{s+c}/\bar{y}_{s+c}) - (d\bar{b}/b)]$$

$$d\hat{x}_s/x_s = [d\bar{y}_{s+c} - (\bar{y}_{s+c} d\bar{b}/b)]/\bar{y}_s$$

Substituting for $d\bar{y}_{s+c}$ and $d\bar{b}/b$ by means of Eqns. 6c and 10,

$$\begin{aligned} d\hat{x}_s/x_s &= (V_y/y_s) + \{W_y \bar{y}_{s+c} [\bar{y}_{s+c} - (bD) - (2b x_s)]/y_s\} \\ &= (V_y/y_s) - \{W_y b [x_s^2 - \bar{x}^2 + (D x_s) + (D \bar{x})]/x_s\} \\ &= (V_y/y_s) + [W_y b \{[r(r+1)\bar{x}] + [D(r+2)]\}/(r+1)] \end{aligned}$$

This differs in the W -term from the conventional expression given in Table 3. The absolute value of this term is larger in this modified method if $D > \bar{x}$; with equally-spaced standards starting at zero (which represents the unspiked sample measurement), then $D = 2\bar{x}$, and so the modified method has the greater sensitivity to quadratic error.

Similarly, the imprecision is given by

$$s_{\hat{x}_s}^2 = [s_{\bar{y}_{s+c}}^2 + (\bar{y}_{s+c} s_{\bar{b}}/b)^2]/b^2$$

and so, from Eqn. 1a and the theory of regression analysis [9],

$$s_{\hat{x}_s}^2 = (s_y/b)^2 \{(N+n)^{-1} + [(x_s + \bar{x})^2 / \sum_1^{N+n} (x - \bar{x})^2]\} = (s_y/b)^2 \{(N+n)^{-1} + [(r+2)^2 E_0]\}.$$

Comparison with the conventional expression in Table 6 shows that precision is better in the modified method at $x_s = 0$, but this is now a minimum, and $s_{\hat{x}_s}$ increases monotonically with increasing x_s . Precision in the two methods is identical when $(1/n) + (r^2 E_0) = (r+2)^2 E_0$, and this is so when $r = [1/(4nE_0)] - 1$. With N equally-spaced nonzero standards, with $n = 1$, this value is equal to $[N(N-9) + 2]/(12N)$, which is negative for $N \leq 8$, positive for $N > 8$ (and > 1 for $N > 16$).

APPENDIX 3: SUMS OF SQUARES OF CONCENTRATIONS

If N standards are equally spaced at concentration intervals I , starting at $x = x_1$, then $x_i = x_1 + [(i-1)I]$. For any x ,

$$\sum_1^N (x - \bar{x})^2 = \sum_1^N x^2 - [(\sum_1^N x)^2/N]$$

Since $\sum_1^N (i-1) = \sum_1^{N-1} i$, and $\sum_1^N (i-1)^2 = \sum_1^{N-1} i^2$, then

$$\sum_1^N x_i = (Nx_1) + (I \sum_1^{N-1} i)$$

$$\sum_1^N x_i^2 = (Nx_1^2) + (I^2 \sum_1^{N-1} i^2) + (2Ix_1 \sum_1^{N-1} i)$$

Here $\sum i$ and $\sum i^2$ are standard expansions; substituting first for these, and then for $\sum x$ and $\sum x^2$, gives

$$\sum_1^N (x - \bar{x})^2 = I^2 \{[N(N-1)(2N-1)/6] + [N(N-1)^2/4]\} = (I^2/12)(N+1)(N-1)N$$

which is independent of x_1 . Note that $\bar{x} = x_1 + [1/2(N-1)I]$ in these circumstances. When $x_1 = 0$, therefore, $E_0 = \bar{x}^2/\Sigma_1^N (x - \bar{x})^2 = 3(N-1)/N(N+1)$.

For any x ,

$$\sum_1^N (x - \bar{x})(x^2 - \bar{x}^2) = \sum_1^N x^3 - [(\sum_1^N x)(\sum_1^N x^2)/N]$$

The sums of x and x^2 are given above; the sum of x^3 is given by

$$\sum_1^N x^3 = (Nx_1^3) + (3Ix_1^2 \sum_1^{N-1} i) + (3I^2x_1 \sum_1^{N-1} i^2) + (I^3 \sum_1^{N-1} i^3)$$

Substituting first for Σi , Σi^2 and Σi^3 with the standard expansions, and then for Σx , Σx^2 and Σx^3 , gives

$$\sum_1^N (x - \bar{x})(x^2 - \bar{x}^2) = (I^2/12)N(N+1)(N-1)\{[(N-1)I] - (2x_1)\}$$

and so for equally spaced standards starting at $x = x_1$,

$$\sum_1^N (x - \bar{x})(x^2 - \bar{x}^2) / \sum_1^N (x - \bar{x})^2 = [I(N-1)] - 2x_1$$

which is equal to $I(N-1)$ when $x_1 = 0$, which is $2\bar{x}$.

Adding n extra zero values of x to $x_1, \dots, x_N$ adds n to N , but leaves Σx and Σx^2 unchanged. The new quantities $\bar{x}_{N+n}$ and $\Sigma_1^{N+n} (x - \bar{x}_{N+n})^2$ can be expressed in terms of the original values, as follows:

$$\bar{x}_{N+n} = (\sum_1^N x)/(N+n) = \bar{x}N/(N+n)$$

$$\sum_1^{N+n} (x - \bar{x}_{N+n})^2 = \sum_1^N x^2 - [(\sum_1^N x)^2/(N+n)] = \sum_1^N (x - \bar{x})^2 + [nN\bar{x}^2/(N+n)]$$

The new value of E_0 , called $E_{0(N+n)}$, is therefore given by

$$E_{0(N+n)} = [N\bar{x}^2/(N+n)] / \{ \sum_1^N (x - \bar{x})^2 + [nN\bar{x}^2/(N+n)] \}$$

$$= [NE_0/(N+n)] / \{ 1 + [nNE_0/(N+n)] \}$$

The new value of r is given by

$$r_{N+n} = (x_s/\bar{x}_{N+n}) - 1 = [x_s(N+n)/(N\bar{x})] - 1 = [(1+r)(N+n)/N] - 1$$

REFERENCES

- 1 L. M. Schwartz, *Anal. Chem.*, **49** (1977) 2062; **51** (1979) 723.
- 2 J. S. Garden, D. G. Mitchell and W. N. Mills, *Anal. Chem.*, **52** (1980) 2310.
- 3 D. G. Mitchell, W. N. Mills, J. S. Garden and M. Zdeb, *Anal. Chem.*, **49** (1977) 1655.
- 4 J. Agterdenbos, F. J. M. J. Maessen and J. Balke, *Anal. Chim. Acta*, **132** (1981) 127.
- 5 I. L. Larsen, N. A. Hartmann and J. J. Wagner, *Anal. Chem.*, **45** (1973) 1511.
- 6 G. J. Kemp, *Clin. Chem.*, **30** (1984) 1163.
- 7 M. J. Cardone, P. J. Palermo and L. B. Sybrandt, *Anal. Chem.*, **52** (1980) 1187.

- 8 G. J. Kemp, *Clin. Chem.*, 30 (1984) 1168.
- 9 P. Möriz, in G. Svehla (Ed.), *Wilson and Wilson's Comprehensive Analytical Chemistry*, Vol. XI, Elsevier, Amsterdam, 1981.
- 10 H. L. Pardue, *Clin. Chem.*, 23 (1977) 2189.
- 11 H. L. Pardue, T. E. Hewitt and M. J. Milano, *Clin. Chem.*, 20 (1974) 1028.
- 12 J. Agterdenbos, *Anal. Chim. Acta*, 108 (1979) 315.
- 13 L. A. Currie, *Anal. Chem.*, 40 (1968) 586.
- 14 A. Hubaux and G. Vox, *Anal. Chem.*, 42 (1970).
- 15 M. Werner, S. H. Brooks and L. B. Knott, *Clin. Chem.*, 24 (1978) 1895.

Short Communication

SIMPLE PROCEDURES FOR OBTAINING CONSTRAINED CALIBRATION EQUATIONS

LOUIS MEITES*

Department of Chemistry, George Mason University, 4400 University Drive, Fairfax, VA 22015 (U.S.A.)

J. J. LEARY

Department of Chemistry, James Madison University, Harrisonburg, VA 22807 (U.S.A.)

(Received 22nd May 1985)

Summary. Simple ways are described of constraining a calibration or other equation so that it will pass through one or more independently selected points and also give the “best” representation of any number of experimental data in terms of the model selected.

A recent paper [1] illustrated the use of Lagrangian multipliers to force a calibration curve obtained by traditional least-squares techniques to pass through one or more independently selected points. This communication has two purposes. One is to discuss the weighting factors that are appropriate to such an application. The other is to illustrate two other ways of achieving the desired end; these do not involve Lagrangian multipliers and may be easier to apply when a simple equation is chosen to represent the curve.

Although these are very general purposes, they will be discussed with reference to the example presented earlier [1]. To permit evaluation of the percentage by volume of ethanol in mixtures with water, the quantity $y = 100 A_{\text{EtOH}} / (A_{\text{EtOH}} + A_{\text{H}_2\text{O}})$, where A denotes the area of the peak obtained by gas chromatography with a thermal conductivity detector, was evaluated experimentally for nine mixtures having compositions evenly spaced from 10 to 90% ethanol by volume. Because the resulting calibration curve was non-linear but evinced no point of inflection, the data were fitted to the quadratic equation

$$y = a_0 + a_1x + a_2x^2 \quad (1)$$

in which y is the quantity defined above while x is the volume percentage of ethanol. Such a fit is of course made by minimizing the quantity

$$Q = \sum w_i [y_i - (a_0 + a_1x + a_2x^2)]^2$$

All of the weighting factors w_i were equated to unity so that maximum attention could be focused on the use of Lagrangian multipliers to force the

regression equation to pass through the points (0,0) and (100,100). In effect that approach assigns infinite weight to those two points.

The values assigned to the w_i should be the reciprocals of the variances of the corresponding y_i . In the present example, the variance of y is equal to zero at both of the extreme points (0,0) and (100,100), and has a maximum at some intermediate value of x . Its numerical values in the region between the extreme points can be obtained either by a standard treatment based on the propagation of uncertainties or by evaluating y repeatedly for each of the different values of x . Without taking its variation in that region into account, only an approximate solution to the problem can be obtained.

In the example being discussed, the two constraining equations and the three minimization equations (for dQ/da_0 , etc.) make it possible to evaluate the three parameters and two Lagrangian multipliers that are involved. Because all five equations are linear, any of these five quantities can be determined independently of the others.

There are two alternative ways of constraining the same fit. Both are simpler, more straightforward, and more economical of human time than the procedure previously described. The numerical values pertain to the data given earlier [1], and all weighting factors are again set equal to unity to facilitate comparisons with the previous results.

The point (0,0) will satisfy Eqn. (1) if and only if $a_0 = 0$, while the point (100,100) will do so if and only if $100 = 100 a_1 + 100^2 a_2$, or

$$a_1 = 1 - 100 a_2 \quad (2)$$

These equations, with slightly different notation, appeared before [1], and the values obtained for a_1 and a_2 (which were 0.588 and 0.00412, respectively) did conform to Eqn. 2.

On this basis Eqn. 1 can be rewritten as

$$y = (1 - 100 a_2)x + a_2 x^2 \quad (3)$$

Colson [3] employed a very similar approach to assigning values to constrained parameters. It is interesting to note that his equation, which may be written in the form

$$y = x/(b_0 + b_1 x + b_2 x^2)$$

yields a better fit to the data of this example than Eqn. 1 does [4].

In combining Eqn. 3 with a general regression program [2], about 45 s was required to type it in the form $Q = X(N) \cdot (1 - (100 - X(N)) \cdot V(1))$, where $V(1)$ denotes the single parameter (a_2) that remains to be evaluated, and to type the coordinates of the nine experimental points. Eighteen seconds of execution on a TRS-80 Model II microcomputer operating in BASIC then gave $V(1) = 0.00412$. Hence $a_0 = 0$, $a_1 = 0.588$, and $a_2 = 0.00412$, which are all identical with the values previously published.

Another approach employs standard techniques of linear regression to obtain an expression for the best value of a_2 . The equation

$$d\sum(y - x + 100xa_2 - x^2a_2)^2/d a_2 = 0 \quad (4)$$

is obtained by combining Eqns. 1 and 2 with the requirement $a_0 = 0$. Its solution is

$$a_2 = (\sum x^2y + 100 \sum x^2 - 100 \sum xy - \sum x^3)/(\sum x^4 - 200 \sum x^3 + 10^4 \sum x^2) \quad (5)$$

A very short program served to yield the value $a_2 = 0.00412$ in less than 1 s of execution time on the same microcomputer.

The choice between these approaches is a matter of taste. Generalized regression onto Eqn. 3 consumes much less human time than is needed to derive Eqn. 4, but demands that a computer and a suitable program be available. Although Lagrangian multipliers had previously been applied to similar problems, we conclude that their use is neither necessary nor markedly advantageous unless the equation chosen to represent the calibration curve is considerably more complicated than Eqn. 1.

It was previously [1] argued that the standard deviation from regression for these data should be calculated on the basis of eight degrees of freedom. The same conclusion can be reached in two other ways. One can consider either that eleven data pairs, including the points at (0,0) and (100,100), are used to evaluate the three parameters in Eqn. 1, or that the nine experimental data pairs are used to evaluate the single parameter a_2 in Eqn. 3. It is immaterial whether the parameters are evaluated simultaneously, as in ordinary regression analysis, or whether values are assigned to one or more of them before evaluating the others, as is done in constraining the fit in the ways described here.

REFERENCES

- 1 J. J. Leary and E. B. Messick, *Anal. Chem.*, 57 (1985) 956.
- 2 L. Meites, *The General Non-Linear Regression Program CFT4A*, The George Mason Institute, Fairfax, Virginia, 1984.
- 3 E. R. Colson, *Anal. Chem.*, 47 (1975) 805.
- 4 E. R. Colson, personal communication, 1985.

Short Communication

PATTERN RECOGNITION OF MULTIVARIATE ANALYTICAL DATA BY PLOTS OF HIGH-DIMENSIONAL DATA

F. L. GEURTS and G. KATEMAN*

Department of Analytical Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen (The Netherlands)

(Received 22nd April 1985)

Summary. The results of an attempt to construct images from multivariate analytical data are described. The method is based on addition of the features of a pattern in a Fourier polynomial and plotting the resulting summation in polar coordinates. The images formed in this way resemble flies, dragonflies, moths, etc. and can be constructed on a personal computer with graphics and printed on a simple matrix printer.

One model of pattern recognition, often used by psychologists, is the visual feature model. An image or pattern is broken down into particular features of the same kind, analogously to those present in a picture. The features are stored and the features of incoming patterns are compared with the features on the list and eventually a match is made. In analytical chemistry, there is usually no picture but data features can be used instead.

Pattern recognition procedures, which mainly comprise statistical or mathematical techniques, divide a population of patterns into classes or direct the patterns to classes. One method that uses human abilities to recognize patterns is the projection of multidimensional patterns in a two-dimensional or pseudo-three-dimensional plane. This requires considerable computational effort in order to find the plane that provides most information, e.g., by principal components analysis. However, there are other ways of grouping data. Chernoff [1] proposed that analytical chemical data should be transformed to pictures that show features comparable to the numerical value of the features. He used faces, attributing features to the size of the mouth, eyes and head, the distance between eyes, nose and mouth, etc. Yeung [2] used sound (pitch, duration, etc.). Andrews [3] investigated the use of Fourier polynomials. The last idea, although easily implemented optically or with a small computer did not achieve much success, probably because the resulting images, which resemble random spectra, can be compared and memorized very badly by man. Chernoff [1] suggested that an improvement could be obtained by constructing the polar representation of the Fourier polynomial. This idea was pursued here because this way of representation creates symmetrical pictures which are quickly described by people as

resembling beetles, butterflies, germinating plants, etc. Such pictures can be sorted and classified quite rapidly. One of the reasons seems to be that symmetry and "credibility" give more information than over-simple or random pictures [4].

Method

In pattern recognition, a pattern is conveniently represented by a vector (pattern vector) A_1 : $A_{i,j} = (A_{i,1}, A_{i,2}, \dots, A_{i,n})$, where n is the dimensionality of the pattern or number of features in the pattern and $A_{i,1}, A_{i,2}, \dots$ are the features. In the method examined, the features are used as the coefficients of a n th Fourier polynomial, so that the polynomial consists of as many terms as there are features in the pattern. This result is then plotted in polar coordinates:

$$F_{i,n}(t) = \sum_{j=1}^n [A_{i,j} \sin(m_j t)] \quad (1)$$

where $A_{i,j}$ is the numerical value of the features after a scaling procedure, i and j are the indices of the pattern and feature, respectively, m_j is the arbitrary numerical value which discriminates between the features of one pattern, and t is the phase. In the pictures, the phase changes from -180 to $+180$ degrees in steps of p degrees. The smaller these steps, the smoother the resulting picture.

The pattern is plotted by

$$\begin{aligned} X\text{-coordinate} &= X(n, t) = F_{i,n}(t) \cos(t) \\ Y\text{-coordinate} &= Y(n, t) = F_{i,n}(t) \sin(t) \end{aligned} \quad (2)$$

The syntax of the n th Fourier polynomial of Eqn. 1 is arbitrary. Many combinations are possible and were tried. For example, the sine term was replaced by other trigonometric relations in the syntax of the polynomial as well as quadratic and other powers [$\sin^2(\)$, $\tan^5(\)$, etc.], absolute values ($\sin$), block functions, and $\ln$ or $\log$ functions.

The feature m_j can consist of odd or even numbers, numbers within the range 0–1, or the series of whole numbers or natural and prime numbers, negative or positive. A logical choice is to give m_j the value of the feature number. If the information content of the feature is known (e.g., by Fisher weighting or principal components analysis) this can form the base of the sequence. The plotting equation 2 can also be changed, e.g., $X(n, t) = F_{i,n}^2(t) \cos(t)$ or $X(n, t) = F_{i,n}(t) \cos^2(t)$, etc.

The criterion for the formula syntax chosen here was the recognizability and discriminating power of the resulting pictures. After many trials with a panel of untrained users, the syntax of Eqns. 1 and 2 seemed the most satisfactory. Obviously these pictures can be memorized and analysed best by human observers.

Data sets

The method was tested on a synthetic data set and on some data sets from the literature. The synthetic data set had two main clusters in a 5-dimensional feature space. Figure 1 shows the hierarchical clustering and the minimal spanning tree clustering together with the picture set of the synthetic data. Figure 2 is based on earlier data [5].

The data set used for testing comprised the distribution percentages of the branched-chain fatty acids in goat milk [6]. The original data matrix consists of 14 patterns (14 fatty acids); each pattern is a 10-dimensional vector (10 goats). Classification by MASLOC produced 6 significant (robust) clusters. These six clusters contained the following acids: (1) acids 1, 3, 6, 7, 11; (2) acids 9, 13; (3) acids 5, 14; (4) acids 4, 8, 12; (5) acid 2; (6) acid 10. Acids 2 and 10 are outliers. Acid 4 is, owing to its very low concentration, only vaguely connected with the acids 8 and 12 in cluster number 4 [6]. Data scaling is necessary because the concentrations of the fatty acids are very different. In this example, range scaling was used (range 0–1). Other scaling procedures would be possible [7]. The resulting images are shown in Fig. 3. Each image corresponds to one fatty acid. In Fig. 4 the images are combined with a minimal spanning tree clustering and a hierarchical clustering.

The images were made on a HP9845B with ROM graphics and a HP7225A plotter. The programs can be obtained from the authors.

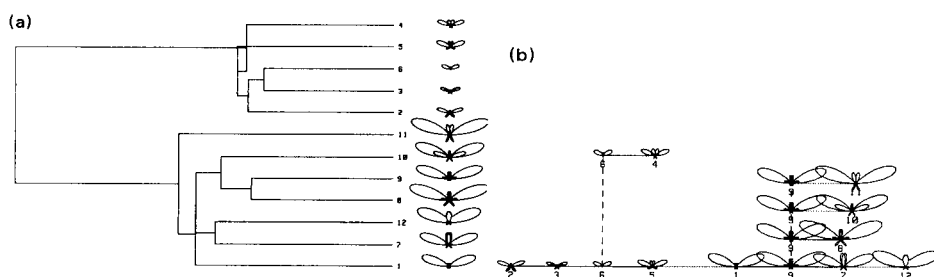


Fig. 1. Synthetic data set: (a) hierarchical clustering; (b) minimal spanning tree.

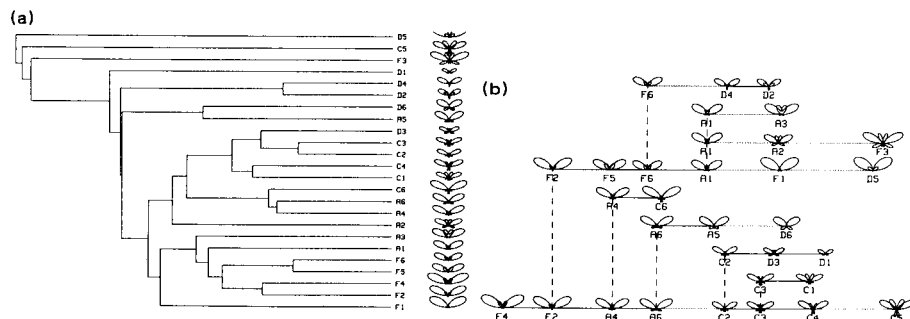


Fig. 2. "Cholesterol" data set [5]: (a) hierarchical clustering; (b) minimal spanning tree.

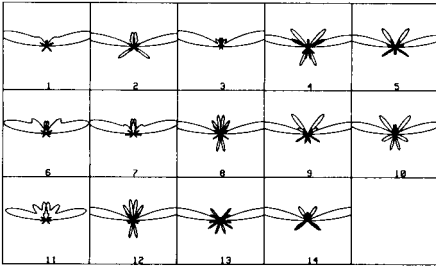


Fig. 3. Images of the 10-dimensional data set for goat milk.

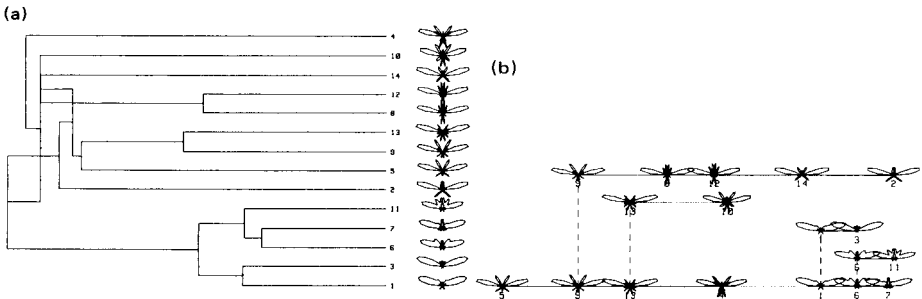


Fig. 4. Goat milk data set: (a) hierarchical clustering; (b) minimal spanning tree.

Results of observation

Every member of a small test panel (10 in all) was asked to make six clusters of the fourteen images in Fig. 3, with each cluster containing at least one image. All the members of the panel combined images 1, 3, 6, 7, 11 (cluster 1); 80% recognized image 2 (cluster 5) as an outlier but only 20% did so for image 10 (cluster 6); 60% combined 9 with 10, and 50% combined 5 and 13 but only 20% combined 9 and 13 (cluster 2); 50% combined 5 and 14; 100% combined 8 and 12, and 50% combined 8, 12 and 4.

Although there was no complete agreement by the panel, it may be concluded there is still a substantial similarity, and in our view no more or less certainty than in the other clustering methods.

Discussion

Pattern recognition provides a comparatively wide range of tools for discovering correlations in multidimensional data sets. Nearly all methods, however, require rather elaborate procedures, complicated computer programs and fast computers with quite extensive memory capacity. The reason is that most pattern recognition algorithms use a distance matrix that undergoes many computational operations.

In the method presented here, no distance matrix is used. After the scaling procedure every pattern is processed simultaneously. Sorting of the images obtained can be done even by untrained people, although some experience

enhances the speed of sorting. As the images can be drawn by a simple graphics program and printed on a simple matrix printer, pattern recognition is possible on a cheap personal computer, either for a quick preview on structure in the data set or as a possible means of unravelling complicated data sets, e.g., nested clusters.

A drawback of the method is that more than about 10 features make the images too complicated and thus more difficult to sort. Preprocessing of the data by correlation-finding techniques, such as Fisher weighting or principal components analysis is possible, but requires the use of other computers. Further data processing by the larger computer then seems more appropriate.

A property of the described method that has not yet been explored fully is its use as an on-line pattern recognition machine. Every new pattern (multi-dimensional data) that emerges can be converted to an image and consigned to one of the classes already obtained, without reprocessing the entire data set.

REFERENCES

- 1 H. Chernoff, *J. Am. Stat. Assoc.*, 68 (1973) 361.
- 2 E. S. Yeung, *Anal. Chem.*, 52 (1980) 1120.
- 3 D. F. Andrews, *Biometrics*, 28 (1972) 125.
- 4 E. L. J. Leeuwenberg, *Am. J. Psychol.*, 84 (1971) 307.
- 5 R. T. P. Jansen, F. W. Pijpers and G. A. J. M. de Valk, *Anal. Chim. Acta*, 133 (1981) 1.
- 6 A. Massart-Leen and D. L. Massart, *Biochem. J.*, 196 (1981) 611.
- 7 D. L. Massart and L. Kaufman, *The Interpretation of Analytical Chemical Data by the Use of Cluster Analysis*, Wiley, New York, 1983.

Short Communication

BEHAVIOUR OF A TETRAHEDRAL NICKEL-TRIOSMIUM CARBONYL CLUSTER AND ITS MONOSUBSTITUTED DERIVATIVES WITH GROUP V DONOR LIGANDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ANTONELLA CASOLI, ALESSANDRO MANGIA* and GIOVANNI PREDIERI

Istituto di Chimica Generale ed Inorganica, Universita' di Parma, Via M. D'Azeglio 85, 43100 Parma (Italy)

ENRICO SAPPA

Istituto di Chimica Generale ed Inorganica, Universita' di Torino, Corso M. D'Azeglio 48, 10125 Torino (Italy)

(Received 24th April 1985)

Summary. The chromatographic behaviour of some monosubstituted derivatives of the tetranuclear cluster $(\text{Cp})\text{NiOs}_3(\mu\text{-H})_3(\text{CO})_9$ (Cp = cyclopentadienyl) with group V donor ligands (benzonitrile tertiary phosphines, triphenylamine and triphenylstibine) is reported. In reversed-phase and adsorption liquid chromatography, the kind of the substituents strongly influences the retention volumes, the dimension of the whole cluster having less effect. Correlations of retention volumes with the nature of the substituents were found only for small series of compounds.

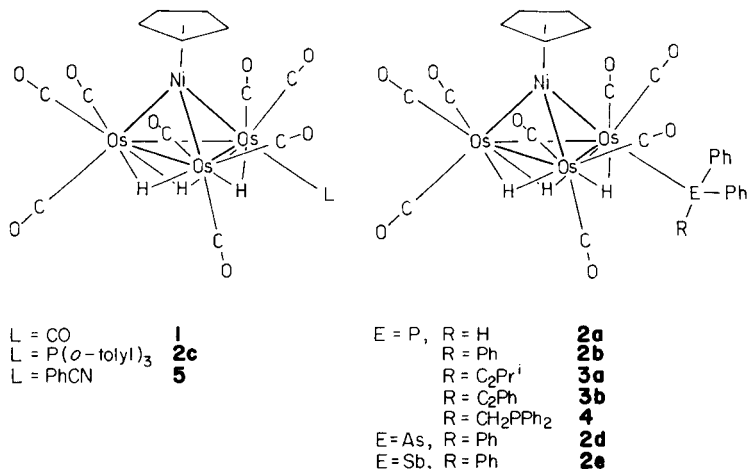
The importance of high-performance liquid chromatography (h.p.l.c.) for separating and quantifying complex mixtures of organometallic complexes is rapidly increasing; recent examples show that this technique may be used even for mixtures of homo- and hetero-metallic clusters [1–4]. As a part of a continuing investigation on the h.p.l.c. of metal complexes [5], the behaviour of bi- and tri-metallic alkyne-carbonyl derivatives of iron, ruthenium and osmium was studied on a reversed-phase system; the complexes containing the same bimetallic unit and different substituents were easily separated [6]. The same was true for homotrimetallic carbonyl and alkyne-carbonyl clusters showing triangular cores [7]. More recently, tri- and tetra-nuclear hetero-metallic clusters containing iron, ruthenium or osmium and nickel and bearing alkyne-derived substituents or sulphur were considered; reversed-phase and adsorption chromatography were studied with a mobile phase of low polarity [8]. These derivatives were better separated on a phase of low polarity, probably because of the high reactivity of the heterometallic M–M' bonds.

Generally, the separations are influenced by the following parameters, the effectiveness of which it is difficult to evaluate separately. For equal homometallic frames, the polarity or bulkiness of the substituents or the

electronegativity of the metals can be important (when the same substituents, but different metals are involved). For similar heterometallic frames, the nature of the heterometal, or/and of the substituents affects separations. For related complexes with different cluster frames, the shape of the metal skeleton plays the major role.

Here, the study reported concerns a series of cluster complexes having the same heterometallic tetrahedral frame NiOs_3 , but differing in the nature of one substituent on one osmium atom. The substituents are benzonitrile, tertiary phosphines, triphenylarsine and stibine, all terminally bound to one osmium atom, in the axial position. In the case of some phosphines, a generally accepted evaluation of the steric hindrance is available [9].

The following complexes were examined: $(\eta^5\text{-C}_5\text{H}_5)\text{NiOs}_3(\mu\text{-H})_3(\text{CO})_8(\text{L})$ (L = CO, complex 1; L = PPh_2H , complex 2a; L = PPh_3 , complex 2b; L = $\text{P}(o\text{-tolyl})_3$, complex 2c; L = AsPh_3 , complex 2d; L = SbPh_3 , complex 2e; L = $\text{PPh}_2\text{C}_2\text{Pr}^i$, complex 3a; L = $\text{PPh}_2\text{C}_2\text{Ph}$, complex 3b; L = bis(diphenylphosphino)methane, DPPM, complex 4; L = PhCN , complex 5). Their syntheses and structural features have been described [10–12]. The separation of



complexes 1–5 was accomplished by either reversed-phase (RP-18) or adsorption (silica) chromatography. The effect of the ligands on the separations is discussed.

Experimental

Complex 1 was obtained by established procedures [10]. Complexes 2–5 were prepared and purified as reported earlier [12]; the complexes were crystallized by cooling hydrocarbon or chloroform/hydrocarbon solutions at -10°C overnight. Their purity was checked by i.r. and ^1H -n.m.r. spectroscopy.

Chromatographic separations were done with a Perkin-Elmer Series 3B chromatograph, equipped with a Rheodyne 7105 injection valve and a LC-75 variable-wavelength u.v.-visible detector. Stainless steel columns (25×0.4 cm

i.d.) were filled with LiChrosorb Si-60 or LiChrosorb RP-18 (Merck); mean particle size 10 μm . The flow rate was 1 $\text{cm}^3 \text{min}^{-1}$. The column eluate was monitored at 265 nm. Solutions (5 μl) of the compounds in acetonitrile or tetrahydrofuran (THF) were injected.

Results and discussion

The chemical inertness of the test compounds towards acetonitrile, methanol and THF was checked by maintaining them in these solvents for several hours at room temperature and by monitoring the i.r. spectra in solution (CO stretching region) before and after the chromatographic runs. Two kinds of columns, RP-18 and silica, were used to test the influence of the nature of one substituent on the chromatographic behaviour. Only compound 4 decomposed during the chromatographic runs, probably owing to the reactivity of the non-bonded phosphorus which is present in the DPPM ligand.

On the RP-18 column, mobile phases ranged from pure acetonitrile to pure methanol, through mixtures of the two solvents. With acetonitrile, separation of the parent compound 1 and the substitution products with phosphines was obtained as shown in Fig. 1A (compounds were injected as THF solutions). The elution order (1, 2a, 2b, 3a, 3b, 2c) does not show any simple relationship, but roughly follows the order of increasing number of carbon atoms, with the exception of 2b, which precedes 3a. A correlation of the whole series with the Tolman's cone angle fails, if it is assumed, as seems correct, that the phosphinoacetylenes have a smaller cone angle than the triphenylphosphines. However, in the series 1, 5, 2a, 2b, the retention volumes (2.0, 2.55, 2.85, 3.8 cm^3) increase, as the number of phenyl groups or the cone angle increases. Again, the elution order of the triarylphosphine derivatives 2b and 2c, which show a relevant difference in retention volume, can be

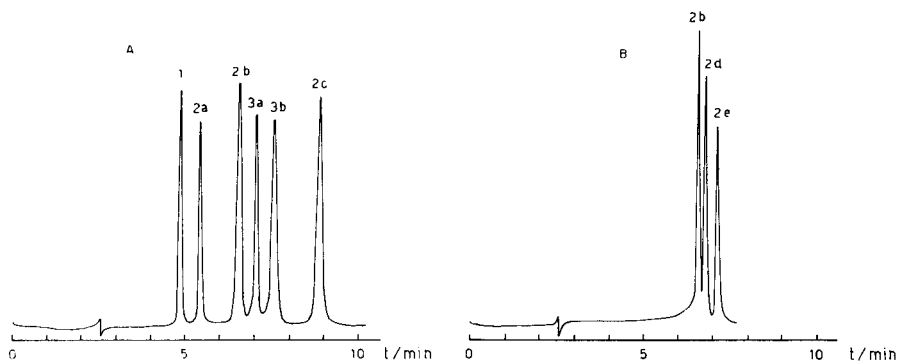


Fig. 1. (A) Separation of the parent cluster $(\text{Cp})\text{NiOs}_3\text{H}_3(\text{CO})_9$, 1, and its phosphine mono-substituted derivatives: PPh_2H , 2a; PPh_3 , 2b; $\text{P}(o\text{-tol})_3$, 2c; $\text{PPh}_2\text{C}_2\text{Pr}^i$, 3a; $\text{PPh}_2\text{C}_2\text{Ph}$, 3b. (B) Separation of the mono-substituted derivatives $(\text{Cp})\text{NiOs}_3\text{H}_3(\text{CO})_8(\text{EPh}_3)$: E = P, 2b; E = As, 2d; E = Sb, 2e. Column, LiChrosorb RP-18; mobile phase, acetonitrile; flow rate, 1 $\text{cm}^3 \text{min}^{-1}$; detection at 265 nm.

related to steric hindrance; the tris(*o*-tolyl) derivative is eluted after, accordingly to the larger cone angle. The phosphinoacetylenes (3a, b) are in the intermediate position within the "smaller" and the "larger" phosphine ligands. In particular, the isopropyl derivative is eluted first before the phenyl one, in accordance with the different number of carbon atoms.

Particularly noteworthy is the separation, even though incomplete, of the substitution products with PPh_3 , AsPh_3 and SbPh_3 (Fig. 1B). In spite of the dimension of the whole cluster, the nature of the heteroatom in the EPh_3 group influences the retention volumes. The elution order follows the order of increasing atomic number and decreasing electronegativity. The same elution order, even though with greater differences in the retention volumes, was obtained on injecting the free ligands PPh_3 , AsPh_3 and SbPh_3 . Under the same experimental conditions, the retention volumes were 1.4, 2.0 and 2.5 cm^3 respectively.

When methanol was used as the mobile phase instead of acetonitrile, a slight reduction in the retention volumes was observed on the RP-18 column. For example, the retention volumes of compounds 2a, b, c were 2.17, 2.85 and 3.90 cm^3 , respectively. However the elution orders were maintained within all series of compounds.

On the silica column, a hexane/THF (90/10) mixture was used. Under these conditions, some differences were observed compared to the behaviour on the reversed-phase column. For example, the three substitution products of EPh_3 had an opposite elution order compared to that on the RP-18 column. For the series of di- and tri-aryl derivatives, however, the elution order $2c \equiv 3a$, 2a, 2b, 3b was obtained; the tri-tolyl derivative is thus eluted first, despite its bulkiness, together with the iso-propyl derivative, while the same order as was observed on the RP-18 column was maintained for the other compounds.

REFERENCES

- 1 C. T. Enos, G. L. Geoffroy and T. H. Risby, *J. Chromatogr. Sci.*, 15 (1977) 83.
- 2 J. R. Fox, W. L. Gladfelter, T. G. Wood, J. A. Smegal, T. K. Foreman, G. L. Geoffroy, I. Tavanaiepour, V. W. Day and C. S. Day, *Inorg. Chem.*, 20 (1981) 3214.
- 3 H. C. Foley and G. L. Geoffroy, *J. Am. Chem. Soc.*, 103 (1981) 7176.
- 4 G. L. Geoffroy, J. R. Fox, E. Burkhardt, H. C. Foley, A. D. Harley, R. Rosen, D. E. Fjare, F. R. Furuya, J. W. Hull Jr., R. Stevens and W. L. Gladfelter, *Inorg. Synth.*, 21 (1982) 58.
- 5 T. Boselli, A. Mangia, C. Pelizzi and G. Predieri, *J. Chromatogr.*, 265 (1983) 347 and references therein.
- 6 A. Mangia, G. Predieri and E. Sappa, *Anal. Chim. Acta*, 149 (1983) 349.
- 7 A. Mangia, G. Predieri and E. Sappa, *Anal. Chim. Acta*, 152 (1983) 289.
- 8 A. Casoli, A. Mangia, G. Predieri and E. Sappa, *J. Chromatogr.*, 303 (1984) 404.
- 9 C. A. Tolman, *Chem. Rev.*, 77 (1977) 314.
- 10 M. Castiglioni, E. Sappa, M. Valle, M. Lanfranchi and A. Tiripicchio, *J. Organomet. Chem.*, 241 (1983) 99.
- 11 M. R. Churchill and C. Bueno, *Inorg. Chem.*, 22 (1983) 510.
- 12 E. Sappa, M. Valle, G. Predieri and A. Tiripicchio, *Inorg. Chim. Acta*, 88 (1984) L23, and unpublished results.

Short Communication

PRECONCENTRATION OF METAL IONS ON SILICA GEL MODIFIED WITH 3(1-IMIDAZOLYL)PROPYL GROUPS

JOSÉ C. MOREIRA and YOSHITAKA GUSHIKEM*

Instituto de Química, Universidade Estadual de Campinas, CP 6154, 13100 Campinas, SP (Brazil)

(Received 3rd April 1985)

Summary. Silica gel functionalized with 3(1-imidazolyl)propyl groups and packed in a glass column is used to adsorb and preconcentrate metal ions from ethanol solution. Elution is done with 0.1 M hydrochloric acid in an ethanol/water mixture having a mole fraction of water of 0.85. The modified silica is applied to preconcentrate metal ions (Cu, Ni, Fe, Zn and Cd) from commercial ethanol, normally used as engine fuel. The method is suitable for quantifying these metals at low $\mu\text{g l}^{-1}$ levels.

Commercial ethanol normally designates a mixture of ethanol and water. Other additives define its use in such diverse fields as the food industry, alcohol chemistry or engine fuels. One aspect of research on ethanol as a fuel, has been the development of methods to determine traces of metals. The presence of some metal ions in ethanol fuel can induce corrosion in the vehicle components in contact with the liquid [1–5]. In the determination of these ions, the first step in the usual procedures has been to evaporate the ethanol solution to dryness, which is obviously time-consuming [6].

Many methods of preconcentration of metal ions from solutions have been described. Of particular interest are those which involve inorganic solid surfaces modified with chelating groups [7–13] and so have the advantage of selectivity. Silica gel modified with 3(1-imidazolyl)propyl groups [$-(\text{CH}_2)_3\text{C}_3\text{H}_3\text{N}_2$] was recently reported to be a good adsorbent of metal ions from ethanol solution [14, 15]. This communication reports results which show that this material is useful for the preconcentration and removal of metal ions from commercial ethanol.

Experimental

The silica with bound 3(1-imidazolyl)propyl groups was prepared as previously described [14, 15]. Silica gel (14 g), previously treated at 150° in high vacuum, was refluxed for 8 h in dry xylene with 7.0 g of 3-chloropropyltrimethoxysilane and 2.4 g of sublimed imidazole. The functionalized silica was then washed with benzene and adsorbed hydrochloric acid was eliminated by treating with ethylene oxide at 0°C . The material was washed with diethyl ether in a Soxhlet extractor for 8 h and finally dried at 150°C in high vacuum.

For preconcentration experiments, the treated silica (2 g) was packed in a glass column (40 cm high, 0.7 cm i.d.) to give a bed height of ca. 10.5 cm). The material was activated for each run by washing with aqueous 0.1 M hydrochloric acid treatment with 0.5% (w/v) potassium hydroxide in ethanol and finally washing with ethanol to remove all hydroxide.

Solutions (5×10^{-3} M) of Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) ions were prepared in dry ethanol. In each run, 5 ml (25×10^{-6} mol) of the solution was percolated through the column at $0.2\text{--}0.5$ ml min^{-1} . The adsorbed ion was eluted with 10 ml of an aqueous ethanolic 0.1 M hydrochloric acid solution in which the ethanol/water ratio was varied. Lower acid concentrations (0.075 and 0.05 M) were less effective in desorbing the metal.

To study the adsorption and recovery of mixtures of ions, several solutions were prepared. Solutions of Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) ions (0.2×10^{-3} M each) were normally used, but some higher concentrations (4×10^{-3} M or 0.4×10^{-3} M) were also tested. For adsorption, 5 ml of the solution was passed through the column; elution was done in every case with 10 ml of 0.1 M hydrochloric acid in ethanol/water with a mole fraction of water of 0.85. The metals were quantified with a Zeiss-FMD3 atomic absorption spectrometer.

Results and discussion

Characteristics of the adsorbent material. The adsorption capacity of the 3(1-imidazolyl)propyl-treated silica gel for each metal was evaluated from adsorption isotherms [14, 15]. This adsorption capacity was not affected after several adsorption/elution cycles during three months of continuous use. The material was resistant to the attack of concentrated acids, such as 6 M HCl, but not the attack of concentrated aqueous alkali solution. Infrared spectra showed that the characteristic bands of the 3(1-imidazolyl)-propyl groups were not modified after treatment with acids. The main characteristics of the adsorbent material are listed in Table 1.

TABLE 1

Characteristics of the adsorbent material

Characteristic	Value
Surface area of functionalized silica (BET)	$266 \text{ m}^2 \text{ g}^{-1}$
Average pore diameter	60 Å
Amount of functionalized groups bound on the silica surface ^a	1.1 mmol g^{-1}
Adsorption capacity for acid	0.72 mmol g^{-1}
Adsorption capacity for individual ions from ethanol solution ^b	Fe(III) = 0.46, Ni(II) = 0.17, Cd(II) = 0.29, Zn(II) = 0.26 and Cu(II) = 0.28 mmol g^{-1}

^aBy elemental analysis. ^bFrom adsorption isotherm.

Adsorption and recovery of individual ions. To confirm that all the metal ion was adsorbed in each run, the effluent solution was collected, evaporated to dryness and the residue was analyzed qualitatively.

The elution of the adsorbed metal was done with 10 ml of a mixture of ethanol and water, 0.1 M in hydrochloric acid. In Fig. 1 are shown the percentages of elution of the individual ions vs. mole fraction of water (χ_B). The extents of elution, except for Ni(II), depend on the ethanol/water ratio. Elution of Cu(II) begins at $\chi_B \approx 0.25$, Zn at $\chi_B \approx 0.4$ and Cd(II) at $\chi_B \approx 0.7$. These three ions are completely eluted from the column at $\chi_B \approx 0.95$.

A different behavior is shown by Fe(III) and Ni(II); Fe(III) is completely eluted at $\chi_B \approx 0.5$ but is incompletely eluted as the amount of water in the mixture increases whereas elution of Ni(II) is almost independent of the ethanol/water ratio. Wada and coworkers [16, 17] concluded that in a binary mixture of methanol and water, the hydrolysis constant of Fe(III) ($K = [\text{Fe}(\text{OH})_2^+][\text{H}^+]/[\text{Fe}^{3+}]$) increases with the mole fraction of methanol in the mixture. If such behavior can be extended to Fe(III) in ethanol/water, it seems likely that the less charged complex ion $\text{Fe}(\text{OH})_2^{2+}$ (present at higher concentration at lower χ_B) will be more easily eluted than the Fe^{3+} ion itself. Nickel(II) was eluted from the column in a similar way whether the silica was modified or not; also, it was not completely retained (Table 1). Presumably, the nickel complex with imidazole groups is very weak.

Adsorption and recovery of a mixture of ions. The effects of mutual interactions between the ions in the process of elution were studied, especially

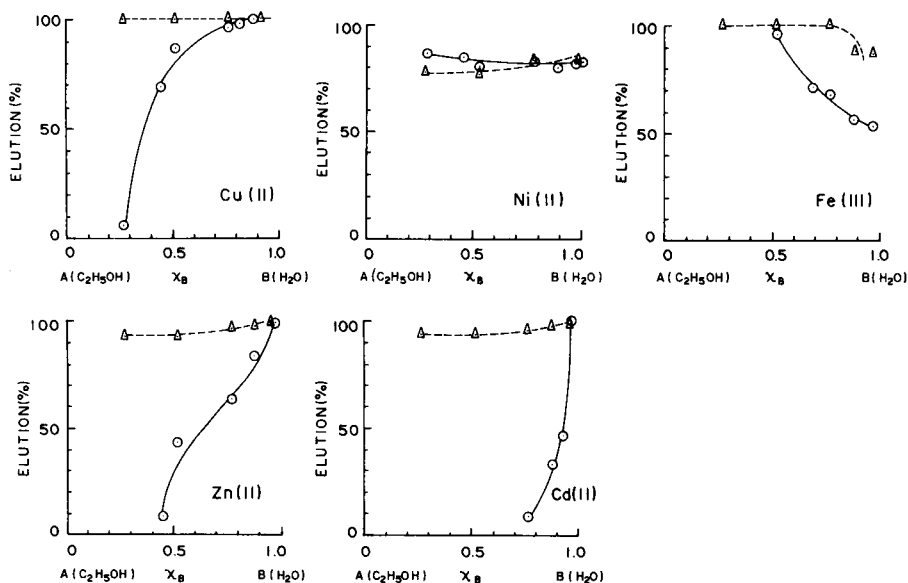


Fig. 1. Elution of the metal ions from different column packings: (—) modified silica; (---) untreated silica.

when one ion was present at higher concentration. For this experiment, a series of solutions was prepared in which the metal ion concentrations were fixed at 0.2×10^{-3} M except for one which was fixed at 4.0×10^{-3} or 0.4×10^{-3} M. The results are shown in Table 2. Elution of Ni(II) was practically complete in every case, because there was no loss of the metal when the mixture was percolated through the column; in the mixture, the Ni(II) concentration was 25 times lower than that used for the individual ion experiment. For Fe(III) and Cd(II), the recoveries were about the same as in the individual ion experiment whereas for Cu(II) and Zn(II), the recoveries were lower. It was also found that the recovery of the individual ions was not significantly affected when an excess of one of the ions was present.

Determination of metals in ethanol fuel. The adsorption method was used to determine the metals present in commercial ethanol from three different plants by passing, depending on the origin, between 0.1 and 5.9 l of sample through the column. The adsorbed metals were eluted with the recommended mixture ($\chi_B = 0.85$); about 50 ml of this solution was sufficient to ensure complete elution of the metals; all the experiments were done in triplicate. The results are listed in Table 3; cadmium was not detected in any sample. The accuracy of the method for Cu(II) in the ethanol from plant B was checked; direct determination, without any preconcentration, showed a concentration of 5.0 mg l^{-1} Cu(II), in agreement with the value of 5.0 mg l^{-1} found with the adsorption method. The high concentration of Cu(II) found in the ethanol from plant B was presumably due to the ethanol being distilled in a copper distillation column whereas plants A and C used stainless steel columns. Cadmium(II) normally does not occur in commercial ethanol. The concentration of Fe(III) depends on the degree of corrosion of the distillation

TABLE 2

Recovery of metal ions from a mixture

Ion	Concn. (10^{-3} M)	Recovery (%) ^a				
		Fe(III)	Ni(II)	Cu(II)	Zn(II)	Cd(II)
Fe(III)	4.0	—	100 ± 3	71 ± 2	21 ± 1	16 ± 1
	0.4	—	80 ± 2	65 ± 2	15 ± 1	23 ± 1
Ni(II)	4.0	73 ± 2	—	79 ± 2	19 ± 1	12 ± 1
	0.4	71 ± 2	—	71 ± 2	17 ± 1	19 ± 1
Cu(II)	4.0	78 ± 2	100 ± 3	—	20 ± 1	5 ± 0.2
	0.4	78 ± 2	100 ± 3	—	15 ± 1	10 ± 1
Zn(II)	4.0	87 ± 3	100 ± 3	69 ± 2	—	18 ± 1
	0.4	89 ± 3	100 ± 3	75 ± 2	—	16 ± 1
Cd(II)	4.0	76 ± 2	100 ± 3	79 ± 2	19 ± 1	—
	0.4	78 ± 2	94 ± 3	69 ± 2	19 ± 1	—

^aConcentration of each ion was 0.1×10^{-4} M.

TABLE 3

Concentration of metal ions in ethanol fuel

Plant	Vol. ^a (l)	Concentration found ($\mu\text{g l}^{-1}$)			
		Fe(III)	Ni(II)	Cu(II)	Zn(II)
A	1-3	— ^b	13 ± 6	200 ± 20	2 ± 0.5
B	0.1-0.6	— ^b	45 ± 15	5000 ± 50	4.8 ± 0.5
C	5-6	9 ± 1	4 ± 0.3	9 ± 3	2.6 ± 0.3

^aVolume percolated through the column containing 2 g of modified silica gel. ^bNot detected.

equipment [6]. The concentration of Ni(II) is that normally found in commercial ethanol (below $50 \mu\text{g l}^{-1}$) and Zn(II) is not detected when the conventional preconcentration method [6] is used.

The authors acknowledge the help of the Usinas Santa Cruz, Maringá and Zanin, which kindly supplied ethanol for this work, and FINEP for financial support.

REFERENCES

- 1 I. M. R. A. Bruning and E. B. Malm, Research and Development Center, CENPES, R.J., 1980, p. 15.
- 2 D. K. Tanaka, S. Wolyne, S. Fairbanks and F. B. P. Pinto, Proceedings of VIIth National Seminar on Corrosion, 1981, Brasil, p. 59.
- 3 E. A. N. Fernandes, MSc. Thesis, School of Agronomy Luiz de Queiroz, Piracicaba, SP, 1981.
- 4 L. Marino, O. R. Crnkovic and D. Spinelli, Proceedings of IXth National Seminar on Corrosion, 1982, Brasil, p. 383.
- 5 D. K. Tanaka and S. Wolyne, Proceedings of IXth National Seminar on Corrosion, 1982, Brasil, p. 166.
- 6 I. M. R. A. Bruning and E. B. Malm, Bol. Tec. PETROBRAS, 25 (1982) 217.
- 7 D. E. Leyden and G. H. Luttrell, Anal. Chem., 47 (1975) 1612.
- 8 S. E. Northcott and D. E. Leyden, Anal. Chim. Acta, 126 (1981) 117.
- 9 M. Grote, A. Schwalk, U. Hueppe and A. Kettrup, Fresenius Z. Anal. Chem., 316 (1983) 247.
- 10 P. Sutthivaiyakit and A. Kettrup, Anal. Chim. Acta, 169 (1985) 331.
- 11 K. Terada, A. Inoue, J. Imamura and T. Kiba, Bull. Chem. Soc. Jpn, 50 (1977) 1060.
- 12 H. Watanabe, K. Goto, S. Taguchi, J. W. McLaren, S. S. Berman and D. S. Russel, Anal. Chem., 53 (1981) 738.
- 13 R. E. Sturgeon, S. S. Berman, S. N. Willie and J. A. H. Desaulniers, Anal. Chem., 53 (1981) 2337.
- 14 Y. Gushikem and J. C. Moreira, J. Colloid Interface Sci., in press.
- 15 Y. Gushikem and M. A. A. da Silva, J. Colloid Interface Sci., in press.
- 16 G. Wada and A. Endo, Bull. Chem. Soc. Jpn, 45 (1972) 1073.
- 17 G. Wada and Y. Kobayashi, Bull. Chem. Soc. Jpn, 48 (1975) 2451.

Short Communication

LIQUID-LIQUID EXTRACTION OF ZIRCONIUM WITH TRI-*n*-BUTYL-ACETOHYDROXAMIC ACID AND SPECTROPHOTOMETRIC DETERMINATION IN THE ORGANIC PHASE WITH XYLENOL ORANGE

DONATELLA DESIDERI, LAMBERTO STACCIOLI and CORRADO TESTA*

Institute of General and Inorganic Chemistry, Faculty of Pharmacy, University of Urbino (Italy)

(Received 11th April 1985)

Summary. Zirconium (6–121 μg) is determined with xylene orange after liquid-liquid extraction by tri-*n*-butylacetohydroxamic acid (TBAH) from 6 M hydrochloric acid. Under optimal conditions (primarily, a 1:1.5 ratio of TBAH to xylene orange, addition of acetic acid, and measurement at 545 nm), the calibration is linear with a molar absorptivity of $34\,650\text{ l mol}^{-1}\text{ cm}^{-1}$. Ions which are not extracted by TBAH or do not form coloured complexes do not interfere; these include uranium and rare-earth elements. Hafnium interferes; interference from thorium is avoided by a preliminary extraction of zirconium with tri-*n*-octylamine.

Direct spectrophotometry of trace elements in an organic phase can solve many analytical problems. The advantages of such a technique are not only the appreciable increase in concentration achieved by liquid-liquid extraction but also avoidance of the stripping necessary when the spectrophotometric determination requires an aqueous phase.

The present paper describes the utilization of tri-*n*-butylacetohydroxamic acid (TBAH) as the extractant for the determination of zirconium by spectrophotometry with xylene orange. Zirconium can be extracted selectively from concentrated acid solutions with TBAH as already reported for liquid-liquid extraction [1–3] and extraction chromatography [4–6]. Xylene orange is a very sensitive and selective spectrophotometric reagent for zirconium in either aqueous [7, 8] or organic [9] phases.

EXPERIMENTAL

Equipment and reagents. A Hitachi model 100.40 spectrophotometer was used with 10-mm glass cells.

The main reagents were $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ (Merck), xylene orange [3,3'-bis-*N,N*-di(carboxymethyl)-aminomethyl-*o*-cresolsulphonphthalein, sodium salt; m.w. = 760.59; BDH Chemicals, and TBAH (m.w. = 243.40), which was synthesized and purified by the Reprocessing Research Laboratory of Casaccia

Center (ENEA, Rome). All the remaining chemicals were of analytical grade (Carlo Erba or Merck).

Solutions. Standard solutions of zirconium were prepared by dissolving zirconyl chloride in 6 M hydrochloric acid. Xylenol orange solutions (0.08% w/v; $1.05 \mu\text{mol ml}^{-1}$) were prepared by shaking the finely powdered reagent for 12 h with absolute alcohol. Solutions of TBAH (0.005, 0.015 and 0.05 M) were made with benzene. Solutions of the other ions were prepared by dissolving the respective salts in 6 M hydrochloric acid. The TNOA solution was 0.1 M in cyclohexane.

Recommended procedure. Zirconium (6–121 μg ; 0.066–1.32 μmol) in 5 ml of 6 M hydrochloric acid was extracted with 5 ml of 0.005 M TBAH in benzene. After shaking for 15 min and phase separation, 3 ml of the organic phase (3.6–72.2 μg of zirconium; 0.04–0.80 μmol) was transferred to a 25-ml calibrated flask. To each sample, 18 ml of the xylenol orange solution and 4 ml of anhydrous acetic acid were added. After 10 min, the absorbance was measured at 545 nm against a reagent blank.

Results and discussion

Preliminary tests. The extractive properties of TBAH for zirconium from concentrated hydrochloric acid solutions were tested first. The range 6–12 M hydrochloric acid was considered in order to extract zirconium quantitatively and selectively. Because of the probable competition of TBAH with the coloured xylenol orange complex, the extraction of zirconium was also examined as a function of the extractant concentration with 0.005, 0.015 and 0.05 M TBAH. For these tests, 5 ml of hydrochloric acid solution containing 0.83 μmol of zirconium was shaken for 15 min with 5 ml of TBAH in benzene; zirconium was then determined in the aqueous phase with xylenol orange. Table 1 shows that 0.005 M TBAH extracts zirconium completely from 6–8 M HCl, but that higher TBAH concentrations are necessary to achieve quantitative extraction in the range 9–11 M HCl. As most other ions

TABLE 1

Extraction of zirconium as a function of TBAH and HCl concentration

HCl (M)	0.005 M TBAH		0.015 M TBAH		0.050 M TBAH	
	Extr. (%)	E_a^0	Extr. (%)	E_a^0	Extr. (%)	E^0
12.0	65.8	1.9	75.5	3.1	87.0	6.7
11.5	67.8	2.1	77.3	3.4	92.9	13.1
11.0	70.0	2.3	80.2	4.0	>99.5	>200
10.5	74.4	2.9	91.8	11.2	>99.5	>200
10.0	83.0	4.9	>99.5	>200	>99.5	>200
9.5	91.9	11.3	>99.5	>200	>99.5	>200
9.0	99.0	99	>99.5	>200	>99.5	>200
8.0–6.0	>99.5	>200	>99.5	>200	>99.5	>200

are not extracted from 6 M HCl [2, 3], 0.005 M TBAH was chosen in order to minimize competition of the extractant with xylenol orange.

Spectrophotometry in the organic phase. A portion (5 ml) of 6 M HCl containing zirconium was shaken for 15 min with 5 ml of TBAH; after phase separation, 3 ml of the organic phase was transferred to a 25 ml volumetric flask for the spectrophotometric determination against a blank. This general procedure was used in studying the best conditions for good sensitivity and reproducibility.

In some papers dealing with the spectrophotometric determination of zirconium in the organic phase [9, 10], pyridine or acetic acid was used as the buffering system. To resolve this, some absorption spectra were measured for the complex in the presence of 50 μmol of TBAH without any buffer and with pyridine or acetic acid present. As shown in Fig. 1(a), in the absence of buffer, the absorption peaks appeared at 560 and 545 nm depending on whether or not xylenol orange was in excess. When 1 ml of pyridine was added, both peaks appeared at 575 nm (Fig. 1b), and when anhydrous acetic acid was present (Fig. 1c), both peaks were found at 545 nm, with much higher absorbance than for pyridine when xylenol orange was in excess. On this basis, the acetic acid system was chosen as the most sensitive.

In further tests, increasing amounts of xylenol orange (0–12 μmol) were added to solutions containing 0.2 μmol of zirconium in 0.2 ml of 0.05 M TBAH in benzene with 1 ml of acetic acid added, and absorbances were measured at 545 nm against an appropriate blank. The absorbance increased steadily up to a xylenol orange/Zr ratio of 20 and levelled off when this ratio exceeded 30 (i.e., 6 μmol of xylenol orange). Then increasing amounts of anhydrous acetic acid (0.1–5.0 ml) were added to solutions containing 0.2 μmol of zirconium in 0.2 ml of 0.05 M TBAH and 10 μmol of xylenol orange. The absorbance increased steadily on increasing the volume of acetic acid up to 3.5 ml, and then remained constant.

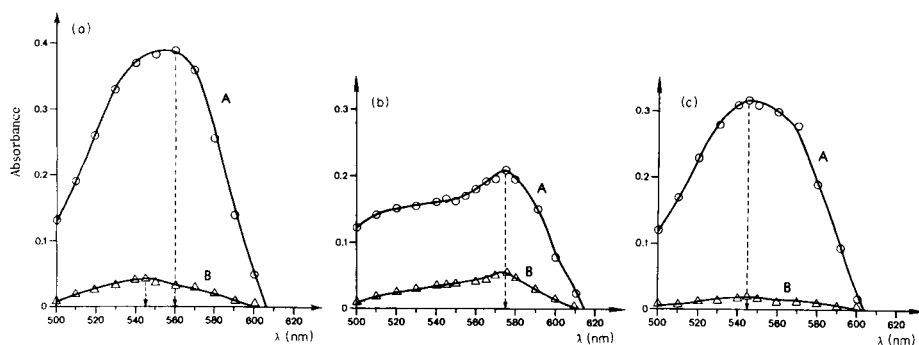


Fig. 1. Absorption spectra for the zirconium/xylenol orange/TBAH system: (a) without buffer; (b) with 1 ml of pyridine added; (c) with 1 ml of anhydrous acetic acid added. Curves: (A) 0.5 μmol Zr, 5.0 μmol xylenol orange, 50 μmol TBAH; (B) 1.0 μmol Zr, 0.5 μmol xylenol orange, 50 μmol TBAH.

To test the effect of TBAH, increasing amounts were added to solutions containing $0.2\ \mu\text{mol}$ of zirconium, $10\ \mu\text{mol}$ of xylenol orange (XO), 4 ml of anhydrous acetic acid and 1 ml of benzene. The absorbance decreased steadily as the TBAH/XO ratio increased because of competition of TBAH for the zirconium (Fig. 2); accordingly, $0.005\ \text{M}$ TBAH was preferred for the final method. Benzene volumes of $0.5\text{--}5\ \text{ml}$ in the final $25\ \text{ml}$ of solution did not affect the absorbance. Under the recommended conditions, the absorbance increased slightly during the first 10 min and then remained constant for 1 h.

The recommended procedure was based on the above features. The calibration plot was linear with a molar absorptivity (ϵ) of $34\ 650\ \text{ml mol}^{-1}\ \text{cm}^{-1}$.

Interferences. To evaluate possible interferences, extractions were done from $5\ \text{ml}$ of $6\ \text{M}$ hydrochloric acid containing $75.7\ \mu\text{g}$ of zirconium ($0.83\ \mu\text{mol}$) and 100–1000-fold amounts of the added ion; the determination of zirconium was then completed in the recommended manner. Ions which were not extracted from $6\ \text{M}$ hydrochloric acid by TBAH [2, 3] and did not interfere in 1000-fold amounts were alkali metals, alkaline-earth metals, rare-earth metals, Al^{3+} , NH_4^+ and Co^{2+} ; similarly, there was no interference from 100-fold amounts of Cr^{3+} , Mn^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , As^{3+} , Sb^{3+} , Bi^{3+} or UO_2^{2+} , or from sulfate, perchlorate or nitrate. Iron(III) was partly extracted, but did not interfere because it did not give complexes absorbing at $545\ \text{nm}$. Tin(II) interfered, causing positive errors, because it is oxidized to Sn^{4+} .

Thorium(IV) caused positive errors because it is partly extracted by TBAH and gives a coloured complex with xylenol orange. Extraction from a $9\ \text{M}$ hydrochloric acid medium decreased the interference somewhat. The problem was solved by a preliminary extraction of zirconium from $12\ \text{M}$ hydrochloric acid by $0.1\ \text{M}$ TNOA in cyclohexane; thorium remains in the aqueous phase, because it does not give anionic chloro complexes, whereas

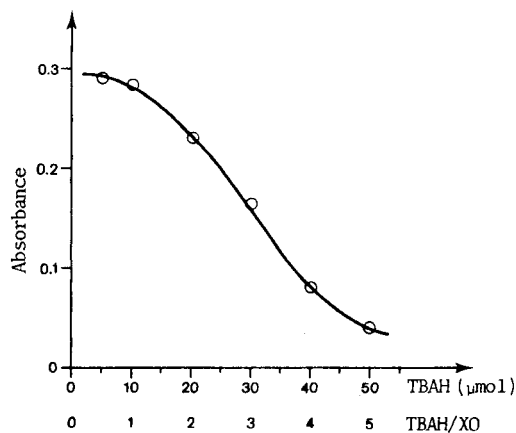


Fig. 2. Effect of TBAH on the absorbance from $0.20\ \mu\text{mol}$ Zr with $10\ \mu\text{mol}$ of xylenol orange and 4 ml of anhydrous acetic acid.

zirconium is quantitatively extracted as $[\text{ZrCl}_6]^{2-}$ [11]; zirconium is then stripped from the organic phase with 6 M hydrochloric acid and extracted with 0.005 M TBAH. Of course, hafnium interferes.

REFERENCES

- 1 G. Grossi, CNEN Report, RT/CHI(70)15, 1970.
- 2 G. M. Gasparini and E. Polidori, J. Chem. Eng. Data, 21/4 (1976) 504.
- 3 G. M. Gasparini and A. Brignocchi, CNEN Report, RT/CHI(78)4, 1978.
- 4 C. Testa, L. Staccioli, D. Desideri and M. Filippini, Studi Urbinati/Fac. Farmacia, 54 (1981) 121.
- 5 C. Testa, L. Staccioli and D. Desideri, Studi Urbinati/Fac. Farmacia, 55 (1982) 7.
- 6 L. Staccioli, D. Desideri and C. Testa, J. Chromatogr., 270 (1983) 324.
- 7 K. L. Cheng, Talanta, 2 (1959) 61; 3 (1959) 81.
- 8 E. Cerrai and C. Testa, Energ. Nucl. Milan, 7 (1960) 53.
- 9 E. Cerrai and C. Testa, Anal. Chim. Acta, 26 (1962) 204.
- 10 J. P. Young and J. C. White, Talanta, 1 (1958) 263.
- 11 C. Testa, J. Chromatogr., 5 (1961) 236.

Short Communication

AN IMPROVED DETERMINATION OF AMMONIA IN KJELDAHL DIGESTS AND ACIDIC SOLUTIONS WITH A BUFFERED BERTHELOT REACTION

P. F. REAY

Applied Biochemistry Division, DSIR, Private Bag, Palmerston North (New Zealand)

(Received 29th May 1985)

Summary. The addition of trisodium citrate to acidic samples and sodium carbonate to an alkaline hypochlorite reagent ensures that up to 100 μmol of hydrogen ion in the sample does not significantly affect the absorbance. Calibration was linear from 0.05–3 μg of ammonia-N. For aliquots (2.5 μg N) of a Kjeldahl digest from gorse leaves, the relative standard deviation was 0.38% ($n = 10$).

The Berthelot reaction is frequently used for the determination of ammonia in solution [1]. Methods based on this reaction are sensitive and relatively selective but the mechanism is complex and several of the steps appear to be pH-dependent [1]. The optimum pH is considered to be between 10.5 and 11.5 with phenol as a reactant and nitroprusside as the catalyst [2]. The optimum pH is lower with higher concentrations of phenol [3]. Buffers such as borate [2], trisodium citrate [3], carbonate/hydrogen-carbonate [4] and sodium phosphate [5] have been used with the reagent solutions to control the pH of the reaction. However, Kjeldahl digests contain sufficient acid that, even with a sodium phosphate/sodium citrate buffer, the samples still require individual neutralisation [5]. In those procedures [2–5] which do include a buffer, precise timing is required between the additions of the hypochlorite and the phenol reagents. With the reverse order, delays of up to 30 min between addition of the reagent solutions has no effect on the final absorbance [6]. This is more convenient for batch analysis. The inclusion of a buffer in this last procedure was investigated for quantifying ammonia in acidic solutions and Kjeldahl digests.

Experimental

Reagents. A solution of ammonium sulphate in 0.01 M sulphuric acid (1 g of $\text{NH}_4^+\text{-N}$ in 100 ml) was diluted 100-fold with 0.01% (v/v) Triton X-100 to provide a working standard. Deionized-distilled water was used throughout. Phenol, trisodium citrate and sodium carbonate were of analytical-reagent grade.

To prepare the phenol reagent [6], sodium nitroprusside (12 mg) was dis-

solved in about 1 ml of water before addition to the aqueous phenol solution (3.1 g, 50 ml). The buffered hypochlorite solution was prepared by adding an equal volume of 1.8 M sodium carbonate containing 0.001% (v/v) Triton X-100 to alkaline hypochlorite [6] and standing for 30 min [4]. Triton X-100 was used in the sample and reagents to improve the repeatability of dispensing and of draining after the reactants had been mixed in a test tube.

Recommended procedure. Into a 10-ml test tube (15-mm diameter polystyrene), pipette up to 0.3 ml of solution containing 0.1–3 μg of $\text{NH}_4^+\text{-N}$. Dilute to 0.3 ml and add 0.2 ml of 1 M trisodium citrate in 0.01% (v/v) Triton X-100. Mix by shaking each rack of tubes. Add 0.5 ml of the phenol/nitroprusside reagent and shake as before after each ten tubes. Add 1 ml of the hypochlorite/buffer reagent within 5 min, mix immediately on a vortex mixer and heat the tubes at 40°C in an aluminium block. After 20–30 min, remove the tubes, allow them to cool, add 3 ml of water and mix (total volume 5 ml). Measure the absorbance at 625 nm in a 1-cm pathlength cell. Include blank solutions and standards with each batch of samples.

Results and discussion

The addition of a borate buffer [2] (0.2 ml) to a sample (0.3 ml, 1 μg of $\text{NH}_4^+\text{-N}$) just prior to the addition of the phenol reagent [6] considerably decreased the absorbance at 625 nm after the Berthelot reaction, whereas a sodium carbonate/hydrogen carbonate buffer (0.2/1, mol/mol) [4] reduced the absorbance by only 3% (data not shown). In a comparison of different ratios of sodium carbonate/hydrogen carbonate, an increase of hydrogen carbonate from 0.2/1 to 1.9/1 decreased the absorbance by a further 18%. Increasing the sodium carbonate from 1/0.2 to 1/0.0 did not significantly change the final absorbance ($p < 0.05$). The use of sodium carbonate alone with the alkaline hypochlorite reagent [6] was investigated further.

The addition of 50 μmol of H^+ to 1 μg of $\text{NH}_4^+\text{-N}$ reduced the absorbance by 10% with unbuffered reagents and the addition of 200 μmol of H^+ reduced it by 70% (Table 1). The absorbance was decreased much less when sodium carbonate (0.9 M) was present in the hypochlorite. To reduce the effect of 100 μmol of H^+ , sufficient sodium hydroxide to neutralise the acid was added to the alkaline hypochlorite reagent with sodium carbonate. This extra alkali increased the absorbance above that found without sodium hydroxide but the value was still not equivalent to that obtained after direct neutralisation of a sample (data not shown).

Direct neutralisation of the sample with sodium citrate was then investigated; the pH of this solution (ca. 7.5) is sufficiently low to reduce ammonia volatilisation when present in excess and it would not affect the final pH significantly. The addition of 0.2 ml of 1 M trisodium citrate to a 0.3-ml sample aliquot and of carbonate to the hypochlorite reagent allowed up to 100 μmol of H^+ to be present without significant effect ($p < 0.05$) whereas 200 μmol caused an error of 3% (Table 1). The addition of citrate with the

TABLE 1

The effect of acid on the absorbance values ($\times 10^{-3}$) at 625 nm for the Berthelot reaction of 1 μg of $\text{NH}_4^+\text{-N}$ in the presence of buffers^a

Buffer added		H^+ added (μmol)				LSD ^c
To sample	To hypochlorite reagent	0	50	100	200	
—	—	337	305	222	89	11
—	Carbonate	324	318	303	181	35
Citrate ^b	Carbonate	324	323	322	316	3
—	Citrate, carbonate	322	319	288	157	12
Citrate	—	333	317	271	117	9

^aThe volume of sample plus buffer was 0.5 ml. The volume of hypochlorite reagent was 1.0 ml with or without sodium carbonate buffer. For citrate, 200 μmol of trisodium citrate was added to each reaction mixture; for carbonate, 0.9 mmol of sodium carbonate was used. Each value is the mean of two. ^bThis is the recommended procedure. ^cLeast significant difference ($p < 0.05$).

alkaline hypochlorite was less effective. Citrate added to the acidic samples without carbonate in the hypochlorite improved the absorbance only for up to 50 μmol of H^+ and did not itself affect ($p < 0.05$) the absorbance (Table 1). The addition of the citrate 1 h before adding the reagents did not significantly reduce the final absorbance. The absorbance of the blank with 0.3 ml of water was about 0.008 and the calibration graph for the recommended procedure was linear from 0.05 to 3 μg of $\text{NH}_4^+\text{-N}$.

The recommended procedure was applied to a Kjeldahl digest of gorse; 100 mg of dried leaves was digested [7] and the digest was diluted to 100 ml. Aliquots (0.2 ml, equivalent to 2.5 μg of $\text{NH}_4^+\text{-N}$) gave an absorbance of 0.9598 ± 0.0036 (mean with standard deviation, $n = 10$, RSD = 0.38%) compared with 0.9591 ± 0.0041 ($n = 10$, RSD = 0.43%) recorded after neutralisation of the sample aliquots with 0.25 ml of 0.2 M sodium hydroxide and without citrate but with carbonate. Without buffering by carbonate or citrate, aliquots of digest neutralised with sodium hydroxide gave an absorbance of 0.9956 ± 0.0052 ($n = 10$, RSD = 0.52%) compared with 0.928 ± 0.02 ($n = 10$, RSD = 2.4%) for samples without neutralisation. The absorbance was thus less variable with the recommended procedure than when unbuffered reagents were used. The use of buffered reagents allowed up to 100 μmol of H^+ to be present without effect on the absorbance. Greater amounts of acid in Kjeldahl digests can be reduced below this amount by the addition of a constant amount of alkali.

In conclusion, the proposed method overcomes a difficulty in using the Berthelot reaction with Kjeldahl digests and other acidic samples and is sufficiently simple and rapid for batch analysis.

REFERENCES

- 1 M. W. Weatherburn, *Anal. Chem.*, 39 (1967) 971.
- 2 C. J. Patton and S. R. Crouch, *Anal. Chem.*, 49 (1977) 464.
- 3 L. Solorzano, *Limnol. Oceanogr.*, 14 (1969) 799.
- 4 D. G. Smith, J. B. MaCaskill, C. D. Stevenson and W. H. L. Edgerley, *Physical and Chemical Methods for Water Quality Analysis*, Water and Soil. Publication No. 38, National Water and Soil Conservation Organisation, Wellington, New Zealand, 1982.
- 5 D. Scheiner, *Water Res.*, 10 (1976) 31.
- 6 A. Kaplan, in D. Glick (Ed.), *Methods of Biochemical Analysis*, Vol. 17, Interscience, New York, 1969, p. 311.
- 7 J. A. Parkinson and S. E. Allen, *Commun. Soil Sci. Plant Anal.*, 6 (1975) 1.

Short Communication

ELECTROCHEMICAL STUDY AND POLAROGRAPHIC DETERMINATION OF RANITIDINE

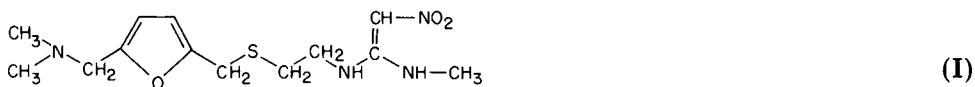
M. DELGADO ZAMARREÑO, J. HERNÁNDEZ MÉNDEZ* and A. SÁNCHEZ PÉREZ

Department of Analytical Chemistry, Faculty of Chemistry, University of Salamanca, Salamanca (Spain)

(Received 13th March 1985)

Summary. In the polarographic reduction of ranitidine, an H_2 -antagonist of histamine, three waves are observed; their half-wave potentials and limiting currents depend strongly on the pH of the solution. The first and second waves are due to reduction of the protonated, $=CH-NO_2H^+$, and unprotonated, $=CH-NO_2$, nitroethene group of ranitidine, respectively; the origin of the third wave is unknown. The characteristics of the second and third waves are studied in acetic acid/acetate buffer at pH 5.5; the first wave does not appear at this pH. The second wave ($E_{1/2} = -0.90$ V, vs. Ag/AgCl) is useful for determining ranitidine in the range $2.4-4.9 \times 10^{-4}$ M by direct current polarography and in the range $2.5 \times 10^{-7}-2.05 \times 10^{-5}$ M by differential pulse polarography.

Ranitidine (I) and cimetidine are powerful inhibitors of gastric acid secretion and are used in the treatment of gastric and duodenal ulcers; apparently, no secondary effects are observed following treatment with ranitidine [1]. A polarographic method has been proposed for the determination of cimetidine, based on the wave at -0.8 V (vs. SCE) caused by the reduction of the cyanoimine group in 1 M hydrochloric acid [2]. Ranitidine differs from cimetidine basically in that a furane ring replaces the imidazole ring and the side-chain of ranitidine contains a nitroethene group in place of the cyanoimine group



In this communication, the reduction of ranitidine at the mercury electrode is described. The mechanism is clarified and the conditions for the determination of ranitidine by direct current (d.c.) and differential pulse (d.p.) polarography are optimized. Previous methods for the determination of ranitidine are based on h.p.l.c. with u.v. spectrophotometric detection [3, 4]; electrochemical methods do not appear to have been applied.

Experimental

Apparatus. A Polarecord (Metrohm E-506) and a E-505 polarographic stand were used with three electrodes: Metrohm EA-1029/1 dropping

mercury electrode (DME), a Metrohm E-427 Ag/AgCl reference electrode and a Metrohm EA-285 platinum counter electrode. Other apparatus used were a Metrohm E-24 coulometer, a Crison pH meter with a Metrohm AG 9100 combined glass electrode, and a P. Selecta thermostat.

Reagents. Solutions of ranitidine were prepared from the solid hydrochloride (Glaxo Laboratories). All other chemicals were of analytical grade.

Preliminary procedure. Different aliquots of aqueous solutions of ranitidine were placed in 50.0-ml flasks; the necessary amounts of acetic acid, sodium acetate, Britton-Robinson buffer or sodium hydroxide were added to obtain the desired pH; potassium chloride was added as supporting electrolyte, and the volume was made up with twice-distilled water. This solution was placed in the polarographic cell and thermostatted; after oxygen had been eliminated with purified nitrogen, the voltage was scanned from 0.0 to -2.0 V (vs. Ag/AgCl). When differential pulse polarography was used, the pulse was -50 mV and the scan rate was 8 mV s^{-1} . Drop time was 2 s/drop in both techniques.

Recommended procedures. Solutions of ranitidine in the concentration range 2.5×10^{-5} – 5×10^{-4} M are prepared in acetic acid/sodium acetate buffer (pH 5.0, 0.1 M) with KCl added to adjust the ionic strength to 0.5 M. After thermostating and removal of oxygen as above, the voltage is scanned from -0.4 to -2.0 V (vs. Ag/AgCl) at a drop time of 2 s. For more dilute solutions (2.6×10^{-7} – 2.0×10^{-5} M), differential pulse polarography is used with a pulse of -50 mV over the same potential range. The limiting current of the polarographic wave (or peak) at -0.86 V is measured.

Results and discussion

Polarographic behaviour of ranitidine. The polarographic behaviour exhibited by ranitidine at pH 2.2 is shown in Fig. 1; three polarographic waves or peaks appear at potentials of about -0.3 , -0.7 and -1.2 V vs. Ag/AgCl.

Influence of pH. The solution pH has a marked influence on the potentials and limiting currents of the waves, some of them disappearing at certain pH

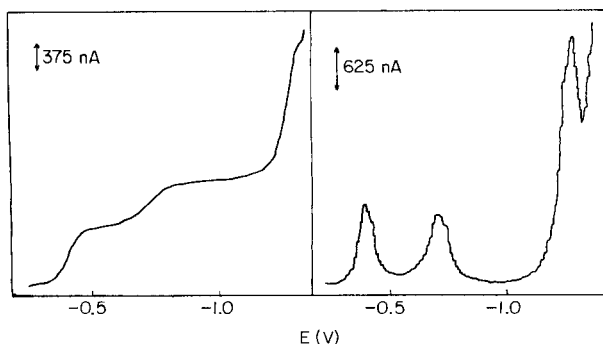


Fig. 1. Direct current and differential pulse polarograms of 2.2×10^{-4} M ranitidine solutions (pH 2.2).

values. To study this effect, a series of polarograms was recorded in solutions of ranitidine (2.2×10^{-4} M) in the 2.2–11.5 pH range. The first wave ($E_{1/2} = -0.3$ V) decreased with increasing pH and disappeared at $\text{pH} > 4$ (Fig. 2). The height of the second wave ($E_{1/2} = -0.7$ V) increased with increasing pH and then remained practically constant within the 4–11 pH range. The third wave ($E_{1/2} = -1.2$ V) was poorly defined at low pH values, owing to overlap with the discharge of the supporting electrolyte; it was better defined within the 5–10 pH range but disappeared at higher pH values.

A more detailed study was made of the influence of acidity on the limiting currents of the first two waves in the 0.8–3.5 pH range (Fig. 2). The plots for the two waves intersect at a pH of about 2.0; this suggests that the waves correspond to the reduction of the species involved in the protonation equilibrium of the diaminonitroethane group. The pK_a for this equilibrium, determined spectrophotometrically, is 2.3 [5].

The $E_{1/2}$ values of the three polarographic waves also changed notably with the pH of the solution. Table 1 shows the $E_{1/2}/\text{pH}$ relationships to which the

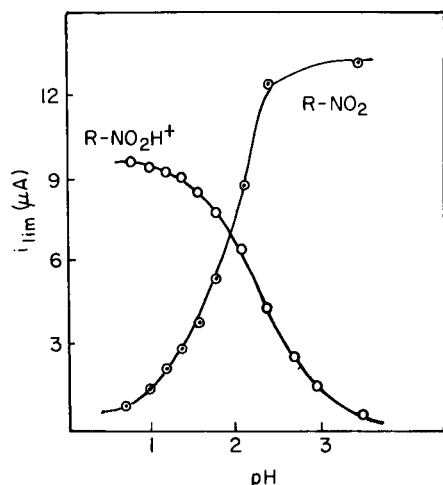


Fig. 2. Effect of pH on the limiting current of the first and second waves of 2.94×10^{-4} M ranitidine solution.

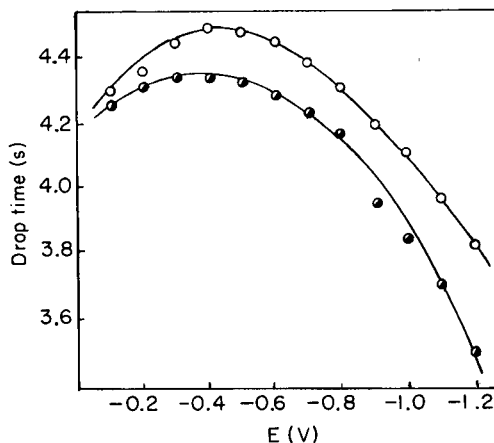


Fig. 3. Electrocapillary curves: (○) blank buffer; (●) 5.6×10^{-4} M ranitidine. (Acetate medium.)

TABLE 1

Study of the influence of pH on the half-wave potential

Wave	Equation for dependence	pH range	H ⁺ involved	$\frac{\alpha n}{\alpha n}$
1	$E_{1/2} = -0.195 - 0.095 \text{ pH}$	0.8–2.7	1.88	1.17
2	$E_{1/2} = -0.57 - 0.067 \text{ pH}$	1.5–11	1.06	0.94
3	$E_{1/2} = -1.09 - 0.052 \text{ pH}$	4.1–7.0	1.4	1.62

experimental data were fitted for the three waves, as well as the number of protons involved in the reduction processes. In subsequent studies, an acetic acid/acetate medium (0.1 M, pH 5.0) was used. At this pH, the first wave does not appear; the second ($E_{1/2} = -0.9$ V) and third ($E_{1/2} = -1.3$ V) waves are well defined.

Influence of the mercury column height and temperature. A series of polarograms for 2.45×10^{-5} M ranitidine in the acetate buffer was recorded; the height of the mercury reservoir was varied between 47 and 80 cm. The plots of the limiting current against the square root of the corrected height, h_c , were straight lines; the equations were $i_1 = -0.59 + 0.29 (h_c)^{1/2}$ for the second wave and $i_1 = -0.09 + 0.20 (h_c)^{1/2}$ for the third wave. These results indicate that the third wave is probably diffusion-controlled, as the intercept at the origin is almost zero [6].

To study the influence of temperature, polarograms were recorded under the same conditions as above, from 20 to 50°C. From the values of the temperature coefficients observed (Table 2) it is deduced that adsorption is involved in the reduction process of the second wave; the third wave exhibits a temperature coefficient which fits a diffusion-controlled process, as indicated by the above mercury column tests.

Electrocapillary curves. In order to examine the possibility of adsorption, electrocapillary curves were obtained for solutions with and without ranitidine (Fig. 3). The curve obtained for ranitidine shows a shift in the electrocapillary maximum towards more positive potentials, and is slightly distorted, thereby confirming the existence of adsorption phenomena.

Influence of ranitidine concentration. Different concentrations of ranitidine (2.44×10^{-5} – 4.88×10^{-4} M) were examined in the acetate buffer. The i_1/C plots for the second and third waves fitted the following calibration equations:

$$\text{second wave } i_1(A) = 6.7 \times 10^{-3} C(M); r^2 = 0.999$$

$$\text{third wave } i_1(A) = 5.3 \times 10^{-3} C(M); r^2 = 0.995$$

Accordingly, either of these waves can be utilized to determine this drug, but the use of the second wave ($E_{1/2} = -0.9$ V) is recommended because of its greater sensitivity and precision and its better definition.

Differential pulse polarography. In order to corroborate the conclusions

TABLE 2

Study of the influence of temperature

ΔT (°C)	22–25	25–29	29–35	35–40	40–44	44–50
Temperature coefficient (w%) ^a						
2nd wave	0.65	0.48	0.62	–0.37	–0.16	–0.13
3rd wave	1.4	2.5	2.0	2.6	2.9	3.3

^a $w = [(2.303/\Delta T) \log i_2/i_1] \times 100$.

reached above with d.c. polarography, those d.p.p. parameters were studied which help to clarify the nature of the polarographic process. The d.p.p. technique also improves the sensitivity of the measurements. Tests on the effect of pulse amplitude (ΔE) on the peak height of the polarograms indicated that a value of -50 mV gave the best responses.

According to theoretical considerations [7] the plot of peak current versus drop time ($\tau^{2/3}$) must be a straight line if the process is controlled by diffusion. As can be seen in Fig. 4, the plot is linear for the third peak ($E_p = -1.2$ V), but not for the second one ($E_p = -0.86$ V), the intensity of which is practically constant for $\tau^{2/3} > 2$. This confirms that adsorption processes are involved in the reduction of the nitroethene group.

The reversibility of the processes was also studied by applying the criteria $E_p^a - E_p^c = \Delta E$ and $i_p^a/i_p^c = 1$ [8]. Neither of the criteria was fulfilled, indicating the irreversibility of both electrode processes.

The d.p.p. technique was also used to determine ranitidine in ten solutions over the concentration range 2.56×10^{-7} – 2.05×10^{-5} M; the calibration based on peak height was linear over that range when the peak current at -0.86 V in acetate buffer was measured. The relative standard deviation, calculated by applying the recommended procedure to 10 solutions containing ranitidine at a concentration of 8.75×10^{-6} M was 2.7%.

Reaction mechanism. Amperostatic coulometry [9] showed a decrease in limiting current corresponding to the nitro group reduction of 16.4% per electron equivalent; therefore, the electrode process involves six electrons, as would be expected for reduction of a nitro group.

The following mechanism seems likely from the data obtained of the n -value, the number of electrons and protons involved in the controlling process (Table 1) [10]:

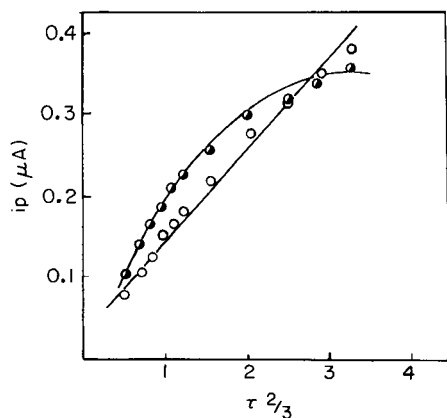
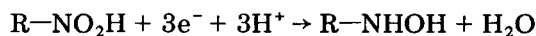
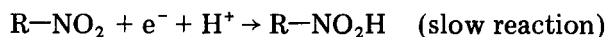
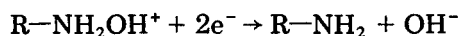
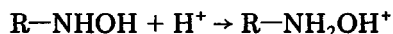


Fig. 4. Plot of peak current vs. drop time ($\tau^{2/3}$) for 1.7×10^{-5} M ranitidine in acetate buffer: (●) second peak; (○) third peak.



The third polarographic wave is very close to the discharge of the supporting electrolyte; the experimental data obtained were inadequate to establish its origin.

REFERENCES

- 1 R. N. Brogden, A. A. Carmine, R. C. Heel, T. M. Speight and G. S. Avery, *Drugs*, 24 (1982) 267.
- 2 A. Sánchez Pérez, J. Hernández Méndez and J. E. Fuentes de Frutos, *J. Assoc. Off. Anal. Chem.*, 68(5) (1985) in press.
- 3 H. M. Van den Berghe and S. M. MacLeod, *Ther. Monit.*, 2 (1980) 379.
- 4 L. E. Martin, J. Oxford and R. J. N. Tanner, *Xenobiotica*, 11 (1981) 831.
- 5 T. J. Cholerton, J. H. Hunt, G. Klinker and M. Martin-Smith, *J. Chem. Soc., Perkin Trans. 2*: (1984) 1761.
- 6 A. A. Vlcek, *Progress in Inorganic Chemistry*, Interscience, New York, 1963, Vol. V, pp. 211-379.
- 7 A. M. Bond, *Modern Polarographic Methods in Analytical Chemistry*, M. Dekker, New York, 1980, p. 252.
- 8 L. Birke, M. Kim and M. Strassfeld, *Anal. Chem.*, 53 (1981) 852.
- 9 E. Bishop and W. Hussein, *Analyst*, 109 (1984) 65, 73, 229.
- 10 P. Zuman, L. Meites and I. M. Kolthoff, *Progress in Polarography*, Wiley-Interscience, New York, 1972, Vol. 3, pp. 127, 261.

Short Communication

THE BEHAVIOUR OF MIXED METAL SULPHIDE/SILVER SULPHIDE SENSING MATERIALS IN SOLUTIONS OF SILVER IONS

VLADISLAVA M. JOVANOVIĆ and MOMIR S. JOVANOVIĆ*

Institute for Analytical Chemistry, Faculty of Technology and Metallurgy, University of Beograd, 11001 Beograd, Karnegieva 4 (Yugoslavia)

(Received 15th July 1984)

Summary. Hydrophobized graphite electrodes activated with CuS/Ag₂S can be used to measure silver ion activities as effectively as silver sulphide electrodes, whereas electrodes activated with PbS/Ag₂S or CdS/Ag₂S are useless for this purpose. The CuS/Ag₂S electrode is also suitable for use in potentiometric titrations involving species that are not contained in the sensing material, but are capable of producing precipitates with silver or sulphide ions.

Silver ions can act as potential-determining species for CuS/Ag₂S, PbS/Ag₂S and CdS/Ag₂S electrode materials, and must be absent when copper, lead or cadmium activities are measured [1, 2]. Because of the dual response of such ion-selective electrodes, any of these sensing materials should be usable for measuring silver or sulphide activities, but sulphide cannot be measured as pointed out by Midgley and Mulcahy [3]. When silver ions are present the conversion $MS + 2 Ag^+ = Ag_2S + M^{+2}$ (M being Cu, Pb or Cd) may cause a potential drift either because of changes in the membrane surface, or because of decreased silver activity when silver enters the solid phase.

Any calculation of conversion equilibria on the basis of solubility products for the corresponding sulphides is dubious, because the solubility of the sulphides depends not only on the pH/pS²⁻ correlation but also on the hydrolysis of the cations and their tendency to form sulphide complexes. Because of the uncertainty about the exact composition of the sensor, as indicated by Heijne and van der Linden [4] who found copper(I) sulphide on the surface of pressed CuS/Ag₂S pellets, a closer approximation to the truth may be obtained by calculating the change in free energy of formation of the sulphides from the corresponding ions, as discussed by Butler [5] and Waggoner [6]. The relevant results are: $\Delta G^0 (Ag_2S) = 280662 \text{ J mol}^{-1}$, $\Delta G^0 (CuS) = 200184 \text{ J mol}^{-1}$, $\Delta G^0 (Cu_2S) = 272797 \text{ J mol}^{-1}$, $\Delta G^0 (PbS) = 154458 \text{ J mol}^{-1}$ and $\Delta G^0 (CdS) = 149204 \text{ J mol}^{-1}$. It is clear that when silver ions are present, all the other sulphides will be converted to silver sulphide, but by no means at the same reaction rate. Such a conversion will make the sensing material richer in silver, i.e., more suitable for measuring silver ion activities if there is no potential drift, or very large change in silver concentration.

In the present work, two sorts of universal Růžička-type selective electrodes [2] were applied; the first was a Radiometer electrode (F3000) and the other was home-made, using polyethylene as the hydrophobizing polymer for the graphite with a carbon brush as the contact to the conducting cable [7].

Experimental

Solutions. Redistilled water, permanent light protection of solutions and plastic vessels were used. Silver solutions down to $10^{-3} \text{ mol l}^{-1}$ were prepared by dilution of a stock $0.1001 \text{ mol l}^{-1}$ solution of silver nitrate. Below this concentration, saturated solutions of silver chloride, bromide and iodide were applied. An ionic strength of 0.1 was maintained throughout by addition of potassium nitrate.

Solutions of sodium sulphide were adjusted to pH 13 and ascorbic acid (1 mg ml^{-1}) was added to prevent oxidation [8]. To avoid any uncertainty in the potentiometric end-point detection with a silver indicator electrode when silver nitrate served as titrant, sulphide solutions were titrated as described by Slanina et al. [9] with addition of standard $0.1003 \text{ mol l}^{-1}$ lead(II) nitrate solution of pH >13. Lead(II) nitrate and potassium chromate solutions were used as primary standards.

Instrumentation. A Radiometer PHM 64 meter was used. Titrants were added from a Metrohm E-415 motor piston-burette. For observations of potential drift as a function of time, a Zeiss K-201 (GDR) recorder was used.

The indicator electrodes were as follows: (1) Radiometer G202B glass, (2) Radiometer F1212S (Ag_2S pellet), (3) home-made Ag_2S -activated membrane (as above), (4) Radiometer F3002 $\text{CuS}/\text{Ag}_2\text{S}$ Selectrode, (5) home-made $\text{CuS}/\text{Ag}_2\text{S}$ -activated membrane, (6) Radiometer F3003 $\text{CdS}/\text{Ag}_2\text{S}$ Selectrode, (7) Radiometer F3004 $\text{PbS}/\text{Ag}_2\text{S}$ Selectrode, and (8) silver (5N) electrode. A Radiometer K701 double-junction saturated calomel reference electrode was used.

Preparation of sensor powders. For activation of the home-made electrodes, the sensing materials were prepared as described by Vesely et al. [10] and Růžička and co-workers [2, 11, 12].

Potential drift tests. The mixed sulphide electrodes were dipped into ca. $10^{-5} \text{ mol l}^{-1}$ silver solutions. In each test, a fresh saturated solution of silver chloride (without solid phase) was examined in the cell formed by F1212S (Ag_2S)/test solution/SCE for 30 min; either the F3000 or the home-made $\text{MS}/\text{Ag}_2\text{S}$ electrode was also dipped in the solution. Recorded potential/time curves (Fig. 1) showed that there was no change in the silver activity when the $\text{CuS}/\text{Ag}_2\text{S}$ electrode was present, whereas a slight permanent decrease in the silver activity occurred when the other two electrodes were present, presumably because of conversion of PbS and CdS to Ag_2S . These three $\text{MS}/\text{Ag}_2\text{S}$ electrodes were also tested for potential drift in silver solutions over silver chloride. Only the $\text{CuS}/\text{Ag}_2\text{S}$ electrode, like the Ag_2S electrode, showed no drift (Fig. 2).

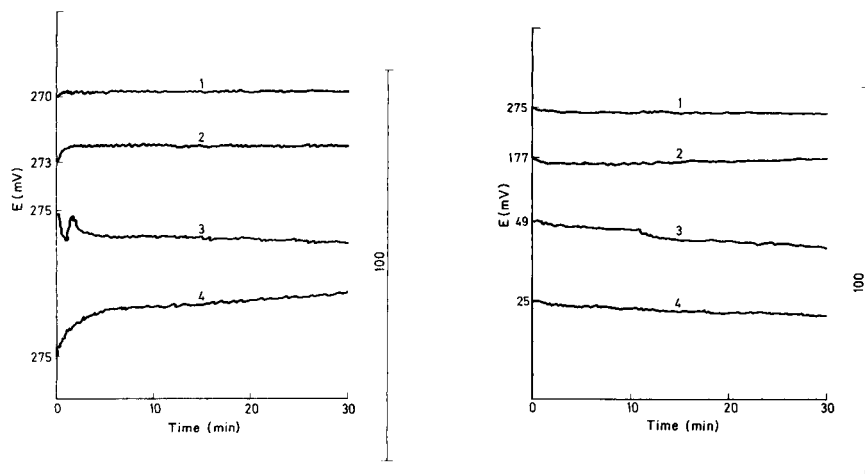


Fig. 1. Time dependence of the F1212S (Ag_2S)/pAg 4.99 solution/SCE cell: (1) alone; (2–4) in the presence of $\text{CuS}/\text{Ag}_2\text{S}$ (2), $\text{PbS}/\text{Ag}_2\text{S}$ (3) and $\text{CdS}/\text{Ag}_2\text{S}$ (4).

Fig. 2. Time dependence of the sensing electrode in the pAg 4.99 solution: (1) F1212S (Ag_2S); (2) $\text{CuS}/\text{Ag}_2\text{S}$; (3) $\text{PbS}/\text{Ag}_2\text{S}$; (4) $\text{CdS}/\text{Ag}_2\text{S}$.

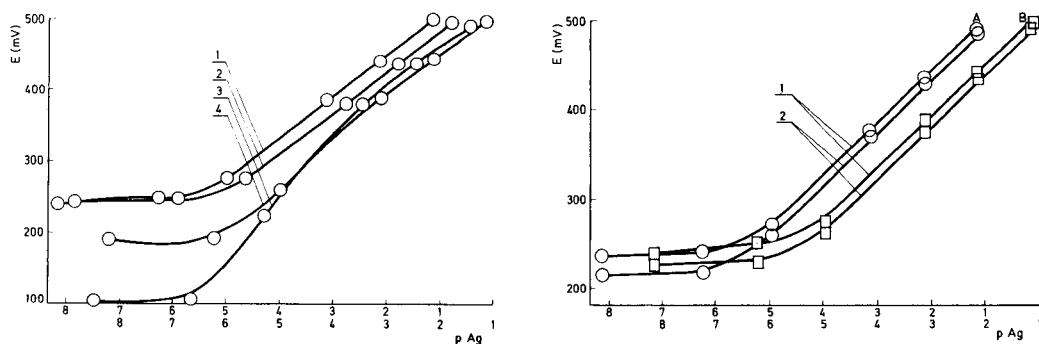


Fig. 3. Calibration graphs for silver ion with different electrodes: (1) F1212S (Ag_2S); (2) $\text{CuS}/\text{Ag}_2\text{S}$; (3) $\text{PbS}/\text{Ag}_2\text{S}$; (4) $\text{CdS}/\text{Ag}_2\text{S}$. The pAg axis is offset for electrodes 3 and 4, for clarity.

Fig. 4. Calibration graphs for silver ion (1) F1212S (Ag_2S); (2) $\text{CuS}/\text{Ag}_2\text{S}$. (A) First measurements; (B) after 72 h. The pAg axis is offset for clarity for the B curves.

Calibration graphs. Calibration graphs were prepared for all four electrodes in silver solutions in the range pAg 1.12–8.16. As can be seen from Fig. 3, the F1212S (Ag_2S) and $\text{CuS}/\text{Ag}_2\text{S}$ powder-activated electrodes behaved very similarly. The $\text{PbS}/\text{Ag}_2\text{S}$ and $\text{CdS}/\text{Ag}_2\text{S}$ electrodes showed responses higher than Nernstian after transference from pAg 3.12 to pAg 4.99 solution. This confirmed that the $\text{PbS}/\text{Ag}_2\text{S}$ and $\text{CdS}/\text{Ag}_2\text{S}$ sensors are unsuitable for measuring silver activities.

The CuS/Ag₂S electrode was also tested for poisoning by silver; after the electrode had been soaked for 72 h in 0.1 mol l⁻¹ silver nitrate, the potentials differed from the initial potentials only by a few mV (Fig. 4). It was concluded that the graphite electrodes activated with CuS/Ag₂S behave in the same way as the Ag₂S electrode towards silver solutions, except that they must be activated in 0.1 mol l⁻¹ silver solution for 30–60 s before immersion into solutions of high pAg, in order to give correct readings (cf. Fig. 3).

Results and discussion

Direct measurements of silver activities with the CuS/Ag₂S electrodes. All the electrodes tested for Fig. 5, and particularly those activated with CuS/Ag₂S, provided Nernstian response down to almost pAg 5.5. The response times varied from seconds to minutes at different pAg in the usual way. Surprisingly, there was very little difference in the E^0 values of the different electrodes.

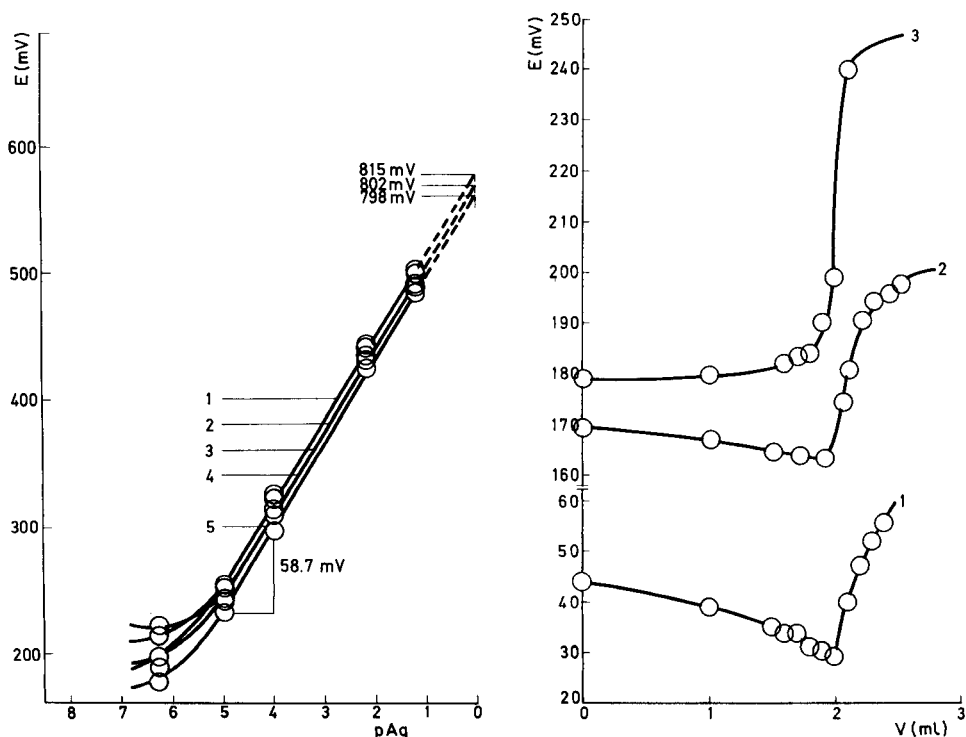


Fig. 5. Calibration graphs for silver ions ($\mu = 0.1$): (1) F1212S (Ag₂S); (2) elemental silver; (3) F3002 (CuS/Ag₂S); (4) home-made Ag₂S; (5) home-made CuS/Ag₂S. E_0 values are given against a standard hydrogen electrode.

Fig. 6. Potentiometric titrations of lead(II) solutions with standard 0.01 M chromate using the home-made electrodes: (1) PbS/Ag₂S; (2) CuS/Ag₂S; (3) Ag₂S.

Potentiometric titrations with the CuS/Ag₂S electrodes. When lead(II) nitrate solutions were titrated with a standard chromate, the smallest potential jump at the end-point was recorded when the PbS/Ag₂S electrode was used (Fig. 6). The reproducibility of the results was also worst. Thus in titrations of 5×10^{-4} M lead(II) nitrate (4.144 mg of lead) with standard 0.01 M chromate solution, the mean results and standard deviations ($n = 9$) for the home-made electrodes were 4.165 ± 0.007 mg with the Ag₂S electrode, 4.186 ± 0.01 mg with the CuS/Ag₂S electrode and 4.206 ± 0.03 mg with the PbS/Ag₂S electrode.

When sulphide solutions were titrated with standard 0.1003 M lead nitrate in a strongly alkaline medium, both the Ag₂S and CuS/Ag₂S electrodes gave similar potential jumps of about 250 mV at the end-point. In the titration of 7.214 mg of sulphide, the results obtained were 7.364 ± 0.06 mg with the Ag₂S electrode (F1212S), 7.473 ± 0.07 mg with the CuS/Ag₂S electrode, and 7.395 ± 0.06 mg with the home-made CuS/Ag₂S (F3002) electrode; these results are the mean and standard deviation for 9 titrations.

Conclusion

The above results prove that CuS/Ag₂S-activated electrodes of the Růžička type can be used to measure silver ion activity in the same way as the Ag₂S electrodes. In solutions of silver, the CuS in the electrode is not converted to Ag₂S during several hours. In contrast, electrodes activated with PbS/Ag₂S or CdS/Ag₂S cannot be used in silver solutions.

REFERENCES

- 1 K. Cammann, *Das Arbeiten mit ionenselektiven Elektroden*, Springer Verlag, 2 Aufl., Berlin, 1977, p. 73.
- 2 E. H. Hansen, G. G. Lamm and J. Růžička, *Anal. Chim. Acta*, 59 (1972) 403.
- 3 D. Midgley and D. E. Mulcahy, *Ion-Selective Electrode Rev.*, 5 (1983) 185.
- 4 G. J. M. Heijne and W. E. van der Linden, *Anal. Chim. Acta*, 93 (1977) 99.
- 5 J. N. Butler, *Ionic Equilibrium, a Mathematical Approach*, Addison-Wesley, Massachusetts, 1964, Chaps. 1–4 and 8–7.
- 6 W. H. Waggoner, *J. Chem. Educ.*, 35 (1958) 339.
- 7 M. S. Jovanović, M. Djikanović, B. D. Vučurović and Z. Avramović, *Proc. 3rd ISE Symposium, Matrafűred (Hungary) Oct. 1980*, Akademiai Kiado, Budapest, 1981, p. 259.
- 8 R. Bork and H. J. Puff, *Fresenius Z. Anal. Chem.*, 240 (1968) 381.
- 9 J. Slanina, E. Buysman, J. Agterdenbos and B. Griepink, *Mikrochim. Acta*, (1971) 657.
- 10 J. Vesely, O. J. Jensen and B. Nicolaisen, *Anal. Chim. Acta*, 62 (1972) 1.
- 11 J. Růžička and E. H. Hansen, *Anal. Chim. Acta*, 63 (1973) 115.
- 12 E. H. Hansen and J. Růžička, *Anal. Chim. Acta*, 72 (1974) 365.

Short Communication

VOLTAMMETRIC DETERMINATION OF SOME ORGANIC COMPOUNDS OF ARSENIC REDUCED AT THE MERCURY ELECTRODE

G. SPINI, A. PROFUMO and T. SOLDI*

Dipartimento di Chimica Generale, Università degli Studi de Pavia, Via Taramelli 12, 27100 Pavia (Italy)

(Received 5th March 1985)

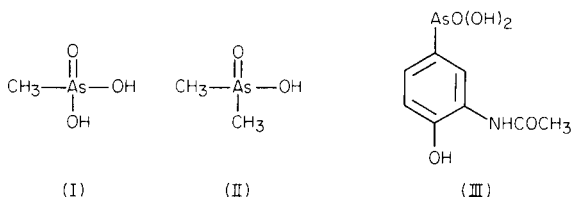
Summary. Cyclic voltammetric scans for methylarsonic acid, dimethylarsinic acid and 3-acetamido-4-hydroxyphenylarsonic acid at a mercury electrode in an acidic medium show anodic adsorption waves when the starting potential is sufficiently negative; the related arsines are formed on the electrode. Detection limits are at the micromolar level for the adsorption peak found at about -0.1 V vs. SCE for all three compounds. Interferences are discussed.

Aryl and alkyl derivatives of arsenic acid are used in agriculture as pesticide and herbicide products. Studies have shown that their reduction products (mainly dimethylarsinic acid) are the principal metabolites of inorganic arsenic [1–3]. The widespread use of arsenical compounds in industry, their importance in the biological and environmental fields, and widespread concern about their toxicity, have prompted the development of many methods for the determination of the organic derivatives. Chromatographic techniques [4], atomic absorption spectrometry [5–8], atomic emission spectrometry [9, 10], and neutron activation analysis [11] have been proposed. Polarographic methods were first used to determine As(III) after mineralization of the organic compounds [12].

Systematic studies on the polarographic behaviour of some derivatives of phenylarsonic acid were undertaken by Watson and Svehla [13], who demonstrated that the first step of the irreversible polarographic process consists of a rapid protonation followed by a one-electron reduction. Dimethylarsinic acid (DMA) and methylarsonic acid (MMA) behave similarly [13, 14], although the mechanism for MMA cannot be demonstrated because the polarographic wave is not well resolved from the supporting electrolyte discharge. For the determination of phenylarsonic acid, Watson and Svehla [13] suggested an acidic medium (pH 1) and concentrations of about 10^{-5} M, whereas DMA and MMA could be quantified only in an alcoholic medium in the presence of guanidinium perchlorate or similar reagent [14]. On the macroscale, the electrochemical process generates the corresponding arsines without,

however, any well defined number of electrons being involved, probably because of partial formation of a phenylarsenoxide which can form an insoluble polymer at higher concentrations.

The voltammetric behaviour of methylarsonic acid (I; MMA), dimethylarsinic acid (II; DMA) and 3-acetamido-4-hydroxyphenylarsonic acid (III; acetarsone) is discussed below.



Experimental

An Amel model 472 polarograph was used with a three-electrode system (dropping mercury electrode, saturated calomel reference electrode and platinum auxiliary electrode. Oxygen removal and operating procedures were all conventional. Measurements were made at $25 \pm 0.2^\circ\text{C}$. For first-harmonic a.c. measurements, a metrohm Polarecord E-506 was used. The potential values reported are always referred to the saturated calomel electrode (SCE).

Solutions were prepared from triply-distilled water and reagents were of analytical grade, Methylarsonic and dimethylarsinic acid (C. Erba) and the acetarsone (SIGMA) were twice crystallized from water. The supporting electrolyte was 0.1 M sulfuric acid.

Results and discussion

Figure 1 shows a cyclic voltammogram of acetarsone. Of the three compounds tested, only acetarsone gave a reduction wave (at -0.8 V) in the cathodic scan. In the subsequent anodic scan, all three compounds gave sharp peaks if an adequate negative potential was reached. In the cathodic scan of acetarsone from 0 to -1.1 V , peak A is due to the reduction of acetarsone; in the anodic scan, there are two peaks, a broad peak at -0.4 V and a sharp one at -0.1 V . In subsequent cathodic scans, these peaks appeared. The peak potential changed with increasing concentrations.

The relationship of peak current and concentration was evaluated for the two peaks obtained from acetarsone in the anodic scan from -1.1 V . Both peaks provided linear relationships over narrow ranges; for obvious reasons, the peak at about -0.1 V was used for quantitative purposes. Figure 2 shows the response curve obtained; the response was linear for the range 10^{-6} – $3 \times 10^{-4}\text{ M}$, reaching saturation values at higher concentrations.

Methylarsonic acid and dimethylarsinic acid showed no peaks in the cathodic scan. In the anodic scan from -1.2 V , MMA showed one peak and DMA two; in the subsequent cathodic scan, DMA showed two sharp peaks and MMA none (Fig. 3). The peaks of all the compounds studied are ascribed to

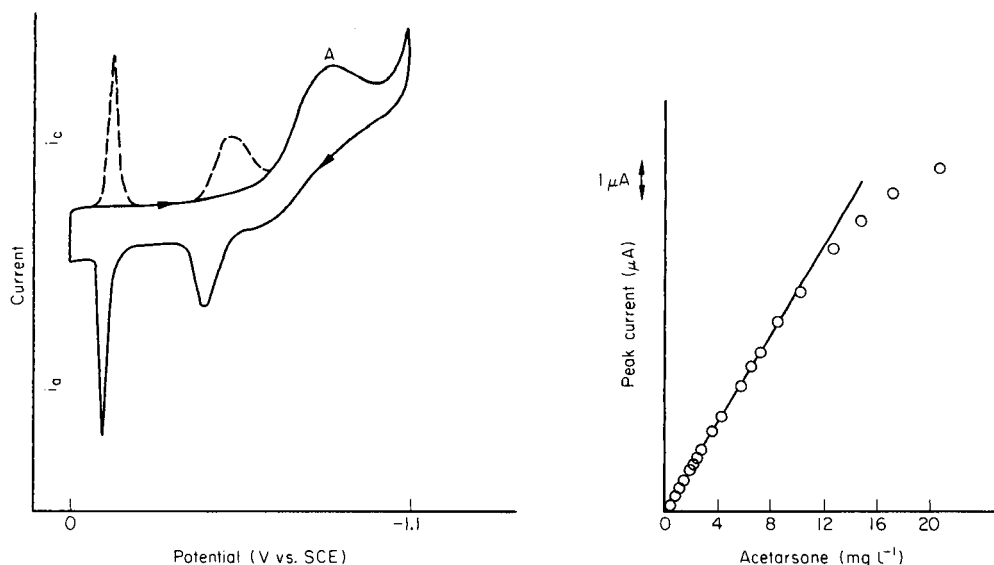


Fig. 1. Cyclic voltammetry of 10^{-5} M acetasone in 0.1 M sulfuric acid (scan rate 100 mV s^{-1} , delay 6 s): (—) anodic scan from 0 V; (----) cathodic scan from -1.1 V.

Fig. 2. Peak current vs. acetasone concentration relationship. Conditions: differential pulse polarography, initial potential -1.1 V, delay 16 s, scan rate 100 mV s^{-1} , pulse amplitude 20 mV, drop time in 0.1 M sulfuric acid (open circuit).

the adsorption of the corresponding arsine formed to the mercury electrode. That was proved by the fact that the voltammograms described above were exactly the same as the voltammograms obtained from the corresponding arsines prepared chemically by reduction with sodium tetrahydroborate. The reduction product of acetasone was obtainable in the polarographic cell by adding tetrahydroborate. Dimethylarsine could also be prepared separately and then tested though its concentration quickly decreased (b.p. = 35.6°C). Methylarsine could not be checked because of its low boiling point (b.p. = 2°C). However, the same mechanism of reaction seems likely for the three products studied, i.e., reduction to the corresponding arsines at -1.1 or -1.2 V, their adsorption on the mercury surface, and desorption at a potential between -0.05 and -0.2 V during the anodic scan. Arguments supporting the idea of an adsorption process [15–18] are as follows: the sharp peaks are typical of adsorption/desorption phenomena, the linear response range is narrow, and the peak potentials vary with concentration; in the case of the arsine obtained chemically from acetasone as described above, the electrocapillarity curve showed that the arsine was adsorbed over a wide voltage range (Fig. 4A); finally, first-harmonic a.c. polarography showed that reduced acetasone depressed the baseline compared to that of the supporting electrolyte (Fig. 4B). The different shapes of the voltammograms of the

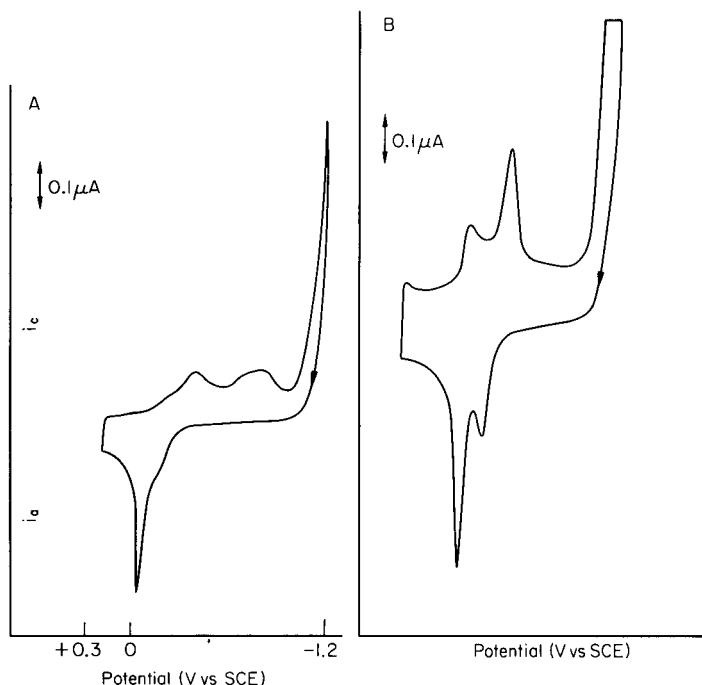


Fig. 3. Cyclic voltammograms: (A) 5×10^{-5} M MMA, starting from -1.2 V; (B) 5×10^{-5} M DMA starting from -1.2 V. Scan rate 100 mV s^{-1} , delay 6 s.

three compounds can be ascribed to their different water solubilities, boiling points, and chemical nature.

Analytical applications

Pulse voltammetry proved to be very sensitive for determining these organic derivatives of arsenic acid. To quantify the compounds, first, the cell was kept at a sufficiently negative potential (-1.1 V for acetarsone, and -1.2 V for MMA and DMA) for a constant time: the peak at the less negative potential (-0.05 to -0.2 V) was used. Table 1 lists the linear response ranges under the conditions outlined for Fig. 2.

The method proposed was tested with good results in the determination of acetarsone in a pharmaceutical product [19] by using standard additions. Addition of MMA and DMA to a filtered ($0.45 \mu\text{m}$) natural water sample, which was then acidified with sulfuric acid, provided realistic recoveries, though it was not possible to distinguish between the two products, because of the similarity of their peak potentials.

Many ions commonly encountered in natural water samples were tested for possible interferences. Chromium(VI) and Cr(III), were tolerated only at concentrations of $<0.2 \text{ mg l}^{-1}$. Copper(II) in 0.1 M sulfuric acid gives a stripping wave at $E_p \approx 0 \text{ V}$; in the determination of DMA, the tolerable limit was

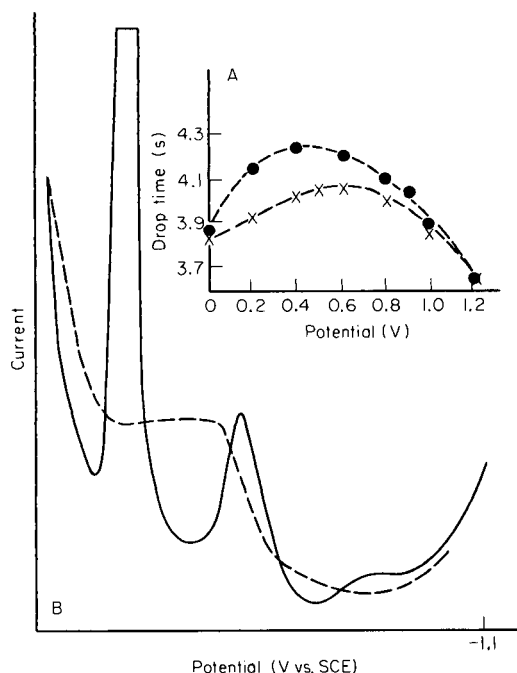


Fig. 4. (A) Electrocapillarity curves: (x) 10^{-4} M acetarsone in 0.1 M sulfuric acid with sodium tetrahydroborate added (50 mg/100 ml) (•) the same mixture without acetarsone. (B) First-harmonic AC polarography: (—) 10^{-5} M acetarsone in acidic tetrahydroborate solution as for (A); (----) the same mixture without acetarsone.

TABLE 1

Linear ranges (i vs. concentration) obtained for the compounds, with initial potentials and peak potentials

Compound	Linear range		Potential (V vs. SCE)	
	(mg l ⁻¹)	(M)	Initial	Peak ^a
MMA	1–45	5×10^{-6} – 1.4×10^{-4}	–1.2	–0.05
DMA	0.3–16	1×10^{-6} – 9×10^{-5}	–1.2	–0.15
Acetarsone	0.2–11	1×10^{-6} – 3.4×10^{-5}	–1.1	–0.10

^a At 5 mg l⁻¹.

2 mg, but only 0.5 mg l⁻¹ was tolerated in the determination of MMA. Lead(II) did not interfere at <4 mg l⁻¹, or arsenic(III) at <2 mg l⁻¹. Anionic surfactants decreased the peak heights of both MMA and DMA, but if standard additions were used, the effect could be corrected for anionic surfactant/organic arsenic ratios of <2.

REFERENCES

- 1 W. Wood, *Science*, 183 (1974) 1049.
- 2 R. W. Braman, *Arsenical Pesticides*, ACS Symposium Series, 1985.
- 3 J. P. Buchet, R. Lanwerys and H. Roels, *Int. Arch. Occup. Environ. Health*, 48 (2) (1981) 111.
- 4 See, e.g., E. H. Daughtrey, A. W. Fitchett and P. Mushak, *Anal. Chim. Acta*, 79 (1975) 199.
- 5 Tagawa Shoji, *Bunseki Kagaku*, 29 (1980) 563.
- 6 J. P. Buchet, R. Lauwerys and H. Roels, *Int. Arch. Occup. Environ. Health*, 46 (1980) 11.
- 7 J. S. Edmonds and W. A. Francesconi, *Anal. Chem.*, 48 (1976) 2019.
- 8 M. O. Andreae, *Anal. Chem.*, 49 (1977) 820.
- 9 R. S. Braman, L. D. Johnson, C. C. Foreback, J. M. Ammons and J. L. Bricker, *Anal. Chem.*, 49 (1977) 621.
- 10 Y. Talmi and D. T. Bostick, *Anal. Chem.*, 47 (1975) 2145.
- 11 W. Holak, *J. Pharm. Sci.*, 61(10) (1972) 1635.
- 12 F. T. Henry and T. M. Thorpe, *Anal. Chem.*, 52 (1980) 80.
- 13 A. Watson and G. Svehla, *Analyst* (London), 100 (1975) 489.
- 14 R. K. Elton and W. E. Geiger, *Anal. Chem.*, 50 (1978) 712.
- 15 H. D. Booth and B. Fleet, *Talanta*, 17 (1970) 491.
- 16 H. Jehring, *J. Electroanal. Chem.*, 21 (1969) 77.
- 17 J. W. Loveland and P. J. Ewing, *J. Phys. Chem.*, 56 (1952) 251, 255, 935, 941, 945.
- 18 B. Breyer and H. H. Bauer, *Alternating Current Polarography and Tensammetry*, Interscience, New York, 1963.
- 19 G. Spini, *Il Farmaco* (Ed.), Pavia, 36 (4) 1981.

ANALYTICA CHIMICA ACTA, VOL. 176 (1985)

AUTHOR INDEX

- Anderegg, R. J.
A selenium-selective chromatographic detector based on isotope clusters 175
- Ang, K. P., see Gunasingham, H. 143
- Ariño, C., see Casassas, E. 113
- Baldwin, R. P., see Chaney, E. N., Jr. 105
- Bednarkiewicz, E.
— and Kublik, Z.
Comparison of the utility of several tensammetric techniques for the determination of traces of surfactants after preconcentration at the hanging mercury drop electrode 133
- Bertels, P. C., see Chakrabarti, C. L. 17
- Bertels, P. C., see Chang, S. B. 1
- Campbell, J. A.
—, Gardner, M. J. and Gunn, A. M.
Short-term stability of trace metals in estuarine water samples 193
- Carabias Martínez, R., see Hernández Méndez, J. 121
- Casassas, E.
—, Ariño, C. and Esteban, M.
Cathodic stripping voltammetry of 2-mercaptoethanol 113
- Casoli, A.
—, Mangia, A., Predieri, G. and Sappa, E.
Behaviour of a tetrahedral nickel-triosmium carbonyl cluster and its mono-substituted derivatives with group V donor ligands in high-performance liquid chromatography 259
- Chakrabarti, C. L., see Chang, S. B. 1
- Chakrabarti, C. L.
—, Chang, S. B., Huston, T. J., Bertels, P. C., Rogers, J. T. and Dick, R.
Capacitive-discharge-heated anisotropic pyrolytic graphite furnace used for atomic absorption spectrometry. Part 2. Experimental verification of temperature distribution of the furnace surface and of the gas phase 17
- Chaney, E. N., Jr.
— and Baldwin, R. P.
Voltammetric determination of doxorubicin in urine by adsorptive preconcentration and flow injection analysis 105
- Chang, S. B.
—, Chakrabarti, C. L., Huston, T. J., Bertels, P. C., Rogers, J. T. and Dick, R.
Capacitive-discharge-heated anisotropic pyrolytic graphite furnace used for atomic absorption spectrometry. Part 1. Theoretical modelling of the heating characteristics of the furnace surface and of the gas phase 1
- Chang, S. B., see Chakrabarti, C. L. 17
- Cromer, M., see Lamy, I. 201
- Cuesta González, M., see Hernández Méndez, J. 121
- Delgado Zamarreño, M.
—, Hernández Méndez, J. and Sánchez Pérez, A.
Electrochemical study and polarographic determination of ranitidine 279
- Desideri, D.
—, Staccioli, L. and Testa, C.
Liquid-liquid extraction of zirconium with tri-n-butylacetohydroxamic acid and spectrophotometric determination in the organic phase with xylenol orange 269
- Dick, R., see Chakrabarti, C. L. 17
- Dick, R., see Chang, S. B. 1
- Esteban, M., see Casassas, E. 113
- Gardner, M. J., see Campbell, J. A. 193
- Geurts, F. L.
— and Kateman, G.
Pattern recognition of multivariate analytical data by plots of high-dimensional data 253
- González López, M. E., see Hernández Méndez, J. 121
- Grishko, V. I.
—, Yudelevich, I. G. and Grishko, V. P.
Variations of thermal-lens spectrophotometry with helium-cadmium laser excitation for the determination of traces of phosphorus 51

- Grishko, V. P., see Grishko, V. I. 51
 Gunasingham, H.
 —, Tay, B. T. and Ang, K. P.
 The electrolytic efficiency of amperometric detection in normal-phase high-performance liquid chromatography 143
 Gunn, A. M., see Campbell, J. A. 193
 Gushikem, Y., see Moreira, J. C. 263
- Hernández Méndez, J., see Delgado Zamarreño, M. 279
 Hernández Méndez, J.
 —, Carabias Martínez, R., González López, M. E. and Cuesta González, M.
 Electroanalytical studies of the pesticide menazon and its hydrolysis products 121
 Huston, T. J., see Chakrabarti, C. L. 17
 Huston, T. J., see Chang, S. B. 1
- Imagawa, T., see Tsuchiya, K. 151
 Ishizuki, T.
 —, Wada, H., Kodama, K. and Nakagawa, G.
 Synthesis of 2-[2-(8-hydroxyquinolyl)-azo]-1-naphthol and its use in the spectrophotometric determination of calcium in potable water 63
- Jankowski, K., see Marczenko, Z. 185
 Jovanović, M. S., see Jovanović, V. M. 285
 Jovanović, V. M.
 — and Jovanović, M. S.
 The behaviour of mixed metal sulphide/silver sulphide sensing materials in solutions of silver ions 285
- Kateman, G., see Geurts, F. L. 253
 Kemp, G. J.
 The susceptibility of calibration methods to errors in the analytical signal 229
 Kodama, K., see Ishizuki, T. 63
 Kraak, J. C., see Tielrooy, J. A. 161
 Kublik, Z., see Bednarkiewicz, E. 133
- Laeven, J. M.
 — and Smit, H. C.
 Optical peak area determination in the presence of noise 77
 Lamy, I.
 —, Seywert, M., Cromer, M. and Scharff, J.-P.
 Simple and mixed ligand complexes of copper(II) with polyfunctional phenolic compounds as models of natural substances 201
- Langmyhr, F. J., see Wibetoe, G. 33
 Leary, J. J., see Meites, L. 249
- Maessen, F. J. M. J., see Tielrooy, J. A. 161
 Mangia, A., see Casoli, A. 259
 Marczenko, Z.
 — and Jankowski, K.
 Sensitive flotation-spectrophotometric determination of gold based on the gold-(III)/bromide/rhodamine 6G system 185
 Meites, L.
 — and Leary, J. J.
 Simple procedures for obtaining constrained calibration equations 249
 Moreira, J. C.
 — and Gushikem, Y.
 Preconcentration of metal ions on silica gel modified with 3(1-imidazolyl)propyl groups 263
- Nakagawa, G., see Ishizuki, T. 63
 Nakagawa, G., see Ohshita, K. 41
- Ohshita, K.
 —, Wada, H. and Nakagawa, G.
 Synthesis of bidentate pyridylazo and thiazolylazo reagents and the spectrophotometric determination of copper in a flow-injection system 41
- Predieri, G., see Casoli, A. 259
 Profumo, A., see Spini, G. 291
 Pszonicki, L.
 Evaluation of analytical interlaboratory comparisons and certification of reference materials 213
- Reay, P. F.
 An improved determination of ammonia in Kjeldahl digests and acidic solutions with a buffered Berthelot reaction 275
 Rogers, J. T., see Chakrabarti, C. L. 17
 Rogers, J. T., see Chang, S. B. 1
- Salvador, G. C., see Tan, H. S. I. 71
 Sánchez Pérez, A., see Delgado Zamarreño, M. 279
 Sappa, E., see Casoli, A. 259
 Scharff, J.-P., see Lamy, I. 201
 Seywert, M., see Lamy, I. 201

- Smit, H. C., see Laeven, J. M. 77
- Soldi, T., see Spini, G. 291
- Spini, G.
- , Profumo, A. and Soldi, T.
Voltammetric determination of some organic compounds of arsenic reduced at the mercury electrode 291
- Staccioli, L., see Desideri, D. 269
- Tan, H. S. I.
- and Salvador, G. C.
Difference spectrophotometric assay of mixtures of phenylpropanolamine hydrochloride with guaifenesin or dextromethorphan hydrobromide in solid cough formulations 71
- Tay, B. T., see Gunasingham, H. 143
- Testa, C., see Desideri, D. 269
- Tielrooy, J. A.
- , Kraak, J. C. and Maessen, F. J. M. J.
High-performance liquid chromatography with post-column reaction detection for the determination of rare-earth elements in phosphoric acids produced for the manufacture of phosphate fertilizers 161
- Tsuchiya, K.
- , Imagawa, T., Yamaya, K. and Yoshida, M.
Separation of microamounts of fluoride coexisting with large amounts of aluminum and silica by improved trimethylsilylating distillation 151
- Wada, H., see Ishizuki, T. 63
- Wada, H., see Ohshita, K. 41
- Wibetoe, G.
- and Langmyhr, F. J.
Spectral interferences and background overcompensation in inverse Zeeman-corrected atomic absorption spectrometry. Part 2. The effect of cobalt, manganese and nickel on 30 elements and 53 element lines 33
- Yamaya, K., see Tsuchiya, K. 151
- Yoshida, M., see Tsuchiya, K. 151
- Yudelevich, I. G., see Grishko, V. I. 51

Separations

separation of microamounts of fluoride coexisting with large amounts of aluminum and silica by improved trimethylsilylating distillation	
K. Tsuchiya, T. Imagawa, K. Yamaya and M. Yoshida (Tokyo, Japan)	151
high-performance liquid chromatography with post-column reaction detection for the determination of rare-earth elements in phosphoric acids produced for the manufacture of phosphate fertilizers	
J. A. Tielrooy, J. C. Kraak and F. J. M. J. Maessen (Amsterdam, The Netherlands)	161
selenium-selective chromatographic detector based on isotope clusters	
R. J. Anderegg (Orono, ME, U.S.A.)	175
sensitive flotation-spectrophotometric determination of gold based on the gold(III)/bromide/rhodamine 6G system	
Z. Marczenko and K. Jankowski (Warsaw, Poland)	185

General Analytical Chemistry

short-term stability of trace metals in estuarine water samples	
J. A. Campbell, M. J. Gardner and A. M. Gunn (Marlow, Gt. Britain)	193
simple and mixed ligand complexes of copper(II) with polyfunctional phenolic compounds as models of natural substances	
I. Lamy, M. Seywert, M. Cromer and J.-P. Scharff (Villeurbanne, France).	201
evaluation of analytical interlaboratory comparisons and certification of reference materials	
L. Pszonicki (Vienna, Austria).	213
the susceptibility of calibration methods to errors in the analytical signal	
G. J. Kemp (Sheffield, Gt. Britain)	229

Short Communications

simple procedures for obtaining constrained calibration equations	
L. Meites (Fairfax, VA, U.S.A.) and J. J. Leary (Harrisonburg, VA, U.S.A.)	249
pattern recognition of multivariate analytical data by plots of high-dimensional data	
F. L. Geurts and G. Kateman (Nijmegen, The Netherlands)	253
behaviour of a tetrahedral nickel-triosmium carbonyl cluster and its monosubstituted derivatives with group V donor ligands in high-performance liquid chromatography	
A. Casoli, A. Mangia, G. Predieri (Parma, Italy) and E. Sappa (Torino, Italy)	259
reconcentration of metal ions on silica gel modified with 3(1-imidazolyl)propyl groups	
J. C. Moreira and Y. Gushikem (Campinas, Brazil)	263
liquid-liquid extraction of zirconium with tri-n-butylacetohydroxamic acid and spectrophotometric determination in the organic phase with xylenol orange	
D. Desideri, L. Staccioli and C. Testa (Urbino, Italy).	269
an improved determination of ammonia in Kjeldahl digests and acidic solutions with a buffered Berthelot reaction	
P. F. Reay (Palmerston North, New Zealand)	275
electrochemical study and polarographic determination of ranitidine	
M. Delgado Zamarreño, J. Hernández Méndez and A. Sánchez Pérez (Salamanca, Spain)	279
the behaviour of mixed metal sulphide/silver sulphide sensing materials in solutions of silver ions	
V. M. Jovanović and M. S. Jovanović (Karnegieva, Yugoslavia)	285
voltammetric determination of some organic compounds of arsenic reduced at the mercury electrode	
G. Spini, A. Profumo and T. Soldi (Pavia, Italy)	291

<i>Author Index</i>	297
-------------------------------	-----

CONTENTS

(Abstracted, Indexed in: Anal. Abstr.; Biol. Abstr.; Chem. Abstr.; Curr. Contents Phys. Chem. Earth Sci.; Life Sci.; Index Med.; Mass Spectrom. Bull.; Sci. Citation Index; Excerpta Med.)

Spectrometric Methods

Capacitive-discharge-heated anisotropic pyrolytic graphite furnace used for atomic absorption spectrometry.

Part 1. Theoretical modelling of the heating characteristics of the furnace surface and of the gas phase
S. B. Chang, C. L. Chakrabarti, T. J. Huston, P. C. Bertels, J. T. Rogers and R. Dick (Ottawa, Ont., Canada) 1

Capacitive-discharge-heated anisotropic pyrolytic graphite furnace used for atomic absorption spectrometry.

Part 2. Experimental verification of temperature distribution of the furnace surface and of the gas phase
C. L. Chakrabarti, S. B. Chang, T. J. Huston, P. C. Bertels, J. T. Rogers and R. Dick (Ottawa, Ont., Canada) 17

Spectral interferences and background overcompensation in inverse Zeeman-corrected atomic absorption spectrometry. Part 2. The effect of cobalt, manganese and nickel on 30 elements and 53 element lines
G. Wibetoe and F. J. Langmyhr (Oslo, Norway) 33

Synthesis of bidentate pyridylazo and thiazolylazo reagents and the spectrophotometric determination of copper in a flow-injection system
K. Ohshita, H. Wada and G. Nakagawa (Nagoya, Japan) 41

Variations of thermal-lens spectrophotometry with helium-cadmium laser excitation for the determination of traces of phosphorus
V. I. Grishko, I. G. Yudelevich and V. P. Grishko (Novosibirsk, U.S.S.R.) 51

Synthesis of 2-[2-(8-hydroxyquinolyl)azo]-1-naphthol and its use in the spectrophotometric determination of calcium in potable water
T. Ishizuki, H. Wada, K. Kodama and G. Nakagawa (Nagoya, Japan). 63

Difference spectrophotometric assay of mixtures of phenylpropanolamine hydrochloride with guaifenesin or dextromethorphan hydrobromide in solid cough formulations
H. S. I. Tan and G. C. Salvador (Cincinnati, OH, U.S.A.) 71

Computer Methods and Applications

Optimal peak area determination in the presence of noise
J. M. Laeven and H. C. Smit (Amsterdam, The Netherlands) 77

Electrometric Methods

Voltammetric determination of doxorubicin in urine by adsorptive preconcentration and flow injection analysis
E. N. Chaney, Jr. and R. P. Baldwin (Louisville, KY, U.S.A.) 105

Cathodic stripping voltammetry of 2-mercaptoethanol
E. Casassas, C. Ariño and M. Esteban (Barcelona, Spain). 113

Electroanalytical studies of the pesticide menazon and its hydrolysis products
J. Hernández Méndez, R. Carabias Martínez, M. E. González López and M. Cuesta González (Salamanca, Spain) 121

Comparison of the utility of several tensammetric techniques for the determination of traces of surfactants after preconcentration at the hanging mercury drop electrode
E. Bednarkiewicz and Z. Kublik (Warsaw, Poland) 133

The electrolytic efficiency of amperometric detection in normal-phase high-performance liquid chromatography
H. Gunasingham, B. T. Tay and K. P. Ang (Kent Ridge, Singapore) 143

(continued on inside back cover)

0003-7001(198510)176:10;1-0