PAGES 1093–1196

ISSN 0003–2654

The Analyst

A monthly international journal dealing with all branches of the theory and practice of analytical chemistry, including instrumentation and sensors, and physical, biochemical, clinical, pharmaceutical, biological, automatic and computer-based methods

Vol. 112 No. 8
August
1987

ROYAL SOCIETY OF CHEMISTRY

The Analyst

The Analytical Journal of The Royal Society of Chemistry

Editor, The Analyst:
Philip C. Weston

Senior Assistant Editors:
Judith Egan, Roger A. Young

Assistant Editors:
Anne Horscroft, Harpal Minhas

Editorial Office: The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-734 9864. Telex No. 268001

Advertisements: Advertisement Department, The Royal Society of Chemistry, Burlington House, Piccadilly, London, W1V 0BN. Telephone 01-437 8656. Telex No. 268001

The Analyst (ISSN 0003–2654) is published monthly by The Royal Society of Chemistry, Burlington House, London W1V 0BN, England. All orders accompanied with payment should be sent directly to The Royal Society of Chemistry, The Distribution Centre, Blackhorse Road, Letchworth, Herts. SG6 1HN, England. 1987 Annual subscription rate UK £160.00, Rest of World £179.00, USA $315.00. Purchased with *Analytical Abstracts* UK £364.00, Rest of World £403.00, USA $709.00. Purchased with *Analytical Abstracts* plus *Analytical Proceedings* UK £411.00, Rest of World £455.00, USA $801.00. Purchased with *Analytical Proceedings* UK £200.00, Rest of World £224.00, USA $394.00. Air freight and mailing in the USA by Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003.
USA Postmaster: Send address changes to: *The Analyst,* Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003. Second class postage paid at Jamaica, NY 11431. All other despatches outside the UK by Bulk Airmail within Europe, Accelerated Surface Post outside Europe. PRINTED IN THE UK.

Information for Authors

Full details of how to submit material for publication in *The Analyst* are given in the Instructions to Authors in the January issue. Separate copies are available on request.

The Analyst publishes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical, biochemical, clinical, pharmaceutical, biological, automatic and computer-based methods. Papers on new approaches to existing methods, new techniques and instrumentation, detectors and sensors, and new areas of application with due attention to overcoming limitations and to underlying principles are all equally welcome. There is no page charge.

The following types of papers will be considered:

Full papers, describing original work.

Short papers: the criteria regarding originality are the same as for full papers, but short papers generally report less extensive investigations or are of limited breadth of subject matter

Communications, which must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications receive priority and are usually published within 5–8 weeks of receipt. They are intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Reviews, which must be a critical evaluation of the existing state of knowledge on a particular facet of analytical chemistry.

Every paper (except Communications) will be submitted to at least two referees, by whose advice the Editorial Board of *The Analyst* will be guided as to its acceptance or rejection. Papers that are accepted must not be published elsewhere except by permission. Submission of a manuscript will be regarded as an undertaking that the same material is not being considered for publication by another journal.

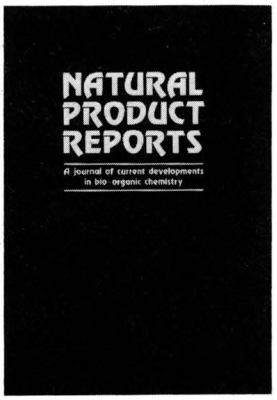
Regional Advisory Editors. For the benefit of potential contributors outside the United Kingdom, a Panel of Regional Advisory Editors exists. Requests for help or advice on any matter related to the preparation of papers and their submission for publication in *The Analyst* can be sent to the nearest member of the Panel. Currently serving Regional Advisory Editors are listed in each issue of *The Analyst*

Manuscripts (three copies typed in double spacing) should be addressed to:
The Editor, *The Analyst,*
Royal Society of Chemistry,
Burlington House,
Piccadilly,
LONDON W1V 0BN, UK

Particular attention should be paid to the use of standard methods of literature citation, including the journal abbreviations defined in Chemical Abstracts Service Source Index. Wherever possible, the nomenclature employed should follow IUPAC recommendations, and units and symbols should be those associated with SI.

All queries relating to the presentation and submission of papers, and any correspondence regarding accepted papers and proofs, should be directed to the Editor, *The Analyst* (address as above). Members of the Analytical Editorial Board (who may be contacted directly or via the Editorial Office) would welcome comments, suggestions and advice on general policy matters concerning *The Analyst.*

Fifty reprints of each published contribution are supplied free of charge, and further copies can be purchased.



Natural Product Reports

A review journal dealing exclusively with natural product chemistry and related topics

6 issues per annum plus index
(approx 90 pages per issue)
1987 Subscription £142.00
($280.00)
Rest of World £159.00
RSC Members £35.50

Natural Product Reports is a bimonthly review journal which commenced publication in 1984 and reviews recent developments in natural product chemistry. Each issue contains approximately 90 pages covering four or five articles; there is an author index and a subject index (cumulative annually) to facilitate location of articles dealing with specific areas.

Natural Product Reports consists of critical reviews of literature that have been published, during well-defined periods, on the topics of general chemistry and biosynthesis of alkaloids, terpenoids, steroids, fatty acids, and O-heterocyclic, aliphatic, aromatic and alicyclic natural products. Occasional reviews provide details of techniques for separation and spectroscopic identification, and describe methodologies that are useful to all chemists and biologists actively engaged in the study of natural products.

Special Issue February 1987

A Centenary Tribute to Sir Robert Robinson 1886-1975

The February 1987 issue of *Natural Product Reports* was produced in honour of Sir Robert Robinson to mark the centenary of his birth on 13 September 1886. The contributions are based largely on lectures that were presented at the RSC Annual Chemical Congress, 1986, held at the University of Warwick. Lectures at the Symposium Tribute, organised by the Historical Group of the RSC, were presented by a mixture of historians of chemistry, chemists (both natural product and theoretical organic), who discussed their subjects in historical perspective, and chemists, who as young people participated in some of the work of Robinson. It was especially pleasing that Professor A. R. Battersby was able to present his Robert Robinson Lecture to the Congress, and also that he kindly agreed to publish his lecture alongside this Tribute.

This Tribute covers the major contributions that Robinson made to organic chemistry, and provides a biography of his life, incorporating reminiscences from those who had known him, together with some of the places with which he was associated.

Contents:

ROYAL SOCIETY OF CHEMISTRY
Information Services

Ordering
Non-RSC Members should send their orders to:
The Royal Society of Chemistry, Distribution Centre, Blackhorse Road, Letchworth, Herts SG6 1HN, UK.

RSC Members should send their orders to:
The Royal Society of Chemistry, Membership Manager, 30 Russell Square, London WC1B 5DT, UK.

BIO '87
Exhibition & Conference

Computer-Aided-Sciences
Exhibition & Conference

MLSE
Medical Laboratory Sciences Exhibition

LABORATORY EXHIBITION
LABORATORY EXHIBITION
LABORATORY EXHIBITION
LABORATORY EXHIBITION

ANALYTICON CONFERENCE
ANALYTICON CONFERENCE
ANALYTICON CONFERENCE
ANALYTICON CONFERENCE

SEE YOU AT "THE WEEK"!

The British Laboratory Week is the major international meeting of its kind in Europe in 1987.

There's no Achema, Analytica, Het Instrument or Salon du Laboratoire this year. They're bi-ennial and tri-ennial, and their schedules coincide in '87.

So plan your visit now!

As you'll appreciate, 'The Week' is an important occasion and, well worth your time and interest. Simply complete and clip the coupon below. We'll make sure all the relevant information is sent to you immediately it becomes available.

The British Laboratory Week is an umbrella title for FOUR exhibitions and THREE conferences. (See panels above.)

Over 750 different companies' products from around the world will be on display.

Heck! With the exhibits and the subjects for discussion we've got lined up, you'd be crazy to stay away.

CUT OUT AND MAIL FOR YOUR FREE TICKET!

To: Brian Curtis, Organiser, British Laboratory Week, The Hub, Emson Close, Saffron Walden, Essex CB10 1HL, United Kingdom.

Please send me_____FREE ticket(s) to "The Week" and information on my principal area of interest:
Laboratory '87 ☐ Medical Laboratory Sciences ☐ Bio '87 ☐
Computer-Aided-Sciences ☐

Title

Company/Organisation

Address

Also, please send me details of the following conferences:
Bio '87 ☐ Analyticon ☐ Computer-Aided-Sciences ☐

THE BRITISH LABORATORY WEEK.

THE COUNTRY'S LARGEST SCIENTIFIC EVENT

13-15 OCTOBER, 1987
OLYMPIA, LONDON, ENGLAND

The British Laboratory Week is organised by Curtis Steadman & Partners Limited, The Hub, Emson Close, Saffron Walden, Essex CB10 1HL, United Kingdom.
Telephone: (0799) 26699.
Telex: 81653 INFORM G.
Fax: (0799) 26088.

42181

Circle 002 for further information

Gas Chromatographic Determination of Carbonates and Sulphides in the Corrosion Products of Metals

Gianrico Castello*
Istituto di Chimica Industriale, Università di Genova, Corso Europa 30, 16132 Genova, Italy
Anna Maria Beccaria and Gildo Poggi
Istituto per la Corrosione Marina dei Metalli, Consiglio Nazionale delle Ricerche, Via della Mercanzia 4, 16123 Genova, Italy

A method has been developed for the gas chromatographic (GC) determination of sulphides in the corrosion products formed on the surface of metals immersed in sea water. The samples are treated at room temperature with 30% glycine solution, which dissolves the compounds of bivalent metals, but leaves unchanged other corrosion products and the metallic matrix. The evolved gases (carbon dioxide and hydrogen sulphide) are stripped with a flow of purified inert gas and trapped at liquid nitrogen temperature in the modified loop of a gas sampling valve. They are then thermally desorbed and injected into a gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector.

The method permits the simultaneous determination of CO_2 and H_2S and the measurement of the amounts of carbonates, oxycarbonates and sulphides in the corrosion layers. The detection limit is about 1 μg of H_2S (with a precision of ±15%). The GC method is therefore about ten times more sensitive than the spectrophotometric method using methylene blue.

The performance of different methods was evaluated by analysis of the corrosion products of copper and iron specimens subjected to corrosion in surface and deep (200 m) sea water.

Keywords: *Metal corrosion products; sulphides; carbonates; gas chromatography; sea water*

The analysis of the oxidation layer formed on the surface of metals immersed in different corrosive solutions yields results that, correlated with other parameters (*e.g.*, electrode impedance, polarisation resistance, equilibrium potential) obtained by electrochemical measurements, permits the corrosion mechanisms and the corrosion resistance to be investigated. Some electrochemical methods are sometimes difficult or impossible to use in the natural environment, *e.g.*, in sea water, mainly owing to long exposure times. In this instance, chemical analysis is the main means of establishing the corrosion mechanism and, by permitting the characterisation of various layers of different thickness formed in sequence, to trace back the changes in the parameters of the corrosive environment such as pH and dissolved oxygen (DO). For example, a first sulphide layer, formed under non-oxidising conditions at pH < 7, may be followed by a layer containing basic carbonates, formed at pH > 8 and with a high DO content (>8 p.p.m.),[1,2] showing that the metal was immersed in a non-oxidising environment owing to decomposition of algae in autumn and winter, followed by a high DO concentration due to algae proliferation in spring and summer.[3]

The analysis of such heterogeneous oxidation layers can be carried out by surface analysis methods such as Auger spectroscopy or ESCA, which permit the analysis of small areas, but sometimes require the use of reference standards which are difficult to obtain. Chemical analysis permits the determination of the ions in the corrosion layer, averaged over greater areas and, correlated with X-ray diffractometry, adds useful information to data obtained by electron spectroscopy.

Chemical analysis methods require the selective dissolution of the different compounds of the oxidation layer with a suitable series of solvents (methanol, glycine) that leave the metallic matrix unchanged.[4–6] The solubilised ions are then determined by atomic absorption spectrometry (AAS) (cations) or ion chromatography (anions), but some problems are encountered in the determination of carbonates and sulphides, which are converted into gaseous CO_2 and H_2S during the treatment with glycine.

These gases can be determined by stripping them from the reaction vessel with a flow of inert gas and measuring their concentrations in a separate manifold. By following classical methods, H_2S is removed from the stripping gas flow using zinc acetate at pH 5, in order to avoid its interference in the titration of CO_2 with sodium methoxide,[7] and determined separately by the methylene blue method.[8,9] Unfortunately, an appreciable amount of CO_2 is absorbed by the zinc sulphide formed in the zinc acetate trap, or is coprecipitated with the zinc sulphide as zinc hydroxycarbonate, and is therefore lost in the subsequent titration.

In a previous paper[10] we described the gas chromatographic (GC) determination of carbonates in the corrosion products by analysis of the CO_2 evolved, in the absence of interferent sulphides. In this paper the simultaneous determination of H_2S and CO_2 is described, and the GC method is compared with the spectrophotometric method.[3,9]

Experimental

Equipment

For the analysis of evolved CO_2 and H_2S a Model 2800 gas chromatograph (Varian, Palo Alto, CA, USA) equipped with columns filled with 80–100-mesh Porapak Q (3 m × 4 mm i.d.) and with a thermal conductivity detector (TCD) was used. The analytical column was connected directly to a six-port gas-sampling valve (Varian, nut-type, stainless steel) and to a trap cooled with liquid nitrogen, in order to freeze and concentrate the CO_2 and H_2S evolved. The trap was made of PTFE tubing (2 m × 2.4 mm i.d.) filled with nylon wire in order to increase the scrubbing surface, the heat exchange and the trapping efficiency. All tubing and connecting pieces were made of PTFE or nylon in order to avoid losses of H_2S due to chemical reaction or adsorption on metal surfaces. Fig. 1 is a schematic diagram of the extraction and trapping apparatus.

The determination of selectively dissolved cations was carried out by using a Uvidec-5 spectrophotometer (Jasco,

* To whom correspondence should be addressed.

Tokyo, Japan), an atomic absorption spectrometer (Varian AA 6) with air - acetylene burner, an anodic stripping differential-pulse polarograph (Multipolarograph 472/WR; Amel, Milan, Italy) and a CGR Cristalloblock 50 X-ray diffractometer (Compagnie Generale de Radiologie, Lyon, France) using Cu Kα radiation. The anions were measured by using an ion chromatograph (Dionex, Sunnyvale, CA, USA) with an HPIC AS 2 column, an HPIC AG 2 pre-column, 3 mM Na_2CO_3 - 2 mM NaOH as the eluent and 0.0125 M H_2SO_4 as the suppressing agent.

Reagents

Zinc acetate solution, 20%.

N,N-*Dimethyl*-p-*phenylenediammonium chloride solution*, 0.25% *in* 6 M *HCl*.

Iron(III) chloride solution, 0.05 g ml^{-1}.

Glycine solution, saturated at 25 °C.

General Procedure

The corroded specimens, with average surface areas ranging from 2 to 20 cm^2, were removed from the corrosive solution, rinsed with doubly distilled water and immediately subjected to selective dissolution in a manifold as described elsewhere,[4] which permits operation in an inert gas atmosphere in order to avoid further oxidation of the specimen. The procedure of the attack is shown in Fig. 2.

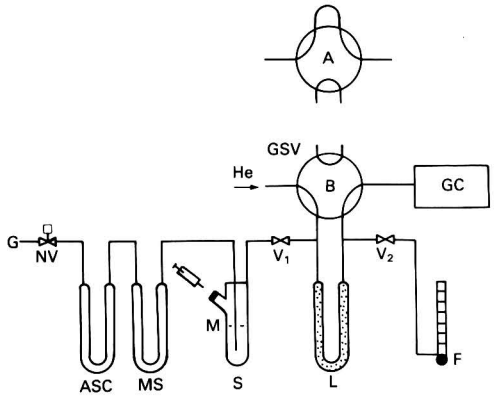


Fig. 1. Schematic diagram of the extraction and analysis apparatus. G, Inert stripping gas inlet; NV, needle valve; ASC, MS, ascarite and molecular sieve traps; S, reaction vessel; M, rubber membrane for injection of reagents; V_1, V_2, on - off valves; L, refrigerated sampling loop; GSV, gas-sampling valve in (A) sample collection and (B) analysis position; F, flow meter; GC, gas chromatograph with thermal conductivity detector

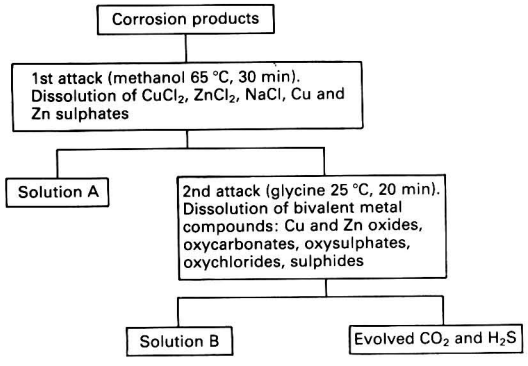


Fig. 2. Schematic diagram of the procedure used for the dissolution of the corrosion products

Solutions A and B were analysed in order to measure the different ions by AAS (Na^+, Mg^{2+}, Cu^{2+}), anodic stripping differential-pulse polarography, ASVPP, (Zn^{2+}, Cu^{2+}) or ion chromatography (Cl^-, SO_4^{2-}) by following previously described methods.[6] The CO_2 and H_2S originating from the dissolution of the carbonates due to the addition of glycine solution to the corroded specimen in the manifold S, and stripped by a nitrogen or helium flow (Fig. 1), were frozen in trap L, cooled with liquid nitrogen. A flow time of 20 min with a stripping gas flow-rate of 20 cm^3 min^{-1} was suitable for nearly complete extraction of the evolved gases. The inlet and outlet of the trap were then closed by means of valves V_1 and V_2 and the trap was heated at room temperature for about 10 min. The gas-sampling valve was switched from position A to position B in order to deliver the evolved gases into the GC column, held at 70 °C. The TCD was operated with a filament current of 155 mA, using a flow-rate of 20 cm^3 min^{-1} of purified helium as the carrier gas. A typical chromatogram is shown in Fig. 3.

Quantitative calibration of the detector response was carried out by using an exponential dilution flask and injecting known amounts of CO_2 and H_2S. The calibration was made within the range 5000–1 p.p.m. with an accuracy of ±2% by measuring the peak heights.

Results and Discussion

The results obtained by measuring CO_2 and H_2S with three procedures (I, II, III), shown in Fig. 4, were compared. In methods I and II, H_2S is measured by the methylene blue spectrophotometric method[8,9] after absorption in a trap filled with zinc acetate solution while the CO_2 is titrated with sodium methoxide[7] (detection limit about 40 μg with ±50% precision and ±10% accuracy) or by GC[10] (detection limit 0.5 μg with ±5% precision and accuracy).

Table 1 shows the results of the calibration of the spectrophotometric method carried out either with sodium sulphide solution or after absorption of known amounts of H_2 in zinc acetate solution. The detection limit of this method is about 10 μg of H_2S (twice the "blank" value of log I_o/I), the precision is ±2.5% and the accuracy is ±10%. The method is not sensitive enough to permit the determination of sulphides

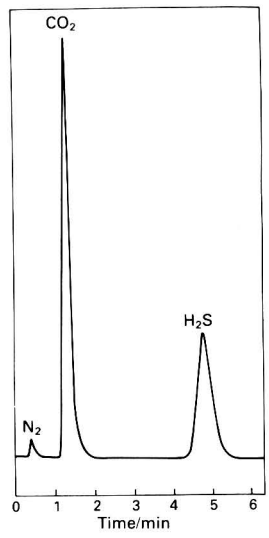


Fig. 3. Chromatogram of the separation of H_2S and CO_2. Column, 80–100-mesh Porapak Q (3 m × 4 mm i.d.); column temperature, 70 °C; helium flow-rate, 20 cm^3 min^{-1}

in the corrosion products of some metals or their alloys (nickel and cupro-nickel), which have a thickness of a few nanometres, the corresponding amount of S^{2-} therefore being about five times less than the detection limit of the methylene blue method, even if the surface area of the sample is taken as 20 cm^2. Further, during the adsorption of H_2S by means of the zinc acetate solution, appreciable amounts of CO_2 are lost. Table 2 shows the CO_2 recovery as a function of the type of treatment followed in method II in Fig. 4 and of the sample composition.

The direct GC of gases evolved from the heated trap permits the simultaneous determination of CO_2 and H_2S without appreciable loss and with good sensitivity (about 1 μg with an average precision of ±15%). Table 3 (column A) shows the results of the calibration obtained by direct injection of H_2S into the GC column. Concentration in the cooled trap was almost completed within 20 min after the injection of the glycine solution; the procedure for heating the trap was critical. On increasing the temperature and the heating time, the amount of H_2S lost (probably by reaction with the metallic surfaces of the gas-sampling valve) increased, as shown in Fig. 5. At room temperature the H_2S recovery was almost complete (>92%) even with long heating times, whereas at 50 °C a large loss was observed. Table 3 also shows the effect of the dead volume of the gas-sampling valve, which was negligible (column B, showing the results of H_2S injections through the trap), and of the dead volume of the on - off valves V_1 and V_2 (column C, showing the results of the complete procedure). The reproducible systematic error of about −8% can be reduced by using microvalves of low dead volume (Model HV, Hamilton, Reno, NE, USA) and shorter connecting lines. The precision of the determination of H_2S by the complete freezing - heating method was ±10%.

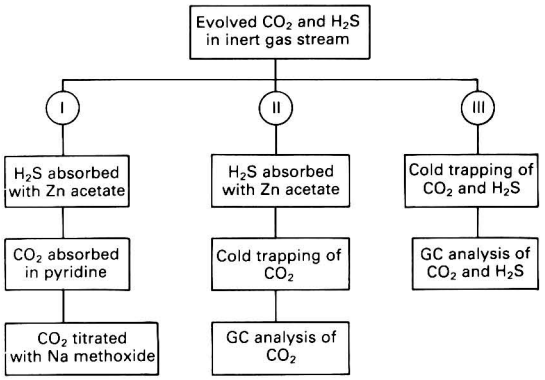


Fig. 4. Schematic diagram of the different methods used for the determination of H_2S and CO_2

Table 1. Spectrophotometric determination of H_2S (methylene blue method). λ = 670 nm; cell path length, 2 cm; final volume of blue methylene solution, 25 ml. Detection limit, 10 μg; correlation coefficient of equation $\log I_o/I = 0.0157Q + 0.446$, 0.992; $n = 5$

Q/μg H_2S	$\log I_o/I$
0.0 (blank)	0.052 ± 0.0076
5.3	0.122 ± 0.010
13.3	0.263 ± 0.0058
26.6	0.456 ± 0.0051
39.8	0.676 ± 0.0060

Analysis of Corrosion Products

By following the described method, the corrosion products formed on Armco iron and copper specimens, immersed offshore for 6 months at different depths (1 and 200 m) with different amounts of DO (8 p.p.m. at the surface, 2 p.p.m. at 200 m) and of S^{2-} (0.0 p.p.m. at the surface, about 0.2 p.p.m. at 200 m in winter), were analysed. The results (Table 4) showed good precision. It was impossible, however, to calculate the accuracy, firstly because no comparison standards were available and secondly because the solubilities of the various compounds depend not only on their structure but also on their physical properties (grain size of the compound, thickness of the oxide film, etc.).

Reasonable agreement between the results of X-ray and chemical analyses was observed (see Table 5) for the main components of the corrosion products (oxides, carbonates, oxychlorides, etc.). The minor constituents (sulphides) were only detected and measured by selective dissolution and GC, which gives no information on the structure of these compounds, but contributes to the knowledge of the composition of the corrosion layer obtained by using other analytical methods.

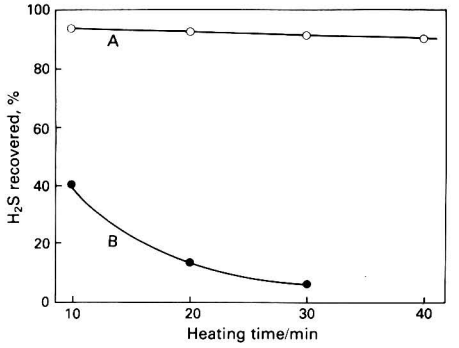


Fig. 5. Effect of desorption temperature on the recovery efficiency of trapped H_2S. (A) 25 °C; (B) 50 °C. Stripping gas flow-rate, 20 cm^3 min^{-1}

Table 3. GC determination of H_2S. $n = 3$

Amount of H_2S		Peak height*		
μl	μg	A	B	C
5	7.6	16 ± 3		
10	15.2	41 ± 3		38 ± 4
25	37.9	104 ± 5	104 ± 5	
50	75.9	220 ± 10	219 ± 8	203 ± 9
75	113.8	316 ± 12		
100	151.8	423 ± 23	427 ± 10	389 ± 37

* A, Direct injection into the column; B, injection through the trap without freezing; C, complete procedure with freezing (see text).

Table 2. Effect of absorption of H_2S in zinc acetate solution on the efficiency of recovery of CO_2. $n = 5$

Sample	Treatment	CO_2 recovery, %
CO_2 (100 μl)	None	100
CO_2 (100 μl)	Absorption in zinc acetate	92 ± 1.3
CO_2 (100 μl) + H_2S (100 μl)	Absorption in zinc acetate	79 ± 2.9
CO_2 (100 μl) + H_2S (200 μl)	Absorption in zinc acetate	56 ± 4.8

Table 4. Chemical analysis of divalent metal compounds in the corrosion layer formed on Cu and Fe exposed in sea water for 6 months. $n = 3$

		Ion/μg cm^{-2}								
Metal	Depth/m	Cu^{2+}	Ca^{2+}	Mg^{2+}	Na^{+}	Fe^{2+}	SO_4^{2-}	Cl^{-}	S^{2-}	CO_3^{2-}
Copper . .	1	200 ± 5	10 ± 0.5	3 ± 0.2	20 ± 2	—	3 ± 0.5	35 ± 2	—	25 ± 2
	200	280 ± 8	14 ± 0.3	5 ± 0.2	25 ± 2	—	14 ± 1	60 ± 3	7 ± 1	32 ± 2
Iron	1	—	8 ± 0.7	4 ± 1	41 ± 2	575 ± 5	—	139.5 ± 11	—	32 ± 2
	200	—	7 ± 0.4	5 ± 1	73 ± 5	183 ± 4	—	153 ± 5	—	40 ± 3

Table 5. Comparison of X-ray and chemical analyses

Metal	Depth/m	Compounds identified by X-ray diffractometry	Stoicheiometrically calculated compounds
Copper	1	CuO, Cu_2O, $CaCO_3$, Cu(OH) (CO_3), NaCl	CuO, Cu_2O, $CaCO_3$, $MgSO_4$, $Cu_x(OH)_y(CO_3)_z$
	200	CuO, Cu_2O, $CaCO_3$, Cu(OH)Cl, $Cu(OH)CO_3$	CuO, Cu_2O, $CaCO_3$, $MgSO_4$, $Cu_x(OH)_y(CO_3)_z$, CuS, $Cu_x(OH)_yCl_z$
Iron	1	$Fe(OH)_2$, NaCl, $CaCO_3$	$Fe(OH)_2$, NaCl, $CaCO_3$, $MgCO_3$, $Fe_x(OH)_yCl_z$
	200	$Fe(OH)_2$, NaCl, $CaCO_3$	FeS, $Fe(OH)_2$, NaCl, $CaCO_3$, $Fe_x(OH)_yCl_z$, $MgCO_3$

Conclusions

The GC method described permits the simultaneous determination of H_2S and CO_2 without previous absorption of the H_2S in zinc acetate solution, a procedure that results in a loss of CO_2. The minimum measurable amounts are 2 μg of H_2S and 0.5 μg of CO_2 when the gases are evolved from the corrosion products of a 20 cm^2 corroded surface. This permits the determination of sulphides, carbonates and oxycarbonates in thin layers (<10 nm) of corrosion products, which cannot be measured with other methods, and adds useful information to the analytical results obtained by electron spectroscopic methods.

References

1. Mor, E. D., and Beccaria, A. M., "4th Congress on Marine Corrosion and Fouling, Juan les Pins (Antibes), France, 1976," Centre de Recherches et d'Etudes Océanographiques, Boulogne, 1977, p. 373.
2. Beccaria, A. M., Mor, E. D., Bruno, G., and Poggi, G., *Werkst. Korros.*, 1982, **33**, 416.
3. Horne, R. A., "Marine Chemistry," Wiley–Interscience, New York, 1969, p. 190.
4. Mor, E. D., and Beccaria, A. M., "Proceedings of the International Congress on Corrosion and Fouling, Washington, DC, 1972," Northwest University Press, Evanston, IL, 1973, p. 477.
5. Beccaria, A. M., and Poggi, G., *Anal. Lett.*, 1985, **18**, 2259.
6. Beccaria, A. M., and Poggi, G., *Anal. Lett.*, 1986, **19**, 1205.
7. Patchorniki, A., and Shalitin, Y., *Anal. Chem.*, 1961, **33**, 1887.
8. Johnson, C. M., and Nishita, H., *Anal. Chem.*, 1952, **24**, 736.
9. Sands, A. E., Grafins, M. A., Wainwright, H. W., and Wilson, M. W., *US Bur. Mines Rep. Invest.*, No. 4547, 1949.
10. Beccaria, A. M., Castello, G., and Poggi, G., *J. Chromatogr.*, 1987, **395**, 641.

Paper A7/40
Received February 9th, 1987
Accepted March 11th, 1987

Determination of Methylmercury in Fish by Gas Chromatography - Direct Current Plasma Atomic Emission Spectrometry*

Kenneth W. Panaro and Donald Erickson
US Food and Drug Administration, Boston District Office, 585 Commercial Street, Boston, MA 02109, USA

and Ira S. Krull†
Department of Chemistry, The Barnett Institute, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA

Gas chromatography (GC) has been interfaced very simply and inexpensively with a direct current plasma (DCP) atomic emission spectrometer in order to perform highly specific and selective determinations of methylmercury (MeHg) in fish samples. A simple, isothermal, low-cost GC was constructed which could be dedicated to the DCP, allowing routine qualitative and quantitative determinations of organomercury species in complex food matrices. Optimisation of the GC - DCP interface was accomplished, followed by a determination of the detection limits, the linearity of the calibration graph and comparison of the results with those obtained by GC - electron-capture detection (ECD) and total mercury by cold-vapour atomic absorption spectrometry. In most instances, qualitative and quantitative results for incurred and spiked levels did not agree for the GC - DCP and GC - ECD approaches. An additional extraction procedure has also been developed for MeHg from fish samples involving extraction with an organic solvent, concentration and injection on to the GC column. Depending on the particular organic solvent employed, artifact formation of MeHg can occur as a result of the extraction - GC conditions. Methods to avoid an artifact situation are suggested, and the possible implications of this for the currently accepted AOAC method involving GC - ECD.

Keywords: *Methylmercury determination; gas chromatography; electron-capture detection; direct current plasma; fish analysis*

The most commonly used method for organomercury speciation is currently gas chromatography (GC) followed by electron-capture detection (ECD). Methylmercury (MeHg) is a misnomer, a non-existent species, that has come to denote a chromatographic peak derived from a methylmercury(II) anion species/salt originally injected. It has been used in the analytical literature for many years, and we follow custom by using this trivial name in place of the more unwieldy alternative. There is some evidence to suggest that dimethylmercury is formed on injection of organomercurials on to the GC column, but only when metal surfaces are present.[1–3] All of the glass column GC evidence has suggested that the peak obtained is the originally injected methylmercury salt. Studies utilising GC - mass spectrometry have indicated that, at least when standard methylmercury(II) chloride is injected, the resultant peak using the GC conditions employed under Experimental is methylmercury(II) chloride and not dimethylmercury.[4,5]

Total mercury levels are usually determined by cold-vapour generation followed by atomic absorption spectrometry (AAS). However, the poor selectivity of ECD for organomercury species and the often time-consuming and elaborate clean-up procedures necessary prior to the injection of many samples, have indicated the need for a simple, more specific approach for the determination of organomercury species.[2,6,7] Specificity in gas chromatographic determinations of mercury has been improved using AAS or microwave induced plasma (MIP) atomic emission detectors.[3,8–10] Although GC - MIP approaches have been demonstrated for MeHg in fish samples, such methods continue to have serious drawbacks, especially an intolerance to large sample volumes injected, plasma instability, poor reproducibility, poor precision and the inability to operate unattended for long periods of time.[11]

There have been no reports of the application of GC - DCP for the determination of MeHg in fish samples, although this approach has been used for numerous other metal species.[11] The earliest and certainly the most work in GC - DCP for organometal speciation has been reported by Uden *et al.*[12]

High-performance liquid chromatography (HPLC) has also been utilised for MeHg speciation, including its interfacing with electrochemical detection,[13] AAS[14–17] and inductively coupled plasma (ICP) atomic emission spectrometry.[18] However, depending on the particular sample, preparation and work-up can be extensive, as in GC, and accurate determination may prove as (or more) difficult. In view of the extreme volatility of MeHg and related organomercury species, GC still appears to be the preferred method of determination. HPLC can serve as a useful and practical alternative or for corroboration and confirmatory purposes when the initial analysis has been caried out by GC.

Element specificity is possible with GC - atomic absorption or GC - atomic emission spectrometry (GC - AAS, GC - DCP, GC - ICP, etc.). We use the term element specificity, as opposed to element selectivity, as the GC - DCP approach used here is specific for mercury-containing species and their emission characteristics. The DCP has now been simply, quickly and inexpensively interfaced with GC to form a dedicated GC - DCP system for methylmercury in fish. A low-cost, isothermal packed-column gas chromatograph has been constructed which can resolve MeHg from the solvent front and ethylmercury (EtHg) in less than 5 min. The interfacing involves a quartz jet tube to convey the GC effluent directly into the DCP plume. This interface allows for prolonged use without replacement.

Experimental

Apparatus and Operating Conditions

Construction of the gas chromatograph for GC - DCP

A schematic diagram of the GC - DCP instrumentation is

* Contribution No. 307 from the Barnett Institute at Northeastern University, Boston, MA, USA.

† To whom correspondence should addressed.

given in Fig. 1. The outside surface of a 3 ft 8 in length of copper tubing was covered with furnace cement. The covered tube was baked overnight in an oven at 200 °C. After the furnace cement had cured, 22 gauge nichrome wire was wound over the cement. The wire was wound with a $\frac{1}{4}$ in space between each turn, except at the injection port end of the tube, where the wire was wound tighter, $\frac{1}{8}$ in space per turn, for 2 in of tubing. The nichrome wire was triple twisted at each end of the wound tubing for electrical lead connections. The triple-twisted nichrome wire had mechanically fastened electrical connectors for connection to a variac for column temperature control. Additional furnace cement was applied to cover all the wound nichrome wire and then baked once more (overnight) at 200 °C.

The cured tubing was installed in a 2.5 in o.d. × 0.5 in i.d. fibre-glass insulation tube with heat-resistant wire insulation covering the triple-twisted nichrome wiring. The entire wired assembly was routed through the 1-in fibre-glass insulation.

The injection port was one leg of a $\frac{1}{4}$ in stainless-steel Swagelok tee. This tee had two septa sandwiched between washers and secured by a Swagelok end-cap, which had a hole drilled in the middle of the cap's plug end for the insertion of a syringe. The second leg of this Swagelok tee was connected to the GC column. The perpendicular leg of the tee was the input for the argon carrier gas. This input ran along the outside of the furnace cement, but inside the fibre-glass insulation, which presumably brought the gas temperature up to that of the column temperature.

The outlet of the GC column had a $\frac{1}{4}$ in stainless-steel Swagelok 90° elbow connection with a $\frac{1}{4}$ in o.d. × 1 mm i.d. quartz tube for transfer of the column effluent to the DCP plume region. The top of the flat-tipped quartz tube was ground into a cone to project the tube higher into the plume. The quartz tube was 6 in long and was wrapped with heating tape to maintain the temperature at 190 °C, with the column maintained at 155 °C.

Direct current plasma

The DCP was a Spectraspan Model IIIb DC argon plasma spectrometer (Spectrametrics/Beckman, Andover, MA), operated in the active diagnostic mode (repeat dial = 0). Other operating conditions included a sleeve pressure of 50 lb in^{-2}; zero nebuliser pressure; nebuliser, spray chamber, sample tube and peristaltic pump removed; a gain of 30; PMT voltage setting of 8 or 9 (900 or 950 V); input slit settings (vertical × horizontal) of 200 × 200 or 300 × 200; and an emission line of 253.652 nm.

Atomic absorption spectrometer

A Perkin-Elmer (Norwalk, CT) Model 403 atomic absorption spectrometer, with digital read-out, was used in the cold-vapour mode for total mercury determinations. A Westinghouse mercury hollow-cathode lamp was operated at 253.6 nm at 15 A rating.

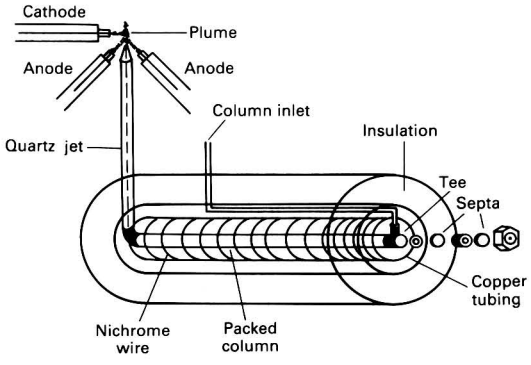


Fig. 1. Schematic diagram of the GC - DCP instrumentation

GC - ECD operating conditions

A Perkin-Elmer Model Sigma 2000 gas chromatograph was used with a ^{63}Ni electron-capture detector. A 6 ft × 2 mm i.d. all-glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport was operated isothermally at 150 °C, with an injection port temperature of 210 °C, a cell temperature of 350 °C and a nitrogen flow-rate through the GC column of 30 ml min^{-1}. An additional 30 ml min^{-1} nitrogen flow was added as the cell make-up gas. Data were collected on a Perkin-Elmer Model LCI-100 laboratory computing integrator. Prior to analysis, the column was conditioned with one 20 µl injection of a 1000 p.p.m. solution of mercury(II) chloride, according to the AOAC, 25.150(b).[19] This is the recommended procedure to passivate the column packing and to improve the chromatographic peak shape for MeHg.

GC - DCP operating conditions

The laboratory-made gas chromatograph was used for all the GC - DCP determinations. A 4 ft × 2 mm i.d. all-glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport was operated isothermally at 155 °C, with an injection port temperature of 175 °C and an argon flow-rate through the column of 100 ml min^{-1}. The DCP quartz jet inlet tube was maintained at 190 °C with external heating tape. Data were collected on a Perkin-Elmer Model LCI-100 laboratory computing integrator. Prior to analysis, the column was conditioned with one 20 µl injection of a 1000 p.p.m. solution of mercury(II) chloride, according to the AOAC, 25.150(b).[19]

Methods and Procedures for Sample Preparation

Total mercury determination

The established sample preparation method according to the AOAC, 25.131–132, 25.134–135[19] was used for cold vapour atomic absorption spectrometry.

GC - ECD sample preparation

The work-up procedure used to extract and determine MeHg in swordfish was that of the AOAC, 25.146–25.152,[19] modified by Hight and co-workers.[6,7] This extraction procedure used toluene instead of benzene as the extracting solvent. It also used a 1.0 g composite sample of fish and 2.5 ml of 1 + 1 hydrochloric acid. The extract of 2–20 ml volumes of toluene was not evaporated, but was instead taken to the final volume of 50 ml for GC - ECD determinations.

GC - DCP sample preparation

The same method of Hight and co-workers[6,7] was used to extract the MeHg from the swordfish. However, the sample preparation now involved concentrating the 50 ml of solution used in the GC - ECD determination step to 5 ml using a Kuderna - Danish (K - D) apparatus. This was performed in a warm water-bath (40–50 °C) under a stream of nitrogen. The concentrated solution was directly injected into the GC - DCP. The evaporation step was one of the problems associated with the final method as it required approximately 90 min to perform.

A second extraction procedure was developed using 20 ml of an extraction solution of 50 + 50 *V/V* of diethyl ether - light petroleum to separate the MeHg from the sample matrix. The extraction solution was evaporated to 5 ml using a K - D apparatus in a warm water-bath (40–50 °C) under a stream of nitrogen. The time needed for this evaporation was less than 10 min. This extraction method was not without problems, in that the diethyl ether portion caused a ghost peak at the same

GC retention time as authentic MeHg when a solvent blank was injected in either GC - DCP or GC - ECD. Even though the results from these GC - ECD determinations compared favourably with the established toluene extraction GC - ECD method, it is recommended that diethyl ether and acetone should not be used. Other solvents may also lead to artifact formation of MeHg under certain GC conditions (see Results and Discussion).

A third extraction procedure was developed using light petroleum as the extraction solvent. The MeHg was completely extracted from swordfish when GC - ECD was used as the determination step (50 ml final volume). However, when the solution was concentrated to 5 ml final volume and determined by GC - DCP, the results were substantially lower than those obtained by GC - ECD. GC - DCP requires this 50 ml to 5 ml concentration step because of the differences in detection limits between ECD and DCP. We believe the differences were due to accidental losses of the MeHg during the evaporation. However, the loss was only seen with light petroleum.

A fourth extraction procedure was attempted; this employed the first portion of the AOAC method[19] with the modifications of Hight and co-workers.[6,7] However, instead of using toluene as the extracting solvent, 50 + 50 *V/V* light petroleum - toluene was used. The results obtained using this method were comparable to the results obtained using the standard AOAC method.[19] No advantages were noted when using GC - ECD as the determination step and, when using GC - DCP, the only advantage noted was the reduction in the time required for the evaporation step.

A fifth extraction procedure was also developed for use by the GC - DCP detection system only, now called the "rapid" method. The major differences in this procedure from that of the modified AOAC method[19] were: a 50 + 50 *V/V* mixture of light petroleum - toluene was used as the extraction solvent; one extraction of the fat from the product with acetone and one with toluene was used; no solvent clean-up of the 1 + 1 HCl was needed; and evaporation of the final solution to 5 ml used a K - D apparatus in a warm water-bath (40–50 °C) under a stream of nitrogen. This rapid method took considerably less time per sample than the standard work-up procedure (2.5 *vs.* 5 h) from the initial sample extraction to final data acquisition.

Recovery studies with MeHg were performed by directly analysing the fish samples and then spiking another portion of the original fish composite at a known level of MeHg and repeating the same determination. All analyses were carried out in duplicate, and each duplicate was injected at least in triplicate with averages ± standard deviations being reported. The same fish sample extracts (standard work-up) used for GC - ECD determinations were concentrated from 50 to 5 ml and directly injected on to the GC - DCP. Separate recovery studies have shown that there was no measurable loss or formation of MeHg in the concentration step.

Fish samples

All of the fish examined were swordfish, except for NBS-RM50, which was tunafish containing a specified amount of Hg from the US National Bureau of Standards (NBS).

Reagents and Solvents

Mercury standard solutions were prepared fresh daily and were stored in a dark cool place when not in use. The GC packing material, 5% DEGS-PS on 100–120 mesh Supelcoport, was obtained from Supelco (Bellefonte, PA). Organic solvents, such as toluene, acetone, light petroleum and diethyl ether, were all purchased as distilled-in-glass grade from Burdick and Jackson (Muskegon, MI). Nitrogen (99.995% pure) and argon were obtained from Associated Gas Products (Everett, MA). The mercury standard for AAS was received as Bethlehem Instrument Mercury, triple distilled (Bethlehem Apparatus, Hellertown, PA). The methylmercury(II) chloride and ethylmercury(II) chloride standards for GC were purchased from K and K Laboratories (Plainview, NY).

American Chemical Society (ACS) certified grade concentrated hydrochloric acid, mercury(II) chloride, tin(II) chloride, sodium chloride, hydroxylamine sulphate and vanadium pentoxide were purchased from Fisher Scientific (Boston, MA). ACS certified grade sodium sulphate was obtained from Mallinckrodt (Paris, KY). Concentrated nitric and sulphuric acids were obtained as Baker Instra-Analyzed grade (J. T. Baker Chemical, Phillipsburg, NJ). The NBS-RM50 mercury in tunafish reference material was obtained from the National Bureau of Standards (US Department of Commerce, Gaithersburg, MD).

Results and Discussion

GC - DCP Limits of Detection, Calibration Graph and Linearity of Calibration Graph

A calibration graph was constructed which was linear from 0.2 to 20 μg per 8 μl injection, with a correlation coefficient of 0.9999. It is possible that linearity would extend beyond this range, but for the purposes of these determinations in fish, this linear dynamic range was adequate. The limit of detection was determined by injecting that concentration of MeHg (0.3 ng per 8 μl) which would produce a signal to noise ratio of about 3:1. Statistically, for 10 replicate injections, the analyte produced an average signal of 7.6 mm with a relative standard deviation (RSD) of 5.8%. At the same time, the background noise level was 2.5 mm (RSD 12.8%).

GC - DCP Determinations of Methylmercury and Ethylmercury in Standards

Although not displayed, standards of methylmercuryl(II) chloride and ethylmercury(II) chloride can be base-line resolved within a total of 5–6 min under the GC - DCP conditions indicated in Fig. 2. The peak shape and symmetry were acceptable. There was no serious solvent response on the DCP and the background noise level was low at the concentrations and attenuation employed.

Fig. 2 is indicative of the GC - DCP chromatograms obtained with a swordfish sample, first analysed by GC - ECD using toluene as the extraction solvent. Four injections are shown: an authentic standard of MeHg, duplicate injections of the fish extract and a second injection of MeHg standard. Once again, peak shape was good, there was no interference from the solvent front and the total chromatographic time was less than 3 min.

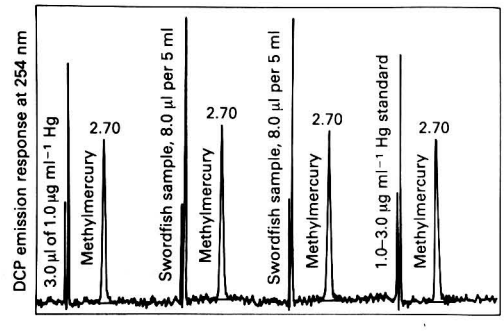


Fig. 2. GC - DCP chromatogram of actual swordfish sample extract using standard (toluene) extraction method, GC - DCP conditions as under Experimental. Retention times in minutes

A rapid sample work-up method was desired to improve the sample throughput and turn-around time. This procedure was developed and optimised. Fig. 3 illustrates a GC - DCP comparison of the MeHg content of the same swordfish sample by the standard method of extraction and the proposed rapid method. There were no obvious differences in the chromatograms for either sample work-up method. There was, however, a significant reduction in the time required (5 *vs.* 2.5 h) for one fish sample analysis from work-up to final GC - DCP chromatogram.

Determination of MeHg in Swordfish and NBS Tunafish Samples

In order to validate the GC - DCP method, a comparison was made between the GC - ECD and GC - DCP methods for incurred and spiked MeHg, using single blind methodology. Tables 1 and 2 summarise these results, including percentage recoveries of the spiked MeHg. The samples were analysed according to the AOAC method 25.146–25.152,[19] modified by Hight and co-workers.[6,7] A poor statistical correlation was found between the two sets of data (Table 3) with one type of Student, 1-tailed, *t*-test correlation term (*t*-value) indicated. Table 3 suggests real differences between the GC - ECD and GC - DCP levels of MeHg found in most fish samples, with the GC - ECD results consistently higher. Thirteen separate samples were compared by both methods, and in the majority of instances (9 out of 13), a significant lack of coincidence or correlation was evident from the *t*-values.

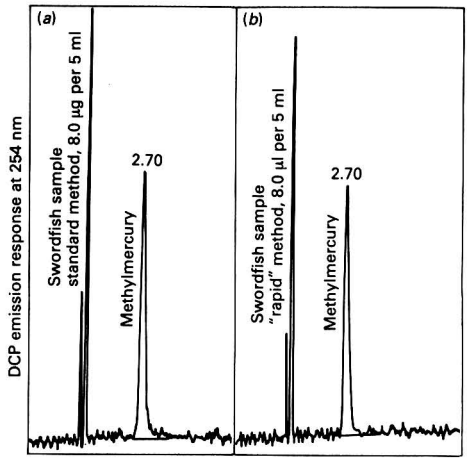


Fig. 3. GC - DCP chromatograms of actual swordfish sample extract using (*a*) standard and (*b*) "rapid" methods of MeHg extraction and work-up. Retention times in minutes

In every single instance, the MeHg levels by GC - ECD were higher than by GC - DCP. Although the differences were minor in some instances (553RBM, 553KWP, 687RBM), in others, there were striking differences (725KWP, 725JRN, 841JRN, 841KWP). Even for the NBS-RM50 sample of tunafish, the GC - ECD level was statistically higher than that obtained by GC - DCP and the stated value (0.93 ± 0.1 p.p.m.). The GC - DCP values have always been equal to or slightly lower than the stated Hg level in NBS-RM50. The NBS has suggested that RM50 contains Hg only as MeHg, but this is not a standard reference material. The statistical approach used through these studies was a comparison of the means of two samples at the 95% ($P = 0.05$) confidence level, for $n - 2$ degrees of freedom.[20]

Using the proposed rapid method of sample work-up and extraction, the same samples of fish were re-analysed by GC - DCP (Table 4). These results were in good agreement with those in Table 2, using the standard work-up, and the percentage recoveries were within acceptable limits. A Student *t*-test analysis of the two sets of data showed good correlation (Table 5), suggesting that the rapid method can provide the same qualitative and quantitative accuracy and precision as the standard work-up method.

Determination of Total Mercury in Swordfish and NBS-RM50 by Cold-Vapour AAS. Comparison with GC - DCP and GC - ECD for MeHg

In order to demonstrate the total mercury levels present in the fish samples, separate determinations were performed using cold-vapour generation atomic absorption spectrometry. Each sample was digested in triplicate. The cold-vapour AAS results are given in Table 6, with the corresponding standard work-up GC - DCP data. The data were compared by a Student *t*-test, with *t*-values at the 95% confidence level indicated. Eleven fish samples were compared, and of these,

Table 1. Determination of methylmercury in fish by standard work-up GC - ECD. Extraction procedure: AOAC 25.146–25.152,[19] modified by Hight and co-workers.[6,7] GC - ECD conditions used: 6 ft × 2 mm i.d. glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport operated at 150 °C, injection at 210 °C, 30 ml min^{-1} N_2 flow-rate in GC plus 30 ml min^{-1} N_2 make-up gas for ECD cell. Only two injections made, results quoted as average ± S.D.

Sample type	Sample	Results ± s.d., p.p.m.	Hg added/ µg	Determination of methylmercury in spiked sample, p.p.m.	Recovery, %
Swordfish ($n = 4$)	458JRN	2.10 ± 0.01	2.0	4.14	98.7
	497JRN	1.07 ± 0.02	2.0	3.30	105.8
	553RBM	1.05 ± 0.05	2.0	3.13	104.4
	553KWP	1.10 ± 0.01	2.0	3.23	106.5
	687RBM	1.15 ± 0.02	1.0	2.27	112.0
	687JRN	1.19 ± 0.05	1.0	2.20	101.0
	720KWP	2.21 ± 0.06	2.0	4.19	98.5
	720JRN	2.12 ± 0.03	2.0	4.15	101.5
	725KWP	2.51 ± 0.04	2.0	4.56	102.5
	725JRN	2.64 ± 0.02	2.0	4.61	98.5
	841JRN	1.99 ± 0.02	2.0	3.93	96.5
	841KWP	2.08 ± 0.02	2.0	4.03	101.5
Tunafish ($n = 3$)	NBS-RM50 (0.93 ± 0.1)	0.97 ± 0.01			
	NBS-RM50 (0.93 ± 0.1)	0.98 ± 0.02			

Table 2. Determination of methylmercury in fish by standard work-up GC - DCP. Extraction procedure: AOAC 25.146–25.152,[19] modified. GC - DCP conditions used: 4 ft × 2 mm glass column packed with 5% DEGS-PS on 100–120 mesh Supelcoport operated at 155 °C, injection at 175 °C, total Ar flow-rate 100 ml min^{-1}, quartz jet at 190 °C; DCP wavelength of emission at 253.652 nm

Sample type	Sample	Results ± s.d., p.p.m.	Hg added/ μg	Determination of methylmercury in spiked sample ± s.d., p.p.m.	Recovery, %
Swordfish (n = 3)	458JRN	1.94 ± 0.11	2.0	3.90 ± 0.08	103.0
	497JRN	1.02 ± 0.02	2.0	3.09 ± 0.03	103.2
	553RBM	1.03 ± 0.07	—	—	—
	553KWP	1.07 ± 0.03	2.0	2.75 ± 0.06*	84.0
	687RBM	1.12 ± 0.05	1.0	2.13 ± 0.01	101.0
	687JRN	0.97 ± 0.06	1.0	2.02 ± 0.06	105.0
	720KWP	2.10 ± 0.06	2.0	4.09 ± 0.06	99.5
	720JRN	2.09 ± 0.06	2.0	3.83 ± 0.03	87.0
	725KWP	2.26 ± 0.04†	2.0	4.64 ± 0.13	119.0
	725JRN	2.22 ± 0.04	2.0	4.10 ± 0.13	94.0
	841JRN	1.88 ± 0.05	2.0	3.91 ± 0.06	101.5
	841KWP	1.96 ± 0.05	2.0	3.83 ± 0.12	93.5
Tunafish (n = 6)	NBS-RM50 (0.93 ± 0.1)	0.89 ± 0.03	—	—	—

* n = 6.
† n = 7.

Table 3. Statistical comparison of MeHg levels in fish by standard work-up GC - ECD and GC - DCP methods

Sample type	Sample	GC - ECD ± s.d., p.p.m. (n = 4)	GC - DCP ± s.d., p.p.m. (n = 6)	t-Value (2.31)*
Swordfish	458JRN	2.10 ± 0.01	1.94 ± 0.11	2.84
	497JRN	1.07 ± 0.02	1.02 ± 0.02	3.87
	553RBM	1.05 ± 0.05	1.03 ± 0.07	0.49
	553KWP	1.10 ± 0.01	1.07 ± 0.03	1.90
	687RBM	1.15 ± 0.02	1.12 ± 0.05	1.12
	687JRN	1.19 ± 0.05	0.97 ± 0.06	6.04
	720KWP	2.21 ± 0.06	2.10 ± 0.06	2.84
	720JRN	2.12 ± 0.03	2.09 ± 0.06	0.91
	725KWP	2.51 ± 0.04	2.26 ± 0.04†	9.97
	725JRN	2.64 ± 0.02	2.22 ± 0.04	19.19
	841JRN	1.99 ± 0.02	1.88 ± 0.05	4.12
	841KWP	2.08 ± 0.02	1.96 ± 0.05	4.50
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.98 ± 0.01	0.89 ± 0.03	6.97

* Student's t-value according to method described in Miller and Miller.[20] For n = 8 degrees of freedom, t = 2.31 at P = 0.05, 95% confidence level.
† n = 7.

Table 4. Determination of methylmercury in fish by GC - DCP using modified "rapid" extraction procedure. Extraction procedure: 50 + 50 toluene - light petroleum, no acid clean-up with organic reagents. GC - DCP conditions as in Table 2

Sample type	Sample	Results ± s.d., p.p.m. (n = 6)	Hg added/ μg	Determination of methylmercury in spiked sample + s.d., p.p.m. (n = 3)	Recovery, %
Swordfish	841JRN	1.87 ± 0.06	2.0	3.94 ± 0.02	103.5
	458JRN	1.93 ± 0.04	2.0	4.03 ± 0.03	105.2
	497JRN	1.04 ± 0.02	2.0	3.03 ± 0.04	99.3
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.91 ± 0.02			

Table 5. Comparison of MeHg levels in fish by standard and "rapid" work-up method with GC - DCP. GC - DCP conditions as in Table 2

Sample type	Sample	Standard method, results ± s.d., p.p.m. (n = 6)	"Rapid" method, results + s.d., p.p.m. (n = 6)	t-Value (2.23)*
Swordfish	458JRN	1.94 ± 0.11	1.93 ± 0.04	0.21
	497JRN	1.02 ± 0.02	1.04 ± 0.02	1.73
	841JRN	1.88 ± 0.05	1.87 ± 0.06	0.31
	841KWP	1.96 ± 0.05	1.87 ± 0.06	2.82
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.89 ± 0.03	0.91 ± 0.02	1.35

* Student's t-value according to method described by Miller and Miller.[20] For n = 10 degrees of freedom, t = 2.23 at P = 0.05, 95% confidence level.

Table 6. Determination of total mercury in swordfish and NBS-RM50 by cold-vapour AAS and comparison with MeHg determined by standard work-up GC - DCP procedure. A wavelength of 253.6 nm was used for cold-vapour AAS (hollow-cathode Hg lamp). Standard work-up and GC - DCP conditions as in Table 2

Sample type	Sample	Total Hg determined by AAS, results ± s.d., p.p.m. ($n = 3$)	MeHg determined by GC - DCP results ± s.d., p.p.m. ($n = 6$)	t-Value (2.36)*
Swordfish	458JRN	1.95 ± 0.01	1.94 ± 0.11	0.15
	497JRN	1.04 ± 0.01	1.02 ± 0.02	1.60
	687RBM	1.04 ± 0.01	1.12 ± 0.05	2.66
	687JRN	1.04 ± 0.01	0.97 ± 0.06	1.94
	720KWP	2.08 ± 0.02	2.10 ± 0.06	0.55
	720JRN	2.08 ± 0.02	2.09 ± 0.06	0.27
	725JRN	2.41 ± 0.01	2.22 ± 0.04	7.85
	725KWP	2.41 ± 0.01	2.26 ± 0.04†	6.21‡
	841JRN	1.91 ± 0.01	1.88 ± 0.05	1.00
	841KWP	1.91 ± 0.01	1.96 ± 0.05	1.66
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.94 ± 0.01	0.89 ± 0.03	2.73

* Student's t-value according to method as described by Miller and Miller.[20] For $n = 7$ degrees of freedom, $t = 2.36$ at $P = 0.05$, 95% confidence level.

† $n = 7$.

‡ For $n = 8$ degrees of freedom, $t = 2.31$ at $P = 0.05$, 95% confidence level.

Table 7. Determination of total mercury in swordfish and NBS-RM50 by cold vapour AAS and comparison with MeHg by "rapid" work-up GC - DCP. AAS and GC - DCP conditions as in Table 2

Sample type	Sample	Total Hg determined by AAS results ± s.d., p.p.m. ($n = 3$)	MeHg determined by GC - DCP results ± s.d., p.p.m. ($n = 6$)	t-Value (2.36)*
Swordfish	458JRN	1.95 ± 0.01	1.93 ± 0.04	0.83
	497JRN	1.04 ± 0.01	1.04 ± 0.02	0.00
	841JRN	1.91 ± 0.01	1.87 ± 0.06	1.11
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.94 ± 0.01	0.91 ± 0.02	2.39

* Student t-value according to method as described by Miller and Miller.[20] For $n = 7$ degrees of freedom, $t = 2.36$ at $P = 0.05$, 95% confidence level.

Table 8. Determination of total mercury in swordfish and NBS-RM50 by cold-vapour AAS and comparison with MeHg by standard work-up GC - ECD. Cold-vapour AAS and GC - DCP conditions as in Table 2

Sample type	Sample	Total Hg determined by AAS results ± s.d., p.p.m. ($n = 3$)	MeHg by determined GC - ECD results ± s.d., p.p.m. ($n = 4$)	t-value (2.57)*
Swordfish	458JRN	1.95 ± 0.01	2.10 ± 0.01	19.6
	497JRN	1.04 ± 0.01	1.07 ± 0.02	2.35
	687RBM	1.04 ± 0.01	1.15 ± 0.02	8.61
	687JRN	1.04 ± 0.01	1.19 ± 0.05	5.00
	720KWP	2.08 ± 0.02	2.21 ± 0.06	3.53
	720JRN	2.08 ± 0.02	2.12 ± 0.03	1.98
	725JRN	2.41 ± 0.01	2.64 ± 0.02	18.00
	725KWP	2.41 ± 0.01	2.51 ± 0.04	4.14
	841JRN	1.91 ± 0.01	1.99 ± 0.02	6.26
	841KWP	1.91 ± 0.01	2.08 ± 0.02	13.3
Tunafish	NBS-RM50 (0.93 ± 0.1)	0.94 ± 0.01	0.97 ± 0.01†	5.66‡

* Student's t-value according to method as described by Miller and Miller.[20] For $n = 7$ degrees of freedom, $t = 2.57$ at $P = 0.05$, 95% confidence level.

† $n = 3$.

‡ For $n = 8$ degrees of freedom, $t = 2.36$ at $P = 0.05$, 95% confidence level.

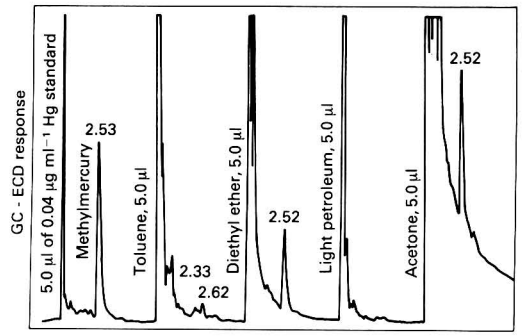


Fig. 4. GC - ECD chromatograms of four different organic solvents. Illustration of artifact formation of MeHg peaks. Retention times in minutes

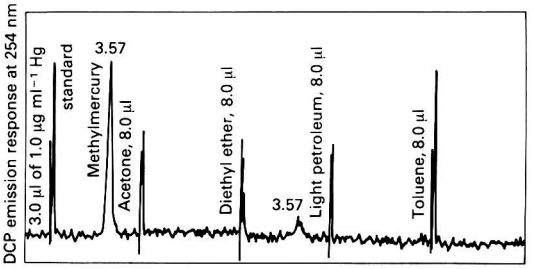


Fig. 5. GC - DCP chromatograms of four different organic solvents. Illustration of reduced artifact formation of MeHg peaks. Retention times in minutes

four samples did not show an overlap (statistical coincidence) of Hg levels (687RBM, 725JRN, 725KWP and NBS-RM50). The other seven samples have Hg levels that are of the same population. There was no reason why there should have been any coincidence or correlation of the data, unless all the Hg present was MeHg.

These results suggested that in most of the fish examined, all or most of the Hg present was MeHg. More importantly, in 10 or 11 instances, the total Hg levels determined by AAS are equal to or higher than MeHg levels by GC - DCP. In only 1 out of 11 instances (687RBM) is the GC - DCP MeHg level statistically higher than total Hg determined by AAS. It is plausible that total Hg levels can be higher than the amount of MeHg present, as it is known that Hg may exist in several forms or species in fish. However, the reverse cannot be possible.

A similar comparison has been made for total Hg levels determined by AAS and the MeHg levels found by the rapid GC - DCP approach (Table 7). Although the number of samples here was lower, 3 out of 4 have t values that indicate equality of the total Hg and the MeHg levels by GC - DCP. The AAS results were always equal to or higher than the GC - DCP values.

A third comparison was made for total Hg levels and the MeHg levels determined by standard work-up GC - ECD. Table 8 summarises the relevant data and Student t-values, using the same statistical treatment as above. Eleven fish samples were compared, but there was no degree of correlation. There were only 2 out of 11 samples that showed any agreement (497JRN and 720JRN), but here and in all the other instances, the GC - ECD MeHg levels are higher than total Hg determined by AAS. This is a striking result, but was expected in view of the general disagreement between MeHg levels determined by GC - ECD and GC - DCP (Table 3). It was clear from Table 3 that one set of data was incorrect, but only when each set has been compared with total Hg levels by the accepted method of determination (cold-vapour AAS), does this become evident. Table 8 suggests that the GC - ECD method can, at times, lead to MeHg levels that are higher than one would expect when compared with the total Hg levels.

Demonstration of Possible Artifacts due to Extraction Solvent and Injection Port Temperature in the GC - ECD and GC - DCP Determinations

What was perhaps most disturbing were the statistically meaningful differences between total Hg determined by cold-vapour AAS and MeHg determined by GC - ECD (Table 8). That the MeHg levels were consistently higher than the total Hg levels, in virtually every sample, suggested a positive artifact formation of MeHg via the standard work-up GC - ECD method. This seemed an inescapable conclusion. The fact that the MeHg levels by GC - DCP were equal to or less than total Hg by cold-vapour AAS suggested that a consistent positive artifact formation was absent.

A possible explanation for the positive artifact formation could be pyrolysis of certain organic solvents within the injection port in GC - ECD, leading to the formation of methyl (alkyl) radicals. This could have been followed by combination with mercury atoms from the mercury(II) chloride coating the GC column support to form an artifact MeHg peak. The injection port temperature in the GC - ECD was about 30–40 °C higher than that used in GC - DCP.

In order to demonstrate a possible accidental (artifact) formation of a peak attributed to MeHg in GC - ECD, two separate studies were performed. In one of these (results in Fig. 4), four different solvents (acetone, diethyl ether, light petroleum and toluene) were separately injected on to the column and determined by GC - ECD. These were just solvent blanks; no sample extraction or work-up was involved. Fig. 5 illustrates a set of similar GC - DCP chromatograms for the same injections of solvent blanks, but first concentrating each solvent from 50 ml to 5 ml. In both of these figures, a separate chromatogram is given for an injection of standard MeHg. Although the absolute amounts (ng) injected were different (higher for DCP), the volumes injected (higher for DCP) partially compensated, so that relative sensitivities for standards injected were about the same (five-fold difference, ECD more sensitive).

A number of significant points were evident. The GC - DCP chromatograms were simpler than those from GC - ECD. This might be expected in view of the DCP's greater element selectivity. Secondly, in two out of the four solvents studied, diethyl ether and acetone, GC - ECD showed the presence of a peak that could be attributed to MeHg. In GC - DCP, a small, broad peak appeared at the correct retention time for MeHg with diethyl ether, but of lower peak height and area than in GC - ECD. Diethyl ether has not been used in any of the sample work-ups for the GC - DCP results reported. It is possible that these same artifact peaks could be formed in the other solvent GC - DCP chromatograms, but the injection port temperature used in GC - DCP was lower by about 40 °C than in GC - ECD.

Another study was carried out in which GC - ECD determination of the solvent blanks was carried out at three different injection port temperatures. With diethyl ether, as the temperature increased, more artifact MeHg formation occurred. At lower temperatures, the GC - ECD artifact level approached that of GC - DCP for the same solvent. With light petroleum and toluene, there was little or no artifact peak at any injection port temperature studied (170–230 °C). For acetone, the solvent front became broader with increasing temperature and artificial appearance was always evident, but was obscured at higher temperatures by the large solvent front.

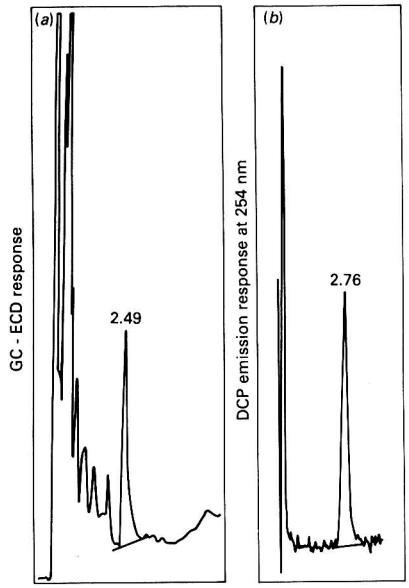


Fig. 6. (*a*) GC - ECD (standard work-up) and (*b*) GC - DCP (standard = "rapid" work-up) chromatograms of NBS-RM50 tunafish. Illustration of improved MeHg specificity by GC - DCP method. Retention times in minutes

It seemed that a higher injection port temperature in GC - ECD could lead to a greater pyrolysis of certain solvents (diethyl ether - acetone) than others (toluene - light petroleum), which then caused artifact formation of MeHg. It is well known that simple alkyl ethers and acetone readily pyrolyse on metal surfaces to release methyl radicals. The presence of a mercury salt on the GC packing, as recommended by the AOAC procedure,[19] may have contributed to this formation. In the future, it might be prudent to change the inorganic salt used to coat GC columns for organomercury determinations. Alternatively, it might be recommended to use a capillary GC column, as recently reported for the determination of MeHg by GC - ECD, even though no actual sample results were reported.[21] In the absence of confirmatory GC - MS data, it is conjecture that the artifact peaks in GC - ECD were MeHg. The one artifact peak for diethyl ether in GC - DCP, at the correct retention time for MeHg and shown to contain Hg, is probably MeHg.

Solvent fronts in GC - ECD are considerably broader and more pronounced than those in GC - DCP (Figs. 4 and 6) thereby increasing the detection limits for MeHg possible using such solvents, *e.g.*, acetone. In Fig. 4, a series of solvent peaks can be seen using GC - ECD, leading to a more complex chromatogram than with GC - DCP. It was not absolutely clear that any of the toluene peaks were due to MeHg. However, the simplicity of the GC - DCP chromatogram for an actual fish extract, in comparison with GC - ECD, is striking (Fig. 6), leading to improved analyte identification.

Explanation of Standard Work-up GC - ECD Results for the Determination of Methylmercury in Fish

How does this all reflect itself in the original comparison of MeHg levels by GC - ECD and GC - DCP (Table 3) where we alluded to differences? In GC - ECD, toluene and acetone alone were both used for sample work-up and extraction, but toluene (Fig. 4) did not cause a significant MeHg artifact peak. However, any residual, unseparated acetone in the final solution injected could have led to artifact MeHg. This was also a function of the age of the GC column, the amount of mercury(II) chloride coating remaining and injection port temperature. It was possible to generate a much larger artifact peak under certain conditions. It was also possible that sample and/or matrix components, injected along with incurred MeHg, may have led to artifact formation.

It is our belief that the GC - ECD results can or may be artifically higher, and that this may be due to the sample components, the solvents used and/or the operating conditions. Such a positive artifact formation can be avoided in the future. GC - ECD has better limits of detection for MeHg in fish but at the expense of selectivity.

Conclusions

These results have demonstrated several unique capabilities and advantages that GC - DCP possesses for metal-containing compounds in fish and related samples. Accurate, precise and reproducible determinations for MeHg in fish have been demonstrated. A comparison of total Hg levels in the same fish samples has shown that, in general, they are higher than those determined by GC - DCP, but often lower than MeHg by GC - ECD. Additional sample preparation was needed for GC - DCP, especially the ability to pre-concentrate the fish extracts before injection. This was not necessary wth GC - ECD, but then there was a serious trade-off in analyte specificity.

Some problems have appeared with the organic solvents used for the extraction step, in that artifact formation of MeHg occurred within the GC. It is possible that simple matrix components also contributed to an artifact formation. Solvent and sample blanks must always be run alongside actual samples in order to demonstrate the presence or absence of artifacts, standard practice in elemental analysis laboratories. Additional problems may arise in the GC - ECD method, wherein co-eluting peaks from the fish sample (interferents) may respond on the ECD as if they were MeHg. The DCP, being Hg-specific, avoids such interferences, and thus can provide more accurate determinations of MeHg. The advantages of element-selective detection for MeHg or other metal species, either by GC or HPLC interfacing, are obvious.

Finally, a very inexpensive, dedicated, isothermal gas chromatograph has been constructed for dedicated DCP interfacing, which could then be used routinely and continuously for the determination of organomercury species. It is hoped that this GC - DCP approach will find utility and application in other trace element analysis and speciation laboratories and applications, perhaps with modification of the metal salt used for column passivation.

This work was performed at the Boston District Office of the US Food and Drug Administration (FDA), Boston, MA. We are grateful to the FDA for the opportunity to perform this work and to report the results. Acknowledgement is made to colleagues within the FDA who prepared blind, spiked fish samples for method validation purposes, including J. Noonan and R. Midwood. W. S. Adams and L. Gershman provided encouragement, time and guidance during these studies. Certain students at Northeastern University, especially C. M. Selavka, read drafts of the manuscript and provided helpful and constructive comments and suggestions.

References

1. Fishbein, L., *Chromatogr. Rev.*, 1970, **13**, 83.
2. Rodriguez-Vazques, J. A., *Talanta*, 1978, **25**, 299.
3. Bzezinska, A., Van Loon, J., Williams, D., Oguma, K., Fuwa, K., and Haraguchi, I. H., *Spectrochim. Acta, Part B*, 1983, **38**, 1339.
4. Johansson, B., Ryhage, R., and Westoo, G., *Acta Chem. Scand.*, 1970, **24**, 2349.
5. Capar, S. G., personal communication.
6. Hight, S. C., and Capar, S. G., *J. Assoc. Off. Anal. Chem.*, 1983, **66**, 1121.

7. Hight, S. C., and Corcoran, M., *J. Assoc. Off. Anal. Chem.*, 1987, **70**, 24.
8. Bye, R., and Paus, P. E., *Anal. Chim. Acta*, 1979, **107**, 169.
9. Talmi, Y., *Anal. Chim. Acta*, 1975, **74**, 107.
10. Sklarew, D. S., Olsen, K. B., and Evans, J. C., Paper presented at the 189th ACS National Meeting, Miami Beach, FL, April 28–May 3, 1965, Anal. 61.
11. Krull, I. S. and Jordan, S. W., *Am. Lab. (Fairfield, CT)*, October 1980, 21.
12. Uden, P. C., Barnes, R. M., and DiSanzo, F. P., *Anal. Chem.*, 1978, **50**, 852.
13. MacCrehan, W. A., and Durst, R. A., *Anal. Chem.*, 1978, **50**, 2108.
14. Holak, W., *Analyst*, 1982, **107**, 1457.
15. Holak, W., *J. Liq. Chromatogr.*, 1985, **8**, 563.
16. Holak, W., Paper presented at Innovative Techniques for the Analysis of Iodine and Methylmercury, 10th Annual Spring Training Workshop, AOAC, Dallas, TX, April 10, 1985.
17. Holak, W., *J. Assoc. Off. Anal. Chem.*, 1983, **66**, 1203.
18. Krull, I. S., Bushee, D. S., Schleicher, R. G., and Smith, S. B., Jr., *Analyst*, 1986, **111**, 345.
19. "Official Methods of Analysis of the Association of Official Analytical Chemists," Fourteenth Edition, Association of Official Analytical Chemists, Washington, DC, 1984.
20. Miller, J. C., and Miller, J. N., "Statistics for Analytical Chemistry," Wiley, New York, 1984, pp. 53–55.
21. Cappon, C. J., and Toribara, T. Y., *LC/GC Mag.*, 1986, **4**, 1010.

Paper A7/13
Received January 19th, 1987
Accepted March 17th, 1987

Acid Dissolution of Soils and Rocks for the Determination of Boron by Inductively Coupled Plasma Atomic Emission Spectrometry

Bernhard A. Zarcinas and Brian Cartwright
CSIRO, Division of Soils, Private Bag No. 2, Glen Osmond, South Australia 5064, Australia

The boron concentration in rocks, soils and standard reference materials was determined using hydrofluoric acid - aqua regia dissolution followed by inductively coupled plasma atomic emission spectrometry (ICP-AES) using the B I 249.773-nm line, corrected for spectral interference by iron. An excess of fluoride was complexed with aluminium to release boron from the stable fluoroborate ion and to protect the borosilicate and quartz components of the instrument. Boron was not lost by volatilisation during volume reduction.

Soil and rock boron values determined using the recommended dissolution procedures were comparable to those obtained using the accepted sodium carbonate fusion procedure and by d.c. arc emission spectrophotometry, and those for standard reference materials showed good agreement and precision with the literature values.

Keywords: *Boron determination; hydrofluoric acid - aqua regia dissolution; inductively coupled plasma atomic emission spectrometry*

The concentration of boron in geological samples ranges from 10 μg g^{-1} in igneous rocks to more than 1000 μg g^{-1} in metamorphic rocks. Soil boron[1] concentrations average about 30 μg g^{-1}. Sea water contains 4.5 μg ml^{-1} of boron. The ability of clay minerals to complex boron has been used as an indicator of paleosalinity[2] and to discriminate between modern marine and fresh water argillaceous sediments and between ancient shales.[3] Boron is an important trace element in plant nutrition but is unusual because of the narrowness of the range of concentration between deficiency and toxicity[4] in plants. Berger and Truog[5] indicated that less than 5% of total soil boron is usually available for crop use.

A number of procedures have been developed for the determination of boron. These have been reviewed by Gladney *et al.*[6] and include a variety of spectrophotometric and spectrometric techniques and also ion-selective electrode and mass spectrometric procedures, all of which require sample dissolution. However, flame insensitivity, serious interferences and tedious manipulations render many of these methods insufficiently sensitive and too time consuming for some geological samples. Owens *et al.*[7] proposed the sodium carbonate fusion procedure of Gupta[8] for the dissolution of geological materials prior to analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES), but reported that the procedure is unsuitable for routine analysis with an unacceptably high detection limit. Walsh[9] described a method for the determination of boron in rocks by ICP-AES on the aqueous leach of a potassium carbonate fusion melt after the bulk of the potassium had been precipitated using perchloric acid. However, Hall and Pelchat[10] reported salt build-up at the top of the torch and clogging of the nebuliser when using the potassium carbonate procedure. Also, platinum crucibles were attacked by the flux and, when nickel crucibles were used, flux creep over the walls occurred.

A simple, rapid and accurate procedure with sufficient sensitivity was required for the determination of boron in silicate rocks and soils. The hydrofluoric acid - aqua regia dissolution method proposed in this paper achieved dissolution of rocks and soils with a good detection limit, without loss of boron by volatilisation during volume reduction, and is suitable for routine chemical analysis. Good sensitivity for boron, relative freedom from interferences and a high rate of sample throughput have made ICP-AES the method of choice for the analysis of many major and trace elements in geological materials.

Experimental

Equipment

PTFE beakers. Capacity 100 ml, 60 × 60 mm, Kartell brand.

Pressure decomposition bombs. 70-ml capacity PTFE-lined bomb manufactured by Uniseal Decomposition Vessels, Haifa, Israel. The bomb was sealed using a closure tool set.

Nickel crucibles. Capacity 100 ml, 64 × 60 mm, supplied by Arthur H. Thomas, Philadelphia, PA, USA.

ICP-AES. A Labtest V-25 direct-reading vacuum spectrometer was used, equipped with 26 analytical channels in the first order, including B (249.773 nm) and Fe (259.939 nm). Digest solutions were introduced into the plasma using a modified Babington pneumatic nebuliser (Type GMK).[11] A Gilson Minipuls 2 peristaltic pump with a Tygon red - red (1.14 mm i.d.) pump tube was used for delivery of solution to the nebuliser.

A stabilisation time of 30 s was followed by three 20-s integrations, the result being calculated as the arithmetic mean of these three readings. Argon flow-rates to the plasma torch were coolant 20 l min^{-1}, nebulisation pressure 280 kPa and UV optics purge 7 l min^{-1}. The viewing height was 15 mm above the load coil with the forward power set at 1.40 kW, producing <2 W of reflected power. The inductively coupled argon plasma source, spectrometer, scanning monochromator, data aquisition system, wavelength profiles of potentially interfering analytes and on-line interference correction procedure have been described elsewhere.[12]

Reagents

All water was doubly distilled. Reagent bottles, volumetric ware and beakers were soaked overnight in 2 M hydrochloric acid, rinsed with water and dried at 60 °C.

Aqua regia. Freshly prepared by mixing 180 ml of nitric acid (Ajax, Univar grade, 70% *m/m*) and 820 ml of hydrochloric acid (BDH Chemicals, AnalaR grade, 31–32% *m/m*).

Hydrofluoric acid, 50% *m/m.* Ajax, Univar grade.

Sodium carbonate. Merck, GR grade.

Aluminium chloride solution, 20% *m/V.* Dissolve 200 g of aluminium chloride (Merck, extra pure) in 1 l of water.

Boron stock standard solution, 1000 μg g^{-1}. Dissolve 5.719 g of boric acid (Merck, GR grade) in 1 l of water. Dilute aliquots of this solution to produce the calibration standards.

Iron stock standard solution, 1000 μg g^{-1}. Dissolve 7.234 g of iron(III) nitrate (Merck, GR grade) in 1 l of 1% *m/V* nitric acid. Dilute aliquots of this solution to produce the calibration standards.

Sample Dissolution

Owens *et al.*[7] proposed using the sodium carbonate fusion procedure for the dissolution of geological materials with determination of boron by ICP-AES. However, this very salty digest solution caused severe calibration drift in the instrument, making the procedure impractical for routine analysis. The boron present in the sodium carbonate of 5–15 μg g^{-1} (also reported by Troll and Saurer[13]) resulted in an unacceptably high solution detection limit.

Hydrofluoric acid, together with various inorganic acids, has been used for the dissolution of geological materials[14–18] prior to analysis for many elements except boron, as residual hydrofluoric acid is complexed with boric acid on completion of the dissolution. Spiers *et al.*[19] evaporated hydrofluoric and hydrochloric acids to dryness for the removal of excess hydrofluoric acid. They reported unreliably high boron analyses but claimed that these were not due to hydrofluoric acid attack on the borosilicate glass nebuliser, spray chamber or quartz torch of their ICP-AES system.

Sample presentation to the nebuliser must be as a liquid. A simple, rapid method for the determination of boron in geological materials required a dissolution technique that would produce a solution low in dissolved salts, to minimise instrumental drift, while realising that some boron-containing minerals, such as tourmaline, are not completely decomposed by hydrofluoric acid.[20] On completion of dissolution, residual fluoride was complexed with aluminium (present as aluminium chloride) to release boron from the stable fluoroborate ion[21] and to protect the borosilicate and quartz components of the instrument.

Procedures

Open beaker

Weigh 1.000 g of rock or soil (ground to <200 μm) into a PTFE beaker. Add 5 ml of aqua regia and 5 ml of hydrofluoric acid. Place the beaker on a hot-plate at 140 °C (measured with a contact thermometer) and evaporate to a minimum volume (1–2 ml) without allowing the digest solution to dry, as loss of boron will result. Repeat with a further 5 ml of each acid and volume reduction. Add 10 ml of aluminium chloride solution and warm the solution for approximately 5 min. Allow to cool and dilute with water to 50 ml in a polyethylene calibrated flask.

Sodium carbonate fusion

Weigh 1.000 g of rock or soil (ground to <200 μm) into a nickel crucible. Mix with 6.0 g of sodium carbonate and heat in a muffle furnace at 1000 °C for 1 h. Remove the mixture from the furnace, cool and cautiously add 5-ml aliquots of 6 M hydrochloric acid (with a covering slip in place to prevent loss) until effervescence ceases. Warm and break up the fusion cake to assist dissolution. Filter into a 100-ml polyethylene calibrated flask using a Whatman No. 2 filter-paper. Wash the silicic acid gel thoroughly with water and then dilute to volume with water.

Pressure decomposition bomb

Weigh 1.000 g of rock or soil (ground to <200 μm) into the bomb and add 5 ml of aqua regia and 5 ml of hydrofluoric acid. Close the bomb using the closure tools provided and heat in an oven at 170 °C for 3 h. Cool, wash the digest solution into a PTFE beaker with water and continue as described for the open beaker procedure.

Two reagent blanks were carried through with each of the decomposition procedures.

Calibration

Boron and iron calibration solutions were prepared separately to match the matrix of the digests, *viz.*, in 4% *m/V* aluminium chloride and 6% *m/V* sodium carbonate solutions. Wallace[22] has shown that of the several spectral lines available for boron determination the 249.773-nm line is the most sensitive. A comparative study using the 249.678-nm spectral line confirmed that both analytical lines were relatively free from spectral interference. Only iron produced a significant interference (Fig. 1). On-line spectral interference correction was calibrated as 1.28 μg ml^{-1} of boron per 1000 μg ml^{-1} of iron for both the hydrofluoric acid and the fusion methods.

Small changes in the profile setting of the spectrometer are known to affect the inter-element interference correction factors, especially if they are large. The interference correction factor was therefore determined daily after the spectrometer was profiled. On-peak correction has an uncertainty equivalent to approximately 10% of the total background interference effect.[23,24] Hence, samples containing 10% of iron (National Bureau of Standards, Standard Reference Material 1633a, coal fly ash, Table 2) would produce an interference of 2.6 μg g^{-1}, implying a detection limit due to the interference correction of 0.26 μg g^{-1}.

The "adaptation effect" described by Maessen *et al.*[25] was investigated for the matrices described above. Net line intensity as a function of time when 1 μg ml^{-1} of boron was aspirated is illustrated in Fig. 2. The results show that it is necessary to aspirate for a minimum period of 30 s to allow the analytical signal to stabilise before integrations are commenced.

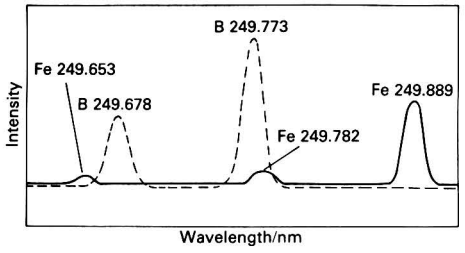


Fig. 1. Wavelength scans in the vicinity of the B I 249.773-nm line. B, 1 μg ml^{-1}; Fe, 100 μg ml^{-1}

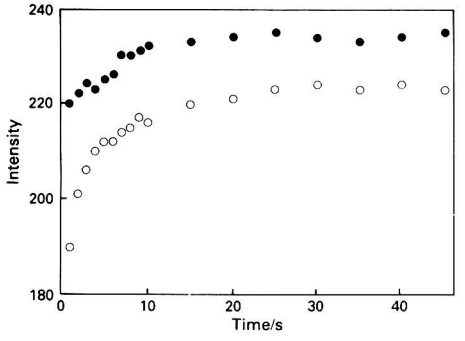


Fig. 2. Intensity of the B I 249.773-nm line as a function of aspiration time. (●) Aluminium chloride; (○) sodium carbonate

Sensitivity

Defining and determining the analytical sensitivity and detection limit of a procedure have been described by the ICP Detection Limits Committee.[26] The 2σ detection limit thus obtained for the hydrofluoric acid procedure was 0.005 μg ml^{-1} and that for the fusion method was 0.010 μg ml^{-1}. When the errors introduced by the correction for spectral interferences are added to these 2σ values, for a sample containing 10% of iron, the new detection limits become 0.265 and 0.275 μg ml^{-1}, respectively. The higher detection limit for the fusion method was attributed to plasma instability induced by (a) the high salt concentration in the digest, (b) probable light scattering, (c) poorer aerosol injection into the plasma due to fouling of the torch injector tube and (d) decreased nebulisation efficiency due to a higher solution viscosity.

For the inter-element interference determined above, the nomograms described by Church[23] indicate that for a sample containing 10% of iron, boron determination is possible only if the sample contains more than 7.5 μg g^{-1}.

Memory Effects

Memory effects are known to be a problem with boron when solutions containing dissolved lithium metaborate fusions[27] are aspirated. A 1-ml volume of 20 μg ml^{-1} of boron (as boric acid), equivalent to 0.1% of boron in a sample, was analysed using the open beaker procedure to assess memory effects. The recovery of 20.2 ± 0.4 μg g^{-1} of boron (n = 10) indicates no memory effects at the maximum concentration of boron generally found in rocks and soils.

Boric acid, generally recommended to complex residual hydrofluoric acid, has been reported not to prevent attack on conventional glass nebulisers.[28] No boron could be detected at the least quantitatively determinable amount, *viz.*, 0.25 μg g^{-1}, when a digest blank solution was aspirated continuously for 15 min.

Results

Recovery of Boron

There have been few reports of the dissolution of geological materials using hydrofluoric acid for the determination of boron since Chapman *et al.*,[29] using hydrofluoric and perchloric acids, claimed a total loss of boron due to volatilisation. Pritchard and Lee[30] added mannitol to their hydrofluoric acid mixture to prevent the loss of boron during volatilisation of silica. The low results indicated that their procedure was unsuccessful. This was probably due to (a) evaporation of the digest solution to dryness, (b) the use of perchloric acid and (c) non-determination of some of the boron as the complexed stable fluoroborate ion.

Volumes of 1 ml of 6 and 10 μg ml^{-1} boron (as boric acid) were analysed using the open beaker procedure to check the quantitative recovery of boron in an essentially matrix-free digest. The results were compared with those obtained using only aqua regia, to assess any losses due to volatilisation, and the classical sodium carbonate fusion procedure (Table 1). The reproducibility of the determinations varied from 3% for the aqua regia procedure to 11% for the fusions.

A small amount of boron was possibly lost using the fusion method. However, considering the experimental error of this very salty matrix due to the inherent difficulties outlined above and experienced by others,[7] the loss was small. The slightly poorer precision of the hydrofluoric acid procedure (compared with the aqua regia procedure) was possibly due to the same plasma and nebuliser instabilities associated with the fusion method. Overall, there was good agreement between the procedures, indicating a negligible loss of boron using hydrofluoric acid.

Evaporation of the digest to dryness for the removal of excess of hydrofluoric acid prior to elemental analysis is often recommended.[15,18] When the hydrofluoric acid and the aqua regia systems were evaporated to dryness at 140 °C, the loss of boron varied from 5 to 25%. Previous experience with plant digestion using nitric acid - perchloric acids at 225 °C resulted in a 20% loss of boron on evaporation to 1 ml. Consequently, a hydrofluoric acid - perchloric acid mixture was not considered for sample dissolution.

Table 1. Recovery of boron in matrix-free and synthetic soil digests

Procedure	Boron concentration*/μg g^{-1} Matrix-free	"Soil"
Hydrofluoric acid - aqua regia	6.1 ± 0.3	5.8 ± 0.3
	10.1 ± 0.5	10.2 ± 0.3
Aqua regia	5.9 ± 0.2	6.1 ± 0.1
	10.1 ± 0.3	9.8 ± 0.2
Sodium carbonate fusion	5.4 ± 0.6	5.6 ± 0.7
	9.5 ± 0.8	9.7 ± 0.9

* Means for three replicates with standard deviations.

Matrix Effects

The reasonable matrix matching of standards with samples for elemental determination by ICP-AES is desirable[31] but usually not possible. A synthetic "soil" consisting of 0.45 g of analytical-reagent grade calcium carbonate, 0.45 g of silica and 0.10 g of goethite with 1 ml of either 6 or 10 μg ml^{-1} boron (as boric acid) was dissolved using the open beaker procedure. The results were compared with those obtained using only aqua regia, to assess any losses due to volatilisation, and the sodium carbonate fusion procedure (Table 1).

As the standards and samples both contained the major matrix components, *viz.*, aluminium chloride for the hydrofluoric acid procedure and sodium carbonate for the fusion procedure, the effective matrix in the synthetic sample consisted of calcium, any silicon in solution and approximately 0.13% of iron.

Analysis of variance using the MINITAB statistical software package[32] showed that the results were not significantly different between the matrix and matrix-free digest solutions after spectral interference due to iron had been taken into account.

Comparison of Dissolution Procedures

The accuracy of the proposed open beaker and bomb procedures for sample dissolution and boron retention was confirmed by the determination of boron in a range of standard reference materials (Table 2). The over-all mean ratio of determined to literature values was 1.02 with a standard deviation of 0.04 for concentrations greater than 2 μg g^{-1}.

Samples of soils commonly found in south-eastern Australia[35] and of Tindelpina Shale (consisting mainly of chlorite, muscovite, quartz, feldspar and calcite)[36] from the Mount Lofty Ranges were chosen to cover a range of boron concentrations.

Tables 3 and 4 report the results obtained using the recommended open beaker procedure with those using bomb dissolution and sodium carbonate fusion. A 1-ml volume of 50 μg ml^{-1} boron (as boric acid) was added to the soil and rock samples prior to dissolution as a check on the recovery of boron in a "real" sample matrix.

For soil samples, analysis of variance of boron values showed no significant differences between the three dissolution methods, except for sample No. 3082, for which the result obtained by the open beaker procedure was 15% lower. This

low result is not attributed to loss of boron by volatilisation but rather to incomplete dissolution of hydrofluoric acid-resistant minerals, as no loss occurred during volume reduction of the bomb digest.

Complete dissolution of rock was not achieved using the open beaker method. Many minerals that are completely decomposed using the bomb procedure are not dissolved in open beakers.[28] Analysis of variance of the boron values showed significant differences between the open beaker dissolution method and the other two methods ($P < 0.001$). There was no significant difference between the boron values of the sodium carbonate fusion and the bomb dissolution procedures.

Quantitative recovery of boron in the standard reference materials and in the spiked soil and rock samples confirmed that (a) no losses occurred due to volatilisation, (b) matrix effects due to soil and rock concomitants were minimal and (c) compensation for the spectral interference due to iron was adequate.

Triplicate analyses of the spiked and unspiked soil and rock samples showed that a precision of about 6% was achieved using the hydrofluoric acid method and about 10% for the sodium carbonate procedure. These are similar to the values obtained by Thompson and Walsh[37] and Owens *et al.*,[7] respectively.

Sample Pre-treatment

Fine grinding of the soil using a Siebtechnik mill and the destruction of soil organic matter by heating at 550 °C for 3 h in a muffle furnace had no significant effect on the recovery of boron.

Comparison between analytical techniques

An independent assessment of the concentration of boron in the soil and rock samples was obtained using d.c. arc emission

Table 2. Boron concentrations (μg g^{-1}) in certified reference materials

Sample	Procedure*	Analysis values†	Literature value	Reference
Fly ash—				
NBS 1633a	1	41.7 ± 1.5	39.7	33
	2	39.3 ± 1.7		
Silcates—				
CCRMP-SY2	1	89.3 ± 2.2	87	34
	2	87.6 ± 2.8		
NBS 278	1	26.0 ± 1.0	25	Cert.‡
	2	27.7 ± 1.5		
NBS 688	1	1.6 ± 0.2	1	33
	2	1.5 ± 0.2		
NBS 91	1	296 ± 4	302	33
	2	295 ± 5		

* Procedure: 1 = open beaker; 2 = bomb dissolution.
† Means for three replicates with standard deviations.
‡ Certificate of analysis.

Table 3. Comparison of boron values in soil obtained by the proposed and other procedures

Sample No.[35]	Soil type[35]	Procedure*	Boron added/ μg g^{-1}	Boron found†/ μg g^{-1}	Boron initially present/μg g^{-1}
588	Calcareous sand	1	—	18 ± 1	18 ± 1
			50.0	70 ± 1	20 ± 1
		2	—	19 ± 2	19 ± 2
			50.0	72 ± 4	22 ± 4
		3	—	19 ± 1	19 ± 1
			50.0	71 ± 1	21 ± 1
574	Calcareous clay	1	—	84 ± 3	84 ± 3
			50.0	128 ± 3	78 ± 3
		2	—	83 ± 6	83 ± 6
			50.0	130 ± 7	80 ± 7
		3	—	88 ± 4	88 ± 4
			50.0	134 ± 4	84 ± 4
3082	Red - brown earth	1	—	244 ± 10	244 ± 10
			50.0	300 ± 15	250 ± 15
		2	—	282 ± 20	282 ± 20
			50.0	351 ± 21	301 ± 21
		3	—	286 ± 15	286 ± 15
			50.0	344 ± 16	294 ± 16
6061	Grey clay	1	—	231 ± 13	231 ± 13
			50.0	290 ± 16	240 ± 16
		2	—	234 ± 21	234 ± 21
			50.0	300 ± 20	250 ± 20
		3	—	238 ± 12	238 ± 12
			50.0	295 ± 13	245 ± 13

* Procedure: 1 = open beaker; 2 = sodium carbonate fusion; 3 = bomb dissolution.
† Means for three replicates with standard deviations.

Table 4. Comparison of boron concentrations in rocks obtained by the proposed and other procedures

Rock No.[36]	Procedure*	Boron added/ μg g^{-1}	Boron found†/ μg g^{-1}	Boron initially present/μg g^{-1}
76	1	—	35 ± 1	35 ± 1
		50.0	84 ± 1	34 ± 1
	2	—	42 ± 3	42 ± 3
		50.0	94 ± 4	44 ± 4
	3	—	46 ± 2	46 ± 2
		50.0	97 ± 2	47 ± 2
24	1	—	100 ± 4	100 ± 4
		50.0	145 ± 4	95 ± 4
	2	—	130 ± 10	130 ± 10
		50.0	183 ± 11	133 ± 11
	3	—	145 ± 6	145 ± 6
		50.0	208 ± 7	158 ± 7
56A	1	—	128 ± 7	128 ± 7
		50.0	195 ± 9	145 ± 9
	2	—	175 ± 13	175 ± 13
		50.0	230 ± 12	180 ± 12
	3	—	182 ± 10	182 ± 10
		50.0	239 ± 11	189 ± 11
46	1	—	213 ± 10	213 ± 10
		50.0	261 ± 11	212 ± 11
	2	—	271 ± 20	271 ± 20
		50.0	326 ± 21	276 ± 21
	3	—	267 ± 13	267 ± 13
		50.0	333 ± 12	273 ± 12

* Procedure: 1 = open beaker; 2 = sodium carbonate fusion; 3 = bomb dissolution.
† Means for three replicates with standard deviations.

spectrophotometry. Thompson[38] has shown that simple linear regression gives accurate estimates of bias between methods if (a) at least ten samples are used, (b) samples cover the concentration range from zero upwards fairly uniformly and (c) results from the method with the smaller variance are used as the independent (abscissa) variable. Boron values in soil and rock determined by d.c. arc spectrophotometry and standard reference materials determined by ICP-AES after dissolution using the open beaker procedure were used as the dependent (y-axis) variables while the boron values in soil and rock determined by ICP-AES after dissolution using the proposed procedures and the literature values reported for the standard reference materials were used as the independent (x-axis) variables to satisfy the conditions for generating the line of best fit using the MINITAB statistical software package,[32] shown in Fig. 3. The regression gave a slope of 1.01 ± 0.02, which is not significantly different from unity, and an intercept of −2.10 ± 5.05, which is not signficantly different from zero, confirming the complete dissolution of soil (using an open beaker), rock (using a bomb) and the standard reference materials (using an open beaker and a bomb) whilst retaining boron during volume reduction.

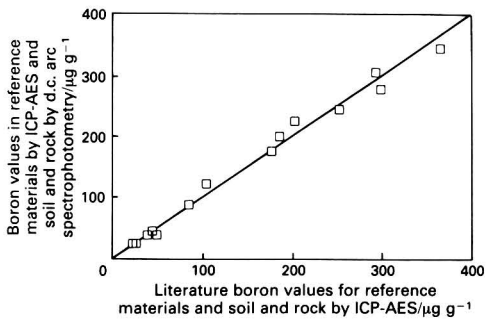


Fig. 3. Boron determined in the standard reference materials by the proposed dissolution procedure and in soil and rock by d.c. arc emission spectrophotometry as a function of the boron values reported in the literature for the standard reference materials and in soil and rock determined by the proposed procedures

Conclusions

The simple and rapid dissolution of silicate materials for the determination of boron was achieved using hydrofluoric acid and aqua regia without a loss of boron by volatilisation during volume reduction. Complexing residual fluoride with aluminium released boron from the stable fluoroborate ion and allowed determination by ICP-AES whilst protecting the borosilicate and quartz components of the spectrometer.

The low salt concentration in the digest solution (compared with the fusion procedure) resulted in minimal calibration drift. Consequently, frequent re-standardisations of the instrument during routine analysis were not necessary.

The increase in detection limit due to the inter-element interference produced by iron, with concentrations ranging up to 10%, did not degrade the quantifiable limit to the extent suggested by Thompson and Walsh,[24] as evidenced by the recovery of boron in the synthetic "soil" and standard reference materials with a precision equal to or better than that suggested by Church.[23] This is probably due to (a) daily monitoring of the spectral inter-element interference after profiling the spectrometer and (b) the use of a modified Babington-type nebuliser (Type GMK) as suggested by Mills.[27]

The boron concentrations in the soils, obtained by the open beaker method, and in rocks, obtained using the bomb procedure, were comparable to those obtained by accepted sodium carbonate fusion (with determination by ICP-AES) and d.c. arc emission spectrophotometry. Determinations of boron in the standard reference materials showed good agreement and precision with the published literature values.

Mills[27] has also shown that, for the standard reference materials investigated, the recovery of boron with adequate precision is achievable using an open beaker procedure. However, dissolution of all silicates for the determination of boron using a single procedure must be treated with caution, as the comparison between methods for the determination of boron in soil, rock and standard reference materials shows.

We express our thanks to J. Eames and N. Morgan of CSIRO, Division of Mineral Physics and Mineralogy, for the d.c. arc emission spectrophotometric analyses, J. C. Mills of the Broken Hill Prop. Co. Ltd. for helpful discussions and V. Gostin of the University of Adelaide for the rock samples.

References

1. Jackson, M. L., *in* Bear, F. E., *Editor,* "Chemistry of the Soil," Second Edition, Reinhold, New York, 1964, p. 134.
2. Dewis, F. J., Levinson, A. A., and Bayliss, P., *Geochim. Cosmochim. Acta,* 1972, **36**, 1359.
3. Potter, P. E., Shimp, N. F., and Witters, J., *Geochim. Cosmochim. Acta,* 1963, **27**, 669.
4. Reisenauer, H. M., Walsh, L. M., and Hoeft, R. G., *in* Walsh, L. M., and Beaton, J. D., *Editors,* "Soil Testing and Plant Analysis," Revised Edition, Soil Science Society of America, Madison, WI, 1973, p. 173.
5. Berger, K. C., and Truog, E., *Soil Sci. Soc. Am. Proc.,* 1945, **10**, 113.
6. Gladney, E. S., Jurney, E. T., and Curtis, D. B., *Anal. Chem.,* 1976, **48**, 2139.
7. Owens, J. W., Gladney, E. S., and Knab, D., *Anal. Chim. Acta,* 1982, **135**, 169.
8. Gupta, U. C., *Soil Sci. Soc. Am. Proc.,* 1966, **30**, 655.
9. Walsh, J. N., *Analyst,* 1985, **110**, 959.
10. Hall, G. E. M., and Pelchat, J.-C., *Analyst,* 1986, **111**, 1255.
11. McKinnon, P. J., Giess, K. C., and Knight, T. V., *in* Barnes, R. M., *Editor,* "Developments in Atomic Plasma Spectrochemical Analysis," Heydon, Philadelphia, 1981, p. 287.
12. Zarcinas, B. A., and Cartwright, B., *CSIRO Aust. Div. Soils Tech. Pap.,* No. 45, 1983.
13. Troll, G., and Sauerer, A., *Analyst,* 1985, **110**, 283.
14. Nadkarni, R. A., *Anal. Chem.,* 1980, **52**, 929.
15. McLaren, J. W., Berman, S. S., Boyko, V. J., and Russell, D. S., *Anal. Chem.,* 1981, **53**, 1802.
16. Lechler, P. J., Roy, W. R., and Leininger, R. K., *Soil Sci.,* 1980, **130**, 238.
17. Uchida, H., Uchida, T., and Iida, C., *Anal. Chim. Acta,* 1979, **108**, 87.
18. Uchida, H., Uchida, T., and Iida, C., *Anal. Chim. Acta,* 1980, **116**, 433.
19. Spiers, G. A., Dudas, M. J., and Hodgins, L. W., *Commun. Soil Sci. Plant Anal.,* 1983, **14**, 629.
20. Barredo, L. B., and Diez, L. P., *Talanta,* 1976, **23**, 859.
21. Siemer, D. D., *Anal. Chem.,* 1982, **54**, 1321.
22. Wallace, G. F., *At. Spectrosc.,* 1981, **2**, 61.
23. Church, S. E., *Geostand. Newsl.,* 1981, **5**, 133.
24. Thnompson, M., and Walsh, J. N., "A Handbook of Inductively Coupled Plasma Spectrometry," Blackie, Glasgow, 1983, p. 126.
25. Maessen, F. J. M. J., Balke, J., and de Boer, J. L. M., *Spectrochim. Acta, Part B,* 1982, **37**, 517.
26. ICP Detection Limits Committee, *ICP Inf. Newsl.,* 1979, **5**, 295.
27. Mills, J. C., *Anal. Chim. Acta,* 1986, **183**, 231.
28. Thompson, M., and Walsh, J. N., "A Handbook of Inductively Coupled Plasma Spectrometry," Blackie, Glasgow, 1983, p. 134.
29. Chapman, F. W., Marvin, G. G., and Tyree, S. Y., *Anal. Chem.,* 1949, **21**, 700.
30. Pritchard, M. W., and Lee, J., *Anal. Chim. Acta,* 1984, **157**, 313.
31. Thompson, M., and Walsh, J. N., "A Handbook of Inductively Coupled Plasma Spectrometry," Blackie, Glasgow, 1983, p. 130.
32. Ryan, T. A., Joiner, B. L., and Ryan, B. F., "MINITAB Student Handbook," Duxbury, Boston, 1976.
33. Gladney, E. S., Burns, C. E., Perrin, D. R., Roelandts, I., and Gills, T. E., *Nat. Bur. Stand. U.S. Spec. Publ.,* No. 260, 1984.
34. Abbey, S., and Gladney, E. S., *Geostand. Newsl.,* 1986, **10**, 3.
35. Stace, H. C. T., Hubble, G. D., Brewer, R., Northcote, K. H., Sleeman, J. R., Mulcahy, M. J., and Hallsworth, E. G., "A Handbook of Australian Soils," Rellim, Glenside, 1968.
36. Sumartojo, J., *PhD Thesis,* University of Adelaide, 1974.
37. Thompson, M., and Walsh, J. N., "A Handbook of Inductively Coupled Plasma Spectrometry," Blackie, Glasgow, 1983, p. 145.
38. Thompson, M., *Analyst,* 1982, **107**, 1169.

Paper A6/294
Received August 21st, 1986
Accepted March 10th, 1987

Rapid Determination of Chromium in Bovine Liver Using an Atomic Absorption Spectrometer with a Modified Carbon Rod Atomiser

John W. Steiner, David C. Moy and Harvey L. Kramer
Department of Primary Industries, Animal Research Institute, Biochemistry Branch, 665 Fairfield Road, Yeerongpilly, Brisbane, Queensland 4105, Australia

Chromium in bovine liver was determined rapidly by atomic absorption spectrometry with a modified carbon rod atomiser. Pre-treatment of samples was minimised by the sequential introduction of mixtures of nitrogen, hydrogen, oxygen and methane into the atomiser. An optical device facilitated increased precision of injection of samples. The effectiveness of the method was confirmed by the determination of chromium in a certified sample.

Keywords: *Chromium determination; matrix modification;* in situ *gaseous pre-treatment; modified carbon rod atomiser; electrothermal atomisation atomic absorption spectrometry*

Chromium is an important trace element for mammalian nutrition, being essential for insulin action and maintenance of normal glucose metabolism.[1,2] Despite the recognition of the biological importance of chromium, its determination is still difficult, especially when older electrothermal atomisation atomic absorption spectrometers are used. Major difficulties of older electrothermal atomisation atomic absorption spectrometry (ETA-AAS) instruments are inherent in the background correction system using a deuterium lamp[3,4] and in the lack of a variable ramp rate facility for the drying and ashing cycles.[5] The determination of chromium in biological materials with older instruments depends largely on the pre-treatment of samples using the tedious and time-consuming chelation and solvent extraction procedure.[6]

We describe here an alternative approach using *in situ* treatment of the acid-digested sample within the atomiser. This utilises a carbon rod atomiser modified as described elsewhere.[7] Use of the modified atomiser involves synchronised introduction of different gases into the atomiser during the drying, ashing and atomising cycle. This facilitates more uniform drying of the sample, which in turn improves the ashing efficiency and provides highly effective atomisation of the sample. Other benefits are an increase in the lifetime of the graphite tube and better precision.

Experimental

Apparatus

A Varian Techtron AA-175 spectrophotometer equipped with a modified carbon rod atomiser (CRA-90) was used. The modifications consisted of the use of an optical device and vertical and transverse gas jets. The optical system facilitated the viewing of sample injection and the gas jets allowed the introduction of gases into the atomiser.[7]

Reagents

Gases

High-purity nitrogen, industrial dry hydrogen and medical-grade oxygen were obtained from Commonwealth Industrial Gases (CIG), Brisbane. Methane (99.9% pure) was obtained from Matheson.

Standard solutions

The concentrations of the working standards were 0, 0.01, 0.02 and 0.03 mg l^{-1} of chromium. These were prepared daily from concentrated stock solutions (BDH Chemicals).

Standard reference material

Freeze-dried, powdered bovine liver standard reference material (SRM 1577) was obtained from the US National Bureau of Standards. The certified concentration for chromium was 0.088 ± 0.012 mg kg^{-1} (95%/95% statistical tolerance limits).

Procedure

Glassware cleaning

New borosilicate glassware was initially de-contaminated by soaking in 10% nitric acid (re-distilled before use). It was subsequently maintained in a clean condition using Decon-90 non-ionic detergent (Decon Laboratories). Prior to use, the glassware was rinsed thoroughly with doubly glass-distilled water.

Preparation of SRM 1577 and test samples

The SRM 1577 and test samples of liver tissue were prepared by weighing suitable amounts (approximately 1.0 g dry or 3.0 g fresh) into glass-stoppered borosilicate digestion tubes of 50-ml capacity. Concentrated doubly distilled, chromium-free nitric acid (5 ml) was added and the tubes were lightly stoppered before being placed in a shaking water-bath at 80 °C for 4 h. The partially digested samples were diluted to 25 ml before analysis. The samples used for recovery studies were fortified with a fraction of the analyte prior to digestion.

Instrument optimisation and operation

The optimisation and operation of the instrument have been described previously,[7] as has the specific order in which absorbance readings were taken to evaluate the method for the determination of chromium in bovine tissue. The instrumental conditions and optimum flow-rates of the gases used for the continuous re-coating of the atomiser and for the *in situ* treatment of the sample are reported in Tables 1 and 2.

Table 1. Instrumental conditions

Parameter	Value
Volume of injection/μl	4
Drying temperature/°C	95
Ashing temperature/°C	60
Atomising temperature/°C	2200
Drying time/s	50
Ashing time/s	30
Hold time/s	3.5
Ramp rate/°C s^{-1}	800
Residence time of transverse gas jet/s	12

Table 2. Optimum flow-rates [mean ± s.d. (n = 6)] for the determination of chromium

Gas introduction system	Gas	Flow-rate/ml min^{-1}
Sheath	Nitrogen	6719 ± 51
	Hydrogen	119 ± 2.1
	Methane	100 ± 1.2
Vertical gas jet	Methane	9.1 ± 0.8
Transverse gas jets	Hydrogen	216 ± 1.2
	Oxygen	177 ± 1.3

Results and Discussion

Evaluation of Method for Determination of Chromium

The proposed method for the determination of chromium was evaluated by regular monitoring of accuracy, precision, repeatability and sensitivity. The effectiveness of using different gases and synchronising their use with the overall operation of the instrument is demonstrated in Table 3. From a sequence of 63 measurements, two intervals of 9 readings (periods 1 and 2) provide the determined chromium concentration.

In conventional operation (Table 3, row 1) good precision but poor accuracy was obtained. However, a significant ($P < 0.05$) improvement in both was achieved (Table 3, row 8) when the appropriate gas mixture, in conjunction with the optical device, was used. Although the concentration of chromium in SRM 1577 is certified as 0.088 ± 0.012 mg kg^{-1}, only 0.081 ± 0.004 mg kg^{-1} was found.

Attempts were made to reduce the difference between the certified and experimentally determined concentrations of chromium in the SRM. This was done by changing the mixing ratios and flow-rates of nitrogen, methane and hydrogen while keeping the flow-rate of oxygen constant. No significant improvement was achieved until the flow-rate of oxygen was elevated from 177 to 220 ml min^{-1} (while keeping the flow-rates of the other gases constant). This provided a value of 0.083 ± 0.014 mg kg^{-1}, but the increase in the oxygen flow-rate resulted in a subsequent reduction of the determined chromium concentration to 0.077 ± 0.010 mg kg^{-1} after 40 firings.

The determined value of 0.081 ± 0.004 mg kg^{-1} lies within the range obtained by other workers[3,4] for SRM 1577 using atomic absorption spectrometry, namely 0.084 ± 0.001 mg kg^{-1} to 0.060 ± 0.006 mg kg^{-1}. The accuracy of our work is further supported by neutron activation analysis studies,[8] which provided a mean value of 0.0806 mg kg^{-1} for a range of 0.0772–0.0840 mg kg^{-1}.

Precision and accuracy tests were performed with the modified procedure on four separate days with four SRM-1577 sub-samples (Table 4). The chromium content found in these samples was 0.081 ± 0.004 mg kg^{-1} (mean ± 95% statistical tolerance limits). Further assessments of precision, accuracy and recovery were performed at regular intervals (approximately every 250 analyses) using the analytical procedures described in this paper. During the course of over 3000 analyses, no variations were observed.

Table 5 compares the results obtained with and without the modified procedure. The absolute error and the relative standard deviation (r.s.d.) are significantly ($P < 0.05$) lower using the modified procedure. The modified procedure also resulted in an improvement in repeatability and sensitivity (slope b_1).

Although the deuterium background correction system was continuously used during the entire investigation, the associated difficulties reported by other workers[3,10] were not experienced, probably because the maximum background absorbance never exceeded 0.025 unit. There are recommendations for using a high and variable ashing temperature to minimise interference.[10] This was not necessary, as the presence of the mixture of gases (nitrogen, methane, hydrogen and oxygen) in the atomiser during ashing effectively eliminated all interferences.

Conclusion

The introduction of different gases into the atomiser effected a direct pre-treatment of partially digested bovine liver samples. A careful synchronisation with the over-all instrumental

Table 4. Recovery of chromium added to bovine liver samples. Four sub-samples, four analyses per sub-sample

Amount of Cr added/μg	Recovery, % ± s.d.
0.3	94.3 ± 3.2
0.6	94.6 ± 1.3
0.9	92.0 ± 1.6

Table 5. Comparison of performance using the modified and unmodified procedures

Analytical parameter	Modification: With	Modification: Without
Absolute error*/mg kg^{-1}	−0.007	−0.021
R.s.d., %	4.1	19.8
Slope (b_1)	6.909	6.220

* The absolute error is defined[9] as the difference between the measured and the certified values for SRM 1577.

Table 3. Experimental conditions and results of the repeated determination of chromium in SRM 1577 certified to contain 0.088 ± 0.012 mg kg^{-1} of chromium

Gas introduction system: Sheath	Vertical jets	Transverse jets	Optical device	Determined chromium concentration/mg kg^{-1}*: Period 1†	Period 2‡
N_2	–	–	–	0.067 ± 0.014	0.065 ± 0.007
$N_2 + H_2$	–	–	–	0.061 ± 0.012	0.110 ± 0.037
$N_2 + H_2 + CH_4$	–	–	–	0.068 ± 0.018	0.057 ± 0.021
$N_2 + H_2 + CH_4$	CH_4	–	–	0.062 ± 0.016	0.063 ± 0.020
$N_2 + H_2 + CH_4$	CH_4	H_2	–	0.068 ± 0.007	0.064 ± 0.009
$N_2 + H_2 + CH_4$	CH_4	O_2	–	0.083 ± 0.014	0.077 ± 0.010
$N_2 + H_2 + CH_4$	CH_4	$H_2 + O_2$	–	0.078 ± 0.007	0.077 ± 0.008
$N_2 + H_2 + CH_4$	CH_4	$H_2 + O_2$	+	0.080 ± 0.003	0.081 ± 0.004

* Mean ± 95%/95% statistical tolerance limits.
† Firing interval 16–24 inclusive (based on n = 9).
‡ Firing interval 52–60 inclusive (based on n = 9).

operation resulted in a significant improvement in the analytical data, shortened the time required for analysis and reduced the deterioration rate of the atomiser.

We thank Mr. F. Duncalfe for the statistical analysis of the data.

References

1. Schwarz, K., and Mertz, W., *Arch. Biochem. Biophys.*, 1959, **85**, 292.
2. Mertz, W., *Physiol. Rev.*, 1963, **49**, 163.
3. Routh, M. W., *Anal. Chem.*, 1980, **52**, 182.
4. Kayne, F. J., Komar, G., Laboda, H., and Vanderline, R. E., *Clin. Chem.*, 1978, **24**, 2151.
5. Matsusaki, K., Yoshino, T., and Yannamoto, Y. *Anal. Chim. Acta*, 1981, **124**, 163.
6. Chao, S. S., and Pickett, E. E., *Anal. Chem.*, 1980, **52**, 335.
7. Steiner, J. W., and Kramer, H. L., *Analyst*, 1983, **108**, 1051.
8. Versieck, J., Hoste, J., De Rudder, J., Barbier, F., and Vanballenberghe, L., *Anal. Lett.*, 1979, **12**, 555.
9. Christian, G. D., "Analytical Chemistry," Xerox College Publishing, Waltham, MA, 1971, p. 456.
10. Guthrie, B. E., Wolf, W. R., and Veillan, C., *Anal. Chem.*, 1978, **40**, 1900.

Paper A6/306
Received September 2nd, 1986
Accepted March 23rd, 1987

Use of Fourier Transform Infrared Spectroscopy for Quantitative Analysis: A Comparative Study of Different Detection Methods

Peter S. Belton, Alfred M. Saffa and Reginald H. Wilson
AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK

The value of Fourier transform infrared spectroscopy in the mid-infrared region for the quantitative analysis of protein - starch mixtures is examined. Attenuated total reflectance, diffuse reflectance and photoacoustic detection are compared. It is concluded that photoacoustic detection is the best method of analysis for protein but that only attenuated total reflectance is of use in wet systems. Diffuse reflectance gave poorly resolved spectra, which severely limited its usefulness.

Keywords: *Infrared spectroscopy; photoacoustic; attenuated total reflectance; diffuse reflectance; detection*

Fourier transform infrared spectroscopy is now a widely used technique in analytical and research laboratories. However, the use of mid-infrared spectra for quantitative analysis has been severely limited. This is in contrast to near-infrared reflectance (NIR), which has been widely exploited in quality control applications for obtaining quantitative information. As the price of Fourier transform instruments continues to drop, however, quality control applications become a more attractive option. The mid-infrared region has much to offer the analyst compared with NIR. Specific bands may be assigned to specific chemical entities and statistical correlation methods are not always necessary, although they are not excluded and may be required in very complicated mixtures.

Several methods of sample presentation are now available that require little or no prior preparation. In this paper, three of the most used methods [attenuated total reflectance (ATR), diffuse reflectance infrared Fourier transform (DRIFT) and photoacoustic detection (PAS)] are compared. The test sample chosen was a mixture of protein and starch, which typifies much of the material currently analysed in the food industry by NIR. The results obtained indicate that, given a careful choice of experimental conditions, useful quantitative data are available on a routine basis.

Experimental

Mixtures of protein and starch were prepared using either a wheat gluten from an industrial supplier or casein (Sigma) with potato starch (BDH Chemicals). The mixtures were made by adding the appropriate mass of protein to starch and mixing well. In most experiments gluten was used, and the term protein will apply to that material unless otherwise designated. The efficiency of mixing and sampling was demonstrated by the reproducibility of replicate samples (three of each) of protein - starch mixtures containing 17.79 and 28.90% of protein. The mean PAS intensities and standard deviations were 13.74 ± 0.037 and 17.87 ± 0.039, respectively. In order to test the potential for quantitative measurements in the presence of water, 1 g of water per gram of dry solid gluten - starch was added and, after thorough stirring, the wet mixtures were allowed to stand overnight before use. The consistency of the wet systems varied from a moist powder at high starch concentration to an elastic mass at high gluten content.

All infrared spectra were obtained on a Digilab FTS60 FTIR spectrometer equipped with a TGS detector and operating at 8 cm^{-1} resolution. The mirror velocity was 0.32 cm s^{-1}, 256 interferograms were co-added before Fourier transformation and triangular apodisation was employed. Acquisition of spectra followed the conventional pattern. A single-beam background was collected with each sampling attachment either empty or with the appropriate reference material. A single-beam spectrum of the sample was then obtained and ratioed to the background before conversion to the relevant quantitative units. ATR spectra are presented in conventional absorbance units. DRIFT spectra are presented in Kubelka-Munk (KM) units.[1,2] PAS spectra are presented in terms of the normalised PAS signal intensity.

DRIFT spectra were obtained with a Spectra-Tech COLLECTOR attachment using a 14 mm diameter sample cup. No attempt was made to block the specular component of reflected light, as a trial carried out using the specular blocker showed little effect other than a considerable reduction in the over-all signal. This observation has been reported by other workers.[3] For the dry mixtures, ground KBr was used as a reference material. However, the wet systems generally had flat, shiny surfaces producing a high degree of specular reflection. In ideal circumstances a reference and a sample with similar surface reflectivity are used in order that a true diffuse reflectance spectrum can be measured. The only reference available with a similar degree of surface reflectance to the wet doughs was an alignment mirror. Unless this was used as the reference, serious spectral distortions resulted. However, even in this instance there was severe distortion in the region 1800–1500 cm^{-1} caused by water vapour.

ATR spectra were obtained with a Spectra-Tech continuously variable angle ATR attachment with a KRS5 crystal (45°, 50 × 20 × 3 mm parallelogram). The best spectra were obtained with the incident angle set at 45°. Powders were spread as evenly as possible over one face of the crystal and on the mounting plate on the opposite side of the crystal. Thus, when the attachment was assembled both sides of the crystal were covered. As much care as possible was taken to ensure both even coverage and even application of pressure. Wet samples were treated in a similar fashion.

PAS measurements were obtained with a Digilab PAS cell fitted with a KBr window (CaF_2 for wet systems) and a 3 mm deep sample cup. All spectra were ratioed to finely powdered carbon black.

Scanning electron micrographs of pure casein and gluten powders were obtained on a Philips SEM 501 instrument.

Results and Discussion

Electron microscopy of gluten - starch mixtures showed the starch to have a bimodal particle size distribution. However, the starch particles were still considerably smaller than the protein particles. There was little indication of significant gluten - starch interaction, except for a few small starch particles lodged in large pores in the gluten. These represented a very small proportion of the total starch content.

Typical spectra obtained using the three techniques are shown in Fig. 1. All spectra, with the exceptions noted below,

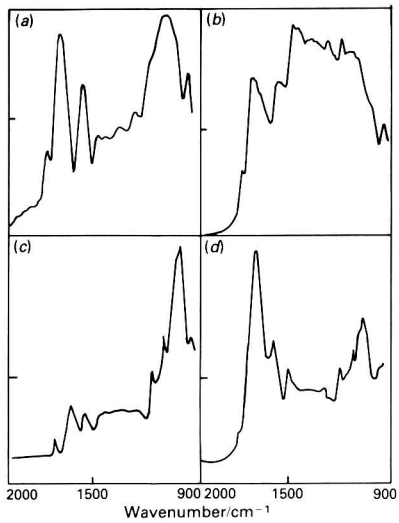


Fig. 1. Representative spectra produced using (*a*) PAS (vertical scale = 8 PAS units per division); (*b*) DRIFTS (vertical scale = 3.2 KM units per division); and (*c*) ATR (vertical scale = 0.1 absorbance units per division) of a dry mixture (50% protein). Also shown (*d*) is the ATR spectrum of the same mixture after the addition of 1 g of water per gram of dry material (vertical scale = 0.75 absorbance unit per division)

show well separated signals from the protein at 1650 cm^{-1} (amide I)[1] and 1540 cm^{-1} (amide II)[4] and from the starch in the region 1180–800 cm^{-1} (C–O and C–C stretching).[5]

It is interesting to compare the general features of these spectra. The ATR spectrum is that most closely resembling a conventional transmission spectrum. The bands in the region 2000–800 cm^{-1} are very well resolved and show a high degree of structure. The PAS spectrum is simlar to the ATR in appearance although the bands are, on the whole, slightly broader and the intensities of the low- and high-frequency peaks are reversed. The broadening is to be expected because of the functional relationship between the PAS intensity and the amount of light absorbed. Both techniques produce spectra with very good signal to noise ratios over the entire region, although there is definite curvature of the base line at lower frequencies. The DRIFT spectrum shows very poor resolution. Most notable is the appearance of a broad, relatively featureless area in the region 1400–900 cm^{-1} with the most intense peak about at 1500 cm^{-1}. However, recent results[3] have suggested that in DRIFT the geometry of the sampling optics is very important in eliminating surface reflection effects and that the optical geometry for DRIFT must be optimised for this purpose. This may not apply with some commercial accessories. Dilution in KBr also reduces[3] specular effects. It has been suggested[3] that the presence of specular effects, caused by either optical geometry or dilution differences, is largely responsible for the variation in the spectral appearance of samples run using different DRIFT attachments and differences between DRIFT spectra and spectra obtained with other techniques. Hence the results reported here may be improved upon by using a different diffuse reflectance cell. In our spectra the protein region from 1700 to 1500 cm^{-1} is also poorly resolved, the amide II band is reduced to a shoulder and the amide I band is broadened. The band at 1750 cm^{-1}, due to lipid, is also reduced to a shoulder.

A typical ATR spectrum of the wetted material is shown in Fig. 1. The presence of a water band at 1650 cm^{-1} obscures the amide I band, but the amide II and starch bands are relatively unaffected. Neither DRIFT nor PAS yielded useful spectra from the wet materials. The DRIFT spectra had severely sloping base lines and strong absorptions due to water vapour in the region 1600–1500 cm^{-1} that obscured the amide I and II bands. A similar problem with wet materials arose with photoacoustic spectra. That these effects were due to water vapour rather than surface liquid water was demonstrated by the sharpness of the spectral lines. In PAS the effect of water vapour was exacerbated by the strong photoacoustic response of vapours. An additional complication with PAS is that the heating effect of the incident radiation led to the generation of extra water vapour within the confined volume of the closed cell.

Ideally, for quantitative analysis, the amount of absorbing material is measured by determination of the area under the absorption peak. However, where there are no line-shape changes or shifts of the peak maxima, simple intensity measurements are sufficient. Whichever type of measurement is selected, it is necessary to choose a correct and consistent base line from which to make measurements. In some spectra this was not straightforward, as the peaks were on sloping or offset base lines. Three different approaches to this problem were tried. The method of final choice was that which provided a calibration graph with the minimum of scatter. These methods were as follows: (A) a value for the base line was estimated from the flat region around 2000 cm^{-1}, and this value was subtracted from subsequent peak-height measurements; (B) an over-all base line as in method A was used and the areas of defined bands above the base line were determined; and (C) a separate base line was defined for each band and a straight line was drawn between defining points on either side of the band and the area measured.

When these techniques were applied to both DRIFT and ATR the over-all measured intensities of the bands were found to vary in an inconsistent manner. This was ascribed to irreproducibility in sample loading. The problems of sample loading in DRIFT have been reported by others[3] and ATR suffers from variations in both crystal coverage and applied pressure. Therefore, in order to overcome these problems an internal ratio method was applied.

For ATR on dry materials, all three quantitative methods were applied and the best calibration graph was obtained using method C. The calibration graph of the ratio (R) of the area (amide I + II) to the area (1180–870 cm^{-1}) *versus* protein to starch ratio is shown in Fig. 2(*a*). Usable spectra from wetted systems were obtained only with ATR. However, a useful calibration graph could not be constructed. The problem was that the amide I band was swamped by the large water peak and in some instances the amide II was superimposed on a severely sloping base line and was considerably offset. Method A was the only method usable for an attempt at quantitative analysis and the plot of the ratio (R) of the absorbance of the amide II to the starch band at 1018 cm^{-1} is shown in Fig. 2(*a*). Clearly, this does not constitute a useable calibration graph.

Distortion of the carbohydrate region in DRIFT meant that the amide II band was effectively lost whereas the amide I band was superimposed on a strongly sloping base line. Further, the large number of overlapping peaks in the starch region coupled with a strongly offset base line made the choice of a base line for method C very difficult. The large number of peaks and the dissimilarity to the ATR or PAS spectra made the choice of band or bands for area measurements problematic. A prominent band was seen, however, in some DRIFT spectra at 1166 cm^{-1}. This peak varied in intensity with starch concentration. Despite the fact that the origin of this peak is unknown, measurements were made at that frequency. Method A was used for quantitative analysis and a calibration graph [Fig. 2(*a*)] of the ratio (R) of the amide I intensity to that at 1166 cm^{-1} was plotted against protein to starch ratio. Dilution in KBr did have an effect on the appearance of the DRIFT spectrum. The starch region showed some improvement in resolution and there was a trend towards the type of spectrum produced by PAS or ATR. However, the improve-

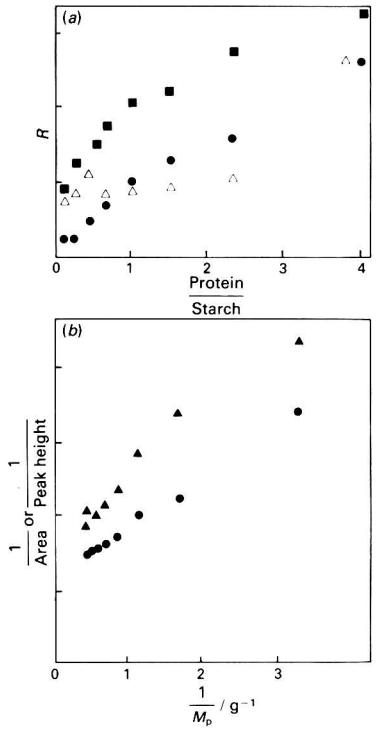


Fig. 2. (*a*) Graphs of R (as defined in text) *versus* protein to starch ratio (*m/m*) for dry powders by DRIFT (■, 0.5 unit per division) and ATR (●, 0.125 unit per division). Also shown is the same plot for the wetted system by ATR (△, 1 unit per division). (*b*) Calibration graphs for PAS presented as the reciprocal protein band area (amide I + II) (▲, 0.65 unit per division) or the reciprocal protein band (amide I) peak height (●, 0.04 unit per division) *versus* the reciprocal of the mass of protein in the mixture, $1/M_p$ (g^{-1})

ment in starch band resolution was not sufficiently marked to allow full quantitative measurement. Dilution only marginally reduced the component at 1500 cm^{-1}. It may be that further dilution with KBr would result in further spectral improvement, but this would have led to very low signals and hence long acquisition times or the need for more sensitive detectors. It was concluded, therefore, that further dilution was not a practical option.

The problems of irreproducible sample loading did not arise with PAS and consequently internal ratioing was not required. PAS has been overlooked as a quantitative method until fairly recently.

A useful and fairly general approximation[6,7] to the equation of Poulet *et al.*[8] is

$$H = \frac{A' \times 2^{0.5} \times \mu\beta}{\mu\beta + 2}$$

where H is the normalised signal intensity resulting from absorption of incident light, A' is a parameter relating the energy absorbed to the final signal intensity[6] in the sample and reference, μ is the thermal diffusion length[7] and β is defined by

$$\beta = \frac{2.303\varepsilon M_p \rho}{M_r M_T}$$

where M_r is the relative molecular mass of the absorbing species of molar absorptivity ε, ρ is the density of the mixture and M_p is the mass of the absorbing species in the mixture of total mass M_T. Assuming that there is no significant variation

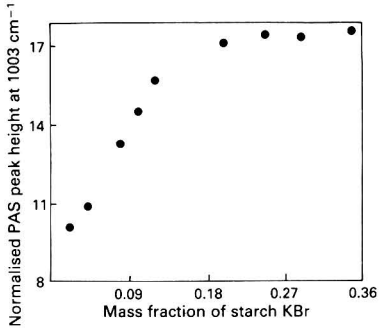


Fig. 3. Variation of peak height at 1003 cm^{-1} starch band with mass fraction of starch in KBr using PAS

in the parameter M_T/ρ, then a plot of $1/H$ *vs.* $1/M_p$ should be linear.[6]

Strictly these equations are valid only for homogeneous materials. However, previous work[6] has shown that they do apply fairly well to powdered systems.

All three methods of calculating intensity were tried on the gluten - starch mixtures. Methods A and B produced linear plots as expected when the reciprocal of the amide I peak height or area of amide (I + II) was plotted against the reciprocal of the mass of gluten in the sample [Fig. 2(*b*)]. Method A produced a plot of slope 0.033, intercept 0.005 and correlation coefficient 0.998. Method B produced a plot of slope 0.054, intercept 0.042 and correlation coefficient 0.995. However, with the starch bands both methods gave intensities independent of concentration and method C showed an over-all decrease in the starch band intensity with increasing starch concentration. The problem was exacerbated by difficulty in obtaining a consistent base line in this region, the shape of which changed with starch concentration. The behaviour of the starch bands in the PAS spectra is due to photoacoustic saturation effects. When the term $\mu\beta$ is large the signal intensity becomes independent of chromophore concentration.[8] In Fig. 3, the effects of dilution of starch with KBr is shown; above 15% by mass of starch there is no variation in band intensity. The general form of the curve is that predicted by Poulet *et al.*[8]

An additional problem in the starch region of the spectrum may be due to phasing effects. Poulet *et al.*[8] suggest that the relative phase of the photoacoustic signal is dependent on the term $\mu\beta$. Phase correction on most Fourier transform instruments is concerned with symmetrisation of the interferogram and is only strictly appropriate for transmission spectra. These phase effects may account for the reversal of the intensity of the protein and starch bands in the PAS spectrum. Further complications in spectral intensities can also arise because the interferometer causes a variation in modulation frequency with wavelength. Hence the thermal escape depth, μ, varies across the spectrum.

A further series of experiments was carried using dry mixtures of starch with casein instead of gluten. Electron microscopy showed that the casein particles were larger than the gluten particles and resistant to grinding. ATR of casein - starch mixtures was not very successful because the protein bands were much weaker that those produced by gluten. This is presumably due to the larger size of the casein particles, which provide a poorer optical contact with the crystal. On the other hand, the PAS spectra of casein - starch mixtures were of very good quality, comparable to those from gluten - starch, showing that PAS is less sensitive to sample morphology.

The three techniques used in this study are those which have become more widely used since the advent of Fourier transform instruments. They are generally considered to be

methods involving a minimum of sample preparation. There are now very few samples that cannot be analysed by at least one of these methods. The difficulty lies in the identification of the best one for a particular problem. Recently, DRIFT has been the first choice for many problems and PAS has been considered only as a last resort. The results presented here, however, suggest that PAS offers a very real alternative to DRIFT as a method of first choice.

Recent work[6,7] has led to a better understanding of the problems associated with quantitative PAS and the potential of the technique is clearly reflected in the calibration graphs that it produced. For many samples, PAS is the only true "no-preparation" method, although it is certainly much better suited to powdered samples. Moreover, it is apparently unaffected by the reflectance phenomena and less sensitive to sample morphology. Both PAS and ATR produce spectra that are useful for spectral interpretation and qualitative analysis. The spectra produced are sufficiently similar to transmission spectra for comparisons to be made with reference spectra and real spectroscopic information can be extracted. This is not always so with DRIFT.

The problem of optical saturation that may be experienced with some absorbers can be overcome by dilution in KBr, although this does require the user to spend more effort in sample preparation. PAS is not alone in this respect. The DRIFT spectra show that in order even to produce a spectrum that approaches the quality of ATR and PAS, dilution in KBr is a necessity. Another problem with DRIFT is the spectral distortion partly due to specular reflectance. The bands in DRIFT spectra are considerably broader than in ATR or PAS, which reduces the spectral discrimination to the extent that the amide II band becomes indistinguishable. DRIFT, like PAS, is not very useful for very wet systems because of the effects of water vapour (although some useful PAS spectra of moist systems such as bread have been obtained by us). With the optical geometry of the DRIFT attachment used in this work, some burning of the surface of powders at the focal point was observed. Another problem was that the DRIFT cell could not be reproducibly loaded so that, unlike the PAS cell, internal ratioing was needed. Irreproducibility in sample loading was also found with ATR, as both crystal coverage and applied pressure are variables that are poorly controlled. ATR was also very sensitive to the nature of the sample. The relatively larger, harder particles of casein provided spectra with weaker protein bands than those from gluten. Materials with large, hard particles may also cause problems of crystal damage. The technique seems best with a material consisting of an even distribution of small particles that give good optical contact. Although ATR was unable to generate a usable calibration graph owing to the presence of a water band at the amide I frequency, it may be possible to improve the calibration by using the technique of absorbance subtraction. If the water band could be subtracted from the spectrum then the distorting effect on the amide II could be eliminated and even the amide I may prove accessible.

Conclusions

Overall, the ATR method proved to have the widest application. This, combined with its relatively low cost, makes it a very valuable quantitative analytical technique. For dry powdered samples or samples with a low vapour pressure of water, despite its relatively higher cost at the moment, PAS should be the technique of first choice. PAS is free from any of the distorting phenomena seen with DRIFT and for most materials is a technique requiring a minimum of preparation. The equipment has developed to the stage where long acquisition times are no longer required; the spectra shown here each took about 10 min to obtain.

The mid-infrared region shows great promise for analytical work in the future. There has recently been an upsurge in near-infrared methods based on reflectance techniques that are of sufficiently low cost that they are becoming widely used in quality control environments. These techniques are hampered by the need for a high degree of statistical manipulation as the bands in this region are broad and overlap strongly. In contrast, the mid-infrared bands are intrinsically narrower and better resolved. There is, therefore, no need for statistical manipulation of data.

The main factor limiting the more widespread use of the method is the relatively high cost of FTIR equipment. Fortunately, the cost is now beginning to be reduced and equipment is commercially available that matches the cost of other conventional quality control techniques. If this downward trend continues, the mid-infrared region should become more widely used.

The authors thank Dr. Mary Parker and Mr. Roger Turner for the electron microscopy. A. M. S. thanks the British Council for a TCTD award.

References

1. Kubelka, P., and Munk, F., *Z. Tech. Phys.*, 1931, **12**, 593.
2. Kubelka, P., *J. Opt. Soc. Am.*, 1948, **38**, 448.
3. Brimmer, P. J., Griffiths, P. R., and Harrick, N. J., *Appl. Spectrosc.*, 1986, **40**, 258.
4. D'Esposito, L., and Koenig, J. L., *in* Ferraro, J. R., and Basile, L. J., *Editors*, "Fourier Transform Infrared Spectroscopy," Volume 1, Academic Press, New York, 1978, Chapter 2, p. 88.
5. Tipson, R. S., and Parker, F. S., *in* Pigman, W., and Horton, D., *Editors*, "The Carbohydrates," Second Edition, Volume 1B, Academic Press, New York, 1980, Chapter 27, p. 1398.
6. Belton, P. S., and Tanner, S. F., *Analyst*, 1983, **108**, 591.
7. Belton, P. S., *in* Chan, H. W-S., *Editor*, "Biophysical Methods in Food Research," Blackwell, Oxford, 1984, p. 123.
8. Poulet, P., Chambron, J., and Unterreiner, R., *J. Appl. Phys.*, 1976, **105**, 1076.

Paper A7/18
Received January 22nd, 1987
Accepted March 16th, 1987

Spectrophotometric and Analogue Derivative Spectrophotometric Determination of Trace Amounts of Iron Using Sulphonated 5-(3,4-Dihydroxyphenyl)-10,15,20-triphenylporphine

Hajime Ishii and Katsunori Kohata
Chemical Research Institute of Non-aqueous Solutions, Tohoku University, Katahira, Sendai, 980, Japan

Sulphonated 5-(3,4-dihydroxyphenyl)-10,15,20-triphenylporphine (SDHP), a water-soluble porphyrin with the ability to complex with metal ions not only inside but also outside the porphine ring, has been synthesised. The analytical applications of the porphyrin, its chromogenic properties and reactivity with metal ions have been investigated. It was found that SDHP forms complexes with metal ions classified as so-called hard acids, such as aluminium(III), iron(III) and tungsten(VI), and also with those classified as soft or border-line acids, such as cadmium(II), copper(II) and lead(II), all these complexation reactions proceeding rapidly at room temperature. As an example, the iron(III) - SDHP system has been studied in detail spectrophotometrically. SDHP reacts with iron(III) to form a 1 : 1 (metal : ligand) complex with a Soret band at 430 nm at pH 4 and a 1 : 2 complex with a Soret band at 415 nm at pH 7. Spectrophotometric and analogue derivative spectrophotometric methods are proposed for the determination of iron at ng ml^{-1} levels utilising these complexations. The apparent molar absorptivities were 1.09×10^5 and 5.37×10^5 l mol^{-1} cm^{-1} for the methods utilising the 1 : 1 and 1 : 2 complex formations, respectively.

Keywords: *Iron determination; sulphonated 5-(3,4-dihydroxyphenyl)-10,15,20-triphenylporphine; spectrophotometry; analogue derivative spectrophotometry*

Water-soluble *meso*-substituted porphyrins such as 5,10,15,20-tetrakis(4-carboxyphenyl)porphine,[1,2] 5,10,15,20-tetrakis(4-sulphophenyl)porphine [T(4-SP)P],[2,3] 5,10,15,20-tetrakis(1-methylpyridinium-3-yl)porphine[4-6] and 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphine[5,7,8] are very useful as highly sensitive colour reagents for metal ions because they possess Soret bands with extremely large molar absorptivities (1×10^5–6×10^5 l mol^{-1} cm^{-1}). However, in general, the complexation reaction of the porphyrins with metal ions in aqueous media is very slow at room temperature. Hence several attempts have been made to accelerate it, including heating,[1-3] the addition of an auxiliary complexing agent such as pyridine or imidazole[4] or a reducing agent such as hydroxylamine or ascorbic acid[5] and the utilisation of the metal-substitution reaction of the cadmium, lead or mercury(II) - porphyrin complex.[6] These attempts were fairly effective for the acceleration of the complexation reaction of the porphyrin with metal ions such as cobalt(II), copper(II), manganese(II), palladium(II) and zinc(II), but were less effective or ineffective for that with many other metal ions. Hence we began to synthesise a series of water-soluble porphyrins with functional groups outside the porphine ring in order to improve the reactivity of the porphyrin and extend its analytical use. Sulphonated 5-(3,4-dihydroxyphenyl)-10,15,20-triphenylporphine (SDHP or H_8L) was the first of these porphyrins to be synthesised.

In this paper an outline of the synthesis of SDHP, its chromogenic properties and its application to the spectrophotometric determination of trace amounts of iron are described.

Experimental

Reagents

All reagents used were of analytical-reagent grade unless stated otherwise. All solutions were prepared with distilled, de-ionised water.

SDHP solution. A 3×10^5 M aqueous solution was prepared using SDHP synthesised as described later. Its concentration was determined by photometric titration with a standard copper(II) solution.

Standard iron(III) and copper(II) solutions. The solutions (1×10^{-2} M) were prepared from ammonium iron(III) sulphate and copper(II) sulphate pentahydrate, respectively. Working solutions were prepared by appropriate dilution.

Apparatus

A Hitachi 139 spectrophotometer and a Hitachi 556 dual-wavelength spectrophotometer were used for absorbance measurements and measurement of the absorption spectrum, respectively, the latter being used as an ordinary double-beam spectrophotometer throughout the measurements. In order to obtain the derivative spectrum, a modified Hitachi 200-0576 derivative unit composed of two analogue differentiation circuits was connected between the latter spectrophotometer's output and a Hitachi 057 X - Y recorder input. The details of this apparatus and the principle and characteristics of the analogue derivative spectrophotometry have been described previously.[6,9]

Proton magnetic resonance spectra were recorded on a JEOL JNM-PS-100 NMR spectrometer and a Bruker CXP-300 pulse Fourier transform NMR spectrometer. The chemical shifts are given in δ values (p.p.m.) from tetramethylsilane (TMS) in $CDCl_3$ and 5-(trimethylsilyl)propionic acid sodium salt (TSP) in D_2O. Infrared spectra were obtained on a JASCO A-3 IR spectrometer. Mass spectra were obtained on a JEOL DX-300 instrument using the fast atom bombardment (FAB) method.

Thin-layer chromatography (TLC) was carried out on 0.2-mm E. Merck 60F-254 pre-coated silica gel plates. For column chromatography, E. Merck silica gel 60 (70–230 mesh) was used.

Recommended Procedures for the Determination of Iron

Ordinary spectrophotometry in acidic media (Procedure A)

To an aliquot containing up to 4 μg of iron(III) in a 25-ml calibrated flask, add 4 ml of 3×10^{-5} M SDHP solution and 2 ml of 1 M acetate buffer (pH 4.1) and dilute to the mark with water. Measure the absorbance of the resultant solution at 442 nm against a reagent blank prepared under the same conditions using 1-cm cells.

Ordinary spectrophotometry in neutral media (Procedure B)

To an aliquot containing up to 1.3 μg of iron(III) in a 25-ml calibrated flask, add 3 ml of 3×10^{-5} M SDHP solution and 2 ml of 0.2 M imidazole buffer (pH 7.2) and dilute to the mark with water. Measure the absorbance at 415 nm in the same manner as Procedure A.

Second-derivative spectrophotometry

When the iron content of the coloured solution prepared by Procedure A is too low to give a measurable absorbance, record the second-derivative spectrum from 500 to 360 nm against a reagent blank using a combination of both first- and second-order differentiation circuits (No. 6 of reference 9) and a scan speed of 150 nm min^{-1} and measure the second-derivative value (the vertical distance from a peak to a trough or that from the base line to a peak).

Procedure for the Synthesis of SDHP

SDHP was obtained in two steps, the synthesis of 5-(3,4-dihydroxyphenyl)-10,15,20-triphenylporphine (DHP) and its sulphonation.

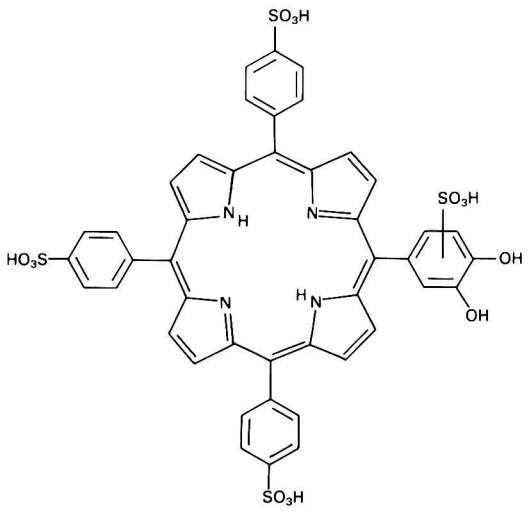
Synthesis of DHP

Benzaldehyde (10.6 g, 0.1 mol) and 3,4-dihydroxybenzaldehyde (6.9 g, 0.05 mol) were dissolved in hot (130 °C) propionic acid (490 ml). The mixture was heated to reflux and a solution of pyrrole (10.1 g, 0.15 mol) in propionic acid (10 ml) was added as rapidly as possible, caution being taken to prevent excessive heating as the reaction is exothermic. The resulting black solution was refluxed for 1 h and then cooled to room temperature. The mixture was allowed to stand in a refrigerator for several days and then filtered with suction to give a black - purple tarry matter. This is a mixture of 5,10,15,20-tetraphenylporphine (TPP) and its mono-, di-, tri- and tetra-substituted derivatives, which have one to four 3,4-dihydroxyphenyl groups in their 5-, 10-, 15- and/or 20-positions [R_F values in TLC: TPP, 1.0; mono-, 0.29 in benzene - diethyl ether (9 + 1); di-, 0.58; tri-, 0.44; tetra-substituted derivative, 0.10 in chloroform - ethyl acetate (1 + 1)]. This mixture was dissolved in a minimum amount of methanol - chloroform, loaded on to a silica gel column (40 × 5 cm i.d. in cyclohexane) and then eluted with benzene. The first band contained TPP, which was confirmed by the following characteristic properties: $\lambda_{max.}$ ($CHCl_3$) 647, 591, 550, 514, 420 nm; $\nu_{max.}$ (KBr) 1590, 1470, 1440, 1350, 1060, 960, 760, 690 cm^{-1}. These are identical with those of TPP independently prepared. After the eluate had become very light red in colour, the column was eluted with benzene - diethyl ether (9 + 1). The conclusion that the second band consisted of the target porphyrin, DHP, was based on the following visible, IR, NMR and mass spectra: $\lambda_{max.}$ ($CHCl_3$) 652, 595, 552, 516, 422 nm; $\nu_{max.}$ (KBr) 3300–3500(m), 2900(w), 1590(s), 1500(m), 1460(s), 1430(s), 1340(s), 1260(s), 1240(s), 1180(s), 1150(sh), 1100(m), 960(s), 930(m), 790(s), 720(s), 690(s) cm^{-1}; ^1H NMR ($CDCl_3$) δ (p.p.m.) 7.6–7.9 (m, 10H, phenyl), 8.0–8.4 (m, 8H, phenyl), 8.8 (s, 8H, pyrrole); FAB - MS, *m/z* (relative intensity) 648 (19, $M^+ + 1$), 647 (47, M^+), 646 (100, $M^+ - 1$). Fractions showing these characteristic properties were collected, evaporated and dried *in vacuo* to yield purple crystals (610 mg, yield 3.8%).

Sulphonation

DHP (0.3 g, 0.46 mmol) and concentrated sulphuric acid (25 ml) were thoroughly mixed in an agate mortar with a pestle. The paste was transfered into a beaker and heated on a steam-bath for 5 h, then cooled in an ice-bath. About 50 ml of iced water was added slowly to precipitate green solids, which were filtered with suction and washed well with acetone. This product was subjected to column chromatography on silica gel. Elution with propan-2-ol - ethyl acetate - water (2 + 3 + 2, *V/V*) gave only one band, fractions of which were collected, evaporated and dried *in vacuo* to yield purple crystals (120 mg, yield 28.2%) with the following properties: R_F 0.24 [propan-2-ol - ethyl acetate - water (2 + 3 + 2, *V/V*)]; $\nu_{max.}$ (KBr) 3200–3500(s), 1620(s), 1590(s), 1550(s), 1450(s), 1430(sh), 1400(sh), 1340(w), 1030–1200(s), 1030(s), 1000(s), 970(m), 790(s), 730(s), 620(s) cm^{-1}; ^1H NMR (D_2O) δ (p.p.m.) 8.1–8.2 (m, 1H), 8.3–8.5 (m, 7H), 8.6–8.9 (m, 10H), 8.9–9.1 (s, 4H). Neither elemental analysis nor the mass spectrum of the product obtained here gave reliable results because the product has a high molecular mass and contains sulphonic acid groups. Hence the positive identification of this product as the desired porphyrin, SDHP, was carried out based on the following TLC tests. Three spots were detected by a TLC test, with propan-2-ol - ethyl acetate - water (2 + 3 + 2, *V/V*) as a developing solvent, on a reaction mixture sulphonated for 2 h and their R_F values almost agreed with those of the di-, tri- and tetra-sulphonated derivatives of TPP (0.65, 0.48 and 0.24, respectively) obtained under similar sulphonation conditions. As the incorporation of a small diphenolic moiety is considered to have almost no influence on the R_F value in a high molecular mass compound such as SDHP (molecular mass 966.99), the three spots detected are considered to correspond to di-, tri- and tetra-substituted derivatives with 2–4 sulphonic acid groups in DHP. In contrast, the spots which corresponded to di- and tri-substituted derivatives completely disappeared and only one spot, which corresponds to the tetra substituted derivative, was present in a TLC test on the product obtained when sulphonated for 5 h.

On the basis of these results, the product obtained by the above procedure is presumed to be SDHP with four sulphonic acid groups. Three of the sulphonic acid groups were concluded to be at the 4-position of each phenyl group based on the analogy of T(4-SP)P,[3] but the binding position in the 3,4-dihydroxyphenyl group could not be deduced. The probable structure of the synthesised SDHP is illustrated below.



Results and Discussion

Properties of SDHP

SDHP is soluble in water and stable in acidic solution, but is gradually oxidised in alkaline solution.

SDHP behaves as a deca-basic acid, although the number of dissociable hydrogens in the molecule is eight. Its proton

dissociation equilibria may be expressed as follows:

$$H_iL^{(8-i)-} \overset{k_i}{\rightleftharpoons} H_{i-1}L^{(9-i)-} + H^+ \quad (i = 1, 2 \cdots, 10) \quad (1)$$

where k_i is the dissociation constant. k_1 and k_2 correspond to the dissociation of two pyrrole hydrogens, k_3 and k_4 the dissociation of two hydroxyl groups, k_5 and k_6 the protonation of two aza nitrogens and k_7, k_8, k_9 and k_{10} the dissociation of four sulpho groups. H_4L^{4-}, H_5L^{3-} and H_6L^{2-} correspond to the so-called free base, mono- and diprotonated forms, respectively. It was attempted to determine these proton dissociation constants spectrophotometrically. However, the only results obtained were that values of pk_5 and pk_6 are very close together (around 5.2) and that pk_4 is 8.52 at 20 °C, at an ionic strength of 0.1 (KNO_3). Their values could not be accurately determined because no clear spectral change corresponding to each individual proton dissociation was observed (*i.e.*, the spectral change was as if one-step dissociation occurred). The values of k_1, k_2 and k_3 were not obtained because SDHP was not stable enough in alkaline solution to permit their exact determination.

Fig. 1 shows the absorption spectra of SDHP at various pH values. Spectra A and B correspond to H_6L^{2-} and H_4L^{4-}, and the Soret bands, which are useful for the determination of trace amounts of metals, are at 438 and 415 nm, respectively. At pH values greater than 10 the Soret band undergoes a slight blue shift.

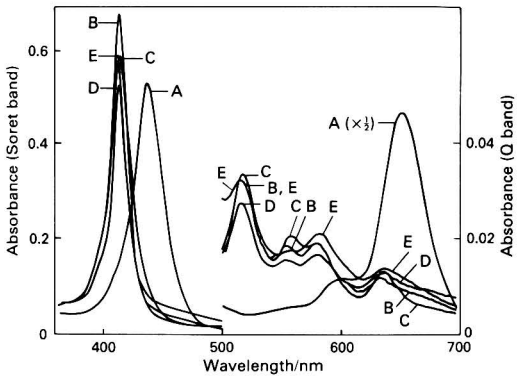


Fig. 1. Absorption spectra of SDHP at various pHs. SDHP, 1.9×10^{-6} M; reference, water. pH: A, 1.3–3.5; B, 6.9–7.6; C, 9.1; D, 11.9–12.1; and E, 13.0–13.5

Reactivity of SDHP with Metal Ions

The reaction of SDHP with metal ions was analogous to that of Tiron (1,2-dihydroxybenzene-3,5-disulphonic acid), which has the same functional groups as SDHP. It formed complexes not only with cadmium(II), cobalt(II), copper(II), lead(II), manganese(II), mercury(II) and zinc(II) but also, as expected, with gallium(III), molybdenum(VI) and tungsten(VI) around pH 4, iron(III), vanadium(IV) and vanadium(V) above pH 4, aluminium(III) and titanium(IV) in the pH range 6–7 and rare earth ions in the pH range 6.5–8, at room temperature. As the complexes with the first seven of these ions gave absorption spectra similar to those of their complexes with porphyrins having no complexing groups outside the porphine ring, *e.g.*, T(4-SP)P, these complexations can be assumed to occur inside the porphine ring, *i.e.*, the metal ion seems to coordinate to four (or two) nitrogens as usual. The absorption spectra of some of these complexes are shown in Fig. 2. On the other hand, the complexations of the other eight ions and the rare earth ions seem to occur outside the porphine ring, *i.e.*, the metal ion seems to coordinate to two oxygens, because (a) these ions, which either scarcely react or do not react at all at room temperature with the porphyrin with no complexing groups outside the porphine ring, rapidly formed complexes with SDHP at room temperature and (b) the spectral change of SDHP on complexation with these ions differed from that when the metal ion coordinates to four nitrogens inside the porphine ring. As an example, the spectral changes in the complexations with gallium(III) at pH 3.8 and aluminium(III) at pH 6.1 are shown in Figs. 3 and 4, respectively. In the former the Soret band shifts from 438 to 428 nm and the Q band from 653 to 680 nm on complexation. On the other hand, in the latter complex the Soret band at 415 nm does not shift and only the decrease in its absorbance is observed on complexation. As for the Q band, its shape changes from four characteristic peaks to three rounded humps with an increase in absorbance.

These two kinds of spectral changes in the complexations differ considerably from those seen when the metal ion forms

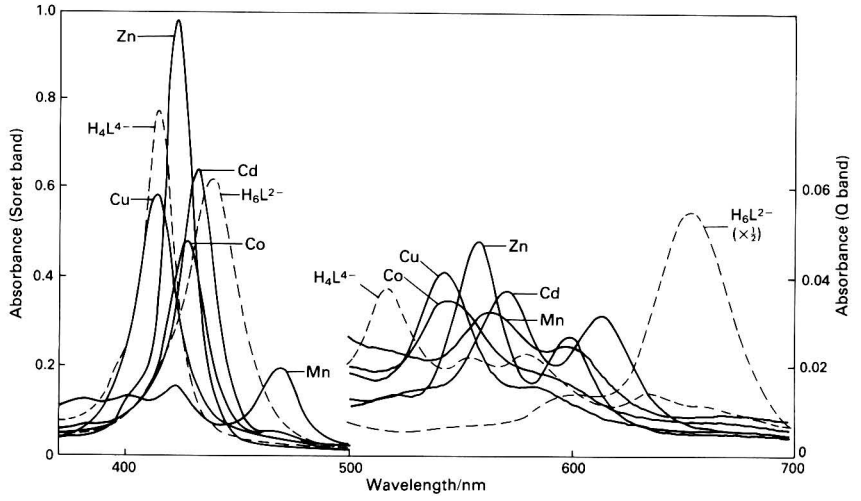


Fig. 2. Absorption spectra of SDHP and its metal complexes. SDHP, 2.2×10^{-6} M; complexes, 2.2×10^{-6} M. pH: H_6L^{2-}, 2.4; H_4L^{4-}, 6.9; complexes, 9.0. Reference, water

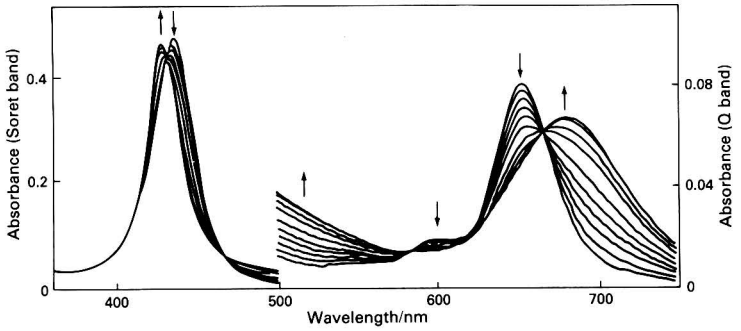


Fig. 3. Absorption spectra of Ga^{III} - SDHP system. SDHP, 1.8×10^{-6} M; pH, 3.8; reference, water. Arrows indicate spectral trends observed when changing the SDHP to Ga ratio from 1:0 to 1:6.4

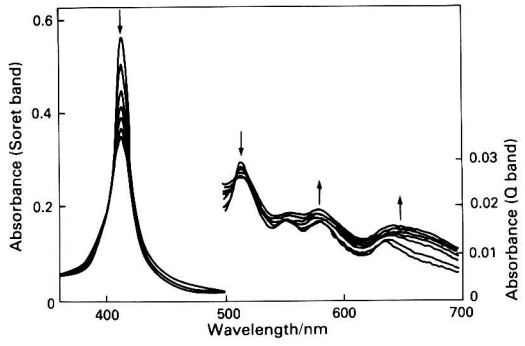


Fig. 4. Absorption spectra of Al^{III} - SDHP system. SDHP, 1.5×10^{-6} M; pH, 6.1; reference, water. Arrows indicate spectral trends observed when changing the SDHP to Al ratio from 1:0 to 1:4.7

a complex by coordinating to four nitrogens inside the porphine ring and are characteristic of the complexation in which the metal ion coordinates with functional groups outside the porphine ring (two oxygens in this instance). In addition, this spectral behaviour scarcely depended on the kind of metal ions, *i.e.*, similar spectral changes to those in Figs. 3 and 4 were also observed in the complexation with iron(III), molybdenum(VI) and tungsten(VI) at pH 4 and with titanium(IV) at pH 7, respectively.

Hence it is finally concluded that for the complexation between SDHP and metal ions, ions classified as so-called hard acids form complexes by coordinating to the two oxygens outside the porphine ring and those classified as soft or border-line acids form complexes by coordinating to four (or two) nitrogens inside the porphine ring. Thus, the reactivity of the porphyrin can be remarkably improved by synthesising SDHP, a water-soluble porphyrin which has complexing groups outside the porphine ring. This suggests that the analytical use of the porphyrin can be considerably extended.

In the subsequent work fundamental conditions for the spectrophotometric and analogue derivative spectrophotometric determination of iron at p.p.b. levels were carried out as an example of the application of the complexation reaction.

Study of the Iron(III) - SDHP System

Absorption spectra

Iron(III) reacts with SDHP to form a 1:1 (metal:ligand) complex in the pH range 3–5.5 and a 1:2 complex in the pH range 5.5–9, as mentioned later. Both complexation reactions

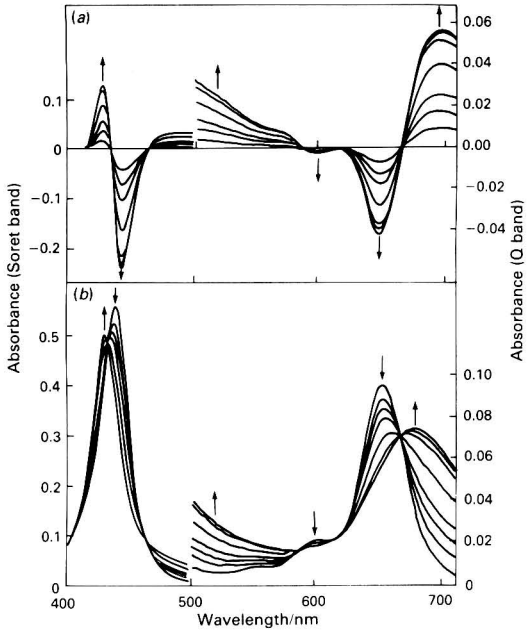


Fig. 5. Absorption spectra of Fe^{III} - SDHP system at pH 4.1. SDHP, 2.0×10^{-6} M. Reference: (*a*), reagent blank; and (*b*), water. Arrows indicate spectral trends observed when changing the SDHP to Fe ratio from 1:0 to 1:4.8

proceed rapidly at room temperature. The spectral changes in the complexations between iron(III) and SDHP at pH 4.1 and 7.2, which are shown in Figs. 5 and 6, respectively, are almost the same as those shown already in Figs. 3 and 4. At pH 4.1 the Soret band shifts from 438 to 430 nm and the Q band from 652 to 676 nm on complexation. The absorption spectra of the complex measured against a reagent blank give maxima at 426 and 694 nm and minima at 442 and 650 nm, 442 nm being the most preferable for the iron determination at this pH as it gives the highest sensitivity. At pH 7.2 the Soret band at 415 nm does not shift and only the decrease in its absorbance is observed by the complexation. The Q band changes in shape from four characteristic peaks to three rounded humps with the increase in absorbance.

Stability of the absorbance

The stability of the absorbance of the system in weakly acidic and neutral media was studied. The colour developed

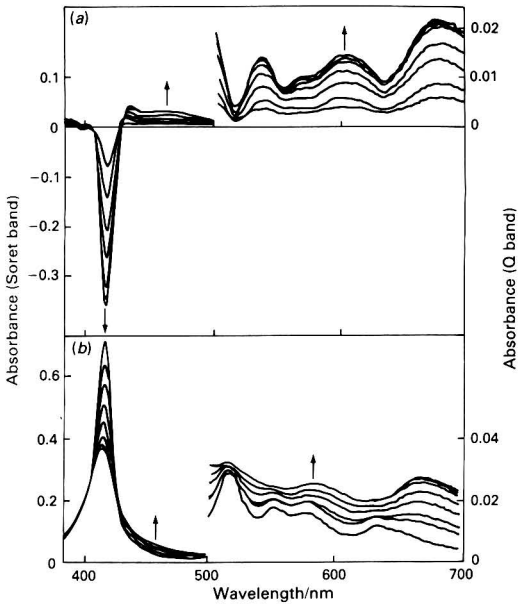


Fig. 6. Absorption spectra of Fe^{III} - SDHP system at pH 7.2. SDHP, 2.0×10^{-6} M. Reference: (*a*), reagent blank; and (*b*), water. Arrows indicate spectral trends observed when changing the SDHP to Fe ratio from 1 : 0 to 1 : 1.7

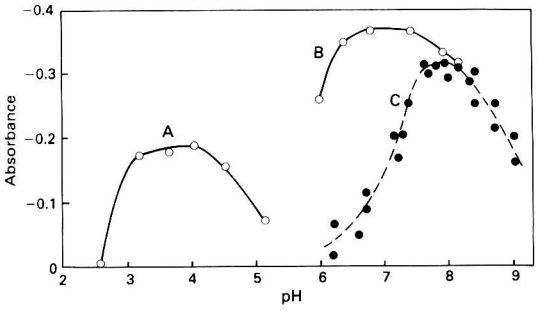


Fig. 7. Effect of pH. SDHP, 3.5×10^{-6} M. Fe: A, 95.9; and B and C, 38.4 ng ml^{-1}. Wavelength: A, 442; and B and C, 415 nm. Reference, reagent blank; B, imidazole buffer used; C, borate or phosphate buffer used

immediately at room temperature in both media. The absorbance measured against the reagent blank remained almost constant for at least 2 h, but after that time it tended to decrease gradually. The oxidation of SDHP in alkaline media could be considerably reduced by shutting out the light.

Effect of pH

Fig. 7 shows the effect of pH on the formation of the iron(III) - SDHP complex. In the pH range 2.6–5.5 a complex with a Soret band at 430 nm is formed, the absorbance of the complex solution measured against a reagent blank at 442 nm being almost independent of pH at 3.6–4.2. Above pH 5.5 another complex with the Soret band at 415 nm is formed, a constant absorbance being obtained at pH 6.4–7.5 when an imidazole - nitric acid buffer was used. However, when phosphate or borate buffer was used, the absorbance of the complex solution changed each time it was measured, probably owing to hydrolysis (see line C); the use of imidazole buffer is recommended in this pH region.

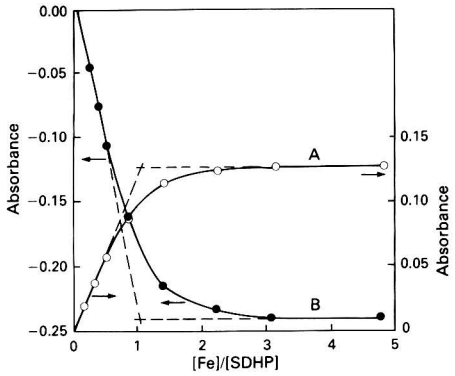


Fig. 8. Molar ratio plots. pH, 4.1; SDHP, 2.0×10^{-6} M; and reference, reagent blank. Wavelength: A, 426; and B, 442 nm

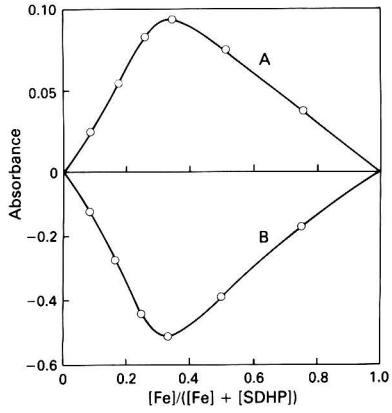


Fig. 9. Job plots. pH, 7.2; [Fe] + [SDHP] = 3.5×10^{-6} M; and reference, reagent blank. Wavelength: A, 434; and B, 415 nm

Effect of SDHP concentration

The effect of SDHP concentration was investigated at pH 4.1 and 7.2 by measuring the absorbance at 442 and 415 nm, respectively, of solutions containing a fixed amount (76.8 and 38.4 ng ml^{-1}, respectively) of iron(III) and various amounts of the reagent. The results revealed that a constant, maximum absorbance is obtained, provided that 1.5–4 and 2.9–4.5 molar excesses of SDHP are added at pH 4.1 and 7.2, respectively, and in both instances a further excess of the reagent tended gradually to decrease the absorbance.

Composition of the complex

The composition of the complex was studied at pH 4.1 and 7.2 by Job's method of continuous variations and the molar-ratio method. Both methods indicated that the ratio of metal ions to ligand molecules was 1 : 1 at pH 4.1 and 1 : 2 at pH 7.2. Some of the results obtained are shown in Figs. 8 and 9.

Calibration graph, sensitivity and precision

Linear calibration graphs through the origin were obtained using the recommended procedures. The equations of the lines obtained by a least-squares treatment were Fe (ng ml^{-1}) = $514A$ in procedure A and Fe (ng ml^{-1}) = $104A$ in Procedure B, where A is the absorbance. The optimum ranges for the determination of iron, the sensitivities for an absorbance of 0.001 and the molar absorptivities calculated from the above

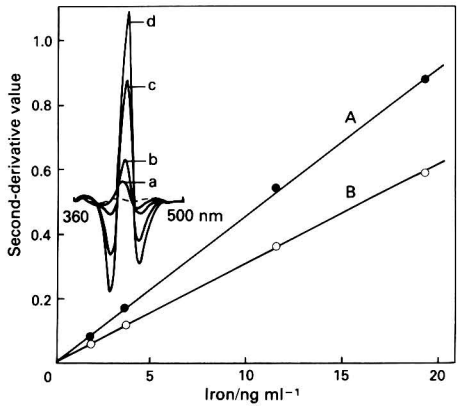


Fig. 10. Calibration graph for iron in second-derivative spectrophotometry. Fe: a, 1.9; b, 3.8; c, 11.5; d, 19.2 ng ml^{-1}. SDHP, 1.1×10^{-6} M; circuits, all No. 6 of reference 9; scan speed, 150 nm min^{-1}; slit width, 1 nm; recorder sensitivity, $\times\frac{1}{2}$; cells, 10 mm; and reference, reagent blank. A, Peak to trough values plotted; and B, base line to trough values plotted

equations were 0.2–4.0 μg, 0.514 ng cm^{-2} and 1.09×10^5 l mol^{-1} cm^{-1} in Procedure A and 0.1–1.3 μg, 0.104 ng cm^{-2} and 5.37×10^5 l mol^{-1} cm^{-1} in Procedure B, respectively.

Two series of ten standard solutions each containing 1.9 and 0.96 μg of iron were analysed by the recommended procedures. The results gave relative standard deviations of 0.9 and 1.0% in procedures A and B, respectively.

Effect of foreign ions

In order to study the effect of various ions on the determination of iron, a fixed amount of iron(III) was taken with different amounts of foreign ions and the recommended procedures were followed. Tolerances for foreign ions in procedure A (at pH 4.1) are summarised in Table 1. Fairly large amounts of cadmium(II), cobalt(II), chromium(III), lead(II), magnesium(II), manganese(II), nickel(II), thorium(IV) and zinc(II) can be tolerated, but the interference of numerous foreign ions restricts the applicability of the reagent, the most important being aluminium(III), copper(II), molybdenum(VI), tungsten(VI), vanadium(IV) and vanadium(V), which also react with SDHP to give complexes, and their Soret bands show maxima at wavelengths close to that of the iron(III) complex. No effective, appropriate masking agent for these interfering ions could be found. Interferences in Procedure B (at pH 7.2), which are not shown here, were severe. The interferences by molybdenum(VI) and tungsten(VI) disappeared, but many transition metal ions interfered. Hence the determination at pH 7.2 is very sensitive, but less selective, prior separation being necessary for the application to real samples.

Analogue Derivative Spectrophotometry to Improve Sensitivity

Procedure A, utilising the 1:1 complex formation, was the most sensitive of the known spectrophotometric methods for iron, but less sensitive compared with Procedure B utilising the 1:2 complex formation. Further sensitisation was therefore tried by introducing an analogue second-derivative spectrophotometric technique.[6,9]

In second-derivative spectrophotometry both the time constant of the analogue differentiation circuit and the scan speed of the spectrophotometer affect the sensitivity and selectivity, so both need to be optimised to give a well resolved large peak. Experimental results indicate that a combination of circuit No. 6 of reference 9 (which has the largest time constant, 2.0 s, the time constant increasing with circuit number in our apparatus) and a scan speed of 150 nm min^{-1} [or that of No. 5 (time constant: 0.96 s) and 300 nm min^{-1}] give the best sensitivity and resolution (*i.e.*, selectivity) for the iron determination. The calibration graph prepared under the recommended conditions by plotting the second-derivative value *versus* the iron concentration was linear and passed through the origin when either the peak to trough values or the base line to trough values were plotted (shown in Fig. 10). The equations for the lines were Fe (ng ml^{-1}) = 21.7D, and Fe(ng ml^{-1}) = 32.3D, where D is the second-derivative value converted into absorbance. It will be seen that iron can easily be determined down to 1.9 ng ml^{-1} in this manner.

Table 1. Tolerance limits for foreign ions in the determination of 76.8 ng ml^{-1} of iron at pH 4.1

Ion	Tolerance limit/ng ml^{-1} 3%*	5%*	Ion	Tolerance limit/ng ml^{-1} 3%*	5%*
Al^{III} ..	7.3	12.2	Ni^{II} ..	810†	
Cd^{II} ..	840†		Pb^{II} ..	510	
Co^{II} ..	820†		Pd^{II} ..	90	150
Cr^{III} ..	860†		Th^{IV} ..	340	560†
Cr^{VI} ..	90	150	Ti^{IV} ..	90	110
Cu^{II} ..	55	65	V^{IV} ..	7.5	12.5
Hg^{II} ..	930†		V^{V} ..	12	20
Mg^{II} ..	460	770	W^{VI} ..	9.4	15.7
Mn^{II} ..	400	660	Zn^{II} ..	630	
Mo^{VI} ..	35	58			

* Tolerable error.
† Maximum tested.

Conclusions

SDHP has been synthesised and its properties and reactivity with metal ions investigated spectrophotometrically. It was found that analytical use of the porphyrin can be extended by introducing complexing groups into the substituent outside the porphine ring. Extremely sensitive spectrophotometric and analogue derivative spectrophotometric methods for the determination of iron have been proposed.

References

1. Ishii, H., Koh, H., and Okuda, Y., *Nippon Kagaku Kaishi*, 1978, 686.
2. Koh, H., Kawamura, K., and Ishii, H., *Nippon Kagaku Kaishi*, 1979, 591.
3. Ishii, H., and Koh, H., *Nippon Kagaku Kaishi*, 1978, 390.
4. Ishii, H., and Koh, H., *Talanta*, 1977, **24**, 417.
5. Ishii, H., and Koh, H., *Bunseki Kagaku*, 1979, **28**, 473.
6. Ishii, H., and Koh, H., *Nippon Kagaku Kaishi*, 1980, 203.
7. Igarashi, S., Kobayashi, J., Yotsuyanagi, T., and Aomura, K., *Nippon Kagaku Kaishi*, 1979, 602.
8. Igarashi, S., Yotsuyanagi, T., and Aomura, K., *Nippon Kagaku Kaishi*, 1981, 60.
9. Ishii, H., and Satoh, K., *Fresenius Z. Anal. Chem.*, 1982, **312**, 114.

Paper A6/304
Received September 1st, 1986
Accepted February 23rd, 1987

Spectrophotometric Determination of Iron in Boiler and Well Waters by Flow Injection Analysis Using 2-Nitroso-5-(*N*-propyl-*N*-sulphopropylamino)phenol

Noriko Ohno and Tadao Sakai*
Department of Chemistry, Asahi University, 1851 Hozumi, Hozumi-cho, Gifu 501–02, Japan

2-Nitroso-5-(*N*-propyl-*N*-sulphopropylamino)phenol (nitroso-PSAP) forms a water-soluble chelate with iron. The molar absorptivity of the complex is 4.3×10^4 l mol^{-1} cm^{-1} at 753 nm. Iron (4–100 μg l^{-1}) is easily and selectively determined in a flow injection system because divalent transition metals (Ni, Co, Cu, Zn and Cd) at the 500 μg l^{-1} level do not interfere. The method has an average recovery error of ±1% and the sampling rate is 25 samples h^{-1}. A simple procedure for the determination of iron in boiler and well waters is described.

Keywords: *Iron determination; flow injection analysis; spectrophotometry; water analysis*

Nitrosophenol and nitrosonaphthol derivatives have been synthesised as selective and sensitive photometric reagents for metals such as cobalt,[1] iron,[2] palladium[3] and nickel.[4] These complexes are designed for use with solvent extraction and the sensitivity for some metals is excellent. Tsurubo and Sakai[5] reported that iron(II) forms a complex cation with 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine (PDT) in aqueous solutions which can be extracted into 1,2-dichloroethane as an ion associate with tetrabromophenolphthalein ethyl ester (TBPE). The calibration graph was linear over the range 0–0.25 mg l^{-1} of iron. However, in order to eliminate the solvent extraction process, water-soluble reagents such as 2-nitroso-5-(*N*-ethyl-*N*-sulphopropylamino)phenol (nitroso-ESAP) and 2-nitroso-5-(*N*-propyl-*N*-sulphopropylamino)-phenol (nitroso-PSAP) have been synthesised by Saito *et al.*[6] These water-soluble reagents are preferable for the determination of trace amounts of metals in a flow injection system. In an earlier paper,[7] we reported the determination of iron with nitroso-ESAP and Wada *et al.*[8] reported the determination of iron with 2-(3,5-dibromo-2-pyridylazo)-5-[*N*-ethyl-*N*-(3-sulphopropyl)amino]phenol (3,5-diBr - PAESPAP) using flow injection analysis. These reagents are very selective, but are not sensitive enough for the analysis of water samples.

This paper reports the highly sensitive and selective determination of iron in boiler and well waters using nitroso-PSAP in a flow injection analysis system.

Experimental

Reagents

All reagents used were of analytical-reagent grade.

Standard iron(II) solution. Iron(II) ammonium sulphate was dissolved in 0.1 M sulphuric acid to give a 1 g l^{-1} stock solution of iron(II), which was standardised by complexometry. The stock solution was diluted to an appropriate concentration as required after adding 0.05 M sulphuric acid.

2-Nitroso-5-(N-propyl-N-sulphopropylamino)phenol (nitroso-PSAP) solution, 2×10^{-4} M. Prepared by dissolving 0.0302 g of nitroso-PSAP (Dojindo Laboratories, Japan) in 500 ml of 5×10^{-3} M sulphuric acid.

Sodium ascorbate solution, 1×10^{-3} M. Prepared by dissolving 0.099 g of sodium ascorbate in 500 ml of distilled water.

Buffer solutions. Buffer solutions (pH 9–12) were made from equal volumes of 0.15 M potassium dihydrogenphosphate and 0.05 M sodium borate, the pH being adjusted with 1 M sodium hydroxide solution.

Apparatus

A Hitachi Model 556 double-beam spectrophotometer with 10 mm quartz cells and a Hitachi Model 057 *X* - *Y* recorder were used for absorption spectra. A Hitachi-Horiba Model F-7II pH meter with a glass electrode was used to measure the pH.

General Procedure (Batch Method)

Mix 3 ml of sample solution containing up to 5 mg l^{-1} of iron, 2 ml of 1% sodium ascorbate, 1 ml of 4×10^{-3} M nitroso-PSAP solution and 5 ml of phosphate buffer solution (pH 8.0) in a 25-ml calibrated flask, dilute to the mark with distilled water and shake thoroughly. Measure the absorbance at 753 nm in a 10-mm cell.

Flow Injection Procedure

The manifold of the flow injection system used is shown in Fig. 1. Two double plunger micro-pumps (Sanuki Kogyo Model DM2U-1026, Japan) are used to pump the solutions. The reagent solution containing 5×10^{-3} M sulphuric acid and the carrier solution of sodium ascorbate are pumped at a flow-rate of 0.8 ml min^{-1} by pump A and the samples (120 μl) containing up to 100 μg l^{-1} of iron are injected into the carrier stream by a six-way injection valve to which a volume control loop is attached. The sample and reagent are mixed in the 150-cm reaction coil. After adding the buffer solution (pH 10.5, 0.35 ml min^{-1}) via pump B, the colour is developed in the 200-cm coil. The absorbance of the complex is monitored by a spectrophotometric detector (Japan Spectroscopic, Model UVIDEC-100-VI) fitted with a micro flow cell (8 μl, 10 mm path length). The absorbance measured at 753 nm is recorded as peak-shaped signals. A Toa Electronics Model FBR-251A recorder is used. The PTFE tubing is of 0.5 mm i.d. except for the back-pressure coil which is 0.25 mm i.d. (5 m long).

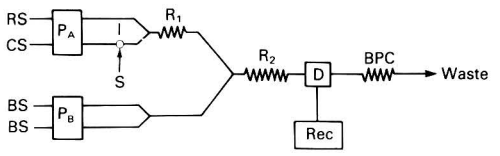


Fig. 1. Schematic diagram of flow system. CS, Carrier solution (1×10^{-3} M sodium ascorbate solution); RS, reagent solution (2×10^{-4} M nitroso-PSAP); BS, buffer solution, pH 10.5; P_A and P_B, pump flow-rates (P_A 0.8 ml min^{-1}, P_B 0.35 ml min^{-1}); R_1 and R_2, reaction coils (R_1, 0.5 mm i.d. × 150 cm, R_2, 0.5 mm i.d. × 200 cm); I, sample injector; S, sample (120 μl); BPC, back-pressure coil (0.25 mm i.d. × 500 cm); D, spectrophotometric detector; Rec, recorder

* To whom correspondence should be addressed.

Results and Discussion

Absorption Spectra and pH of Formation of Iron(II) Chelate Compounds

A 1.5-ml portion of a 10 mg l^{-1} iron(II) standard solution was placed in a 25-ml calibrated flask and the reagents were added. The mixture was diluted according to the general procedure described under Experimental. After standing for 5 min, the absorption spectra and pH on formation of the chelate were measured against a reagent blank. Iron(II) reacted with nitroso-PSAP to form a chromogenic chelate compound, which had absorption maxima at 425 and 753 nm. The molar absorptivities of the iron chelate compound were 13400 l mol^{-1} cm^{-1} at 425 nm and 43000 l mol^{-1} cm^{-1} at 753 nm. However, other metals such as cobalt(II), copper(II) and nickel reacted with nitroso-PSAP to form complexes which had absorption maxima at 390–450 nm. As a result, iron was determined sensitively and selectively at 753 nm.

Fig. 2 shows the effect of pH on the formation of the iron complex by the batch method. Graph 1 was obtained when the reagents were added in the order iron, sodium ascorbate, buffer solution and nitroso-PSAP. The absorbance decreased considerably in the pH range 8–9, but at higher pH (9.7–10.3) maximum and constant absorbance was obtained after 1 h. The effect may be attributed to the dissociation constant of nitroso-PSAP (8.4),[9] the hydrolysis product or hydroxo complex formed and the very slow reaction rate of complex formation in the above pH range. At 80 °C the maximum absorbance was quickly obtained even at pH 8–9. As a result, the order of reagent addition was seen to be important for the rapid formation of chelate compounds.

Effect of Nitroso-PSAP Concentration in FIA System

The effect of the nitroso-PSAP concentration on the colour development was studied for 50 and 100 μg l^{-1} of iron(II). The nitroso-PSAP concentration was varied from 1×10^{-5} to 3×10^{-4} M. The peak signals were maximum and constant at nitroso-PSAP concentrations above 1×10^{-4} M, as shown in Fig. 3. In this work, a 2×10^{-4} M nitroso-PSAP solution was used.

Effect of pH on Iron Complex Formation in FIA System

The dependence on pH of the complex formation with nitroso-PSAP was investigated (Fig. 4). The peak signals were maximum and constant in the pH range 9–12. The optimum pH range was very wide and acceptable. Above pH 12, the peak signal decreased because the iron was hydrolysed. The pH of the buffer solution in the FIA system was adjusted to 10.5.

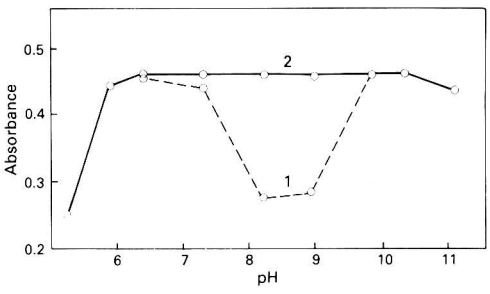


Fig. 2. Effects of pH and order of reagent addition on formation of iron chelate compounds by the batch method. (1) Iron + sodium ascorbate + buffer + nitroso-PSAP, absorbance measured after 1 h; (2) iron + sodium ascorbate + nitroso-PSAP + buffer, absorbance measured after 5 min. Iron concentration, 0.6 mg l^{-1}; sodium ascorbate concentration, 0.08%; nitroso-PSAP concentration, 1.6×10^{-4} M; wavelength, 753 nm

However, the complex was quickly formed without hydrolysis when the reagents were added in the order iron, sodium ascorbate, nitroso-PSAP, buffer solution, in the pH range 6.4–10.3 (Fig. 2). Accordingly, as can be seen in Fig. 1, the buffer solution was added after mixing the chromogenic reagent and samples. The iron(II) - nitroso-PSAP chelate compound was stable for at least 3 h.

Effect of the Reaction Coil Lengths

The effect of the lengths of the reaction coils (R_1 and R_2 in Fig. 1) was examined and the R_1 coil length was varied in the range 50–400 cm. The reagent and iron(II) react to form the coloured chelate compound in R_1 (0.5 mm i.d.). The largest peak height was obtained in the range 50–150 cm, but the reproducibility was poor when the R_1 coil length was less than 100 cm. A 150-cm coil is recommended. The same flow-rates were used for both the reagent and the sample channels (0.8 ml min^{-1}).

The effect of the R_2 coil length was studied over the range 100–600 cm. The peak heights decreased with increasing R_2 length because of dispersion. Accordingly, a 200-cm reaction coil length was used for R_2. A flow-rate of 0.35 ml min^{-1} was recommended and 120 μl was found to be the most suitable sample volume.

Effect of Reductant

Iron(III) does not form a complex with nitroso-PSAP, but it is able to form a chelate in the presence of a reductant. The effect of the concentration of sodium ascorbate as a reductant was examined. The reduction of iron(III) was complete above 3×10^{-4} M sodium ascorbate. Therefore, a 1×10^{-3} M sodium ascorbate solution was chosen.

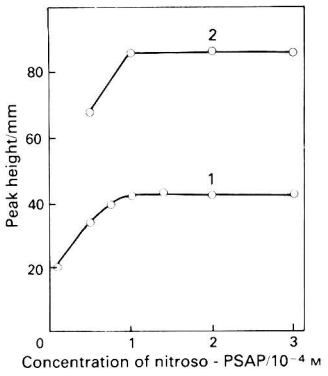


Fig. 3. Effect of nitroso-PSAP concentration in FIA. (1) 50 μg l^{-1} iron(II); (2) 100 μg l^{-1} iron(II). Sodium ascorbate concentration, 1×10^{-3} M; pH, 10.5; wavelength, 753 nm; sample volume, 120 μl

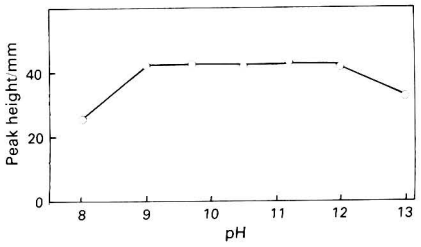


Fig. 4. Effect of pH on iron complex formation in FIA. Iron(II) concentration, 50 μg l^{-1}; nitroso-PSAP concentration, 2×10^{-4} M; sodium ascorbate concentration, 1×10^{-3} M; wavelength, 753 nm

Table 1. Effect of foreign ions on the determination of 50 μg l^{-1} of iron(II)

Foreign ion	Added as	Amount added/ mg l^{-1}	Recovery, %	Foreign ion	Added as	Amount added/ mg l^{-1}	Recovery, %
F^-	KF	50	99.6	Pt^{IV}	H_2PtCl_6	10	101.2
Cl^-	KCl	50	99.0	Tartaric acid		10	98.0
Br^-	KBr	50	99.0	Pd^{II}	$PdCl_2$	5	100.0
I^-	KI	50	100.4	W^{VI}	Na_2WO_4	5	99.0
NO_3^-	$NaNO_3$	50	98.0	Cr^{III}	$CrCl_3$	5	100.3
CO_3^{2-}	Na_2CO_3	50	100.1	Sb^{III}	$SbCl_3$	5	100.2
CH_3COO^-	CH_3COONa	50	100.0	Pb^{II}	$Pb(NO_3)_2$	2.5	99.6
ClO_4^-	$NaClO_4$	50	98.1			25	100.0*
NH_3	Aqueous ammonia	50	100.0	Ba^{II}	$BaCl_2$	2.5	99.2
Citric acid		50	98.0	Se^{IV}	$SeCl_4$	1	100.0
Li^{I}	LiCl	25	100.1			25	100.0*
Si^{IV}	Na_2SiO_3	25	100.0	Co^{II}	$CoCl_2$	1	98.7
As^{III}	$NaAsO_2$	25	99.8	Cu^{II}	$CuCl_2$	0.5	100.6
Sr^{II}	$SrCl_2$	25	99.5			5	98.9†
Hg^{I}	$HgNO_3$	25	99.0	Ni^{II}	$NiSO_4$	0.5	100.0
Hg^{II}	$Hg(CH_3COO)_2$	25	98.6	Al^{III}	$AlCl_3$	0.5	98.5
Cd^{II}	$Cd(NO_3)_2$	16	100.9			2.5	98.9*
Ca^{II}	$CaCl_2$	10	100.4	Bi^{III}	$Bi(NO_3)_3$	0.005	100.0
Zn^{II}	$ZnSO_4$	10	99.3			25	100.0*
Mg^{II}	$MgCl_2$	10	101.4				

* 0.04% Tiron was added to the buffer solution.
† 4×10^{-4} M potassium cyanate or 2×10^{-4} M bathocuproinedisulphonic acid was added to the sample solution.

Table 2. Determination of total iron in boiler and well waters

Sample	Iron found/mg l^{-1} Proposed method	TPTZ method*
Boiler water 1	0.46	0.47
Boiler water 2	0.51	0.50
Boiler water 3	0.07	0.06
Well water 1	0.03	0.04
Well water 2	0.01	—

* Spectrophotometry using 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) chelate reagent.

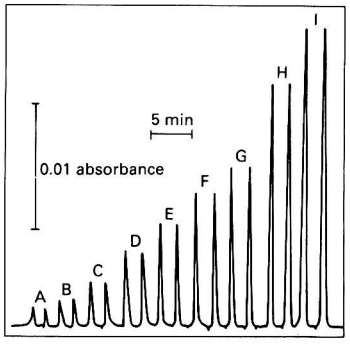


Fig. 5. Flow signals for iron(II). Iron(II) concentration (μg l^{-1}): A, 0; B, 4; C, 10; D, 20; E, 30; F, 40; G, 50; H, 80; and I, 100

Effect of Flow-rate

The flow-rate of pump A was varied between 0.5 and 2 ml min^{-1} with the flow-rate of pump B constant, and also with the flow-rate of pump B varied between 0.2 and 1 ml min^{-1}. When the flow-rate of pump A increased, the peak height of the signal became larger because of the small dispersion of the complex. When the flow-rate of pump B increased with a constant flow-rate of pump A, the peak height became smaller owing to the dilution effect on the complex.

When the flow-rate of pump A was 1.6 ml min^{-1} and the flow-rate of pump B was 0.35 ml min^{-1}, that is, when the ratio (P_A/P_B) was 4.5, the peak height was maximum and constant. When P_A/P_B was >5, the effect of the buffer decreased, the colour development was incomplete and double peaks appeared.

Calibration Graph

Fig. 5 shows the recorder traces for a series of runs in duplicate. The calibration graph was linear for 4–100 μg l^{-1} of iron and the dynamic range was wide. The detection limit was 1 μg l^{-1}(signal to noise ratio = 2). The relative standard deviation was below 1% for ten runs on a 50 μg l^{-1} iron sample. The sampling rate was about 25 samples h^{-1} in the proposed manifold.

Interferences

For each interference, solutions were prepared which contained fixed concentrations of other ions and their interferences were investigated by the recommended flow injection analysis procedure. The tolerable amounts of various ions were taken as that amount which caused an error of ±2% on each peak height and the tolerance limits for various metals ions are listed in Table 1. There was no interference from 50 mg l^{-1} of Cl^-, I^-, NO_3^-, SO_4^{2-}, CH_3COO^-, ClO_4^- and citric acid. Most metal ions did not interfere at levels from 2.5 to 25 mg l^{-1} (50–500-fold for iron). Cu^{II} and Ni^{II} at 0.5 mg l^{-1} and Co^{II} at 1 mg l^{-1} had no significant effect because the absorbance was measured at 753 nm, where the absorption spectra did not overlap each other. When a 0.04% Tiron concentration was added to the buffer solution, the interference of Bi^{III} was reduced and the tolerance limit raised considerably for Al^{III}, Pb^{II} and Se^{IV}. Moreover, 5 mg l^{-1} of Cu^{II} (100-fold for iron) could be tolerated by the addition of potassium cyanate or bathocuproinedisulphonic acid. Consequently, the method was shown to be selective and sensitive for the determination of iron.

Determination of Total Iron in Boiler and Well Waters

Total iron in three boiler- and two well-water samples was determined by the proposed flow injection method. The results obtained are summarised in Table 2. It is important to monitor the corrosion of the boiler so that the iron in boiler

and well waters is determined. The boiler water and/or well water was heated for 30 min at 80 °C after the addition of sulphuric acid to give a final concentration of 0.05 M. A 120-μl portion of the sample solution was injected into the flow injection system. The same samples were also concentrated to one-tenth of the original volume and determined by spectrophotometry using 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ, Dojindo Laboratories, Japan).

The results obtained by the proposed method were in good agreement with those obtained in TPTZ batch spectrophotometry.

In conclusion, micro-amounts of iron in boiler and well waters can be determined spectrophotometrically using water-soluble nitroso-PSAP. The method was applied successfully to flow injection analysis. This method is very selective for the determination of micro-amounts of iron without interference from other metal ions.

References

1. Toei, K., and Motomizu, S., *Bunseki Kagaku*, 1973, **22**, 1079.
2. Korenaga, T., Motomizu, S., and Toei, K., *Anal. Chim. Acta*, 1973, **65**, 335.
3. Toei, K., Motomizu, S., and Hamada, S., *Anal. Chim. Acta*, 1978, **101**, 169.
4. Motomizu, S., and Toei, K., *Anal. Chim. Acta*, 1978, **97**, 335.
5. Tsurubo, S., and Sakai, T., *Analyst*, 1984, **109**, 1397.
6. Saito, M., Horiguchi, D., and Kina, K., *Bunseki Kagaku*, 1981, **30**, 635.
7. Sakai, T., and Ohno, N., *Bunseki Kagaku*, 1984, **33**, 331.
8. Wada, H., Nakagawa, G., and Ohshita, K., *Anal. Chim. Acta*, 1983, **153**, 199.
9. Yoshida, I., and Ueno, K., *Bunseki Kagaku*, 1985, **34**, 77.

Paper A7/44
Received February 9th, 1987
Accepted March 16th, 1987

Extraction and Spectrophotometric Determination of Tungsten with Thiocyanate and Amides

Neera Mishra, S. K. Sinha, K. S. Patel and R. K. Mishra
Department of Chemistry, Ravishankar University, Raipur-492 010, M.P., India

A spectrophotometric method for the determination of microgram amounts of tungsten(VI) is described. The method is based on the formation of trithiocyanatotungsten(V) by the reduction of the metal with tin(II) chloride in strong hydrochloric acid solution, the reaction of tungsten(V) with thiocyanate and its extraction with amide into benzene as an adduct in 4.0–8.0 M HCl. The extraction of the metal with various amides was examined and ten amides were found to be suitable extraction reagents for the spectrophotometric determination. The molar absorptivities of the adducts lie in the range 0.92×10^4–1.43×10^4 l mol^{-1} cm^{-1} at 405 nm. When the method was modified as a qualitative test for tungsten, the detection limit was 0.005 μg ml^{-1}. The method is simple, convenient, reproducible and applicable to alloy steels containing up to 0.05% of tungsten. The effect of diverse ions was examined and the results indicated that Fe, Co, Ni, Cu, Zn, Cd, Mn, Al, Sb, Bi, Ti, Hf, V, Cr, Mo, Re, Pd, etc., did not interfere. Niobium was found to interfere but was removed by masking with oxalate.

Keywords: *Tungsten determination; spectrophotometry; amide extraction; thiocyanate; steel analysis*

Numerous spectrophotometric methods for the determination of tungsten have been reported.[1–12] Of these, the classical thiocyanate method is widely used for the spectrophotometric determination of the metal; however, it suffers from interferences from many metal ions and colour inhibition with F^- or NO_3^-.[1–3] Donaldson[4] claimed that the thiocyanate method did not yield compatible results for the ores when measurements were carried out in aqueous media and after extraction of the complex into polar organic solvents. It was also subject to interference from Ti, V, Nb or Mo. Many organic reagents, such as tribenzylamine, diantipyrylmethane, tetraphenylarsonium chloride and diethazine hydrochloride, were reported to enhance the selectivity of the method. Of these, tribenzylamine[5] is a sensitive reagent. However, it has many drawbacks, *e.g.*, a very narrow acidity range of *ca.* 4 M HCl, colour inhibition with F^- or NO_3^-, the requirement for a modified procedure for the determination of the metal in the presence of Ti and V and many metal ions are tolerated only at microgram levels. Diantipyrylmethane[4] and tetraphenylarsonium chloride[6] are selective extractants but their sensitivities are low (ε 1510 and 1550 l mol^{-1} cm^{-1}, respectively). Diethazine hydrochloride is also a sensitive reagent, although it suffers from serious interference from Ti, V, Nb, Ta and Mo.[7]

The acidic reagents quinolin-8-ol and toluene-3,4-dithiol have been proposed for the spectrophotometric determination of tungsten. Quinolin-8-ol is a less sensitive and selective reagent and the complex formed exhibits an absorption maximum in the ultraviolet region (370 nm).[3,8] Toluene-3,4-dithiol is a sensitive reagent but its use involves some experimental difficulties, *e.g.*, the extraction of the metal at high temperature to speed up the reaction in strong hydrochloric acid media of *ca.* 10–11 M and oxidation of the reagent into disulphide by atmospheric oxygen.[3,9]

Polyphenols such as pyrocatechol and 3,5-dinitropyrocatechol in the absence or presence of complexing agents such as amines or dyes are also sensitive reagents.[10] Of these, the colour reaction of 3,5-dinitropyrocatechol with tungsten(VI) in the presence of brilliant green is the most sensitive (ε 132 000 l mol^{-1} cm^{-1}); however, it requires three analytical steps, *viz.*, formation of the floated complex in light petroleum, discarding of the organic layer and dissolution of the floated complex into chloroform to improve the selectivity.[11] The determination of tungsten with 6,7-dihydroxy-2,4-diphenylbenzopyranol is also a sensitive method, although it suffers from serious interference from Fe, Al, Sn, Ge, Ti, Zr, Hf and V.[12]

In order to overcome most of these difficulties, a convenient and reproducible spectrophotometric method for the determination of tungsten with thiocyanate and amides is proposed. Tungsten(VI) is reduced by tin(II) chloride in strong hydrochloric acid solution, the metal is reacted with thiocyanate and the resulting thiocyanato complex is extracted with a solution of amide in benzene containing 4.0–8.0 M HCl. Of ten different *N*-arylacetamides used, the simplest compound, *N*-phenylacetamide (PAA), was chosen for detailed studies in this investigation.

The proposed method is free from interference from the metals (*e.g.*, Ti, V, Mo or Re) which were generally found to interfere in most of the reported methods, the extractants employed are common laboratory reagents and the overlapping of the spectrum of the reagent blank with that of the adduct is almost negligible. Moreover, it widens the optimum acidity range of the classical thiocyanate method and shifts the working range towards lower acidity. Hence the method proposed here removes most of the drawbacks of the above established methods.[1–12]

Experimental

Apparatus

An ECIL Model GS-865 UV - visible spectrophotometer and a Carl Zeiss Jena Spekol instrument equipped with 1-cm quartz cells were employed for measuring the absorbance values.

Reagents

All chemicals used for the preparation of solutions were of analytical-reagent grade (BDH Chemicals or Merck). All the aqueous solutions were pre-saturated with benzene before extraction.

Standard tungsten(VI) solution, 1 mg ml^{-1}. Prepared by dissolving 1.7941 g of $Na_2WO_4.2H_2O$ in doubly distilled water and diluting to 1 l with water.

Solutions of amides in benzene, 2% *m/V.*

Potassium thiocyanate solution, 40% *m/V.*

Tin(II) chloride in concentrated hydrochloric acid, 40% *mV.*

Procedure

An aliquot of the solution containing 15–150 μg of W^{VI} was placed in a 150-ml separating funnel and 1 ml of the tin(II) chloride solution and 4 ml of concentrated hydrochloric acid were added. After 20 min, the solution was reacted with 1 ml of the thiocyanate solution and diluted to 10 ml with distilled water. The aqueous solution was then equilibrated with 15 ml of the solution of amide in benzene for 2 min. The separated organic layer was dried over 2 g of anhydrous sodium sulphate in a 25-ml beaker. The absorbance of the extract was measured at $\lambda_{max.}$ using benzene as the reference.

Results and Discussion

Absorption Spectra

Fig. 1 shows the absorption spectra of some adducts formed with $[WO(SCN)_3]$ and amides in benzene and also of the reagent blank, obtained by the proposed procedure. The absorption spectra of all adducts gave an absorption maximum around 405 nm in benzene, and remained constant whatever the acids, solvents or salts in the aqueous solutions. The absorption of the reagent blank at this wavelength was almost negligible and hence benzene was employed as a reference for all the measurements.

Effect of Solvents

The influence of various water-immiscible solvents such as pentan-1-ol, isobutyl methyl ketone, ethyl acetate, chloroform, carbon tetrachloride, benzene and toluene were examined for the extraction of the trithiocyanatotungsten(V) complex with *N*-phenylacetamide. The adduct was quantitatively extracted only into the first four solvents with different values for molar absorptivity (pentan-1-ol, 11 500; isobutyl methyl ketone, 12 700; ethyl acetate, 19 700; and benzene, 12 000 l mol^{-1} cm^{-1} at $\lambda_{max.}$ 405 nm). Carbon tetrachloride was unable to extract the adduct whereas chloroform and toluene partially extracted it. Of these, benzene was chosen for further experimental work owing to its selective extraction of the metal.

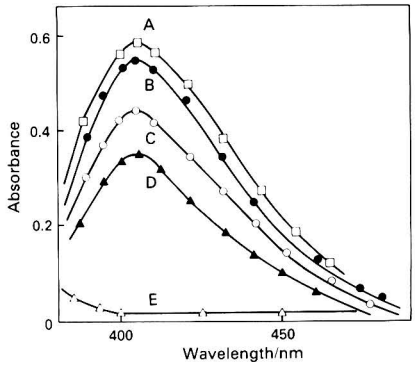


Fig. 1. Absorption spectra of some adducts of $WO(SCN)_3$ with amides and that of the reagent blank. (A) *N*-(3,4-Dimethylphenyl)-acetamide adduct, [W] = 4.98 × 10^{-5} M; (B) *N*-(2,3-dimethylphenyl)-acetamide adduct, [W] = 4.18 × 10^{-5} M; (C) *N*-(4)-methylphenyl)-acetamide adduct, [W] = 3.08 × 10^{-5} M; (D) *N*-phenylacetamide adduct, [W] = 2.92 × 10^{-5} M; and (E) 2% PAA at a volume ratio of 2:3 (aqueous to benzene)

Effect of Reducing Agent and Acids

The reduction of tungsten(VI) by means of tin(II) chloride was examined in both 10 M hydrochloric and sulphuric acid. The results indicated that a waiting time of 15 min was satisfactory over the range 18–27 °C. The rate of reaction was influenced by temperature and waiting times of 10 and 7 min were satisfactory at 35 and 45 °C respectively. The colour intensity of the adduct in both acids at the absorption maximum (405 nm) was the same. Of these, hydrochloric acid was selected for detailed studies because the preparation of 40% *m/V* tin(II) chloride solution in sulphuric acid is difficult.

The effects of the amount of tin(II) chloride on the reduction of the metal and on the extraction of the adduct were examined at a volume ratio of the two phases of unity. Fig. 2 shows that the optimum concentration of tin(II) chloride in the aqueous phase for the complete extraction of the metal should be between 0.03 and 0.35 M. The optimum hydrochloric acid concentration observed for maximum and constant colour development of the adduct in the organic solution is 4.0–8.0 M (Fig. 2). Beyond 8 M HCl turbidity appears owing to the miscibility of the two phases.

Effect of Thiocyanate and Amide

The effect of the amount of potassium thiocyanate and *N*-phenylacetamide on the extraction of the metal at a volume ratio of the two phases of unity was examined. Fig. 2 shows that over a wide concentration range potassium thiocyanate did not affect the extraction of the metal. Concentrations of at least 0.07 M PAA in benzene and 0.30 M KSCN solution were necessary for full colour development and the addition of higher concentrations of the reagents (*ca.* 0.25 M PAA and 1.5 M KSCN) caused no adverse effects. The order of addition of reagents is important. The dilution of the solution must be effected after the reduction of the metal. In order to avoid the use of a larger amount of the organic compound for the recovery of the metal, a volume ratio of 2:3 (aqueous to benzene) was employed.

Effect of Time, Temperature and Dilution

A shaking time of 1 min was sufficient for the complete extraction of the metal and a time of 2 min was therefore employed in all experimental work. The extraction of the adduct was not affected by variation in temperature from 20 to 45 °C. The dilution effect of the aqueous phase was examined by keeping the concentration of potassium thiocyanate (0.4 M) and hydrochloric acid (6 M) constant in all instances. The

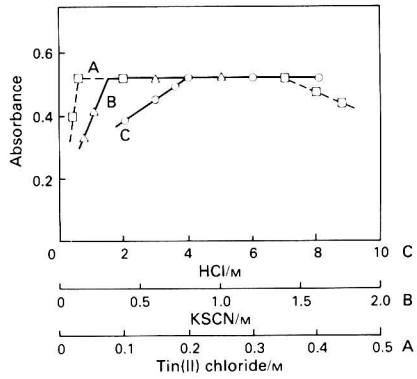


Fig. 2. Effect of tin(II) chloride, thiocyanate and hydrochloric acid on extraction of the metal. [W^{VI}], 4.35 × 10^{-5} M; [Sn^{II}], 0.17 M; [HCl], 6.0 M; [KSCN], 0.4 M; and [PAA], 0.15 M. Absorbance *versus* molar concentration of (A) tin(II) chloride, (B) potassium thiocyanate and (C) hydrochloric acid

results indicated that a variation in the volume ratio of the organic to the aqueous phase from 2 : 1 to 1 : 2.5 did not affect the absorbance of the extract. The extract was stable for at least 2 h at room temperature.

Qualitative Test for Tungsten

A rapid procedure for the detection of the metal was developed by reducing trace amounts of tungsten(VI) with 0.2 ml of tin(II) chloride in a hot 5–6 M HCl solution by keeping the total aqeuous volume between 5 and 10 ml. The solution was cooled and treated with 0.2 ml of the potassium thiocyanate solution and 0.3 g of *N*-phenylacetamide, and then shaken with a few drops of benzene. The detection limit for this qualitative test is 0.005 μg ml^{-1} of W.

Optimum Concentration Range and Precision of the Method for the Determination of Tungsten(VI)

The calibration graph was plotted as described under Procedure and the optimum concentration range on the basis of Beer's law was 1.0–13.5 μg ml^{-1} of W. The precision of the method was evaluated by making ten measurements on a sample containing 80 μg of W^{VI} per 15 ml, and the relative standard deviation was found to be 1.1%.

Composition of the Complex

An attempt was made to determine the ratio of reagents to W^{V} in the adduct. The distribution ratio (log *D*) of the metal was plotted *versus* the logarithm of the molar concentration of the reagent at a volume ratio of the two phases of unity. On the basis of the slopes of 2.9 and 1.85 in Fig. 3, it is assumed that 3

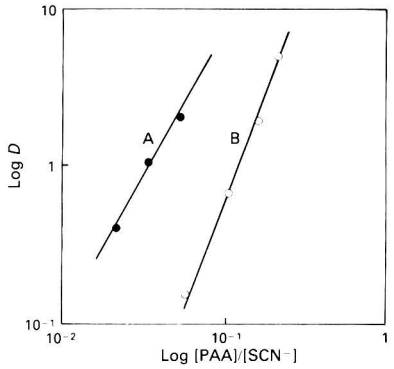


Fig. 3. Curve-fitting method for determination of ratio of the variables in the adduct. [W^{VI}], 4.35 × 10^{-5} M; [Sn^{II}], 0.17 M; HCl, 6 M; [KSCN], 0.4 M; and [PAA], 0.15 M. (A) Log *D versus* log (molar concentration of PAA in benzene) and (B) log *D versus* log (molar concentration of KSCN in aqueous solution)

mol of SCN^- and 2 mol of PAA react with WO^{3+} to give a neutral benzene-extractable species. The reaction sequence can be expresssed as follows:

(1) reduction of the metal:

$$2H_2WO_4 + SnCl_2 + 8HCl \rightarrow 2[WOCl_3] + SnCl_4 + 6H_2O$$

(2) formation of thiocyanato complex:

$$WOCl_3 + 3SCN^- \rightleftharpoons [WO(SCN)_3] + 3Cl^-$$

(3) formation of benzene-extractable adduct with amide:

$$[WO(SCN)_3] + 2PAA_0 \rightleftharpoons [WO(SCN)_3]2PAA_0$$

where the subscript 0 denotes benzene.

Amides as Extraction Reagents

The extraction of trithiocyanatotungsten(V) with 2% *m/V* solutions of various amides in benzene was examined and the spectral data are summarised in Table 1. All *N*-arylacetamides tested react with [$WO(SCN)_3$] in a similar manner to give the benzene-extractable adduct. The molar absorptivity and Sandell's sensitivity of the adducts are in the range 0.92 × 10^4–1.43 × 10^4 l mol^{-1} cm^{-1} and 2.00 × 10^{-2}–1.28 × 10^{-2} μg cm^{-2} of W at $\lambda_{max.}$ 405 nm, respectively. The nature of the amides greatly affects the formation and the colour intensity of the adduct in the organic solution. The acetamide and aryl derivatives of an amide (*e.g.*, *N*-arylbenzamides, *N*-arylmethylbenzamides, *N*-arylchlorobenzamides) could not extract the complex into benzene whereas benzamide showed a partial extraction. The *N*-arylacetamides are very useful for the extraction of the metal (Table 1). The nature of the substituents in the *N*-ring of the amide greatly influence the colour intensity of the adduct in the organic solution but no trend is observed with respect to inductivity, degree and position of substituents.

Effect of Diverse Ions

The effect of diverse ions on the determination of 80 μg of W^{VI} was examined with PAA as described under Procedure. Small amounts of Mo^{VI} and Re^{VII} did not interfere because they are reduced to lower oxidation states by tin(II) chloride in strong hydrochloric acid media. However, niobium(V) interfered seriously in the determination of the metal and was removed by masking with oxalate. The three anions mentioned (F^-, NO_3^- and PO_4^{3-}) interfered when present in amounts greater than 10 mg. The tolerance limits (in milligrams) of diverse ions causing an error of less than ±2% are summarised in Table 2.

Application of the Method

The method was applied to standard alloy steels. A weighed amount of the sample (0.3–1.0 g) was dissolved in 25 ml of aqua regia in a 400-ml beaker and heated to remove the oxides of nitrogen. The product was digested separately with 10 ml of

Table 1. Spectral data for trithiocyanatotungsten(V) with *N*-arylacetamides in benzene

Amide	Molar absorptivity/ l mol^{-1} cm^{-1}	Sandell's sensitivity/ μg cm^{-2} of W
N-Phenylacetamide	12 000	0.0153
N-(2-Methylphenyl)acetamide	12 000	0.0153
N-(4-Methylphenyl)acetamide	14 300	0.0128
N-(2-Chlorophenyl)acetamide	9 600	0.0191
N-(4-Chlorophenyl)acetamide	12 400	0.0148
N-(3-Chloro-4-methylphenyl)acetamide	9 200	0.0200
N-(2,3-Dimethylphenyl)acetamide	13 050	0.0140
N-(2,5-Dimethylphenyl)acetamide	12 400	0.0148
N-(2,6-Dimethylphenyl)acetamide	13 100	0.0140
N-(3,4-Dimethylphenyl)acetamide	11 650	0.0157

Table 2. Tolerance limit of various ions in the determination of 80 μg of W^{VI}

Ion added	Amount/mg	Ion added	Amount/mg
Fe^{III}	100	La^{III}	5
Co^{II}	15	Bi^{III}	1
Ni^{II}	25	Sb^{III}	30
Cu^{II}	1(10)*	Pb^{II}	20
Mn^{II}	100	Zn^{II}	20
Cr^{III}	25	Cd^{II}	20
Ti^{IV}	40†	Al^{III}	20
Zr^{IV}	20	Be^{II}	1
Hf^{IV}	15	Mg^{II}	20
V^{V}	5	Ca^{II}	20
Nb^{V}	5‡	Ag^{I}	1
Mo^{VI}	2	Hg^{II}	1
U^{VI}	15	PO_4^{3-}	10
F^-	10	AsO_4^{3-}	5
NO_3^-	10	$C_2O_4^{2-}$	60
EDTA	40	NH_2CSNH_2	40

* In the presence of 1 ml of thiourea (3% *m/V*).
† As the oxalate.
‡ In the presence of 1 ml of sodium oxalate (5% *m/V*).

concentrated hydrochloric acid and then with 5 ml of concentrated sulphuric acid, being heated to dryness in each instance. The sample was dissolved in 15 ml of 5 M hydrochloric acid, and the cold solution was treated with 20 ml of tartaric acid solution (50% *m/V*) and neutralised with 20 ml of sodium hydroxide solution (50% *m/V*). The solution was diluted to the desired volume with distilled water in a calibrated flask. The metal content of the solution was determined as described under Procedure. The results are presented in Table 3.

Table 3. Determination of tungsten in alloy steels

Sample*	Composition, %	Tungsten found,† %	Relative standard deviation, %
2H	C, 0.63; Mn, 0.82; S, 0.029; P, 0.040; Ni, 0.38; Cr, 2.38; Mo, 0.34; W, 0.05	0.049	1.4
21b	C, 0.84; Si, 0.12; S, 0.044; P, 0.036; Mn, 0.43; W, 0.46	0.458	1.1
64a	C, 0.80; Cr, 4.40; Mo, 4.11; V, 1.57; W, 5.66	5.672	1.0

* 2H = Himmat Steel Foundry Standard, Kumbhari, Raipur, India; 21b and 64a = British Chemical Standards, Middlesbrough, UK.
† Average of six determinations.

References

1. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience, New York, 1959, pp. 886 and 887.
2. Marczenko, Z., "Spectrophotometric Determination of Elements," Ellis Horwood, Chichester, 1976, pp. 568–571.
3. Fogg, A. G., Marriott, D. R., and Thorburn Burns, D., *Analyst*, 1970, **95**, 848.
4. Donaldson, E. M., *Talanta*, 1975, **22**, 837.
5. Yatirajam, V., and Dhamija, S., *Talanta*, 1975, **22**, 760.
6. Affsprung, H. E., and Murphy, J. W., *Anal. Chim. Acta*, 1964, **30**, 501.
7. Puzanowska-Tarasiewicz, H., Grudniewska, A., and Tarasiewicz, M., *Mikrochim. Acta*, 1981, **I**, 19.
8. Eberle, A. R., *Anal. Chem.*, 1963, **35**, 669.
9. Greenberg, P., *Anal. Chem.*, 1957, **29**, 896.
10. Busev, A. I., and Sokolova, T. A., *Zh. Anal. Khim.*, 1975, **30**, 1784.
11. Nazarenko, V. A., Poluektova, E. N., and Shitareva, G. G., *Zh. Anal. Khim.*, 1973, **28**, 1966.
12. Poluektova, E. N., *Zh. Anal. Khim.*, 1966, **21**, 187.

Paper A7/4
Received January 7th, 1987
Accepted March 9th, 1987

Spectrophotometric Microdetermination of Tin: Use of a Sensitiser and Kalman Filtering to Improve the Sensitivity and Selectivity

Yi-Ming Liu and Ru-Qin Yu
Department of Chemistry and Chemical Engineering, Hunan University, Changsha, People's Republic of China

A spectrophotometric method for the microdetermination of tin with *o*-nitrophenyl fluorone(*O*-NPF), using a sensitiser and Kalman filtering is described. The use of *N*-[1-(1′-chloro-2′-hydroxylpropyl)polyoxyethenyl-2-hydroxylpropyl]-*N*-hexadecyl-*N*,*N*-dimethylammonium chloride (PHDAC), synthesised in this laboratory, improved the sensitivity of the colour reaction. In the absence of PHDAC the absorption spectrum of tin - *o*-NPF solution was essentially the same as that of the reagent blank; however, strong bathochromic and hyperchromic effects occurred when PHDAC was added and the tin - *o*-NPF complex had a maximum absorption at 515 nm with a molar absorptivity of 1.85×10^5 l cm^{-1} mol^{-1}. Kalman filtering was used as a "chemometrics filter," filtering out the interferences from relatively large amounts of molybdenum and tungsten. The method was applied to the determination of tin in metallurgical samples with satisfactory results.

Keywords: *Tin determination; photometric sensitising agent; Kalman filtering; o-nitrophenylfluorone; spectrophotometry*

Several kinds of dyes have been used for the spectrophotometric determination of micro-amounts of tin. The sensitivity of most of these determinations can be improved by adding surfactants to solutions of the tin - dye complex.[1–4] The most commonly used surfactants are quaternary ammonium salts such as cetyltrimethylammonium bromide (CTMAB) and cetylpyridinium bromide (CPB). It is known that these surfactants are not the most satisfactory sensitising agents because of their limited sensitising efficiency, poor solubility at low temperature, and adherence to the vessel wall. The derivatives of 2,3,7-trihydroxyfluorone, first proposed by Nazarenko *et al.*[5] as photometric reagents for tin, were widely used for the determination of this element. Shen *et al.*[6] recently studied the colour reaction of *o*-nitrophenylfluorone (*o*-NPF) with tin(IV) in the presence of CTMAB. This paper proposes a sensitiser *N*-[1-(1′-chloro-2′-hydroxylpropyl)polyoxyethenyl-2-hydroxylpropyl]-*N*-hexadecyl-*N*,*N*-dimethylammonium chloride (PHDAC), which makes the determination very sensitive and the experimental conditions more easily controllable.

Kalman filtering has found widespread use in analytical chemistry.[7] It has been applied to resolve overlapped responses in both electrochemistry and spectroscopy,[8–10] to process data in flow injection analysis[11] and to tackle the drift problem[12] and model errors[13] in multi-component analysis. In this study, Kalman filtering is applied as a "chemometrics filter," which, in combination with chemical masking reagents, suppresses the interferences from relatively large amounts of Mo^{VI} and W^{VI} in the determination of tin, making the method more selective. The described procedure is a rapid, accurate, selective and sensitive spectrophotometric method for the microdetermination of tin, and has been applied to the determination of tin in copper and aluminium alloys and steel samples with satisfactory results.

Experimental

The absorbance was measured with either a Model DU-7 spectrophotometer (Beckman) or a XG-125 spectrophotometer (Xiamen), using 1-cm quartz cells. An IBM PC-XT microcomputer was used for running the Kalman filtering program (written in BASIC).

Reagents

o-NPF(Tianjin, analytical-reagent grade) was used as an alcoholic 1×10^{-3} M solution. The standard tin solution (9.85×10^{-5} M) in 0.5 M H_2SO_4 was prepared from spectrally pure tin metal. PHDAC was synthesised in this laboratory[14] and used as an aqueous 2% *m*/*V* solution. Other solutions used were: CTMAB (2.5×10^{-3} M), CPB (2.5×10^{-3} M) and Triton X-100 (5% *V*/*V*).

Procedure

Pipette 0.30 ml of tin solution into a 10-ml calibrated flask. Add 1 ml of 5% *m*/*V* ascorbic acid solution, 0.5 ml of dilute lactic acid (1 + 1) and 1 ml of 0.02 M trisodium citrate solution. After mixing add 1.5 ml of 5 M H_2SO_4, 0.2 ml of 0.2% *m*/*V* ammonium oxalate solution, 1.5 ml of PHDAC solution and 0.5 ml of *o*-NPF solution. Dilute the solution to volume. Allow to stand for about 10 min, then measure the absorbance at 515 nm against a reagent blank and determine the concentration of tin using the calibration equation (see under Results and Discussion). In the presence of large amounts of Mo^{VI} and/or W^{VI}, measure the absorbances between 490 and 540 nm at 1 nm intervals and treat the data using the Kalman filtering algorithm.

Results and Discussion

Sensitising Effect of PHDAC on the Tin - *o*-NPF System

The presence of PHDAC had little effect on the absorption spectrum of the tin - *o*-NPF solution was essentially the same as that of the reagent blank. The addition of PHDAC drastically changed the pattern, showing strong bathochromic and hyperchromic shifts. The sensitised spectrophotometric system showed an absorption maximum at 515 nm, and this wavelength was employed for further measurements. A comparison of the sensitising effect of PHDAC with those of some other surfactants is given in Table 1.

wavelength was employed for further measurements. A comparison of the sensitising effect of PHDAC with those of some other surfactants is given in Table 1.

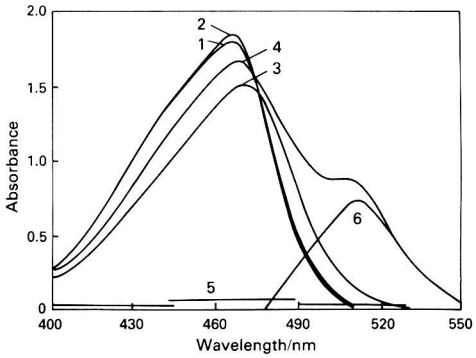


Fig. 1. Absorption spectra as measured against water: (1) *o*-NPF; (2) Sn - *o*-NPF; (3) *o*-NPF-PHDAC; (4) Sn - *o*-NPF-PHDAC. Absorption spectra as measured against the reagent blank: (5) Sn - *o*-NPF; (6) Sn - *o*-NPF-PHDAC. [Sn] = 3.83×10^{-6} M, [*o*-NPF] = 5.0×10^{-5} M, [PHDAC] = 0.3% *m/V*, [H_2SO_4] = 0.75 M

Table 1. Spectrophotometric characteristics of several sensitised Sn^{4+} - *o*-NPF reactions

System	λ_{max}/nm	Molar absorptivity/ l cm^{-1} mol^{-1}
Sn - *o*-NPF - PHDAC	515	1.85×10^5
Sn - *o*-NPF - CTMAB	515	1.61×10^5
Sn - *o*-NPF - CPB	515	1.52×10^5
Sn - *o*-NPF - Triton X-100	515	1.10×10^5

Effects of Experimental Conditions on Complex Formation

The absorbance of the complex was essentially constant over the acidity range 0.25–1.0 M in H_2SO_4. Maximum complex formation was achieved by adding more than 0.4 ml of *o*-NPF solution; when more than 2.5 ml was added, the absorbance decreased. In order to obtain the maximum absorbance at least 0.5 ml of PHDAC solution had to be added. The absorbance remained unchanged for at least 10 h.

Composition of the Complex

The Sn : *o*-NPF ratio was found to be 1 : 2 both by the method of continuous variations and by the method of Bent and French.[15]

Calibration

The absorbance data were measured according to the recommended procedure by taking appropriate aliquots of the standard tin solution. The data were fitted by standard least-squares treatment, and the following calibration equation was obtained: $Y = 0.01 + 1.56X$, $r = 0.996$, where Y is the absorbance, X is the content of tin in μg ml^{-1} and r is the correlation coefficient. Beer's law was obeyed over the range 0.2–5.0 μg of tin in 10 ml of solution, with a molar absorptivity of 1.85×10^5 l cm^{-1} mol^{-1} at 515 nm.

Effect of Foreign Ions

The effects of interferences from foreign ions were investigated in a 10-ml solution containing 3.4 μg of tin. There was no interference from 20 mg of Al^{III}, Zn^{II}, Cu^{II}, 5 mg of Fe^{III}, Mn^{II}, Pb^{II}, 1 mg of Mg^{II}, Cd^{II}, 0.1 mg of Si^{IV}, Cr^{VI}, V^{V} or 0.02 mg of Nb^{V}, Ti^{IV}, Sb^{III}, W^{VI} and Zr^{IV}. The tolerable amount of Mo^{VI} was 0.002 mg. Germanium interfered with the tin determination.

Table 2. Determination of tin in solutions containing Mo^{VI} and/or W^{VI} with or without Kalman filtering. Initial estimates: $X(0) = 0$; $P(0) = 10^{-2} \cdot I_3$. Measurement noise variance $R(k) = 10^{-6}$ for all wavelengths

Mo added/ μg	W added/ μg	Sn added/ μg	Sn found: Without filtering/μg	Sn found: With filtering/μg
0.0	0.0	1.71	1.71	1.70
0.0	0.0	4.54	4.53	4.51
10.0	0.0	0.00	2.04	0.002
0.0	50.0	0.00	1.52	0.007
10.0	0.0	2.27	4.28	2.20
0.0	50.0	1.71	3.28	1.74
0.0	100.0	2.27	†	2.21
15.0	0.0	1.14	4.10	1.12
10.0	50.0	2.27	†	2.29

† The absorbance lay beyond the linear range of the calibration graph.

Kalman Filtering as a "Chemometrics Filter"

Tungsten and molybdenum are common elements in steel samples, and the chemical means of overcoming interferences from these elements were not very satisfactory. A Kalman filter was designed to determine the concentration of tin and to suppress the effects of tungsten and/or molybdenum.

As mentioned above, the theory of Kalman filtering for multi-component analysis has been extensively treated elsewhere. Only the essentials of this technique for the case described are covered here. The concentrations of the components do not change during the absorbance measurement, and if the model error is negligible, the system model can be expressed as

$$X(k) = I \cdot X(k-1) \quad . . \quad . . \quad . . \quad (1)$$

where $X(k)$ is the vector of concentrations of Sn^{IV}, Mo^{VI} and W^{VI} at wavelength k in the spectrum and I is the identity matrix. The measurement process model

$$Z(k) = S^T(k) \cdot X(k) + \mathbf{v}(k) \quad . . \quad . . \quad (2)$$

relates the measurement of absorbance, $Z(k)$, to the component concentrations by a 3-row vector with the specific absorptivities (ml cm^{-1} μg^{-1}) defining the measurement function, $S^T(k)$, and $\mathbf{v}(k)$ is the noise contribution to the measurement of absorbance at wavelength k. The basic algorithm equations are as follows:

$$X(k|k-1) = X(k-1|k-1) \quad . . \quad . . \quad (3)$$

$$P(k|k-1) = P(k-1|k-1) \quad . . \quad . . \quad (4)$$

$$K(k) = P(k|k-1) \cdot S(k) \cdot [S^T(k) \cdot P(k|k-1) \cdot S(k) + R(k)]^{-1} \quad (5)$$

$$X(k|k) = X(k-1|k-1) + K(k) \cdot [Z(k) - S^T(k) \cdot X(k|k-1)] \quad (6)$$

$$P(k|k) = [I - K(k) \cdot S^T(k)] \cdot P(k|k-1) \cdot [I - K(k) \cdot S^T(k)]^T + K(k) \cdot R(k) \cdot K^T(k) \quad (7)$$

where $X(k|k)$ is the concentration estimate update, $P(k|k)$ the error covariance update, $K(k)$ the Kalman gain and $R(k)$ the absorbance measurement variance at wavelength k. T stands for the transpose of the matrix. The measurement function, $S^T(k)$, was calculated from the single-component spectrum. The measurement range of the wavelength of the spectrum lay between 490 and 540 nm. The measurements were made every 1 nm, and processed from lower to higher wavelengths. Table 2 shows some results obtained which indicate that the tolerable amounts of both Mo^{VI} and W^{VI} can be increased at least six times. Similarly, the interference from other co-existing ions can also be eliminated by using a corresponding Kalman filter, if necessary.

Table 3. Determination of tin in metallurgical samples

Sample	Tin content, %	Tin found, %	S.D.	Number of determinations
Copper alloy	0.089	0.090	0.0021	5
Aluminium alloy	0.079	0.078	0.0035	5
Steel	0.0064	0.0061	0.00026	6

Applications

Tin was determined in samples from various sources without any preliminary separation. The steel samples were analysed by the spectrophotometric method with the aid of Kalman filtering. Table 3 shows the results of analysing several metallurgical standard reference materials.

Analysis of copper or aluminium alloys

Dissolve the weighed sample in a mixture of 5 ml of 5 M H_2SO_4 solution and 1 ml of hydrogen peroxide. Dilute with water and boil the solution. Cool, and dilute the solution to 50 ml in a calibrated flask. Pipette 0.5 ml of the solution into a 10-ml calibrated flask and determine tin using the recommended procedure.

Analysis of steel samples

Dissolve the sample in a minimum amount of dilute nitric acid (1 + 3) with several drops of 15% *m/V* $(NH_4)_2S_2O_8$ solution added. Evaporate the solution to almost dryness. Add 10 ml of 5 M H_2SO_4 solution and boil the solution for several minutes. Transfer the solution into a 50-ml calibrated flask and dilute to the mark with water. Pipette a 0.5-ml aliquot of the solution into a 10-ml calibrated flask and proceed as outlined for copper and aluminium. Treat the absorbance data using the Kalman filtering algorithm to eliminate the interference from molybdenum and tungsten.

This work was supported by the National Natural Science Foundation, PRC.

References

1. Kulkarni, V. H., and Good, M. L., *Anal. Chem.*, 1978, **50**, 973.
2. Dagnall, R. M., West, T. S., and Young, P., *Analyst*, 1967, **92**, 27.
3. Mori, I., *Bunseki Kagaku*, 1970, **19**, 455.
4. Con, T. H., Nemcova, I., Nemec, I., and Suk, V., *Anal. Chim. Acta*, 1980, **115**, 279.
5. Nazarenko, V. A., and Lebedeva, N. V., *Zh. Anal. Khim.*, 1955, **10**, 289.
6. Shen, H. X., and Wang, L. S., *Huaxue Shiji*, 1983, **5**, 262.
7. Lilley, T., *Anal. Proc.*, 1984, **21**, 147.
8. Poulisse, H. N. J., *Anal. Chim. Acta*, 1979, **112**, 361.
9. Brown, T. F., and Brown, S. D., *Anal. Chem.*, 1981, **53**, 1410.
10. Scolari, C. A., and Brown, S. D., *Anal. Chim. Acta*, 1984, **166**, 253.
11. Thijssen, P. C., Prop, L. T. M., Kateman, G., and Smit, H. C., *Anal. Chim. Acta*, 1985, **174**, 27.
12. Poulisse, H. N. J., and Engelen, P., *Anal. Lett.*, 1980, **13** (A14), 1211.
13. Rutan, S. C., and Brown, S. D., *Anal. Chim. Acta*, 1984, **160**, 99.
14. Liu, Y. M., and Yu, R. Q., paper presented at the "Microtechniques and Trace Analysis Symposium, Wuhan, 1986," to be published.
15. Bent, H. E., and French, C. L., *J. Am. Chem. Soc.*, 1941, **63**, 568.

Paper A7/12
Received January 14th, 1987
Accepted March 2nd, 1987

Extraction - Spectrophotometric Determination of Sulphur Dioxide

P. Selvapathy
Centre for Environmental Studies, Anna University, Madras-600 025, India

T. V. Ramakrishna
Department of Chemistry, Indian Institute of Technology, Madras-600 036, India

N. Balasubramanian and R. Pitchai
Centre for Environmental Studies, Anna University, Madras-600 025, India

A sensitive spectrophotometric method for the determination of trace amounts of sulphur dioxide, after fixation in buffered formaldehyde solution, is described. The method is based on the reaction between iodate and sulphur dioxide in the presence of an excess of chloride ions. The resulting ICl_4^- species forms an ion pair with pyronine-G, which is then extracted into benzene. The colour system obeys Beer's law in the range 0–4 μg of sulphur dioxide. The coefficient of variation is 4.3% for 10 determinations of 2 μg of sulphur dioxide. The effect of interfering gases is discussed. The method has been applied to the determination of sulphur dioxide in ambient air in the vicinity of a coal-fired power station and the results have been compared with the widely used pararosaniline method.

Keywords: *Sulphur dioxide determination; extraction - spectrophotometry; ion pair formation; pyronine-G*

Air pollution due to sulphur dioxide has arisen mainly as a consequence of the widespread use of sulphur and its compounds in manufacturing and industrial processes and the increased use of fossil fuels as a source of energy. It has been estimated that the combustion of coal and petroleum products contribute 70 and 16%, respectively, of man-made sulphur dioxide pollution.[1] As the presence of sulphur dioxide in ambient air is known to be a health hazard, the development of analytical methods for its determination has attracted considerable attention.

The formation of a red complex between sulphur dioxide trapped in tetrachloromercurate(II) solution and acid-bleached pararosaniline in the presence of formaldehyde is widely used for the spectrophotometric determination of sulphur dioxide.[2] Although this method is reasonably specific and sensitive, it was reported to lack reproducibility, particularly when dyes from different sources were used.[3]

A highly sensitive method for the determination of sulphur dioxide, after fixation as sulphite in an alkaline medium, has been reported by Ramakrishna and Balasubramanian.[4] The reaction of sulphur dioxide with iodate in an acidic medium containing chloride ions to produce the ICl_4^- species formed the basis of this method. The ICl_4^- species formed an ion pair with rhodamine-6G and was extracted into benzene for spectrophotometric measurement at 540 nm. This approach, however, has the disadvantage that an alkaline medium for fixing atmospheric sulphur dioxide can also collect other acidic impurities and interfere in the determination. The use of tetrachloromercurate(II) for trapping sulphur dioxide, on the other hand, can lead to a serious interference arising from interaction of the dye cation with the $HgCl_4^{2-}$ species.

The use of a buffered formaldehyde solution for trapping sulphur dioxide has been advocated by Dasgupta *et al.*[5] Our studies of this trapping solution for sulphur dioxide revealed that the reaction leading to the formation of ICl_4^- can be exploited for the reliable determination of very low concentrations of sulphur dioxide in ambient air when the interaction is with the pyronine-G cation rather than the rhodamine-6G cation. The use of pyronine-G provided not only superior sensitivity but also low blank values. This paper describes the study and evaluation of the variables which govern the interaction with the pyronine-G cation to provide a basis for a selective spectrophotometric method for the determination of sulphur dioxide.

Experimental

Apparatus

The absorbance was measured using a CECIL Model CE 373, linear read-out grating spectrophotometer with 10 mm quartz cells. Fritted glass bubblers, with suitable suction devices, were used for trapping sulphur dioxide from air. The air flow-rate was measured using a rotameter.

Reagents

All chemicals used were of analytical-reagent grade and distilled water was used for preparing the reagent solutions.

Standard sulphur dioxide solution, ca. 350 p.p.m. Prepared by dissolving 0.4 g of anhydrous sodium sulphite in 500 ml of water and standardising iodimetrically.[6] A suitable volume of this solution was diluted using a 7 mM solution of formaldehyde to give a solution containing 1 μg ml^{-1} of sulphur dioxide. This solution remained stable for at least one month.

Potassium iodate solution, 0.003%.

Pyronine-G solution, 0.03%.

Sodium chloride solution, 15%.

Sodium hydroxide solution, 4.5 N.

The above solutions were prepared by dissolving appropriate amounts of the reagents in water.

Sulphuric acid, 5.5 N. Prepared by suitable dilution of concentrated sulphuric acid.

Buffered formaldehyde solution. Prepared by diluting 530 μl of formaldehyde solution (37%) and 204 mg of potassium hydrogen phthalate to 1 l with water.[5] The solution, which was 7 mM in formaldehyde and 1 mM in potassium hydrogen phthalate, had a pH of 4.2 at 25 °C.

Benzene. This solvent, free from thiophene,[7] was used for extraction purposes.

Procedure

Sampling

Air samples were collected by drawing 10–100 l of air through a fritted glass bubbler containing 15 ml of buffered formaldehyde solution for a period of 25–250 min at a rate of 0.4 l min^{-1}. Sulphur dioxide fixed in formaldehyde solution is stable for at least one month. The volume of the solution was made up to 15 ml with water prior to determination.

Determination

Into a 50-ml calibrated flask were placed 5 ml solutions of 0.003% potassium iodate, 0.03% pyronine-G, 5.5 N sulphuric acid and 15% sodium chloride. An aliquot of the formaldehyde solution, containing not more than 4 μg of sulphur dioxide, was treated with 1 ml of 4.5 N sodium hydroxide solution. The solution was then introduced into the 50-ml calibrated flask through a long-stem funnel with the tip kept well immersed in the reagent solution. The solution was diluted to volume, mixed well and allowed to stand for 5 min. It was then transferred into a 125-ml separating funnel and extracted with 5 ml of benzene for 15 s. The organic layer was separated and transferred into a test-tube and treated with about 1 g of anhydrous sodium sulphate to remove trace amounts of water before stoppering. The absorbance of the benzene extract was measured at 535 nm in 10 mm cells against a reagent blank run through the entire procedure. The concentration of sulphur dioxide was established by reference to a calibration graph prepared by treating 0–4 ml of standard sulphur dioxide solution (0–4 μg of sulphur dioxide) with 15 ml of buffered formaldehyde solution and following the described procedure.

Results and Discussion

Preliminary studies were carried out using 5 ml solutions of 0.003% potassium iodate, 0.03% pyronine-G, 5.5 N sulphuric acid and 15% sodium chloride. The resulting solution was reacted with 3 μg of sulphur dioxide in 15 ml of buffered formaldehyde solution after treating with 1 ml of 4.5 N sodium hydroxide solution to break the formaldehyde - bisulphite complex. The extractions were carried out after diluting to 50 ml with water and then equilibrating for 15 s with 5 ml of benzene. The results indicated that the extraction of the ion pair into benzene was fairly selective as the absorbance of the blank at 535 nm, the maximum absorbance of the ion pair, was found to be low.

The optimum acidity for the formation of the ICl_4^- species for subsequent association with the pyronine-G cation was first established. A constant and maximum absorbance was obtained when the over-all acidity of the reaction medium containing the optimum concentration of other reagents was greater than 0.4 N. The results are shown in Fig. 1. Subsequent studies were carried out using solutions maintained at 0.45 N for the formation and extraction of the ion pair.

The effect of potassium iodate concentration on the formation of the ion pair was investigated using 1–8 ml of a 0.003% solution of potassium iodate in a final volume of 50 ml. For the maximum interaction, the presence of at least 3 ml of a 0.003% *m/V* solution of potassium iodate was found to be essential. Similar studies on the effect of pyronine-G and sodium chloride concentrations revealed that 4 ml of a 0.03% *m/V* solution of pyronine-G and 4.5 ml of 15% *m/V* sodium chloride were sufficient to provide a constant and maximum absorbance. The presence of higher concentrations of pyronine-G and sodium chloride did not affect the recovery of sulphur dioxide, but caused a slight increase in the blank value.

The formation of the ion pair was found to be almost instantaneous and mixing the phases for about 15 s was found to be sufficient for its quantitative extraction into benzene. The colour system, after extraction, was found to remain stable for 10 min, after which a gradual decrease in the absorbance was noticed.

Benzene, toluene, hexane, cyclohexane, carbon tetrachloride, chloroform, butyl acetate and isoamyl acetate were investigated as extraction solvents; however, only benzene proved to be satisfactory as the extraction of the ion pair was found to be maximum only in this instance.

A linear calibration graph was obtained over the concentration range 0–4 μg of sulphur dioxide when the ion pair was extracted into 5 ml of benzene from 50 ml of the diluted solution, as described in the recommended procedure. The precision of the proposed procedure was checked by establishing the concentration of 10 samples containing 2 μg of sulphur dioxide. The mean recovery was found to be 1.99 μg with a coefficient of variation of 4.3%.

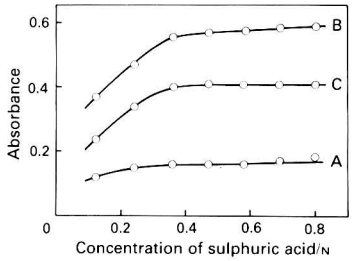


Fig. 1. Effect of acidity on absorbance. (A) 15 ml of 7 mM HCHO, 1 ml of 4.5 N NaOH, 0–8 ml of 5.5 N H_2SO_4, 5 ml each of 0.003% KIO_3, 0.03% pyronine-G and 15% NaCl solutions, diluted to 50 ml, extracted with 5 ml of benzene and measured against benzene at 535 nm in 10 mm cells. (B) As in (A), with 3 μg of SO_2. (C) As in (B), measured against reagent blank (A)

Effect of Interfering Gases

The effect of other common air pollutants in the determination of 3 μg of sulphur dioxide was studied by introducing the gas under examination with sulphur dioxide into an air stream passing through the trapping medium at 0.4 l min^{-1} and then determining sulphur dioxide by the recommended procedure. Up to 500 μg of carbon monoxide and acetylene did not interfere in the determination of 3 μg of sulphur dioxide. Nitrogen dioxide, when present in excess of 5 μg, caused a low recovery. The negative interference of up to 200 μg of nitrogen dioxide was overcome by the incorporation of 2 ml of a 5% solution of sulphamic acid with the potassium iodate and other reagent solutions. Hydrogen sulphide interfered seriously at all levels causing positive errors. Attempts to overcome the interference of hydrogen sulphide either by precipitating out as insoluble metal sulphides of cadmium, zinc, silver, etc., or by selective oxidation of the trapped sulphide with oxidising agents such as hydrogen peroxide and bromine water, prior to reaction with potassium iodate, were not successful as the recoveries were found to be erratic. Only the use of a pre-trap packed with silver sulphate coated glass beads[8] was found to be effective in overcoming its interference. Using such a trap it was possible to overcome the interference of up to 20 μg of hydrogen sulphide in the determination of 3 μg of sulphur dioxide.

Application of the Method to Field Samples

Air samples in the vicinity of a coal-fired power station were collected and analysed by the recommended procedure. The results were compared with those obtained using the standard pararosaniline procedure.[5] Whereas the aliquots used for determination by the proposed procedure ranged from 2 to 4 ml, 10-ml aliquots of the sampled solution were used for determination by the pararosaniline method. A pre-trap packed with silver sulphate coated glass beads was used to overcome the interference of hydrogen sulphide, if any. A 2-ml volume of a 5% solution of sulphamic acid was incorporated with the potassium iodate and other reagent solutions to overcome the interference of nitrogen dioxide. The results obtained are shown in Table 1, from which it is clear that the results obtained by both the methods are in good agreement.

Table 1. Analysis of air near a coal-fired power station

Sample No.	Sulphur dioxide found*/μg	
	Pararosaniline method	Pyronine-G method
1	11.25 (44.77)	11.6 (46.17)
2	15.00 (59.69)	15.0 (59.69)
3	8.20 (32.62)	8.0 (31.83)
4	10.00 (39.79)	10.3 (40.99)
5	12.40 (49.34)	12.2 (48.54)
6	16.00 (63.67)	16.1 (64.06)

* The data in parentheses gives the concentration of sulphur dioxide in p.p.b.

Conclusions

Sulphur dioxide can be precisely determined down to 0.4 μg using the proposed procedure. The calibration graph is rectilinear in the range 0–4 μg of sulphur dioxide. The coefficient of variation is 4.3% for 10 determinations of 2 μg of sulphur dioxide. The application of this method to the determination of sulphur dioxide in atmospheric samples has demonstrated the usefulness of the method for determinations at very low levels.

References

1. Robinson, E., and Robbins, R. A., "Sources, Abundance and Fate of Gaseous Atmospheric Pollutants," Final Report, SRI Project SCC-8501, Standford Research Institute, Menlo Park, CA, 1968.
2. West, P. W., and Gaeke, G. C., *Anal. Chem.*, 1956, **28**, 1816.
3. Stephens, B. G., and Suddeth, H. A., *Analyst*, 1970, **95**, 70.
4. Ramakrishna, T. V., and Balasubramanian, N., *Indian J. Chem.*, 1982, **21A**, 217.
5. Dasgupta, P. K., Cesare, K. D., and Ullrey, J. C., *Anal. Chem.*, 1980, **52**, 1912.
6. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," 2nd Edition, Longman, London, 1957, p. 354.
7. Vogel, A. I., "A Text Book of Practical Organic Chemistry Including Qualitative Organic Analysis," Longman, London, 1971, pp. 172–173.
8. Buck, M., and Gies, H., *Staub*, 1966, **26**, 379.

Paper A7/37
Received February 14th, 1987
Accepted March 18th, 1987

Glass Transition Temperatures of Poly(Vinyl Chloride) and Polyacrylate Materials and Calcium Ion-selective Electrode Properties*

G. J. Moody, B. Saad and J. D. R. Thomas†
Department of Applied Chemistry, Redwood Building, UWIST, P.O. Box 13, Cardiff CF1 3XF, UK

The glass transition temperatures of eight poly(vinyl chloride) (PVC) samples of different relative molecular masses in addition to poly(methyl acrylate), poly(methyl methacrylate) and poly(2-methylpropyl methacrylate) were determined, in some instances as electrode master membranes with incorporated sensor and solvent mediators. They were also investigated as support matrices for calcium ion-selective electrodes (ISEs) based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} with dioctylphenyl phosphonate or other liquids as solvent mediator. No important difference in terms of response times, selectivity coefficients, Nernstian slopes, membrane resistances or pH - e.m.f. profiles were exhibited by the eight PVC-based electrodes. Functional calcium electrodes were also fabricated with a poly(2-methylpropyl methacrylate) matrix but not with corresponding poly(butyl methacrylate), poly(methyl methacrylate) or poly(methyl acrylate) matrices.

Keywords: *Calcium ion-selective electrodes; glass transition temperatures; poly(vinyl chloride) and polyacrylate matrix ion-selective electrode membranes*

Poly(vinyl chloride) (PVC) has been the most commonly used polymer support in the fabrication of polymer membrane and coated-wire ion-selective electrodes since it was pioneered at UWIST on the simple design principle of dissolving PVC in a solution of sensing cocktail in tetrahydrofuran and removal of the tetrahydrofuran by evaporation.[1] A few alternative polymer matrices, such as poly(methyl acrylate),[2] poly(vinyl 2-methylpropyl ether),[3] Urushi[4] and block copolymers of poly(bisphenol A carbonate) with poly(dimethylsiloxane)[5] or polyurethane[6] are also suitable but none seriously challenges the established popularity of PVC. This relates in part to its high tensile strength, chemical inertness, glass transition temperature, availability, low cost and easy fabrication. However, high-performance PVC ion-selective electrodes with long lifetimes and rapid response times are now available for many ions, yet little is known about the nature of the viable PVC itself.

It has been suggested[6] that polymers such as polyurethane and silicone rubber are suitable matrices for making membranes because they are rubbery and the glass transition temperatures of their master membranes are below room temperature. However, in response to this, there is little information relating ion-selective electrode performance to glass transition temperatures of the polymers used as membrane support matrices in liquid membrane electrodes; nor is there information on the extent to which the plasticising solvent mediators lower the glass transition temperature.

This study concerns an evaluation of calcium ion-selective electrodes based on a cocktail of calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} and solvent mediator when incorporated in eight different PVCs, poly(methyl acrylate), several polymethacrylates and also the measurement of the glass transition temperatures of various polymers. In addition, the glass transition temperatures of some master membranes, that is, with incorporated solvent mediators and calcium sensors, were measured.

Experimental

Reagents and Materials

All chemicals and materials were of the best laboratory-reagent grade, except calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate}, which was synthesised.[7]

PVC samples 1–7 were the polymers of various molecular masses (Table 1) determined by gel permeation chromatography at RAPRA, Shrewsbury, produced for the IUPAC Working Party on the fine structure and thermal stability of PVC (gifts from Dr. G. S. Park, UWIST). PVC 8 was the Breon Resin III EP obtained from British Petroleum and used at UWIST since 1970.

The poly(methyl acrylate) was prepared as described by Hassan *et al.*[2] Poly(methyl methacrylate) was a medium relative molecular mass polymer (M_w 90 000) obtained from Aldrich. Poly(butyl methacrylate) and poly(2-methylpropyl methacrylate) were obtained from Monomer-Polymer (Leominster, MA).

All solutions were prepared using doubly de-ionised water and chlorides of the metals.

Apparatus

Glass transition temperatures, T_g, of PVC, poly(methyl acrylate) and several polymethacrylate powders were determined using a Perkin-Elmer DSC 2-C differential scanning calorimeter. The samples (*ca.* 5 mg) were placed in an aluminium pan sample holder, properly sealed and run under air, with a heating rate of 40 °C min^{-1}, range 5 mcal s^{-1} and chart speed 40 mm min^{-1}.

The T_g values of polymer membranes incorporating the plasticising solvent and the calcium sensor were kindly determined by British Petroleum Chemicals (Grangemouth) over the range −140 to 130 °C at 10 °C min^{-1} using a Du Pont 1090 thermal analyser.

The membrane resistances were measured on the membrane of assembled electrodes, not just the PVC membrane, after storage for 48 h in 10^{-1} M calcium chloride solution using a Bruker E130M potentio/galvanostat.

The e.m.f. measurements were made at 25 ± 1 °C relative to a Corning ceramic-junction saturated calomel reference electrode with a Beckman Model 4500 digital pH - millivoltmeter coupled to a Servoscribe 1S potentiometric recorder.

Electrodes

Calcium ion-selective electrode membranes were cast from 0.04 g of calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]-phosphate} and 0.36 g of dioctylphenyl phosphonate in 0.17 g

* Presented at the International Symposium on Electroanalysis and Sensors in Biomedical, Environmental and Industrial Sciences, Cardiff, UK, April 6–9, 1987.

† To whom correspondence should be addressed.

of PVC (or other polymers) in 6 cm^3 of tetrahydrofuran using established procedures.[8] For conditioning purposes, the e.m.f. was monitored immediately after filling with the internal reference solution (0.1 M calcium chloride). Except for initial air storage for 24 h, the electrodes were always kept in 0.1 M calcium chloride between measurements. The selectivity coefficients were determined using the separate solution method[9] at a 10^{-2} M concentration of cations. The pH interference was obtained by adjusting the pH of 20 cm^3 of 10^{-2} M calcium chloride to *ca.* 10 with 0.1 M sodium hydroxide and measuring the e.m.f. after successive spiking with 0.1 M hydrochloric acid to a final pH of *ca.* 3.

Results and Discussion

Poly(Vinyl Chloride) Systems

Glass transition temperatures and relative molecular masses of eight PVCs, and the resistance and calcium ion-selective electrode properties of membranes made using the appropriate proportions of sensor, solvent mediator and the various PVCs with tetrahydrofuran as casting solvent, are shown in Table 1. All master membranes were clean, transparent and flexible. PVCs with high relative molecular masses and high T_g values gave strong master membranes (especially PVCs 1 and 8), which did not stick to the glass plate. However, there were no problems in fabricating any electrode even with the stickiest PVC membrane (PVC 3).

The spread of relative molecular masses of PVCs 1–7 (Table 1) is indicated by the heterogeneity index $\overline{M}_w/\overline{M}_n$, where a value of 2.0 indicates an ideal distribution of relative molecular mass. The T_g and $\overline{M}_n$ values for PVC 8 indicate that the sample should have a high average relative molecular mass. Here, $\overline{M}_w$ and $\overline{M}_n$ are the weight-average relative molecular mass (given by light-scattering measurements) and number-average relative molecular mass (given by osmometry), respectively; $\overline{M}_w = \Sigma M_i^2 n_i/\Sigma M_i$ and $\overline{M}_n = \Sigma n_i M_i/\Sigma n_i$, where n_i is the number of molecules of the ith species, all of relative molecular mass M_i.

The freshly prepared electrodes were conditioned in 10^{-1} M calcium chloride solution and the responses during this process are shown in Fig. 1. All the electrodes gave a steady potential after a maximum of 2 h in calcium chloride solution. After air storage for 24 h, the electrodes were again immersed in fresh 10^{-1} M $CaCl_2$, when a steady e.m.f. was reached within 1 h. Hence for PVC calcium ion-selective electrodes, air storage could be practised if necessary as an alternative to the usual storage techniques. However, with other ion-selective electrodes where leaching is a problem, air storage may be the best prospect.

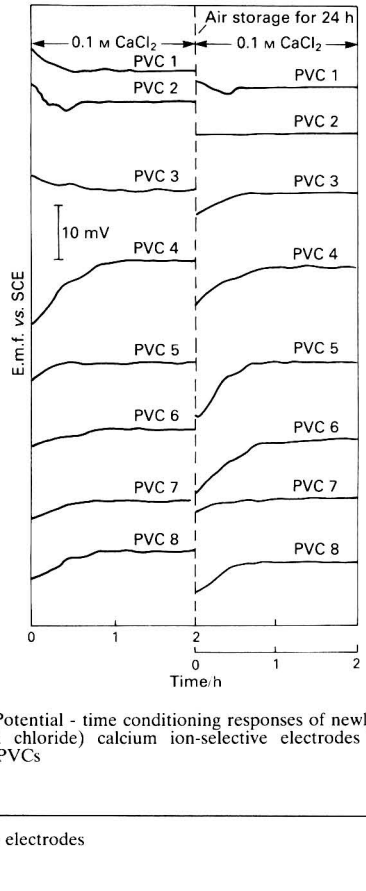


Fig. 1. Potential - time conditioning responses of newly fabricated poly(vinyl chloride) calcium ion-selective electrodes from eight different PVCs

Table 1. Some characteristics of IUPAC PVCs and associated calcium ion-selective electrodes

PVC and membrane number	$\overline{M}_n$	$\overline{M}_w$	T_g/°C	Electrode membrane resistance/MΩ	Time after electrode fabrication/weeks	Slope/mV decade^{-1}	Limit of detection/M	Selectivity coefficient,* $k^{pot}_{Ca,B}$ B = Mg	B = Na	B = K
1	47 150	175 300	102	2.02	1	32.0	2.5×10^{-6}	1.28×10^{-3}	3.90×10^{-2}	4.83×10^{-2}
					12	28.3	1.0×10^{-5}	5.72×10^{-4}	3.12×10^{-2}	2.61×10^{-2}
2	39 770	86 810	85	1.63	1	29.6	2.2×10^{-6}	9.81×10^{-4}	4.85×10^{-2}	3.53×10^{-2}
					12	30.0	1.1×10^{-5}	6.31×10^{-4}	3.83×10^{-2}	3.68×10^{-2}
3	25 900	81 330	86	1.89	1	30.0	7.8×10^{-6}	3.50×10^{-3}	0.13	0.11
					12	30.5	7.9×10^{-6}	8.98×10^{-4}	3.18×10^{-2}	5.90×10^{-2}
4	39 350	104 900	86	1.11	1	30.8	4.5×10^{-5}	1.04×10^{-3}	3.72×10^{-2}	7.59×10^{-2}
					12	30.4	1.1×10^{-5}	8.20×10^{-4}	6.14×10^{-2}	4.01×10^{-2}
5	48 520	104 800	85	2.09	1	27.0	4.5×10^{-6}	1.28×10^{-3}	3.80×10^{-2}	4.74×10^{-2}
					12	30.5	5.0×10^{-6}	1.30×10^{-3}	4.64×10^{-2}	5.93×10^{-2}
6	36 310	101 300	88	2.46	1	26.3	1.8×10^{-5}	1.31×10^{-3}	4.03×10^{-2}	7.51×10^{-2}
					12	30.8	1.6×10^{-5}	2.74×10^{-3}	0.12	0.13
7	43 700	103 400	86	1.28	1	26.5	1.2×10^{-5}	2.22×10^{-3}	4.80×10^{-2}	9.99×10^{-2}
					12	28.5	8.9×10^{-6}	1.86×10^{-3}	6.25×10^{-2}	0.12
8 (Breon) III EP)	77 000	—†	98	3.02	1	28.0	2.8×10^{-6}	2.18×10^{-3}	9.66×10^{-2}	1.03×10^{-2}
					12	30.8	3.2×10^{-6}	1.47×10^{-3}	5.57×10^{-2}	6.46×10^{-2}

* Separate solution method.
† Unavailable.

The average resistances of all the PVC electrodes are about the same after storage for 2 d in 10^{-1} M calcium chloride solution. These values are lower than the resistances of various commercially available calcium ISEs[9] and the long-lived potassium ion-selective electrodes prepared by LeBlanc and Grubb.[5]

A comparison was made between the response characteristics of 1-week- and 12-week-old PVC calcium ion-selective electrodes. The new and older electrodes gave good Nernstian responses (Table 1). There was also no significant difference in the limit of detection as determined using the recommended IUPAC method.[10] There was also no noticeable difference between the dynamic response times of both the 1-week- and 12-week-old electrodes, the dynamic values all being <10 s.

The average selectivity coefficients as determined by the separate solution method showed no significant change in selectivity between the 1-week- and 12-week-old electrodes (Table 1). Griffin and Christian[11] reported that PVC valinomycin electrodes plasticised by dipentyl phthalate showed no significant decrease in selectivity over 2 years. The pH - e.m.f. profiles of the newly fabricated PVC electrodes are shown in Fig. 2.

The characteristics of PVC electrodes with a thicker sensor membrane cut from a master membrane consisting of twice the standard amounts of PVC 8, CaX_2 and DOPP, respectively, were almost identical with all those mentioned for the standard PVC model.

The glass transition temperature, T_g, is linked to the temperature at which the PVC was prepared and it rises with decreasing polymerisation temperature.[12] As expected, PVC 1 (prepared at −30 °C) has a high T_g value (102 °C) (Table 1), whereas the values of 85–88 °C for the PVC samples 2–7 (prepared at 55 °C) are similar to the values reported by Brennan[13] using the DSC technique. The DSC profiles of the various PVC samples used in this study are shown in Fig. 3.

The T_g values were measured at the onset of the endothermic transition.

The PVC master membrane 8, incorporating PVC Breon III EP and the calcium salt with dioctylphenyl phosphonate, constitutes the standard calcium ISE membrane material for the purpose of this study. The T_g value of this reference sensor membrane (−65 °C) is substantially lower than that of the parent polymer (98 °C) but close to that of the master membrane cast without the calcium salt (−67 °C), as shown in Fig. 4.

It is interesting that viable PVC calcium ISEs could not be fabricated with the Aldrich low relative molecular mass PVC incorporating the same phosphate sensor and mediator utilised in this work.[14]

Polyacrylate Systems

As indicated above, little attention has been given to the prospect of the suitability of polymers other than poly(vinyl chloride) for ISE fabrication. Hence, master membranes 9–27 (Tables 2–5) were also cast from four different polyacrylates, calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate} sensor and several commonly used solvent mediators including dioctylphenyl phosphonate by employing the classical prescription[1,8] of mass contents.

Trials with poly(butyl methacrylate) and just solvent mediators to conserve calcium salt produced unmanageable master membranes (18–22 in Table 4) and no further studies were made. The master membranes with either poly(methyl acrylate) or poly(methyl methacrylate) plus CaX_2 and various mediators also failed to produce viable electrochemical sensor materials, although the T_g values of their dioctylphenyl phosphonate based membranes (12 and 16) were very low, namely −90 and −86 °C, respectively (Tables 2 and 3).

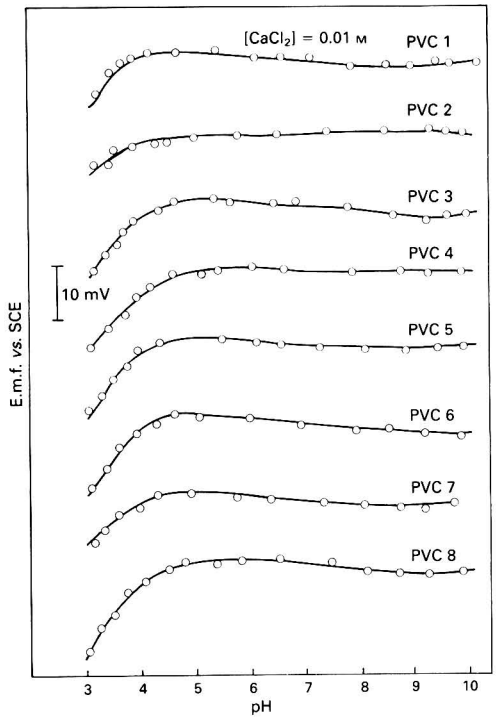


Fig. 2. Potential - pH responses of conditioned poly(vinyl chloride) calcium ion-selective electrodes for eight different PVCs

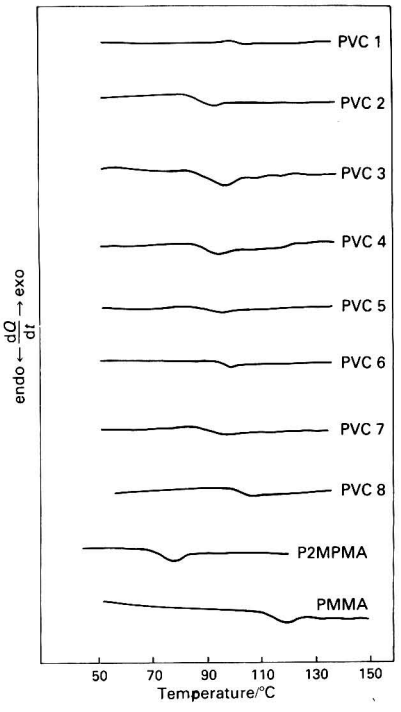


Fig. 3. DSC profiles of polymer samples. PVC = poly(vinyl chloride); P2MPMA = poly(2-methylpropyl methacrylate); and PMMA = poly(methyl methacrylate)

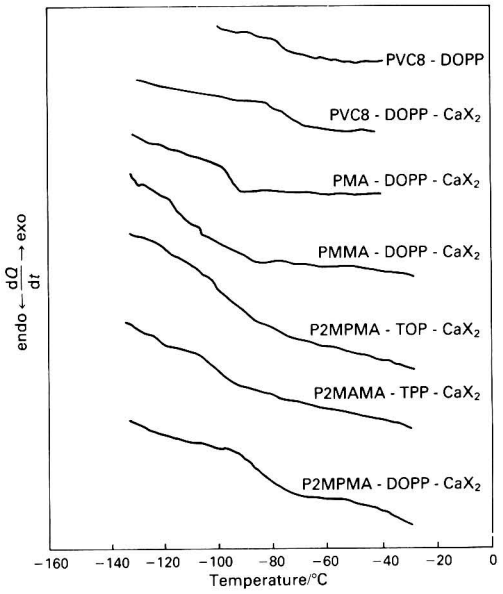


Fig. 4. DSC profiles of Breon III EP poly(vinyl chloride) (PVC 8) and polyacrylate matrices incorporating sensor cocktails. PVC = poly(vinyl chloride); PMA = poly(methyl acrylate); PMMA = poly(methyl methacrylate); P2MPMA = poly(2-methylpropyl methacrylate); DOPP = dioctylphenyl phosphonate; TOP = trioctyl phosphate; TPP = tripentyl phosphate; and CaX_2 = calcium bis-{di[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate}

However, functional calcium ISEs could be fabricated from poly(2-methylpropyl methacrylate) in conjunction with CaX_2 and either dioctylphenyl phosphonate or tripentyl phosphate (membranes 25 and 26 in Tables 5 and 6), but the selectivity quality was inferior to that of the corresponding Breon III EP model (Table 1).

The glass transition temperatures of the Breon III EP model and the polyacrylate master membranes in Tables 1–3 and 5 are much lower than that of the parent polymer alone, yet only PVC and poly(2-methylpropyl methacrylate) provide viable sensor membranes. In this respect, it is interesting that the T_g value of poly(2-methylpropyl methacrylate) (75 °C) is close to the range of values (85–102 °C) observed for the various PVCs (Tables 1 and 5). On the other hand, poly(methyl methacrylate) with a T_g of 108 °C yields a non-functional ISE membrane whereas poly(methyl acrylate) and poly(butyl methacrylate) are relatively soft polymers. Therefore, although poly(2-methylpropyl methacrylate) gives reasonable ISEs, the properties other than just the glass transition temperatures of polymers have a role in providing a matrix for the master membrane with the appropriate calcium electrode function.

Conclusion

There were no important differences in terms of the parameters evaluated for the PVC electrodes assembled from the eight PVC materials. However, the Breon III EP PVC will remain our first choice simply because it is readily available in large amounts. Even the best of the alternative acrylate polymers, namely poly(2-methylpropyl methacrylate), provides an inferior sensor matrix for the organophosphate-based liquid ion-exchanger calcium cocktail used.

Table 2. Composition and characteristics of poly(methyl acrylate)-based master membrane. T_g of poly(methyl acrylate): 8 °C

Membrane number	Solvent mediator	Mass of component/g: Mediator	Sensor	Polymer	Properties of master membrane
9	Trioctyl phosphate	0.360	0.04	0.170	Opalescent, stiff. Non-functional
10	Trioctyl phosphate				
11	Tripentyl phosphate			0.300	
12	Dioctylphenyl phosphonate				Clear, transparent, T_g = −90 °C. Non-functional
13	Dibutyl phthalate				Flexible, milky appearance and sticky. Non-functional

Table 3. Composition and characteristics of poly(methyl methacrylate)-based master membrane. T_g of poly(methyl methacrylate): 108 °C

Membrane number	Solvent mediator	Mass of component/g: Mediator	Sensor	Polymer	Properties of master membrane
14	Trioctyl phosphate	0.360	0.04	0.170	Opalescent, hard. Non-functional
15	Tripentyl phosphate				
16	Dioctylphenyl phosphonate				Opalescent, hard. T_g = −86 °C. Non-functional
17	Dibutyl phthalate				Very soft, transparent and very sticky. Too weak to hold inner reference solution. Non-functional

Table 4. Composition and characteristics of poly(butyl methacrylate)-based master membrane. T_g of poly(butyl methacrylate): 22 °C

Membrane number	Solvent mediator	Mass of component/g			Properties of master membrane
		Mediator	Sensor	Polymer	
18	Trioctyl phosphate	0.180	—	0.300	Each membrane was very soft, very sticky and too weak to hold the inner reference solution
19	Trioctyl phosphate	0.360	—		
20	Tripentyl phosphate		—		
21	Dioctylphenyl phosphonate		—		
22	Dibutyl phthalate		—		

Table 5. Composition and characteristics of poly(2-methylpropyl methacrylate)-based master membrane. T_g of poly(2-methylpropyl methacrylate): 75 °C

Membrane number	Solvent mediator	Mass of component/g			Properties of master membrane
		Mediator	Sensor	Polymer	
23	Trioctyl phosphate	0.360	0.04	0.170	Clear, transparent but too thin to handle
24	Trioctyl phosphate			0.300	Clear, transparent, sticky. $T_g = -83$ °C. Noisy response
25	Tripentyl phosphate				Clear, transparent, sticky. $T_g = -90$ °C. Functional
26	Dioctylphenyl phosphonate				Clear, transparent, sticky. $T_g = -73$ °C. Functional
27	Dibutyl phthalate				Clear, transparent, very sticky. Too weak to hold inner reference solution

Table 6. Some electrochemical characteristics of poly(2-methylpropyl methacrylate)-based calcium ISEs

Membrane number	Limit of detection/, M	Slope/ mV decade^{-1}	Useful pH range	Lifetime/ d	Selectivity coefficient, $*k^{pot}_{Ca,B}$			Resistance/ MΩ
					B = Mg	B = Na	B = K	
24	5.6×10^{-6}	19.5	—	3	—	—	—	558
25	7.1×10^{-6}	26.3	3.5–10	10	7.4×10^{-3}	0.168	0.138	12.7
26	6.0×10^{-6}	27.4	3.5–10	24	8.4×10^{-3}	0.193	0.170	26.2

* [B] = 10^{-2} M.

The authors are grateful to Dr. G. S. Park, UWIST, for valuable discussions concerning PVC characteristics and to Dr. K. Wilkinson and S. H. McMurdo, British Petroleum Chemicals plc (Grangemouth) for the low-temperature glass transition measurements. Financial assistance to B. Saad from the Government of Malaysia and Universiti Sains Malaysia is also gratefully acknowledged.

References

1. Moody, G. J., Oke, R. B., and Thomas, J. D. R., *Analyst*, 1970, **95**, 910.
2. Hassan, S. K. A. G., Moody, G. J., and Thomas, J. D. R., *Analyst*, 1980, **105**, 147.
3. Schäfer, O. F., *Anal. Chim. Acta*, 1976, **87**, 495.
4. Hiiro, K., Kawahara, A., and Tanaka, T., *Anal. Chim. Acta*, 1979, **110**, 321.
5. LeBlanc, O. H., and Grubb, W. T., *Anal. Chem.*, 1976, **48**, 1658.
6. Fiedler, U., and Růžička, J., *Anal. Chim. Acta*, 1973, **67**, 179.
7. Craggs, A., Delduca, P. G., Keil, L., Key, B. J., Moody, G. J., and Thomas, J. D. R., *J. Inorg. Nucl. Chem.*, 1978, **40**, 1483.
8. Craggs, A., Moody, G. J., and Thomas, J. D. R., *J. Chem. Educ.*, 1974, **51**, 541.
9. Moody, G. J., and Thomas, J. D. R., "Selective Ion-sensitive Electrodes," Merrow, Watford, 1971.
10. Guilbault, G. G., Durst, R. A., Frank, S., Freiser, H., Hansen, E. H., Light, T. S., Pungor, E., Rechnitz, G. A., Rice, N. M., Rohm, T. J., Simon, W., and Thomas, J. D. R., *Pure Appl. Chem.*, 1976, **48**, 127.
11. Griffin, J. J., and Christian, G. D., *Talanta*, 1983, **30**, 201.
12. Reding, F. P., Walter, E. R., and Welch, F. J., *J. Polym. Sci.*, 1962, **56**, 225.
13. Brennan, W. P., *Perkin-Elmer Thermal Analysis Application Studies*, 1977, **22**, 5.
14. Lakshminarayanaih, N., *J. Membr. Sci.*, 1981, **8**, 255.

Paper A6/448
Received November 24th, 1986
Accepted March 5th, 1987

Determination of Camazepam and Bromazepam in Human Serum by Adsorptive Stripping Voltammetry

Lucas Hernández, Antonio Zapardiel, José Antonio Pérez López and Esperanza Bermejo
Department of Analytical Chemistry, Autonoma University, 28049 Madrid, Spain

Adsorptive stripping voltammetry was used for the determination of trace amounts of the benzodiazepines camazepam and bromazepam. This very sensitive method is based on controlled adsorptive pre-concentration of the drugs on the hanging mercury drop electrode. Measurements were taken by differential-pulse voltammetry after determination of the optimum accumulation conditions. The response was linear in the range 3×10^{-9}–9×10^{-9} M (270 s and −0.60 V for the pre-concentration) for camazepam and 1×10^{-8}–8×10^{-8} M (90 s and −0.40 V) for bromazepam. In the concentration ranges investigated, the relative standard deviation was lower than 6%. The method is applicable to the determination of the drugs in human serum, with detection limits of 20 ng ml^{-1} of serum (30 s and −0.62 V for the pre-concentration) for camazepam and 200 ng ml^{-1} of serum (10 s and −0.41 V) for bromazepam.

Keywords: *Camazepam determination; bromazepam determination, adsorptive stripping voltammetry*

The ansyolitic, sedative and tranquillising properties of benzodiazepines make them of great interest as therapeutic agents. They are readily and quickly absorbed by organisms and are present in biological fluids just hours after administration. Benzodiazepines are clinically effective at doses of 0.5–30 mg, giving blood concentrations in the range 10–500 ng ml^{-1}. The metabolites of these drugs are usually equally active, thus contributing to the total duration of the action and accumulation processes in the organism.

From a clinical viewpoint, benzodiazepines are important drugs which are widely used both in psychological and somatic diseases. They greatly increase muscular relaxation, depress medular reflexes and, at high doses, lower blood pressure, with a varied additional toxicity. The widespread use of these compounds and the need for clinical, pharmacological and toxicological study, require fast and sensitive analytical techniques to determine the presence of the drug in several biological fluids.

Camazepam and bromazepam (Fig. 1) are two widely used benzodiazepines.

Bromazepam and some of its metabolites[1] [3-hydroxybromazepam, 2-(2-amino-5-bromobenzoyl)pyridine, bromazepam *N*(Py)-oxide, 2-(2-amino-5-bromo-3-hydroxybenzoyl)pyridine] have been found in blood, serum and urine. The biotransformation of camazepam gives rise to temazepam and oxazepam,[2] which can be identified in urine a few hours after oral administration.

Several techniques have been used to determine benzodiazepines in biological fluids. Gas - liquid chromatography, although highly sensitive, requires lengthy sample treatment procedures and can cause the loss of volatile compounds or heat-induced decomposition of some benzodiazepines. Differential-pulse polarography offers advantages in terms of speed but low sensitivity and specificity are important drawbacks. Because of these drawbacks, analysts have turned their attention towards the development of other techniques capable of achieving sensitivities similar to gas - liquid chromatography but free from its associated problems.

Polarography,[3–10] voltammetry,[5–7,11] gas[7,10,12–17] and liquid[9,10] chromatography and other techniques,[18–22] have all previously been used for the detection and determination of camazepam and bromazepam.

Differential-pulse polarography of bromazepam at the dropping mercury electrode gives three peaks. The higher potential peak is due to the 4,5-azomethine group; the other two are believed to be caused by a reduction process occurring in the pyridine group.[4] Camazepam shows a peak due to the 4,5-azomethine group plus a breaking down of the ether dimethylcarbamate group.[5–6]

Several electrochemical studies carried out on benzodiazepines, including electrocapillary curves and capacity - potential curves using the mercury electrode, have pointed to the existence of adsorption processes of the drugs. The determination of small amounts is possible by combination of the voltammetric response with interfacial accumulation of the drug on the electrode. In this way, low concentrations can be accurately determined by adsorptive stripping voltammetry. Pre-concentration is achieved by the adsorption of the analyte on the electrode surface and the determination of surface-active species by the application of a voltammetric sweep method.

The aim of this study was to establish suitable experimental conditions for the determination of camazepam and bromazepam by differential-pulse voltammetry using adsorptive stripping voltammetry at the hanging mercury drop electrode and its specific application to determination of the drugs in human serum.

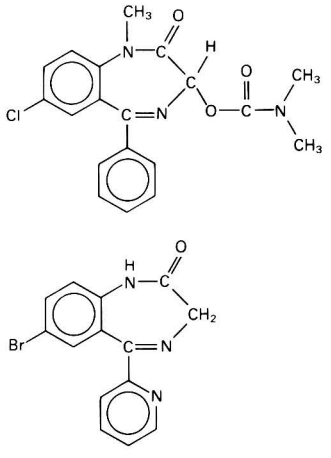


Fig. 1. Structures of 7-chloro-2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl dimethylcarbamate (camazepam) and 7-bromo-1,3-dihydro-5-(2-pyridyl)-2*H*-benzodiazepin-2-one (bromazepam)

The technique used has recently been applied in this laboratory to the determination of several benzodiazepines and tienodiazepines,[23] and various pharmaceutical compounds including diazepam and nitrazepam,[24] cardiac glycosides[25] and methotrexate.[26] A summary of all the investigated applications of this technique has recently been given by Wang.[27]

Experimental

Apparatus

A Metrohm 646 VA processor in conjunction with a 647 VA stand was used, and this allowed the total automation of the stripping procedure. A multi-mode mercury drop electrode (Metrohm 6.1246.020) was used, which served as the working electrode when in the hanging mercury drop electrode (HMDE) mode. The average drop size was 0.44 mm^2. An Ag - AgCl electrode (KCl, 3 M) was used as the reference electrode and a glassy carbon rod (2 × 65 mm) as the auxiliary electrode. The solution was stirred in the cell by means of a built-in rotor. Metrohm glass polarographic cells were used. All measurements were performed at room temperature.

Reagents and Solutions

Stock solutions (1.00×10^{-3} M) of pure camazepam (Farmasimes) and bromazepam (Hoffman La Roche) were prepared by dissolving the compounds in methanol. The solutions were stored in the dark under refrigeration to minimise the risk of decomposition.

The supporting electrolytes were Britton - Robinson (a mixture of boric, phosphoric and acetic acids at 0.04 M concentration), phosphate and acetate buffers of different ionic strength, prepared according to the procedure recommended by Mongay and Cerda.[28]

All chemicals used were of analytical-reagent grade from E. Merck. Aqueous solutions were prepared in purified water (Milli-Q and Milli-Ro, Millipore).

The human serum samples were pools from five subjects.

Procedures

Adsorptive stripping voltammetry

De-oxygenation with nitrogen was applied for 10 min in the initial cycle and then for 30 s in each successive cycle. An accumulation potential was applied to the working electrode while the solution was stirred continuously. After a 15-s rest time, a negative-going differential-pulse scan was initiated, the resulting voltammograms being recorded for different operational parameters.

Pre-conditioning of column and extraction procedure for determination of the drugs in human serum

A Waters Associates Sep-Pak C_{18} extraction cartridge was pre-washed once with 5 ml of methanol and twice with 3 ml of water. After the last rinse, the cartridge was buffered to pH 9.12 with 2 ml of 0.01 M sodium tetraborate (pH 9.12). For the extraction, 1 ml of human serum containing 25–2500 ng of camazepam or bromazepam was passed through the Sep-Pak cartridge, the drugs being adsorbed on the Sep-Pak matrix. The matrix was then rinsed twice with 2 ml of water. For elution of the drugs, two 2-ml portions of diethyl ether were passed through the Sep-Pak column. The total eluent (4.0 ml) was dried under a stream of nitrogen. The residue was reconstituted with 100 µl of methanol and diluted to 25.0 ml with 0.04 M acetate buffer (pH 5.0) for camazepam or with 0.04 M Britton - Robinson buffer (pH 5.0) for bromazepam. The voltammograms were recorded under the optimum instrumental conditions.

Results and Discussion

Spontaneous adsorption of camazepam and bromazepam on the surface of the HMDE was observed by differential-pulse voltammetry. This adsorption can be used as an effective pre-concentration step prior to voltammetric measurement. In this way, highly sensitive determinations of the compounds can be achieved by adsorptive stripping voltammetry.

Fig. 2 shows the voltammograms obtained for solutions of (*a*) 1.29×10^{-8} M camazepam and (*b*) 6.02×10^{-8} M bromazepam, with pre-concentration periods of 270 and 90 s, respectively. The corresponding response with no accumulation is also shown. Well defined stripping peaks were observed for both drugs, with peak potentials of −0.90 V for camazepam and −0.55 V for bromazepam, and peak half-widths of 45 and 38 mV, respectively; these peaks are attributed to the reduction of the 4,5-azomethine group.[3,5,29,30]

For the relatively short accumulation period used, significant peak-current enhancements are observed compared with conventional pulse voltammetry and hence all subsequent work was directed towards the optimisation of the accumulation conditions, the nature of the solvent, ionic strength, mass transport, temperature, pH, potential, time and other instrumental parameters, which directly affect the voltammetric response. The response was examined in the presence of various supporting electrolytes, *e.g.*, phosphate, acetate and Britton - Robinson buffers of differing ionic strength in the range 0.04–0.20 M.

Fig. 3 shows the dependence of peak current on ionic strength for several electrolytes. This parameter has a strong effect on the adsorptive stripping response.

Maximum size peaks were obtained using 0.04 M acetate buffer (pH 5.0) for camazepam and 0.04 M Britton - Robinson buffer (pH 5.0) for bromazepam, and these buffers were therefore employed throughout the study.

It was observed that the response increased when the pH of the solution was raised from 2 to 5, but that it remained constant from pH 5 to 8 and decreased progressively at pH 8. A negative shift in peak potential from −0.80 to −1.08 V (camazepam) and −0.41 to −0.96 V (bromazepam) was

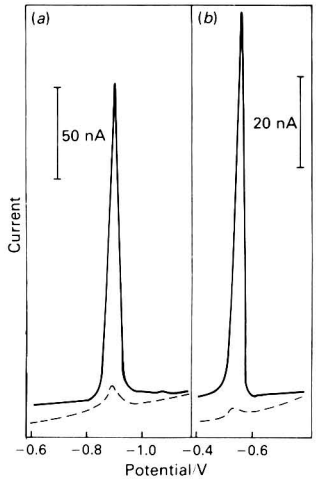


Fig. 2. Differential-pulse adsorptive stripping voltammograms with stirring at 1920 rev min^{-1} for: (*a*) 1.29×10^{-8} M camazepam, 270 s accumulation period at −0.60 V (Ag - AgCl - 3 M KCl) in 0.04 M acetate buffer, pH = 5.0; (*b*) 6.02×10^{-8} M bromazepam, 90 s accumulation period at −0.40 V (Ag - AgCl - 3 M KCl) in 0.04 M Britton - Robinson buffer, pH = 5.0. Scan rate, 15 mV s^{-1}; pulse amplitude, 70 mV. The discontinuous line represents voltammetric response without accumulation

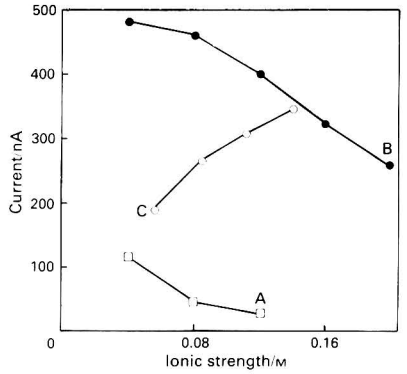


Fig. 3. Influence of ionic strength on the voltammogram peak current for different buffers at pH = 5.0. (A) Phosphate buffer; (B) acetate buffer; and (C) Britton - Robinson buffer. All solutions contain 9.40×10^{-8} M camazepam. Other conditions as in Fig. 2, except a 105-s accumulation time and a pulse amplitude of 50 mV were used

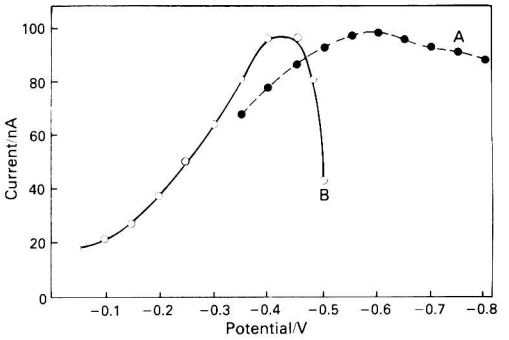


Fig. 5. Dependence of accumulation potential on peak current. (A) 9.20×10^{-9} M camazepam; and (B) 8.00×10^{-8} M bromazepam. Other conditions as in Fig. 2

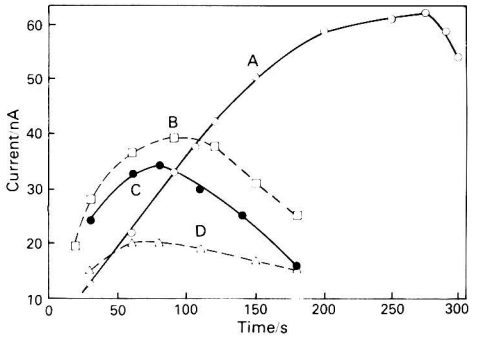


Fig. 4. Influence of accumulation time on peak current. (A) 6.40×10^{-9} M camazepam in 0.04 M acetate buffer, pH = 5.0; (B), (C) and (D) 3.00×10^{-8} M bromazepam in 0.04, 0.08 and 0.16 M Britton - Robinson buffer, respectively. Other conditions as in Fig. 2

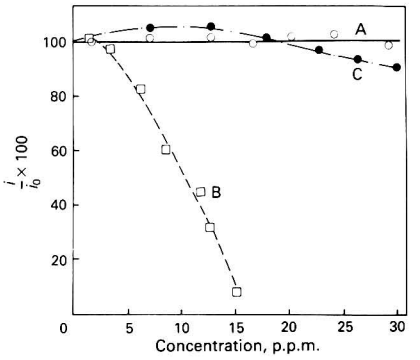


Fig. 6. Effect of various surfactants on the stripping peak current for 2.40×10^{-8} M camazepam, 105-s accumulation time. (A) Chloride ion; (B) gelatin; and (C) albumin. Other conditions as in Fig. 2

observed on increasing the pH from 2.5 to 10.0. The peak potential decreased linearly with increasing pH owing to the reduction of the double bond in the 4,5-azomethine group.[4-6]

The dependence of the stripping peak current on the pre-concentration time was examined. Fig. 4 shows the dependence of the adsorptive stripping peak current on the accumulation time for both benzodiazepines.

A linear dependence was observed for 6.40×10^{-9} M camazepam up to 120 s accumulation time, with a slope of 0.32 nA s^{-1} and for 3.00×10^{-8} M bromazepam up to 50-s accumulation time with a slope of 0.69 nA s^{-1}. The selection of the optimum accumulation time depends on the concentration ranges studied. Experiments showed that a 270 s accumulation time is the optimum for the determination of camazepam between 3×10^{-9} and 9×10^{-9} M. For bromazepam between 1×10^{-8} and 8×10^{-8} M a 90-s period is required.

The dependence of the stripping peak current on the accumulation potential was examined over the range −0.35 to −0.80 V for camazepam and 0.00 to −0.50 V for bromazepam. The results are shown in Fig. 5. The highest peaks were obtained using −0.60 V (camazepam) and −0.40 V (bromazepam).

Several instrumental parameters directly affect the voltammetric response, mainly in the form and reproducibility of the waves.

It was observed that the peak current increased linearly with drop size; forced convection during the accumulation step also affects the resulting stripping peak current, remaining almost constant on changing the stirring speed from 1220 to 2620 rev min^{-1}. The best conditions were found to be 1920 rev min^{-1} for a 0.44 mm^2 drop size.

The peak current remained virtually constant with rest time. A rest time of 15 s was employed throughout the study. The peak height changed linearly with pulse amplitude up to 100 mV. However, as both peak height and width must be considered, pulse amplitudes greater than 80 mV are not recommended. In order to obtain an adequate response, the following parameters were chosen: scan speed 10–25 mV s^{-1}, scan amplitudes lower than 0.6–0.7 V and pulse repetition 0.4 s. The peak half-widths observed in the voltammograms at 10^{-7} M concentration and under the optimised conditions are always lower than 60 mV. The determination is based on the fact that the peak current is directly dependent on the concentration of the drug.

Table 1 summarises the optimum conditions for the determination of the two benzodiazepines, the range of linear response, the slopes of the calibration graphs and the corresponding correlation coefficients and detection limits.

Well defined stripping peaks were observed for these concentration ranges. Higher concentrations or longer accumulation times resulted in deviation from linearity as full

surface coverage was approached. It is also possible to use this method for higher concentrations, but a change in working conditions is necessary, especially with regard to accumulation time.

The standard additions method can be used for the determination as long as linearity is maintained.

The detection limits increase with accumulation time, *i.e.*, for camazepam at 270 s the detection limit was found to be 3.4×10^{-10} M and at 120 s the detection limit was 1.5×10^{-9} M.

Relative standard deviations, calculated from ten successive measurements on a stirred 6.06×10^{-9} M camazepam solution and 4.02×10^{-8} M bromazepam solution were 2.0 and 2.2%,

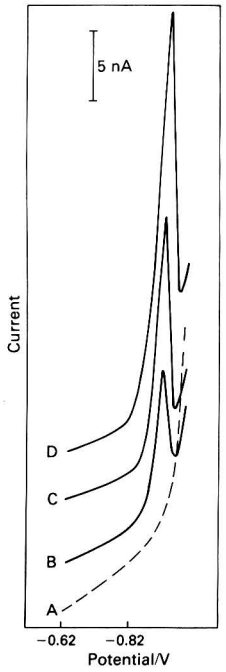


Fig. 7. Stripping curves for different concentrations of camazepam extracted from human serum. (A) Without camazepam; (B) 100 ng ml^{-1} of serum; (C) 200 ng ml^{-1} of serum; and (D) 400 ng ml^{-1} of serum. Conditions: 30-s accumulation time, −0.62 V accumulation potential. Other conditions as in Fig. 2

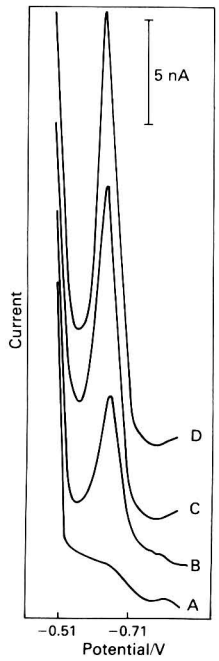


Fig. 8. Stripping curves for different concentrations of bromazepam extracted from human serum. (A) Without bromazepam; (B) 258 ng ml^{-1} of serum; (C) 515 ng ml^{-1} of serum; and (D) 741 ng ml^{-1} of serum. Conditions: 10 s and −0.41 V as accumulation time and potential, respectively. Other conditions as in Fig. 2

Table 1. Optimum conditions and results obtained for determination of camazepam and bromazepam

Compound	Accumulation Time/s	Accumulation Potential/V	Linear response/M	Sensitivity/ nA l mol^{-1}	Intercept/ nA	Correlation coefficient	Detection limit/M
Camazepam . .	270	−0.60	3×10^{-9}–9×10^{-9}	1.1×10^{10}	−0.87	0.9998	3.4×10^{-10}
Bromazepam . .	90	−0.40	1×10^{-8}–8×10^{-8}	1.2×10^{9}	1.26	0.9996	3.5×10^{-9}

Table 2. Results obtained for the determination of camazepam and bromazepam in human serum at −0.62 and −0.41 V for the accumulation, respectively.

Compound	Parameter	Accumulation time/s: 5	10	15	20	25	30	40	60
Camazepam . .	Sensitivity/nA ng^{-1} ml of serum	0.044	0.055	0.066	0.078	0.083	0.085	0.072	0.019
	Correlation coefficient	0.997	0.998	0.997	0.997	0.998	0.998	0.980	0.977
	Determination limit/ ng ml^{-1} of serum	60	30	30	30	25	20	30	190
	Linear response/ng ml^{-1} of serum	100–1000	50–1000	50–1000	50–1000	50–1000	50–1000	50–1000	250–1000
Bromazepam . .	Sensitivity/nA ng^{-1} ml of serum	0.015	0.019	0.017	0.016	0.015	—	—	—
	Correlation coefficient	0.997	0.999	0.997	0.996	0.996	—	—	—
	Determination limit/ng ml^{-1} of serum	190	180	200	220	220	—	—	—
	Linear response/ng ml^{-1} of serum	200–2500	200–2500	200–2500	250–2000	250–2000	—	—	—

respectively. Such a low deviation can be directly attributed to the automatic control provided by the VA 646 processor, which ensures the reproducibility of the results. For camazepam concentrations between 3×10^{-9} and 9×10^{-9} M, the relative standard deviation ranges between 5.8 and 0.8%, and for bromazepam concentrations between 1×10^{-8} and 8×10^{-8} M the relative standard deviation ranges between 6.0 and 1.4%.

The presence of other surface-active compounds can affect the adsorptive stripping response of benzodiazepines, in particular through competitive coverage, and was therefore investigated. The influence of surfactants, commonly found in biological samples, was also tested.

Fig. 6 shows the effect of chloride ion, gelatin and albumin on the adsorptive stripping peak of 2.40×10^{-8} M camazepam. Although chloride ion does not seem to affect the adsorption of this drug, gelatin (even in small amounts) causes significant depression of the peak current. Among the interferences caused by albumin, slight decreases in peak current were observed. Such interferences can be minimised by changing the accumulation potential. Bromazepam showed similar results.

Taking into account the above results, a determination of the drugs in human serum was carried out. The direct determination of camazepam and bromazepam in human serum is not possible owing to the high surfactant concentration in the sample which effectively inhibits the pre-concentration process on the electrode. In order to avoid such an inhibition, Sep-Pak C_{18} cartridges were used for the extraction of the drugs. Serum samples with differing camazepam and bromazepam contents were tested following the procedure described under Experimental.

Figs. 7 and 8 show the feasibility of measuring camazepam and bromazepam in serum samples. The stripping peaks are in accordance with those obtained for the pure drugs. The peak current continues to increase with accumulation time, although a shorter time is required. An increase in the amount of the drug shows a corresponding increase in the peak current.

Table 2 summarises the optimum conditions for the determination of camazepam and bromazepam in human serum samples. It has been proved that the standard additions method can be used for the determination of these drugs in serum. In the concentration range 50–1000 ng ml^{-1} of serum for camazepam and 200–2500 ng ml^{-1} of serum for bromazepam, the recovery was 95–97% for camazepam and 94–96% for bromazepam.

It is concluded that adsorptive stripping voltammetry at the hanging mercury drop electrode is suitable for the determination of trace amounts of benzodiazepines, as the drugs are adsorbed spontaneously, and the method can be applied to the determination of the drugs in biological fluids by adsorptive stripping voltammetry in conjunction with a Sep-Pak C_{18} cartridge.

The authors thank CAICYT for the financial support awarded to this project (No. 2077–83).

References

1. Schutz, H., *Editor*, "Benzodiazepines, A Handbook," Springer Verlag, Heidelberg, 1982.
2. Schutz, H., *Ärztl. Lab.*, 1979, **25**, 75.
3. Smyth, W. F., Smyth, M. R., Groves, J. A., and Tan, S. B., *Analyst*, 1978, **103**, 497.
4. Brooks, M. A., *Bioelectrochem. Bioenerg.*, 1983, **10**, 37.
5. Bermejo, E., *PhD Thesis*, Autonoma University of Madrid, 1986.
6. Sengun, F. I., *Chim. Acta Turc.*, 1983, **11**, 301.
7. Sengun, F. I., Ari-Urubelen, A., Ulas, K., and Fedai, I., *Sci. Pharm.*, 1984, **52**, 66.
8. Smyth, M. R., Tan Beng, S., and Smyth, W. F., *Anal. Chim. Acta*, 1977, **92**, 129.
9. Lund, W., Hannisad, M., and Greibrokk, P., *J. Chromatogr.*, 1979, **173**, 248.
10. De Silva, J. A., Bekersky, I., Brooks, M. A., Weinfeld, R. E., Glover, W., and Puglisi, C. V., *J. Pharm. Sci.*, 1974, **63**, 1140.
11. Smyth, F., Scannell, R., Goggin, T. K. and Lucas-Hernandez, D., *Anal. Chim. Acta*, 1982, **141**, 321.
12. Maurer, H., and Pfleger, K., *J. Chromatogr.*, 1981, **222**, 409.
13. Marcucci, F., Mussini, E., Cotellesa, L., Ghirardi, P., Parenti, M., Riva, R., and Salva, P., *J. Pharm. Sci.*, 1978, **67**, 1470.
14. Quaglio, M. P., and Bellini, A. M., *Farmaco Ed. Prat.*, 1981, **31**, 487.
15. Riva, R., Albani, F., and Baruzzi, A., *Farmaco Ed. Prat.*, 1982, **37**, 15.
16. Cuisinaud, G., Peillon, E., and Ferry, N., *J. Chromatogr.*, 1979, **178**, 314.
17. Violon, C., and Vercruysse, A., *J. Chromatogr.*, 1980, **189**, 94.
18. Maurer, H., and Pfleger, K., *J. Chromatogr.*, 1981, **222**, 409.
19. Hoogmortens, J., *J. Chromatogr.*, 1980, **194**, 262.
20. Schutz, H., *J. Anal. Toxicol.*, 1978, **2**, 148.
21. Schutz, H., *Fresenius Z. Anal. Chem.*, 1979, **294**, 135.
22. Pala, F., De Cosmo, G., Sabato, A., and Bondolia, F., *Resuscitacion*, 1981, **9**, 125.
23. Hernandez, L., Zapardiel, A., Perez Lopez, J. A., and Rodriguez, V., *in* Smyth, M. R., and Vos, J. G., *Editors*, "Electrochemistry, Sensors and Analysis," Elsevier, Amsterdam, 1986, pp. 385–390.
24. Kalvoda, R., *Anal. Chim. Acta*, 1983, **148**, 79.
25. Wang, J., Mahmoud, J. S., and Farias, A. M., *Analyst*, 1985, **110**, 855.
26. Wang, J., Tuzhi, P., Lin, M., and Tapia, T., *Talanta*, 1986, **33**, 707.
27. Wang, J., *Am. Lab.*, 1985, **17**(5), 41.
28. Mongay, C., and Cerda, V., *Talanta*, 1977, **24**, 747.
29. Brooks, M. A., *in* Smyth, W. F., *Editor*, "Polarography of Molecules of Biological Significance," Academic Press, London, 1979, Chapter 3.
30. Oelschlager, H., *Bioelectrochem. Bioenerg.*, 1983, **10**, 25.

Paper A6/473
Received December 16th, 1986
Accepted February 27th, 1987

Enzyme Sensor for the Determination of Lactate and Lactate Dehydrogenase Activity

Dietmar Weigelt
Marienberg District Hospital, DDR 9340 Marienberg, GDR

and Florian Schubert* and Frieder Scheller
Central Institute of Molecular Biology, Academy of Sciences of the GDR, Department of Applied Enzymology, DDR 1115 Berlin-Buch, GDR

L-Lactate monooxygenase was immobilised by gelatin entrapment and fixed to a Clark-type oxygen probe. The resulting enzyme sensor was utilised in a Glukometer, an enzyme electrode based analyser. With this combination L-lactate was determined with a linear range between 10^{-5} and 6×10^{-4} M. The relative standard deviation was below 1% and the stability of the sensor was 55 d. By addition of NADH and pyruvate to the measuring solution, sequential determination of lactate and lactate dehydrogenase activity was possible. The linear measuring range for the latter was 4.5×10^{-8}–9×10^{-7} mol s^{-1} l^{-1} (2.7–54.0 U l^{-1}). With human plasma and serum good agreement between the sensor and spectrophotometric methods was obtained.

Keywords: *Lactate monooxygenase; lactate sensor; lactate dehydrogenase; sequential determination*

In clinical laboratories there is a strong demand for the determination of lactate and lactate dehydrogenase activity. Elevated levels of these parameters are indicative of several diseases. In addition to the well established spectrophotometric procedures a number of biosensors for lactate have been developed. In early attempts in this direction, lactate dehydrogenase[1] and cytochrome b_2[2] were used. These enzymes require the addition of cofactors, *i.e.*, they do not work "reagentless." In order to improve lactate sensors, oxygen-dependent lactate oxidising enzymes have been employed, namely lactate oxidase[3] and lactate monooxygenase,[4,5] the latter catalysing the reaction

$$\text{L-lactate} + O_2 \rightarrow \text{acetate} + CO_2 + H_2O$$

The incorporation of these enzymes in lactate electrodes permits the use of the highly selective Clark-type oxygen probe as the indicator sensor.

A lactate oxidase electrode for the determination of lactate and lactate dehydrogenase activity has also been reported.[6] This paper describes the use of lactate monooxygenase in a sensor for the sequential measurement of lactate and lactate dehydrogenase. The enzyme membrane was designed so as to achieve diffusional control, which was demonstrated using a sandwich arrangement of lactate monooxygenase and cytochrome b_2.

Owing to the highly effective immobilisation used, this sensor exhibits excellent operational characteristics. It has been tested in a flow-through analyser and applied to biological samples.

Experimental

Materials

Lactate monooxygenase (LMO, 10 U mg^{-1}) from *Mycobacterium smegmatis* was purchased from Boehringer (Mannheim, FRG) and lactate dehydrogenase (LDH, 110 U mg^{-1}) and NADH from VEB Arzneimittelwerk (Dresden, GDR). Cytochrome b_2 was prepared from *Hansenula anomala* according to Labeyrie *et al.*[7] with an activity of 4100 U ml^{-1}. L-(+)-Lactate (lithium salt) was obtained from Serva Biochemica (Heidelberg, FRG) and gelatin from VEB Gelatinewerk (Calbe, GDR).

The buffers used were citrate and phosphate buffers with variable concentrations and pH values.

* To whom correspondence should be addressed.

Apparatus and Procedures

Lactate monooxygenase membranes were prepared by gelatin entrapment.[8] A 5% gelatin solution was prepared by dissolving the gelatin in doubly distilled water at 37 °C. Variable amounts of LMO were dissolved in 200 µl of the gelatin solution and the solution obtained was cast on 5 cm^2 of a plane polyethylene support and dried at 4 °C for at least 12 h. The membrane was then removed from the support and stored at 4 °C until taken for use. For cytochrome b_2 membrane preparation, 29 U cm^{-2} of gelatin membrane were used. These layers were stored at −20 °C until taken for use. For lactate sensor preparation, a 3 × 3 mm piece of LMO membrane was placed between a polyethylene membrane and a dialysis membrane and fixed to a Pt - Ag - AgCl electrode so that the polyethylene membrane was next to the Pt electrode. This electrode is part of the Glukometer enzyme electrode analyser (ZWG, Berlin, GDR), which consists of a thermostat including the measuring cell with the enzyme sensor inserted from the side, and a separate electronic part containing an amplifier and display. The Glukometer was used for most lactate measurements. Its measuring cell was equipped with a magnetic stirrer and contained 2 ml of the appropriate buffer.

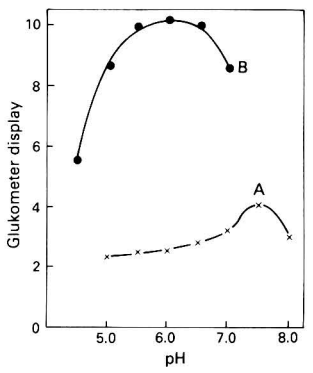


Fig. 1. Effect of pH of (A) 0.1 M phosphate buffer and (B) 0.1 M citrate buffer on the response of the lactate sensor; 2.5×10^{-4} M lactate. The Glukometer reading was adjusted to 10 mM using citrate buffer (pH 6.0) as background solution

Lactate samples of 50 µl were added. The maximum of the first derivative (di/dt) of the current - time curve is displayed by the device in mmol l^{-1}. Usually the Glukometer was calibrated by injecting a 10 mM lactate standard and adjusting the reading to this value.

For flow-through determination, the Glukometer was combined with a flow-through cell, an APS 4 automatic sampler, a DP 2-2 peristaltic pump (both from VEB MLW, Medingen, GDR) and a strip-chart recorder. The flow-rate was 1.2 ml min^{-1}. Samples were diluted 50-fold in the measuring buffer. Before these measurements, both the sample and the buffer were air-saturated by shaking or stirring. LDH determination was carried out using the Glukometer measuring cell in combination with pO_2 meter (VEB Metra, Radebeul, GDR) and a recorder. For these and the sequential lactate and LDH measurements the current - time graphs were recorded. For experiments with cytochrome b_2 membranes these were placed in front of the electrode and covered by a dialysis membrane, an LMO layer and another dialysis membrane. A potential of +0.25 V *vs.* Ag - AgCl was applied to this electrode.

Results and Discussion

Sensor Characterisation

The response of the LMO electrode to L-lactate was optimised with respect to pH and buffer concentration using potassium phosphate - sodium phosphate and sodium citrate - sodium hydroxide buffers, respectively (Figs. 1 and 2). A membrane with 1 mg cm^{-2} of LMO was used. The sensitivity with citrate buffer was about double that with phosphate buffer, which is in close agreement with results obtained with the enzyme covalently bound to nylon.[4] With citrate buffer the optimum pH was pH 6.0. The sensitivity increased with increasing buffer concentration, a plateau being reached at 0.2 M. In contrast, using phosphate buffer the optimum pH was 7.5 and no further activation was observed above a concentration of 0.1 M.

The influence of enzyme loading on the calibration graph for lactate in the sensor was studied in 0.1 M citrate buffer between 0.1 and 15 U cm^{-2} (Fig. 3). As expected, with increasing loading the sensitivity increased. A plateau was reached with 2.5 U cm^{-2}, *i.e.*, with higher enzyme concentrations no substantial increase in sensitivity was obtained. Increasing the stirrer speed also did not bring about any enhancement of sensitivity. These results indicate that the sensor is controlled by internal substrate diffusion.

With the exception of the lowest enzyme loading, the upper limit of linearity decreased with increasing enzyme loading, being 6×10^{-4} M lactate when enzyme saturation was reached. At the plateau of the calibration graphs the oxygen tension in the membranes was effectively zero, indicating that the linearity limit was set by oxygen depletion.

To prove the diffusional limitation of the lactate sensor, the capacity of the LMO membrane to oxidise lactate was investigated. The membrane (10 U cm^{-2}) was fixed to the electrode in a sandwich arrangement together with a cytochrome b_2 membrane. The measuring solution contained 10^{-3} M potassium hexacyanoferrate(III) and the electrode was polarised to +0.25 V *vs.* Ag-AgCl to indicate whether hexacyanoferrate(II) is produced in the cytochrome b_2 layer. Fig. 4 shows the signal indicated by the sensor in terms of dependence on lactate concentration. With citrate buffer no lactate reaches the cytochrome b_2 layer at concentrations up to 6×10^{-4} M. Evidently all lactate below this value is completely oxidised in the LMO layer, *i.e.*, the LMO sensor is actually controlled by lactate diffusion. Above 6×10^{-4} M an anodic oxidation current appears, reflecting the formation of hexacyanoferrate(II) by cytochrome b_2. This means that lactate permeates through the LMO membrane and reaches the cytochrome b_2 membrane. The oxygen limitation, becoming effective at lactate concentrations higher than 6×10^{-4} M, now prevents LMO from oxidising all the lactate, *i.e.*, the over-all process in the LMO membrane is no longer controlled by lactate diffusion.

In contrast, when using phosphate buffer a signal is observed above 10^{-4} M lactate. Obviously under these measuring conditions the LMO activity is too low for complete substrate conversion, *i.e.*, to maintain diffusion control. Therefore, with independently prepared sensors different sensitivity ratios between measurements in citrate buffer (diffusion controlled) and phosphate buffer (kinetically controlled) were found, as can be seen from Figs. 1 and 2.

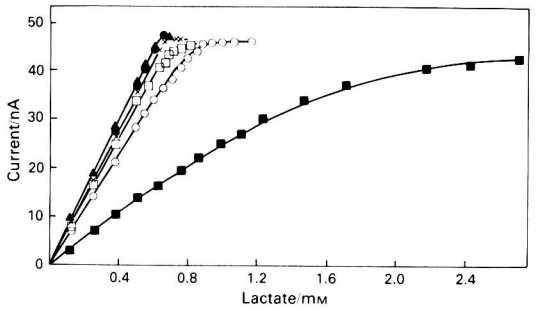


Fig. 3. Effect of enzyme loading on the current - concentration graph of the lactate sensor. (■) 0.1 U cm^{-2}; (○) 0.5 U cm^{-2}; (□) 1.0 U cm^{-2}; (×) 2.5 and 5.0 U cm^{-2}; (●) 10.0 U cm^{-2}; and (▲) 15.0 U cm^{-2}

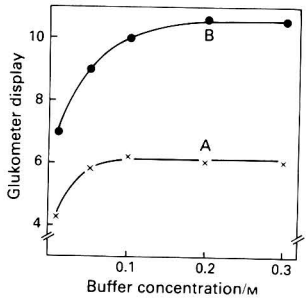


Fig. 2. Effect of the concentration of (A) phosphate buffer (pH 7.5) and (B) citrate buffer (pH 6.0) on the response of the lactate sensor; 2.5×10^{-4} M lactate. The Glukometer reading was adjusted to 10 mM using 0.1 M citrate buffer as background solution

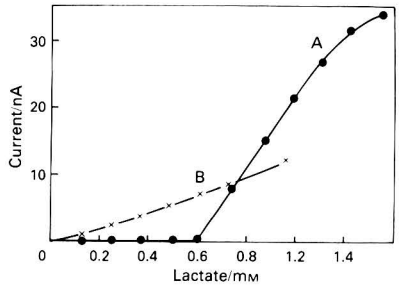


Fig. 4. Current signal of the electrode assembled with a cytochrome b_2 and a LMO membrane in terms of dependence on the concentration of lactate. (A) 0.1 M citrate buffer (pH 6.0); (B) 0.1 M phosphate buffer (pH 7.5)

Lactate Determination

For all further measurements 0.1 M citrate buffer (pH 6.0) and LMO membranes with an activity of 10 U cm^{-2} were used. The detection limit of the sensor was 10^{-5} M. The linear range, which extends up to 6×10^{-4} M, is sufficient to determine even non-physiologically high lactate levels.

The sample lactate concentration is displayed by the Glukometer 8 s after sample addition. Rinsing and equilibration with fresh background buffer takes 40 s; therefore, 60 determinations per hour can be carried out with the sensor. This high measuring frequency can be ascribed to the extremely thin gelatin membrane used for enzyme immobilisation, allowing very fast substrate and product diffusion.

The relative standard deviation of the method using 5×10^{-3} M aqueous lactate sample solution was usually below 1% ($n = 20$). Lactate in 30 blood plasma samples collected from hospital patients was determined with the sensor and the results were compared with those obtained using the Boehringer monotest procedure (Fig. 5). The correlation between the two methods is excellent. For flow-through lactate measurement, 20 µl of sample solution were diluted with 1 ml of buffer. Linearity was obtained up to 5×10^{-2} M lactate in the sample. The measuring frequency was 40 per hour, one cycle including a 20-s sampling time and a 70-s rinsing time. The precision of the method was as good as that of the manual method. The stability of the lactate sensor is shown in Fig. 6. Within 55 d, during which more than 600 measurements were performed, no decrease in sensitivity was observed. This is comparable to data obtained with nylon-bound LMO by Mascini *et al.*,[4] who found, however, a lifetime of only 10 d with the enzyme simply retained by a dialysis membrane.

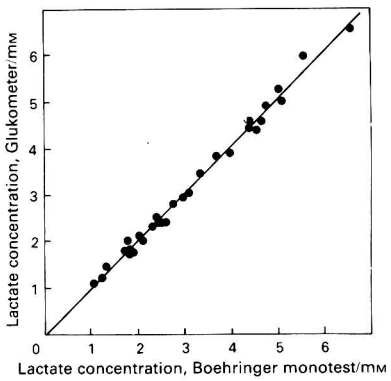


Fig. 5. Correlation of plasma lactate measurement with the Glukometer equipped with the proposed sensor and the spectrophotometric method. $y = -0.1 + 1.03x$; $r = 0.998$; $n = 30$

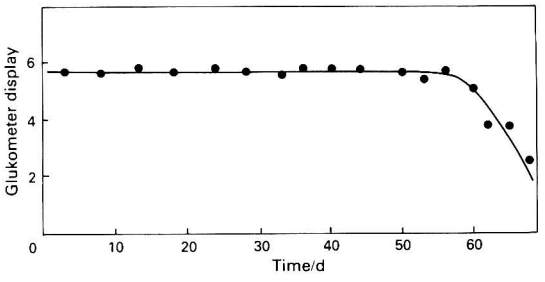


Fig. 6. Stability of the lactate sensor

Obviously the proteinaceous nature of the gelatin matrix used for the present membrane contributes to the high stability of LMO.

Determination of Lactate and LDH

A typical recording of sequential lactate and LDH measurements is shown in Fig. 7. The sample (100 µl) added to 2 ml of buffer contained 2.8×10^{-3} M lactate and LDH, corresponding to an activity of 4.06×10^{-6} mol s^{-1} l^{-1}. When the current decrease resulting from lactate oxidation was completed, NADH was added to the measuring cell and after exactly 1 min the reaction was started by injection of pyruvate. The subsequent current decrease reflects the lactate formation by the LDH-catalysed reaction. The optimum concentrations were determined spectrophotometrically to be between 2×10^{-4} and 4×10^{-4} M for NADH and 5×10^{-4} M for pyruvate, which are in agreement with recommended values.[9] Under these conditions, lactate at concentrations up to 2×10^{-3} M in the measuring solution did not affect the LDH reaction.

After pyruvate addition, the current - time graph was linear for at least 2 min. From the slope of this graph and the current decrease on lactate oxidation, which is used as a calibration value, the LDH activity can be calculated directly. The slope depends linearly on LDH activity up to 9×10^{-7} mol l^{-1} s^{-1}.

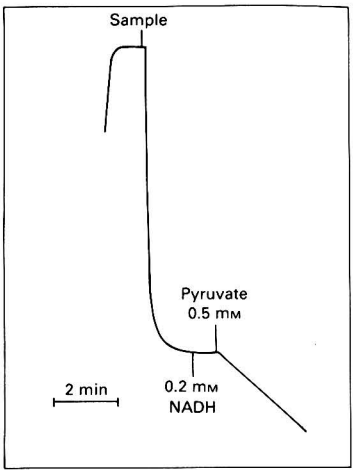


Fig. 7. Response curve of sequential determination of lactate and LDH with the proposed sensor

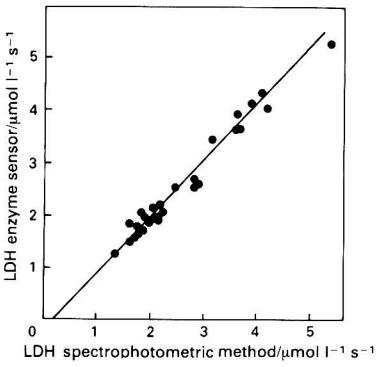


Fig. 8. Correlation of serum LDH measurement with the proposed sensor and the spectrophotometric method. $y = 0.29 + 1.11x$; $r = 0.999$; and $n = 30$

The detection limit is 4.5×10^{-8} mol l^{-1} s^{-1}, which corresponds to 10^{-6} mol l^{-1} s^{-1} in the sample. The relative standard deviation of LDH measurement was 1.2% ($n = 20$) using an amount of enzyme equivalent to 4.17×10^{-6} mol l^{-1} s^{-1} in the sample. Sequential determination of lactate and LDH can be carried out with up to 10^{-2} M lactate in the sample without influencing the linearity of the LDH-dependent signal *vs.* activity relationship. The time required for one sequential measurement is as short as 4 min and 15 samples can be processed per hour.

The sensor was applied to the measurement of LDH in human sera. Good agreement with the results of the standard spectrophotometric method was obtained (Fig. 8).

Conclusions

The proposed lactate sensor based on immobilised lactate monooxygenase allows the rapid and accurate measurement of L-lactate in human plasma. Further, by a simple step of addition of NADH and pyruvate, the subsequent determination of LDH activity is possible. The short response and equilibration times of the enzyme electrode, resulting in high sample frequencies, are obviously provided by the very thin, though highly active, gelatin - enzyme membrane ensuring a rapid and diffusion-limited response. A further effect of the gelatin matrix is its capability to stabilise the lactate monooxygenase enzyme. Compared with sensors using LDH or cytochrome b_2, the present electrode is insensitive to interferences from the sample because the specific oxygen electrode is used as the base probe.

References

1. Blaedel, W. J., and Jenkins, R. A., *Anal. Chem.*, 1976, **48**, 1240.
2. Guarnaccia, R., and Racine, P., *Biomed. Technol.*, 1976, **21**, 189.
3. Tsuchida, T., Takasugi, H., Yoda, K., Takizawa, K., and Kobayashi, S., *Biotechnol. Bioeng.*, 1985, **27**, 837.
4. Mascini, M., Moscone, D., and Palleschi, A., *Anal. Chim. Acta*, 1984, **157**, 45.
5. Weaver, M. R., and Vadgama, P. M., *Clin. Chim. Acta*, 1986, **155**, 295.
6. Mizutani, F., Sasaki, K., and Shimura, Y., *Anal. Chem.*, 1983, **55**, 35.
7. Labeyrie, F., Baudras, A., and Lederer, F., *Methods Enzymol.*, 1978, **53**, 238.
8. Scheller, F., Pfeiffer, D., and Jänchen, M., *GDR Pat.*, G 01 N/217 843, 1978.
9. *Z. Klin. Chem. Klin. Biochem.*, 1972, **10**, 182.

Paper A6/441
Received November 11th, 1986
Accepted March 23rd, 1987

Single Fibre Optic Fluorescence pH Probe

Ming-Ren S. Fuh, Lloyd W. Burgess, Tomas Hirschfeld and Gary D. Christian
Center for Process Analytical Chemistry, Department of Chemistry, BG-10, University of Washington, Seattle, WA 98195, USA

and Francis Wang
Lawrence Livermore National Laboratory, University of California, Livermore, CA 94550, USA

Fibre optic sensing is a growing technology in analytical chemistry. Scattering, absorbance, reflectance and luminescence spectroscopic measurements have been made using fibre waveguides. Fluorescence is particularly suited for use in fibre sensing because of its sensitivity and versatility, and the ease with which this type of measurement may be implemented with a single fibre optic.

The probe configuration used in this study for pH measurement consists of fluorescein isothiocyanate (FITC) covalently bonded to a porous glass bead. This bead is attached to a single, multi-mode optical fibre, which conducts both the excitation and emission radiations. The increase in fluorescence intensity, as the acid form of the immobilised FITC is converted to its basic form, is related to the pH. The dynamic range of this miniature probe is pH 3–7 and this can be extended by decreasing the dye loading. The response time is between 20 and 35 s, depending on the capacity.

Keywords: *Fibre optic fluorescence pH probe; pH measurement; fibre optic*

One of the most interesting and valuable applications of fibre optic sensing technology is the development of miniature probes for remote sensing.[1,2] The measurement of pH is very important in chemical systems and the limitations of conventional pH electrodes make them unsuitable for certain applications. Several fibre optic pH sensors based on absorption dyes and fluorescent compounds have been described.[3–6] Most of these sensors employ a membrane to retain an immobilised reagent on a polymer or porous glass support located at the end of the probe. Such sensors are typically constructed on a bifurcated fibre optic system. The response time for these devices is long, owing to the requirement that the analytes diffuse across the membrane and into a layer of immobilised reagent. The sensor must be large enough to accommodate the bifurcated bundle and membrane holder system. In addition, the bifurcated optics are less efficient than a single optical fibre as the volume being excited and the emission observation regions do not coincide. Here, we describe a fluorescence-based pH sensor which consists of an individual porous glass bead fixed to a single optical fibre.

The probe response relies on changes of the fluorescence characteristics of an immobilised reagent, fluorescein isothiocyanate (FITC), with pH. Fluorescein has been widely used as a labelling agent because of its stability and high fluorescence quantum yield. Immobilisation technology is well developed for this reagent for a variety of supports. The strongest fluorescence of fluorescein appears in alkaline solutions; lowering the pH reduces the fluorescence intensity. The absorption and fluorescence spectra of fluorescein at different pH values in aqueous solution have been studied and related to the multiple proteolytic forms of the dye shown in Fig. 1.[7] Fluorescein isothiocyanate was used in this study because it has similar spectral properties to those of fluorescein and the isothiocyanate group provides a site for easy immobilisation of the molecule on to a porous glass bead.

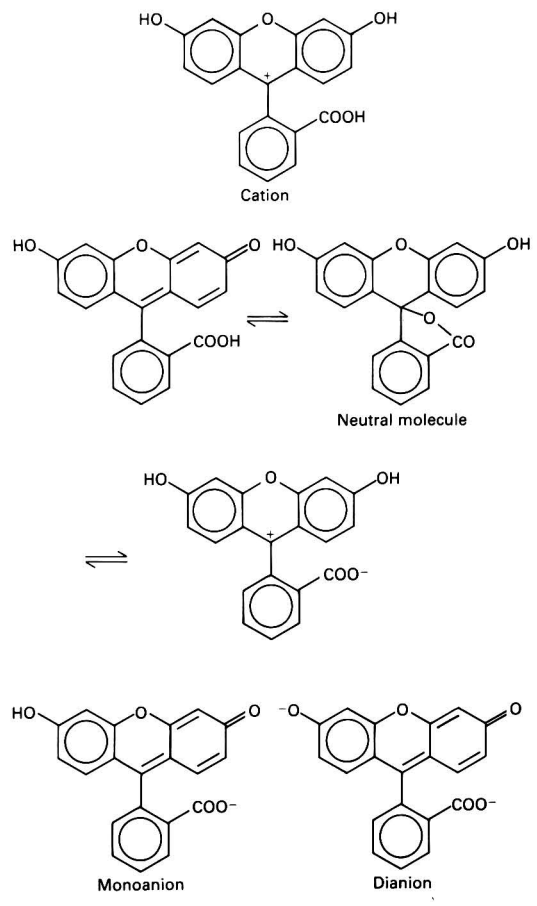


Fig. 1. Different proteolytic forms of fluorescein isothiocyanate (FITC)

Experimental

Materials

Silanised controlled-porosity glass (CPG) beads containing a long-chain alkylamine were obtained from Pierce Chemical (Catalogue No. 24875). These beads range in size from 125 to 177 μm, have average pore diameters of 50 nm and a surface

area of 70 m^2 g^{-1}. Fluorescein isothiocyanate was purchased from Polysciences. The reagents magnesium chloride hexahydrate, manganese(IV) chloride tetrahydrate, lithium chloride, sodium nitrate, magnesium nitrate and sodium hydroxide were Baker Analyzed reagents. Calcium chloride and sodium chloride were obtained from MCB Manufacturing Chemists. Hydrochloric acid, boric acid, citric acid and disodium hydrogen phosphate were purchased from Mallinckrodt.

Citric acid and phosphate buffers[8] were used in all the measurements and calibrated by pH electrode with a Beckman SelectIon 5000 pH meter (Beckman, Irvine, CA).

Radiant Communications SG-820 multi-mode optical communications fibres were used as the waveguides. These fibres have a 105 μm fused silica core surrounded by a 125 μm o.d. doped silica cladding and a readily strippable 500 μm protective polymer buffer. The numeric aperture is 0.24. The beads were cemented to the fibre end-faces using Norland No. 63 UV-curable epoxy resin.

Apparatus

The experimental measurements were obtained using the instrumental configuration shown in Fig. 2. It consists of an argon ion laser (Spectra Physics, Model 162), a double monochromator (Instruments, DH-10), a photomultiplier tube (Hamamatsu, R1140) and a photometer (Pacific Instruments, Model 126). A photocell (United Detector Technology, UDT-555D) is used to monitor the laser output. The outputs from the two detectors are recorded using a strip-chart recorder (Linear Instruments) and an IBM PC/XT computer containing an 8-channel 12-bit A/D converter (Metra Byte, DASH-16).

In order to use the same optical fibre to conduct both the excitation and fluorescence radiations, a coupler is required to direct the excitation light from the source into the fibre and the fluorescence from the sensing area to the detection system. A holed-mirror coupler, which has been described previously, was constructed for this purpose.[9] Briefly, it consists of a long focal length lens which focuses the excitation radiation through the hole in the mirror on to the fibre optic end-face and under-fills its numeric aperture. The returning fluorescence fills the numeric aperture of the fibre optic and is reflected to the detection system by the mirror. The collection efficiency, which is defined as the ratio of the area of fluorescence on the mirror minus the area of the hole divided by the area of the fluorescence on the mirror, is about 95%.

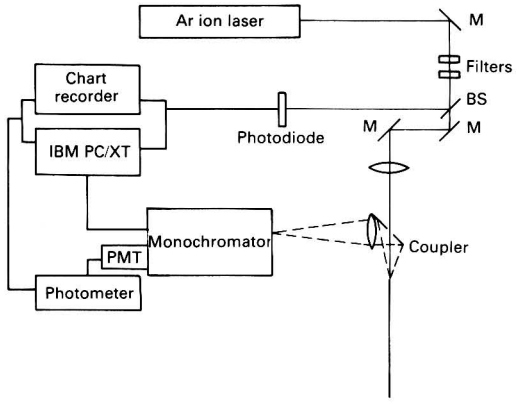


Fig. 2. Experimental set-up. See text for details

Procedure

FITC was immobilised on the controlled-porosity glass beads via the long-chain amine group using a modification of a previously described procedure.[10,11] One gram of the beads is soaked in 10 ml of saturated FITC in pH 8.6 borate buffer for 5 d at 35 °C. The beads were washed first with acetone and then with distilled water several times until no FITC was visible in the wash solution. In order to assure the removal of non-covalently bonded FITC, the beads were left in distilled water for two additional days at 30 °C. The beads were then dried by suction and stored in a desiccator until used. A modified immobilisation procedure was used to examine the effect of reduced dye loading on the pH response of the bead.

In the dye loading study, the porous glass bead was first glued on to the end of the fibre optic. Then the bead was dipped into a dye solution (containing either 2.6×10^{-3} or 1.3×10^{-5} M FITC) for 20–30 min. This was followed by soaking in the solvent of the dye solution for 30 min, and then in water for 1 h.

A schematic diagram of the pH probe is shown in Fig. 3. Because the porous glass beads are not uniform in shape, size or diameter, nearly round beads which covered the fibre optic core were selected. The FITC-immobilised glass bead is attached to the end of an approximately 1-m length of fibre optic by placing one drop of the optical epoxy resin at the fibre tip using a small capillary tube. Light from a long-wavelength UV lamp is introduced from the other end and propagated through the fibre to harden the adhesive. In order to minimise the amount of epoxy resin absorbed by the porous glass bead, the epoxy resin is partially cross-linked until it becomes very viscous before bringing the bead into contact with the glue.

Results

Fig. 4 shows the solution absorption spectra of 1×10^{-4} M FITC in the pH range 3–7 using an HP 8450A UV - visible spectrometer. It demonstrates that changes in absorptivity, as well as spectral shifts, occur with changing pH. Solution emission spectra, taken through an optical fibre attached to a 5 mm path length cell and with excitation at 488 nm, are shown in Fig. 5. Again, changes in fluorescence efficiencies and spectral shifts are observed. The relatively sharp features at 497, 505 and 585 nm are the fused silica Raman scattering peaks from the optical fibre. Once the FITC is immobilised on the bead, a small spectral shift is observed, as shown in Fig. 6. The Raman bands of the optical fibre are no longer visible, being swamped by the bead fluorescence. Because of the pH-dependent absorptivity and quantum efficiency of the FITC, the over-all response of the pH sensor is a function of the excitation and observation wavelengths.

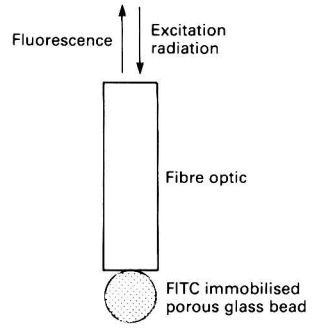


Fig. 3. Configuration of the probe. It consists of a 105/125 mm fused silica fibre optic and an FITC-immobilised porous glass bead

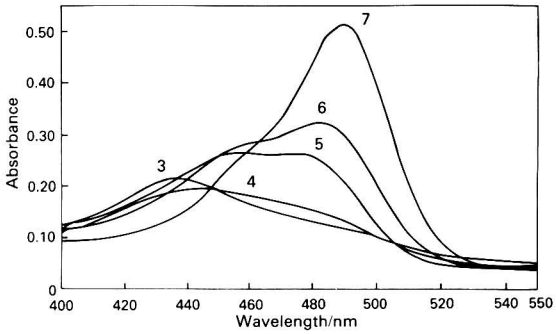


Fig. 4. Absorption spectra of 1×10^{-4} M FITC in 0.1 M citric acid buffers (pH 3–7). The numbers on the curves represent pH

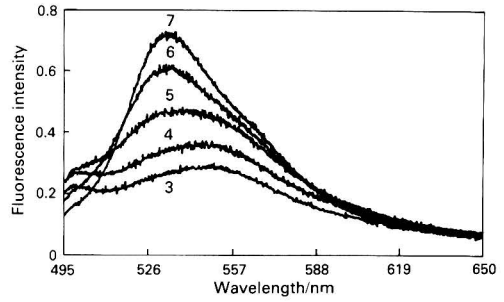


Fig. 6. Fluorescence spectra of the FITC-immobilised glass bead in 0.1 M citric acid buffers (pH 3–7). The numbers on the curves represent pH. These spectra are taken through 2 m of fibre optic. Excitation at 488 nm; 0.1 mW

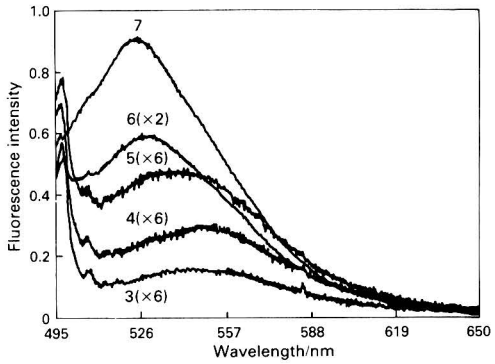


Fig. 5. Fluorescence spectra of 1×10^{-4} M FITC in 0.1 M citric acid buffers (pH 3–7). The numbers on the curves represent pH and are multiplied by the relative detector gain. These spectra are taken through 2 m of single fibre with a 5-mm path length cell. Excitation at 488 nm; 0.1 mW

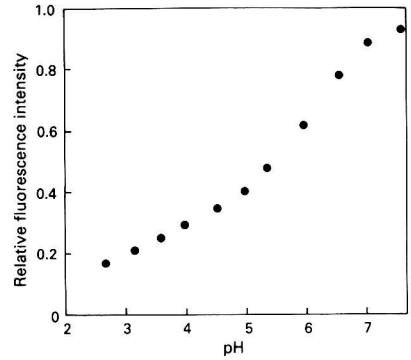


Fig. 7. pH response curve of the FITC-immobilised optrode

Fig. 7 illustrates the pH response between pH 2.8 and 7.5 when exciting at 488 nm and monitoring at 530 nm with a 3-nm band pass. Two essentially linear response regions, with correlation coefficients of 0.998 and 0.999, are observed, one from pH 2.8 to 5 and the second with a steeper slope between pH 5.5 and 7. In solution, fluorescein is a proteolytic acid with $pK_{a_1} = 2.2$, $pK_{a_2} = 4.4$ and $pK_{a_3} = 6.7$. The major species present between pH 2.8 and 5.5 are the neutral molecule and the monoanion, but the monoanion is not excited at 488 nm.[12] The monoanion and dianion are the major species between pH 5.5 and 7.0. The dianion has a high quantum yield, 0.9, and an absorption maximum at 491 nm, near the excitation wavelength. Therefore, we can expect two different response regions for the probe, with the region between pH 5.5 and 7.0 being more sensitive.

It has been reported[13] that the dianion of fluorescein can form dimers if the concentration is higher than 10^{-3} M. The dimer has two absorption peaks, at 468 and 508 nm, and a minimum between these peaks around 488 nm. The dimer should exhibit a lower fluorescence than the dianion monomer when excited with 488 nm light. Therefore, the pH responses of fluorescein above pH 7, where most fluorescein molecules are dianions, will strongly depend upon the dimerisation of the dianion. Fig. 8 shows the pH responses when using two different FITC concentrations for immobilisation on glass beads. The response of the probe between pH 7 and 8 can be enhanced by the elimination of the dimerisation effect when using 1.3×10^{-5} M FITC for immobilisation.

The rate of response for the probe between pH 3 and 7 is recorded in Fig. 9. The results indicate that the response time is independent of the pH and the difference between the initial and final pH values, and is about 20 s to a steady-state reading at relatively high (0.1 M) buffer concentrations. In 1×10^{-3} M buffer solutions the response time slows to about 35 s, as would be expected, but is still shorter than that of other pH electrodes and optrodes reported.

The responses of the pH optrode at three different temperatures is shown in Fig. 10. The numbers on the curves represent the temperature of the buffer solutions. The local probe temperature is expected to be higher than the solution temperature, owing to absorption of the excitation radiation. The relative fluorescence intensity decreases as the solution temperature increases. This decrease is much larger than the pH change (<1%) measured for these buffers using a pH glass electrode. A larger temperature effect was observed in the higher pH region and may be attributed to differential effects on various proteolytic forms of fluorescein isothiocyanate.

Fluorescence indicators have been shown to form chelated complexes with metal ions which can alter their pH calibration graph.[13,14] The influence of various metal ions on the FITC probe was examined, and the results are shown in Table 1. The fluorescence intensity decreases with increasing concentration for a given salt. Although the results indicate that there is no salt effect on pH responses with the 10^{-5} M salt solutions, at the 10^{-3} M concentration, a salt effect in the pH reading is observed. However, no spectral shifts were observed that can be related to any specific metal ions.

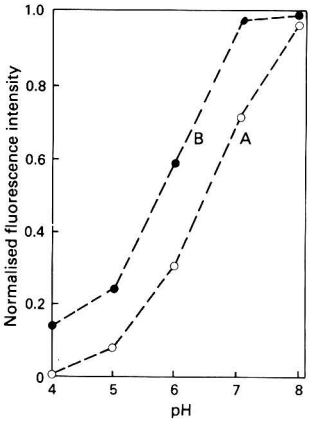


Fig. 8. pH responses of the FITC-immobilised probes prepared using low concentrations. (A) 1.3×10^{-5} M and (B) 2.6×10^{-3} M of FITC for immobilisation

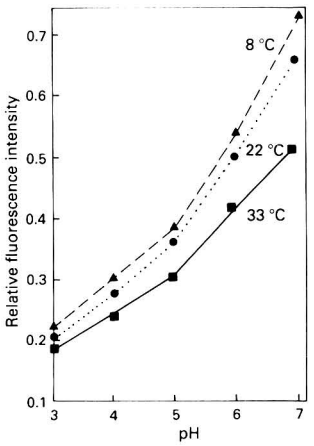


Fig. 10. Responses of the FITC-immobilised pH optrode at three different solution temperatures

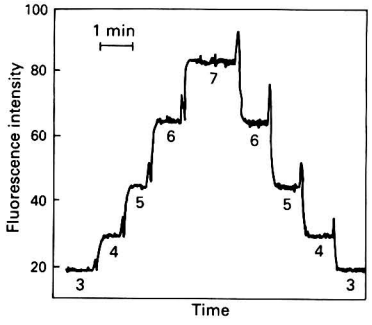


Fig. 9. Time - response curve of the FITC-immobolised pH probe. The numbers on the curve represent pH

Table 1. Response of the FITC pH probe in different salt solutions

Solution*	Concentration/ M	pH†	Relative fluorescence intensity
HCl	1×10^{-4}	3.765	0.58 ± 0.03
LiCl	1×10^{-5}	3.798	0.59 ± 0.03
NaCl	1×10^{-5}	3.803	0.58 ± 0.02
$MgCl_2$	1×10^{-5}	3.802	0.59 ± 0.02
$CaCl_2$	1×10^{-5}	3.792	0.59 ± 0.02
$MnCl_2$	1×10^{-5}	3.796	0.59 ± 0.03
$NaNO_3$	1×10^{-5}	3.812	0.57 ± 0.03
$Mg(NO_3)_2$	1×10^{-5}	3.808	0.56 ± 0.03
LiCl	1×10^{-3}	3.775	0.50 ± 0.02
NaCl	1×10^{-3}	3.796	0.50 ± 0.02
$MgCl_2$	1×10^{-3}	3.792	0.48 ± 0.03
$CaCl_2$	1×10^{-3}	3.725	0.48 ± 0.02
$MnCl_2$	1×10^{-3}	3.744	0.48 ± 0.03
$NaNO_3$	1×10^{-3}	3.815	0.52 ± 0.02
$Mg(NO_3)_2$	1×10^{-3}	3.805	0.49 ± 0.03

* All salt solutions are prepared in 0.0001 M HCl.

† The pH is measured by a pH electrode with a Beckman SelectIon 5000 pH meter.

Discussion

One drawback of using this CPG bead pH sensor is that each device must be calibrated. A considerable variation in the absolute response to pH was found for a number of probes constructed from the same batch of FITC immobilised glass beads. This is caused by the irregular shapes and the range of sizes of the beads and the fact that some pore volume is occupied by the epoxy resin used to glue the bead to the fibre. The relative response observed from beads within the same immobilisation batch, however, was constant. Beads with FITC immobilised from different batches exhibited varying relative responses under a given set of observation conditions. Once a probe was constructed and calibrated, the readings did not vary over short-term use.

Although this pH probe is far from being a universal pH sensor, it has several advantages over other devices. The response time is faster than for previously reported optical sensors (0.7–2 min), particularly in low capacity buffer systems. The very small probe volume (about 2 nl) makes it attractive for special applications. The dynamic range for the optrode is wider than other pH sensors based on coloured indicators, owing to the numbers of proteolytic forms of fluorescein. Decreasing the dye loading has the effect of extending the probe response in the region between pH 7 and 8, making it useful in the region of physiological measurements.

Limitations exist with respect to meaurements at high pH, owing to the susceptibility to hydrolysis of the covalent bond between the porous glass support and the indicator. Changes in indicator fluorescence caused by non-specific alterations are also a potential problem. Co-immobilisation of an additional dye to correct for the effects of salt concentration is a possible solution.[15] Isolation of the bead in a thin ion-permeable membrane may also prove necessary for some applications, at the expense of sensor response time.

References

1. Seitz, W. R., *Anal. Chem.*, 1984, **56**, 16A.
2. Wolfbeis, O. S., *Trends Anal. Chem.*, 1985, **4**, 184.
3. Kirkbright, G. F., Narayanaswamy, R., and Welti, N. A., *Analyst*, 1984, **109**, 1025.
4. Peterson, J. I., Goldstein, S. R., and Fitzgerald, R. V., *Anal. Chem.*, 1980, **52**, 864.

5. Saari, L. A., and Seitz, W. R., *Anal. Chem.*, 1982, **54**, 821.
6. Munkholm, C., Walt, D. R., Milanovich, F. P., and Klainer, S., *Anal. Chem.*, 1986, **58**, 1430.
7. Martin, M. M., and Lindqvist, L., *J. Luminescence*, 1975, **10**, 381.
8. Perrin, D. D., and Dempsey, B., "Buffers for pH and Ion Control," Halstead Press, New York, 1974.
9. Hirschfeld, T., Deaton, T., Milanovich, F., and Klainer, S., *Opt. Eng.*, 1983, **22**, 527.
10. Twining, S. S., *Anal. Biochem.*, 1984, **143**, 30.
11. Mosbach, K., *Editor*, "Methods in Enzymology," Volume 44, Academic Press, New York, 1976.
12. Rozwadowski, M., *Acta Phys. Pol.*, 1961, **20**, 1005.
13. Lavorel, J., *J. Phys. Chem.*, 1957, **61**, 1600.
14. Bishop, E., *Editor*, "Analytical Chemistry," Volume 51, "Indicators," Pergamon Press, Oxford, 1972.
15. Lübbers, D. W., and Optiz, N., *in* Seiyama, T., *Editor*, "Proceedings of the International Meeting on Chemical Sensors, Fukuoka, Japan, September 19–22, 1983," Elsevier, Amsterdam, 1983, pp. 609–619.

Paper A6/475
Received December 17th, 1986
Accepted March 10th, 1987

Automation of Simple Instrumentation for Langmuir - Blodgett Technology

R. Stephen Brown and Ulrich J. Krull*
Chemical Sensors Group, Department of Chemistry, University of Toronto, Erindale College, Mississauga Road, Mississauga, Ontario L5L 1C6, Canada

Simple and sensitive electronic circuitry suitable for automated monolayer analysis and constant pressure deposition at an air - water interface is described. The performance criteria are considered with respect to instrumental response and the resolution of chemical effects observed during the acquisition of pressure - area isotherms. The advantages of the circuitry, the Wilhelmy plate pressure transducer and the cost are compared with a popular commercial trough system.

Keywords: *Langmuir - Blodgett trough; Wilhelmy plate; thin-layer deposition*

Current interest in the study of surfactant and lipid monolayers at air - water interfaces has resulted in an increased demand for trough assemblies suitable for Langmuir - Blodgett monolayer pressure - area studies.[1,2] Such studies include classical compression isotherms and important new applications based on the transfer of the surface monolayer to a solid substrate.[3–9] In most instances, it is desirable that the deposition process provides a surface monolayer which maintains the original molecular order established on the trough. Such criteria necessitate careful control of monolayer surface conditions, especially during transfer of the film from the sub-phase on to the substrate. Previous monolayer studies and monolayer deposition results reported by Krull and co-workers[10–12] were derived from commercial trough devices capable of accurately maintaining surface characteristics by feedback control of surface pressure (Lauda Model 1974 thin-film balance, Sybron-Brinkmann, Toronto, Canada). Recently, a relatively simple trough design was introduced to provide an accurate and inexpensive instrumental alternative to conventional commercial equipment.[13] This trough made use of a motor-driven moveable polytetrafluoroethylene (PTFE) barrier to vary the surface area and a Wilhelmy plate, coupled to a sensitive position sensor, as a film pressure transducer. The total cost of this assembly was less than one tenth of that of the commercial system. The shortcomings of the proposed system were: the operation was fully manual, requiring the operator to continually adjust the barrier position during constant-pressure operation; there was no mechanism for the measurement of barrier position or trough area, except by the manual recording of position values; and pressure measurement used an expensive pH meter as a millivolt meter, which made for awkward zero and calibration adjustments of the output.

This paper describes the development of a simple, inexpensive electronic measurement and control system for the automation of the manual trough assembly. The circuitry provides automated barrier position and pressure detection measurements, which allows continuous monitoring of experimental parameters on an X - Y plotter and supports the operation of a feedback proportional pressure control circuit. The electronics are described such that they could easily be adapted to operate on similar manual troughs using a variety of different components. An evaluation of the performance of the system compared with the commercial trough assembly is presented.

Experimental

Trough Design

The rectangular trough was machined from a solid block of PTFE to provide an internal solution compartment which measured 400 × 200 × 25 mm. A sweep barrier of PTFE measuring 250 × 20 × 10 mm spanned the trough and was moved to vary its surface area. One point of a closed loop of wire was attached to the edge of the barrier, and movement was achieved with a high-torque d.c. motor (Hankscraft Motors, Reedsburg, WI, USA) which drove the cable loop between an apex at the motor hub and a pulley at the other end of the trough (Fig. 1). A more detailed description of the trough fabrication may be found in reference 13.

Barrier Position Measurement

A 10-turn, precision (linearity ±0.25%) potentiometer was coupled to the motion of the sweep barrier by means of a second belt-drive system as shown in Fig. 1. V_{in} was taken from a 6 V regulated power supply. R1 and R2 were chosen to provide a 0.9 V range over a complete barrier sweep, which was suitable for the X - Y plotter, where

$$V = \text{turn fraction} \times \frac{R2}{R1 + R2} \times V_{in} \quad \ldots \quad (1)$$

The results reported here were for a turn fraction of 3/10 (three turns over a barrier sweep) and R1 and R2 values of 20 kΩ. The potentiometer was originally positioned so that the output provided zero volts at a sweep barrier position

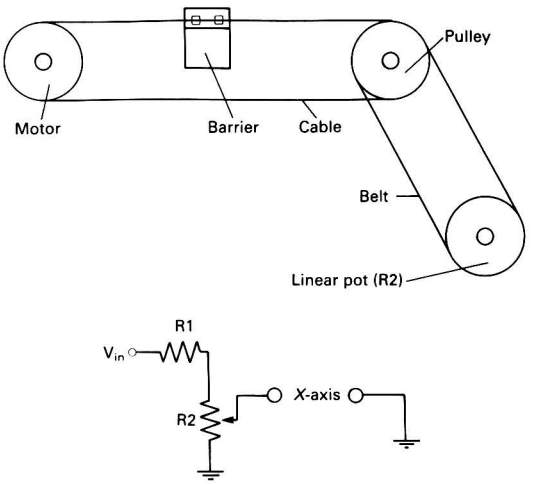


Fig. 1. Schematic diagram of barrier drive with X-position potentiometer and electronic circuit

* To whom correspondence should be addressed.

equivalent to zero surface area. The barrier was then swept to a position of known surface area, and the variable calibration adjustment on the *X*-axis of the recorder was used to set a positional display representative of that area. All further area measurements between these two extremes were indicated by the linear voltage output change which correlated directly with trough area. Calibration in terms of area per molecule, where desired, was simply achieved by utilisation of a scaling factor of the *X*-axis derived from the number of surfactant molecules which were quantitatively deposited at the aqueous surface to prepare the monolayer.

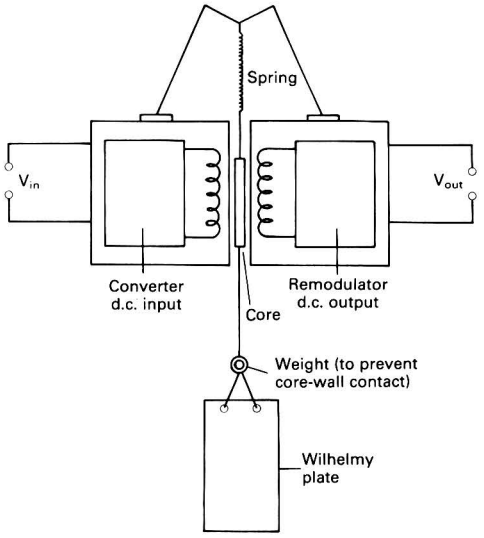


Fig. 2. Surface pressure transducer with filter-paper as a Wilhelmy plate

Pressure Measurement and Control

The pressure sensor was a Wilhelmy plate made of Whatman student grade filter-paper, suspended from the iron core of a vertically mounted inductive motion transducer (Model SS-101, G. L. Collins, Long Beach, CA, USA), as illustrated in Fig. 2. The converter d.c. input was taken from a 6 V regulated power supply. The plate was positioned so as to span the air - sub-phase interface vertically. The surface pressure of a monolayer on the sub-phase was measured by the proportional vertical displacement of the plate.

Circuitry based on operational amplifiers was constructed as shown in Fig. 3. The circuitry was divided into two major sections. The pressure measuring section was used to drive the *Y*-axis of an *X* - *Y* plotter. The pressure control section was used to drive the motor, and subsequently the sweep barrier of the trough. Each operational amplifier (op amp) in the circuit was a standard 741 device (Motorola MC1741 or equivalent). All resistors were 0.25 W ±5% rated components.

The pressure measuring section consisted of three op amps arranged in a differential configuration. The inputs to be compared were taken from the pressure transducer (P) and from a zero compensating adjustment (Rz2). A fourth op amp served to amplify linearly the difference output by a gain factor of R4/R3, where R4 was variable to allow for calibration. In the controlling device, R1 was 10 kΩ, R2 was 26.7 kΩ, R3 was 10 kΩ and R4 was a 50 kΩ, 10-turn precision (linearity ±0.25%) potentiometer. R1 and R2 were chosen to give a stable output from the differential configuration of the three amplifiers. Each set of resistors, R1 and R2, was of equal value to give a common mode rejection ratio of unity. If the ratio was not unity, the gain of the amplifier circuit would vary with the zero adjustment, making calibration very difficult. Resistors of ±5% precision were experimentally observed to

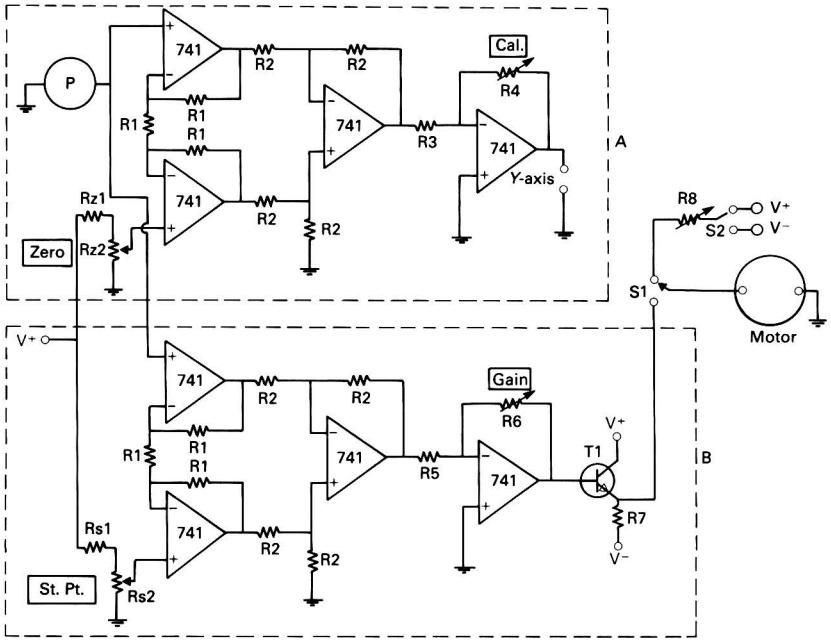


Fig. 3. Schematic diagram of electronic automation circuit, indicating pressure transducer (P) input, surface pressure zero (Rz2) and calibration (R4) potentiometers, pressure set-point (Rs2) and gain (R6) potentiometers, *Y*-axis output and sweep barrier motor control (S1), speed (R8) and direction (S2) adjustments. The pressure measuring sector (A) and the pressure control sector (B) are outlined

exhibit no gain dependence on the zero level. R3 and R4 gave a calibration gain which was continuously variable from a factor of 0 to 5 (R4/R3). This was suitable for adjustment of the *Y*-axis sensitivity of the plotter.

The initial core position within the pressure transducer was adjusted so that the transducer output was approximately +0.5 V, and so that the output voltage increased with an upward motion of the Wilhelmy plate. The zero-adjustment circuitry employed a value of V^+ of +12 V, a resistor Rz1 of 200 kΩ and a 10-turn, 20 kΩ potentiometer Rz2. This choice of components provided a zero correction range from 0 to 1.09 V [from equation (1), where the turn ratio is unity, R1 = Rz1 and R2 = Rz2] corresponding to the range of the output of the transducer.

Calibration of surface pressure was performed relative to the commercial instrument. Stearic acid monolayers were compressed to collapse on the commercial trough, and collapse was reproducibly achieved at a surface pressure of 38.8 ± 0.3 mN m^{-1}. An identical sub-phase solution without a monolayer was placed in the uncalibrated trough, and the zero position was then set using the zero adjustment. Stearic acid monolayers of equivalent mass to those used on the commercial device were then applied to the trough. Monolayers were compressed to collapse, and the *Y*-axis was set to 38.8 mN m^{-1} by adjustment of the calibration potentiometer (R4).

The use of three op amps in a differential configuration allowed for an unloaded (MΩ impedance) input for the transducer. The linearity of the transducer output with core displacement was maximised by preventing the loading of the induction coil, as a minimum current was required by the measuring device.

The circuit used for pressure control was virtually identical with that for pressure measurement, except that the output was used to control the barrier drive motor. R1, R2 and V^+ were as previously described. The resistance of R5 was 1 kΩ, and R6 was a 20 kΩ, 10-turn, PC board-mounted potentiometer. T1 was a 2N2055 power transistor, with a heat sink. R7 consisted of two 280 Ω, 5 W resistors in series. The voltage supplies V^+ and V^- applied to the transistor were +12 and −12 V, respectively, and were derived from a power supply capable of delivering 250 mA. Rs1 was 200 kΩ and Rs2 was a 10-turn, 20 kΩ potentiometer. The control circuit continuously monitored the difference between the output of the pressure transducer (P) and the output of the set-point potentiometer (Rs2). The voltage difference was proportionally amplified and then applied to move the sweep barrier in the appropriate direction. The sweep barrier movement served to reduce the voltage difference, and movement terminated only when the difference attained a value of zero volts. The polarity of the sweep barrier drive motor was set so that a negative deviation of pressure from the set-point would cause inward movement (compression), and a positive deviation would result in outward movement (expansion).

The pressure set-point was adjusted by turning the set-point potentiometer (Rs2) and observing the pressure response on the calibrated *Y*-axis of the recorder. Initially, the gain was adjusted by setting the pressure to an intermediate value, and then increasing the value of R4 until the motor control became unstable (began oscillating about the set-point, without coming to rest). The gain potentiometer was then set back a quarter turn, and was not subsequently re-adjusted. This method of optimisation provided maximum stable proportional pressure control. A value of 20 for R4 : R3 was found to be large enough to achieve instability by adjustment of the gain. A larger gain could be achieved by increasing the ratio of R4 : R3.

A switching network was used to shunt between manual and pressure control operation for recording pressure - area isotherms and to control manual sweep speed and direction. This required a single-pole double-throw (SPDT) switch (S1) to select the motor-driving source and a SPDT switch (S2) in series with a 5 kΩ variable potentiometer (R8) to control manual motor speed and direction. Stable operation of all circuits was enabled by ensuring that all device grounds were common between power supplies, the controller and the recorder. The op amps were all driven by ±12 V regulated power supplies.

Results and Discussion

Pressure Measurement

The sensitivity of the Wilhelmy plate to surface pressure increased with plate width and therefore surface contact area. The linearity of the output decreased with increased plate size. A suitable compromise, allowing adequate vertical displacement without significant loss of linearity, was achieved using a plate which measured 20 mm high by 10 mm wide.

The Wilhelmy transducer output was evaluated directly against the Langmuir - Blodgett design of the commercial instrument by temporarily mounting the Wilhelmy plate in the commercial trough. Pressure measurements were acquired concurrently from the two transducers, and the Wilhelmy plate output was correlated with the commercial instrument values (Fig. 4). A regression analysis yielded a linearity coefficient of 1.001 ± 0.002, and an off-set constant of −0.013 ± 0.008 mN m^{-1}. The correlation coefficient of the fit was 0.998. This indicated that the Wilhelmy plate transducer, in association with the described measuring electronics, was able to function well within the requirements of most monolayer deposition and pressure - area experiments. Possible detrimental effects of the physical disruption of the monolayer by the protruding plate have not been determined.

The Wilhelmy plate was easier to clean and less sensitive to extraneous environmental vibration than the commercial pressure sensing barrier. Its sensitivity to air currents was compensated by situating the trough in a large Perspex box. Dislodging and re-positioning of the transducer core position, either by mechanical contact or by changing the sub-phase, was observed to have an effect on the zero position of the pressure output, but no significant effect on the calibration. This suggested that transducer sensitivity to surface pressure was relatively independent of core position. An occasional problem was the attraction and physical contact of the transducer core to the inner wall of the induction column. Core - wall contact provided resistance to core motion, and eradicated the reliability of pressure determinations. The occurrence of core - wall contact was largely eliminated by hanging a small, non-magnetic weight (0.1 g) from the core assembly to increase resistance to horizontal motion. Experimentally, no detrimental effects were observed in the transducer output as a result of the extraneous weight.

Compression curves recorded for the trough (Fig. 5) quantitatively and qualitatively compared favourably with

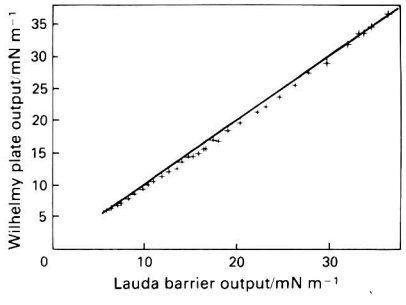


Fig. 4. Comparison of Wilhelmy plate pressure measurements with commercial (Lauda Model 1974) pressure results. +, Experimental results; solid line, ideal pressure agreement

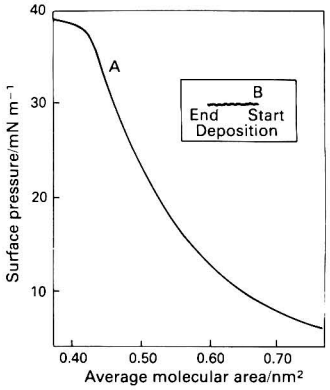


Fig. 5. (A) Compression curve for phosphatidylcholine - cholesterol (1 + 1) obtained on the described trough. (B) Pressure - area monitoring during the constant-pressure deposition of a monolayer from the trough, indicating pressure control of ±0.1 mN m^{-1}

those obtained from the commercial instrument, and with results in the literature. Monolayer structure and purity could be readily evaluated by features such as collapse pressure and area per molecule, and by fine structure such as shoulder peaks and inflections observed in pressure - area analyses.

Pressure Control

The trough control system was observed in most instances to behave identically with the commercial system. The operation of both devices under constant pressure deposition yielded a pressure controlled to ±0.1 mN m^{-1}. Each trough occasionally showed a tendency to leak monolayer material out of the enclosed surface compartment, generally owing to wear at the sweep barrier - trough contact point. Such leakage was found to occur around both the moving barrier and the stationary, pressure-sensing barrier for the commercial instrument. Loss under the moving barrier of either trough was incidental, and simply resulted in a loss of the calibration of the area per molecule without influencing the pressure calibration. This type of loss of surfactant was easily alleviated by the application of fresh PTFE tape between the sweep barrier and the trough edge. Leakage under the pressure-sensing barrier of the commercial trough, however, resulted in an alteration of the pressure calibration, which was a function of the difference of the surface pressure on either side of that barrier. Deposition results which were obtained from the commercial trough while this type of leakage was occurring were necessarily discarded owing to the loss of surface pressure characterisation. Repairing this type of leakage was possible only by a re-taping of the barrier system, which was awkward, and often introduced impurities into the apparatus. The physical mechanism of operation of the Wilhelmy plate did not rely on a pressure difference measurement, and therefore any leakage which occurred could not have had an effect on the surface pressure calibration.

The larger sub-phase dimensions of the new trough were ideally suited for many deposition experiments which were not possible with the smaller commercial instrument sub-phase. Of particular interest was the ability to deposit monolayers on to long optical glass and quartz fibres by casting at an angle for the purpose of intrinsic optrode development.[12]

Table 1. Comparison of the operational characteristics of the proposed trough with a commercial system [Lauda Model 1974 thin-film balance (Sybron-Brinkmann, Toronto, Canada)]

Parameter	Proposed device	Commercial device
Measuring range	0–50 mN m^{-1}	0–100 mN m^{-1}
Pressure sensitivity	0.5 mN m^{-1}	0.3 mN m^{-1}
Barrier speed	1–9 cm min^{-1}	0.9–6.5 cm min^{-1}
Area calibration	±0.03 nm^2	±0.01 nm^2
Pressure control	±0.1 mN m^{-1}	±0.1 mN m^{-1}
Cost factor	1	10

Quantitative evaluation of deposition from a monolayer held under constant pressure was achieved by observation of barrier movement during monolayer extraction. This movement confirmed expected deposition results, such as inward movement during withdrawal of a polar substrate, or during insertion of a non-polar substrate. The total change in surface area was used to calculate the transfer ratio of surface monolayer to the substrate, generally yielding results close to unity, as expected.

Conclusion

A comparison of the performance of the trough and control system with the commercial system is presented in Table 1. In general, the characteristics of the trough and control assembly were adequate with respect to the Langmuir - Blodgett trough specifications. Surface pressure measurement was possible after completion of a simple calibration procedure, and the results obtained agreed well with the commercial instrument pressure measurements. During deposition experiments, surface pressure was controlled to ±0.1 mN m^{-1}, which was adequate to ensure proper transfer of monolayer structure from the trough to a substrate, and was equivalent to the controlling ability of the commercial instrument. The cost of this trough assembly was maintained at less than one-tenth of that of the commercial version.

References

1. Langmuir, I., *J. Am. Chem. Soc.*, 1917, **39**, 1848.
2. Blodgett, K. B., and Langmuir, I., *Phys. Rev.*, 1937, **51**, 964.
3. Pitt, C. W., and Walpita, L. M., *Thin Solid Films*, 1980, **68**, 101.
4. Furtlehner, J. P., and Messier, J., *Thin Solid Films*, 1980, **68**, 233.
5. Sagiv, J., *J. Am. Chem. Soc.*, 1980, **102**, 92.
6. Weis, R. M., Bakkrishnan, K., Smith, B. A., and McConnell, H. M., *J. Biol. Chem.*, 1982, **257**, 6440.
7. Fischer, A., and Sackmann, E., *J. Phys. (Paris)*, 1984, **45**, 517.
8. Bowen, P. J., and Lewis, T. J., *Thin Solid Films*, 1983, **99**, 157.
9. Smaby, J. M., Hermetter, A., Schmid, P. C., Paltauf, F., and Brockman, H. L., *Biochemistry*, 1983, **22**, 5808.
10. Arya, A., Krull, U. J., Thompson, M., and Wong, H. E., *Anal. Chim. Acta*, 1985, **173**, 331.
11. Krull, U. J., Thompson, M., and Wong, H. E., *J. Electroanal. Chem.*, 1986, **211**, 371.
12. Krull, U. J., Bloore, C., and Gumbs, G., *Analyst*, 1986, **111**, 259.
13. Krull, U. J., Thompson, M., and Wong, H. E., *Analyst*, 1985, **110**, 1299.

Paper A7/41
Received February 9th, 1987
Accepted March 24th, 1987

Hydroxyproline in Pork

Analytical Methods Committee*
Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN, UK

In a previous report, figures were given for the nitrogen factors of pork, based on the analyses of various joints of pork taken from five selected carcases. The hydroxyproline contents of various samples were determined at the same time as the other measurements were made but, because of difficulties in preparing homogeneous samples of some tissues, the results were withheld for confirmation. These results are presented in this paper.

Keywords: *Hydroxyproline; pork*

The Analytical Methods Committee has received and has approved for publication the following report from its Meat Factors Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this report was Prof. R. A. Lawrie (Chairman), Mr. N. Craddock (until December 1986), Mr. R. A. Evans, Mr. D. Favell, Dr. G. Finney (from December 1986), Mr. N. M. Griffiths, Prof. R. S. Hannan, Mr. A. J. Harrison, Mr. N. Harrison, Dr. R. B. Hughes, Dr. A. J. Kempster, Mr. R. S. Kirk, Dr. R. L. S. Patterson and Dr. R. Wood with Mr. J. J. Wilson as Secretary.

Introduction

The Meat Factors Sub-Committee[1] was appointed in November 1983 to review the nitrogen factor† for pork recommended in 1961 by the Meat Products Sub-Committee.[2] The figure 3.45 was confirmed as the nitrogen factor (on a fat-free basis) most appropriate for the entire comminuted raw meat (including intermuscular fat) for the dressed side of pork.

Subsidiary to the main trials, which involved the analysis (for nitrogen, moisture, ash and fat) of samples from the rind, subcutaneous fat and lean of pork, hydroxyproline was also measured. As this amino acid is characteristic of connective tissue and is absent from muscular tissue, its determination is particularly important in meat analysis. The location of the joints and the methods of sample preparation and analysis were published in a previous report.[1]

Because of difficulties in preparing homogeneous samples of rind, data on the hydroxyproline content of the tissues and joints were withheld for confirmation. These results are presented in this paper.

Statistical Methodology

The data were analysed using an analysis of variance[3] which included terms for joint and side.

Initially, separate analyses were carried out within each tissue to calculate mean percentage hydroxyproline contents for each joint. Subsequent analyses weighted each joint for the component tissues. The Newman - Keuls multiple range test[4] was then conducted on joint means to see if any anatomical patterns could be identified. Multiple range tests are used to identify groupings that occur in a data set by systematically testing differences between pairs of means.

* Correspondence should be addressed to the Secretary, Analytical Methods Committee, Analytical Division, Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN, UK.

† In common usage the term "nitrogen factor" signifies the percentage of nitrogen in a sample (on a fat-free basis).

Results and Discussion

Fig. 1 illustrates the positions on the carcase of joint means, on a fat-free basis, for (i) lean only, (ii) the lean with its associated subcutaneous fat and (iii) the lean with its associated subcutaneous fat and rind. It also shows the corresponding calculated values for the four middle joints and for the side as a whole.

Table 1 shows the results of multiple range tests on joint means for individual tissues (both with associated fat and fat-free). As would be expected, the content of hydroxyproline is lowest in the lean samples, intermediate in subcutaneous fat (which is held in a framework of connective tissue) and highest in rind. The relatively low percentage in the lean from the leg, despite the tendonous nature of the distal muscles of this joint, must be presumed to reflect the predominance of the large muscles in the ham area which have a low content of connective tissue.[5]

It is evident that the hydroxyproline (on a fat-free basis) is lower in the lean of the leg and the rump loin than in the rib loin, rib belly and hand, and that there is no significant difference in the hydroxyproline content of the subcutaneous fat between any of the seven joints. On the other hand,

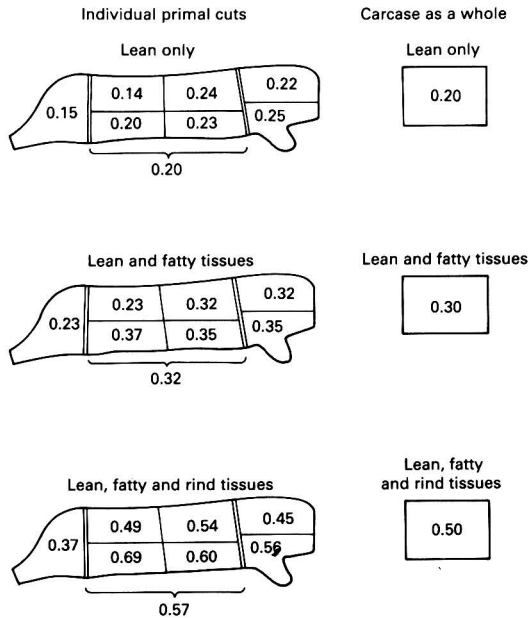


Fig. 1. Hydroxyproline (% fat-free basis) in porcine joints

Table 1. Hydroxyproline contents of component tissues of joints of pork. Least-squares means, %

Tissue	Basis	n*	Joint†							S.E. of means
			Leg	Rump loin	Rump belly	Rib loin	Rib belly	Collar	Hand	
Rind . .	With associated fat	10	$3.40^{a,b}$	3.99^{d}	$3.50^{a,b,c}$	$3.68^{b,c}$	3.75^{c}	$3.59^{b,c}$	3.26^{a}	0.084
	Fat-free	10	3.82^{a}	4.28^{b}	3.77^{a}	$4.16^{b,c}$	$4.06^{a,b,c}$	4.37^{b}	$3.87^{a,c}$	0.088
Sub fat . .	With associated fat	5	0.43^{b}	$0.31^{a,b}$	$0.34^{a,b}$	0.26^{a}	$0.32^{a,b}$	$0.38^{a,b}$	0.41^{b}	0.030
	Fat-free	5	1.65^{a}	1.65^{a}	1.27^{a}	1.46^{a}	1.32^{a}	1.84^{a}	1.46^{a}	0.137
Lean . .	With associated fat	5	$0.14^{a,b}$	0.13^{a}	$0.18^{a,b}$	$0.21^{a,b}$	$0.20^{a,b}$	$0.20^{a,b}$	0.23^{b}	0.020
	Fat-free	5	$0.15^{a,b}$	0.14^{a}	$0.20^{a,b,c}$	0.24^{c}	0.23^{c}	$0.22^{b,c}$	0.25^{c}	0.020

* Number of replicates.
† Values in a horizontal line bearing the same superscript are not significantly different at the 5% level.

Table 2. Hydroxyproline contents of joints of pork weighted for component tissues. Least-squares means, %

Basis	n*	Joint†							S.E. of means
		Leg	Rump loin	Rump belly	Rib loin	Rib belly	Collar	Hand	
With associated fat	5	0.31^{a}	$0.38^{a,b}$	0.47^{c}	$0.39^{a,b}$	$0.42^{b,c}$	0.34^{a}	$0.44^{b,c}$	0.020
Fat-free	5	0.37^{a}	$0.49^{b,c}$	0.69^{e}	$0.54^{b,c,d}$	0.60^{d}	0.45^{b}	$0.56^{c,d}$	0.026

* Number of replicates (using the mean figure for hydroxyproline in the rind, *i.e.*, the average from the two sets of data).
† Values in a horizontal line bearing the same superscript are not significantly different at the 5% level.

although there are some significant differences between the rinds of certain joints in this parameter, there is no systematic anatomical pattern.

Table 2 shows the results of multiple range tests on joint means weighted for the component tissues (both with associated fat and fat-free).

It will be noted that the value for the leg, when expressed on a fat-free basis, is significantly lower than those in the other six joints, whereas that from the rump belly is significantly higher.

Considerably more data on the hydroxyproline content of different porcine joints will become available from the further investigations recommended by the Meat Factors Sub-Committee.[1]

The statistical analysis was carried out by Miss Karen Solly of the Meat and Livestock Commissions's Planning and Development Group.

References

1. Analytical Methods Committee, *Analyst*, 1986, **111**, 969.
2. Analytical Methods Committee, *Analyst*, 1961, **86**, 557.
3. Winer, B. J., "Statistical Principles in Experimental Design," McGraw-Hill, New York, 1971, p. 309.
4. Winer, B. J., "Statistical Principles in Experimental Design," McGraw-Hill, New York, 1971, p. 216.
5. Lawrie, R. A., Pomeroy, R. W., and Cuthbertson, A., *J. Agric. Sci.*, 1963, **60**, 195.

Paper A7/107
Received March 17th, 1987

SHORT PAPERS

Rapid Application of Samples to Thin-layer Chromatographic Plates

J. Stephen Cridland
Department of Pharmacology, University of Cape Town Medical School, Observatory, 7925 South Africa

An inexpensive device for 16–21-s application of samples to thin-layer chromatographic plates is described. The reproducibility of application is comparable to that of standard methods and is adequate for quantification. Samples may be dissolved in a variety of solvents; those tested were chloroform, diisopropyl ether, ethyl acetate, butan-1-ol and plasma - water (1 + 1).

Keywords: *Thin-layer chromatography; sample application; assay apparatus*

Thin-layer chromatographic densitometry (TLCD) or fluorimetry is the most inexpensive versatile analytical technique for determining nanogram amounts of organic compounds such as drugs in blood. However, it is under-utilised because of lack of sensitivity,[1] the expensive equipment needed for sample application and the inaccuracy and tedium of application using hand-held capillaries. This paper describes and validates the use of a laboratory-made applicator (Fig. 1).

The applicator was designed for the loading of solutions of a drug in a solvent held in full 5-μl capillaries, followed by transfer of their contents on to a line of origin 1.5 cm from the edge of a thin-layer plate. The interval of application between samples was chosen to be 7 mm (the diameter of the spot formed by 5 μl of solvent), although this is unnecessarily wide for most applications; if the solvent is chosen so that it scarcely elutes the drug, the spot formed by the latter after drying is about the same in diameter as the capillary, about 1 mm. Typically, for paracetamol, such solvents are diisopropyl ether, chloroform and ethyl acetate but not butan-1-ol or plasma - water.

A prototype device allowed only vertical loading but it is often convenient to use solvents that do not stay fully within a vertically supported capillary, *e.g.*, chloroform. Such solvents will not flow out if the capillary is held at about 70° from the upright.

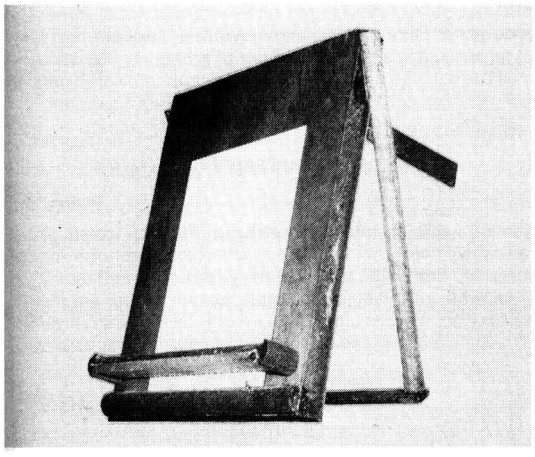


Fig. 1. Applicator (with TLC plate *in situ*) in semi-vertical position for loading

Experimental

Apparatus

To hold the thin-layer plate, a base 25 cm square is used; to prevent the plate from sliding off, a lip is attached at the edge parallel to the proposed line of origin. The base is fitted with legs so that it is stable in the horizontal position and at 70° from the horizontal. Along the proposed line of origin and 0.75 cm above are fixed an array of 27 glass tubes glued side by side. Each tube is 2.5 cm × 7 mm o.d. × 1 mm i.d., to accommodate capillaries (*e.g.*, 5-μl micropipettes; Modulohm, Herlev, Denmark) typically about 0.6 mm in diameter and 3 cm in length. Each tube is perpendicular in two dimensions to the base, so that when the latter is 70° from the horizontal, the tubes are at 20°.

To use the apparatus, a thin-layer plate is placed on the base with an edge firmly against the lip so that the tube array is above the notional line of origin 1.5 cm "up" the plate. The apparatus is then tipped into the 70° position. Each capillary is filled (in a near-horizontal position) from a solution, then withdrawn. The "wet end" is then raised higher than the other (but still maintaining near-horizontal orientation) and is wiped dry (if necessary). The "dry end" is inserted into the hole running through the appropriate glass tube and the capillary slides down under its own mass or is stroked gently down into contact with the plate using a strip of writing paper. It may drain at this point. When loading is complete, the apparatus is returned to the horizontal position when other capillaries will drain their contents on to the plate. Should this not occur (rarely, *e.g.*, with some polar solvents and with small capillaries) they can be induced to drain by raising and dropping them. The plate should be labelled at this stage at the latest, as it is easy to forget to do so. Holding the plate firmly, the apparatus is inverted so that the capillaries, now empty, fall out. The plate can then be removed without the risk of scratching the silica surface.

The device is built of Pyrex glass (Corning, Stone, UK), wood, Araldite epoxy resin (Ciba-Geigy, Basle, Switzerland) and Wood Seal polyurethane varnish (Gypkor, Cape Town, South Africa) so that it may be cleaned, when necessary, using technical alcohol or a similar organic solvent.

Validation

To obtain at least a pessimistic estimate of the reproducibility of sample application, 27 applications of 1.3 μg of paracetamol in 5 μl of each of the following solvents were made: chloroform, diisopropyl ether, ethyl acetate, butan-1-ol and human blood plasma - water (1 + 1) (as in toxicological assay). These solvents were chosen for their range of volatility, polarity and viscosity. After drying, the plates (HPTLC

Table 1. Validation of thin-layer applicator. Results are for 1.3 μg of paracetamol (27 applications)

Solvent	Mean peak height/cm	Coefficient of variation, %	Time of application per sample/s
Chloroform	13.4	2.2	16.3
Diisopropyl ether . .	19.7	3.1	16.5
Ethyl acetate	13.8	2.9	16.1
Butan-1-ol	6.3	4.9	20.9
Plasma - water (1 + 1)	4.5	7.9	19.4

Fertigplatten, Kieselgel 60; Merck, Darmstadt, FRG) were developed through 3.5 cm with chloroform - propan-2-ol (9 + 1); the R_F value of paracetamol was 0.35. Each series of applications was timed. After drying, each plate was scanned using a densitometer (KM3 chromatogram spectrophotometer; Zeiss, Oberkochen, FRG) at 280 nm in the absorption mode. The peak heights and the coefficients of variation were calculated.

The results are shown in Table 1.

Discussion

Table 1 shows that the reproducibility was adequate. As expected, the coefficients of variation were lowest when the solvents were least polar and the peaks leanest. The volatility of the solvent does not seem to be a problem.

The duration of application is shorter than for manual injection into chromatographs. This, and the simple *modus operandi*, lead to great simplification for the experimenter and technician compared with manual spotting.

Close examination of the thin-layer plates used revealed no damage by capillaries, but it was evident that the plasma - water mixture dissolves silica slightly.

Reference

1. Cridland, J. S., and White, N. J., *Clin. Chem.*, 1983, **29**, 403.

Paper A6/428
Received November 10th, 1986
Accepted March 2nd, 1987

Routine Spectrophotometric Determination of Citric Acid in Milk Powders

Harvey E. Indyk and Andreas Kurmann
New Zealand Co-operative Dairy Co. Ltd., P.O. Box 7, Waitoa, New Zealand

A modified method for the determination of citric acid based on the chromophore formed between pyridine, acetic anhydride and citrate is described. The technique facilitates the simple, rapid and routine determination of citric acid in milk powder products. The recovery and precision (C.V.) are 95.2 and 2.3%, respectively, and comparison with a specific enzyme assay shows a mean difference between the two methods of 1.5%. The method may also be applied to liquid milks and whey.

Keywords: *Milk powder; citric acid determination; spectrophotometry*

The proteins of bovine milk are probably the best characterised of all food systems. However, although several models for the casein micelle structure have been proposed, none is as yet completely satisfactory. It is, however, well accepted that the structural integrity of the casein complex is intimately dependent on the availability of colloidal calcium phosphate.[1–5] Citrate would seem to be multi-functional in milk and in the present context it is likely to behave as an endogenous chelator of the calcium ion, thereby influencing both the heat stability and viscosity of milk protein.[6–8]

Several analytical methods have been developed for the determination of citrate. Traditionally, spectrophotometric techniques utilising the formation of a chromophore between pyridine, acetic anhydride and citrate have been employed in liquid milk[9] and adapted for cheese.[10] Enzyme-catalysed reactions have been applied to citrate with proven specificity and are considered a suitable reference method.[11,12] Gas - liquid chromatography has been used in the determination of organic acids, including tricarboxylic acids, subsequent to sample purification and derivatisation either with *N,O*-bis-(trimethylsilyl)acetamide[13,14] or by alkylation.[15,16] Currently, high-performance liquid chromatography is increasingly applied to this type of determination although sample clean-up still remains a problem in many instances.[17–23]

In this, as in many industrial laboratories, chromatographic equipment is often fully committed to other problems, making it unavailable for the routine quality control of citrate levels in products. Limitations of sample throughput and tediousness similarly make the enzyme assay unattractive for high frequency determinations. This paper therefore describes the modifications and improvements made to the spectrophotometric determination in fluid milk,[9] thereby facilitating the routine determination of citric acid in milk powder products.

Experimental

Equipment

A Shimadzu UV-160 spectrophotometer (Tokyo, Japan) was used for measurements at 428 nm. Two water-baths were used, one thermostatically controlled at 32 °C and the second containing an ice - water slurry. Two pipettors were set to deliver 1.7 and 5.8 ml. Pipettes with volumes of 5,10 and 15 ml were used for the standards preparation.

Reagents

Trisodium citrate, pyridine and trichloroacetic acid (TCA) were of analytical-reagent grade and were obtained from BDH Chemicals (Poole, Dorset, UK). Acetic anhydride (analytical-reagent grade) was obtained from Reidel-de Haen (Seelze, Hannover, FRG) and purified Milli-Q water was used in the preparation of sample and reagent solutions where required.

Standard Preparation

A stock solution of citric acid (1000 μg ml^{-1}) was prepared by dissolving exactly 1.5318 g of trisodium citrate in 1 l of water. Volumes of 0, 5, 10 and 15 ml of the stock solution were accurately dispensed into 50-ml calibrated flasks. At the same point in the procedure as for the reconstituted samples (see below), 20 ml of TCA (30% *m/V*) were added to each flask which was then diluted to the mark with water.

Sample Preparation

Samples of approximately 2.0 g of the milk powders were accurately weighed into 100-ml calibrated flasks and reconstituted by adding 40 ml of warm water to each. The flasks were shaken and left to stand for 30 min. A 40-ml aliquot of TCA (30% *m/V*) was added to each flask, which was then diluted to the mark with water, shaken, and left to stand for 45 min. The contents were filtered through Whatman 540 paper, discarding the first 10 ml.

Derivatisation

Aliquots of 1.0 ml of each working standard and sample extract were accurately pipetted into appropriately labelled boiling-tubes fitted with ground-glass stoppers. All the tubes were placed in the ice - water slurry bath and 1.7 ml of pyridine were added to the contents of each tube. Following swirling, 5.8 ml of acetic anhydride were immediately added to each tube. The tubes were stoppered, shaken and placed in the 32 °C water-bath without delay for exactly 30 min, after which they were cooled to room temperature (*ca.* 20 °C).

Determination of Citrate

A calibration graph was established at 428 nm for the working standards (0, 100, 200 and 300 μg ml^{-1}) against the standard blank. The absorbance for each sample was then measured and the concentration (μg ml^{-1}) calculated with reference to the linear calibration graph.

Concentration of anhydrous citric acid (mg per 100 g of sample) =

$$\frac{X}{1000} \times \frac{100}{1} \times \frac{100}{\text{sample mass}}$$

where X = concentration of citrate in sample extract (μg ml^{-1}).

Table 1. Recovery of citric acid from a spiked milk powder formulation. Basal value of 937 mg per 100 g

Citric acid added/μg	Citric acid recovered/μg	Recovery,*%
50	48.2	96.4
100	94.3	94.3
150	142.4	94.9

* Results are mean of duplicate determinations.

Table 2. Comparison of test method with enzyme assay for the determination of citric acid in milk powder. Figures in parentheses are standard deviations

Sample	Test method/ mg per 100 g	Enzyme method/ mg per 100 g	Difference, %
1*	717 (5.7)	724 (1.4)	0.98
2*	749 (5.6)	756 (5.7)	0.93
3†	957 (6.4)	942 (0.7)	1.57
4‡	1463 (23.3)	1430 (2.8)	2.26
5*	726 (7.1)	739 (3.5)	1.79
6†	982 (24.0)	972 (15.5)	1.02
7†	937 (0.4)	955 (20.5)	1.92

* Standardised with de-mineralised whey to final ash level of 2.0–3.0% *m/m*.

† Standardised with de-mineralised whey to final ash level of 3.5–4.0% *m/m*.

‡ Whole milk powder, ash level of 6.0% *m/m*.

Results and Discussion

It was evident from early experiments that there were difficulties in controlling the kinetics of chromophore formation at the relatively low temperature of 32 °C. This was further complicated by the sequential preparation of samples and standards and the differing time that each would stand prior to incubation. These problems were simply resolved by the use of an ice-bath during reagent addition and the optimisation of the incubation time (30 min). Plateau absorbance was achieved after approximately 80 min, however, this would result in excessively long assay times, and termination at 30 min (96% of maximum absorbance) returned satisfactory quantitative information. One further variable which required optimisation was the volume of pyridine (1.7 ml); it has been demonstrated that this amount gave superior stability characteristics combined with maximum spectral intensity.

The absorption spectrum for the complex has been confirmed as exhibiting a single pronounced maximum at 428 nm. The nature of the complex remains unknown although previous reports suggest a condensation reaction between pyridine and citraconic anhydride.[9]

The method was subjected to the usual assessment of criteria for reliability and the following results were obtained.

Linearity

Excellent linearity was observed over the acid concentration range 0–300 μg ml^{-1}.

Recovery

Table 1 illustrates the recovery for a typical milk powder formulation spiked with citrate.

Precision

Replicate determinations (n = 7) for a typical sample indicated a mean, standard deviation and coefficient of variance of 928 mg per 100 g, 21.4 and 2.3%, respectively, and illustrated satisfactory precision for this determination.

Accuracy

Several samples were analysed for citric acid by the modified spectrophotometric method. Comparison was made with the reference enzymatic procedure[11] after dissolution of the milk powder, protein removal with TCA solution (10% *m/V*) and filtration. The results correlated well and are presented in Table 2.

It is of interest that those products low in citrate are standardised with partially de-mineralised whey, a process which must remove citrate along with other ionic species.

Conclusions

The above data demonstrate the suitability of the proposed technique and illustrate that the recommended modifications to the original strategy[9] ensure the integrity of experimental data. Mutzelburg[12] has also reported similar data for fluid milk in validating the use of an enzyme assay, however, the enhanced control of experimental variables in the proposed modified procedure returns a lower coefficient of variance (2.3% *vs.* 4.4%) and is directly applicable to milk powders. It has further been demonstrated that the techniques may be identically and successfully applied to both liquid milk and whey (2.0 ml sample volume).

One concern may relate to the use of pyridine, however, the authors routinely perform the entire determination under a fume-hood. Such precautions are equally advisable in other procedures employing pyridine such as carbohydrate and carboxylic acid derviatisation prior to GLC analysis.

Perhaps the principal advantages of the modified spectrophotometric determination as compared to alternatives are the significantly reduced costs, short analysis time and the convenience of a simple technique in industrial laboratories where highly skilled staff are often committed to more sophisticated instrumental determinations.

The authors thank the New Zealand Co-operative Dairy Co. Ltd. for the facilities provided and permission to report the results of this work.

References

1. Waugh, D. F., *in* McKenzie, H. A., *Editor*, "Milk Proteins, Chemistry and Molecular Biology," Volume 11, Academic Press, London, 1971, Chapter 2, pp. 3–85.
2. Farrell, H. M., and Thompson, M. P., *in* Webb, B. H., Johnson, A. H., and Alford, J. A., *Editors*, "Fundamentals of Dairy Chemistry," Second Edition, Avi Publishers, Westport, CT, Chapter 9, pp. 442–473.
3. Cheeseman, G. C., *J. Soc. Dairy Tech.*, 1975, **28**, 181.
4. Fox, P. F., *J. Dairy Sci.*, 1981, **64**, 2127.
5. Neville, M. C., and Watters, C. D., *J. Dairy Sci.*, 1983, **66**, 371.
6. Fox, P. F., and Mulvihill, D. M., *J. Dairy Res.*, 1982, **49**, 679.
7. Mohammad, K. S., and Fox, P. F., *J. Soc. Dairy Tech.*, 1983, **36**, 112.
8. Kjaergaard Jensen, G., and Nielsen, P., *J. Dairy Res.*, 1982, **49**, 515.
9. White, J. C. D., and Davies, D. T., *J. Dairy Res.*, 1963, **30**, 171.
10. "Determination of the Citric Acid Content of Cheese and Processed Cheese Products," International Dairy Federation, Brussels, 1971, FIL-IDF 34 B.
11. "UV-method for the Determination of Citric Acid in Foodstuffs," Boehringer Mannheim, Mannheim, Catalogue No. 139076.
12. Mutzelburg, I. D., *Aust. J. Dairy Tech.*, 1979, 82.
13. Stumpf, D. K., and Burris, R. H., *Anal. Biochem.*, 1979, **95**, 311.
14. Tsuda, T., Nakanishi, H., Morita, T., and Takebayashi, J., *J. Assoc. Off. Anal. Chem.*, 1985, **68**, 902.
15. Littman, S., Schulte, E., and Acker, L., *Z. Lebensm. - Unters. Forsch.*, 1982, **175**, 101.
16. Molnar-Perl, I., and Pinter-Szakacs, M., *J. Chromatogr.*, 1986, **365**, 171.

17. Marsili, R. T., Ostapenko, H., Simmons, R. E., and Green, D. E., *J. Food Sci.*, 1981, **46**, 52.
18. Ashoor, S. H., and Knox, M. J., *J. Chromatogr.*, 1984, **299**, 288.
19. Buchanan, D. N., and Thoene, J. G., *J. Chromatogr., (Biomed. Appl.)*, 1985, **344**, 23.
20. Mentasti, E., Gennaro, M. C., Sarzanini, C., Baioochi, C., and Savigliano, M., *J. Chromatogr.*, 1985, **322**, 177.
21. Caccamo, F., Carfagnini, G., Di Corcia, A., and Samperi, R., *J. Chromatogr.*, 1986, **362**, 47.
22. Panari, G., *Milchwissenschaft*, 1986, **41**, 214.
23. Association of Official Analytical Chemists, *J. Assoc. Off. Anal. Chem.*, 1986, **69**, 359.

Paper A7/42
Received February 9th, 1987
Accepted March 11th, 1987

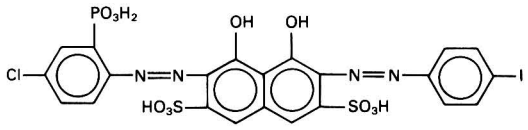
Studies of the β-Type Binuclear Chelates of the Lanthanides with *p*-Iodochlorophosphonazo

Jinzhang Gao,* Ruyao Chen, Jingou Hou and Guangbi Bai
Institute of Chemistry, Northwestern Teachers College, Lanzhou, Gansu, The People's Republic of China

This paper describes work carried out on the colour-forming reaction between *p*-iodochlorophosphonazo (*p*-ICP) and the lanthanides. In the pH range 3.0–4.0 (λ = 700–800 nm) β-type mononuclear chelates are formed between Eu^{3+}, Gd^{3+}, Tb^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+} or Yb^{3+} and *p*-ICP. A synergistic colour effect is clearly observed when equimolar amounts of other lanthanide ions are added to chelate solutions of Er^{3+}, Tm^{3+} or Yb^{3+}, *i.e.*, a β-type binuclear complex is formed. Maximum molar absorptivities were obtained with gadolinium. The calibration graph is linear in the 0–8 × 10^{-6} M range for Ce^{3+}, Pr^{3+}, Ho^{3+}, Tb^{3+}, Dy^{3+}, Tm^{3+} and Lu^{3+}, and the 0–1.0 × 10^{-5} M range for Nd^{3+} and Sm^{3+} when Yb^{3+} = 8.0 × 10^{-6} M.

Keywords: *Lanthanide β-type binuclear chelates; p-iodochlorophosphonazo; synergistic colour; lanthanide determination*

Much attention has been paid to β-type chelates since Taketatsu[1] reported the reaction of chlorophosphonazo III (CPA-III) with the heavier lanthanoid (rare earth) ions to give a chelate with a maximum absorption in the 700–800 nm region. This chelate was defined as a β-type chelate. In a recent paper,[2] we reported the formation of such chelates between rare earth ions and CPA-III. Such chelates can be formed with both light and heavy rare earth elements and can be used for the determination of trace amounts of light rare earths in the presence of heavy rare earths. This paper describes the reaction between *p*-iodochlorophosphonazo (*p*-ICP, see below) and the rare earth ions which produces coloured β-type chelates that could be used in analytical applications.



Structure of *p*-iodochlorophosphonazo

Experimental

Standard solutions of lanthanide chlorides were obtained from the respective oxides of 99.99% purity; *p*-iodochlorophosphonazo was purchased from Wuhan University[3] and used without further purification. A buffer solution, pH 3.80, was prepared from monochloroacetic acid and sodium hydroxide solutions.

All absorption spectra were measured with a Model UV-300 automatic recording spectrophotometer (Shimadzu, Japan). The pH was determined with a pHS-2 meter (Shanghai, China). Experimental details have been described elsewhere.[2]

Results and Discussion

Absorption Spectra

The reactions of 14 lanthanide ions with *p*-ICP at pH 3.0–4.0 were studied and the results obtained are given in Fig. 1 and Table 1. Over the 700–800 nm region only the eight ions from Eu^{3+} to Yb^{3+} were able to form a β-type complex, with absorption maxima ranging from 742 to 751 nm. A hyperchromic effect was observed when an equimolar amount of another lanthanide ion was added to the above chelate solutions to form a β-type binuclear chelate. From Table 2 it can be seen that the probability of hyperchromic effects being observed increases with an increase in atomic number from Eu to Yb. It is interesting to note that the maximum molar absorptivity in all the β-type binuclear chelates is observed for gadolinium. The molar absorptivities of the β-type binuclear chelates are higher than those of the β-type mononuclear chelates. The maximum molar absorptivity is 2.56 × 10^5 l mol^{-1} cm^{-1}.

Conditions of Formation of β-Type Binuclear Chelates

In the same way as chlorophosphonazo-III, *p*-ICP reacts with light and heavy rare earths to form a β-type binuclear chelate.

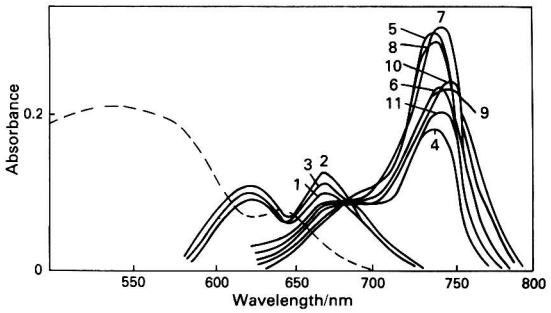


Fig. 1. Absorption spectra of Ln^{3+}-*p*-ICP chelates; reference, distilled water. [Ln^{3+}] = 6.0 × 10^{-6} M, [*p*-ICP] = 1.2 × 10^{-5} M, pH = 3.2–3.4. (1) La, Nd; (2) Ce, Sm; (3) Pr, Lu; (4) Eu; (5) Gd; (6) Tb; (7) Dy; (8) Ho; (9) Er; (10) Tm; (11) Yb; (broken line) *p*-ICP

Table 1. Absorption characteristics of β-type mononuclear chelates in the Ln^{3+} - *p*-ICP system

System	λ_{max}/nm	Absorbance	Molar absorptivity (ε)/l mol^{-1} cm^{-1}
Eu-*p*-ICP ..	742	0.197	3.28 × 10^4
Gd-*p*-ICP ..	744	0.282	4.70 × 10^4
Tb-*p*-ICP ..	745	0.245	4.08 × 10^4
Dy-*p*-ICP ..	745.5	0.284	4.73 × 10^4
Ho-*p*-ICP ..	747	0.271	4.52 × 10^4
Er-*p*-ICP ..	748	0.245	4.08 × 10^4
Tm-*p*-ICP ..	751	0.247	4.12 × 10^4
Yb-*p*-ICP ..	752	0.218	3.63 × 10^4

* To whom correspondence should be addressed.

Table 2. Molar absorptivity of β-type binuclear chelates

System X	Molar absorptivity/10^{-5} l mol^{-1} cm^{-1}													
	X	X-Ce	X-Pr	X-Nd	X-Sm	X-Eu	X-Gd	X-Tb	X-Dy	X-Ho	X-Er	X-Tm	X-Yb	X-Lu
Eu-*p*-ICP ..	0.45	—	—	—	—	—	2.38	1.54	1.82	1.88	1.72	1.69	1.66	—
Gd-*p*-ICP ..	0.88	—	—	—	2.07	2.38	—	2.56	2.47	2.26	2.22	2.12	2.11	—
Tb-*p*-ICP ..	0.56	—	—	—	1.32	1.54	2.56	—	1.82	1.79	1.78	1.74	1.71	—
Dy-*p*-ICP ..	0.66	—	—	—	1.52	1.82	2.47	1.82	—	1.78	1.76	1.72	1.69	0.75
Ho-*p*-ICP ..	0.58	—	—	0.65	1.66	1.88	2.26	1.79	1.78	—	1.73	1.69	1.65	0.74
Er-*p*-ICP ..	0.64	0.86	0.90	1.05	1.66	1.72	2.22	1.78	1.76	1.73	—	1.52	1.37	0.73
Tm-*p*-ICP ..	0.60	0.93	1.04	1.29	1.55	1.69	2.12	1.74	1.72	1.69	1.52	—	1.35	0.66
Yb-*p*-ICP ..	0.65	1.18	1.28	1.32	1.54	1.66	2.11	1.71	1.69	1.65	1.37	1.35	—	0.65

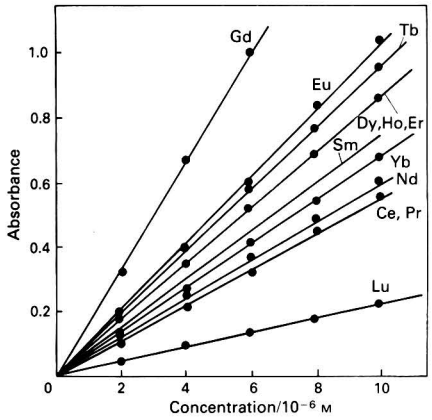


Fig. 2. Effect of various ions as matrix solutions on the absorbance of the Tm^{3+} - *p*-ICP - Ln^{3+} system. $[Tm^{3+}] = 8 \times 10^{-6}$ M; [*p*-ICP] = 6.4×10^{-5} M

This chelate has been determined to be Ln-Ln′-*p*-ICP (1:1:3) (where Ln and Ln′ are light and heavy lanthanides, respectively) by the continuous variation and equilibrium shift methods. The binuclear chelates were stable in the pH range 3.7–4.0 and the colour reaction was complete in 30 min at room temperature.

Beer's Law

A constant amount of Ho^{3+}, Er^{3+}, Tm^{3+} or Yb^{3+} was selected as a matrix solution and another lanthanide ion was added as an artificial sample. The optimum conditions were pH 3.8 and an 8.0×10^{-6} M matrix solution (Ln^{3+}). The results given in Fig. 2 indicate that the slope of the calibration graph increases from Ce^{3+} to Gd^{3+} and decreases from Tb^{3+} to Lu^{3+}. Beer's law is obeyed in the 0–8.0×10^{-6} M concentration range for Ce, Pr, Nd, Sm, Tb, Dy, Ho, Er and Lu, 0–6.0×10^{-6} M for Gd and 0–1.0×10^{-5} M for Eu and Yb when an 8.0×10^{-6} M solution of Tm^{3+} was used as a matrix. Similar results were obtained for other matrix solutions. The coefficient of variation was found to be approximately 0.4%.

References

1. Taketatsu, T., *Bull. Chem. Soc. Jpn.*, 1977, **50**, 1758.
2. Gao, J.-Z., Zhu, X.-M., Kang, J.-W., and Bai, G.-B., *Anal. Lett.*, 1985, **18 (A12)**, 1507.
3. Zhang, H.-S., Chen, Z.H., and Zeng, Y., *J. Wuhan Univ. (Nat. Sci. Ed.)*, 1982, No. 3, 37.

Paper A6/412
Received October 29th, 1986
Accepted December 30th, 1986

Separation of Uranium from Neodymium in a Mixture of Their Oxides

Dawood M. Mohammed
Iraqi Atomic Energy Commission, NRC, P.O. Box 765, Tuwaitha, Baghdad, Iraq

A detailed study has been made of the extraction and separation of uranium and neodymium by poly(hydroxamic acid) resin and tributyl phosphate (TBP) from solutions containing mixtures of these elements. Satisfactory separation schemes were achieved after determining the ion-exchange capacity of the resin for both elements in addition to the distribution coefficient of the elements between TBP and the aqueous solution. After separation, uranium was measured potentiometrically or spectrophotometrically and neodymium was measured by atomic absorption spectrometry.

Keywords: *Uranium separation; neodymium; ion exchange; solvent extraction*

The growth of nuclear technology for power reactor applications stimulated the investigation of urania as a fuel material. The development of thoria - urania[1] and of zirconia - calcia - urania[2] compositions for use as nuclear fuels heightened interest in other oxide sytems. The oxides of interest are those which form extensive solid solutions with urania, *e.g.*, neodymium oxide.[3] The analysis of such solutions is necessary and atomic absorption spectrometry (AAS) is a useful technique for the determination of neodymium. Uranium can interfere in the determination of metals by AAS. Direct aspiration of uranium solution into a spectrometer causes radioactive contamination; in addition, >1 mg ml^{-1} of uranium absorbs at the wavelengths used for these analyses.[4,5] Uranium can be separated from most other metals by ion exchange or solvent extraction.[6] Many solvent extraction procedures have been adopted to separate uranium from other elements.[4,7] In this work tributyl phosphate (TBP) was chosen as a simple and efficient extractant for the separation of uranium.

Ion exchange has also been widely used to separate uranium. Strelow and Winert[8] selectively separated uranium from other elements by cation-exchange chromatography. Philips and Fritz[9] chromatographically separated uranium from thorium and some other elements using poly(hydroxamic acid) resin (PHA).

This paper reports a comparative study of solvent extraction (TBP - HNO_3) and ion exchange (PHA resin) for the separation of uranium from neodymium for their determination in mixtures of their oxides.

Experimental

Materials, Reagents and Apparatus

The polymer PHA was prepared by the method described by Zin[10] and purified by washing with water, 1 M hydrochloric acid and finally with water until free from chloride.

Analytical-reagent grade neodymium oxide, uranyl nitrate, hydrochloric acid, nitric acid, tributyl phosphate and carbon tetrachloride were used. TBP was washed as previously described.[6] De-ionised water was used throughout.

The instruments used were a Perkin-Elmer Model 460 atomic absorption spectrometer, a Metrohm E-536 potentiometer, a Shimadzu Model H240 UV - visible spectrophotometer and a Radiometer pH82 pH meter.

Ion-exchange Separation

Sorption of metal ions by PHA resin

The total capacities of the resin for U^{VI} and Nd^{III} were determined by equilibrating about 0.5 g of the moist resin (known water content) with aqueous solutions containing the metal ions at known concentrations. For the adjustment of pH, an acetate buffer and hydrochloric acid were used in the pH ranges 3–7 and 1–2, respectively. The resin was then filtered and the ion concentrations in the filtrate were measured by AAS for Nd and by spectrophotometry[11,12] for U. The total capacity of the resin for each ion was calculated from the difference in metal concentrations before and after equilibrium.

Separation of uranium from neodymium

The moist resin was packed into a glass tube (20 × 1.2 cm i.d.) and conditioned with 30 bed-volumes of acetate buffer (pH 3). After conditioning the resin, 5 ml of solution containing uranium(VI) and neodymium(III) were allowed to pass through the column at a flow-rate of 1 ml min^{-1}. The column was then washed with acetate buffer and the uranium retained on the resin was eluted with 100 ml of 2 M hydrochloric acid.

Solvent Extraction Separation of Uranium from Neodymium Using TBP

Determination of distribution coefficients of uranium and neodymium

The distribution coefficients of uranium and neodymium in water - TBP systems were determined at different molarities of nitric acid. A 20% solution of TBP in carbon tetrachloride was prepared by diluting 20 ml of washed TBP to 100 ml with carbon tetrachloride. This solution was equilibrated for 3 min with an equal amount of nitric acid (known molarity). After the phases separated, the aqueous phase was discarded.

Equal volumes (10 ml) of both the aqueous phase (uranium or neodymium solutions) and the equilibrated organic phase were mixed in separating funnels at ambient temperature, then shaken mechanically for 10 min. Preliminary experiments showed that this period was sufficient to attain equilibrium. After equilibration, aliquots of both phases were analysed for uranium and neodymium as described above. The distribution coefficient was deduced as the ratio of element concentration in the organic phase to that in the aqueous phase.

Separation of uranium from neodymium using TBP - 6 M nitric acid

Solutions containing various amounts of uranium and neodymium were prepared and extracted with TBP - 6 M nitric acid as described above. Uranium in the organic phase was either directly measured or stripped with 0.1 M nitric acid and measured potentiometrically or spectrophotometrically, whereas neodymium in the aqueous phase was measured by AAS.

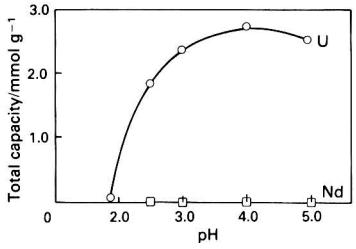


Fig. 1. Capacity *versus* pH for PHA resin

Table 2. Effect of initial nitric acid concentration on the distribution coefficients (*D*) of uranium and neodymium (1 + 1 by volume). Organic phase: 20% TBP - chloroform

HNO_3/ M	*D* U	*D* Nd
1	25	A*
3	253	A
6	617	A
8	439	A
10	287	A
12	14	A

*A = Almost completely in the aqueous phase.

Table 1. Separation of U from Nd using PHA resin in pH 4 acetate buffer

Metal	Added/ mg	Recovered/ mg	Recovery, %
Nd	30.0	28.9	96.3
U	600.0	605	100.8
Nd	300.0	303	101.0
U	600.0	601	100.2
Nd	600.0	595	99.2
U	600.0	601	100.2
Nd	600.0	602	100.3
U	300.0	304	101.3
Nd	600.0	598	99.6
U	30.0	31	103.3

Table 3. Separation of U from Nd using TBP - HNO_3. Organic phase: 20% TBP - chloroform

Metal	Added/ mg	Recovered/ mg	Recovery, %
Nd	30.0	31.8	106.0
U	600.0	607.2	101.2
Nd	300.0	308.7	102.9
U	600.0	602.4	100.4
Nd	600.0	594.0	99.0
U	600.0	601.0	100.2
Nd	600.0	597.6	99.6
U	300.0	301.0	100.6
Nd	600.0	596.4	99.4
U	30.0	30.2	100.7

Table 4. Analysis of Nd_2O_3 and UO_2 mixtures

Sample No.	Mass of mixture/ g	Metal oxide	PHA resin method Added, %	PHA resin method Found, %	TBP - HNO_3 method Added, %	TBP - HNO_3 method Found, %
1	1.106	Nd_2O_3	80.00	79.20	80.00	78.00
		UO_2	20.00	19.82	20.00	19.90
2	1.004	Nd_2O_3	50.00	49.02	50.00	52.00
		UO_2	50.00	50.50	50.00	50.00
3	1.132	Nd_2O_3	10.00	10.20	10.00	9.50
		UO_2	90.00	88.20	90.00	92.00

Analysis of a mixture of uranium(IV) oxide and neodymium(II) oxide

About 1 g of a mixture of the oxides was dissolved in 5 ml of 8 M nitric acid. The solution was evaporated to dryness and the residue was dissolved in acetate buffer of pH 4 or 6 M nitric acid. Uranium and neodymium were separated using ion-exchange and solvent extraction procedures and determined as described above.

Results and Discussion

The PHA resin is a white, macroporous, microbead polymer. It was tested for the sorption of uranium and neodymium from solutions in the pH range 3–7. The resin did not show any sorption of neodymium whereas the maximum sorption of uranium was observed at pH 4 (Fig. 1). The resin is clearly selective for uranium in the presence of neodymium and separation of the two metals can thereby be effected. The resin was used to separate uranium and neodymium from a solution of pH 4 containing U^{VI} and Nd^{III} species, U^{VI} being retained on the resin bed. The results in Table 1 indicate that efficient separation was obtained.

The extraction of U and Nd from aqueous solutions into TBP was tested using various concentrations of nitric acid. No neodymium was extracted under any of the conditions studied. However, uranium was largely extracted, the maximum distribution ratio being obtained from 6 M nitric acid (Table 2). The results are in agreement with those obtained by previous workers.[13] The extractant is selective for uranium, optimum extraction occurring when the concentration of nitric acid in the aqueous phase is 6 M. Therefore, the TBP - 6 M HNO_3 system was applied to the separation of uranium from neodymium. Uranium was completely extracted into the organic phase whereas neodymium remained in the aqueous phase and was measured by AAS (Table 3).

Three samples [prepared by mixing neodymium(III) oxide and uranium(IV) oxide in various proportions] were analysed for uranium and neodymium using the procedures established above. After dissolution of the sample, uranium and neodymium were separated either by ion exchange using PHA resin at pH 4, or by solvent extraction into TBP from 6 M nitric acid solution. Uranium was measured spectrophotometrically and neodymium by AAS. The results are shown in Table 4.

Conclusion

It can be concluded that uranium can be separated from neodymium by ion-exchange or solvent extraction procedures. In general, ion exchange is the more practicable technique and PHA resin is recommended for the separation of uranium and neodymium as part of the analysis of a mixture of their oxides.

References

1. Handwerk, J. H., and Noland, R. A., "Fabrication of Fuel Elements for the BORAX-IV Reactor, Progress in Nuclear Energy, Series V, Volume 2, Metallurgy and Fuels," Pergamon Press, London, 1959.
2. Lied, R. C., Lynch, G. D., and Handwerk, J. H., "The ZrO_2 - CaO - UO_2 Ceramic Fuel Fabrication for EBWR, Spiked Core Elements," Argonne National Laboratory, Argonne, IL, ANL-6168, 1961.
3. Kolar, D., Handwerk, J. H., and Beals, R. J., "Investigation in the System Urania - Neodymia," Argonne National Laboratory, Argonne, IL, ANL-631, 1962.
4. Sparks, R. W., Vita, O. A., and Walker, C. R., *Anal. Chim. Acta,* 1972, **60**, 222.
5. Walker, C. R., and Vita, O. A., *Anal. Chim. Acta,* 1968, **43**, 27.
6. Tavares, M. D., Baros, J. S., and Maros, F., *Talanta,* 1977, **24**, 326.
7. Walker, C. R., Vita, O. A., and Sparks, R. W., *Talanta,* 1969, **47**, 1.
8. Strelow, F. W. E., and Weinert, C. H. S., *Talanta,* 1973, **20**, 1127.
9. Philips, R. S., and Fritz, J. S., *Anal. Chim. Acta,* 1982, **139**, 237.
10. Zin, W. M., *PhD Thesis,* Salford University, 1981.
11. Maeckl, W. J., Elliott, G. B. M., and Erien, J., *Anal. Chem.,* 1959, **31**, 1130.
12. Davies, W., and Gray, W., *Talanta,* 1964, **11**, 1203.
13. Keller, C., "The Chemistry of the Transuranium Elements," Verlag Chemie, Weinheim, 1971.

Paper A6/121
Received March 17th, 1986
Accepted March 5th, 1987

Extraction - Spectrophotometric Determination of Hydrazine with 2-Hydroxy-1-Naphthaldehyde

J. Mañes, P. Campillos and G. Font
Department of Bromatology, Toxicology and Analytical Chemistry, Faculty of Pharmacy, 46010 Valencia, Spain

H. Martre and P. Prognon
Laboratory of Analytical Chemistry II, Faculty of Pharmacy, 92290 Châtenay-Malabry, France

An extraction - spectrophotometric method for the determination of hydrazine, based on its reaction with 2-hydroxy-1-naphthaldehyde at 100 °C, is described. Beer's law is obeyed between 35 and 700 ng ml^{-1} of hydrazine. The molar absorptivity at 412 nm is 27 000 l mol^{-1} cm^{-1}. The method described has been applied satisfactorily to the determination of hydrazine in feed waters for steam-generating boilers.

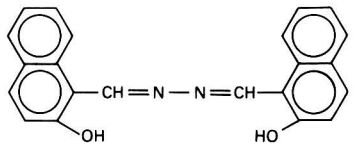
Keywords: *Hydrazine determination; 2-hydroxy-1-naphthaldehyde reagent; spectrophotometry; water; boiler feed waters*

Hydrazine is widely used as a scavenger to remove traces of oxygen in boiler feed water systems where it is maintained within the concentration range 0.5–1.0 μg ml^{-1}.

Recently, the determination of hydrazine has been extended as a consequence of its use in organic synthesis. Furthermore, hydrazine has produced carcinogenic and mutagenic effects in laboratory animals.[1]

Several methods have been described for the determination of trace amounts of hydrazine, including spectrophotometry, gas chromatography and high-performance liquid chromatography. Most of these are based on the reaction of hydrazine with aromatic aldehydes such as benzaldehyde,[2,3] salicylaldehyde,[4–6] *p*-dimethylaminobenzaldehyde,[7,8] pentafluorobenzaldehyde[9] and 5-nitro-2-hydroxybenzaldehyde[10] to form aldazines.

In this paper we propose an extraction - spectrophotometric method for the determination of hydrazine based on its reaction with 2-hydroxy-1-naphthaldehyde to form a water-insoluble yellow aldazine, 2,2′-dihydroxy-1-naphthaldazine (DHND):



Experimental

Apparatus

A Pye Unicam SP8 100 spectrophotometer with 1-cm quartz cells and a Crison Digit 501 pH meter were used.

Reagents and Solutions

All chemicals used were of analytical-reagent grade.

Hydrazine stock solution, 0.01 M. Prepared from hydrazine sulphate and standardised by bromate titration. This solution is stable for at least 1 week in a refrigerator.

Hydrazine standard solution, 1 μg ml^{-1}. Prepared by appropriate dilution of the stock solution immediately before use.

2-Hydroxy-1-naphthaldehyde, 0.012 M *in ethanol.*

Buffer solution, 1 M *sodium acetate* - 0.1 M *acetic acid.*

$EDTANa_2$ solution, 0.2 M.

Chloroform.

Procedure

Pipette a portion of the sample solution containing up to 7 μg of hydrazine into a glass-stoppered tube and dilute to 10 ml. Add 1 ml of acetate buffer solution and 1 ml of 2-hydroxy-1-naphthaldehyde solution. Warm the reactants in a boiling water-bath for 20 min. After cooling, extract the DHND by manual shaking for 2 min with 5 ml of chloroform. Allow the phases to separate and measure the absorbance of the chloroform extract at 412 nm against a reagent blank prepared in the same way but free from hydrazine.

Results and Discussion

Absorption Spectra

The absorption spectrum of DHND in the chloroform phase shows three absorption maxima at 332, 412 and 434 nm (Fig. 1) for which the molar absorptivities are 12 700, 27 000 and 22 500 l mol^{-1} cm^{-1}, respectively. Similar results were obtained in dichloromethane and in isobutyl methyl ketone.

Colour Development

The reaction between hydrazine and 2-hydroxy-1-naphthaldehyde is slow at room temperature. The effect of temperature and time were studied. The best results were obtained by heating at 100 °C for at least 15 min.

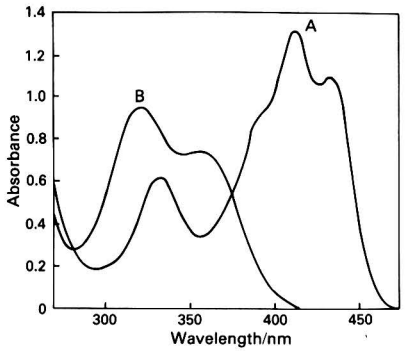


Fig. 1. Absorption spectra of (A) DHND (4.8×10^{-5} M) and (B) reagent (1.4×10^{-4} M) in chloroform

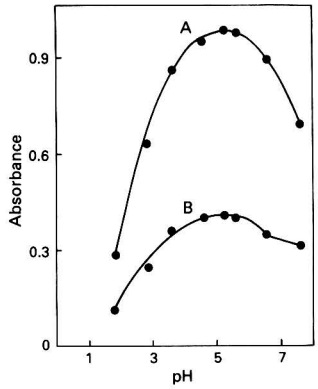


Fig. 2. Variation of absorbance with pH for the reaction product. (A) 580 ng ml^{-1} and (B) 240 ng ml^{-1} of hydrazine

Effect of pH

The effect of pH on the reaction was investigated over the range 1.8–8.4. As shown in Fig. 2 the optimum pH range is 4.6–6.6. A constant absorbance was observed for the reaction product in the acetate buffer concentration range studied (0.55–2.20 mM).

Effect of 2-Hydroxy-1-naphthaldehyde Concentration

The variation in the absorbance of DHND was investigated as a function of reagent concentration. A constant absorbance was observed in the reactive concentration range 9–13 mM. An excess of the reagent increased the blank absorbance but had no effect on the absorbance of DHND.

Effect of Shaking and Standing Time

The extraction time was varied from 15 s to 5 min, extraction being complete within 1 min.

The colour of the DHND in the chloroform was stable for more than 48 h.

Effect of the Volume of Aqueous Phase

When the volume of aqueous phase varied between 5 and 50 ml (5 ml of chloroform), the absorption remained constant up to a volume of approximately 17 ml. Above this volume the absorption was not quantitative.

Validity of Beer's Law and Precision

Beer's law was obeyed over the range 0.35–7 μg of hydrazine in the extract. The equation of the calibration graph was $A = 1.676 \times 10^{-3} x + 1.5538 \times 10^{-3}$, where A is the measured absorbance and x the concentration of hydrazine solution in ng ml^{-1}. The regression coefficient was 0.9998.

The standard deviation calculated from five determinations on a solution containing 320 ng ml^{-1} of hydrazine is 7.4 ng ml^{-1}, the relative standard deviation is 1.16% and the relative error ($P = 0.05$) of the method is 1.14%.

Table 1. Tolerance limits for various ions in the determination of 0.32 μg ml^{-1} of hydrazine

Foreign ion	Tolerance limit/μg ml^{-1}
As^{III}, As^{V}, Cd^{II}, K^{I}, La^{III}, Li^{I}, Mg^{II}, Mo^{VI}, Sn^{II}, Sr^{II}, Tl^{I}, W^{VI}, Zn^{II}, F^-, Cl^-, Br^-, I^-, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, $H_2BO_3^-$, $C_2O_4^{2-}$, citrate	≥1000†
Ba^{II}, Ca^{II},* Pb^{II},* Hg^{II},* $S_2O_5^{2-}$	500
Cu^{II},* Te^{IV}	200
Al^{III}, Bi^{III},* Co^{II},* Ni^{II},* V^{V}, NH_4^+	100
Se^{IV}	50
Fe^{III},* Mn^{II}, Zr^{IV}, NH_2OH, NO_2^-	10
Cr^{III},* UO_2^{2+}	1
Ti^{IV}	0.4

* With EDTA.
† Maximum amount studied.

Table 2. Determination of hydrazine in steam boiler waters

Sample	Proposed method/ng ml^{-1}*	*p*-DAB method/ng ml^{-1}*
A	417 ± 5.6	388 ± 9.2
B	758 ± 12.3	734 ± 17.8

* Mean of three determinations.

Effect of Foreign Ions

Sample solutions containing 320 ng ml^{-1} of hydrazine and various foreign ions were prepared and the effect of foreign ions on the determination of hydrazine was studied. The results are summarised in Table 1. The tolerance limit of an ion was fixed as the maximum amount causing an error in the absorbance of not greater than 2%. Ionic interference was effectively masked by the addition of EDTA. EDTA concentrations of up to 20 mM did not affect the colouration of the reaction product.

Application to Real Samples

In order to assess the value of the proposed method it was applied to the determination of hydrazine in two steam boiler water samples extracted from the boiler. Table 2 shows the results and those obtained by the *p*-dimethylaminobenzaldehyde (*p*-DAB) method. The agreement was satisfactory.

References

1. Vernot, E. H., MacEwen, J. D., Bruner, R. H., Haus, C. C., and Kinkead, E. R., *Fundam. Appl. Toxicol.*, 1985, **5**, 1050.
2. Matsui, F., Robertson, D. L., and Lovering, E. G., *J. Pharm. Sci.*, 1983, **72**, 948.
3. Butterfield, A. G., Curran, N. M., Lovering, E. G., Matsui, F., Robertson, D. L., and Sears, R. W., *Can. J. Pharm. Sci.*, 1981, **16**, 15.
4. Bailey, L. C., and Medwick, T., *Anal. Chim. Acta*, 1966, **35**, 330.
5. Abdou, H. M., Medwick, T., and Bailey, L. C., *Anal. Chim. Acta*, 1977, **93**, 221.
6. Kester, P. E. and Danielson, N. D., *Chromatographia*, 1984, **18**, 125.
7. Watt, G. W., and Chrisp, J. D., *Anal. Chem.*, 1952, **24**, 2006.
8. Rodier, J., "L'Analyse de l'Eau," Seventh Edition, Dunod, Paris, 1984.
9. Liu, Y. Y., Schemeltz, J., and Hoffman, D., *Anal. Chem.*, 1974, **46**, 885.
10. Neurath, G., and Luttich, W. J., *J. Chromatogr.*, 1968, **34**, 257.

Paper A6/351
Received September 17th, 1986
Accepted March 18th, 1987

Microcomputer-aided Control of a Titrator Applied to Precipitation Titrations

Shuko Fujieda and Nobue Nishi
Department of Chemistry, Ochanomizu University, Bunkyo-ku, Tokyo 112, Japan

A microcomputer-aided control system is proposed for a titrator which is applicable to precipitation reactions. The titrant is added in drops and the passage of a droplet is detected by means of a light-emitting diode - phototransistor combination to give a stop signal to the electrically operated burette. When the precipitate formation has been equilibrated, as confirmed potentiometrically, a control signal is fed to resume the addition of the titrant. The validity of the whole system is demonstrated for potentiometric precipitation titrations of silver ion with a chloride titrant. Satisfactorily reproducible and precise results were obtained.

Keywords: *Automatic titrator; microcomputer; photosensor; potentiometry; precipitation titration*

An experienced operator can skillfully manipulate a conventional burette so that the titration procedure can be followed under the optimum conditions. In recent years, however, automatic titrators that are controlled by a microcomputer have become commercially available, and even inexperienced beginners can also obtain end-points with almost the same precision and accuracy as those obtained by an experienced operator. However, these sophisticated instruments are expensive and sometimes even an automated titrator is inadequate for application to such a time-consuming reaction as a precipitation reaction. After the addition of a small amount of titrant, the time necessary for the complete formation of a precipitate may differ in different steps of a titration, and may also depend on the nature and conditions of the reaction. Therefore, a uniform time interval of titrant addition may result in inaccuracy or wastage of time. In addition, the burette tip should be placed out of contact with the solution to be titrated, so that no reaction may proceed during the waiting interval. Pehrsson and Ingman[1] also suggested that the burette tip should not be immersed in the solution to be titrated.

For these reasons, in this work the titrant was added drop by drop. A microcomputer-aided control system was assembled for executing titrations under optimum conditions, in which the passage of a titrant droplet was detected directly by a combination of a light-emitting diode (LED) and a phototransistor for controlling the progress of the titration. Popular and inexpensive devices were selected for setting up the system. The validity of the proposed system was demonstrated and evaluated by applying it to potentiometric titrations of silver nitrate with sodium chloride as an example.

Experimental

Reagents

Silver nitrate and sodium chloride were of guaranteed grade from Wako Junyaku.

Potentiometric Titration Cell

The potentiometric cell was assembled to monitor the progress of precipitate formation in a conventional manner.[2] A silver bar dipped in the solution to be titrated was used as a monitoring electrode, which was connected electrically to a saturated calomel electrode (SCE), serving as a reference electrode, by a salt bridge composed of potassium nitrate and agar.

Hardware

A schematic diagram of the whole system is shown in Fig. 1, including circuits for control of a motor-driven burette, drop detection and measurement of potential difference. A microcomputer (M5, SORD) working with an 8-bit central processing unit (Z-80A, Zilog) was used with an extension box in which a floating-point BASIC, read-only memory and a 32-kbyte random access memory card were installed, in addition to an interface board constructed by us. Start and stop signals of the electrically driven burette (HS-1B, Toa Dempa) operated with a 12 V direct current were given from the microcomputer via a programmable peripheral interface (PPI, i8255, Intel) in the form of on - off switching to control a solid-state relay (SSR) for a direct current (D2P-050, JEL System).

The LED (TLN101, Toshiba) and phototransistor (TPS601, Toshiba) were placed facing each other on opposite sides of the path of titrant drops. The passage through the infrared light beam from the LED, which has a peak wavelength at 940 nm, of a falling titrant drop was monitored with the phototransistor. When a droplet of titrant reached the light path, the drop detection signal was latched in the flip - flop circuit via a Schmitt trigger circuit (74LS14, Texas Instruments), as shown in Fig. 2. After the signal had been acquired by the microcomputer, the latter gave a reset signal.

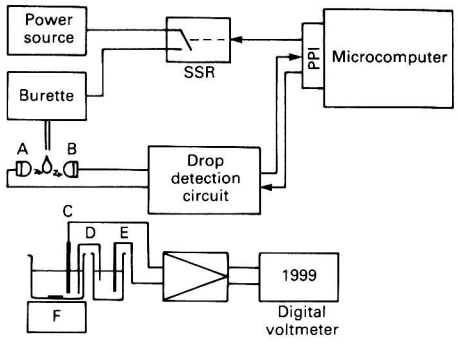


Fig. 1. Schematic diagram of the whole system. A, LED; B, phototransistor; C, indicator electrode; D, salt bridge; E, reference electrode; and F, magnetic stirrer. PPI = programmable periferal interface

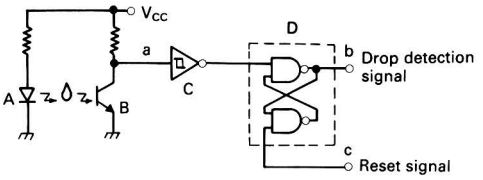


Fig. 2. Drop detection circuit. A, LED; B, phototransistor; C, Schmitt trigger circuit; and D, flip - flop circuit. a, Detected signal at phototransistor; b, drop detection signal; and c, reset signal for flip - flop circuit. V_{cc} represents the 5 V d.c. supply voltage

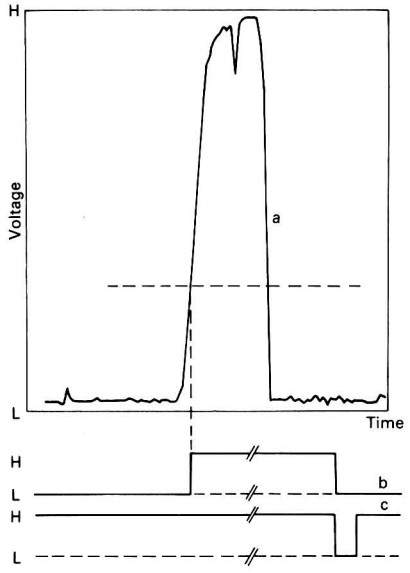


Fig. 3. Drop detection signal. a, Detected signal at phototransistor; b, drop detection signal; and c, reset signal for flip - flop circuit. H, high level voltage; and L, low level voltage

Potential differences between the monitoring electrode and the SCE were read as the output voltage of a digital voltmeter (SC-51, Thinky), to which the maximum input voltage range was ±1.999 V, and the nominal input impedance exceeded 1000 MΩ. An amplification circuit with an operational amplifier was utilised, if necessary, to amplify the input voltage range to the voltmeter if the observed potential difference was too small to feed to the voltmeter. The electrical burette was equipped with a 1-ml glass syringe.

Software

All the program was written in BASIC, except that part of the program for acquiring data at such a high speed as shown in Fig. 3(a), which was written in Assembly language.[3] The equivalence point was estimated by calculating the differential values of $\Delta V/\Delta v$ and $\Delta^2 V/\Delta v^2$ utilising the titrant volume added (v, ml) and the potential difference observed (V, mV) in the titration.[2]

Procedure

The driving of the electric burette was started by applying a control signal from the microcomputer to the SSR to switch on the 12 V d.c. power supply. The delivery speed of the burette was 0.008 ml s^{-1}. On detecting the fall of a titrant drop by the phototransistor, followed by the Schmitt trigger and flip - flop circuits, the microcomputer gave a stop signal to the burette via the SSR. Then the potential difference between the monitoring and reference electrodes was measured until the complete formation of the precipitate was confirmed by the potentiometric signal. The stable value was fed to the microcomputer via the keyboard and the equivalence point was calculated and displayed.

Results and Discussion

For detailed knowledge of the fall of a titrant drop, the behaviour of the output voltage at point a in Fig. 2 was monitored on-line in a separate system by acquisition via an analogue to digital converter, instead of C and D in Fig. 2, by the microcomputer, executed by a machine language program. A typical plot of the acquired data is shown Fig. 3a.[3] Detail has been omitted for the sake of clarity. For the sampling interval executed by BASIC in this work, the delay time was *ca.* 2 ms or less. Therefore, introduction of a Schmitt trigger circuit as shown in Fig. 2 may be preferred. When a drop arrived at the light path being monitored by the LED - phototransistor combination, the output voltage of the phototransistor, which was used as the drop detection signal, exceeded the threshold value shown by the broken line in Fig. 3a, a set signal was latched in the flip - flop circuit shown in Fig. 2. After appropriate sequences, a reset signal was sent to the flip - flop circuit from the microcomputer and the whole control system was brought to a stand-by state for the following drop. The signals at points b and c in Fig. 2 are shown as b and c in Fig. 3, respectively. The axes in Fig. 3 are in arbitrary units to demonstrate the relationships.

The electrically driven burette was equipped with a multi-turn variable resistor, the resistance of which was to be correlated with the movement of the syringe piston, the maximum volume of which was 1 ml. However, the linearity observed between the volume discharged from the burette and the corresponding resistance was not satisfactory for the present purpose. Therefore, the volume of titrant addition was calculated from the volume of a droplet and the number of drops added, instead of using electrical values involving the movement of the piston. Good reproducibility of the volume of each droplet was essential for calculating accurately the volume of titrant added from the number of droplets and the use of a clear burette tip may also be necessary for reproducible dropping. The suitability of the detection circuit was examined by weighing a number of droplets leaving the burette tip. The mean volume of about 100 droplets, weighing every droplet, was 0.0365 ml for water, the standard deviation being 0.0001_8 ml.

Control of the electrically driven burette was subject to increased errors when the delivery speed was increased. Therefore, the delivery speed indicated under Procedure is considered reasonable for the proposed system. Differences in droplet size were not found to be significant between the automated control proposed here and the manual procedure.

Aqueous solutions containing 18.51, 18.97, 19.43, 19.43, 20.50 and 21.40 μmol of silver ion were titrated with sodium chloride solution, the equivalence points calculated by the Newtonian method being 18.40, 18.87, 19.40, 19.41, 20.30 and 21.30 μmol, respectively. The mean error was −0.47%. As the reproducibility for six samplings with a micro-syringe with a full-scale reading of less than 100 μl was 0.27% (standard error), the main part of the error in titrations might be due to the use of small volumes. To treat titrant volumes greater than the maximum volume of the built-in glass syringe, silver nitrate solutions of 76.25, 199.9, 201.8, 204.6, 213.9, 213.9, 222.2, 256.2 and 260.3 μmol were titrated. The observed equivalence points, with percentage errors in parentheses, were 76.47 (+0.29), 199.6 (−0.15), 200.1 (−0.84), 203.4 (−0.59), 213.4 (−0.23), 213.8 (−0.05), 222.9 (+0.32), 256.9

(+0.27) and 260.8 (+0.19) μmol, respectively, and the simple arithmetic mean error was −0.088%. The proposed microcomputer-aided control system was thus demonstrated to be valid by this execution of a common precipitation reaction.

Moreover, the solubility product of 1.0×10^{-10} calculated from the potential difference observed at 23.8 °C by using the Nernst equation agreed reasonably with the literature values.[4] Therefore, the suitability of measuring potential difference was also demonstrated.

References

1. Pehrsson, L., and Ingman, F., *Talanta*, 1977, **24**, 79 and 87.
2. Day, R. A., and Underwood, A. L., "Quantitative Analysis," Fourth Edition, Prentice Hall, Englewood Cliffs, NJ, 1980.
3. Fujieda, S., Nishi, N., and Nakanishi, M., "Proceedings of the 34th Annual Meeting of the Japan Society for Analytical Chemistry, Kobe, 1985," Japan Society of Analytical Chemistry, Shinagawa, Tokyo, p. 564 (in Japanese).
4. Sillén, L. G., and Martell, A. E., "Stability Constants of Metal Ion Complexes," Chemical Society, London, 1964, p. 286; Supplement No. 1 (Special Publication No. 25), 1971, p. 177.

Paper A6/382
Received October 13th, 1986
Accepted March 9th, 1987

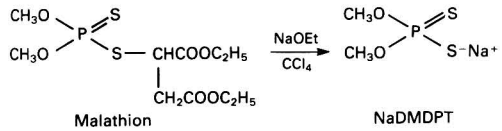
Spectrophotometric Determination of Malathion with Methylene Blue

Kalluru Seshaiah and Pratapa Mowli*
Environmental Engineering Division, Engineering College, S.V. University, Tirupati 517 502, India

A spectrophotometric method is described for the determination of malathion with methylene blue. Malathion is decomposed with alkali and the resultant dimethyldithiophosphate is extracted with methylene blue into chloroform. The colour of the organic layer is measured at 652.1 nm. The method is both simple and sensitive.

Keywords: *Malathion determination; extraction - spectrophotometry; organophosphorus pesticides; dimethyldithiophosphate; methylene blue*

A spectrophotometric method for the determination of malathion was first introduced by Norris *et al.*[1] In this method malathion is decomposed by alkali to sodium dimethyldithiophosphate (NaDMDTP), fumarate and ethanol.



Malathion NaDMDPT

+ fumarate + ethanol

The NaDMDTP is converted to a yellow copper complex, extracted into carbon tetrachloride and determined spectrophotometrically; unfortunately, the colour of the copper - DMDTP complex fades very quickly. Roussow,[2] Hill[3] and Wayne *et al.*[4] have unsuccessfully tried to overcome this drawback.

Clark and Qazi[5] reported a modified spectrophotometric method for the determination of malathion. In this method they employed bismuth instead of copper for the complexation of DMDTP and stated that the Bi - DMDTP complex is more stable than the Cu - DMDTP complex. The absorbance of the Bi - DMDTP complex lies in the ultraviolet region (325 nm). Clark and Qazi[6] further modified their method by a ligand-exchange reaction in which the Bi - DMDTP complex is transformed into a bismuth - dithizone complex, which absorbs in the visible region (495 nm). Although the further modified method of Clark and Qazi[6] has improved the sensitivity and the absorbance is in the visible region, it requires a larger number of reagents and one more stage of extraction for the ligand-exchange reaction. Hence we have attempted to extract DMDTP with cationic dyes, the absorbances of which are in the visible region.

Methylene blue and safranine, which are insoluble in chloroform, form soluble complexes with DMDTP that can be quantitatively extracted into chloroform. The molar absorptivity of the methylene blue - DMDTP complex (1522.4 l mol^{-1} cm^{-1}) is greater than that of the safranine - DMDTP complex (739.2 l mol^{-1} cm^{-1}). In this paper we report a spectrophotometric method for the determination of malathion with methylene blue.

Experimental

Apparatus

A Hitachi U3400 UV - visible NIR spectrophotometer with stoppered 10-mm glass cells was used.

Reagents

Malathion standard solution, 82.5 μg ml^{-1}, 2.5 × 10^{-4} M. A 0.16737 g mass of 98.6% standard malathion solution (American Cyanamid) is dissolved in 100 ml of carbon tetrachloride. A 5-ml aliquot of this solution is diluted to 100 ml.

Malathion, 50% emulsifiable concentrate, 82.5 μg ml, 2.5 × 10^{-4} M. A 0.3300-g mass of the 50% *m/m* emulsifiable concentrate (Cyanamid India) is dissolved in 100 ml of carbon tetrachloride and 5 ml of this solution are diluted to 100 ml.

Dimethyldithiophosphate, 2.5 × 10^{-4} M. A 0.2190-g mass of the purified ammonium salt (Fluka) is dissolved in 250 ml of distilled water. A 5-ml aliquot of this solution is diluted to 100 ml with distilled water.

Methylene blue. Methylene blue (0.2 g) (S.D. India) is dissolved in 1000 ml of distilled water.

Safranine. Safranine (0.2 g) (BDH Chemicals) is dissolved in 1000 ml of distilled water.

Sodium, 1% solution in ethanol. Freshly cut sodium (1 g) is dissolved in 100 ml of ethanol. This solution is prepared fresh daily.

Buffer solution, pH 6. Concentrated sulphuric acid (3.4 ml) is added to 250 ml of distilled water in a 500-ml flask. Monosodium dihydrogen phosphate monohydrate (25 g) is added, and the flask is shaken until dissolution is complete and then diluted to 500 ml.

Carbon tetrachloride. Glaxo ExcelaR, India.

Chloroform. Glaxo ExcelaR, India.

Procedure

Extraction of DMDTP with methylene blue and safranine

Volumes of 10 ml of water, 1 ml of DMDTP solution and 2 ml of buffer solution, followed by 10 ml of methylene blue or safranine solution and 10 ml of chloroform are placed in a 50-ml separating funnel. The funnel is stoppered and shaken vigorously for 1 min. The organic layer is allowed to separate and then transfered into a spectrophotometric cell via a cotton-wool plug placed in the stem of the funnel. The absorption spectrum is recorded immediately against a chloroform blank (see Fig. 1). The absorbance of the methylene blue - DMDTP complex is measured at 652.1 nm using various concentrations of DMDTP. The absorbances are plotted against concentration of DMDTP and the results obtained are shown in Fig. 2.

The absorbances of different concentrations of DMDTP are measured as described by Clark and Qazi.[6] These values are also presented graphically in Fig. 2.

* To whom correspondence should be addressed.

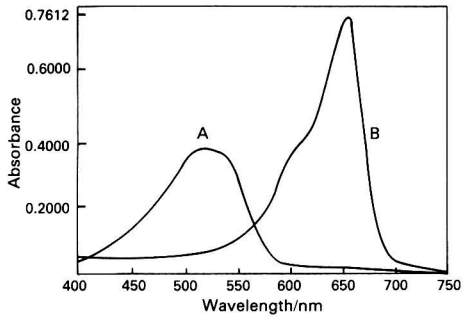


Fig. 1. Absorption spectra of safranine and methylene blue complexes of DMDTP extracted into 10 ml of chloroform. (A) 2 ml of 2.5 × 10^{-4} M DMDTP with excess safranine and (B) 2 ml of 2.5 × 10^{-4} M DMDTP with excess methylene blue

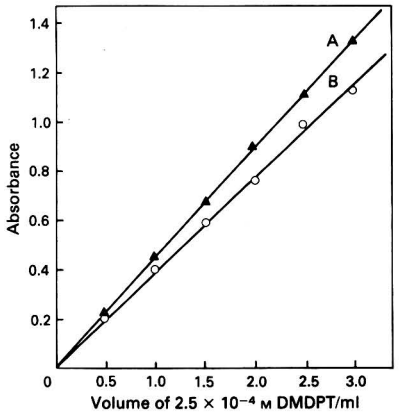


Fig. 2. Absorbance *versus* concentration graph for DMDTP. (A) Bi - DMDTP complex transformed to Bi - dithizone (λ = 495 nm). (B) Methylene blue - DMDTP complex (λ = 652.1 nm)

Determination of malathion in formulations

Volumes of 10 ml of carbon tetrachloride, 1 ml of standard or 50% emulsifiable concentrate malathion solution, together with 1 ml of sodium in ethanol, are added to a 50-ml separating funnel, and the solution is swirled gently for 1 min. Distilled water (10 ml) is added, followed by vigorous shaking. The layers are separated and the carbon tetrachloride solution discarded. The aqueous layer is washed with 5 ml of carbon tetrachloride. One drop of phenolphthalein indicator is added to the aqueous layer and 2 M sulphuric acid added dropwise until the pink colour just disappears. A 2-ml aliquot of buffer solution is added, followed by 10 ml of methylene blue and 10 ml of chloroform and the solution is shaken vigorously for 1 min. The chloroform solution is transferred into the spectrophotometer cell and the absorption is measured at 652.1 nm.

Results and Discussion

The absorption spectra of 2.0 ml of 2.5 × 10^{-4} M DMDTP (with an excess of methylene blue) and safranine extracted into 10 ml of chloroform are shown in Fig. 1. It is evident that methylene blue gives better results than safranine because its absorption is greater. The methylene blue - DMDTP complex is stable and there was no change in the absorbance over more than 24 h. Hence methylene blue was used for the extraction of DMDTP.

Table 1. Determination of malathion in standard solutions

	Malathion standard solution, 98.6%			Malathion, 50% emulsifiable concentrate		
Sample No.	Taken/ μg	Found/ μg	Recovery, %	Taken/ μg	Found/ μg	Recovery, %
1	82.5	82.8	100.4	82.5	81.5	98.8
		83.0	100.6		83.6	101.3
		82.3	99.8		85.2	103.3
2	123.8	124.3	100.4	123.8	126.3	102.0
		124.2	100.3		120.6	97.4
		124.5	100.6		122.6	99.0
3	165.0	165.4	100.2	165.0	159.6	96.7
		166.2	100.7		168.5	102.1
		166.8	101.1		169.4	102.7
4	206.3	205.6	99.7	206.3	208.7	101.2
		207.2	100.4		214.7	104.0
		204.5	99.1		203.2	98.5
5	247.5	249.4	100.8	247.5	252.7	102.1
		247.6	100.0		239.0	96.6
		246.8	99.7		255.4	103.2

Graphs of absorbance *versus* concentration of DMDTP extracted with (A) Bi and Bi - DMDTP converted to Bi - dithizone and (B) with methylene blue are shown in Fig. 2. These graphs show that the DMDTP can be extracted quantitatively with methylene blue into chloroform. These results further show that extraction with methylene blue gives almost the same sensitivity as the further improved method of Clark and Qazi.[6]

The method is applicable to the determination of malathion in malathion formulations. In order to study the interference of other ingredients such as emulsifiers and solvents, a technical grade malathion and 50% emulsifiable concentrate (50% *m/m* active malathion and 50% *m/m* solvent and emulsifiers) were analysed and the percentage recoveries are reported in Table 1. These results demonstrate the suitability of the method for the determination of malathion in formulations. The minimum detection limit for the determination of malathion with this method is 82.5 μg with a standard deviation of 0.44 μg.

Further studies showed that the method can be applied to other organophosphorus pesticides which undergo quantitative hydrolysis with sodium ethoxide in carbon tetrachloride.

Hence the determination of malathion with methylene blue has distinct advantages over previously reported methods. It yields results similar to those of the further improved method of Clark and Qazi,[6] but requires fewer reagents and is more simple. The extraction involves only one stage and there is no ligand-exchange step.

References

1. Norris, M. V., Vail, W. A., and Averell, P. R., *J. Agric. Food Chem.*, 1954, **2**, 570.
2. Roussow, S. D., *Afr. J. Agric. Sci.*, 1961, **4**, 435.
3. Hill, A. C., *J. Sci. Food Agric.*, 1969, **20**, 4.
4. Wayne, R. S., Groth, W. C., Miles, J. W., and Guerrant, G. O., *J. Assoc. Off. Anal. Chem.*, 1972, **55**, 926.
5. Clark, E. R., and Qazi, I. A., *Analyst*, 1979, **104**, 1129.
6. Clark, E. R., and Qazi, I. A., *Analyst*, 1980, **105**, 564.

Paper A7/23
Received January 26th, 1987
Accepted March 31st, 1987

COMMUNICATION

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications receive priority and are usually published within 5–8 weeks of receipt. They are intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are usually examined by one referee and inclusion of a Communication is at the Editor's discretion.

An Optical Potassium Ion Sensor

John F. Alder, David C. Ashworth and Ramaier Narayanaswamy*

Department of Instrumentation and Analytical Science, UMIST, P.O. Box 88, Manchester M60 1QD, UK

and Richard E. Moss and Ian O. Sutherland

Department of Organic Chemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, UK

An optical fibre sensor is described which is sensitive to potassium ions in aqueous solution in the concentration range 10^{-3}–10^{-1} M.

Keywords: *Optical fibre; immobilised reagent; crown ether; sensor; potassium determination*

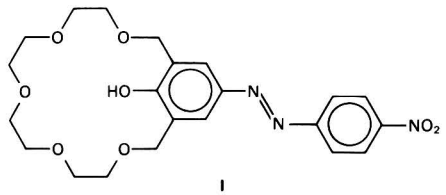
Over the past few years there has been increasing interest in the development of optical sensors for chemical species as an alternative to the established electrochemical devices. Optical sensors do not need a separate reference device, do not suffer from electrical interference or "cross-talk" and, in biomedical applications, are considered safer as there is no electrical connection to the body.

This work was carried out as part of a study ultimately to develop an optical fibre probe for the *in vivo* determination of potassium ions. Potassium ions are of interest as an early manifestation of post-operative shock is an increase in the blood potassium level from the normal concentration of 4.5 mM, and the ability to monitor this level continuously is obviously advantageous. The determination of potassium ions by spectrophotometry has been accomplished (using solvent extraction) with crown ether - pH dye ternary complexes[1–3] and chromogenic crown ethers (for excellent reviews see references 4 and 5), but there have been no previous reports of potassium-selective sensors (having the configuration of a probe) utilising chromogenic crown ethers. We report here an optical fibre potassium sensor capable of determining aqueous potassium ions in the range 10^{-3}–10^{-1} M.

Experimental

Synthesis

The reagent used (**I**) was synthesised by preparing 2-hydroxy-1,3-xylyl-18-crown-5 according to the literature[6] and allowing it to react with the diazonium salt derived from 4-nitroaniline.



I

* To whom correspondence should be addressed.

Probe Construction

The probe hardware was similar to that described elsewhere.[7] The tip of an optical fibre (Crofon, Du Pont) of core diameter 1.0 mm was polished and the sheathing was cut away to expose *ca.* 0.25 mm of core. On the tip of this core was placed *ca.* 1 mg of ground (200–300 mesh) Amberlite XAD-2 resin that had previously been treated[8] by washing with methanol, followed by 0.1 M HCl, then de-ionised water (Elga nuclear grade mixed-bed resin) until free from chloride, and finally dried at 40 °C. The resin was held in place by encapsulating the tip in a porous PTFE membrane (Millipore FHUP, 0.5 μm), which was in turn held in place by means of carefully applied heat-shrinkable sleeving.

The probe was sensitised by dipping the tip in a solution of **I** (0.1% in methanol) overnight. After removal the probe was placed in de-ionised water for 2 h and was then ready for use.

Instrumentation

The instrumentation associated with the probe was similar to that described elsewhere.[9] The determination was carried out at 557 nm and the instrument gain settings were 10 mV full-scale deflection for 0–10^{-1} M KCl and 3 mV full-scale deflection for 0–10^{-2} M KCl.

The response change of the probe was determined on going from aqueous solution containing no potassium to that containing potassium ions at a known concentration. As the colour of the reagent was pH dependent, it was necessary to buffer the aqueous solutions to an appropriate pH, and in this work *N,N,N′,N′*-tetramethyl-1,2-diaminoethane - HCl was employed for this purpose.

Results and Discussion

The sensitivity of the probe (at constant pH) was found to be markedly pH dependent. Fig. 1 shows the effect of pH on the sensitivity of the probe to potassium ions. The greatest sensitivity to potassium was shown at pH 8, although 5×10^{-3} M K^+ could easily be determined throughout the pH range 7–9.

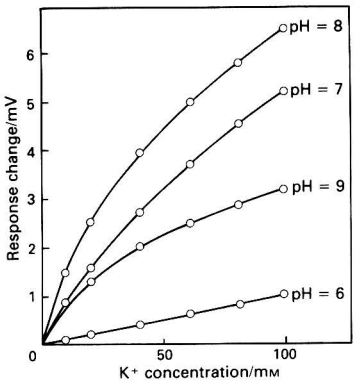


Fig. 1. Effect of pH on probe sensitivity

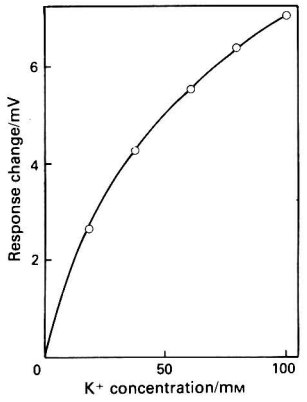


Fig. 2. Sensor response for K^+ concentrations in the range $0–10^{-1}$ M

The response of the probe to potassium ions (KCl) in the ranges $0–10^{-1}$ M (Fig. 2) and $0–10^{-2}$ M (Fig. 3) is shown at a nominal pH of 8.0. The signal to noise ratio at 10^{-3} M K^+ was 5, which implies that 0.5 mM K^+ is the limit of detection with this probe.

The measured response time (95% response) of the probe at a concentration of 10^{-3} M K^+ was 2–3 min, and at a concentration of 10^{-2} M K^+ was 5–7 min. These are similar to response times reported for an optical sodium ion sensor.[10]

The sensor also responds to sodium, with an K^+/Na^+ selectivity ratio of 6.4. This is an improvement on the sodium ion sensor which, although reported to be selective for sodium, has an Na^+/K^+ selectivity ratio of only 1.2.

Although the probe can be used for the determination of potassium at concentrations found in human blood plasma (blood $K^+ \approx 4.5$ mM), the Na^+/K^+ ratio found in blood is approximately 30. The K^+/Na^+ selectivity ratio of the present probe is far too low to determine K^+ levels in blood. Work is currently in hand to improve the selectivity by designing and

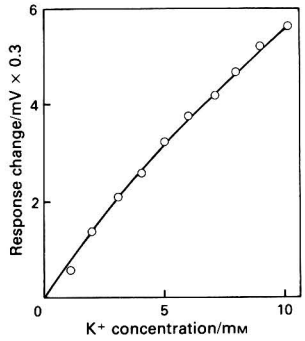


Fig. 3. Sensor response for K^+ concentrations in the range $0–10^{-2}$ M

synthesising compounds having an inherently high K^+/Na^+ selectivity ratio. Such compounds may incorporate one of two structural features: a three-dimensional cage structure of the appropriate cavity size,[11] or a large ring system containing electronegative atoms (as a valinomycin mimic).[12] Both of these approaches are being attempted.

Conclusions

We have demonstrated the feasibility of using immobilised crown ethers to determine potassium in aqueous solution. An optical fibre probe has been constructed, utilising a chromogenic crown ether, which responds reversibly to aqueous potassium ions in the concentration range $10^{-3}–10^{-1}$ M, with a K^+/Na^+ selectivity ratio of 6.4.

The authors thank the Science and Engineering Research Council for financial support of this work.

References

1. Sumiyoshi, H., and Nakahara, K., *Talanta*, 1977, **24**, 763.
2. Abrodo, P. A., Gomis, D. B., and Sanz-Medel, A., *Microchem. J.*, 1984, **30**, 58.
3. Sanz-Medel, A., Gomis, D. B., and Alvarez, J. R. G., *Talanta*, 1981, **28**, 425.
4. Takagi, M., and Ueno, K., *Top. Curr. Chem.*, 1984, **121**, 39.
5. Lohr, H. G., and Vogtle, F., *Acc. Chem. Res.*, 1985, **18**, 65.
6. Brown, C. M., Fergusson, G., McKervey, M. A., Lindsay Mulholland, D., O'Connor, T., and Parvez, M., *J. Am. Chem. Soc.*, 1985, **107**, 2703.
7. Kirkbright, G. F., Narayanaswamy, R., Welti, N. A., *Analyst*, 1984, **109**, 1025.
8. Narayanaswamy, R., and Sevilla, F., III, *Analyst*, 1986, **111**, 1085.
9. Narayanaswamy, R., and Sevilla, F., III, *J. Opt. Sensors*, 1986, **1**, 403.
10. Zhujun, Z., Mullin, J. L., and Seitz, W. R., *Anal. Chim. Acta*, 1986, **184**, 251.
11. Cram, D. J., and Ho, S. P., *J. Am. Chem. Soc.*, 1986, **108**, 2998.
12. Grootenhuis, P. D. J., Van der Wal, P. D., and Reinhoudt, D. N., *Tetrahedron*, 1987, **43**, 397.

Paper A7/246
Received June 15th, 1987
Accepted June 29th, 1987

BOOK REVIEWS

Fundamentals and Applications of Chemical Sensors
Edited by D. Schuetzle, Robert Hammerle and James Butler. *ACS Symposium Series*, 309. Pp. x + 394. American Chemical Society. 1986. Price $74.95 (US and Canada); $89.95 (export). ISBN 0 8412 0973 1.

When a research field is developing as fast as that of chemical sensors, there is always the risk that any book published on the subject is going to be out of date before it reaches the bookshelves. This particular series was founded to provide a medium for publishing symposia quickly and the submission dates on the papers presented in the book range from the end of October 1985 to the end of February 1986. One can, therefore, consider this volume as being pretty much up to date. An important feature of the papers in the book, which were presented at the 1984 International Chemical Congress of Pacific Basin Societies, is that they cover not only the applications of chemical sensors and the results obtained, but also the fundamentals of their operation. In this respect it is not only a good state-of-the-art review but also a very useful text from which to learn the principles behind many of the different types of sensor currently being studied. All of the papers in the book are of really very high quality in both content and presentation. Some are outstanding and I will leave it to the individual reader to judge for himself which they are.

The book starts with two extensive papers on recent advances in chemically sensitive electronic devices and recent advances in gas sensors in Japan, which give a detailed overview of many of the approaches to chemical sensing currently being studied. It is clear from these papers that much of the impetus, to gas sensing particularly, has been from the motor industry and for environmental monitoring, particularly of humidity and fuel gases in Japan. The next major section, which takes up half of the remainder of the book, is dedicated to gas sensors, and covers not only the range of tin oxide gas sensors, perovskite-type oxide, thick-film zirconium oxide and Schottky barrier diode and metal oxide semiconductor capacitor gas sensors, but also microsensors based on phthalocyanine film sensors and chemical microsensors based on surface impedance changes. I found this section particularly educational as many of the fundamentals of the sensors were discussed as well as the results obtained. Each paper is terminated by a conclusion, which in all instances sums up nicely the present status of the individual sensors.

The next section, on sensors for liquids and solids, starts with a significant paper on the advances in atmospheric gas composition determinations using infrared spectroscopy. It has always been debatable whether such a complex optical system as infrared could be construed as a chemical sensor and by its inclusion one can make some interesting comparisons between the sort of data available from such a system and from the more specific, smaller scale devices demonstrated elsewhere in the book. The other papers in this section include discussions of chemically modified electrode sensors, coated-wire ion-selective electrodes and chemical sensing using near-IR reflectance analysis. Overall, this is a very interesting group of papers but the last two sections on environmental sensors and biosensors were by no means lacking in interest. A very interesting paper on electrochemical sensors, sensor arrays and computer algorithms for the detection and identification of airborne chemicals is followed by a discussion of coating materials for surface acoustic wave and optical waveguide devices, which sets down some very useful specifications for materials in this field. The paper on microbial sensors for process and environmental control came as a pleasant surprise, giving a very interesting insight into a completely different approach to chemical analysis using biologically active materials. The last three papers in the biosensors section include papers on receptor action in stabilised lipid membranes, designer-sensitive drug sensors and the development of subcutaneous glucose sensors, rounding off a text which has taken one through most of the modern scientific disciplines in the pursuit of chemical sensing devices and processes.

Overall, I was very impressed by this book. It will serve as a very useful teaching and reference text, and source of new ideas for many years to come. I found it so interesting that I am now going to sit down and read it again!

John F. Alder

Near Infrared Spectroscopy in Food Analysis
B. G. Osborne and T. Fearn. Pp. viii + 200. Longman. 1986. Price £29.95. ISBN 0 582 49489 3.

This is an important book for all analysts because it is the first book on the subject of the modern analytical application of near-infrared (NIR) spectroscopy. Although some food analysts, who have no previous knowledge of the subject, may be disappointed to discover the difficulties of applying NIR methods, analysts from other areas will be interested to learn that the technique is expanding rapidly to encompass major analytes (>1%) in all areas of analysis. In spite of its title, eight of the nine chapters are concerned with a general exposition of NIR spectroscopy, which covers the history of NIR analysis, theory and physics, instrumentation, data handling and calibration, problems and future prospects and a single chapter on food analysis applications. The book is well produced, clearly illustrated and has a small index.

NIR analysis relies on statistical techniques to relate absorbance to composition. The theoretical basis for the underlying assumptions is put forward with clarity, as are the statistical methods. With this knowledge, it is readily understood why NIR method development is very time consuming and hence expensive. However, the benefits of rapid analysis will often make NIR a viable method. Although the method has made a major impact in agricultural and food analysis, the book contains a section covering other uses of NIR analysis in animal feeds, forages, pharmaceuticals, textiles and tobacco, which demonstrate that NIR analysis is not limited to natural products.

The book contains an appendix of official methods using NIR analysis; although they are few in number, future editions of the book will be able to expand this section. The authors could have capitalised on this idea by including a commentary on these methods, explaining the reasoning behind the instructions. As it is, readers are left to draw their own conclusions rather than being able to test their new understanding of NIR analysis gained from the book. In its present form the appendix gives equal weight to an AOAC method for utilising NIR for the determination of moisture in vegetables by extraction into methanol and the ICC method for the determination of moisture and protein in wheat. The first is used rarely, whereas the latter is the basis for the world-wide use and major success of NIR analysis.

Near-infrared analysis is likely to become much more widely used in the next 5–10 years. Until a more detailed treatise on NIR analysis is published, this book will fill a very important gap and it is recommended to all analysts who think that NIR methods may be the answer to their requirements for rapid and precise analysis.

A. M. C. Davies

Electrochemistry, Sensors and Analysis. Proceedings of the International Conference "Electroanalysis na h'Éireann," Dublin, Ireland, June 10–12, 1986
Edited by Malcolm R. Smyth and Johannes G. Vos. *Analytical Chemistry Symposia Series, Volume* 25. Pp. xviii + 419. Elsevier. 1987. Price $119; Dfl295. ISBN 0 444 42719 8.

Books devoted to the texts of lectures presented at Conferences and Symposia are mementoes of usually enjoyable and educative events, and give non-attenders belated access to the proceedings. Just a few months have elapsed between "Electroanalysis na h'Éireann" and the appearance of the book. Although many of the papers highlight speakers' researches rather than being based on original, hitherto unpublished work, they nevertheless provide readers with a reasonably representative view of the state of the art of the theme areas of the event.

This book leans to a rather voltammetric viewpoint that is characteristic of modern electroanalytical chemistry. However, this is to be expected from the interests of the Conference organisers, and to follow this trend the book has a first section of 15 papers devoted to analytical voltammetry, followed by 6 papers on potentiometry. The rest of the book reflects developments on the biomedical side with 6 papers on bioelectrochemistry and a few more within the block of 12 papers devoted to modified electrodes and sensors. The book ends with 9 papers directed at clinical and pharmaceutical chemistry.

Some of the papers cover a broad area, as does that of the opening paper on analytical pulse voltammetry by the Osteryoungs, which gives a good broad introduction. However, it is arguable that such treatments can be better given in review-type books with a limited number of authors who are then able to discuss the subject in greater depth. Apart from papers devoted to determinations, such as the electroanalysis of drug materials, many are concerned with mechanistic studies, such as electron transport and energy transduction in mitochondria. Modified electrodes now attract much attention, and papers such as that on the oxidation of hexacyanoferrate(II) mediated by electrodes modified with ruthenium-containing polymers merit the attention of readers, as these are cornerstones of present day research areas and can lead to more effective electrochemical approaches to analysis.

Finally, the volume stands as a worthy tribute to the memory of Professor Hans Wolfgang Nürnberg, whose untimely death at about the time the Conference was announced represents a great loss to all of analytical chemistry, and especially to electroanalysis.

As with earlier members of this series, the book is strongly bound in an attractive jacket, but it is a pity that the Editors have not produced a keyword index. To assemble an index would have postponed the unusually fast publication by just two days!

J. D. R. Thomas

A Handbook of Silicate Rock Analysis
P. J. Potts. Pp. x + 622. Blackie. 1987. Price £128. ISBN 0 216 91794 8; 0 412 00881 5 (USA).

This is the first modern book to undertake a comprehensive survey of instrumental and classical chemical analytical techniques for silicate rocks. The book is divided into 20 chapters in a logical sequence to cover basic concepts in analytical chemistry, procedures based on wet chemistry and optical spectroscopy, ion-selective electrodes, X-ray and beam procedures, nuclear techniques, ion exchange and other chromatographic procedures, mass spectrometry and specialised topics such as fire assay. The preliminary material on basic concepts consists of excellent introductions to the important topics of statistics, sampling strategies, contamination, reference materials and the selection of appropriate techniques for particular elements.

The sections on instrumental techniques deal with basic aspects in considerable depth with commendable clarity, which makes this text of much wider general interest than the title might imply. A deal of practical detail is complemented by parametric data including most sensitive lines, interference effects and detection limit data, which will make this volume an essential laboratory handbook for those engaged in the analysis of natural or man-made mineral materials.

The production, layout, typography and diagrams are all of excellent quality, as befits the text overall.

This is a superb book and will in due course become to be regarded as a classic text in the field. It is therefore highly recommended for library purchases and, for the reason stated earlier, to all practising professional analytical chemists with interests in major and trace element analyses.

D. Thorburn Burns

Principles of Electroanalytical Methods
T. Riley and C. Tomlinson. *Analytical Chemistry by Open Learning.* Pp. xx + 252. Wiley. 1987. Price £9.95 (softback); £28; $47.75 (cloth). ISBN 0 471 91330 8 (softback); 0 471 92329 4 (cloth).

This book has as much to do with the learning approach as with subject content. It is, of course, one of a series of texts produced as a result of the "Open Tech" Project initiative of the Committee of Heads of Polytechnic Chemistry Departments of the United Kingdom. As a "Distance Learner" or rather "Teacher," it is concerned with the electroanalytical part of the basics of analytical chemistry and instrumental techniques and is aimed at those undertaking courses leading to BTEC (Levels IV and V), Royal Society of Chemistry (Certificates of Applied Chemistry) or other qualifications, that is, it is aimed at senior technician level.

A wider readership will benefit from reading this book, which sets the broad scene for the further, more specialised books on "Potentiometry and Ion-selective Electrodes" and "Polarography and Other Voltammetric Methods."

The book opens with a good basics chapter on the principles of solution chemistry and electrochemistry, covering ionisation and the various interactions of electrolytes in solution, prior to introducing electrodes and electrochemical cells. This first chapter naturally leads on to the treatment of galvanic cells and electroanalysis in the core second and third chapters. It is interesting that the authors lead on quickly from a simple description of galvanic cells to a broad survey of the various electrode types now available. This approach leads readers directly to an appreciation of the "tools of the trade" and encourages them to the essential background knowledge of making these function properly by giving details of the IUPAC Convention, Nernst equation, liquid junction and e.m.f. measurement sections which follow later.

The material is extremely readable and very well set out, with frequent and useful self-assessment questions (SAQ) and clear definitions of objectives. If there is a criticism, it has to be with an over-use of non-routine acronyms, such as "dvm" for digital voltmeter. While on digital voltmeters, the reviewer would have been happier with them being considered after the potentiometer. The second chapter then goes on to pH measurements and buffers and ends with analytical potentiometry, where the principles of calibration plots and interpretation of titration curves are laid down.

Electroanalysis as the basis of voltammetry is similarly well

treated in Chapter 3 and covers the principles of over-potential and micro- and macro-electrolysis before embarking on the essentials of three-electrode circuitry, the potentiostat and cell design. Thus, again, the reader is effectively "talked" gently through that which he is meant to learn and with such careful choice of phraseology and clear use of diagrams that he cannot fail to understand and to learn. All is now set for the electrodes, solvent and supporting electrolytes which are what the technique really has to deal with. Of course, he also learns to know what is meant by d.c. and a.c. polarography, solid electrode voltammetry, amperometric titrations, linear sweep, voltammetry, cyclic voltammetry, stripping voltammetry, sampled d.c. polarography, normal-pulse polarography and differential-pulse polarography. Yes, the authors have done well in explaining all these, and the final review chapter concerning methods of electroanalytical chemistry puts everything in perspective by a diagram before the brief discussion on electrogravimetry and coulometry to complete 200 pages of learner-orientated material.

Finally, there are 44 pages of attractively presented, self-assessment questions and responses, followed by 6 pages of units of measurement, glossary of terms, etc. If this book typifies the ACOL series, formal lectures will become poorly attended unless teachers can at least emulate the approaches of this book.

J. D. R. Thomas

Assessment and Control of Biochemical Methods

T. Hector. *Analytical Chemistry by Open Learning.* Pp. xviii + 150. Wiley. 1986. Price £9.95 (softback); £28; $47.75 (cloth). ISBN 0 471 91279 4.

This volume is one of a series which aims "to provide a uniquely comprehensive and integrated coverage of analytical chemistry" at the Senior Technician level. On the back cover it says, "This book deals with the problems associated with selecting, evaluating and monitoring biochemical assays. Examples are taken from assays used in clinical chemistry laboratories, but the principles described can be applied to many other routine laboratories or research situations."

Certainly it is appropriate that a book of this type should have clinical chemistry as its *point d'appui.* One gets the impression that the meticulous technique engendered in the classical analytical chemist is less important nowadays with the advent of certain instrumental methods that require little or no sample preparation. Biological samples and reagents, however, are notoriously unstable and subject to matrix effects and thus still require painstaking manipulation.

There are four chapters, each containing a number of self-assessment questions (SAQs), which are of variable difficulty, but all of which graphically and effectively reinforce their point. These SAQs are repeated in a "chapter" of 44 pages at the end of the book, together with "suggested responses." The chapter titles are as follows: (1) Selection and Evaluation of Biochemical Assay Methods; (2) Calibration and Control Materials; (3) Internal Quality Control; and (4) External Quality Assessment. Finally, there is a rather unnecessary appendix of SI units, abbreviations and physical constants.

Chapter 1, at 36 pages, is by far the longest chapter, and gives a reasonably thorough overview of the factors one needs to consider in selecting a new analytical method. Hence consideration is given to the specification of the reagents (stability, special storage conditions, health and safety aspects) and the instrumentation required. Most of the chapter is devoted to a discussion of accuracy and precision, these concepts being differentiated by analogy with the sport of archery. There is an SAQ illustrating four targets on which the reader has to indicate the likely distribution of five arrows released by skilled/unskilled archers using correctly/incorrectly-sighted bows, and state whether these conditions affect the accuracy and/or precision. As accuracy and precision are the hallmarks (and motto!) of the analytical chemist, this diagram appropriately forms part of the front-cover design. Precision is described in classical fashion by reference to standard deviation and coefficient of variation. Two approaches to the assessment of accuracy are described in detail, these being the "comparison method" to obtain a correlation coefficient, and a description of a "recovery experiment." Both approaches are backed up with an SAQ.

Chapter 2 distinguishes between "calibration" and "control" samples. The importance of meticulously following manufacturers' instructions in reconstituting control samples is emphasised by reference to certain enzymes whose activities vary according to reconstitution temperature (creatine kinase) or whose activities decline or increase gradually after reconstitution (acid phosphatase and alkaline phosphatase, respectively).

Chapter 3 stresses the importance of day-to-day assurance in producing reliable results, and describes the use of Shewhart plots and CUSUM charts for monitoring the accuracy of a given method.

Chapter 4 details the importance of EQA in providing a retrospective check on bias. The *modus operandi* of EQA schemes is described by reference to the contrasting UK External Assessment Scheme and Wellcome Diagnostics Scheme, together with relevant SAQs.

I found this book to be particularly "user friendly," being at once clear, concise and informative. The various pitfalls associated with the analysis of biochemical substances made absorbing reading. However, although this book is predominantly an Open-Learning text, it can be used as a conventional text book, and hence a short index would have been useful. I feel also that a chapter describing the use of analysis of variance in assessing intra- and inter-laboratory reproducibility would have been pertinent. Finally, I am always disappointed when a short biography of the author is not included.

This book will undoubtedly be very helpful to those at whom it is directed (BTech Levels IV and V), and not only those on Open-Learning Schemes!

Alan Crooks

The Manipulation of Air-sensitive Compounds. Second Edition.

D. F. Shriver and M. A. Drezdzon. Pp. x + 326. Wiley-Interscience. 1986. Price £43.25. ISBN 0 471 86773 X.

This monograph owes a great deal to the First Edition, as much of the text and many of the drawings reappear verbatim. However, this is more a reflection of the rate of change in technology in the intervening years, rather than any fault of the authors.

Having said this, there have been significant improvements in the presentation of this Second Edition. Probably the most important of these is the emphasis given to matters of safety. Throughout the book the reader's attention is immediately drawn to these items, as they are presented in emboldened type. This kind of presentation, particularly in a book dealing very much with practical aspects of a subject, can only be applauded and used as an example to other authors.

For readers not familiar with the earlier edition, the book contains information on the following aspects of the subject. Part 1 covers the use of inert atmosphere techniques, including chapters on bench-top inert atmosphere techniques; glove-boxes; inert gases and their purification; and purification of solvents and reagents. Part 2 covers the use of vacuum line manipulations, including chapters on vacuum line design and operation; vacuum pumps, pressure, flow and leak measurement; joints, stopcocks and valves; specialised operations;

and metal systems. There are also six appendices containing information on safety, glassblowing, plastics, metals, vapour pressures and pressure/flow conversions.

The book has clearly not been written for the analytical chemist but for the chemist dealing with air-sensitive compounds *per se*. However, there is a great deal of useful information for the analytical chemist; it is just that he has to spend time looking for it, as it is spread throughout the book. There is information on sampling, separation, filtration and weighing of air-sensitive compounds. Similarly, there is information on the use of the optical microscope and the preparation of samples for infrared, ultraviolet, NMR and X-ray diffraction spectroscopy and gas chromatography.

The authors claim to have set out ". . . to make the book useful to the chemist who is beginning work in the field. In addition, considerable technical data and information are given that should aid chemists at all levels of proficiency in the design of experiments." Throughout the book the presentation is at a practical level with many detailed examples included, but above all it is in a very readable form. The contents and index in the book also make the information readily accessible. It is considered that the authors have achieved their aims and have produced a most useful book.

C. W. Fuller

Advanced Agricultural Instrumentation. Design and Use Edited by William G. Gensler. *NATO ASI Series. Series E: No.* 111. Pp. viii + 480. Martinus Nijhoff. 1986. Price Dfl 215; $94.50; £65.95. ISBN 90 247 3329 4.

This text consists of the Proceedings of the NATO Advanced Study Institute on "Advanced Agricultural Instrumentation" held in May - June 1984, when personnel from industry, government and academia pooled their concepts and experience. The 16 chapters are divided between photosynthesis-related instrumentation, environmental instrumentation (*e.g.*, neutron probes, thermocouple psychrometers), theoretical framework, growth and nutrient status and water status instrumentation.

The book has several features of merit. It combines theoretical concepts with applied research and agriculture, so we move from isotopic cross-sections for neutron interactions to a detailed account of how to insert a neutron probe up to 10 m deep in rocky soils, and its applications to estimate drainage beneath forest and grassland areas. The articles unambiguously define the units of measurement with particular reference to SI units and their equivalents. Constructional diagrams are clearly presented and safety precautions to be observed on operating instruments such as the neutron probe are carefully spelled out. The authors have taken pains to describe not only the advantages but also, almost more importantly, the disadvantages and shortcomings of certain equipment with operational precautions that are seldom found in published papers in scientific journals. The references are up to date (1984) and most useful.

The chapters on photosynthesis should particularly appeal to biochemists and biophysicists and those on water status and radiation thermometry to agriculturalists and agronomists. Of special interest to plant nutritionists and agricultural analytical chemists is the section on growth and nutrient status. Details of laboratory analytical tests are presented and, although rather sketchy, references are given for further study. Reference should have been made, in the subsection on sample preparation, to the fact that for certain analyses, such as water-soluble carbohydrate and Van Soest detergent fibre determinations, the herbage should be freeze-dried rather than oven-dried at 80 °C. There is an interesting chapter on the development of membrane, ion-selective, enzyme and other types of electrodes and their application to the nutrient film technique of greenhouse culture as practised at the Glasshouse Crops Research Institute, Littlehampton.

A stimulating aspect of the book is that one can think of areas in Britain where one could apply a variation of the latest American research. For example, we could apply the sensing of water stress in vines or cotton plants in Arizona linked with an automatic irrigation system to thirsty crops such as potatoes in Pembrokeshire.

The printed index is useful but could have had more page references per subject. The rest of the book is produced from typescript prepared for photography with an unjustified right-hand margin. It is clearly readable and well bound. The queue of my colleagues already waiting to borrow it underlines the recommendation that it should be purchased by every library accessed by agricultural scientists.

N. T. Faithfull

NEW BOOKS FOR ORGANIC CHEMISTS AND BIOCHEMISTS

Amino Acids and Peptides Vol. 18

This series was previously entitled 'Amino Acids, Peptides, and Proteins.' The latest volume in the series covers the literature published on the subject during 1985.

Brief Contents:
Amino Acids; Peptide Synthesis; Analogue and Conformational Studies on Peptide Hormones and Other Biologically Active Peptides; Cyclic Modified and Conjugate Peptides; ß-Lactam Antibiotic Chemistry; Metal Complexes of Amino Acids and Peptides.

Specialist Periodical Report

Senior Reporter: J. H. Jones,
University of Oxford

Hardcover 314pp
ISBN 0 85186 164 4
Price £64.00 ($96.00)
RSC Members price £42.00

General and Synthetic Methods Vol. 9

Hardcover 734pp
ISBN 0 85186 904 1
Price £120.00 ($230.00)
RSC Members price £65.00

A review of the literature published during 1984.

Brief Contents:
Saturated and Unsaturated Hydrocarbons; Aldehydes and Ketones; Carboxylic Acids and Derivatives; Alcohols, Halogeno-compounds, and Ethers; Amines, Nitriles and Other Nitrogen-containing Functional Groups; Organometallics in Synthesis; Saturated Carbocyclic Ring Synthesis; Saturated Heterocyclic Ring Synthesis; Highlights in Total Synthesis of Natural Products; Reviews on General and Synthetic Methods.

Specialist Periodical Report

Senior Reporter:
G. Pattenden,
University of Nottingham

Topics in Lipid Research

From structural elucidation to biological function

Over the last decade many lipid chemists and biochemists have made a conscious effort to move from purely structural investigations to those which throw more light on the biological function of lipid molecules.

For many years lipids were considered to be molecules containing just C, H and O, related to fatty acids and hydrocarbons, with high oil-water partition co-efficients; more recently, however, it has been realized that the more complex structures such as glycolipids and lipoproteins, and indeed glycolipoproteins, are fascinating both as structural challenges and as functional elements of the cell.

The book includes both reviews and research papers dealing with recent developments in lipid research.

Brief Contents:
Platelet Activating Factor; Eicosanoids; Glycolipids; Probes and Anaesthetics; Membrane Structure and Function; Environmental Adaptation.

Edited by R. A. Klein
and B. Schmitz
Molteno Inst.
University of Cambridge

Hardcover 348pp
ISBN 0 85186 353 1
Price £37.50 ($65.00)
RSC Members price £24.50

Chemical Aspects of Food Enzymes

Softcover 326pp
ISBN 0 85186 686 7
Price £37.50 ($72.00)
RSC Members price £24.50

This publication reviews current applications of enzymes in food processes. It should stimulate interest and point the way forward in this area of vital importance.

Contents:

Enzyme Structure and Specifity: Factors Affecting Enzyme Activity and Kinetics; Genetic Engineering: Basic Considerations; The Potential for Chemical modifications of Enzymes; Applications of Immobilized Enzymes and Cells in the Food Industry; Encapsulation of Enzymes and other Agents in Liposomes; Developments in the Microencapsulation of Enzymes in Food Technology; Protein Engineering of Food Enzymes; Extending Enzyme Applications in the Food Industry; Enzymatic Modification of Lipids; Enzymatic Modification of Food Carbohydrates; Genetic Engineering of Lactic Acid Bacteria; The Molecular Biology of Plant Thiol Proteinases; Production of Heterologous Products in *Escherichia coli* and Yeast; Enzymic Modification of Dairy and Other Food Proteins; Biosensors: Principles and Potential; The SERC Biotechnology Directorate's Programme in Protein Engineering; Food Enzymes: Industrial Potential and Scientific Challenges; Subject Index.

Edited by A. T. Andrews
AFRC Institute of Food Research, Reading

ORDERING:

RSC Members should send their orders to: The Royal Society of Chemistry, Membership Manager, 30 Russell Square, London WC1B 5DT, UK. Non-RSC Members should send their orders to: The Royal Society of Chemistry, Distribution Centre, Blackhorse Road, Letchworth, Herts SG6 1HN, UK.

ROYAL SOCIETY OF CHEMISTRY
Information Services

FIRST FOLD HERE

THE ANALYST READER ENQUIRY SERVICE
For further information about any of the products featured in the advertisements in this issue, write the appropriate number on the postcard, detach and post.

FOLD HERE

THE ANALYST READER ENQUIRY SERVICE

AUG'87

For further information about any of the products featured in the advertisements in this issue, please write the appropriate number in one of the boxes below.
Postage paid if posted in the British Isles but overseas readers must affix a stamp.

PLEASE USE BLOCK CAPITALS LEAVING A SPACE BETWEEN WORDS *Valid 12 months*

1 NAME

2 COMPANY

PLEASE GIVE YOUR BUSINESS ADDRESS IF POSSIBLE. IF NOT, PLEASE TICK HERE ☐

3 STREET

4 TOWN

5 COUNTY
POST CODE

6 COUNTRY

7 DEPARTMENT/
DIVISION

8 YOUR JOB TITLE/
POSITION

9 TELEPHONE NO

OFFICE USE ONLY RECD PROC'D

FOLD HERE

Postage will be paid by Licensee

Do not affix Postage Stamps if posted in Gt. Britain, Channel Islands, N. Ireland or the Isle of Man

BUSINESS REPLY SERVICE
Licence No. WD 106

Reader Enquiry Service
The Analyst
The Royal Society of Chemistry
Burlington House, Piccadilly
LONDON
W1E 6WF
England

ANALAO 112(8) 1093–1196 (1987) August 1987

The Analyst

The Analytical Journal of The Royal Society of Chemistry

CONTENTS

Typeset and printed by Black Bear Press Limited, Cambridge, England