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Directly Coupled Chromatography - Atomic Spectroscopy

Part 2.* Directly Coupled Liquid Chromatography - Atomic Spectroscopy

A Review

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Summary of Contents

1. Introduction
2. Choice of atomic spectroscopic technique
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4. Electrothermal atomisation atomic absorption spectrometry
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7. Conclusion
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Keywords: *Coupled techniques; liquid chromatography; atomic spectroscopy; trace metal speciation; review*

1. Introduction

In Part 1 of this review[1] we critically evaluated the direct coupling of gas chromatography with analytical atomic spectroscopy, particularly as a means of providing quantitative information on trace element speciation. Whereas the sensitivity and selectivity of atomic spectroscopic techniques for determining trace element concentrations in a wide variety of samples was acknowledged, it was indicated that this information is limited to total metal concentrations and that there is a growing demand for quantitative information on the form in which elements are present, the so-called speciation. In this review speciation is defined as the quantification of the different chemical forms of an element in a sample. In several situations, *e.g.*, toxicology, environmental modelling, clinical chemistry, food science, forensic science and industrial analysis, a knowledge of the chemical form and the relative amounts of trace elements is vital. Characteristically metal-containing organic species occur at very low concentrations (typically sub-nanogram per gram levels) in highly polar solvents, such as biotic fluids or natural waters. A wide variety of chemical types are encountered in trace metal speciation studies, for example, comparatively volatile neutral molecules (*e.g.*, Me_4Pb, Me_3As), involatile charged organometallic species (*e.g.*, Bu_3Sn^+), large or low relative molecular mass complexes, chelates (both volatile and involatile) and different valent states (*e.g.*, Fe and Cr). A number of approaches have been suggested for trace metal speciation, including relatively inexpensive approaches such as electrochemical techniques. Unfortunately it is often difficult to obtain unambiguous information from such methods, and considerable experience is often required to eliminate responses that are instrumental artefacts. More expensive approaches such as coupled chromatography - mass spectrometry may also be used. These are indeed powerful tools for trace metal speciation as they also yield information on molecular structure, but the complexity of the spectra and instrumentation, in addition to the high cost, mitigate against routine use. This is particularly true of liquid chromatography - mass spectrometry at this time. Whereas atomic spectroscopic detectors cannot by themselves identify different species, coupled with chromatographic techniques and the use of appropriate standards, they can yield unequivocal identification at the levels of interest, on-line in real time and using simple interfaces between readily available instrumentation. Thus the growing interest in trace metal speciation has provoked studies of the coupling of the separative power of chromatographic techniques with the sensitivity and selectivity of various atomic spectroscopic techniques. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are clearly the most suitable chromatographic techniques if complex samples are involved. There is a wider choice of which spectroscopic technique to use as the detector, and some influences on this choice are discussed below.

Although gas chromatography has been coupled with analytical atomic spectrometry for trace metal speciation,[1] it is limited to volatile and thermally stable organometallic species or metal chelates. The use of liquid chromatography (LC) considerably expands the type of chemical and physical species that may be studied. The separation of ions and involatile high molar mass organometallic species, in addition to volatile species, is possible using one or another of the popular LC configurations. Adsorption, ion-exchange, gel-permeation, normal- and reversed-phase chromatography have all been used in conjunction with atomic spectroscopy.

2. Choice of Atomic Spectroscopic Technique

The coupling of LC with atomic spectroscopy has been reviewed in a number of publications,[2–7] and frequently the complication of atomising large volumes of a liquid mobile phase is noted. Thus the atom cells used, *e.g.*, flame, furnace or plasma, must be capable of handling solvent flow, typically 0.1–3.0 ml min^{-1}, which may be aqueous or organic in nature. Usually nebulisation is used to convert the liquid effluent from

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Table 1. Coupled liquid chromatography - flame atomic absorption spectrometry

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
Flame AAS. The column is connected to the nebuliser and the venturi effect pulls the eluent through the column	Chelex ion-exchange resin, 100–120 mesh, packed into a 1-ml syringe, resin volume 0.5 ml. 0.1–0.5 ml samples. Resin washed with 0.1 M $CuCl_2$ to generate Cu form	Solutions of EDTA and NTA; pH 4–9	Chelates strip Cu from the column, which is monitored by AAS, Cu signal then related to chelate concentration. Linear up to 50×10^{-6} mM EDTA or NTA	Cu	13
Flame AAS with column connected directly to nebuliser	60 cm × 1.0 cm i.d. Sephadex G-15 column. Eluent: 0.1 M NaCl or 0.1 M NaCl + 0.001 M EDTA. 1 ml sample volume	Detection of Mg and K in $MgCl_2$ - KCl solution	To balance column flow with aspiration rate, a T-piece used with third arm placed in water reservoir. Linear from 10^{-5} to 1.7×10^{-4} M	Mg K	10
Flame AAS directly coupled through the nebuliser by PTFE tubing (0.023 in i.d.)	60 cm × 2 mm i.d. Porasil A column. Eluent 0.5% *V/V* pyridine in toluene. Sample volume = 10 µl	Cr as the $Cr(acac)_3$, $Cr(HAP)_3$ and $Cr(HFAA)_3$ chelates	Adjustment of oxidant and fuel flows were made to accommodate the solvent in the flame. Detection limit of 40 ng	Cr	16
Flame AAS, see ref. 10	94.5 cm × 1.5 cm Sephadex G-25 column. NH_3 solution - NH_4Cl (pH 10) eluent 0.02 M, at 1.83 ml min^{-1}. Column pre-equilibrated with $MgCl_2$ solution	Determination of various condensed phosphates by on-column complexation with Mg	Phosphates elute in order tetra, tri, di, mono, with free magnesium eluting last after 73 min. $W\frac{1}{2}$ = 5 min. Linear up to 20 µg phosphate as triphosphate	Mg (285.2)	11
Flame AAS, direct coupling through nebuliser. Flow spoiler removed from chamber, *ca.* 80% of eluent reaching flame	50 cm × 2.6 mm i.d. ODS column. T_c = 50 °C. Eluent, 3 : 2 *V/V* H_2O - MeOH at 1.0 ml min^{-1} at 1200 lb in^{-2}. 1 µl sample	Tetraalkyllead compounds in petrol	No background problems found, possibly due to the large and constant amount of MeOH in eluent. Linear from 0.25 to 50 µg	Pb	17
Flame AAS, direct coupling, *c.f.*, ref. 16. Air - C_2H_2 flame	5 cm × 2.1 mm i.d. Aminex A-14 resin, 4% cross-linked with SDVB, 20 + 3 µm. 0.5 M $(NH_4)_2SO_4$ eluent at 2.0 ml min^{-1}	Separation of $Cu_2(EGTA)$, $Cu(NTAO)^-$, $Cu(EDTA)^{2-}$ and $Cu(CDTA)^{2-}$	pH of sample affects formation of $Cu_2(EGTA)$ but not of other complexes. Detection limits (ng Cu): EGTA, 13.5; NTA, 16.2; EDTA, 29.4; and CDTA, 450 in order of elution	Cu	15
Flame AAS, direct coupling through nebuliser. See ref. 15	Identical with ref. 15	Copper chelates of aminocarboxylate ions in spiked sewage effluents	Assumptions made as to detection limits and hence feasibility of method. Detection limits (ng Cu): NTA, 10.7 and EDTA, 23.6	Cu	15
Flame AAS, column coupled directly to nebuliser. Air - C_2H_2 flame	Partisil-10 SC X cation-exchange column. T_c = 55 °C. 1 M NH_4NO_3 at 4.0 ml min^{-1} as eluent. 25 µl sample size	Separation of Cu - EDTA, Cu - trien and Cu - glycine complexes	Use of UV - visible detection enabled only Cu - trien to be monitored, with reduced sensitivity compared to AAS	Cu	19
	25-cm ODS-SILXI column. Eluent: 50–100% methanol - water gradient in 10 min	Alkyl and aryl Zn additives in lubricating oils. Samples diluted in CH_2Cl_2	AA detector shown to be superior to UV - visible detection	Zn (213.9)	
Flame AAS. Use of column directly coupled to nebuliser. Aspiration rate controls flow of eluent through column	Basic anion-exchange Dowex 2X-8 column soaked overnight in 3 M HCl followed by water rinsing. Pt and Au complexes eluted with NH_3 (75%)	Pt and Au in aqueous solutions	Pt and Au solutions (pH 6) passed through the column, the metals retained and then eluted with NH_3 into nebuliser. Linear from 2 to 10 µg for Au and from 35 to 175 µg for Pt	Au Pt	20
Flame AAS; see refs. 10 and 11 for interface	97.5 cm × 1.5 mm i.d., Sephadex G-25 column	Monitoring of Kurrol's salt, $(KPO_3)_n$, di-, tri- and orthophosphate as Mg complexes	Kurrol's salt used as useful marker for void volume of column. Estimation of stability constants also made	Mg (285.2)	12

Table 1—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
Flame AAS using standard flame conditions. Eluent passed into nebuliser	25 × 0.46 cm Partisil-10 SCX column. Eluent, NH_4NO_3 at various molarities and pH, 1–2 ml min^{-1}	Use of metal labelling to determine amino acids, in this instance histidine as copper complex	Nebuliser operated in starved mode by use of injection cup (see ref. 23). 100-µl drops from column into cup. Detection limit of 48.5 ng	Cu	21
Flame AAS using N_2O - C_2H_2. Directly coupled through nebuliser or hydride generation followed by electro-thermal quartz furnace AAS	250 × 3.0 mm i.d., ODS Spherisorb S5W. $T_c = 23 \pm 0.1$ °C. Eluent: acetone - pentane (3 + 2) at 1.0 ml min^{-1} for methyltin compounds; acetone - pentane (7 + 3) at 1.2 ml min^{-1} for ethyltin compounds	Methyl and ethyl tin compounds, both SnR_4 and $SnR_{4-n}Cl_n$.	The design of a miniature, continuous flow hydride generation system given. Linear up to 50 µg using flame and up to 100 ng for hydride generation	Sn (286.3)	40
Flame AAS, using air - C_2H_2 flame directly coupled through nebuliser	300 mm × 3.9 mm i.d., µBondapak C_{18} column. Eluent: acetonitrile - water (70 + 30) at 3.0 ml min^{-1}. 20 µl injections	Tetraalkyllead compounds in petrol	The relative merits of UV and AAS detection discussed with latter proving more suitable for this application. Linear from 1.1 to 11 µg	Pb (283.3)	18
Flame AAS using flow injection sample manipulator (FISM) interface with fuel-rich air - C_2H_2 flame	100 mm × 7.5 mm i.d. Spherogel TSK 2000SW (10 µm). Eluent: 130 mmol NaCl, 6.8 mmol NaOH, 3 mmol NaN_3, 4 mmol KCl and 10 mmol TES at 0.4 ml min^{-1}, pH = 7.43 at 37 °C	Study of metal - ligand binding in clinical samples	FISM interface described enabled La - HCl to be mixed with eluent prior to introduction through nebuliser. Linear up to 3.75 mmol Ca	Ca (422.7) Mg (285.2)	36
Flame AAS using hydride generation and electro-thermal quartz tube atomisation. $T = 800$ °C	500 × 3 mm standard Dionex anion column, Eluent: 2.6 ml min^{-1} 0.0024 M $NaHCO_3$ - 0.0019 M Na_2CO_3 - 0.001 M $Na_2B_4O_7$	Speciation of As^V, MMA, *p*-APA	Miniature hydride generation system, see ref. 11. 1 h re-equilibration time between eluent systems. Detection limit 10 ng ml^{-1}	As (193.7)	41
	0.005 M $Na_2B_4O_7$	Speciation of As^{III} and DMA			
Flame AAS using fraction collector as interface	33 × 1.0 cm Sephadex G-15 column. Eluent: 0.2 M $NaClO_4$ (pH 2) at 40 ml h^{-1}. 46 × 10 cm Sephadex G-10, eluent 0.1 M $HClO_4$ at 19 ml h^{-1}	Separation of successive Cr^{III} isothiocyanato complexes with SCN - Cr ratio of 1:6		Chromium	48
Flame AAS using continuous flow hydride generation and heated quartz tube atomisation	Zipax ion-exchange pre-column attached in series to strong base anion-exchange BAX 10 resin (5 µm, 250 × 5 mm column). Eluent 10^{-4}% sulphuric acid (flow-rate 4.0 ml min^{-1}), switched to 0.01 M ammonium carbonate (flow-rate 4.0 ml min^{-1})	Arsenic speciation in soil pore waters	Pre-column acted as a guard column and enabled pre-concentration step to be incorporated in the determination. Arsenate, arsenite and monomethylarsonic acid characterised in soil pore water	Arsenic (193.6)	24
UV detector or atomic absorption spectrometer connected directly via the nebuliser. Various types of tubing used for the interface	Column, HS-3 C-18 (10 cm × 0.46 cm i.d.); solvent A, MeOH; solvent B, 0.01 M; Na_3PO_4 in water; gradient, linear from 0 to 70% MeOH for 10 min; and flow-rate, 2 ml min^{-1}	Determination of iron in blood	Main aim of paper is an investigation of peak dispersion in a coupled LC - AAS system. Three types of tubing examined: (i) serpentine tube of 0.25 mm i.d.; (ii) straight tube 1.27 mm i.d.; (iii) polyethylene tube 0.55 mm i.d. All tubes 49 cm long	Iron (248.3)	54

Table 1—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
UV detector of atomic absorption spectrometer connected directly via the nebuliser. Various types of tubing used for the interface	P.E. 3 × 3 column (0.3 cm × 4.6 mm i.d.) packed with octadecyl-bonded silica gel (3 μm). Mobile phase MeOH, flow-rate 2 ml min^{-1}. Solute $Mg(NO_3)_2$		System used for peak dispersion measurements	Mg	54
Directly coupled flame atomic absorption utilising pulse nebulisation and a slotted tube atom trap	Partisil-10-SCX (250 × 4.6 mm i.d.) column. Mobile phase of 80 : 20 methanol - water in 0.1 M NH_4OAc	Tributyltin determination in coastal waters	Organotin compounds quantitatively extracted from sea water into chloroform and then into methanol to facilitate injection on to the HPLC column. Total analysis within 8 min	Sn (224.6)	25

the chromatograph into an aerosol suitable for introduction into a flame or plasma. This step is often regarded as the most inefficient process in atomic spectroscopy and considerable attention has been given to modifying and optimising this aspect of the coupling. These efforts are critically reviewed below.

3. Flame Atomic Absorption Spectrometry

In addition to offering excellent inter-metal selectivity, flame atomic absorption spectrometry (FAAS) has the advantage that it readily accepts liquid samples. In a study of the effect of various mobile phases on nebulisation efficiency, Jones *et al.*[8] found that with methanol, ethanol, chloroform and benzene, 100% nebulisation efficiencies could be achieved at flow-rates of 1 ml min^{-1}, whereas for water at the same flow-rate only 32% was nebulised into the flame. The use of organic mobile phases requires some modification of air - fuel ratios as the organic eluent can act as a secondary fuel. Koropchak and Coleman[9] found that operating a nebuliser at a slight backpressure not only negated the use of a post-column diluter to match LC flow-rate with nebuliser uptake rate, but also gave improved signal to noise ratios with a standard nebuliser arrangement. The various LC - FAAS couplings reported in the literature are surveyed in Table 1.

The group at Kyushu University, Japan, utilised the sensitivity and selectivity of AAS to monitor Mg and K in chloride solutions after separation.[10] They used a T-piece with one end placed in a water reservoir to balance the LC and aspiration flow-rates. This group[10–12] also utilised the same coupling to study the formation of various phosphates from orthophosphate up to Kurrol's salt $(KPO_3)_n$. Manahan and Jones used Cu-specific detection to monitor EDTA and NTA concentrations by passing the column eluent into the nebuliser of the spectrometer[13] in mixed solutions and also in spiked sewage effluents.[14] They expanded the range of amino carboxylic acid - copper chelates that could be monitored[15] to include EGTA and CDTA. The same workers demonstrated that organic mobile phases (toluene - pyridine) could be used for the separation of various chromium chelates.[16] Botre *et al.*,[17] along with Messman and Rains,[18] used the separation of tetraalkyllead compounds in petrol to demonstrate LC - FAAS couplings using organic mobile phases. For this particular application, however, the extensive study of GC - AAS systems for the speciation of tetraalkyllead compounds has yielded more sensitive and rapid analysis.[1]

Van Loon *et al.*[19] used direct coupling of the column eluent to the nebuliser to monitor copper - amino acid complexes used in the treatment of metal poisoning and also to study zinc aryl and alkyl compounds in lubricating oils. Kahn and Van Loon[20] used a similar coupling to pre-concentrate and speciate Au and Pt complexes from aqueous solutions.

Slavin and Schmidt,[21] in their LC - FAAS coupling, operated the nebuliser in a starved mode by using an injection cup[22] for the determination of amino acids after metal labelling. The concept of metal labelling of species to permit determination by atomic spectrometry has great potential; however, the low sensitivity of flame AAS would cause problems in the determination of amino acids in body fluids at the levels at which they occur. A flow injection sample manipulator (FISM) was used by Renoe *et al.*[23] as an interface between the chromatograph and spectrometer. This FISM allowed the addition of matrix modifiers, in this instance HCl - La_2O_3, to the HPLC eluent prior to introduction to the nebuliser of the spectrometer.

Several of the most recent publications on coupled LC - FAAS have stressed relatively simple interface systems and have reported increased sensitivity by attention to the atom cell. Three approaches have been reported by workers in these laboratories. The first has been used in the study of arsenic speciation in soil pore waters.[24] The arsenic species are first pre-concentrated on an anion-exchange column and then swept off by changing the mobile phase from sulphuric acid to ammonium carbonate. After passing the effluent into a continuous-flow hydride generator, the volatile arsines produced are atomised in a flame-heated quartz tube. The second technique[25] is a modified version of Slavin and Schmidt's direct coupling[21] utilising starved nebulisation via a directly coupled vented tube from the column to the nebuliser. Sensitivity is increased using a slotted-tube atom trap to increase the residence time in the flame. This system has been extensively used for the determination of tributyltin in coastal waters and oyster tissue, and demonstrates the advantage of this technique in instances where the species of interest cannot easily be detected by UV and fluorescence monitors conventionally used as HPLC detectors. In our most recent developments[26] we have reported the use of a series of rotating platinum spirals, which under microprocessor control transport the effluent in discrete drops from the end of a mini-bore column successively to a cool flame, to dry the spiral, and a conventional air - acetylene flame to atomise the sample. Again a quartz tube is used as an atom trap to enhance the sensitivity.

4. Electrothermal Atomisation Atomic Absorption Spectrometry

Electrothermal atomisation (ETA), mainly using graphite furnaces, offers the advantage of high sensitivity for a single atomisation; however, the necessity to dry and ash a sample

Table 2. Coupled liquid chromatography - electrothermal atomisation atomic absorption spectrometry

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ETA-AAS, HPLC eluent passed into a sample well then 10–50 µl sampled into a standard furnace. Programs:	250 × 4.6 mm columns		Coupling operated in either a pulsed mode, where the eluent was passed into a PTFE flow-through cup periodically sampled, or in a survey mode where the eluent was collected by an auto-sampler and each fraction analysed		27
Dry at 80 °C for 15 s; atomise at 2700 °C for 5 s.	LiChrosorb C_{18} RP on 10-µm silica. Eluent MeOH at 0.12 ml min^{-1}	Triphenylarsine.		As (193.3)	
Dry at 100 °C for 10 s; char at 100 °C for 10 s, atomise at 2500 °C for 15 s	LiChrosorb C_2 RP. 1.5 ml min^{-1} of MeOH	Ph_3SnCl, Pr_3SnCl, Bu_3SnCl		Sn (253.7)	
Dry at 80 °C for 25 s; atomise at 750 °C for 12 s	LiChrosorb C_8 RP. Eluent, (a) 0.01 M NH_4OAc, (b) 25 p.p.m. mercaptoethanol in MeOH. Flow a + b (96 + 4) for 25 min then gradient, 10% min^{-1}, to 100% b at 0.30 ml min^{-1}	MeHgCl, EtHgCl, PhHgCl ^{n}PrHgCl, in 1 + 1 H_2O - MeOH		Hg (253.7)	
Dry at 25 °C for 20 s; char at 80 °C for 10 s; atomise at 2000 °C for 10 s	LiChrosorb Si-100 10-µm silica. Eluent hexane - CH_2Cl_2 (95 + 5), 0.33 ml min^{-1}	Ph_6Pb_2		Pb (283.3)	
ETA-AAS using Zeeman background correction. Dry at 100 °C for 25 s; ash at 1000 °C for 1 s; atomise at 3000 °C 5 s using $NiNO_3$ as co-analyte and Ar shield gas (4 l min^{-1}). 37 µl injections	10 cm Partisil-PXS-ODS column. Eluent: MeOH - H_2O (2 + 1) at 0.3 ml min^{-1}. 20 µl injection.	Se-specific detection of $Me_2NC(Se)NH_2$ and $(C_6H_5CH_2)_2Se$	Design and operation of interfacing device consisting of sampling valve, timing circuit and co-analyte addition described. Linear from 10 to 100 p.p.b. for a single atomisation	Se (196)	30
ETA-AAS. Dry at 100 °C for 20 s; char at 700 °C for 30 s; atomise at 2500 °C for 10 s	8-cm Bio-Rex 70, weak acidic cation-exchange resin	Cu - amino acid complexes in human serum	The eluent from the column collected by an autosampler and then automatically injected into furnace	Cu (324.7)	43
	24 cm, silica gel (100–120 mesh, ASTM D1314-61T, grade 923). Flow-rate = 0.40 ml min^{-1} of acetone - water (60 + 40), pH 7–8	Naturally occurring Cu - amino acids, Cu - histidine and Cu - glutamine from an aqueous mixture			
ETA-AAS using Zeeman-effect background correction	500 × 2.5 mm column, Hitachi gel no. 3010. Eluent: MeOH at 0.67 ml min^{-1}	Tetraalkyllead compounds in petrol	10 µl samples from each 250 µl of eluent injected into furnace	Pb (283.3)	29
ETA-AAS using auto-sampler as interface, see ref. 24. T_{at} = 2700 °C	300 × 7.8 mm column, SDVB copolymer (10 µm) Eluent: THF at 1 ml min^{-1} or THF - CH_3CN (19 + 1).	SEC used for organo-metallic polymers, OMP-1, OMP-2, OMP-4	A 50 s interval exists between injections, thus at 1 ml min^{-1} only 2.4% of eluent sampled.	Sn (286.3)	
	LiChrosorb C_{18} (10 µm) 250 × 3.2 mm column. Eluent: ethanol at 0.25 ml min^{-1}	RPC used for organotin and silicates	Linear up to 20 ng Sn or Si for a 20 µl injection	Si (251.6)	28
ETA-AAS using Zeeman-effect background correction. Automated interface that controls eluent sampling, co-analyte addition, injection and furnace operation	µBondapak (C_{18}) RPC column. Eluent: H_2O - acetonitrile - acetic acid and 0.005 M heptane - sulphonic acid (95 + 5 + 6)	Separation of arseno-betaine, arsenocholine and inorganic arsenic	Chromatograms illustrating separation of arsenic compounds at 1 µg level given	As	34

Table 2—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ETA-AAS with microprocessor-controlled interface, details of interface and computer control program given	Partisil SCX cation-exchange column. Eluent: 0.1 M acetate buffer (pH 4.3)	Separation of Cr^{III} and Cr^{VI}	Pulsed-mode operation, eluent sampled for ETA-AAS only every 30–120 s	Cr	31
Dry at 60 °C for 20 s; ash at 250 °C for 12 s; atomise at 2400 °C for 5 s	LiChrosorb C_{18} (10 µm) Eluent: MeOH - H_2O 90 + 10) at 0.5 ml min^{-1}, 20 µl injection	Tetraphenyllead	Total consumption mode, peak containing eluent stream is stored prior to ETA-AAS analysis	Pb (283.3)	31
ETA-AAS using Zeeman-effect background correction. Dry at 60 °C for 25 s; ash at 500 °C for 12 s; atomise at 2400 °C for 5 s	25 cm LiChrospher (10 µm) RPC column. Eluent: MeOH - H_2O (90 + 10)	Tetraphenyllead	Eluent stream containing lead compound is stored after separation in tubing (10 ft × 0.05 cm), prior to injection into furnace	Pb (283.3)	32
Microprocessor-controlled interface, see ref. 29. 37 µl injection from each 100 or 220 µl sample of eluent			Detection limit of 480 pg		
ETA-AAS, 20 µl injections every 45 s. Dry at 150 °C for 15 s and 200 °C for 5 s, atomise at 2700 ° for 10 s. No background correction	25 cm × 3.2 mm i.d. LiChrosorb SAX (10 µm) column. Eluent: 0.05 M NaH_2PO_4 at 0.5 ml min^{-1}	Speciation of DMA, MMA and arsenilic acid	HPLC separation schemes were employed for As speciation work with several soil and drinking water samples. Linear from 0.1 to 10 ng As	As (193.7)	35
	Same column but with 0.03 M ammonium acetate - 0.045 M acetic acid. Eluent at 0.25 ml min^{-1}	Speciation of MMA, DMA and As^{III}			
No background correction	25 cm × 3.2 mm i.d. Altex SCX column (10 µm) with 0.0375 M ammonium acetate - acetic acid	Speciation of As^{III} and As^{V}			
Zeeman-effect background correction	Eluent at 0.15 ml min^{-1}. 30 cm × 4 mm i.d., µBondapak C_{18} RPC (10 µm) column, H_2O - MeOH (95 + 5), 0.005 M w.r.t. TBA, at pH 7.3 adjusted with phosphoric acid	Speciation of As^{III} and As^{V}	The use of the ion-pair reagents THAN or TBAP requires the superior background correction afforded by the Zeeman effect. Linear up to 500 ng As		35
	25 cm × 4.6 mm i.d., Altex Chromosorb RP-18 column (10 µm). H_2O - MeOH saturated with THAN for 23 min then MeOH, at 1.0 ml min^{-1}	Speciation of As^{III}, DMA, MMA and As^{V}			
ETA-AAS, using PTFE flow through sampling cup as interface, 20 µl injections at 43 s intervals. Dry at 110 °C for 8 s; char at 1200 °C for 7 s; atomise at 2500 °C for 8 s. 20 s furnace cooling period	25 cm × 3 mm i.d., low capacity anion-exchange column (Dionex) gradient elution from H_2O - MeOH (80 + 20) to 0.02 M $(NH_4)_2CO_3$ - MeOH (85 + 15) at 1.2 ml min^{-1}. 5–25 µl injections. 8–12 min equilibration time	Separation of DMA, MMA, As^{III} and As^{V}	The column packing prepared by passing a suspension of a high capacity strong anion-exchange latex over a cation-exchange resin Linear from 5 to 200 ng of As	As (193.7)	42
ETA-AAS, see ref. 42	HPLC column and conditions same as ref. 41	Arsenical residues, DMA, MMA, As^{III} and As^{V} in soils	Extraction and extensive clean-up procedure is given	As (193.7)	36

Table 2—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ETA-AAS using Zeeman-effect background correction. Dry at 80 °C for 20 s; ash at 370 °C for 10 s; atomise at 2300 °C for 5 s	25 cm LiChrosorb 10 µm C-18 ODS column. Eluent 0.5 ml min^{-1}, 80 + 20 MeOH - H_2O for 28 min, followed by a step gradient to 100% MeOH	Tetraalkyllead compounds	Addition of iodine found to enhance signal and precision	Pb	33
Dry at 80 °C for 20 s; ash at 400 °C for 10 s; atomise at 2300 °C for 5 s	Same column. Eluent MeOH - H_2O (97.5 + 2.5) isocratic at 0.1 ml min^{-1}	Organotin compounds	Increased signal and precision found when Zr-coated graphite cuvettes were used	Sn (224.6)	
ETA-AAS, see ref. 42	8% cross-linked quaternary ammonium type anion-exchange resin (Bio-Rad Aminex A-27) column with solvent program from H_2O to 0.2 M $(NH_4)_2CO_3$	As compounds in air, water and soil samples	The effluent stream was sampled every 43 s during the 45 min analysis time	As	38
ETA-AAS. Dry at 100 °C for 10 s; ash at 200 °C for 10 s; atomise at 2700 °C for 10 s	Column 300 × 7.8 mm o.d. µStyragel (10 µm). Mobile phase, THF. Flow-rate 1.0 and 0.5 ml min^{-1}	Triorganotin copolymers	Aliquots of 20 µl withdrawn at 49 s intervals. At 1.0 ml min^{-1}, 2.43% of total effluent sampled	Sn (224.6)	39
ETA-AAS, 10–100 µl injections	35 × 1 cm i.d. column 9 cm AG 50W-XB (100–120 mesh) cation-exchange resin, 26 cm AG 1-X8 (100–120 mesh) anion-exchange resin	As^{III}, As^{V}, MMA, and DMA in arsenic contaminated sediment interstitial water, up to 2 ml injected	The separated As species were collected in fractions from which injections were made into furnace	As	44
	Column conditioned with 50 µg of each arsenic species		Detection limit 10 p.p.b. in original sample		
ETA-AAS using a fraction collector as interface. Dry at 100 °C for 30 s; char at 1300 °C 30 s; atomise at 2700 °C for 10 s. 20 µl injections	25 cm × 2.6 mm, ODS-HC Sil-X-I. Eluent: either gradient from 50% to 100% MeOH in 25 min or 20% MeOH for 10 min then gradient to 100% in 30 min	Organophosphorus compounds in lubricating oil	Chromatographic analysis time = 25–40 min, whereas ETA-AAS analysis time = 110–120 min. Detection limit of 0.3 mg l^{-1}	P (213.6)	45
ETA-AAS, see refs. 27, 34, 35	See ref. 42	Inorganic and organo-arsenic compounds in oil shale retort and process waters	Compounds found were: arsenite, arsenate, methylarsonic acid, phenylarsonic acid, along with one unidentified compound	As (193.7)	46
ETA-AAS using fraction collector as interface with manual injections	Anion-exchange resin Dowex 1-X4, 200–400 mesh in acetate form, 115 mm × 10 mm. Eluent: 0.1% acetic acid for 65 min, 5% acetic acid for 130 min then 1 M HCl for 65 min. Flow-rate, 20 drops min^{-1}	Separation of DMA, MMA and As^{III} - As^{V}. As^{III} levels found separately in soil polluted with As	Extraction procedure given for soils. The chromatographic separation does not speciate As^{III} and As^{V}	As (193.7)	47
ETA-AAS rapid-scan UV - visible detector connected to furnace via autosampler	Reversed-phase HPLC - ETA-AAS ODS C-18 column (Altex 6 mm i.d. × 250 mm length) with guard column (Waters 3.2 mm i.d × 40 mm length). Size exclusion chromatography using a series combination of 50/100/1000 Å µSpherogel column (Altex, 8.0 mm i.d. × 300 mm length) with swollen divinylbenzene as the packing	Vanadyl and nickel compounds in heavy crude petroleums and asphaltenes	Spectra from the rapid-scanning detector were stored by microcomputer. ETA-AAS histogram data were recorded by both strip-chart recorder and digital integrator	V (318.4) Ni (232.0)	49, 50

Table 2—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ETA-AAS with micro-processor-controlled interface based on previous design. See ref. 34	Either single column, Hypersil 5 μm ODS, (250 mm × 5 mm i.d.) or 5 μm ODS and one Hamilton 10 μm PRP 1 (250 mm × 5 mm i.d.) in series in a constant temperature (28.5 °C) enclosure	Determination of organocopper species in soil pore waters	Injector consists of a pneumatic slider injection valve and a solenoid-controlled stainless-steel syringe needle. N_2 used to deliver the sample via sample loop, through syringe needle into the cuvette. Injection sequence, valve operation and activation of solenoid controlled by microcomputer	Cu	52
ETA-AAS using Zeeman-effect background correction. Conductivity detector in series with automated interface based on ref. 34	Dionex system consisting of 50 × 3 mm anion pre-column (Dionex 30008), 150 × 3 mm anion separator column (Dionex 30589) and a 250 × 3 mm anion suppressor column (Dionex 30066), in series. Mobile phase 0.0080 M Na_2CO_3 at 0.46 ml min^{-1}	Distilled water, synthetic river water and Texas river water spiked with selenite and selenate	Dionex Model 16 ion chromatograph used. Detection limit 20 ng Se for each selenium compound. Pre-concentration from a max. of 4 ml of an anion-rich water sample extends detection limit to 5 ng of Se	Se	53

prior to atomisation makes it practically impossible to couple the eluent from a chromatograph directly to a furnace. Thus various indirect couplings have been used to overcome this problem, and these are summarised in Table 2.

Brinckman's group, at the National Bureau of Standards, developed two such indirect couplings.[27] The first utilised a PTFE flow-through cell from which the eluent was periodically sampled and injected into a graphite furnace, the so-called pulsed-mode operation. In the second, termed survey-mode, the eluent was collected by an auto-sampler and each collected fraction analysed by ETA-AAS. These two sampling modes were demonstrated for the speciation of various Sn, Hg, As and Pb compounds[27]; the detection limits quoted were not evaluated by any conventional method and should therefore be treated with care. The survey mode of operation was also used for the speciation of organometallic polymers and organotin and silicates by the same group.[28]

Koizumi *et al.*[29] used HPLC - Zeeman ETA-AAS for the speciation of tetraalkyllead compounds in gasoline. The eluent was sampled every 250 μl while the flow was stopped and the sample vaporised in a high temperature furnace. The interference caused by background absorption was avoided by using Zeeman-effect background correction. Vickrey's group also used Zeeman-effect background correction in their couplings.[30–32] They described an interface device, which consisted of a sampling valve, timing circuit and automatic co-analyte addition, in this instance nickel ions[30] for selenium speciation. This interface was later controlled by a microprocessor[32] and 37 μl samples were injected into the furnace from each 100 or 220 μl of eluent. They also used stream splitting of chromatographic peaks[31] prior to atomisation for the speciation of tetraphenyllead and pulsed-mode operation for the speciation of Cr^{III} and Cr^{VI},[31] where the eluent was sampled every 30 or 120 s. The coupling was also used for tetraalkyllead and organotin speciation.[33] With the former, the addition of iodine prior to atomisation was found to enhance both the signal and the precision. A similar effect was found by using zirconium-coated cuvettes in the speciation of organo-tin compounds.[33] Irgolic's group at the same institution used a similar automated interface for the speciation of arsenobetaine, arsenocholine and inorganic arsenic at the microgram level.[34] In a joint study, Brinckman's and Irgolic's groups[35] demonstrated various chromatographic separations for the speciation of arsenic compounds in soil and water samples. The extremely high background molecular absorption levels encountered when ion-pair reagents, such as tetraheptylammonium nitrate (THAN), were used required Zeeman-effect background correction, as normal deuterium arc correction proved insufficient.

Workers at the US Department of Agriculture[17,36] utilised a flow-through PTFE sampling cup as an interface between a low-capacity anion-exchange column and a graphite furnace. They speciated organic and inorganic reducible forms of arsenic in pesticide residues and gave details of a clean-up procedure for use in the speciation of soil arsenical residues by the same procedure.[36] This flow-through PTFE sampling cup is now commercially available and a data sheet is available on its application to arsenic speciation studies.[37]

Another indirect form of coupling was utilised by Burns *et al.*[40] and Ricci *et al.*,[41] namely, hydride generation prior to atomisation. In their comprehensive study of organotin compounds, the former group[40] found a 1000-fold increase in the response to tin when using hydride generation followed by ETA as opposed to coupling the eluent directly to the nebuliser for flame atomisation. The speciation of reducible forms of arsenic was achieved by Ricci *et al.*,[41] using hydride generation prior to atomisation by a heated quartz tube. The use of hydride generation circumvents the problems of low nebulisation efficiency normally encountered with FAAS, thus enabling sensitive detection along with "real time" detection.

Recent reports of coupled LC - graphite furnace systems have either reported dual detector systems or emphasised the use of microprocessors. Fish *et al.*[49–51] have used ETA-AAS in conjunction with a rapid-scan UV-visible detector to investigate vanadyl and nickel compounds in heavy crude petroleum and asphaltenes, although here the sample was loaded into the furnace in an auto-sampler.

5. Flame Atomic Fluorescence Spectrometry

The advantages of AFS as a chromatographic detector have been extolled by Van Loon *et al.*[55] as offering simultaneous multi-element detection with greater sensitivity than AAS.

Table 3. Coupled liquid chromatography - flame atomic fluorescence spectrometry

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
FAFS using a N_2 shielded circular air - C_2H_2 flame, eluent passed directly into nebuliser	10 cm × 1 cm column, 80 mesh Chelex 100 washed with HCl (40 ml) and water (40 ml) at 1 ml min^{-1}. Eluent H_2O (pH 6) for 4 min then 2 M HNO_3	Speciation of Cr^{III}, Cr^{VI}, Ag^{I}, Mn^{II} and Mn^{VII} in standards and synthetic sea water	In the sea water a scattering peak, due to NaCl, appears well before Cr^{III}, Mn^{II} or Ag^{I} elutes	Cr Mn Ag	56
As above	Partisil-10 SCX column at 55 °C. Eluent water until first peak eluted then a 5 min convex gradient to 100% 1 M NH_4NO_3 at 4.0 ml min^{-1}	Separation of Cu, Ni and Zn EDTA, trien and glycine complexes	The glycine and EDTA complexes have almost identical retention times, however multi-element AFS gives excellent resolution	Cu Ni Zn	57
FAFS using air - C_2H_2 capillary tube burner, Ar shielded, Xe continuum lamp sources, direct coupling to nebuliser	50 cm × 2 mm Chromosep S column packed with pellicular 10 µm silica gel. Eluent diethyl ether - methanol (40 + 1) at 0.5–2.0 ml min^{-1}	Investigation of acetylation reaction of ferrocene by acetic anhydride	Progress of reaction more specifically followed using AFS than using normal UV detection	Fe (283.3 and 252.2)	58
FAFS; see ref. 56	6 mm column of XAD-2 resin. Various elution systems used	Study of absorption of trace metals on Amberlite resins	Metals are not desorbed by MeOH but by methanolic HCl, methanolic NH_3 and Na_2H_2EDTA. Linear up to 1 mg l^{-1} of Cu, 1.6 mg l^{-1} of Fe and 0.6 mg l^{-1} of Zn	Cu Fe Mg Zn	59

Table 4. Coupled liquid chromatography - direct current plasma atomic emission spectrometry

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
DCP, using Spectraspan III instrument	250 × 4 mm i.d., 8 µm Spherisorb SEP. Eluent 5 : 15 : 80 acetonitrile, diethyl ether and Skelly B at 2.2 ml min^{-1}. Column washed prior to use with 0.5% pyridine in Skelly B	Separation of metal diethyldithiocarbamates	DCP detector in series with UV detector used to confirm metal content of eluted peaks. Linear from 5 to 500 ng Co and from 10 to 500 ng Cu	Co Ni Cu	67
DCP (Spectraspan III) For reversed-phase and ion-exchange chromatography, eluent passed directly into standard nebuliser system	250 mm Partisil ODS column. Eluent H_2O - acetonitrile (60 + 40) at 0.65 ml min^{-1}. 250 mm, 10 µm Partisil 10 silica. Eluent 8% CH_2Cl_2 in Skelly-solve B	Speciation of $Cu(enAA_2)$, $Cu(enTFA_2)$ and their Ni analogues. $Cr(HFA)_3$ and various mixed-ligand chelates formed by reaction of Cr with TFA and HFA	Nebulisation of eluents used for adsorption chromatography caused rapid C build-up and thus required a new design of of nebuliser. Eluent was directed at chamber wall in a fine jet and resulting mist swept into plasma.	Cu (324.7) Ni (341.5) Cr (267.7)	68
	250 mm, 8 µm Spherisorb, eluent: 5 + 20 + 75 acetonitrile - diethyl-ether - Skelly-solve B	$Hg(DEDTC)_2$, $Cr(DEDTC)_2$	Nebulisation efficiency of 20–25% was attained with no C build-up over 10 h period. Linear from 30 to 4000 ng Cu and from 60 ng to 2.5 µg Cr	Hg (253.7) Cr (267.7)	
DCP plasma, same interface for hydrocarbon eluents as ref. 68	300 × 4 mm i.d., 10 µm Partisil silica. Eluent 6% acetonitrile in CH_2Cl_2, 1.5 ml min^{-1}	Speciation of mer and fac isomers of $Co(BAA)_3$ and $Co(PAM)_3$	Detection limit of 100 ng for Cr	Cr (267.7)	69
	8% CH_2Cl_2 in hexane	Mixed ligand complexes of $Cr(HFA)_n$ - $(TFA)_{3-n}$ and the mer/fac isomers of Cr - $(TFA)_3$			
	Concave gradient of 3–20% CH_2Cl_2 in hexane	As for above only better peak shape and shorter analysis time achieved			

Table 4—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
DCP using cross-flow nebuliser with direct introduction of eluent	500 mm × 5 mm, Sephadex 10 column. Eluent H_2O at 2.0 ml min^{-1}	Separation of Cd sulphate, bromide and acetate	Examination of nebulisation parameters concerned with coupling reported	Cd (228.8)	70
DCP (SpectraSpan IIIB). The end of the analytical column was attached to the DCP via a short length of stainless-steel tubing connected to a section of flexible plastic inlet tubing	The HPLC separations of the two Cr ions was achieved using paired ion, reversed-phase conditions, with either a tetrabutylammonium counterion or a camphor sulphonate counter-ion in solution	Speciation of Cr^{VI} and and Cr^{III} in various water, biological and tannery samples	The order of elution of the two Cr ions is completely reversed going from one counter ion to the other in the mobile phase Detection limits for both Cr species in the range 5–10 p.p.b. with at least 2–4 orders of magnitude linearity in the calibration graphs	Cr (425.4)	73
	The analytical columns used were as follows: 1, 5 µm, 15 cm × 4.6 mm i.d. C_{18} Resolv column (Waters); 2, 5 µm, 15 cm × 4.6 mm i.d., C_{18} Altex column (Altex/Beckman); 3, 10 µm, 25 cm × 4.6 mm i.d., C_{18} column (Altex) and 4, 10 µm, 25 cm × 4.6 mm i.d., C_8 column (Altex)				
On-line detection with SpectraSpan IIIB DCP. Outlet of column connected directly to the cross-flow nebuliser	Separation by gradient elution HPLC, using either a Du Pont Zorbax ODS (4.6 mm × 250 mm) column or a Hamilton PRP-1 (4.1 mm × 150 mm) column previously equilibrated with the tetra-alkylammonium ion selected for use	Polyphosphate oligomers	Linear oligomers ranging from P_1 (orthophosphate) to P_{12} can be observed in neutralised polyphosphate samples. A detection limit of 0.2 µg of P is observed with the 214.9 nm emission line. Precision for each of the major oligomers (P_1–P_{10}) is in the 1–5% RSD range	P (214.9, 213.6)	71
SpectraSpan IV three electrode direct current argon plasma equipped with an échelle grating spectrometer, and ceramic cross-flow nebuliser fitted with a PTFE collar to a modified 10-ml Pyrex round-bottomed flask spray chamber	Silica 5.0 µm column (250 mm × 4.5 mm i.d.) Mobile phase 100% CH_2Cl_2 at 1.0 ml min^{-1}	Various aqueous and organic solvent systems Copper hexafluoroacetyl-acetonate	Paper based on the design and characterisation of a nebuliser - spray chamber interface. The entire interface was situated in the SpectraSpan IV so that the chimney tip was 15 mm below the plasma excitation region, placing the solvent - analyte spray in the same vertical plane as the plasma	Cu (324.7)	74
DCP (SpectraSpan IIIB). Wavelength scan achieved using a DBC-33 system (Spectrametrics)	Protein fractionation carried out on a 2.6 × 100 cm column packed with Sephacryl S-300	Protein-bound metals in serum and intravenous infusion fluids	Detection limits: Cu, 3.2 µg l^{-1}; Fe, 3.9 µg l^{-1}; and Zn, 9.3 µg l^{-1}	Cu (324.7) Fe (373.4) Zn (213.8)	75
DCP (SpectraSpan IIIB)	15 or 25 cm × 4.1 mm i.d., 10 µm PRP-1 (Hamilton) column. Various mobile phases used	Speciation of methylated organotins in water samples, tuna fish and various food products	On-line hydride generation used	Sn (303.4)	76

Such detectors have been utilised a little more with LC (Table 3) than with GC. Van Loon's own group have used non-dispersive simultaneous multi-element FAFS for the speciation of Cr^{III}, Ag^{I}, Mn^{II} and Mn^{VII} in synthetic sea water.[56] Excellent resolution was demonstrated for a mixed solution (10 mg l^{-1} of each species, 10-ml injection); however, the high sensitivity of AFS was not tested. This group also demonstrated the multi-element capability of coupled LC - FAFS for the speciation of Cu, Ni and Zn amino acids and amino carboxylic acids.[57] Unfortunately no mention of the metal concentrations was made.

Siemer *et al.*[58] reported the use of continuum source FAFS in the study of the acetylation reaction of ferrocene by acetic anhydride. They found it much easier to follow the reaction by Fe-specific detection than by conventional UV detection. Mackey,[59] in a study of the interactions of simple cations (Cu, Fe and Zn) with macroreticular resins, used multichannel FAFS but quantified the results by batch measurements using graphite furnace AAS. The LC - FAFS system was found to be linear up to 1.0, 1.6 and 0.6 mg l^{-1} for Cu, Fe and Zn, respectively; deviation from linearity was said to occur at 20 times the detection limit as defined by Larkins.[60]

Clearly the availability of intense, stable line sources that encourage the greater use of AFS and perhaps the advantages outlined by Van Loon would be appreciated by more workers.

6. Atomic Emission Spectrometry

The relatively low excitation temperature of the various atomic spectroscopic flames limits their usefulness as atom cells for coupled LC - AES applications. Flames have been used in various configurations as molecular emission detectors; for example, McGuffin and Novotny[61] monitored HPO bands for phosphorus-selective detection of various compounds eluting from a microbore LC column. Similarly Cope and Townshend[62] have used a phosphorus-sensitive molecular emission cavity analysis (MECA) detector as a detector for HPLC.

The low neutral temperature of the microwave induced plasma (MIP) makes it very sensitive to large solvent flow-rates. Although several workers have devised continuous nebulisation systems[63–66] for this type of plasma, it has proved singularly unpopular in LC applications. In contrast, both the direct current plasma (DCP) and inductively coupled plasma (ICP), with their ability to withstand both organic and aqueous solvent flows, have found various applications as LC detectors.

Direct Current Plasma

The group at Amherst, Massachusetts, have been one of the main exponents of coupled LC - DCP-AES (Table 4), using both two and three electrode plasmas.[65–68] They found[68] that the standard nebulisation arrangement was sufficient for eluents used in ion-exchange and reversed-phase chromatography, but when used in conjunction with the organic solvents used for adsorption chromatography a rapid build-up of carbon resulted. Thus they designed a novel nebuliser, which had an efficiency of 20–25% and could be run continuously for up to 10 h with no carbon deposits forming. The couplings were used in the speciation of diethyldithiocarbamate complexes of Co, Cu and Ni,[67] and Hg and Cr.[68] The study of mixed ligand complexes of the type $Cr(HFA)_n$ - $(TFA)_{3-n}$, for n = 0, 1, 2 and 3,[69] was aided by the metal specific detection afforded by the coupled system.

Koropchak and Coleman[70] used a cross-flow nebuliser in their LC - DCP coupling. They studied nebulisation parameters in order to optimise the plasma detection capabilities when interfaced to a liquid chromatograph. They demonstrated its capability in the speciation of three cadmium salts; however, the hope that the DCP could provide sensitive specific detection for the halogens was not realised.

The group at the University of Massachusetts[71] have also studied the characteristics of the nebuliser - spray chamber interface. They again used a cross-flow nebuliser but fitted a modified spray chamber and located the entire interface directly below the excitation region with the solvent - analyte spray in the same vertical plane as the plasma. The performance of this arrangement was investigated using a range of aqueous and organic solvents containing copper hexafluoroacetyl acetonate and advantages were demonstrated.

Other workers using coupled LC - DCP have paid more attention to the chromatography using direct connection of the column to the nebuliser. Krull *et al.*[71,73] have studied the speciation of chromium in various water, biological and tannery samples, reporting detection limits of 5–10 p.p.b. with at least 3–4 orders of magnitude linearity in the calibration plots. Chromatographic details were given for a number of columns used in the investigations.

The well known tolerance of the DCP to a wide variety of different solvents obviously aids its adaptation as an LC detector. Given the multi-element capability of the DCP it is easy to see why it is gaining in popularity as an LC detector.

Inductively Coupled Plasma

The coupling of LC with ICP-AES (Table 5) is normally directly through standard nebuliser arrangements. Browner *et al.*[77] considered the effect of nebulisation chamber position using both Meinhard[78] and fixed cross-flow[79] nebulisers for LC - ICP couplings. Although they only studied aqueous eluents, they found that peak broadening and distortion occurred when the chamber was placed inside the ICP gas box, owing to extended liquid transport. If, however, the chamber is sited outside the gas box, then a loss in signal commensurate with aerosol transport over an equivalent distance occurred. Thus it can be seen once again that the dead volume in any LC detector coupling must be kept to a minimum.

Fraley *et al.*[80,81] built on their experience with hybrid techniques and compared FAAS and ICP-AES as HPLC detectors for the speciation of copper aminocarboxylic acid chelates.[80] Both techniques were found to yield a similar response; however, the multi-element facility of ICP-AES was demonstrated using a dummy column to simulate chromatographic conditions. The simultaneous detection of Ca, Cu, Mg and Zn aminocarboxylic acid chelates with linearity up to 1 µg[81] illustrates another advantage of AES over AAS, *i.e.*, long linear calibrations.

Gast *et al.*[82] demonstrated a coupling using a fixed cross-flow nebuliser for the speciation of carbonyl complexes of Fe and Mo, various forms of As, dialkylmercury compounds, tetraalkyllead compounds and various ferrocene derivatives. The ICP-AES detector was evaluated by injecting small samples into the nebuliser. They studied the effect of solvent composition and determined both linear ranges and detection limits by this method. Morita *et al.*[83] used direct sampling of the eluent to the nebuliser for the determination of Co : P : C ratios in vitamin B_{12} and also in the simultaneous multi-element detection of various proteins. The same group also determined arsenic compounds in biological samples using this technique,[84] as did Kurosawa *et al.*[85] for the unequivocal identification of arsenobetaine in shark liver and muscle. Arsenic speciation studies have also been made by Bushee *et al.*[86] using on-line hydride generation.

Hausler and Taylor[87,88] used ICP-AES in conjunction with size-exclusion chromatography and evaluated a number of spray chamber designs. Using toluene as eluent[87] it was found that cooling the chamber to 0 °C resulted in a better sensitivity being obtained. This evaluation, along with the determination of detection limits, was performed in the absence of the chromatographic column. When pyridine was used in the eluent,[88] the best sensitivity was achieved with the chamber thermostated at 20 °C. Detection limits, found by the same

Table 5. Coupled liquid chromatography - inductively coupled plasma atomic emission spectrometry

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ICP, eluent from column passed directly into nebuliser. All-Ar plasma. For FAAS work air - C_2H_2 flame used	Aminex A-14 column; see ref. 15	Separation of EDTA and NTA chelates	Compared with FAAS detection for Cu chelates and found both gave similar response. Also used dummy column to simulate chromatography for various metals	Cu (324.7)	80
ICP all-Ar plasma, outlet of column connected by capillary PTFE tubing to nebuliser of cross-flow design	250 × 4.6 mm i.d. Zorbax-C8 column, eluent: 70% *V/V* ethanol Eluent: 5 min linear gradient of 50–55% ethanol then 5 min linear gradient up to 80% EtOH, 1 ml min^{-1}, 20 µl injection	Separation of iron carbonyl complexes Separation of various molybdenum carbonyl complexes	ICP was tested as an HPLC detector by injecting small samples through an injector into the nebuliser, to evaluate effect of various solvents, sensitivity, linearity and detection limits	Fe (259.94) Mo (281.615)	82
	Hypersil (6 µm), 100 × 4.6 mm i.d. Eluent 30% MeOH, 1% *m/m* n-hexadecyltrimethyl-ammonium bromide, 0.08 M, pH 5, at 1.2 ml min^{-1}	Separation of DMA, MMA, *p*-APA, As^{V}, phenylarsonic acid		As (278.022)	82
	Eluent EtOH - 0.05 M NaBr (1 + 2), pH 3, 1.2 ml min^{-1}	Separation of Hg^{II} methylmercury, ethylmercury and propylmercury		Hg (253.652)	
	Eluent 75% EtOH 1.4 ml min^{-1}. 30 µl injection	Tetraalkyllead compounds in petrol		Pb (283.306)	
Ar ICP, see ref. 87. Spray chamber thermostated to 20 °C	100-Å µStyragel Waters column. Eluent pyridine at 0.5 or 1.0 ml min^{-1} or toluene at same flow-rate	Separation of a 21-element standard (see ref. 87), ferrocene and derivatives, copper and cobalt complexes and organically bound metals in solvent-refined coal	Detection limits in pyridine, determined by same method as ref. 87, are generally slightly worse than those found using toluene	See ref. 87	88
Ar ICP, eluent taken from UV detector directly to cross-flow nebuliser. 32-element polychromator used for simultaneous detection, or monochromator for single channel operation	250 × 1.6 mm i.d., AG 1-X4 (<400 mesh) anion-exchange resin. Eluent 0.05 M $(NH_4)_2SO_4$	Separation of NTA and EDTA chelates of Cu, Zn, Ca and Mg	The data acquisition storage and output is microprocessor-controlled. Linear up to 1 µg for all elements	Cu Ca Mg Zn	81
Ar plasma, see ref. 80	600 × 7.5 i.d., TSK 3000 SW size exclusion column, or a 500 × 7.5 mm i.d., TSK 2000 SW column. Eluent H_2O at 1.0 or 1.5 ml min^{-1}	Speciation of dissolved Ca and Mg in natural water filtrates	By using UV detection and ICP-AES, inference as to the organic binding made	Ca Mg	89
ICP, the eluent from the the column being fed to a cross-flow nebuliser via PTFE tubing (0.5 mm i.d. × 300 mm long)	Strong cation-exchange resin (UIPX-210 SC from Toyo Soda, Japan). Step gradient elution trom 0.2 M NaH_2PO_4, pH 0.2, to 0.2 M NaH_2PO_4, pH 4.3, column temperature 0 °C	Amino acids	In the detection of sulphur, a simple Ar purge system was used to reduce light absorption by oxygen. Detection limits of 30–50 µg ml^{-1} and 1–3 µg ml^{-1} as amino acids were obtained for carbon and sulphur, respectively	C (193.09) S (180.73)	90

Table 5—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ICP, the HPLC column being connected to a Meinhard concentric nebuliser rated at 3 ml min^{-1} by 2 ft of 1/16 in o.d., 3/64 in i.d. PTFE tubing	Hamilton PRP-1 resin-based, reversed-phase column	Various arsenic, selenium and phosphorus compounds	The standard software was modified to allow the chromatogram to be displayed graphically on-line. Detection limit for As 130 μg l^{-1} at 100 μl injection volumes	As (189.0) C (247.8) P (214.9) S (180.7) Se (203.9)	91
ICP, the eluent from the micro-HPLC is carried into the nebuliser using either water or methyl isobutyl ketone as carrier, via a simple or modified stainless T-type connector. Series UV detection also used	Micro-HPLC. The columns were PTFE tubing of 0.5 mm i.d. by 12 cm length packed with Jasco SC-01 (ODS-silica, 5 μm) for reversed-phase mode, and PTFE tubing 0.5 mm i.d. by 15 cm length packed with Jasco Fine Sil-5 (silica, 5 μm) for normal-phase mode. Mobile phases were MeOH or MeOH - H_2O mixture for reversed-phase mode and toluene for normal-phase mode	Cu, Zn, Fe and Co organometallic compounds	Application of micro HPLC with simple interface for LC - ICP previously reported (ref. 93)	Cu (324.7) Zn (213.8) Co (228.6) Fe (259.9)	54
	250 × 3 mm i.d., silica gel Si60 (8 μm). Eluent toluene, 1.4 ml min^{-1}	Separation of various ferrocene compounds		Fe (259.94)	82
All-Ar ICP, eluent passed directly into nebuliser	600 × 2 mm, TSK GEL 3000 SW. Eluent 0.9% NaCl, 1.0 ml min^{-1}	Separation of vitamin B_{12}. Separation of various proteins; ferritin, catalase, aldolase, albumin, cytochrome C and chymotrypsinogen A	Multi-element detection used to calculate Co : P : C ratio. Simultaneous multi-element detection of Cu, Fe, Mn, P and Zn	C (246.7) Co (228.6) Cu (324.9) Fe (259.9) Mn (257.6) P (241.9) Zn (213.8)	83
All-Ar ICP, eluent passed directly into nebuliser	Either: Nagel-Nucleosil 10-SA cation- or 10-SB anion-exchange resin. Eluent 0.25 M phosphate buffer, pH 7.4	Identification of arsenobetaine in shark muscle and liver by comparison with standard chromatogram of arsenobetaine, DMA, MMA, As^{III} and As^{V}	Arsenobetaine matched, on both resins, the main As compound found in the shark tissues	As (193.7)	87

Table 5—*continued*

Detector	Chromatography	Sample	Comments	Element (wavelength/nm)	Reference
ICP, all-Ar plasma, eluent passed directly to nebuliser; various spray chamber designs evaluated with and without cooling to 0 °C	100-Å Styragel Waters column at a flow-rate of 1.0 or 0.5 ml min^{-1} of toluene. Bio-Beads SX-2 size exclusion column. Eluent toluene at same flow-rates, 200 µl injected	Separation of various Si, Pb, Sn and Ge organometallic compounds. Separation of of a 21-element standard, metal salts of dialkylbenzene sulphonates, in an organic matrix	Various spray chambers and detection limits were evaluated without the chromatographic column being used. Detection levels are comparable to those found for aqueous solutions	Al, Ag, Ba, Cd, Cu, Fe, Mg, Mn, Ni, Pb, Si, Sn, Ti, V and Zn	
ICP, eluent from the column fed to a cross-flow nebuliser using directly coupled PTFE tubing (0.5 mm i.d. × 300 nm long)	Strong cation-exchange resin (IEX-2 10 SC - Toyo Soda, Japan) 250 mm × 4 mm i.d. at 50 °C. Mobile phase 0.4–1.0 M ammonium lactate (pH 4.22)	Rare earth elements in geological samples	Similar interface to ref. 90. Detection limits for system 0.001–0.3 µg ml^{-1} with 100 µl sample injection	Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu	97
ICP using a micro-centric nebuliser, which is inserted directly into the tip of a conventional sample introduction tube of an ICP torch	Ion-pairing, reversed-phase separation. Microbore 1 mm i.d. × 50 cm C_{18} column (HRSM-50-C_{18}, C-M Laboratories, Nutley, NJ). Mobile phase: Cr, 5 mM sodium pentane-sulphonate in MeOH - H_2O (20 + 80), pH 3 at 120 µl min^{-1}; As, 5 mM tetrabutyl-ammonium phosphate in MeOH - H_2O (5 + 95), pH 7.2 at 140 µl min^{-1}	Inorganic and organo-metallic compounds in various solvents	First departure from using conventional cross-flow, concentric or Babington-type pneumatic nebulisers. At present some problems with low residence times of analyte species in the plasma and possible solvent interactions in the excitation process thus impairing detection limits	Cr (205.6) As (193.7)	98
ICP, with cross-flow nebuliser attached directly to the micro-column by PTFE tubing. The sample gas carries the eluent as a fog into the plasma torch	Microcolumn gel permeation chromato-graphy. Column made of Teflon tubing 1 mm i.d. × 20 cm packed with Fine GEL SC-220 (11.7 µm, Jasco, Japan). Mobile phase is distilled water	Analysis of carbon-containing materials, example of saccharides	Comparison made with previously reported system using various T-type connectors in the interface, ref. 4	C	96
ICP, with cross-flow nebuliser. Interface arrangement as in ref. 97	Anion-exchange resin IEX-260-SA-SIL at 60 °C. Mobile phase 0.1–0.73 M $HCOONH_4$, pH 3.0	Determination of 12 common 5′-ribo-nucleotides	A UV detector was used in series (260 nm) The twelve nucleotides were separated using a 2 h gradient elution	P (213.6)	99

procedure as above, were slightly worse than those obtained with toluene. Gardner *et al.*[89] used ICP-AES in series with UV detection to monitor the speciation of Ca and Mg in natural water filtrates. If both detectors gave a response, the tenuous suggestion that the metal was organically bound was made. This example illustrates one of the main advantages of hybrid chromatographic techniques, *i.e.*, they provide unambiguous identification of a metal. The most definite conclusion from the chromatographic data obtained[89] was that a species contained Ca, Mg, or neither; the nature of the organic moiety remained speculative.

Recent papers on coupled LC - ICP have shown a tendency to move towards the use of microbore HPLC. The group at Toyohashi University of Technology have used micro-columns in their studies on various interface couplings[92–96] utilised for the determination of Cu, Zn, Fe and Co in organometallic compounds and the analysis of carbon-containing materials such as saccharides. Lawrence *et al.*[98] have also used microbore columns, although they have in addition reported the first departure from using conventional cross-flow, concentric or Babington-type pneumatic nebulisers and have developed a microconcentric nebuliser, which is inserted directly into the tip of a conventional sample introduction tube of an ICP torch. However, at the present time problems with low residence times of the analyte species in the plasma and possible solvent interaction in the excitation process have been reported to impair detection limits.

Whereas the exploitation of the ICP as an LC detector is undoubtedly limited by the availability and high cost of instrumentation, it offers major advantages in terms of multi-element detection, linear working range, versatility (metals and non-metals can be determined) and sensitivity.

7. Conclusion

Atomic absorption, although the most inherently metal-specific of the atomic spectroscopic techniques, introduces restrictions to the potential couplings available with liquid chromatography. In LC - FAAS using reversed-phase systems, *i.e.*, mainly aqueous eluents, low nebulisation efficiency may limit the sensitivity of the technique. However, operation of the nebuliser in a starved mode, for example by using modified injection cup devices, has been shown to alleviate much of this problem. When normal phase, *i.e.*, organic, eluents are used, then higher nebulisation efficiencies are possible; however, transport of large amounts of organic solvents to the flame can have adverse effects on its properties, *e.g.*, increased background levels from carbon particles and band spectra. Therefore, sample transport systems are being developed in our laboratory that do not use

conventional nebulisation. As such systems allow the sample to be desolvated prior to reaching the flame for atomisation, they offer much promise for the future. The advantages of directly coupled LC - FAAS systems that offer on-line, real-time analysis with simple, cheap, readily demountable interface systems largely offset the lower detection limits obtained compared with electrothermal atomisation.

The use of electrothermal atomisation should circumvent the problem of low nebulisation efficiency; however, the time required to run through an atomiser dry - ash - atomise - cool cycle results in only infrequent samples being analysed out of the flowing chromatographic stream. In order to minimise the possibility of missing a species, very low flow-rates are normally used, although much of the eluent is still not monitored. To overcome this problem in direct interfaces, devices such as autosampler systems are often required and "real time" chromatographic interpretation is not possible. This is a major disadvantage when optimisation of the chromatography is required. Thus the advantage of high sensitivity detection obtained when using electrothermal atomisation is only achieved at the expense of real-time analysis, and often involves expensive and complicated interface systems. The advent of microbore HPLC may provide some solution to problems of interfacing with electrothermal atomisers. The low flow-rates ($\mu l\ min^{-1}$) encountered in microbore HPLC mean that the volume containing a species is very small and providing the peak resolution is good, injection into the furnace of the whole chromatographic peak may be feasible. Another possible, though expensive, way of making coupled HPLC - ETA-AAS a real-time method would be to use a dual furnace system.

The same problems beset coupled LC - AFS as afflict any AFS method, namely, a lack of suitably stable and intense line sources. However, the availability of atomic fluorescence instruments using an ICP as the atom cell will perhaps encourage a renewed interest in this technique and enable the advantages of multi-element low level detection afforded by AFS to be utilised.

The plasma emission techniques offer the possibility of multi-element detection and long linear ranges. With reversed-phase eluents, both DCP-and ICP-AES, like FAAS, suffer from low nebulisation efficiencies and to increase detectability this efficiency must be increased. The use of normal phase eluents affords high nebulisation efficiencies but, as a result, a higher background emission level, and hence an increase in detection limits. This may be offset by the increased analyte flow into the plasma. The ability of plasmas to monitor not only metal emission lines, but also carbon lines, could, so long as non-carbon containing eluents are used, offer a universal LC detector. Although emphasis has been placed on the advantages of specific or at least selective detection, the value of a universal detector should not be underestimated, as the wide usage of FID in GC shows. By using a simultaneous multi-element facility, DCP- or ICP-AES could offer such universality.

Recent emphasis on studies into characterising the processes that take place within an interface, *i.e.*, within the nebuliser - spray chamber, connectors or even connecting tubing itself,[54] should lead to a better understanding of the ideal sample transport system and hence the development of interface techniques that fully realise the potential of coupled techniques. The trend towards microbore columns,[92–98] thus decreasing the eluent flow, also allows experimentation with low flow but high efficiency nebulisers, such as the frit nebuliser.[100]

The arrival of commercial ICP-MS systems that provide detection limits significantly lower than ICP-AES, and approaching those obtained by ETA-AAS offers an exciting new detection mode for coupled HPLC systems. ICP-MS systems operate in a real-time mode on flows of liquid similar to the elution rates from mini-bore HPLC systems. Thus several groups of workers are already experimenting with coupled HPLC - ICP-MS, which may offer the sensitivity in a multi-element mode necessary for trace metal speciation in real samples with the on-line detection capability necessary to enable optimisation of the separation and routine operation.

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Di- and Tributyltin Species in Marine and Estuarine Waters. Inter-laboratory Comparison of Two Ultratrace Analytical Methods Employing Hydride Generation and Atomic Absorption or Flame Photometric Detection

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Di- and tributyltin compounds present in marine and estuarine waters at sub-parts per billion (<μg l^{-1}) levels were determined using two different chemical speciation procedures. Generally, good analytical agreement was obtained from split samples independently analysed by a simultaneous hydride generation - dichloromethane extraction procedure followed by gas chromatographic separation and flame photometric detection (GC - FPD, performed at the National Bureau of Standards) and by a hydride generation procedure followed by purge and trap collection with boiling-point separation and atomic absorption detection (HG-AA, performed at the Naval Ocean Systems Center). Sea water samples containing tributyltin at sub-p.p.b. levels can be stored frozen (−20 °C) in polycarbonate containers for up to 2–3 months without any serious loss of analyte.

Keywords: *Antifouling marine biocides; atomic absorption and flame photometric detection; hydride generation; inter-laboratory comparisons; organotin speciation*

Organotin compounds are increasingly used industrially as catalysts, plastics stabilisers and biocides.[1] Tributyltin species are among the most effective organotin biocides and their worldwide use as active agents in antifouling coatings, especially for ships,[2] is rapidly expanding.

The increasing use of tributyltin-based antifouling coatings has raised concern about their environmental fate and the effects on non-target organisms of the toxic tributyltin species released from the coatings. Bioassays with algae,[3] oyster,[4] crab[5] and mussel larvae,[6] mysid shrimp,[7] copepods[8] and fish[9] have shown sub-lethal and lethal effects of tributyltin at parts per billion (μg l^{-1}) and lower levels in water. Consequently, some nations have issued regulations (France) or proposed regulations (UK) to control the use of tributyltin-based antifouling coatings on small craft in an attempt to protect marine life (generally shellfish) near harbours and marinas. The US Environmental Protection Agency recently initiated a special review on the environmental use of organotin compounds.[10]

In order to evaluate effectively the environmental risks associated with tributyltin biocide usage and to develop monitoring strategies, analytical methods capable of the detection and speciation of key diagnostic butyltin species in environmental waters at concentrations as low as parts per trillion (ng l^{-1}) levels must be developed and employed. The determination of the toxic tributyltin species and its less toxic[3,11,12] primary degradation product, dibutyltin, is of paramount importance. The relative abundances of these species offer clues to their persistence and toxic impacts at ambient levels in environmental systems. However, the chemical determinations of these species in natural waters are difficult and cannot be achieved by conventional total tin analysis. Only recently have methods been described for the requisite sub-μg l^{-1} speciation of butyltins in aquatic environments. These include chromatographic[13–16] or boiling-point[17–20] separation of butyltin species, followed by atomic absorption,[16–18,20] mass spectrometric,[14] or flame photometric[13,15,19] detection. Sample derivatisation by Grignard reaction[14–16] or hydride generation[13,17–20] is usually employed.

Unfortunately, there have been no standards on which to base assessments of the relative accuracy of these analytical methods. Consequently, an inaugural inter-laboratory comparison of methods for the detection of tributyltin in deionised water was organised and recently performed by the National Bureau of Standards (NBS), employing a stable, chromatographically purified aqueous tributyltin research material.[21] In general, the 35 participant laboratories performed well in determining total tin (as tributyltin) at the parts per million (mg l^{-1}) level, but speciation of the tin at sub-μg l^{-1} levels is an entirely different problem. A new intercomparison is planned using a more dilute mixed butyltin species research material.

It is also necessary to analyse butyltin species in natural water samples to determine if it is possible to compare data from different laboratories at low, ambient levels (ng l^{-1}) in marine and estuarine waters. Similarly, sample preservation procedures such as freezing must also be evaluated in order to determine their applicability for the storage and exchange of environmental samples. Such procedures were effective in the storage of sea water samples containing organoarsenic species, with respect to distribution and stability, for a period of three months at −20 °C.[22]

For these reasons, and for reporting preliminary results on concentrations of butyltin species in a variety of coastal marine and estuarine waters, the Naval Ocean Systems Center (NOSC) and the NBS undertook a joint intercomparison to measure tributyltin and its primary degradation product, dibutyltin, in shared marine and estuarine water samples collected on the east and west coasts of the USA and in England.

Experimental

Glassware or plasticware (polycarbonate) was used directly if new, or else it was leached with 10% nitric acid solutions for 8–12 h and rinsed repeatedly with de-ionised water. Samples were collected from ships or piers in new polycarbonate (8–20 l) containers. Sampling sites included the west (San Diego Bay, CA, nine sites) and east (Chesapeake Bay, MD, three sites) coasts of the USA and the east coast of England (two sites).

The sampling containers were submerged to 0.5–1.0 m depth, the caps were removed by hand, the bottles filled and the caps replaced underwater prior to removal from the water. In this manner, we avoided collecting surface microlayer films, which can contain butyltin compounds in relatively high concentrations.[13,15]

On return to the laboratory, samples were either immediately frozen (−20 °C) and shipped, or aliquots were transferred into 1-l polycarbonate containers, frozen and shipped. Prior to analysis, the samples were thawed at room temperature or by gentle warming (40 °C), but were not allowed to exceed room temperature and were analysed while still cool.

Frozen Sample Storage Evaluation

An effective sample storage procedure was considered essential to allow successful comparisons of the determination of butyltin concentrations measured in split samples, and for the development of monitoring procedures. Frozen sea water samples containing tributyltin at sub-μg l^{-1} concentrations were evaluated in order to document their long-term stability.

A large volume of sea water, circulated over panels painted with antifouling paint containing tributyltin, was collected in a 20-l polycarbonate bottle. This unfiltered source solution was poured into individual 500-ml polycarbonate bottles and frozen at −20 °C. Individual bottles were then removed at various times and analysed by hydride generation - atomic absorption (HG - AA) for tributyltin. Very little, if any, dibutyltin or monobutyltin was initially present. Subsequent analysis did not, therefore, address these species, as concentrations were very near or below the detection limits (5 ng l^{-1}).

Hydride Generation - Atomic Absorption Method

At NOSC, the HG - AA method[17,18] for producing volatile tin species with detection by modified hydrogen flame atomic absorption spectrometry was an adaptation of methods described by Braman and Tompkins[19] and Hodge *et al.*[20] Inorganic tin and organotin compounds are derivatised to stannane and the respective alkyltin hydrides by sodium borohydride before detection.

Briefly, a sample was placed into a 500-ml modified gas washing bottle and acidified to pH 5.0–5.5 with 2 M acetic acid. Hydride derivatives were formed by the addition of 4% *m/V* sodium tetrahydroborate(III) prepared in a 1% *m/V* sodium hydroxide solution in distilled water. A ratio of 1 ml of sodium tetrahydroborate(III) solution to 100 ml of sample was used to generate hydride species, which were purged from solution with helium carrier gas and trapped in a glass U-tube (2 mm i.d.) packed with 0.01–0.015 g of 3% OV-1 on Chromosorb W HP (80–100 mesh) and immersed in liquid nitrogen. Inlet and outlet lines to the U-trap and detector were made of FEP Teflon. The solution was purged for a total of 5 min after the addition of sodium tetrahydroborate(III) to ensure the maximum removal of tin hydrides from solution. The trap was then removed from the liquid nitrogen bath and the tin species were separated and detected sequentially according to their boiling-points as they distilled from the trap. The tin species were carried into a quartz tube, atomised in a hydrogen - air flame and detected by an atomic absorption spectrometer at 286.3 nm. Gas flow-rates with respect to hydrogen, air and helium were 220, 140 and 40 ml min^{-1}, respectively.

The volatilisation of tributyltin hydride (Bu_3SnH) required heating the trap in an oil-bath (180 °C). Standardisation was accomplished by the addition of an appropriate alkyltin standard (in ethanol) to the unknown, or by calibration graphs with the values calculated by peak integration. Concentrations of butyltin species are reported as ng l^{-1} of tributyltin chloride or dibutyltin dichloride.

The detection limit for dibutyltin and tributyltin hydrides was 5 ng l^{-1}. We demonstrated a relative standard deviation of 6.3% (standard deviation of a single measurement divided by the mean) for the analysis of five replicate determinations prepared in sea water at a concentration of 10 ng l^{-1} of tributyltin chloride. The redistribution of butyltin groups resulting from the sodium tetrahydroborate(III) derivatisation process has not been detected in butyltin standards analysed at NOSC. The recent analysis of a tributyltin reference material prepared in distilled water at the NBS, containing tin only as the tributyl species,[21] has shown no evidence of re-distribution.

Gas Chromatographic Separation - Flame Photometric Detection Method

At the NBS, 100–200-ml aliquots of sea water samples were analysed by a simultaneous extraction (dichloromethane) and hydride generation method followed by gas chromatography - flame photometric detection (GC - FPD).[13] Briefly, for a typical analysis of saline water with a butyltin concentration in the sub-μg l^{-1} range, the following procedure was used. To 100 ml of sample in a 125-ml glass separating funnel, equipped with a Teflon stopcock and Teflon-lined screw-top, were added 2.8 ml of dichloromethane and 2.0 ml of 4% *m/V* aqueous $NaBH_4$. In addition, a 10-μl spike of a 0.5 p.p.m. aqueous solution of dipropyltin dichloride was added as an internal standard. The funnel was capped and shaken by hand for 1 min, vented and then shaken (240 strokes min^{-1}) on a wrist-action shaker for 10 min. After a 5-min settling period, the lower organic layer was removed. An additional 1.4 ml of dichloromethane was added and the extraction procedure repeated. The organic layers were combined (approximately 2 ml) in polypropylene centrifuge tubes and evaporated to 100–200 μl or less under a gentle stream of air. Appropriate reagent blanks were carried through the entire procedure.

A gas chromatograph equipped with a flame photometric detector was used for the determinations. Chromatographic separations were carried out on a 1.83 m (6 ft) × 2 mm i.d. glass column packed with 1.5% OV-101 (liquid methylsilicone) on Chromosorb G HP (100–120 mesh). A hydrogen-rich flame was employed, supported by H_2 flowing at the measured rate of 110 ml min^{-1}, air at 70 ml min^{-1} and N_2 (zero grade) carrier gas at 20 ml min^{-1}. The FPD was equipped with a 600-nm cut-on interference filter (band pass 600–2000 nm) to monitor the SnH molecular emission. The output signal from the FPD was recorded simultaneously on a strip-chart recorder and an integrator - plotter. For all runs reported here, the column temperature was programmed at 23 °C for 2 min and then heated to 170 °C at 32 °C min^{-1}. The detector temperature was maintained at 200 °C and the injection port at 150 °C.

The determination of butyltin species was performed either by the method of standard additions or from calibration graphs using sea water or estuarine samples containing little or no measurable levels of butyltin species. Butyltin and dipropyltin chlorides were used as received for the preparation of standard solutions for determination. Concentrations of butyltin species are reported as nanograms of tributyltin chloride or dibutyltin dichloride per litre. The GC - FPD method gives detection limits for di- and tributyltin species of

approximately 5 ng l^{-1}, with a relative standard deviation of 10–15% at 10 ng l^{-1}.[13]

Results

Typical chromatograms for the HG - AA and GC - FPD methods are shown in Fig. 1. Di- and tributyltin species were detected in the 14 coastal marine and estuarine surface water samples in concentrations ranging from trace to several hundred ng l^{-1}. Tables 1 and 2 show the means (X) for 2–5 replicate analyses (depending on the sites), standard deviations of a single measurement (S_x) and the percentage relative standard deviations (RSD) of single measurements. With a few exceptions, the concentrations of the dibutyltin species were lower than those of the tributyltin species.

In general, agreement was good between the two analytical methods employed. In all but five samples the NBS and NOSC values were within 20% of their mean concentration for tributyltin. With dibutyltin, only four of the values varied by more than 20% of the means. The RSDs for replicate analyses were also similar for the two methods, being in the range 11–15% for the two tin species.

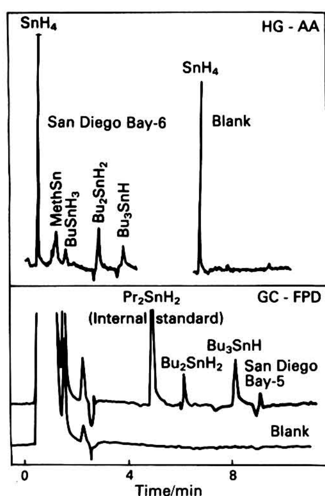

Fig. 1. Chromatograms from analysis of environmental samples by HG - AA and GC - FPD methods. For HG - AA, Bu_2SnH_2 = 16 ng l^{-1} and Bu_3SnH = 9 ng l^{-1}. For GC - FPD, Bu_2SnH_2 = 28 ng l^{-1} and Bu_3SnH = 90 ng l^{-1}

There was no consistent trend for one method yielding a higher concentration of butyltins than the other. In 5 of 14 instances (36%), the GC - FPD values were greater than the HG - AA values for tributyltin. Also, 5 of 14 analyses showed greater values of dibutyltin with GC - FPD.

Only two samples showed serious discrepancies (more than a two-fold difference) for tributyltin concentrations: the San Diego Bay-5 (four-fold) and the Bradwell Ck. marina (which barely exceeded two-fold) sites. Three sites showed such discrepancies with dibutyltin: the San Diego sites 8 and 9 (two- and three-fold, respectively) and the MAFF Laboratory (five-fold) site.

Plots of HG - AA values compared with GC - FPD values for dibutyltin and for tributyltin showed good agreements with the theoretical line of slope 1.0 (Figs. 2 and 3).

The analytical results (using the HG - AA method) for three frozen sample storage sets measured at different times and containing tributyltin at sub-µg l^{-1} concentrations are presented in Table 3. Individual analytical values represent single determinations performed by one of three analysts on one of two instruments. Data from set II show that a slow loss of tributyltin occurs during the frozen storage of sea water samples containing tributyltin at sub-µg l^{-1} levels. The regression line for these data has a slope of −0.00149 µg l^{-1} per week, with a standard deviation of 0.00027. This corresponds to a tributyltin loss of about 1.4% per week of the fitted time zero value (0.112 µg l^{-1}). After 41 weeks of storage, the tributyltin level was less than 50% of the initial concentration (Table 3). In this study, replicate analyses of sea water samples containing di- and tributyltin gave average RSDs of 11–15%. Given this level of uncertainty, the frozen storage of samples for up to 2–3 months should not cause serious analytical problems. A significant negative slope was not obtained from tributyltin values regressed with storage time using data from sets I and III. This is probably due to the relatively short storage times of sets I and III, which were only one-third and one-sixth of the time of set II storage.

Discussion

This study evaluated the determination of dibutyl- and tributyltin species at ng l^{-1} concentrations in natural waters

Table 1. Concentrations of tributyltin detected in marine and estuarine waters

	GC - FPD (NBS)			HG - AA (NOSC)		
	X	S_x	R.S.D., %	X	S_x	R.S.D., %
San Diego Bay-6	5*	—†	—	9	2.0	22
Bay Bridge, MD	6	1.2	20	NM‡	—	—
Baltimore Harbor, MD	11	0.8	7	12	—†	—
San Diego Bay-7	22	1.1	5	21	1.5	7
San Diego Bay-4	23	2.5	11	18	2.1	12
MAFF Lab., England	37	11	30	68	0	0
San Diego Bay-9	50	8.1	16	93	24	26
San Diego Bay-8	79	8.2	10	95	10	11
San Diego Bay-5	90	33	37	19	7.1	37
Annapolis, MD	97	5.1	5	103	5.2	5
San Diego Bay-3	162	2.7	2	209	13	6
San Diego Bay-1	184	30	16	270	26	10
San Diego Bay-2	338	60	18	369	38	10
Bradwell Ck., England	732	—†	—	332	43	13
Average			15			13

* Tributyltin chloride, ng l^{-1}.
† Single analysis.
‡ Not measurable.

Table 2. Concentrations of dibutyltin detected in marine and estuarine waters

	GC - FPD (NBS)			HG - AA (NOSC)		
	X	S_x	R.S.D., %	X	S_x	R.S.D., %
MAFF Lab., England	2*	—†	—	11	1.1	10
Bay Bridge, MD	5	0	0	NM‡	—	—
Baltimore Harbor, MD	11	1.6	15	11	—	—
San Diego Bay-6	11	1.5	14	16	1.2	8
San Diego Bay-7	13	0.3	2	12	0.6	5
San Diego Bay-8	17	2.8	16	52	9.5	18
Annapolis, MD	19	1.0	5	29	4.1	14
San Diego Bay-4	20	5.1	26	23	4.6	20
San Diego Bay-9	20	2.7	14	49	4.2	9
Bradwell Ck., England	23	—	—	19	4.0	21
San Diego Bay-5	28	7.9	28	25	2.1	8
San Diego Bay-3	60	7.7	13	75	0	0
San Diego Bay-2	263	18	7	211	14	7
San Diego Bay-1	270	48	18	270	21	8
Average			13			11

* Dibutyltin dichloride, ng l^{-1}.
† Single analysis.
‡ Not measurable.

Table 3. Frozen storage of sea water samples containing tributyltin at sub-µg l^{-1} concentrations

Set I: April–August 1984		Set II: April–September 1985				Set III: September–November 1985	
Weeks	Concentration*	Weeks	Concentration*	Weeks	Concentration*	Weeks	Concentration*
0	0.13	0	0.112	5.3	0.105	0	0.063
0.3	0.13	0.1	0.070	5.4	0.085	0.3	0.071
0.9	0.14	1.0	0.093	5.6	0.090	4.1	0.058
1.1	0.11	2.1	0.086	5.6	0.080	4.3	0.061
2.1	0.13	2.1	0.130	5.7	0.071	4.3	0.068
3.3	0.09	2.3	0.120	5.7	0.082	4.4	0.068
4.1	0.13	2.3	0.128	7.6	0.097	4.7	0.088
4.9	0.10	2.4	0.102	7.6	0.097	5.3	0.069
6.0	0.11	2.9	0.102	11.4	0.073	5.4	0.110
6.9	0.10	3.0	0.103	16.6	0.057	5.6	0.076
7.0	0.10	3.0	0.133	16.7	0.060	5.7	0.092
7.9	0.10	3.1	0.110	16.9	0.075	6.4	0.086
8.9	0.11	3.3	0.121	17.9	0.089		
9.9	0.09	4.1	0.080	18.4	0.073		
11.7	0.13	4.1	0.090	18.4	0.103		
13.7	0.12	4.9	0.111	18.7	0.054		
13.9	0.15	4.9	0.090	18.9	0.071		
14.0	0.10	5.0	0.090	20.9	0.099		
		5.0	0.087	41.4	0.043		
		5.1	0.083	41.6	0.046		

* Tributyltin chloride, µg l^{-1}.

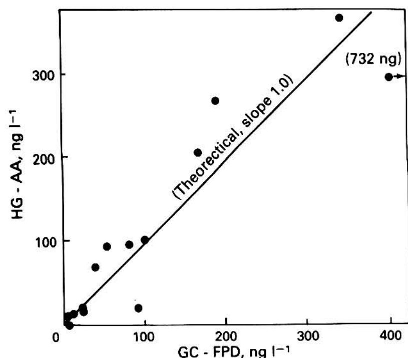

Fig. 2. Graph of HG - AA values *versus* GC - FPD values for tributyltin species from environmental sea water samples. The line is the theoretical slope, 1.0

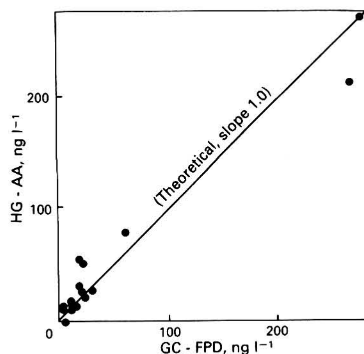

Fig. 3. Graph of HG - AA values *versus* GC - FPD values for dibutyltin species from environmental sea water samples. The line is the theoretical slope, 1.0

using two different measurement methods. The HG - AA method employs the direct hydridisation of the bulk sample, purging by an inert gas, trapping on a chromatographic substrate at liquid nitrogen temperatures and butyltin detection by highly element-specific atomic absorption spectrometry following boiling-point elution from the trap. The GC - FPD method employs hydride generation coupled with simultaneous extraction into an organic solvent (CH_2Cl_2). It is clear from this study that both methods give similar analytical results for di- and tributyltin species in marine and estuarine waters. It is possible that the few instances of disagreement (showing a 2-fold or greater difference in results) resulted from factors related to the chemical or physical composition of samples that affected one method more than the other. However, no obvious anomalies (oil pollution, excessive particulates, algal blooms, etc.) were noted in these samples.

Frozen Sample Storage

The data presented in Table 3 indicate that frozen storage of sea water samples containing tributyltin is a reasonably effective method of sample preservation for a period of approximately 2–3 months. Data from set II, which was obtained over a 10-month period, showed that a slow loss of tributyltin occurred during frozen storage. The mechanism of this loss may not have involved de-alkylation as increases in degradation products (mono- and dibutyltin) were not noted. About half of the initial tributyltin concentration was present after a 10-month storage period. Data from sets I and III, representing 6.4–14.0 weeks of frozen storage, did not show statistically significant negative slopes, and also confirm that frozen storage for several weeks does not result in significant losses of tributyltin.

Frozen storage in polycarbonate bottles has proved to be effective in preserving sea water samples. Previous work has shown that storage of sea water samples containing tributyltin in polyethylene plastic containers resulted in substantial (62%) adsorptive losses from initial values after a one-week period at 4 °C.[23] Samples stored in polycarbonate plastic, Pyrex glass and Teflon containers exhibited adsorptive losses of 3, 4 and 7%, respectively.[23] Efforts are continuing to evaluate the stability of frozen sea water samples containing tributyltin for a period of approximately 1 year.

Chemical speciation of butyltins in marine and estuarine waters at ambient (ng l^{-1}) levels is difficult and as a result there is little data available for toxicologists and environmental agencies to evaluate. As legislation restricting the use of organotin antifouling paints is growing worldwide, there is a rapidly increasing need for monitoring data for the toxic tributyltin species and its less toxic degradation product, dibutyltin. This study is the first effort to make inter-laboratory comparisons of methods for butyltin speciation in natural waters. Efforts of this type are important to establish intercomparability of methods and data as environmental monitoring programmes are undertaken.

A related, parallel effort at NBS[21] has chromatographically generated a stable aqueous tributyltin research material, used in conducting the first worldwide methods intercomparison for organotin measurements. The next phase of this effort is underway and will generate a mixed butyltin species research material for use in a inter-laboratory comparison involving speciation of the material diluted to sub-$\mu g\ l^{-1}$ levels.

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Portions of this work will be included in the dissertation of C. L. M. to be submitted as a requirement for the PhD degree from the University of Maryland.

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Determination of Scandium in Coal Fly Ash and Geological Materials by Graphite Furnace Atomic Absorption Spectrometry and Inductively Coupled Plasma Atomic Emission Spectrometry

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Two procedures for the determination of scandium in coal fly ash and other geological materials have been developed. The first procedure consists in dissolution of the samples with a mixture of nitric, perchloric and hydrofluoric acids in a sealed PTFE vessel followed by determination by graphite furnace atomic absorption spectrometry. In the second procedure, the sample is decomposed by fusion with lithium tetraborate, the melt is dissolved in 5% nitric acid and scandium is determined by inductively coupled plasma atomic emission spectrometry using an argon plasma. The limits of determination are 0.80 and 0.63 μg g^{-1}, respectively, for the two methods. The scandium concentration in eight coal ash samples obtained by GFAAS and ICP-AES compare well with those determined previously by NAA.

Keywords: *Scańdium determination; coal fly ash; geological materials; graphite furnace atomic absorption spectrometry; inductively coupled plasma atomic emission spectrometry*

Studies of the particle size dependence of element concentrations in fly ash can be classified into two categories.[1] The first category consists of those studies that relate the elemental concentrations to the particle size of size-classified material (sufficient mass of size-classified material must be collected in order to allow gravimetric determination prior to elemental analysis). The second category consists of the many studies that have employed inertial cascade impactor systems for aerodynamic size classification. Because only small masses of material may be collected on the stages of the cascade impactor, the specific elemental masses of the particles deposited on each stage are often ratioed to the mass of an element that does not demonstrate a marked dependence of concentration on particle size.

One of the elements that has been frequently used for this purpose is scandium. This element was selected because it is present at very low levels in the natural environment, it is essentially non-volatile at furnace temperatures, and also because most fly ash analyses are carried out by neutron activation analysis (NAA), which gives a detection limit of about 0.002 μg of Sc.[2] Gladney *et al.*,[3] for example, reported the concentration of Sc in several geological environmental NBS standard materials, but referred only to NAA and flame atomic absorption spectrometric (FAAS) techniques.

More recently, ICP-AES and DCP-AES techniques have been successfully employed to determine trace amounts of elements in geological and related materials, including Sc and rare earth elements.[4–13] In contrast, very few papers have been published on the determination of Sc by ETA-AAS.[14–20]

Sen Gupta[16] determined Sc in silicate rocks after coprecipitation with calcium oxalate and hydrated iron(III) oxide. Working in the peak-height mode ($T_{atom.}$ = 2500 °C) using pyrolytically coated graphite tubes, the sensitivity for Sc was 37 pg per 0.0044 A, superior to that found (50 pg per 0.0044 A) in a tantalum-lined furnace.[17] Sen Gupta later showed[18] that, at 2000 °C, greater sensitivity can be achieved by using a tantalum foil-lined graphite furnace (1.2 pg per 0.0044 A) instead of a pyrolytically coated furnace (13 pg per 0.0044 A).

Wu and Ma[19] reported the direct determination of Sc in soils by graphite furnace AAS (GFAAS) with a pyrolytically coated graphite tube lined with both tungsten and tantalum foil. Sample aliquots (10 μl) were atomised at 2730 °C for 10 s in a 0.5 l min^{-1} argon gas flow. The absolute limit of determination was 4.6 pg of Sc.

Atnashev *et al.*[20] reported the determination of Sc by ETA-AAS using a tungsten coil atomiser, carrying out the pulsed atomisation in a laminar flow of Ar - N_2 (10 + 1). However, no characteristic amounts data for Sc were reported by Atnashev *et al.*,[20] but it was reported that the results compare well with corresponding values obtained with a L'vov graphite cell.

In this work we compared two different procedures for sample dissolution: acid attack with nitric - perchloric - hydrofluoric acids and lithium tetraborate fusion. Scandium was determined in the solution derived from the mixed acid digestion procedure by GFAAS, whereas in the fusion solution we used the ICP-AES technique.

Experimental

Reagents

Standard solutions of Sc were prepared from a 1000 mg l^{-1} stock standard solution for atomic absorption spectrometry (Aldrich-Chemie, Steinheim, FRG) by dilution with de-ionised water or lithium tetraborate. Perchloric acid (70% *m/V*), nitric acid (65% *m/V*) and hydrofluoric acid (40% *m/V*) were all Suprapur reagents (E. Merck, Darmstadt, FRG). Lithium tetraborate solution (5 g l^{-1}) was prepared from Baker Analyzed reagent flux grade material (J. T. Baker Chemicals, Denveter, The Netherlands). Water was purified in a Milli-Q system (Millipore, S.P.Q. Milan, Italy).

Apparatus

A Perkin-Elmer 5000 atomic absorption spectrometer equipped with a D_2 arc background corrector, an AS-40 autosampler and an HGA-500 graphite furnace was used for the Sc determinations. A 7500 data station was used for the display and storage of the fast atomisation signals. Peak absorbance and integrated absorbance signals were calculated using the HGA Graphics II software and PR 210 printer - plotters were used for printing out the analytical information and the high-resolution peak profiles. The optimum instrumental parameters for Sc determinations (Table 1) were established after extensive investigations. Pyrolytically coated graphite tubes were used in all determinations.

A Perkin-Elmer ICP/6000 inductively coupled plasma atomic emission spectrometer was used for all Sc determina-

Table 1. AAS instrumental operating conditions

Model 5000 spectrophotometer:

Wavelength	391.2 nm
Spectral slit width	0.2 nm
Calibration mode	Peak area
Integration time	6 s
Background corrector	Deuterium arc

HGA-500 graphite furnace:

Step, *n*	Temperature/°C	Ramp time/s	Hold time/s
1	80	1	4
2	120	10	10
3	500	10	10
4	1700	20	10
5	2700	0*	6
6	2800	1	3

Purge gas	Argon, interrupted
Sample volume	20 µl
Alternative volume	20 µl

* Maximum power heating mode; read activated at −2 s.

Table 2. ICP-AES instrumental operating conditions

Incident RF power	1250 W
Reflected RF power	<5 W
Plasma gas flow-rate	15 l min^{-1}
Auxiliary gas flow-rate	0.3 l min^{-1}
Nebuliser gas pressure	26 lb in^{-2}
Viewing height	14 mm above load coil

tions in lithium tetraborate solutions; the operating conditions used are given in Table 2.

For sample decomposition, a Perkin-Elmer Autoclave 3 was used. Neutron activation analyses were performed with the TRIGA Mark II reactor at the University of Pavia. Standards and samples were irradiated for 50 h at a flux of 1.2 × 10^{12} neutrons cm^{-2} s^{-1}; the radionuclide used was ^{46}Sc with a half-life of 83.80 d and a characteristic peak at 889 keV.

Acid Dissolution Procedure

About 0.25 g of sample was taken with 4.0 ml of 65% nitric acid, 2.0 ml of 70% perchloric acid and 4.0 ml of 40% hydrofluoric acid in the PTFE beaker of the Autoclave-3 and heated in a drying oven for 3–5 h at 150 °C.

After cooling the contents of the PTFE beaker were slowly evaporated to dryness on a low-temperature (200 °C) aluminium block. After the addition of 1 ml of nitric acid and 10 ml of water, the solution was transferred into a polypropylene bottle and diluted to 100 ml with de-ionised water. If any undissolved residue was visible at this stage, the solution was filtered through a 0.5 µm Fluoropore membrane filter and the residue solubilised with 2.0 ml of 70% perchloric acid and 5.0 ml of 65% nitric acid.

The mixture was heated on the plate nearly to dryness and, after washing with 5.0 ml of water, was then heated until the evolution of dense white fumes. The PTFE beaker was then cooled, 1.0 ml of nitric acid plus 10 ml of water were added to dissolve the salts and the solution was combined with that in the polypropylene bottle. This final solution was then diluted to 100 ml.

Fusion Procedure

Approximately 0.25 g of sample and 0.5 g of lithium tetraborate were placed in a 50-ml platinum crucible and thoroughly mixed. The sample - flux mixture was fused in a muffle furnace at 1000 ± 50 °C for 45 min.

When the fusion was complete, the cooled crucible was placed in a 100-ml beaker, a small PTFE-coated stirrer was inserted and 25 ml of 5% nitric acid were added.

The solution was heated for 15–20 min at 50–60 °C on a magnetic stirrer and then transferred into a 100-ml polypropylene flask.

The same procedure was repeated with a second aliquot of nitric acid and, when dissolution was complete, the contents of the flask were adjusted to volume with de-ionised water. Blanks containing only lithium tetraborate were also fused in the manner described above.

These solutions are stable over a period of several months and may be used for the determination of ten major elements, plus some trace elements.

Results and Discussion

Optimisation of HGA Operating Parameters

In order to select the optimum ashing temperature, a study of the effect of this parameter on the absorption signal of Sc was carried out.

Aliquots of 20 µl of a 50 µg l^{-1} Sc solution were injected into the pyrolytically coated graphite tubes and drying and atomisation cycles of 120 °C for 10 s and 2700 °C for 6 s, respectively, were employed. The integrated absorption signal produced during the atomisation step was detected at 391.2 nm and recorded by the HGA Graphics II software.

On varying the ashing temperature between 1000 and 2000 °C, we observed that the signal remained constant at temperatures up to 1800 °C (Fig. 1). Hence, an ashing temperature of 1700 °C was selected for all further determinations.

Interferences

Sen Gupta[18] reported that there are no inter-element interferences in the determination of lanthanides in GFAAS and that interferences from associated common elements can be eliminated by heating the sample at about 1800–2000 °C before atomisation.

The final solution in work of the Sen Gupta[18] is, however, relatively free from other elements because it is first submitted to a double calcium oxalate and a single hydrous iron(III) oxide co-precipitation step. Our final solution, in contrast (250 mg of sample diluted to 100 ml), contains several milligrams of matrix elements such as Si, Al, Fe, Mg, Ca, Na and K.

For this reason we verified the interference effects of a synthetic solution containing 100 mg l^{-1} of Fe, Mg, Na, K and P, 500 mg l^{-1} of Al and 1000 mg l^{-1} of Ca and Si on the absorbance signal of 20 µg l^{-1} of Sc. Fig. 2 shows that under the experimental conditions reported in Table 1 the interferences due to the principal elements in our matrices are negligible.

GFAAS Determinations

According to the concept of "characteristic mass" (m_o) developed by Slavin and Carnrick,[21] we calculated this quantity by injection of the analyte in aqueous solution (1% HNO_3) and by the method of standard additions.

The instrumental parameters and the experimental conditions are given in Table 1 and the results for m_o are reported in Table 3.

The characteristic mass (m_o) averaged on ten scandium determinations in aqueous solution (1% HNO_3) was 31.2 ± 4.0 pg per 0.0044 A s (CV = 12.7%) but with two different values, 34.6 ± 1.9 pg per 0.0044 A s (n = 5, CV = 5.6%) and 27.9 ± 1.2 pg per 0.0044 A s (n = 5, CV = 4.4%) for two different lots of pyrolytically coated graphite tubes.

The characteristic amounts found by the standard additions method for NBS 1633, 1633a, 1645, 1646, 278 and 688 standards compare very well with those in water. The precisions for the reported characteristic mass data appear to be better than 10–20%.

Table 3. Characteristic mass data (pg per 0.0044 A s) for Sc in aqueous solution and in different NBS materials

Sample	Number of samples (n)	Characteristic mass (m_0) Lot A*	Lot B*	Average	CV, %
Water (1% HNO_3)	5	34.6 ± 1.9	27.9 ± 1.2	31.2 ± 4.0	12.7
NBS 1633	3	32.4 ± 1.7	25.3 ± 0.8	30.0 ± 5.9	19.7
NBS 1633a	3	33.3 ± 1.2	25.3 ± 1.8	29.2 ± 4.5	15.4
NBS 1645	3	33.9 ± 1.5	24.7 ± 1.7	29.3 ± 5.2	17.9
NBS 1646	3	32.5 ± 2.5	24.8 ± 2.6	28.7 ± 4.8	16.7
NBS 278	3	34.3 ± 2.0	29.2 ± 1.1	31.8 ± 3.1	9.8
NBS 688	3	32.8 ± 1.9	25.1 ± 2.6	28.9 ± 4.6	16.0

* Lots A and B are two different lots of pyrolytically coated graphite tubes.

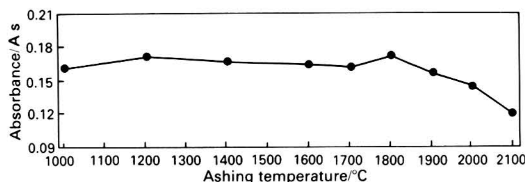

Fig. 1. Typical ashing curve for 1 ng of Sc in 1% HNO_3

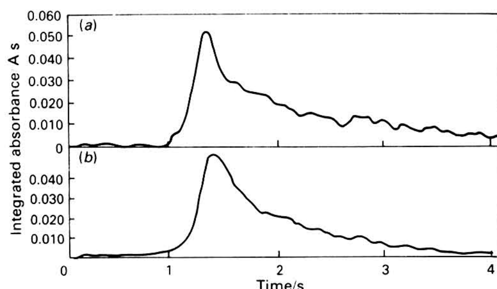

Fig. 2. High-resolution peak profiles for (a) 20 µg l^{-1} of Sc and (b) 20 µg l^{-1} of Sc plus Fe, Mg, Na, K and P (100 mg l^{-1}), Al (500 mg l^{-1}) and Ca and Si (1000 mg l^{-1})

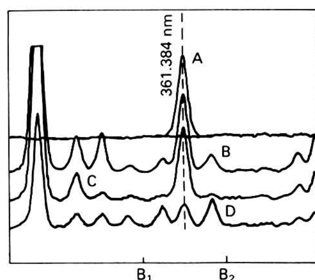

Fig. 3. Graphic spectral scans of Sc II at 361.384 nm. Conditions: 100 µg l^{-1} of Sc in A, lithium tetraborate; B, NBS 1633a solution; C, NBS 688 solution; and D, NBS 1646 solution. Background positions: B1, −0.126 nm and B2, +0.140 nm

After about 50–70 firings of a new pyrolytically coated graphite tube at the recommended temperatures, the integrated absorbances decrease because of the destruction or loss of some of the pyrolytic coating and the tube should be discharged. The detection limit, DL (calculated as the concentration corresponding to three times the standard deviation of the blank), was 2.0 µg l^{-1}, whereas the lowest quantitatively determinable concentration, LQD (defined as the concentration corresponding to ten times the standard deviation of the blank and a dilution factor of 400), was 2.7 µg g^{-1} of Sc.

Table 4. Results for NBS reference materials (µg g^{-1})

NBS standard	This work GFAAS	ICP-AES	Certified values	Other values[3]
Coal fly ash:				
1633	25.1 ± 3.5	28.7 ± 2.0	—	26.6 ± 1.7
1633a	40.6 ± 5.1	41.3 ± 4.8	(40)*	38 ± 3
River sediment:				
1645	(1.8)	(1.6)	(2)	2.6
Estuarine sediment:				
1646	9.4 ± 3.1	10.9 ± 1.9	(10.8)	10.4
Obsidian rock:				
278	6.1 ± 1.3	3.5 ± 0.1	(5.1)	4.9 ± 0.5
Basalt rock:				
688	39.1 ± 2.4	40.4 ± 0.7	(38.1)*	36.2

* Values reported but not certified by NBS.

Table 5. Comparative concentrations of Sc (µg g^{-1}) in different coal ash samples

Sample	This work GFAAS	ICP-AES	NAA
BMA 1	26 ± 1.7	29 ± 0.6	27 ± 1.4
BMA 2	20 ± 1.1	20 ± 0.4	19 ± 1.0
GMA 1	33 ± 2.0	36 ± 0.6	34 ± 1.7
GMA 2	3.5 ± 0.08	2.8 ± 0.03	3 ± 0.2
BCL 1	8 ± 0.09	9 ± 0.07	10 ± 0.5
BCL 2	9 ± 1.0	10 ± 0.4	11 ± 0.5
BMI 1	25 ± 1.3	28 ± 0.6	25 ± 1.3
BMI 2	24 ± 0.7	25 ± 0.3	29 ± 1.5

ICP Determinations

The choice of the Sc II line at 361.384 nm was based on a systematic consideration of the detection limit, the expected elemental concentration in the sample solution and spectral interferences.

Fig. 3 shows a scan from 0.5 nm below to 0.5 nm above the scandium 361.384 nm line, overlaid with scans of three NBS reference materials.

These, and other scans not reported in the figure, indicate that, for these and similar types of samples, the Sc emission line is sufficiently free from background interferences to be utilised in a reasonably straightforward manner.

Background correction must be carried out accurately because the background under the analyte line is extremely structured and could cause spectral interference problems, particularly if there were any broadening as the concentrations increased.

The detection limit was 1.6 μg l^{-1}, whereas the lowest quantitatively determinable concentration was 2.1 μg g^{-1} of Sc.

Comparison of GFAAS and ICP Results

Results for scandium determinations obtained in this work compare well with NBS reference values (reported but not certified) and literature values. The accuracy data are given only for those samples for which the Sc concentration exceeded the LQD value, as quantitative determinations can usually be made with satisfactory accuracy and precision only above this concentration level.

The scandium concentration determined by ICP-AES for the NBS 1645 sample was found to be less than the estimated DL, whereas the value for NBS 278 appears to be biased low. The poor recoveries also obtained for this element using the acid dissolution - HGA technique indicate that incomplete solubilisation (dissolution or fusion) for the NBS 1645 sample was responsible for the low scandium results.

It is evident from these data that accurate determinations can be carried out by both the methods; the fusion - ICP-AES method gives a better precision then the acid digestion - GFAAS procedure. Similar conclusions can be drawn from the data in Table 5 relative to other coal ash samples analysed for their Sc content either by mixed acid digestion - GFAAS and fusion - ICP-AES procedures or by neutron activation analysis.

Conclusions

Both the fusion - ICP-AES and acid digestion - GFAAS procedures are capable of the successful routine determination of Sc in coal fly ashes, provided that the samples contain relatively high concentrations of this element (*i.e.*, 10–20 times the LQD). The major source of error in the experimental reproducibility lies (mostly for acid solubilisation) in the preparation of the sample powder solution.

In general, the data obtained for fusion - ICP-AES or acid digestion - GFAAS determinations are as good as those obtained for NAA determinations.

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Multi-element Pre-concentration by Solvent Extraction Compatible with an Aqua Regia Digestion for Geochemical Exploration Samples

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The interference of nitric acid in the organic solvent extraction of metal iodide complexes as ion associates with the trioctylmethyl ammonium ion was investigated. The results show that the interference is caused by nitric oxide extracted into the organic solvent in the form of nitrosyl compounds and that this can be eliminated by the addition of sulphamic acid or urea. A multi-element extraction procedure was applied to geochemical exploration samples digested with aqua regia. Ag, Cd, Se, Te and Tl were determined by AAS in the extracts down to 100 p.p.b. levels.

Keywords: *Multi-element extraction; nitric acid interference; trace element determination; geochemical samples; trioctylmethyl ammonium chloride*

Geochemical exploration often involves the determination of a number of trace elements, many of which are well below the detection limits of AAS or ICP-AES. For such elements a multi-element organic solvent extraction would be an attractive solution[1] if it could be made sufficiently simple and could cover all the elements of interest. Most of the trace elements of interest in geochemical exploration are easily solubilised by digesting the samples with boiling aqua regia and this is probably the most widely used decomposition procedure when looking for primary mineralisation. It may be performed on a large scale with very simple equipment, *i.e.*, test tubes, an aluminium block and a hot-plate, and even fairly resistant minerals such as pyrite and cinnabar are attacked.

For samples with a high iron content, one of the most promising multi-element extraction systems is the extraction of iodide or bromide complexes into 4-methylpentan-2-one (IBMK), possibly as ion associates with long chain aliphatic polyamines or polyphosphines. Two reagents of this kind have been extensively applied, namely, trioctylmethylammonium (TOMA) chloride[2–5] and trioctylphosphine oxide (TOPO).[6–9]

Unfortunately, the extraction of iodide or bromide complexes is incompatible with an aqua regia digestion of the samples unless the solutions are dried and then re-dissolved in, for example, HCl. This would make the procedure prohibitively time and labour consuming for geochemical exploration.

In the course of establishing analytical procedures for the geochemical exploration of epithermal gold deposits, 16 trace elements had to be determined, possibly from a single sample decomposition. Seven of these (Cu, Pb, Zn, Co, Ni, Mn and Mo) had sufficient ratios of abundances to detection limits to provide meaningful analyses by direct flame AAS of the sample digest and four after hydride (As, Bi and Sb) or cold vapour (Hg) generation. For the five remaining (Ag, Cd, Se, Te and Tl), the possibility of applying the extraction of iodide complexes as ion associates with TOMA or TOPO were explored. Reactions causing interference were identified as being due to nitric oxide and an extraction procedure appropriate for the geochemical analyses of soil, stream sediment and rock-chip samples, where some precision may be sacrificed for greater speed, was established.

Experimental

Sample solutions were prepared by boiling 2 g samples in a test-tube with 8 ml of aqua regia for 30 min. After cooling, the volume was adjusted to 20 ml with distilled water. The final concentration was not critical and varied between 3 and 4 M HCl. After settling, 10 ml of this solution were drawn into a polypropylene syringe fitted with a 10 cm × 1.2 mm i.d. plastic capillary tube extension to the inlet, followed by 2 ml of ascorbic acid (30%), 2 ml of a 2 M KI solution and 3 ml of IBMK containing 5% *V/V* TOMA or 5% *m/V* TOPO. After shaking for about 1 min the syringes were left to stand, and after the separation of the phases the organic layer was transferred into glass vials, which were then capped. This solution was then used for measuring several different trace elements by AAS.

A Varian-Techtron Model 1475 AA spectrometer was used, either with a microsampling flame attachement or a GTA 95 graphite furnace. For flame AAS, 50 µl of the extract were delivered to the nebuliser with a micropipette. A 3 s integration time was used, giving the operator enough time to keep the whole absorbance signal within the integrating period. Ag and Cd were determined down to 0.1 p.p.m. in the sample.

Se, Te and Tl, which have insufficient detection limits by flame AAS, were determined in a graphite furnace using pyrolytic graphite coated tubes for Se and Te and a pyrolytic graphite platform for Tl. Te and Tl were determined down to 0.1 p.p.m. and Se down to 0.2 p.p.m. from integrated absorbance readings. A vessel containing IBMK was kept in the carousel of the automatic sampler to slow down the evaporation of the organic solvent from the sample extracts loaded in open vials. The carousel was always kept covered. Instrumental conditions are given in Table 1.[10]

Results and Discussion

Preliminary experiments using extraction from 3 M HCl with the addition of up to 1 ml of nitric acid to the 10 ml of extracted

Table 1. Conditions of measurement in graphite furnace AAS

Element	Se	Te	Tl
Analytical line/nm	196.0	214.3	276.8
Atomisation surface	Wall	Wall	Platform
Sample volume/µl	10	10	5
Modifier volume/µl	3	3	2
Temperature programme/(°C; s_{ramp} + s_{hold})			
Drying	110; 5 + 10	110; 5 + 10	500; 5
Pyrolysis	500; 8	500; 8	
	1200; 1 + 3	1200; 1 + 3	600; 10 + 5
Atomisation	2800; 1 + 1.2	2600; 1 + 1.2	2600; 1 + 2
Modifier for Se and Te	10% HNO_3 + 0.5% Cu + 0.5% Mg		
Modifier for Tl	1% H_2SO_4 + 0.5% Mg		

solution showed that extraction with TOMA is less affected by the nitric acid than extraction with TOPO and the former was therefore used for further experiments.

It soon became evident that the presence of nitric acid in the limited amount used did not affect the extraction itself, but rather the stability of the extracts. If determined immediately after separation, the correct amount of extracted metals was usually found. Metals in the extract showed different stabilities. Tellurium was very rapidly lost and was therefore used for checking the stability of the extracts.

In sample extracts with a high metal content a yellow precipitate was formed within a few hours and the original brown colour became orange. Iodine, Pb, Cu, Zn and Ga were identified in the precipitate by XRF. Extracts with a lower metal content turned bright yellow, and even if no precipitate was visible the Te content fell markedly. If the extract was stirred just before introduction into the graphite furnace with an automatic sampler, highly scattered readings for Te were observed. As the determinations were repeated the higher values eventually disappeared. This behaviour indicated that Te was still present but was in the form of particles that were gradually settling out after stirring.

If after separation the organic phase was stored undisturbed in a glass vial, the colour change from brown to yellow always began from the top surface. Discolouration was particularly frequent with samples high in iron. The extracts also emitted vapours, highly irritant to the eye.

From these observations and some basic chemistry the following sequence of events may be seen: 1, Nitric acid oxidises iodide in the aqueous phase with the production of nitrous acid and nitric oxide. The latter forms nitrosyl compounds that are extracted into IBMK. 2, In the IBMK, nitric oxide catalyses the oxidation of iodide to iodine by atmospheric oxygen. 3, As iodide is lost, soluble metal iodide complexes turn into less soluble forms and precipitate. 4, Iodine reacts with IBMK in the enolic form, giving an iodinated ketone. Halogenated ketones are well known tear gas compounds.

If this sequence of reactions is a correct description of the processes involved, the instability of the extracts is not due to the nitric acid itself but rather to the products of its reduction, *i.e.*, nitrous acid and nitric oxide. These may be eliminated by the addition of urea, sulphamic acid or ammonia in general. The presence of the ammonium ion in TOMA explains why extracts with this reagent were more stable than with TOPO. Both urea and sulphamic acid were investigated. The addition of 1 ml of a 1% solution of these reagents was found to be sufficient to secure the stability of the extracts.

A correlation between the instability of extracts and a high iron content in the samples pointed to an iron nitrosyl halide as the NO-containing species extracted. The amount of NO extracted, and presumably the stability of extracts, could therefore be assessed from the amount of iron in the organic phase. Iron extracted under different conditions, *i.e.*, with and without the addition of ascorbic and/or sulphamic acids and with increasing concentrations of nitric oxide, was determined by microsampling flame AAS using the Fe line at 372 nm. Nitric oxide was added as sodium nitrite, which is reduced to nitric oxide by the excess iodide. The solutions contained 25 mg of iron added as $FeCl_3$. Other conditions of the extraction were identical with those used for the sample solutions, *i.e.*, the final concentrations were 2 M HCl, 0.266 M KI and 4% ascorbic acid.

The results show that in the presence of nitric oxide iron is extracted both in the absence and the presence of ascorbic acid. The addition of ascorbic acid reduces the amount extracted but less so than sulphamic acid. With an increase in the amount of nitrite, the amount of Fe extracted in the absence of ascorbic acid levels off at about 0.003 M NO_2^-, but increases further in its presence (Fig. 1). The addition of ascorbic acid alone thus cannot prevent the extraction of iron and nitric oxide into the organic phase. Only a combination of ascorbic and sulphamic acids reduces iron extraction to a negligible level.

Whether nitric oxide is also extracted in some other form is difficult to assess. However, if so, the amount must be limited as no instability of extracts unconnected with a high iron content in the samples was ever observed. It is also difficult to assign any valence to the iron in the nitrosyl compounds extracted. Iron nitrosyl halides with metal valencies I–III are known. It is plausible that the nitrosyl compound extracted is a negatively-charged ferrous complex that forms an ion associate with TOMA or protonated IBMK. This is suggested by the observation that when carrying out the extraction under identical conditions but without the addition of sulphamic acid and using KBr instead of KI, sample solutions high in iron turned green on addition of ascorbic acid. This green compound is partially extracted into IBMK, as seen by the intense green of the organic layer. On standing, the green gradually fades and a red - brown precipitate forms at the contact with the aqueous layer. It is known that negatively charged nitrosyl halides of Fe^{II} are green, neutral halides are red and cationic halides are brown.[11] A less intensive green colour is observed when adding ascorbic acid to the iodide solutions. It is, however, obscured by the brown of the extracts.

The elements Ag, Cd, Se, Te and Tl were determined in the same extracts in order to check the expected correlation between the amount of iron extracted and the stability of the extract. Ag and Cd showed no systematic variation with the amount of nitrite added; the scatter of values was within 5%.

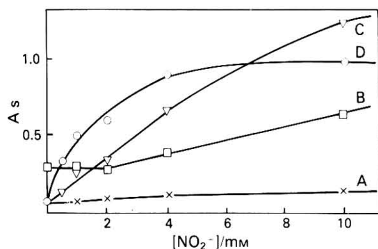

Fig. 1. Signal of iron extracted into IBMK - TOMA in presence of increasing amounts of NO_2^- from 2 M HCl, 0.266 M KI and: A, ascorbic acid (4%) + sulphamic acid (0.066%); B, sulphamic acid only; C, ascorbic acid only; and D, neither

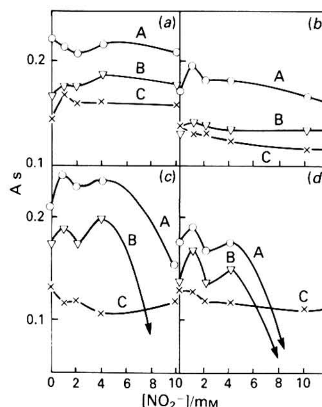

Fig. 2. Signals of Se, Te and Tl measured from same solutions as in Fig. 1. (*a*) Solution A; (*b*) solution B; (*c*) solution C; and (*d*) solution D of Fig. 1. A, Se; B; Te; and C, Tl. Amounts extracted: Te and Tl, 1 µg; Se, 2.5 µg

Signals of Se, Te and Tl determined in the graphite furnace are plotted in Fig. 2. Thallium, in a similar manner to Ag and Cd, does not show any systematic variation. Se and Te, at the highest NO_2^- concentration and both with the addition of ascorbic acid only and without any addition, show significantly lower values indicating the instability of the extracts. The same two solutions also have the highest Fe content. The signal of Te in the extract without the addition of ascorbic acid, determined 30 min after extraction, was 0.166 A s and fell to 0.02 A s within 1 h. If the two readings for the unstable extracts are excluded there is no systematic variation. The scatter of readings has a relative standard deviation of about 10%, which is not statistically significant.

Although 1 ml of 1% sulphamic acid removes all instability up to the concentration level of NO investigated, a 2% solution was used in the determinations. Out of more than 2000 samples analysed by this procedure, only about 10 showed any instability by decolourising. These were repeated using a smaller aliquot of the sample solution and dilution with 3 M HCl. The relative standard deviation of the determinations is about 10% for Ag and Cd, 15% for Se and Te and 20% for Tl, which is adequate for geochemical exploration purposes.

Conclusions

The results reported in this paper indicate that it is possible to apply the extraction of iodide and/or bromide complexes of metals as ion associates with TOMA to the analysis of geochemical exploration samples, even in the presence of up to 10% *V/V* HNO_3 if certain provisions are made. In particular, the time during which the nitric acid and iodide are in intimate contact must be short in order to minimise the amount of nitrous acid formed. The sample solution extracted should, therefore, always be clear as the presence of clay colloids slows down the separation of phases. If the sample solutions are clear the separation is very fast because of the high density of the aqueous layer.

As the instability of the extracts is due to nitric oxide extracted in the form of nitrosyl compounds, it may be eliminated by reducing both nitrous acid and nitric oxide (which are in equilibrium) to nitrogen by the addition of sulphamic acid or urea to the extracted solutions.

Ascorbic acid must be added to counteract the oxidation of iodide to iodine, which would make the separation of phases difficult. It is also needed to reduce some of the extracted elements to their lower valencies that form the iodide complexes.

Extracts prepared under these conditions with the addition of sulphamic acid and kept in a refrigerator have been found to be stable for many days. As many elements of interest in geochemical exploration form relatively stable iodide and/or bromide complexes (*e.g.*, Ag, Au, Bi, Cd, Cu, Ga, Hg, In, Pb, Sb, Se, Sn, Te, Tl and Zn) this extraction system has potentially very broad applications.[3,10]

The enrichment factor attainable is evidently limited by the presence of common base metals (Cu, Pb and Zn), which use up the reagents and may saturate the organic phase. This is not generally a limiting factor for geochemical exploration samples.

The main advantage of the method is that the extraction may be applied to samples digested with aqua regia, which is a much simpler, and for geochemical exploration more widely used, oxidative decomposition procedure than HCl + $KClO_3$,[2] HCl + H_2O_2[5] or fusion with potassium pyrosulphate.[12] All these have been used in order to make the extraction of iodide complexes with TOMA applicable to geochemical exploration samples.

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Analytical Reference Materials

Part VI.* Development and Certification of a Sediment Reference Material for Selected Polynuclear Aromatic Hydrocarbons†

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A naturally contaminated environmental sediment reference material (EC-1) was developed and analysed for selected polynuclear aromatic hydrocarbons (PAH). Freeze-dried and homogenised subsamples of EC-1 were Soxhlet extracted and the extracts were cleaned-up on activated silica gel and alumina columns. The levels of PAH in this material were determined by using the following three independent analytical methods: (1) GC - FID, (2) GC - MS and (3) reversed-phase HPLC with fluorescence and UV detectors. Up to a total of 72 replicate determinations were performed and the results obtained by each method were in good agreement with each other. Interlaboratory PAH results for EC-1 obtained in a round-robin study also confirmed the in-house data. The results for ten PAH of which the between-method difference was less than ±10% were pooled to generate the certified values.

Keywords: *Polynuclear aromatic hydrocarbon determination; certified reference material; quality assurance; sediment samples*

Polynuclear aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants as they are naturally occurring and are also formed by the pyrolysis of carbonaceous materials at high temperatures. The routine determination and monitoring of PAH in environmental samples are essential because of their persistence and carcinogenic and mutagenic properties.[1] Although PAH levels in open lake or marine surface waters are low, they are readily adsorbed and accumulated by sediments and particulate matter and μg g^{-1}–ng g^{-1} levels of PAH have been reported in many sediment samples.[2–5]

Methods for the determination of PAH in environmental samples have been reviewed previously.[6] The most popular detection techniques involve either gas chromatography (GC) or high-performance liquid chromatography (HPLC). The determination of PAH by HPLC with a fluorescence detector has been used by many workers[7–9] as many PAH are highly sensitive to this detector. The judicious selection of excitation and emission wavelengths also makes the detector highly specific, which minimises the interferences from unresolved co-eluting PAH resulting from highly complicated mixtures.[7,10] In many instances the fluorescence detector is complemented by a variable-wavelength UV detector for the detection of those PAH with a low sensitivity to the former.[11–13]

GC determinations of PAH are usually carried out with high-resolution capillary columns.[2–5,14,15] The quality of these chromatograms is much higher than those obtained by packed columns and therefore capillary columns are considered essential for complicated environmental samples. Although PAH are generally detected by a flame ionisation detector (FID), electron-capture detectors[16,17] can also be used under special circumstances for these compounds. The determination of PAH by a sensitive and selective mass spectrometer interfaced to a high resolution capillary column is currently the most powerful approach.[18,19] In this instance, mass spectra of a sample provide positive identification of known PAH or structural information for unknown PAH. By using the selected ion monitoring (SIM) technique, sub-nanogram amounts of PAH can easily be detected.

* For Part V of this series, see reference 26.
† This material is currently not for sale and not available for general distribution.

Two interlaboratory studies on the determination of PAH in sediment samples have been reported.[20,21] The results of these studies and that organised by our section[22] indicated that widespread results were obtained from different laboratories. For many parameters, interlaboratory relative standard deviations (after the rejection of outliers) ranged from 30 to 60%, suggesting that there was a need to improve the accuracy of data obtained from PAH determination in environmental samples. Although several certified reference materials (CRM) for PAH determination have been prepared by the US National Bureau of Standards,[23] only one of them, a sample of urban particulate matter, was in an environmental matrix. In order to fulfil quality assurance and method research requirements, the development of sediment CRMs for PAH is therefore necessary.

In this paper, we describe the development and certification of the first in a series of sediment reference materials for method evaluation and other in-house and interlaboratory quality assurance applications. Certified or reference values for the following 14 PAH are reported: phenanthrene (phen), anthracene (anth), fluoranthene (F), pyrene (Py), benzo[*a*]anthracene (B[*a*]A), chrysene (chry), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*e*]pyrene (B[*e*]P), benzo[*a*]pyrene (B[*a*]P), perylene (pery), indeno[123*cd*]pyrene (I[*cd*]P), dibenz[*ah*]anthracene (D[*ah*]A) and benzo[*ghi*]perylene (B[*ghi*]P). All of the above, except B[*e*]P and perylene, are listed as US EPA priority pollutants.[24]

Experimental

Preparation of Sediment Reference Material

Approximately 450 kg of wet sediment were collected from a landfill site in Hamilton Bay, Ontario, Canada. The sample, coded EC-1, was freeze-dried, crushed, sieved, blended and subsampled. Further details of this procedure have been published elsewhere.[25,26]

Extraction of PAH in Sediments

A 10.00 g aliquot of EC-1 was extracted in a Soxhlet apparatus with 350 ml of 59 + 41 *V*/*V* acetone - hexane for 8 h at a rate of 8 cycles h^{-1}. For the comparison of recoveries, Soxhlet

extraction with other solvent systems and extraction using a sonicator[25,26] were also evaluated. The results of this comparison are given under Results and Discussion.

Clean-up of Sediment Extracts

The combined organic extract was shaken with 400 ml of 2% $KHCO_3$ in a 1-l separating funnel for 1 min with frequent venting. After the layers had separated, the aqueous layer was drained into a 500-ml separating funnel and then discarded. The organic layers in the two separating funnels were combined and passed through 100 g of anhydrous Na_2SO_4 in a column. The funnels were washed with 2 × 10 ml of hexane and the washings were again applied to the column. After the last trace of solvent was removed from the Na_2SO_4 column by vacuum suction, the dry extract was evaporated down to *ca.* 5 ml using a rotary evaporator with a 35 °C water-bath.

A 400 × 10 mm i.d. glass clean-up column with either a coarse porosity fritted disc or a glass-wool plug was filled with a freshly prepared slurry of 10.0 g of silica gel (Davison grade 923, 100–200 mesh activated at 130 °C for 18 h before use) in hexane with 1 cm of anhydrous Na_2SO_4 at the top. The concentrated sediment extract in hexane was quantitatively transferred on to the column and drained just into the Na_2SO_4 layer. The sample flask was rinsed with 2 ml of hexane and the rinsing again applied to the column. This process was repeated twice. The column was then eluted with 50 ml of hexane and the eluate was discarded. This fraction contains chlorobenzenes, PCBs and several chlorinated insecticides if they are also present in the sample. The column was further eluted with 60 ml of 40 + 60 *V/V* dichloromethane - hexane. This fraction was collected in a 250-ml round-bottomed flask and was evaporated down to *ca.* 5 ml using a rotary evaporator as described above. After the addition of 20 ml of hexane and 3 ml of isooctane, the evaporation was repeated until the volume was *ca.* 3 ml.

A second clean-up column was prepared by filling a 230 × 5 mm i.d. disposable Pasteur pipette having a glass-wool plug at the bottom with 5 cm of activated neutral alumina (Woelm, Brockmann activity 1, 100–200 mesh) and 5 mm of anhydrous sodium sulphate at the top. This column was eluted with 5 ml of hexane and the eluate was discarded. The concentrated extract, after silica gel column clean-up, was applied to the column, rinsing through with 3 × 1 ml of hexane. The column was further eluted with hexane until a total of 10.0 ml of hexane was collected. This fraction contained aliphatic hydrocarbons and other non-polar co-extractives that had not been removed by the silica gel column. The PAH were removed from the alumina column by elution with toluene until a volume of 10.0 ml was collected.

Gas Chromatography with Flame Ionisation Detection (GC - FID)

A Hewlett-Packard 5880A gas chromatography equipped with a Grob-type split - splitless capillary injection port, a flame ionisation detector, a 7671A autosampler and Level IV terminals was used. A 30 m × 0.25 mm i.d. DB-5 fused-silica capillary column of 0.1 µm film thickness (J and W Scientific) operating under the following conditions was used for PAH analysis. Temperatures: injection port and detector, 275 °C; oven initial temperature 70 °C, hold 1.5 min at 70 °C, programming rate 1, 30 °C min^{-1} (from 70 to 160 °C), rate 2, 2 °C min^{-1} (from 160 ° to 260 °C), hold 10 min at 260 °C. Flow-rates: hydrogen, 30 ml min^{-1}; air, 240 ml min^{-1}; detector make-up gas (helium), 25 ml min^{-1}. Carrier gas, helium; column head pressure, 15 lb in^{-2}. Splitless valve on for 90 s. A 2-µl aliquot of the final extract was injected in the splitless mode without dilution.

Gas Chromatography with Mass Spectrometry (GC - MS)

The system consisted of a Hewlett-Packard 5880A gas chromatograph, as described above, a 5970B mass-selective detector (MSD) a 9816S computer and a 9133XV disc drive. The DB-5 capillary column was directly interfaced with the electron-impact ion source (70 eV) for maximum sensitivity. The GC operating conditions were identical to those used in the FID determination, except that the detector gases were not needed and the column head pressure was 4 lb in^{-2}. A 2-µl aliquot of a 20-fold diluted sample extract was analysed. The data were acquired by the following two modes: (a), linear scanning from *m/z* 50 to 300 in order to obtain abundance data of major fragments for compound identification purposes; and (b), selected ion monitoring (SIM) for quantitative analysis. In the latter instance, the following molecular ions characteristic of PAH were monitored: 1, *m/z* 178 for phen and anth; 2, *m/z* 202 for F and Py; 3, *m/z* 228 for B[*a*]A and chry; 4, *m/z* 252 for B[*b*]F, B[*k*]F, B[*e*]P, B[*a*]P and pery; 5, *m/z* 276 for I[*cd*]P and B[*ghi*]P; and 6, *m/z* 278 for D[*ah*]A. The dwell time for each ion was 100 ms. Three labelled internal standards, *i.e.*, phen-d_{10}, chry-d_{12} and B[*ghi*]P-$^{13}C_{12}$ were used for the calibration of response factors.

High-performance Liquid Chromatography (HPLC)

A system including a Waters Model 510 pump, a Rheodyne 7125 loop injector and 20- µl loop, a 4.6 mm i.d. × 25 cm long Zorbax ODS column (DuPont, 5–6 µm particle size) and a Schoffel Model FS 970 fluorescence detector were used. Mobile phase (isocratic), 85 + 15 acetonitrile - water; flow-rate, 1.0 ml min^{-1}. The detector wavelength was set at 280 nm (excitation) and 389 nm (emission). A 20-µl aliquot of a 100-fold diluted sample extract was injected.

Standards and Standard Solutions

Most PAH standards are available from Aldrich Chemical or Eastman Kodak. Certified reference materials of PAH are also available from the Commission of European Communities, BCR, Brussels. Individual stock solutions were prepared by dissolving 50.0 mg of each PAH in a 100-ml low actinic calibrated flask with toluene; some PAH required gentle heating or sonification to dissolve. Appropriate amounts of the 14 PAH stock solutions in proportions similar to those found in EC-1 were pipetted into a low actinic calibrated flask and diluted to volume with toluene. This solution, which contained PAH at µg ml^{-1} levels, was used in the GC analyses as an external standard. A standard for the HPLC analysis of EC-1 was prepared by diluting the above solution with the HPLC mobile phase.

Results and Discussion

The sediment reference material EC-1 was originally prepared and analysed for PCBs.[25] It is a fine (200–325 mesh), silty clay sediment naturally contaminated with many toxic organics and metals. Although homogeneity tests were not performed for PAH before subsampling, subsequent determinations on various lots of sediment subsamples did not reveal any inhomogeneity for PAH (Table 1). Therefore, EC-1 is considered sufficiently homogeneous for use as a PAH reference material.

Soxhlet extraction has been used by many workers for the determination of organics, including PAH, in sediments. Although most of the extraction in this work was carried out with 41 + 59 hexane - acetone, comparative extraction was also investigated using solvent systems such as 1 + 1 benzene - methanol, cyclohexane and hexane. The results for the

determination of PAH in EC-1 under various extraction conditions are given in Table 2. No difference in PAH recoveries from EC-1 subsamples was observed between the four solvent systems, although the non-polar solvents (cyclohexane and hexane) gave extracts much lighter in colour. Further experiments by Soxhlet extraction were all carried out with the 41 + 59 hexane - acetone because this solvent was easy to evaporate and was also used in our multi-residue extraction procedure. A longer extraction time (24 *vs.* 8 h) did not produce higher results (Table 2). Ultrasonic extractions[25] of EC-1 with 1 + 1 hexane - acetone were also carried out, and again recoveries of PAH by this technique were identical to those obtained by the Soxhlet method (Table 2). As different solvent systems and different extraction methods gave the same results, it was therefore concluded that the extraction recoveries of PAH from EC-1 were quantitative in these experiments.

Table 1. Homogeneity test of various lots of EC-1 subsamples for PAH. Concentrations in μg g^{-1}

Bottling sequence	Start		Middle		End	
Bottle no.	144	434	1014	2029	3769	4784
F	19.6	21.9	20.5	20.9	21.2	20.6
B[*a*]A	8.9	7.5	8.3	7.8	8.0	8.5
B[*a*]P	5.2	4.6	4.5	4.9	4.7	4.4
I[*cd*]P	5.0	5.0	4.3	5.6	4.5	4.7

A rotary evaporator was used to evaporate organic extracts containing PAH. At a water-bath temperature of 40 °C, this technique was found to be satisfactory for the 14 PAH determined in this work. Quantitative recoveries of the hydrocarbons were obtained unless the solution was evaporated to dryness. However, if the determination of the more volatile PAH, such as naphthalene, is required, a Kuderna - Danish evaporator equipped with a three-stage Snyder column should be used to minimise evaporative losses of the volatiles.

Silica gel, neutral alumina and Florisil have been commonly used for the clean-up of sediment extracts containing PAH.[2,11,27,28] Fully activated silica gel and neutral alumina both gave ≥95% recoveries of all PAH when microgram amounts of the hydrocarbons were spiked directly on to the columns. Activated Florisil also worked well for most PAH, however, as reported earlier, the satisfactory recovery of B[*a*]P could not be obtained on this column.[11] Gel permeation chromatography with Sephadex LH-20 is also a popular approach to the clean-up of sediment extracts for PAH determination,[2,20,28,29] especially for the separation of aliphatic and aromatic hydrocarbons. In this work, clean-up of sediment extracts was carried out using a 20-g Sephadex LH-20 column with a 1 + 1 benzene - methanol elution system according to the method of Giger and Schaffner.[2] However, it was found that in our work the Sephadex column did not further improve the clean-up of the EC-1 extracts after they were subjected to the silica gel and alumina columns.

Table 2. Concentrations of PAH (μg g^{-1}) in EC-1 obtained under various extraction conditions. Average of three analyses

Solvent system*	A	B	C	D	A	E
Extraction method	Soxhlet	Soxhlet	Soxhlet	Soxhlet	Soxhlet	Ultrasonic
Extraction time	8 h	8 h	8 h	8 h	24 h	3 × 3 min
Replicates	3	3	3	3	3	3
Phen	15.9	16.0	14.7	15.9	15.2	14.2
Anth	1.3	1.7	1.2	1.3	1.5	1.0
F	22.2	21.5	23.3	22.7	22.4	21.4
Py	17.2	15.1	15.8	16.8	16.2	16.8
B[*a*]A	8.5	8.3	8.2	8.0	8.2	10.2
Chry	8.4	8.3	8.2	7.5	8.7	7.5
B[*b*]F	9.0	7.9	8.3	7.8	7.8	8.2
B[*k*]F	4.2	4.4	5.2	4.0	4.6	3.6
B[*e*]P	5.3	4.9	5.1	4.6	5.0	4.4
B[*a*]P	5.0	4.8	5.1	4.9	5.1	4.9
Pery	0.8	0.7	1.0	0.8	0.8	0.8
I[*cd*]P	5.2	5.2	5.3	2.9	5.3	5.4
D[*ah*]A	1.4	0.8	1.5	1.3	1.5	1.2
B[*ghi*]P	4.3	2.6	4.8	2.9	4.4	4.4

* A = 41 + 59 hexane - acetone; B = 1 + 1 benzene - methanol; C = cyclohexane; D = hexane; and E = 1 + 1 hexane - acetone.

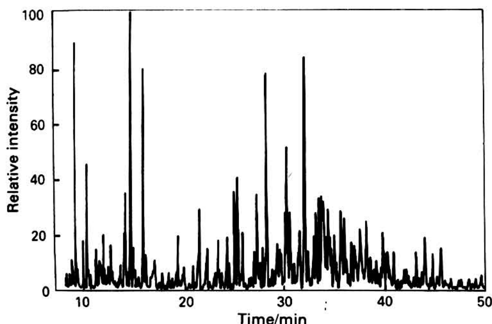

Fig. 1. Total ion current chromatogram of EC-1 extract after silica gel and alumina column clean-up

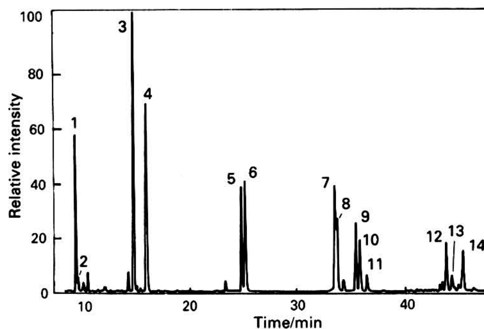

Fig. 2. Reconstructed multi-ion current profile of EC-1. See under Experimental for the ions monitored. Identity of peaks: (1) phen, (2) anth, (3) F, (4) Py, (5) B[*a*]A, (6) chry, (7) B[*b*]F, (8) B[*k*]F, (9) B[*e*]P, (10) B[*a*]P, (11) Pery, (12) I[*cd*]P, (13) D[*ah*]A and (14) B[*ghi*]P

Table 3. Mean concentrations of selected PAH (μg g^{-1}, dry mass) in reference material EC-1. Uncertainty is one standard deviation

	GC-FID	GC-MSD	HPLC*
No. of analyses	30	12	30
Phen	15.8 ± 1.2	16.2 ± 1.5	14.9 ± 0.4‡
Anth	1.3 ± 0.3	1.2 ± 0.3	0.8 ± 0.1‡
F	22.5 ± 2.0	23.6 ± 1.9	23.8 ± 2.1
Py	16.8 ± 1.9	17.8 ± 1.5	16.2 ± 2.1
B[*a*]A	8.5 ± 0.9	8.7 ± 1.0	8.8 ± 0.6
Chry	9.2 ± 0.9†	9.6 ± 1.1†	7.9 ± 0.8‡
B[*b*]F	7.6 ± 1.2	8.5 ± 0.9	8.0 ± 0.5
B[*k*]F	4.5 ± 0.6	4.4 ± 0.6	4.4 ± 0.3
B[*e*]P	5.2 ± 0.6	5.7 ± 0.8	5.4 ± 0.3‡
B[*a*]P	5.4 ± 0.7	5.8 ± 0.7	5.0 ± 0.6
Pery	0.8 ± 0.2	1.7 ± 0.2	1.7 ± 0.1‡
I[*cd*]P	5.8 ± 0.5	6.1 ± 1.0	5.5 ± 0.4
D[*ah*]A	1.3 ± 0.2	1.5 ± 0.2	1.1 ± 0.2
B[*ghi*]P	4.6 ± 0.7	5.4 ± 1.0	4.9 ± 0.4

* Results obtained by fluorescence detection except phen, anth, chry, B[*e*]P and pery.

† Results include triphenylene.

‡ Results obtained by UV detection and no. of replicates was five.

Table 4. Interlaboratory results on selected PAH (μg g^{-1}) in EC-1

Parameter	No. of results*	Range	Median	Mean ± s.d.
Phen	11	9.9–24.35	16.80	16.57 ± 4.59
Anth	9	0.35–13.18	1.50	3.92 ± 4.70
F	15	14.87–45.3	21.81	23.45 ± 7.39
Py	13	9.58–26.0	18.50	18.42 ± 5.21
B[*a*]A	11	4.6–15.6	7.60	8.41 ± 3.04
Chry	10	6.7–44.0	8.80	13.70 ± 11.86
B[*b*]F	11	3.68–15.2	6.75	8.08 ± 3.64
B[*k*]F	11	2.8–16.61	3.63	5.58 ± 4.17
B[*e*]P	8	3.12–7.76	5.36	5.55 ± 1.50
B[*a*]P	15	2.61–30.0	4.50	6.58 ± 6.77
Pery	7	1.05–2.19	1.16	1.49 ± 0.48
I[*cd*]P	11	3.12–7.6	4.90	5.10 ± 1.36
D[*ah*]A	10	1.44–11.0	2.35	3.62 ± 2.35
B[*ghi*]P	13	0.45–20.31	4.73	7.32 ± 6.48

* Some laboratories did not provide all the results obtained.

Therefore, Sephadex and Florisil column clean-ups were not employed.

At the early stage of PAH determination, a 12-m OV-1 capillary column was used. This column successfully resolved all 14 PAH of interest, including the following isomeric pairs: phen and anth, B[*a*]A and chry, and B[*b*]F and B[*k*]F. However, even better resolution of the PAH could be obtained by using a 30-m, thin-film DB-5 column. In the latter instance, base-line resolution was observed for all 14 PAH except B[*b*]F and B[*k*]F. This efficient column provided adequate resolution of the PAH and co-extractives in this complex EC-1 sample (Fig. 1).

Initially, the PAH in EC-1 were identified by their retention times as they were chromatographed on the OV-1 and DB-5 columns. Their identities were positively confirmed by operating the MSD in the scanning mode and comparing the mass spectra with authentic standards. For all 14 PAH, the match quality were better than 9 800 (best match = 10 000). As chrysene and triphenylene were not resolved on our GC columns and their mass spectra were very similar, we were not able to tell which one of the two, or whether a mixture of both PAH was present in EC-1. Nevertheless, this peak was quantitated against a chrysene standard in subsequent determinations. By monitoring only the characteristic molecular ions of the PAH, the MSD was extremely selective, as indicated by the reconstructed multi-ion current profile of an EC-1 extract (Fig. 2) *versus* the total ion current chromatogram (Fig. 1). As the molecular ion is the most abundant ion for each PAH, SIM quantitation of these PAH was also highly sensitive. Under the conditions used the MSD was approximately 100 times more sensitive than the FID in these PAH determinations.

Table 5. Certified concentration of selected PAH in reference material EC-1. Uncertainty is one standard deviation

Parameter	Concentration/ μg g^{-1}
Phen	15.8 ± 1.2
F	23.2 ± 2.0
Py	16.7 ± 2.0
B[*a*]A	8.7 ± 0.8
B[*b*]F	7.9 ± 0.9
B[*k*]F	4.4 ± 0.5
B[*e*]P	5.3 ± 0.6
B[*a*]P	5.3 ± 0.7
I[*cd*]P	5.7 ± 0.6
B[*ghi*]P	4.9 ± 0.7

Because of availability of equipment, only nine of the 14 PAH in EC-1 were determined by HPLC. The samples were chromatographed on a reversed-phase C_{18} column and the nine PAH were detected by a filter fluorimeter (Table 3). Under isocratic conditions, several PAH eluted closely with each other and were not separated by modification of mobile phase composition. For example, B[*a*]A and chry, and B[*b*]F, pery and B[*e*]P were the two groups of co-eluting PAH. However, by operating the fluorescence detector at λ_{ex} 280 and λ_{em} 389 nm, B[*a*]A was selectively detected in the presence of chry as the latter had very little fluorescence sensitivity at such wavelengths.[7,10] For similar reasons, the determination of B[*b*]F was not significantly interfered with by the presence of B[*e*]P and perylene in the same sample. Extracts of EC-1 were also determined by an independent laboratory for the 14 PAH using the gradient elution HPLC technique detailed in the US EPA Method 610.[24] Under these conditions, all 14 PAH were resolved and they were quantitated by the fluorescence and UV detectors connected in series.

The GC-FID, GC-MSD and HPLC results of the PAH in EC-1 are summarised in Table 3.

Recently, we organised an interlaboratory study on the determination of PAH in sediment samples, including EC-1.[22] The interlaboratory results submitted by 14 Canadian participants were generated by a variety of extraction, clean-up and detection methods. Interlaboratory round-robin results alone are generally considered insufficient to certify environmental reference materials as the precision and accuracy of these results are obtained in an uncontrolled manner. Nevertheless, based on what we have learned in the 100 or more interlaboratory studies organised by us, the interlaboratory mean or median results are usually good estimates of the true values in unknown samples. As shown in Table 4, the interlaboratory results further confirmed our in-house results as the two were in good agreement with each other.

Levels for ten PAH (phen, F, Py, B[*a*]A, B[*b*]F, B[*k*]F, B[*e*]P, B[*a*]P, I[*cd*]P and B[*ghi*]P) in EC-1 were certified and their values are listed in Table 5. In these instances, the agreement between the in-house results using three independent detection techniques (Table 3) were all better than ±10%. The certified values were obtained by calculating the weighted averages of the pooled in-house results[26] and the uncertainty was one standard deviation. Values for chrysene could not be ascertained because both GC methods were unable to resolve chrysene and triphenylene. The other three PAH are relatively minor components in EC-1 and the results listed in Table 3 are for information only.

Currently, this sediment reference material is kept at −20 °C in the dark. No degradation of PAH in the sample has

been detected since the work was initiated approximately two years ago.

In conclusion, a naturally contaminated lake sediment certified reference material was developed and certified for ten PAH at the μg g^{-1} level. The certified values were derived by repetitive in-house analysis using three different methodologies, *i.e.*, GC - FID, GC - MS and HPLC techniques. These values were further confirmed by an interlaboratory study. This material is a valuable tool in the development and evaluation of analytical methods for PAH, and for the generation of accuracy statements in in-house and interlaboratory quality assurance activities in such analysis.

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Analytical Reference Materials

Part VII.* Development and Certification of a Sediment Reference Material for Total Polychlorinated Biphenyls†

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A lake sediment reference material naturally contaminated with PCBs was prepared. Subsamples of this material were subjected to repetitive in-house analyses for total PCBs. The quantitative recovery of PCBs was demonstrated by performing ultrasonic and Soxhlet extractions under various conditions. Sample extracts were usually cleaned-up with Florisil and the cleaned extracts were shown to be free from other major interferences, except for sulphur, which was later removed by mercury. The presence of PCBs in the samples was confirmed by the perchlorination of the sample extract and by GC - MS techniques. Most of the sample extracts were quantitated against a 1 + 1 + 1 mixture of Aroclors 1242, 1254 and 1260 on a 3% OV-1 packed column using the technique described by Webb and McCall. These results were further confirmed by the quantitative GC - MS analyses of EC-1 and by interlaboratory results provided by independent laboratories. The reference material showed no signs of degradation of its PCB content over a six-year storage period at −20 °C in the dark.

Keywords: *Polychlorinated biphenyl determination; certified reference materials; quality assurance; lake sediment samples*

Aroclors or mixtures of polychlorinated biphenyls (PCBs) with various percentages of chlorine by weight were manufactured in the United States by Monsanto Chemical Company. Because of their general inertness, PCBs were widely used as transformer dielectric fluids, plasticisers and flame retardants, etc., in industry. Although the production of Aroclors has been curtailed in recent years owing to government regulations, the disposal, dump leaching and accidental spillage of the millions of pounds of these persistent Aroclors manufactured in the preceding years still cause environmental pollution problems in air, water and biota samples.

Aroclors are classified as priority pollutants by the US Environmental Protection Agency and environmental monitoring of the compounds was begun in the 1960s because of their toxicity and persistence. Although PCB determinations are considered routine, the results from many naturally contaminated samples are often unsatisfactory, as indicated by the many interlaboratory round-robin studies organised by our section[1,2] and by other parties.[3,4] Typically, the interlaboratory relative standard deviation of PCB results in naturally contaminated sediment samples at low μg g^{-1} levels is between 25 and 50%. Hence, there is a need to develop a real-life reference material in order to monitor the performance of laboratories involved in PCB determinations.

Several years ago, our section initiated the research and development of a number of sediment certified reference materials (CRMs) for toxic organics such as PCBs,[5] chlorobenzenes[6] and polynuclear aromatic hydrocarbons,[7] in order to fulfil the requirements of our on-going quality assurance programmes. Two marine sediment CRMs were recently available commercially, however, no details were given on how these CRMs were characterised. In this paper, we report our rigorous approach to analyse and certify the total PCB contents in our sediment CRM coded EC-1.

* For Part VI of this series, see p. 31.

† This material is currently not for sale and not available for general distribution.

Experimental

Preparation of Sediment Reference Material

Detailed procedures for sample collection, preliminary drying, freeze-drying, blending, bottling and homogenity testing before and after subsampling have been described in previous publications.[5,6]

Extraction and Clean-up of Sediment Samples

The Soxhlet and ultrasonic extractions of PCBs in sediment samples and the partitioning and evaporation steps were identical to those employed for the chlorobenzene determinations.[6] The clean-up of sediment extracts was carried out in a 500 × 19 mm i.d. glass column filled with 20.0 g of activated Florisil and 10 mm of anhydrous sodium sulphate at the top. The PCBs in the extract were eluted by 200 ml of hexane. After the addition of 3 ml of iso-octane and a few boiling chips, this hexane fraction was evaporated down to *ca.* 3–5 ml using a three-stage macro Snyder column and a heating mantle. The concentrated extract was diluted to 10.0 ml with iso-octane. Sulphur and sulphur compounds in the cleaned-up extract were removed by vigorous agitation with mercury until the metal remained shiny.

Gas Chromatography with Electron-capture Detection (GC - ECD)

A Hewlett Packard 5700 series gas chromatograph equipped with a Ni-63 electron-capture detector, a Model 7671A autosampler and a Model 3390 reporting integrator was used. The column was a 1.8 m × 2 mm i.d. glass column packed with 3% OV-1 on 100–120 mesh Gas Chrom Q. The temperatures were: injection port, 250 °C; detector, 300 °C; and column, 185 °C. The flow-rate was 25 ml min^{-1} and the carrier gas argon - methane (95 + 5). Aroclors 1242, 1254 and 1260 were supplied by the US Environmental Protection Agency. The working standard was a mixture of 1 + 1 + 1 Aroclors 1242, 1254 and 1260 in iso-octane with a total concentration of 600 pg μl^{-1}. The quantitation of PCBs was carried out by the peak matching technique described by Webb and McCall.[8]

Table 1. Ions, congeners and concentrations of calibration standards used in the capillary column GC - MSD analysis of EC-1

Homologue	m/z		Calibration congener	
	Quantitate ion	Confirm ion	Congener no.*	Concentration/ pg µl^{-1}
Cl_1	188	190	1	50
Cl_2	222	224	5	50
Cl_3	256	258	29	50
Cl_4	292	290	50	100
Cl_5	326	328	87	100
Cl_6	360	362	154	100
Cl_7	394	396	181	150
Cl_8	430	432	200	150
Cl_9	464	466	209	250
Cl_{10}	498	500	209	250
Chrysene-d_{12}	240	—	—	250

* Adopted from reference 9.

Table 2. Summary of results (µg g^{-1} total PCBs) from EC-1 sediment reference material by packed column Webb - McCall quantitation method

Extraction	Ultrasonic	Soxhlet
No. of analyses	97	72
Range/µg g^{-1}	1.85–2.15	1.88–2.17
Mean ± SD	2.02 ± 0.07	1.97 ± 0.08

Gas Chromatography with Mass-selective Detection (GC - MSD)

A Hewlett Packard 5880A gas chromatograph equipped with a split - splitless injection port, a Level II terminal, a Model 7671A autosampler, a Model 5970B mass-selective detector (MSD), a Model 9816S computer and a Model 9133XV 15 megabytes disc drive were used, together with a 30 m × 0.25 mm i.d. DB-5 fused-silica capillary column, which was directly interfaced to the electron impact ion source for maximum sensitivity. The GC conditions were: injection port, 275 °C; interface, 280 °C; column initial temperature, 70 °C (held for 1.5 min); programming rate, 30 °C min^{-1} (70–170 °C), 2.5 °C min^{-1} (170–260 °C); and oven temperature held at 260 °C for 15 min. The splitless valve was on for 1.5 min and the column head pressure was 4 lb in^{-2}. Fully automated sample injection, data acquisition, data editing and report generation was made possible with the existing "Sequencing" software and a keystroke program on the GC terminal. The detector was operated in the selected ion monitoring (SIM) mode for both confirmation and quantitation. In both instances, two characteristic ions (one for quantitation and the other for confirmation) were monitored for each PCB homologous series (Table 1). For quantitative work, the procedure described by Budde and co-workers[10,11] was used, except that the 2,2′,3,4,4′,5,6-heptachlorobiphenyl (congener 181) instead of 2,2′,3,4′,5,6,6′-heptachlorobiphenyl (congener 188) was used owing to availability. Chrysene-d_{12} was used as an internal standard and the dwell time for each ion was 100 ms.

Sediment extracts were quantitated against an iso-octane mixture of nine congeners of various concentrations (Table 1). At each level of chlorination, one PCB congener in the calibration mixture was used as the concentration standard for all isomers in that group, *e.g.*, congener 29 was used to quantitate all trichlorobiphenyls in the sample. The only exception was that decachlorobiphenyl was used as the concentration calibration standard for both nona- and deca-chlorobiphenyls in the sample. The total PCB concentration was obtained by the summation of all concentrations in each homologous series. All PCB congeners were obtained from Ultra Scientific, Hope, RI, USA. Chrysene-d_{12} was obtained from Aldrich Chemical, Milwaukee, WI, USA.

Results and Discussion

Efforts were made to ensure that the PCBs in EC-1 were quantitatively recovered. The ultrasonic and Soxhlet extraction techniques that are routinely used for the extraction of organics in sediments were employed and compared. In order to achieve a valid comparison between the extraction methods, identical clean-up (Florisil column) and quantitation (packed column Webb - McCall) procedures (see later discussions) were used in these samples.

A total of 97 PCB determinations were carried out on EC-1 by the ultrasonic extraction technique using a 1 + 1 mixture of acetone and hexane. A total of 72 determinations were also carried out on the same certified reference material by Soxhlet extraction using 59 + 41 acetone - hexane. All of these results are summarised in Table 2. It is obvious from these data that nearly identical results were obtained from both techniques. As the presence of moisture has been reported to provide better recoveries of some organochlorines in soil samples,[12] EC-1 samples with 0 or 30% moisture content were Soxhlet extracted simultaneously for comparison. A *t*-test was applied to the means of these results and no difference was found in these means at the 95% significance level, indicating that a moisture content of 0 or 30% in the sediment samples had no effect on the recovery of PCBs. Another *t*-test was applied to the sample results obtained by ultrasonic extraction under similar conditions and again no difference in PCB recovery was observed.

As similar PCB results were obtained from samples Soxhlet extracted for 3, 7, 20 and 72 h, it was implied that PCBs were exhaustively removed from this sediment after 3 h of Soxhlet extraction. Different solvents were also employed to see if the recovery of PCBs was solvent dependent. The results indicated that both acetone and methylene chloride gave similar recoveries of PCBs to 59 + 41 acetone - hexane, whereas non-polar solvents such as hexane and benzene gave slightly lower (*ca.* 90%) recovery.

Florisil has been used by many workers[13] to remove co-extractives in sediment samples before organochlorine and PCB determinations. Although some workers have preferred to use Florisil deactivated with a few percent. of water for the column clean-up, activated Florisil was used in this study because it gave a better separation of some organochlorines and polynuclear aromatic hydrocarbons from PCBs.

In order to ensure that the Florisil clean-up was effective, some of the samples were subjected to additional clean-up steps. In triplicate runs, the PCB fraction of the Florisil-cleaned EC-1 extract was further cleaned-up on a Celite and 3% deactivated silica-gel column[14] and, in another instance, on an activated neutral alumina column,[15] according to published methods. In both instances, no change in the PCB profile and amount could be observed in the EC-1 extracts

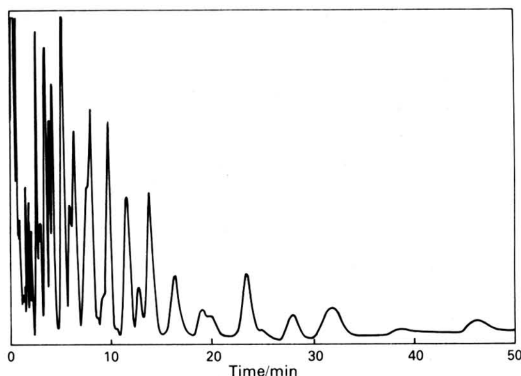

Fig. 1. GC - ECD chromatogram of the PCB fraction in EC-1. A 1.8 m × 2 mm i.d. 3% OV-1 column was used

Table 3. Composition of PCBs in EC-1 as determined by quantitative GC - MS analyses (six replicates). Uncertainty is one standard deviation

Homologue	EC-1 Concentration/ ng g^{-1}
Cl_1	Nd*
Cl_2	Nd
Cl_3	184 ± 12 (9.9)†
Cl_4	453 ± 24 (24.3)
Cl_5	688 ± 33 (36.9)
Cl_6	280 ± 21 (15.0)
Cl_7	161 ± 6.7 (8.6)
Cl_8	98 ± 7.6 (5.3)
Cl_9	Nd
Cl_{10}	Nd
Total concentration	1864

* Nd = None detected.
† Figures in parentheses show % *m/m* of each chlorobiphenyl in EC-1.

after additional clean-up. The Florisil-cleaned EC-1 extract was also subjected to an ethanolic KOH treatment at 80–90 °C for 30 min. Again, no change in the PCB components could be observed before and after the additional clean-up. These experiments indicated that Florisil-cleaned EC-1 extracts were free from any major interference from other organochlorines. It should be noted that a few chlorobenzenes, *p,p'*-DDE and Mirex present in EC-1 could not be separated from the PCBs by column chromatography. Their presence, however, would not affect the PCB results as their concentrations were relatively low compared to those of the PCBs.

Most of the EC-1 extracts were chromatographed on a 6-ft 3% OV-1 column operated at 185 °C. The PCBs were quantitated by the established peak-matching technique developed by Webb and McCall.[8] This method was used as it has been demonstrated to be better than other packed column techniques in a collaborative study.[16] It is still the official method for the quantitation of total PCBs in our Water Quality laboratories and is also approved by the US EPA (Method 608).[17] Samples were quantitated against a 1 + 1 + 1 mixture of Aroclors 1242, 1254 and 1260; preliminary runs of sample extracts had indicated that the PCB components in EC-1 were very similar to this mixture (Fig. 1).

Extracts of EC-1 were subjected to the perchlorination procedure described by Armour[18] using antimony pentachloride. The formation of decachlorobiphenyl in these reactions confirmed the presence of PCBs in the EC-1 extracts. The perchlorination results were not used quantitatively to determine the PCB concentration in EC-1 as the latter was a complicated mixture of several Aroclors. A concentrated extract of EC-1 (containing approximately 3.0 µg ml^{-1} of total PCBs) was analysed on a 30-m DB-5 column interfaced to a mass-selective detector. Data were acquired in the selected ion monitoring mode for the detection of the ten chlorobiphenyl homologous series, *i.e.*, from mono- to decachlorobiphenyl. Two characteristic ions were used for each homologous series: one for quantitation and the other for confirmation, as shown in Table 1. Although the mono-, di-, nona- and decachlorobiphenyls were not present in EC-1 in detectable amounts, the presence of tri-, tetra-, penta-, hexa-, hepta- and octachlorobiphenyls in this reference material was confirmed by the presence of both characteristic ions at the right retention times and in the expected ratios for each of the six homologous series listed above.

Table 4. Summary of interlaboratory results for total PCBs in EC-1

Study number	N-27	DQC-3
No. of laboratories	15	14
No. of results used*	25	12
Range of results/µg g^{-1}	0.96–3.41	1.11–3.26
Median/µg g^{-1}	1.96	1.75
Mean ± s.d./µg g^{-1}	2.05 ± 0.61	1.98 ± 0.69

* After rejection of outliers.

PCBs in EC-1 extracts were also quantitatively determined by GC - MSD using the method described by Budde and co-workers.[10,11] As PCB homologues have overlapping retention time windows, special precautions were taken to avoid interferences by PCB congeners containing more chlorines. Under the electron ionisation mode, a PCB molecule undergoes fragmentation by the loss of two chlorines, and to a lesser extent by the loss of HCl and Cl,[19,20] thus causing interference in the determination of PCBs with one or two less chlorine atoms. In this work, the level of chlorination in each PCB peak was previously determined by a full scan run of a concentrated Aroclor mixture. The level of chlorination in a sample PCB peak was first assigned by the observed relative abundance of the two corresponding characteristic ions. This, together with the information obtained in the full scan run, was generally sufficient to eliminate interference generated by fragmentation ions produced by co-eluting PCBs with more chlorines.

The results of the quantitative GC - MSD determination of PCBs in EC-1 are shown in Table 3. In this instance, the concentration of each PCB homologous series and the total PCB concentration were obtained. As indicated, the GC - MS results further confirmed the GC - ECD results as the total PCB concentrations obtained by these two different quantitative methods (Tables 2 and 3) varied by less than 10%. The over-all lower sensitivity of the MSD to most PCBs, especially the hepta- and higher chlorobiphenyls, rendered some PCB peaks undetected by this detector at low concentrations. This could be the reason why, in the example of EC-1, that the total PCB results obtained by mass-selective detection were slightly lower than those obtained by electron-capture detection.

Reference material EC-1 was used in two interlaboratory round-robin studies in two different years. In both instances, the participants were requested to analyse the material for total PCBs by using their own in-house methods and standards. The interlaboratory results (Table 4) were diversified because of the different extraction and clean-up methodologies and the calibration standards and quantitation techniques employed by various participants.[1,2] Despite all these variations, the interlaboratory medians and means of the PCB results in both studies were in excellent agreement with the in-house results as summarised in Tables 2 and 4.

The stability of PCBs in EC-1 under cold storage conditions (−20 °C in the dark) was monitored twice annually. The

results give no evidence of degradation during storage over the last six years.[21]

In conclusion, we have successfully prepared and certified a naturally contaminated lake sediment reference material (EC-1) for total PCB contents on the basis of 169 in-house determinations. The reference value, 2.00 ± 0.05 μg g^{-1} (uncertainty is one standard deviation), generated by combining all the GC - ECD results (Table 2), was further supported by GC - MS results and interlaboratory results in two round-robin studies. This certified reference material is currently being used in many of our intralaboratory and interlaboratory quality assurance programs for PCB determinations.

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Determination of Uranium(VI) in Process Liquors by Ion Chromatography

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Two methods are described for the determination of U^{VI} in process liquors using ion chromatography based on cation separation and cation - anion separation with ammonium sulphate - sulphuric acid as the eluent. The U^{VI} species is detected spectrophotometrically at 520 nm after post-column reaction with 4-(2-pyridylazo)resorcinol. Chromatographic and detector variables, such as eluent composition and concentration, metallochromic indicator concentration and eluent and indicator flow-rates, are discussed. The method is linear for peak heights up to 15 μg ml^{-1} and has a quantitation limit of 0.04 μg ml^{-1} using direct injection.

Keywords: *Uranium(VI) determination; ion chromatography; 4-(2-pyridylazo)resorcinol; process liquors; mixed-mode column*

An important requirement of the hydrometallurgical industry is the rapid, inexpensive and reliable determination of metallic species in process liquors and mill effluents. These analytical requirements have generally been met using a number of instrumental spectroscopic techniques, including flame emission spectrometry (FES), atomic absorption spectrometry (AAS) and atomic emission spectrometry (AES). When these and other related methods are combined with various excitation sources and aqueous solution handling procedures, at least 75 elements can be determined. It is to be expected that with this broad range of applicability particular examples will arise where an alternative technique should be considered. Uranium and certain other metals that form refractory oxides in a flame are difficult to determine by atomic absorption spectrometry. This problem is not serious when atomic emission spectrometry is applied in conjunction with direct and inductively coupled plasma excitation sources. Satisfactory determination of aqueous uranium in the μg ml^{-1} range is possible. Lynch *et al.*[1] have reported a flow injection method involving solvent extraction and adsorptiometric determination of uranium in leachates and effluents which gave a detection limit of 0.1 μg ml^{-1}.

Neutron activation analysis is a popular non-spectroscopic method for the determination of uranium in aqueous solution; however, the availability of a neutron source and the need for a preliminary de-watering step are important considerations. The detection limit claimed by commercial suppliers for this method using standard procedures is 0.01 μg ml^{-1}.

Our research has been directed towards studying the absorption - desorption characteristics of aqueous metallic species in contact with various microorganisms.[2] Of particular interest has been the behaviour of the uranium(VI) present in acid process leach liquor when in contact with biomass. The process liquor contains uranium(VI) at levels of the order of 200 μg ml^{-1} and substantial levels of other species, particularly iron(III). Metals such as thorium, zinc, copper, nickel and cobalt are also present in the liquor, which contains about 2.5 g l^{-1} of sulphate.

In the absorption - desorption studies of uranium(VI) in contact with biomass, both equilibrium and kinetic experiments are required. The large number of samples taken in these experiments were initially analysed for uranium(VI) using neutron activation analysis. However, reproducibility problems and the absence of a convenient neutron source prompted a search for an alternative method. Recently a number of reports[3-5] have indicated that ion chromatography can be applied to the determination of many transition metal species in aqueous solution. The method relies on the use of low-capacity cation- and anion-exchange materials. Cation-exchange separations are obtained using a surface-sulphonated microporous polystyrene - divinylbenzene resin. Anion-exchange separations are accomplished using the surface-sulphonated resin coated with aminated latex particles. This exchanger does exhibit a residual cation-exchange capacity. Both exchangers are reported to offer a high efficiency and excellent pH stability. A wide range of selectivity for transition metals can be achieved by a variation in particle size, functional groups, degree of latex cross-linking and, most importantly, by the use of both neutral and anionic complexing agents in the eluent. The determination of U^{VI} as UO_2^{2+} by ion chromatography was reported by Riviello using a sulphate eluent.[6,7] This paper describes the application of ion chromatography, post-column derivatisation and spectrophotometric detection[8,9] for the determination of U^{VI} in process leach liquors.

Experimental

All ion chromatographic analyses reported were carried out with a Dionex Series 2010i ion chromatograph equipped with a Dionex Ionpac membrane reactor for post-column derivatisation. Detection was at 520 nm using a Cary 219 UV-visible spectrophotometer fitted with a 10 mm (8 μl) flow cell. Two chromatographic columns were employed for the U^{VI} determinations, an HPIC-CS2 cation separator and HPIC-CS5 cation - anion separator (Dionex). Both separators were operated with the appropriate guard columns. Eluent reagents, ammonium sulphate and sulphuric acid were of analytical-reagent grade and were obtained from J.T. Baker Chemical Company. The metallochromic indicator, 4-(2-pyridylazo)resorcinol (PAR), was supplied as the mono-sodium salt monohydrate by Aldrich Chemical, Milwaukee, WI, USA. The indicator was supplied to the membrane reactor from a nitrogen pressurised reservoir. Other components of the indicator solution, ammonia solution and acetic acid were of analytical-reagent grade (J. T. Baker Chemical Company). Synthetic uranium solutions used for this study were prepared from analytical-reagent grade uranyl nitrate (BDH, Canada). A large carboy of biologically produced uranium process liquor was obtained from Dennison Mines, Elliott Lake, Ontario, Canada. The analysis of this bulk sample for U^{VI} was carried out by Dennison Mines. The reported U^{VI} concentration was confirmed by a commercial laboratory using neutron activation. The addition of other

metal species to synthetic uranium solutions was carried out using atomic absorption standards. The metal content of process liquors and synthetic solutions was determined using atomic absorption spectrometry.

Results and Discussion

Typical chromatograms of synthetic solutions containing 2.0 and 1.0 μg ml^{-1} of U^{VI} as UO_2^{2+} are shown in Fig. 1. These chromatograms were obtained using an anion - cation mixed-mode separator (Dionex HPIC-CS5) and a cation separator (Dionex HPIC-CS2) followed by post-column derivatisation and spectrophotometric detection. The chromatogram for the CS5 column exhibits an early peak (above and below the absorption base line), which results from water in the sample and unretained ionic species eluting in the void volume. This early dip has been eliminated in the chromatogram for the CS2 column by matching the sample matrix with the eluent. This was found to be good practice whenever possible, but is especially significant when separating ions at low levels.

In comparing the two columns, it was observed that the cation separator produced a UO_2^{2+} response that was 30% higher than the anion-cation separator under the same chromatographic conditions. Table 1 summarises the conditions used for operating both columns and the post-column detection system. These conditions are similar to those reported by Riviello[6,7] for the cation separator and were routinely used for U^{VI} determination. Alternative conditions were often used for certain analytical requirements; the effects of these variations on column performance serve to characterise the columns.

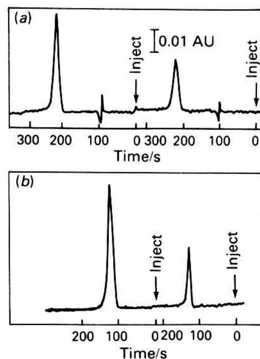

Fig. 1. Typical chromatograms for synthetic U^{VI} solutions, 1.0 and 2.0 μg ml^{-1}. (*a*) CS5 column and (*b*) CS2 column. Conditions as in Table 1

Table 1. Operating conditions for cation (Dionex HPIC-CS2) and cation - anion (Dionex HPIC-CS5) columns

Eluent	0.02 M $(NH_4)_2SO_4$ and 0.20 M H_2SO_4
Eluent flow-rate	1.0 ml min^{-1}
Column pressure drop	720–740 lb in^{-2} = 4960–5100 kPa (CS5) 480–510 lb in^{-2} = 3310–3520 kPa (CS2)
Metallochromic indicator	4 × 10^{-4} M 4-(2-pyridylazo)resorcinol 3.0 M NH_3 solution 1.0 M CH_3COOH
Indicator flow-rate	0.4 ml min^{-1}
Sample matrix	Variable
Sample volume	50 μl
Absorption scale	0.20 absorbance units full scale (a.u.f.s.)
Wavelength	520 nm

Table 2 summarises the column performance for the determination of U^{VI} employing both cation and cation - anion separation modes. Peak heights were normally used as an indication of U^{VI} concentration.

Fig. 2 shows chromatograms obtained by direct sample injections of 5.0, 2.0 and 1.0 μg ml^{-1} U^{VI} standards using the cation - anion separator column (CS5) under standard operating conditions. Similar performance was obtained using the cation separator column (CS2). Both columns produced chromatograms that verify the linearity of the chromatography up to 10 μg ml^{-1}.

An extension of the linear range was possible with an adjustment in operating conditions. An increase in the metallochromic indicator flow-rate was effective in increasing the range, but a much higher base-line variation was observed. A decrease in the eluent flow-rate at constant indicator flow-rate extended the linear range to 15 μg ml^{-1} with no adverse effect on the base-line stability, but the peak heights were slightly reduced.

As noted in Table 2 the retention times observed for the two separation modes were somewhat different. For the cation column separation, which shows a shorter retention time (125 s), it is possible that the complex formed with UO_2^{2+} in the eluent stream dissociates on the column and moves through as a simple aquated species. If the cation - anion column is used, it is possible that the anionic uranyl sulphate species present undergo anion exchange and that the corresponding aquated species are retained on the substrate resin by cation exchange. The dual functionality of this column would be expected to provide a superior selectivity for transition metals and may be the reason for the longer retention times.

The performance of the system was investigated using routine techniques and minor alterations in the column and detection conditions. As noted in Table 2, the quantitation limit is estimated to be 0.090–0.120 μg ml^{-1} using modest spectrophotometric sensitivity. The limit of detection is lower than the quantitation limit. An increase in injection volume from 50 to 100 μl reduced the detection limit proportionally. Larger injection volumes may be used in some instances, but when process samples containing high concentrations of other ionic species that are readily retained by the column are injected, the intervals between sample injection must be greatly increased to avoid interference from these slow eluting species.

An improvement in the quantitation limit is more readily achieved by increasing the sensitivity of the detecting instrument and reducing the base-line variation. In the case of the cation separator, using a full-scale absorbance range of 0.02 and a reduced metallochromic indicator concentration (1 × 10^{-4} M), with all other conditions remaining the same, the direct injection of a standard containing 0.10 μg ml^{-1} of U^{VI} yielded a peak height of about 11.5% of the full scale and a base-line variation of 2%. Fig. 3 shows the chromatograms obtained for three standard U^{VI} solutions containing 0.20,

Table 2. Column performance. Operating conditions as in Table 1

	CS2	CS5
Retention time*	125 s	225 s
Linear range	≤10 μg ml^{-1}	≤10 μg ml^{-1}
Peak height	13% full scale at 1.0 μg ml^{-1} (0.20 a.u.f.s.)	10% full scale at 1.0 μg ml^{-1} (0.20 a.u.f.s.)
Quantitation limit (direct injection)	0.090 μg ml^{-1}	0.120 μg ml^{-1}
Base-line variation	0.4% of full scale (0.20 a.u.f.s.)	0.4% of full scale (0.20 a.u.f.s.)
Signal to noise ratio	3 : 1	3 : 1

* Retention time reported as the time from sample injection to elution peak.

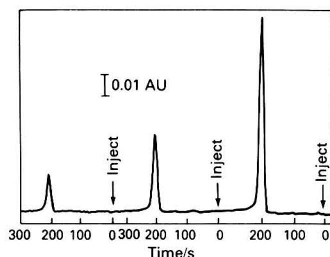

Fig. 2. Linearity verification, CS5 column. Synthetic U^{VI} solutions, 5.0, 2.0 and 1.0 μg ml^{-1}; conditions as in Table 1

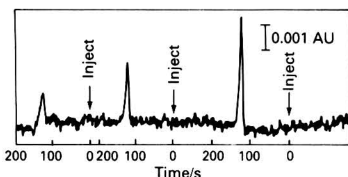

Fig. 3. Low-level chromatograms using CS2 column. Synthetic U^{VI} solutions, 0.20, 0.10 and 0.05 μg ml^{-1}; reagent, 1×10^{-4} M; other conditions as in Table 1

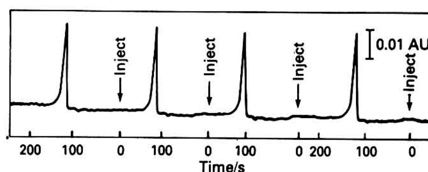

Fig. 4. Evidence of reproducibility of CS2 column. Synthetic U^{VI} solution, 1.10 μg ml^{-1}; conditions as in Table 1

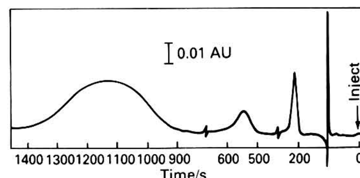

Fig. 5. Uranium process liquor chromatogram using CS5 column; conditions as in Table 1

0.10 and 0.05 μg ml^{-1} of U^{VI} using the above conditions. The quantitation limit if a signal to noise ratio of 3 : 1 is assumed is about 0.05 μg ml^{-1}. It was observed that if the $(NH_4)_2SO_4$ concentration in the eluent was increased to 0.08 M the peak height was enhanced by about 25% with no effect on the base-line variation but with the retention time reduced to about 100 s. Under these conditions the quantitation limit may be decreased to 0.4 μg ml^{-1}. The performance of the system using the cation - anion separator was found to be similar to this, but the reduced peak height response for this column resulted in a proportionately higher quantitation limit. The quantitation and detection limits of both columns could be greatly improved by the substitution of a concentrator column in place of the direct injection sample loop.

When analysing samples containing U^{VI} concentrations below about 0.50 μg ml^{-1} it was necessary to be particularly careful in flushing the entire system, otherwise sample to sample contamination resulted in memory effects. It was generally observed that the optimum chromatographic performance was achieved when the sample matrix was matched to the eluent. This was particularly true in the determination of low level samples. If the sample matrix is perfectly matched (which is not always possible) to the eluent, the water dip is eliminated.

Both the cation and cation - anion separator modes showed excellent reproducibility. Fig. 4 shows the chromatograms of four successive injections of a standard 1.1 μg ml^{-1} U^{VI} sample using the cation separator column. Taking the peak heights of the four peaks shown and a further four injections, the digitally recorded data gave a mean peak height absorbance above base line of 0.0283 and a standard deviation of 0.0005. The matrix of the sample used in this series was matched to the eluent, although minor mismatch was found to have little influence on reproducibility.

The delivery of a sufficient and constant supply of derivatisation reagent (PAR) to the post-column reactor was an important factor in achieving a linear response, acceptable reproducibility and minimum base-line variation. This did not normally present a problem. Some consideration was given to the question of the kinetics of colour development. Experiments conducted independently of the flow system showed that complete derivatisation occurred in less than 2 s. In our flow system, the time interval from the post-column contact of reagent and eluted U^{VI} to spectrophotometric detection was about 2 s. Increasing this time interval by 50% had no detectable effect on the analytical results if the tendency for peak broadening was ignored. It should be noted that this behaviour cannot be assumed for all metal species where post-column derivatisation and downstream spectrophotometric detection is employed. Work being carried out by the authors indicates that the detection of Th^{IV} by spectrophotometry using PAR requires a residence time of more than 40 s for full colour development.[10]

Synthetic samples containing U^{VI} (2.0 μg ml^{-1}) and Ni^{II}, Co^{II}, Cu^{II}, Zn^{II}, Fe^{II} (10.0 μg ml^{-1}), Fe^{III} (20.0 μg ml^{-1}) and Th^{IV} (2.0 μg ml^{-1}), both individually and in combination, were prepared in sulphate solution. Passing through the cation anion separator the bivalent metals appeared as one peak with a retention time of about 550 s. The elution of Fe^{III} did not begin until 800 s and resulted in a very broad peak with an estimated retention time of 1130 s. Chromatograms of samples containing U^{VI} and each metal in turn verified the makeup of the bivalent metal peak. Retention times varied from 530 s for Zn^{II} to 565 s for Co^{II}. As expected, Fe^{III} was strongly retained and was eluted at the same time as observed when present in the composite sample.

The presence of Th^{IV} was not observed on any of the chromatograms. This is probably because it was not sufficiently retained under the chromatographic conditions, or because the conditions for derivatisation were not appropriate for its detection. The reproducibility and linearity of the U^{VI} peak heights after many injections of composite samples was comparable to the performance observed for pure U^{VI} samples, indicating minimal impurity accumulation and column capacity loss.

An acid leach uranium process liquor (pH 2.3) was reported by Dennison Mines to contain 160 μg ml^{-1} of U^{VI}, 1120 μg ml^{-1} of Fe^{III}, 9.0 μg ml^{-1} of Zn^{II}, 2.6 μg ml^{-1} of Cu^{II}, 2.3 μg ml^{-1} of Co^{II} and 2.0 μg ml^{-1} of Ni^{II}, together with unreported amounts of aluminium, calcium, magnesium and thorium. The U^{VI} concentration was confirmed using neutron activation analysis by NAS, Hamilton, Ontario. This liquor was diluted 100-fold with the eluent and injected repeatedly into the system. Fig. 5 shows the chromatogram obtained for the sample of process liquor. This chromatogram was similar to that obtained for the synthetic composite sample. All the peaks were reproducible and there was no indication of ionic accumulation.

The presence of large amounts of Fe^{III} in uranium process liquors requires an interval at least 25 min between injections

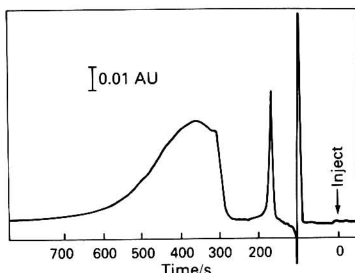

Fig. 6. Uranium process liquor chromatogram using CS5 column. Eluent, 0.08 M $(NH_4)_2SO_4$, 0.2 M H_2SO_4; other conditions as in Table 1

to ensure the complete elution of Fe^{III}. The Fe^{III} could be rapidly eluted by using oxalate, although re-establishing equilibrium with the original eluent would then need to precede a further determination. As a reasonable compromise, if the $(NH_4)_2SO_4$ level was increased to 0.08 M, the elution of Fe^{III} was complete within 12 min. Fig. 6 shows the chromatogram obtained.

The use of ion chromatography for the determination of U^{VI} in a wide range of aqueous solutions, both synthetic and industrial, is now routine practice in our laboratory. Over 500 samples have been analysed using the system described with no apparent loss of column response. Biological processes related to the sample create no observable problems in the procedure. The method deserves consideration for the determination of U^{VI} in the low μg ml^{-1} range and could be used successfully in the ng ml^{-1} range with pre-concentration.

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Gas Chromatographic Determination of Acrolein in Rain Water Using Bromination of *O*-Methyloxime

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A gas chromatographic method using an electron-capture detector was developed for the determination of acrolein based on the bromination of *O*-methyloxime. Acrolein was determined by gas chromatography with a 3% silicone Ge XE-60 packed column and the calibration graph showed good linearity in the range 0–0.06 μg ml^{-1} of acrolein in aqueous solution. The detection limit was 0.4 ng ml^{-1} of acrolein (signal to noise ratio = 2) and the relative standard deviation from five determinations of 0.04 μg ml^{-1} of acrolein in aqueous solution was 4.5%. This method is satisfactory for the selective and reproducible determination of trace amounts of acrolein in rain water.

Keywords: *Acrolein determination; gas chromatography; acrolein bromination;* O*-methyloxime; rain water*

Aldehydes are present in vehicle exhaust gases and are formed by photochemical reactions with hydrocarbons in air. As aldehydes are therefore related to photochemical oxidant concentrations, it is very important to be able to determine them in air, as an indication of air pollution levels. Acrolein is a particularly important aldehyde and spectrophotometric and fluorimetric methods[1–5] are generally used for its determination. Several methods using gas and liquid chromatography also have been reported for the determination of aldehydes, including acrolein,[6–8] but these methods are usually limited in either sensitivity or selectivity.

Oxime derivatives used in the gas chromatographic determination of carbonyls are methoximes,[9,10] benzyloximes,[11,12] *p*-nitrobenzyloximes[11] and pentafluorobenzyloximes.[13–15] For these methods, except for pentafluorobenzyloximes, flame-ionisation or nitrogen-specific detector systems are used.

Although a gas chromatographic method based on the bromination of acrolein has been reported in earlier papers,[16,17] the brominated product of acrolein is unstable and the reproducibility of the determination is poor. This paper reports a method for the sensitive and selective determination of acrolein involving the bromination of acrolein *O*-methyloxime and gas chromatography with electron-capture detection.

Experimental

Reagents and Materials

Methoxylamine hydrochloride (MOA.HCl) (Wako Pure Chemical Industries, Osaka, Japan) was dried under reduced pressure. Other reagents used were of analytical-reagent grade. A standard solution of acrolein was prepared by dissolving 100 mg of the purest grade of acrolein available in distilled water and diluting to 100 ml.

The brominated derivative of acrolein *O*-methyloxime was supplied by Tokyo Kasei Kogyo (Tokyo, Japan), and the Sep-PAK C_{18} (SP) cartridge was from Waters Associates (Milford, MA, USA).

Apparatus and Conditions

A Hitachi 073 gas chromatograph (GC) with a ^{63}Ni electron-capture detector (ECD) and a Hitachi 663 GC with a flame-ionisation detector (FID) and a flame thermionic detector (FTD) were used. The following conditions were used for the GC with ECD: a 2 m glass column packed with 3% silicone GE XE-60 on 60–80 mesh Chromosorb W AW DMCS; column temperature, 90 °C; injection and detector temperature, 170 °C; and carrier gas (nitrogen) flow-rate, 40 ml min^{-1}. The GC with FID conditions were as follows: 1, a 2 m glass column packed with 20% TCP on 60–80 mesh Chromosorb W AW DMCS; a column temperature of 80 °C; an injection and detector temperature of 120 °C; and a carrier gas (nitrogen) flow-rate of 40 ml min^{-1}; 2, a 2 m glass column packed with 10% DEGS on 60–80 mesh Chromosorb W AW DMCS; a column temperature of 140 °C; an injection and detector temperature of 170 °C; and a carrier gas (nitrogen) flow-rate of 40 ml min^{-1}. The GC with FTD conditions were the same as the GC-FID conditions except for the carrier gas, which was helium, also with a flow-rate of 40 ml min^{-1}.

A Hitachi M52S GC - MS was used with a 10–20 eV ionisation energy.

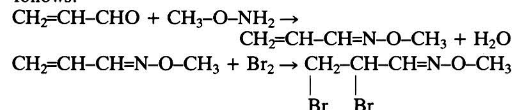
General Procedure

A 1 ml aliquot of 2 M sodium acetate and 1 ml of MOA.HCl (5 mg ml^{-1}) are added to 5 ml of sample solution containing about 0.3 μg of acrolein. The mixture is allowed to stand for 10 min at room temperature and then 1 ml of 1.5 M sulphuric acid, 0.2 ml of 0.2 M potassium bromate and 2 g of potassium bromide are added and dissolved with stirring. After standing for 15 min at room temperature, the excess of bromine is reduced with 0.05 M sodium thiosulphate. The solution is forced through an SP cartridge and the derivative in the cartridge is eluted with 1.5 ml of diethyl ether. A 4 μl portion of the eluate is measured by GC with ECD and the peak-height method is used for the determination of acrolein.

Results and Discussion

Derivatisation Reaction

Aldehydes are known to react with MOA to form *O*-methyloxime.[10] In this work, we investigated a method based on the bromination of acrolein *O*-methyloxime, followed by GC with ECD to determine acrolein with a high sensitivity and selectivity. It is assumed that the reaction proceeded as follows:



$$CH_2{=}CH{-}CHO + CH_3{-}O{-}NH_2 \rightarrow CH_2{=}CH{-}CH{=}N{-}O{-}CH_3 + H_2O$$

$$CH_2{=}CH{-}CH{=}N{-}O{-}CH_3 + Br_2 \rightarrow CH_2Br{-}CHBr{-}CH{=}N{-}O{-}CH_3$$

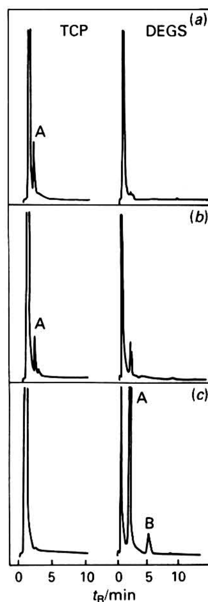


Fig. 1. Chromatograms of acrolein and its derivatives by GC-FID. 20% TCP: column temperature, 80 °C; carrier gas (N_2), 40 ml min^{-1}. 10% DEGS: column temperature, 140 °C; carrier gas (N_2), 40 ml min^{-1}. (*a*) Pre-reaction, A = acrolein; (*b*) *O*-methyloxime derivative, A = acrolein - MO; and (*c*) brominated derivative of *O*-methyloxime, A = acetic acid, B = brominated derivative of acrolein - MO

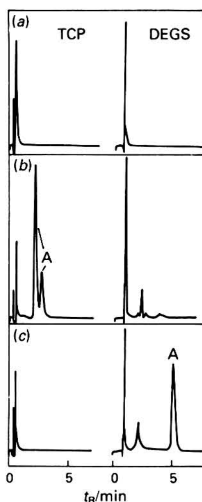


Fig. 2. Chromatograms of acrolein and its derivatives by GC-FTD. 20% TCP: column temperature, 80 °C; carrier gas (He), 40 ml min^{-1}. 10% DEGS: column temperature, 140 °C; carrier gas (He), 40 ml min^{-1}. (*a*) Pre-reaction; (*b*) *O*-methyloxime derivative, A = acrolein - MO; and (*c*) brominated derivative of *O*-methyloxime, A = brominated derivative of acrolein - MO

In order to identify the reaction procedure described above, the solutions extracted with diethyl ether (*i.e.*, the pre-reaction solution, the solution containing *O*-methyloxime and that containing the brominated derivative of *O*-methyloxime) were measured by GC-FID and GC-FTD (Figs. 1 and 2).

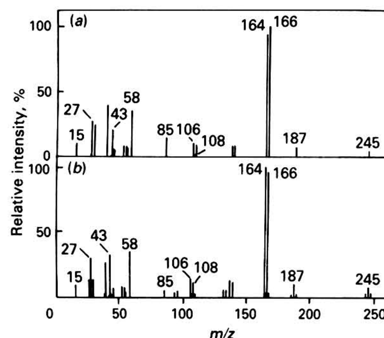


Fig. 3. Mass spectrum of acrolein - MO.Br derivative. (*a*) Brominated derivative of acrolein *O*-methyloxime and (*b*) synthesised 2,3-dibromopropionaldehyde *O*-methyloxime

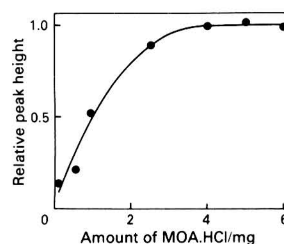


Fig. 4. Effect of amount of MOA on the formation of acrolein - MO. Acrolein, 1.0 µg; sodium acetate (2 M), 1.0 ml

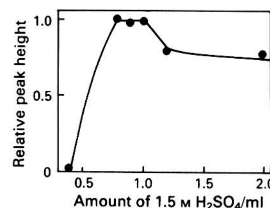


Fig. 5. Effect of volume of 1.5 M H_2SO_4 on bromination of acrolein - MO. Acrolein, 1.0 µg; MOA.HCl, 5 mg

On the chromatograms obtained by GC-FID, the acrolein peak [peak A in Fig. 1 (*a*)] disappeared and two new peaks [Fig. 1 (*b*)] appeared with the formation of *O*-methyloxime. Furthermore, in Fig. 1 (*c*), two of the peaks decreased and a new peak (peak B) appeared. This new peak is assumed to be caused by the bromination of *O*-methyloxime. Peak A in Fig. 1 (*c*) was identified as acetic acid by GC - MS measurement.

On the chromatograms obtained by GC-FTD (Fig. 2), the acrolein peak did not appear, but two large peaks appeared as the *O*-methyloxime derivative was formed. Then, as can be seen in Fig. 2 (*c*), these peaks disappeared and a new large peak appeared as bromination took place.

From these results, it is assumed that the nitrogenous compound was formed and converted to different nitrogenous compounds by bromination. The two peaks of *O*-methyloxime may be due to *syn*- and *anti*-isomers. The peaks resulting from the brominated derivative of *O*-methyloxime could not be separated under the proposed conditions. The extent of the reaction, determined by GC-FID from those compounds remaining after each reaction period, was about 92% for 1 mg of acrolein in 5 ml of aqueous solution.

The mass of the brominated derivative of acrolein *O*-methyloxime is shown in Fig. 3. The highest peak (*m*/*z* 245) of the

molecular ion peaks (m/z 243, 245 and 247) appeared with an ionisation energy of 10 eV. The fragment peaks are assumed to be (M − Br) at m/z 164 and 166, and (M − 2Br) at m/z 85. The mass spectrum agreed with that of the standard 2,3-dibromopropionaldehyde *O*-methyloxime obtained from Tokyo Kasei Kogyo, Tokyo, Japan.

As a result, the brominated derivative of acrolein *O*-methyloxime is assumed to be 2,3-dibromopropionaldehyde *O*-methyloxime.

O-Methyloxime Formation

Levine *et al.* have reported the formation of *O*-methyloxime in methanol solution.[10] In this work, the formation of acrolein *O*-methyloxime in aqueous solution was studied in order to be able to dissolve potassium bromide adequately in the second step.

Fig. 4 shows the effect of MOA.HCl in sodium acetate buffer solution. The yield of the product was maximum and constant in the range 4–6 mg of MOA.HCl. The temperature of the reaction had no influence, even at room temperature (15–20 °C), 40 and 60 °C. The minimum time for the completion of the reaction was found to be 10 min.

Bromination of *O*-Methyloxime

The effect of acidity on the bromination of acrolein *O*-methyloxime is shown in Fig. 5. The yield was maximum in the range 0.8–1.0 ml of 1.5 M sulphuric acid, corresponding to pH 1.3–1.7.

The peak height was constant in the range 2–4 g of KBr, and the yield was constant in the range 0.1–2.0 ml of 0.2 M potassium bromate. The minimum reaction time for complete bromination was found to be 10 min after the addition of the reagents. The derivative was stable for at least 5 d.

Use of Sep-PAK C_{18} Cartridge

The brominated derivative of *O*-methyloxime was completely extracted with one 5 ml aliquot of diethyl ether. An SP cartridge was used to enrich and clean up the acrolein derivative, and the following procedure was established. Pump the sample solution after reaction through the cartridge with a 10 ml syringe. Remove the cartridge from one syringe to another and pump 1.5 ml of diethyl ether through to elute the acrolein derivative.

The recovery of the derivative obtained from the cartridge using 0.2 µg of acrolein was 98%.

Fig. 6. Typical chromatogram of rain water by GC-ECD. A = Acrolein derivative

Table 1. Retention time of derivatives

Parent compound	Retention time/ min	Relative retention time
Acrolein	7.39	1.00
Methacrolein	5.39*	0.73
	6.95†	0.94
Crotonaldehyde	9.07	1.23

* Peak of brominated derivative of methacrolein.
† Peak of brominated derivative of methacrolein *O*-methyloxime.

Selection of GC Column

In order to separate the acrolein peak from those compounds with ethylenic bonds, such as methacrolein and crotonaldehyde, five different columns were tested, namely, 1% PEG-HT, 10% TCEP, 10% DEGS, 3% silicone GE XE-60 and 5% silicone OV-225. As a result, it was found that the 3% silicone GE XE-60 column was the most efficient and effective for the separation of the peaks. The retention times are shown in Table 1. It was found by GC - MS that two peaks (5.39 and 6.95 min) of methacrolein were of the brominated derivative of methacrolein and the brominated derivative of methacrolein *O*-methyloxime, respectively. This result shows that the *O*-methyloxime formation from methacrolein had not proceeded to completion.

Calibration Graph and Precision

A calibration graph was prepared with an acrolein standard solution under the optimum experimental conditions. The relationship between peak height and the concentration of acrolein in aqueous solution was linear over the range 0–0.06 µg ml^{-1}. The relative standard deviation from five replicates was estimated to be 4.5% for 0.04 µg ml^{-1} of acrolein. The detection limit (signal to noise ratio = 2) was 0.4 ng ml^{-1} of acrolein.

Recovery of Acrolein from Rain Water

In order to assess the proposed method, recovery experiments were carried out on mixtures of rain water and acrolein standard solutions. The results obtained are shown in Table 2. The proposed method can be satisfactorily applied to the determination of acrolein in rain water, as the recovery obtained was in the range 90–101%.

Table 2. Recovery of acrolein from rain water

		Acrolein		
Rain water sample	pH	Added/ µg	Found/ µg	Recovery, %
A	4.5	—	ND*	—
		0.050	0.045	90
		0.200	0.202	101
B	4.8	—	ND	—
		0.050	0.047	94
		0.200	0.192	96

* ND = Not detected.

Table 3. Results obtained for the determination of acrolein in rain water

	Acrolein*/ng ml^{-1}	
Rain water sample	Mean	Range
A-1	1.8	1.5–2.0
A-2	ND†	
A-3	2.8	2.5–3.1
A-4	2.2	2.1–2.2
B-1	ND	

* Mean of three determinations.
† ND = Not detected.

Determination of Acrolein in Rain Water

Acrolein was determined in rain water samples by the proposed method. Table 3 shows the results obtained, and Fig. 6 shows a typical chromatogram of acrolein in rain water.

Conclusion

An improved method for the determination of acrolein in aqueous solutions, based on the bromination of *O*-methyloxime followed by GC with ECD, has been established. This method is highly sensitive and selective for the determination of acrolein in rain water compared with previous methods.

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Simple Gas Chromatographic Determination of the Distribution of Normal Alkanes in the Kerosene Fraction of Petroleum

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An internal standard technique has been applied to the determination of the distribution of normal alkanes in the kerosene fraction of petroleum using a capillary column. The applicability of packed columns for such a determination has also been studied and compared with the existing Universal Oil Products (UOP) method.

Keywords: *Normal alkanes determination; subtractive gas chromatography; internal standards technique; molecular sieves*

The determination of the concentration and concentration distribution of normal alkanes in hydrocarbon mixtures is of considerable importance in the petroleum and petrochemicals industries. Normal alkanes from petroleum sources are an important feed stock for the petrochemical industries; the long chain alkanes can be converted to lubricant and fuel additives, plasticisers, industrial surfactants, flotation agents, solvents and raw materials for protein synthesis by means of oxidation, halogenation, esterification, fermentation, etc. Such wide applications have generated a new interest in the refinery processes for recovering long chain alkanes from petroleum. Flow properties, such as viscosity, viscosity index, fluidity, pour point, etc., of heavy petroleum fractions largely depend on the n-alkane content. The distribution of n-C_{11}–n-C_{14} alkanes obtained from the kerosene fraction has immense potential in the manufacture of biodegradable detergents. In view of this importance, various methods and techniques have been proposed to determine the distribution of n-alkanes in the kerosene fraction of petroleum.

The determination of n-alkanes in complex hydrocarbon systems by their selective adsorption on molecular sieve 5A was suggested by Brenner and Coats[1] as early as 1958. Since then the molecular adsorption technique has been invariably used by several workers[2,3] in spite of its limitations. The mechanism of selective adsorption and the structure and properties of molecular sieves have been discussed in detail by many workers. Nelson[4] determined the n-alkane content of petroleum distillates by calculating the difference in mass of the zeolite before and after adsorption. O'Connor and co-workers[5,6] suggested recovering adsorbed n-alkanes from the sieve by a diffusion-controlled process for quantitative determination. Larson and Becker[7] used volumetric techniques for the determination of n-alkanes in olefin-free petroleum distillates.

Whitham[8,9] used a subtractive method using a conventional GLC column with and without a molecular sieve and the n-alkanes were determined by the difference between the two chromatograms. These methods, however, were inadequate for low concentrations of n-alkanes. Eggertsen and Groennings[10] and later Blytas and Peterson[11] modified this method so that n-alkanes were desorbed from the molecular sieve by heating and were then determined on a GLC column. Hydrofluoric acid followed by isooctane extraction[12,13] was used for the recovery of adsorbed n-alkanes by the destruction of the molecular sieve. Petrovic and Vitorovic[14] reported the direct gas chromatographic determination of C_9–C_{14} n-alkanes in the kerosene fraction using an open tubular column of Apiezon L. Hine[15] used an open tubular column for the determination of the total n-alkane content in petroleum fractions. Johanson *et al.*[16] described the possibility of determining hydrocarbons by structural group types in gasoline and distillates.

There is no standard analytical method for the determination of n-alkanes in the kerosene fraction, except for the Universal Oil Products (UOP) method.[17] This method is based on a subtraction technique using two gas chromatographs in series separated by a molecular sieve column, but has certain inherent limitations.

In this paper we propose a simple and straightforward capillary gas chromatographic method for the determination of the n-alkane distribution in straight-run kerosene fractions. The method makes use of the high resolution capability of an open tubular column (WCOT) to separate the n-alkanes from branched components and an internal standard technique[18] for fast, reliable and accurate determinations. A simplified procedure is also discussed utilising the applicability of packed columns for such determinations and is suggested as an alternative to the UOP method.[17]

Experimental

Two gas chromatographs with flame-ionisation detectors were employed, one for the capillary and one for the packed-column studies. The former was a Varian gas chromatograph (Model 3700) with a chromatographic data system (CDS-111) and potentiometric recorder (Model 9176). The provision to install the capillary column was used in order to achieve the separation of individual n-alkanes from branched peaks. A fused-silica open tubular column of 50 m × 0.2 mm with methylsilicone phase of 0.2 µm film thickness was used. The injector and detector blocks were set at 300 and 320 °C, respectively, and the column was programmed from 85 to 250 °C at 4 °C min^{-1} with 4 min of initial hold-up time. Nitrogen was used as a carrier gas at an average linear velocity of 18.5 cm s^{-1}, corresponding to a flow-rate (uncorrected) of 1.5 ml min^{-1}. A 0.1-µl sample was injected with a split ratio of 70 : 1.

The second gas chromatograph (Perkin-Elmer Model 3920 B) was installed with a 3 m × 2 mm i.d. packed column of 5% SE-30 (Methyl E-301) on Chromosorb P, 80–100 mesh. The injector and detector temperatures were kept at 300 and 320 °C, respectively. The initial column temperature was 45 °C and it was temperature-programmed at a rate of 4 °C min^{-1}, with an initial hold-up time of 8 min, to a final temperature of 220 °C. Nitrogen was used as the carrier gas with flow-rate of 30 ml min^{-1}, and a 0.2-µl sample was used for the determination. A Spectra-Physics minigrator and recorder were used for computing the data. High purity n-alkanes were used to prepare reference blends and internal standard samples. A de-normalised reference stock was prepared from kerosene samples in two stages for making the calibration blends. The kerosene sample was subjected to urea adduction and the last traces of n-alkanes were removed by molecular sieve adsorp-

tion.[19] The calibration blend was prepared by mixing a known amount of de-normalised reference stock with a pure n-alkane reference blend.

Results and Discussion

The proposed method is based on a wall-coated open tubular column of SE-30 (methylsilicone), which has the best solvent characteristics of a non-polar phase for separating complex hydrocarbon mixtures according to boiling points. Some properties of this capillary column were determined in order to show the efficiency of the stationary phase. The number of theoretical plates of the capillary column used was 254×10^3 with a coating efficiency of 69.8% and a capacity ratio of 5.1 for n-tridecane. The separation number (Trennzahl) for consecutive pairs of n-C_{13} and n-C_{14} was found to be 49.3. This has been used as a measure of column efficiency under temperature-programmed conditions and gives the maximum number of peaks that can be separated between two sequential homologues.

Fig. 1 shows a typical chromatogram of a kerosene sample obtained under the operating conditions outlined above. Each n-alkane has been well separated from the neighbouring branched components by utilising the high resolving power of the capillary column. Two different chromatograms of the sample were recorded; one as above and the other with n-hexadecane added as an internal standard. Area and base-line sensitivity parameters were taken into account for the accurate peak detection using the CDS-111 data system. The values obtained for individual n-alkane concentrations (obtained by area normalisation and internal standard techniques) were found to be in good agreement. The concentration of n-C_{16} in the original kerosene sample was determined by the area normalisation technique for calculating the individual concentration of n-hexadecane. The precision of the above proposed internal standard method was examined by determining five replicate injections of the sample and the standard deviation and coefficient of variation were found to be 0.4726 and 1.5766, respectively, with an average total n-alkane concentration of 29.976%. The reproducibility of individual n-alkane concentrations from run to run is shown in Table 1.

The peak subtraction technique using a molecular sieve column was applied in order to investigate whether the n-alkanes were masked by branched alkanes. The technique can quantify the extent of contribution of branched to the n-alkanes and the concentration of individual n-alkanes can therefore be determined with high precision. The subtraction was achieved using a Linde molecular sieve 5A packed in the quartz liner of the split injector of the gas chromatograph. The molecular sieve was activated in the injector port itself by heating at 300–350 °C. A section of the subtracted and unsubtracted chromatograms obtained using the capillary column is shown in Fig. 2; it was noticed that the number of branched alkanes obscured by n-alkanes was negligible owing to the high resolution of the capillary column.

Packed-column investigations using a single gas chromatograph with a flame-ionisation detector appear to be promising as an alternative to the UOP method. Data from individual n-alkanes in the same kerosene sample were obtained using a packed column with internal standards. The total concentration of n-alkanes in the sample (Table 2) was 31.372%, which was about 1.396% higher than the value reported when using the fused-silica capillary column. This higher value was expected, owing to the limitations of the packed column in resolving n-alkane peaks from the branched-chain components. The concentration of branched components obscured by n-alkanes was calculated using chromatograms of the sample obtained with and without molecular sieve subtraction (Fig. 3). The deviation in the values obtained for total n-alkane concentration in the kerosene sample using packed and capillary columns does not exceed the error expected in routine GC determinations.

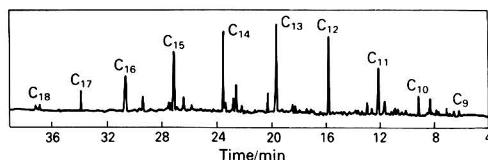

Fig. 1. Kerosene sample on fused-silica capillary column

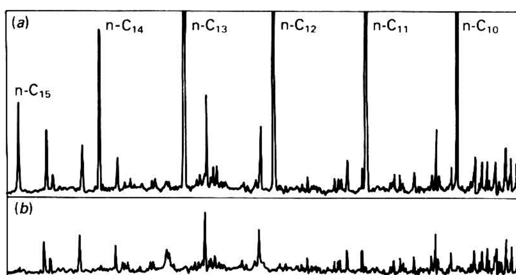

Fig. 2. Section of chromatogram of kerosene sample on capillary column (*a*) without molecular sieve and (*b*) with molecular sieve

Table 1. Repeatability and precision in capillary-column method

n-Alkane components	Concentration, % *m/m* 1	2	3	4	5	Average, % *m/m*	Standard deviation, % *m/m*	Coefficient of variation, %
C-8	0.28	0.26	0.29	0.29	0.30	0.2840	0.0152	5.3401
C-9	0.84	0.85	0.88	0.88	0.91	0.8720	0.0277	3.1822
C-10	2.42	2.45	2.52	2.52	2.57	2.4960	0.0602	2.4138
C-11	5.09	5.25	5.14	5.15	5.00	5.1260	0.0913	1.7805
C-12	6.31	6.20	6.37	6.39	6.51	6.3560	0.1135	1.7856
C-13	6.00	5.89	6.06	6.10	6.21	6.0520	0.1186	1.9600
C-14	4.53	4.45	4.58	4.66	4.68	4.5800	0.0946	2.0655
C-15	2.28	2.31	2.37	2.40	2.44	2.3600	0.0652	2.7624
C-16	0.78	0.79	0.81	0.82	0.84	0.8080	0.0239	2.9548
C-17	0.36	0.35	0.37	0.37	0.38	0.3660	0.0114	3.1152
C-18	0.32	0.31	0.33	0.33	0.34	0.3260	0.0114	3.4975
C-19	0.19	0.19	0.20	0.20	0.21	0.1980	0.0084	4.2256
C-20	0.15	0.14	0.15	0.16	0.16	0.1520	0.0084	5.5043
Total	29.55	29.44	30.07	30.27	30.55	29.976	0.4726	1.5766

Table 2. Repeatability and precision in packed-column method

n-Alkane components	Concentration, % *m*/*m*					Average, % *m*/*m*	Standard deviation, % *m*/*m*	Coefficient of variation, %
	1	2	3	4	5			
C-8	0.27	0.29	0.31	0.30	0.33	0.3000	0.0224	7.4536
C-9	0.89	0.93	0.98	1.01	1.05	0.9720	0.0634	6.5230
C-10	2.49	2.56	2.63	2.67	2.70	2.6100	0.0851	3.2623
C-11	5.13	5.22	5.24	5.32	5.40	5.2620	0.1026	1.9492
C-12	6.38	6.31	6.53	6.44	6.60	6.4520	0.1157	1.7917
C-13	6.08	6.2	6.19	6.38	6.38	6.246	0.1310	2.098
C-14	4.68	4.75	4.84	4.89	4.93	4.818	0.1022	2.1217
C-15	2.59	2.61	2.60	2.72	2.75	2.654	0.0750	2.8272
C-16	0.87	0.89	0.90	0.83	0.86	0.87	0.0274	3.1478
C-17	0.54	0.51	0.52	0.49	0.51	0.514	0.0181	3.5300
C-18	0.29	0.28	0.31	0.30	0.31	0.2980	0.0130	4.3753
C-19	0.24	0.21	0.23	0.23	0.25	0.2320	0.0148	6.3933
C-20	0.13	0.14	0.16	0.14	0.15	0.1440	0.0114	7.9179
Total	30.58	30.90	31.44	31.72	32.22	31.372	0.65093	2.075

Table 3. Accuracy and coefficient of variation in packed-column method

n-Alkane components	Actual concentration, % *m*/*m*	Observed concentration, % *m*/*m*					Coefficient of variation, %	Accuracy, %
		1	2	3	4	5		
C-11	4.18	4.25	4.35	4.4	4.1	4.3	2.69	2.39
C-12	4.80	4.90	4.94	5.0	4.75	4.88	1.89	1.87
C-13	3.87	4.0	3.95	3.85	4.15	4.10	2.98	3.61
C-14	2.08	2.10	2.15	2.2	2.3	2.22	3.44	5.48
C-15	0.75	0.72	0.77	0.70	0.67	0.75	5.40	4.50
Total	15.68	15.97	16.16	16.15	15.97	16.25	0.78	2.64

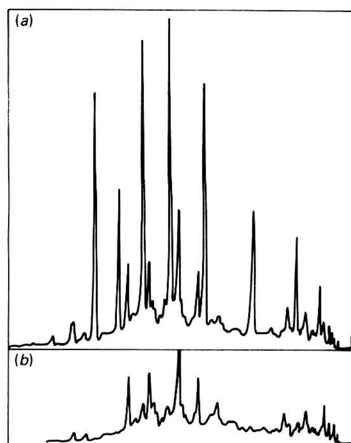

Fig. 3. Kerosene sample on packed column (*a*) without molecular sieve and (*b*) with molecular sieve

In order to check the precision and accuracy of the packed-column method, a high purity calibration blend was prepared by weighing portions of a de-normalised kerosene reference stock and a blend of pure n-alkanes from n-undecane to n-pentadecane. The calibration blend was determined on the packed column and the concentration of each n-alkane was calculated and compared with the actual values. The agreement between the actual and observed concentrations was found to be between 1.875 and 5.48%, as shown in Table 3, and the coefficient of variation was 0.78%.

The proposed packed-column method has many advantages over the UOP method.[17] The flame-ionisation detector that was used in place of the thermal-conductivity detector, apart from being more sensitive, has the distinct advantage of having the same quantitative response to equal masses of any hydrocarbon, thus avoiding having to account for the response factors of individual components. Peak broadening, which may be caused by the use of a transfer line in the UOP method,[17] is eliminated by using a single gas chromatograph. Also, the optimisation of the detector current in both gas chromatographs in order to match the detector signals for the relative distribution of an isoalkane blend is not required in the proposed method. The total n-alkane concentration depends on the factor used for the conversion of area percent. to mass percent. in the non-distributive mode. Any error in preparing the calibration blend will affect the total concentration of n-alkanes obtained for the sample. As the sample is injected twice in the proposed method (with and without the molecular sieve) the accuracy in a sample injection of 0.2 µl is well within acceptable limits.

Conclusion

The proposed internal standard method for the determination of normal alkanes using a fused-silica capillary column is both fast and reliable. The proposed method using a packed column is simpler and more sensitive than the existing UOP method[17] for the determination of normal alkanes and gives reasonable accuracy compared with an open tubular column.

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Determination of Polychlorinated Biphenyls in Waste Oil by Gas - Liquid Chromatography

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A 0.1 g portion of waste oil is cleaned up by silica gel chromatography followed by dimethylformamide - hexane partitioning and chromatography on a de-activated neutral alumina column. The purified extract is examined for PCBs by either packed-column or capillary-column gas chromatography using either electon-capture or Hall electrolytic conductivity detectors. Recoveries at the 10 mg kg^{-1} level are in the range 66–98% and the coefficient of variation is typically 12%. A reporting limit of 5 mg kg^{-1} is readily attainable and PCBs can be detected down to about 1 mg kg^{-1}.

Keywords: *Polychlorinated biphenyl determination; waste oil; gas - liquid chromatography*

During the last few years there has been an interest in the determination of polychlorinated biphenyls (PCBs) in waste oils. The US Environmental Protection Agency[1] have proposed a limit of 50 mg l^{-1} for PCBs in used oil that can be marketed for energy recovery by combustion. Also, there is a need to examine waste oil for PCBs under the EC Commission proposal for an amendment to Directive 75/439 on the disposal of waste oils.[2]

There are very few published procedures for the determination of PCBs in mineral oils. Copland and Gohmann[3] proposed a method based on a treatment with concentrated sulphuric acid followed by digestion with potassium permanganate and then determination by chromatography on Florisil, but quoted only limited recovery data for used oil samples. Sonchik *et al.*[4] evaluated the procedures available for the determination of PCBs in waste oil, although they only examined oils with PCBs added at 50 and 500 mg kg^{-1}. For the determination of PCBs in transformer oils, Nero and Hudson[5] described an HPLC clean-up procedure followed by determination using gas chromatography with mass spectrometry; however, the authors did not report any recovery or repeatability data.

In this paper a procedure is proposed that allows PCBs to be determined in waste oils down to 5 mg kg^{-1} and which does not require treatment of the oil with potentially hazardous reagents such as sulphuric acid or potassium permanganate. The method has been used for the determination of PCBs in waste oil from transport and industrial sources and in processed waste oils.

Experimental

Apparatus

Chromatography tubes, 100 mm × 20 mm and 150 mm × 12 mm, fitted with PTFE stopcocks and four 100-ml and one 500-ml separating funnels were used. A sintered-glass (porosity 1) filter tube (200 mm × 30 mm) and a rotary evaporator (water-bath at 40 °C) or a Kuderna - Danish evaporator (boiling water-bath) were also employed.

A gas chromatograph featuring one of the following systems, or similar, can be used for the determination of PCBs.

Packed column

1.5 m × 3 mm, 3% stabilised DEGS on Gas-Chrom Q, 80–100 mesh. Oven temperature 195 °C, nitrogen carrier gas (80 ml min^{-1}). Electron-capture detector, 250 °C. Injection, 5 µl on column. Injector temperature 190 °C.

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Capillary column

25 m × 0.22 mm, OV-101, 0.1 × 10^{-6} m film thickness, chemically bonded (Chrompack). Oven temperature 100 °C, 1 min; raised to 235 °C at 30 °C min^{-1}, hold 12 min. Argon - methane (9 + 1) carrier gas (1.6 kg cm^{-2}; 1–2 ml min^{-1}). Electron-capture detector, 300 °C; make-up gas, argon - methane (9 + 1), 20 ml min^{-1}. Injection, 1 µl. Grob-type splitless (splitless period 0.5 min), injector temperature 325 °C.

Capillary column

25 m × 0.53 mm BP-10 (equivalent to OV1701), 1 × 10^{-6} m film thickness, chemically bonded (SGE). Oven temperature 180 °C, 3 min; raised to 225 °C at 20 °C min^{-1}, hold 30 min. Helium carrier gas (0.7 kg cm^{-2}; 10 ml min^{-1}). Hall electrolytic conductivity detector (halogen mode); base 250 °C; nickel tube 970 °C; propan-1-ol electrolyte 0.4 ml min^{-1}; hydrogen 30 ml min^{-1}. Vent time 1 min. Injection, 5 µl direct; injector temperature, 300 °C.

Reagents

Silica gel. Merck, Kieselgel 60, 0.063–0.2 mm, Art 7734.
Neutral alumina. Woelm, N, Akt. 1.
Standard solutions. Aroclor 1242, Aroclor 1254 and Aroclor 1260, all 1 µg ml^{-1} in hexane.
Dimethylformamide. Hexane saturated.
Hexane. Dimethylformamide saturated.
Hexane.
Sodium sulphate. Anhydrous, granular.
Sodium sulphate solution, 20 g l^{-1}.
Cotton-wool. Washed with hexane.

Procedure

A reagent blank should always be carried through the procedure at the same time as the samples are run.

Preparation of Chromatography Columns

Silica gel column

Heat the silica gel at 130 °C overnight and then store in a stoppered container prior to use. The silica gel may be stored for at least 1 month without deterioration. Place a plug of cotton-wool in the bottom of a 100 mm × 20 mm chromatography tube and two-thirds fill the column with hexane. Expel air from the tube by tapping the cotton-wool with a glass rod and allowing a few millilitres of hexane to flow out through the

tap. Weigh 10 g of silica into a 250-ml beaker, add 20–30 ml of hexane and mix to form a uniform slurry. Pour the slurry into the tube while allowing the solvent to run out through the stop-cock. Packing may be assisted by tapping the column gently. Allow the solvent to fall until the meniscus is just above the top of the bed and then wash down the walls of the tube with hexane. With a 20–30 mm depth of solvent above the bed, add anhydrous sodium sulphate to form a layer about 10 mm deep. Allow 250 ml of hexane to pass through the column and discard the hexane. Throughout these operations do not allow the column bed to dry out.

Alumina column

Heat the alumina for 3–4 h at 500 °C and allow to cool in a desiccator. Weigh a suitable amount (100–200 g) into a stoppered container, rapidly add distilled water (10 g for each 100 g of alumina) and stopper the container securely. Mix the contents thoroughly for 1.5 h on a rotary mixer or similar apparatus. Store the prepared alumina in a well stoppered container prior to use. The alumina may be stored for about 2 weeks if necessary. Place a plug of cotton-wool in the bottom of a 150 mm × 12 mm chromatography tube, fill the column with hexane and expel air in a similar way to that described for the silica gel column. Weigh 10 g of alumina into a beaker and pour the alumina into the column in a steady stream, allowing the hexane to flow out of the tap at the same time. Assist the packing of the column by gentle tapping, wash the walls of the column with solvent and add a 10-mm layer of anhydrous sodium sulphate to the top of the column. Again, do not allow the column to dry out.

Sample Preparation

Weigh, to 0.01 g, about 1 g of the well mixed oil into a 10-ml calibrated flask. Dilute to volume with hexane and mix the contents thoroughly.

Sample Clean-up

Allow the solvent level in the silica gel chromatography tube to pass just into the sodium sulphate layer. Transfer 1.0 ml of the sample solution on to the column and allow the sample to drain into the bed without allowing the bed to dry out. Wash the column with 20 ml of hexane using the first 2 ml to wash the walls of the tube; allow this 2 ml to pass into the bed. Add the remainder of the hexane, taking care to avoid disturbing the top of the column bed. Allow the hexane to run through the column at about 3 ml min^{-1} and discard the hexane. Elute the column with 100 ml of hexane in a similar manner and retain the eluate. Reduce the volume of the solvent to about 25 ml by evaporation.

Extract the hexane with three successive 10-ml portions of (hexane-saturated) dimethylformamide (DMF) and then wash the combined DMF extracts with 10 ml of hexane (DMF-saturated). Separate the hexane and wash it with 10 ml of DMF; discard the hexane and add the DMF to the original combined 30 ml of DMF extract. Transfer this extract into a 500-ml separating funnel, add 200 ml of sodium sulphate solution and 10 ml of hexane and shake well. Allow the layers to separate, discard the lower aqueous layer and pass the hexane through 20–30 g of anhydrous sodium sulphate contained in a sintered-glass filter tube. Rinse the tube with 10 ml of hexane, combining the rinsings with the original hexane extract. Reduce the volume of the extract to about 1 ml by evaporation.

Allow the solvent level in the alumina chromatography tube to fall to just below the surface of the sodium sulphate layer and place a suitable container under the tap. Transfer the hexane extract on to the column and allow it to pass into the bed without allowing the column to dry out. Rinse the extract container with 1 ml of hexane and transfer this on to the column, allowing it to pass into the bed. Elute the column with 15 ml of hexane taking care to avoid disturbing the top of the column. Adjust the volume of the eluate to 1.0 ml by evaporation.

Examination of the Purified Extract

Inject an aliquot (1–5 µl) of the extract on to the gas chromatograph and compare the chromatogram with those obtained from standard solutions of PCB. The presence of PCBs in the sample is indicated when the pattern of peaks resembles that observed in a standard material. Retention times must agree closely (within ±0.2 min) and relative peak heights must be broadly similar, although some departures from those values observed in the standard may be encountered.

Estimation of PCB Content

An estimate of the PCBs present in the sample is made by comparing the total peak area of the PCB peaks with the total area of the peaks in the standard.

Results and Discussion

Aroclors 1242, 1254 and 1260 were added to various types of waste oil corresponding to a concentration of 10 mg kg^{-1} in the oil. These samples were then treated as described under Procedure. The recoveries obtained are shown in Table 1. There is no significant difference in the recoveries observed for the different types of PCB, indicating that no over-all fractionation of these compounds occurs during the three clean-up steps.

The hexane - DMF partition procedure[6] and the alumina column procedure[7,8] are standard techniques in pesticide residue analysis. The silica gel column has proved useful in this laboratory for the preliminary clean-up of various types of raw sample extracts containing relatively high levels (*ca.* 0.1–0.2 g) of co-extractives. It is necessary to wash the silica gel with hexane (250 ml) before use as the gel may be a source of PCBs.[9] We have found hexane washings of the silica gel to contain peaks corresponding to Aroclors 1242 and 1254, which, if attributed to the sample, would indicate an apparent PCB content of about 5 mg kg^{-1}. This impurity is removed by 100 ml of hexane; use of 250 ml provides a good safety margin to allow for possible batch variations in the silica gel. Reagent blanks for the entire procedure, when the silica gel washing is carried out, are equivalent to about 0.2 mg kg^{-1} of PCBs. A reporting limit for PCBs in waste oil of 5 mg kg^{-1} is, therefore, readily attainable.

The silica gel column retains the dark brown matter in used oil whereas the eluate contains, in addition to PCBs, a clear oily component (*ca.* 50 µl) from the sample. Although the presence of this material in a 1.0 ml extract does no permanent harm to the gas chromatographic column or detector, it does interfere with the determination of PCBs as it can give rise to distorted peak shapes and reduced detector sensitivity. This oily matter is not retained by de-activated alumina and therefore the hexane - DMF procedure is required for its removal.

The final clean-up on the de-activated alumina column removes a number of interfering peaks from the DMF-treated extracts so that the identification and quantitation of any PCB is made more reliable.

Figs. 1–3 show typical chromatograms obtained from samples of "spiked" oil. Although the electron-capture detector is more sensitive than the Hall detector the latter has an advantage in that, in the halogen mode, it responds selectively to halogen-containing species. A further advantage of the Hall detector is that the response is based only on the amount of chlorine present and thus a more reliable estimate of PCB content is obtained when the PCB pattern in a sample

Table 1. Recoveries from used and waste oils

Sample	Aroclor PCB added at 10 mg kg^{-1}	Number of determinations (n)	Mean recovery, %	Standard deviation, %
Transport and industrial oil . .	1242	7	71	8
Transport and industrial oil . .	1254	8	84	9
Transport and industrial oil . .	1260	7	66	12
Transport oil	1242	4	98	15
Transport oil	1260	4	92	10
Processed waste oil	1242	4	71	7
Processed waste oil	1260	4	78	9

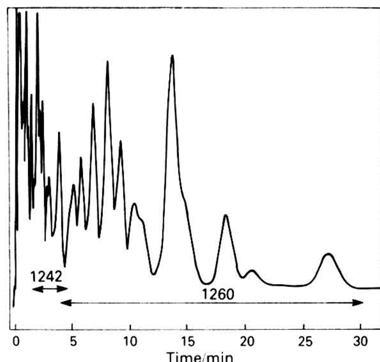

Fig. 1. Chromatogram of waste transport and industrial oil spiked with Aroclors 1242 and 1260 (10 mg kg^{-1} of each). Packed column with an electron-capture detector

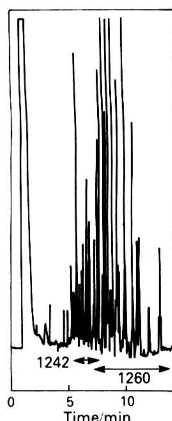

Fig. 2. Chromatogram of processed waste oil, spiked with Aroclors 1242 and 1260 (10 mg kg^{-1} of each). Capillary column with an electron-capture detector

is not an exact match of that in the standard. The latter situation will inevitably lead to some errors when an electron-capture detector is used as the different PCB isomers have different electron-capturing powers.[10]

Because of this, the determination of the PCB content of sample extracts by electron-capture detection is a difficult problem and various approaches are available. Quantitation may be carried out (as in this work) using a commerical PCB preparation, *e.g.*, an Aroclor, as a standard material.[11] An extension of this approach involves firstly determining the proportion, by mass, that each peak in an Aroclor standard chromatogram contributes to the total PCB in that stan-

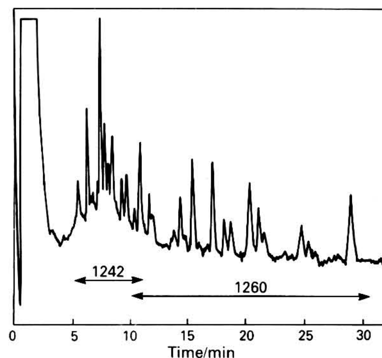

Fig. 3. Chromatogram of processed waste oil, spiked with Aroclors 1242 and 1260 (10 mg kg^{-1} of each). Capillary column with a Hall electrolytic conductivity detector

dard.[11,12] PCBs in a sample extract may then be quantified on a peak-to-peak basis followed by summing the individual PCB components to give the total amount of PCBs. The problem of non-uniform detector responses is eliminated when an isomer-specific determination is carried out[13] so that each PCB peak is quantitated using the appropriate single PCB isomer standard. An alternative to quantifying every isomer in a sample extract is to use seven representative PCB isomer standards.[14] For further discussion on the problems of PCB quantitation (and PCB determination in general) the reader is referred to the recent work of Erickson.[15]

The method presented allows about four analyses to be carried out in 1.5 working days. It may prove possible to reduce this time period by replacing the DMF - hexane partition procedure with a column clean-up using gel permeation chromatography. We are currently investigating this possibility.

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Simultaneous Measurement of Phenobarbital, Carbamazepine, Phenytoin and 5-(4-Hydroxyphenyl)-5-phenylhydantoin in Serum by High-performance Liquid Chromatography

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A rapid, highly sensitive high-performance liquid chromatographic method has been developed for the determination of phenobarbital, carbamazepine, phenytoin and its main metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin, in 50 µl of serum. Serum protein was precipitated with an acetonitrile solution containing 5-(4-methylphenyl)-5-phenylhydantoin as the internal standard. The drugs were eluted from a 5 µm, C-18 reversed-phase column at 40 °C with a mobile phase consisting of an acetonitrile - methanol - phosphate buffer of pH 4.8 (22 + 28 + 50% *V*/*V*), at a flow-rate of 1 ml min^{-1} with UV detection at 214 nm. Each analysis required no longer than 12 min. Quantitation was achieved by the measurement of the peak-height ratio and the relative and absolute recoveries varied from 94 to 109%. Within-day coefficients of variation ranged from 1.2 to 3.22% and between-day coefficients of variation from 2.0 to 3.4% in subtherapeutic, therapeutic and toxic concentrations.

Keywords: *High-performance liquid chromatography; phenobarbital; carbamazepine; phenytoin; 5-(4-hydroxyphenyl)-5-phenylhydantoin*

Serum level monitoring of anticonvulsant drugs is important for optimum drug therapy and the relationship between serum levels and therapeutic and toxic effects have been clearly established for several drugs. Routine serum level monitoring based on pharmacokinetic and clinical knowledge has improved the treatment of epileptic patients. Whereas phenobarbital and phenytoin remain the two most commonly prescribed anticonvulsant drugs, patients are frequently coadministered other anticonvulsants. Thus, comprehensive therapeutic monitoring of anticonvulsant therapy demands the availability of suitable methods of separating and determining the various combinations of these drugs. The ideal procedure for the assay of these drugs and metabolites should provide reliable, rapid and precise determinations. Of the many methods available for monitoring anticonvulsant therapy, high-performance liquid chromatography (HLPC) offers the potential for the rapid simultaneous analysis of drugs and metabolites.[1] Most of the reported methods require extensive sample preparations prior to chromatography, and some apply only to the assay of one or more specific anticonvulsant drugs.[2-14] Our objective was to develop a simple, isocratic HPLC method for the simultaneous determination of phenobarbital, carbamazepine, phenytoin and its main metabolite, 5-(4-hydroxyphenyl)-5-phenylhydantoin, in serum.

Experimental

Apparatus

The following apparatus from Beckman Instruments (Geneva, Switzerland) was used: a Model 112 solvent delivery module, a Model 160 absorbance detector operating at 214 nm, a 45 × 4.6 mm i.d. Ultrasphere ODS pre-column, a 250 × 4.6 mm i.d. Ultrasphere ODS 5-µm column and an electric column heater for controlling column temperature. A WISP 710 B autosampler injector from Waters Associates (Milford, MA, USA) was also used. The recorder was a Model BD41 dual pen recorder from Kipp and Zonen (New York, USA).

Reagents and Standards

Purified water was used throughout (Milli-Q water purification system; Millipore, Bedford, MA, USA) and HPLC-grade acetonitrile and methanol (Fisher Scientific, Pittsburgh, PA, USA).

Phosphate buffer. Potassium phosphate (monobasic, 10 mM) was prepared and adjusted to pH 4.8 with 0.9 M phosphoric acid.

Mobile phase. This consisted of potassium phosphate buffer - methanol - acetonitrile (50 + 28 + 22, % *V*/*V*). The mobile phase was degassed daily by passing it through a 0.45 µm membrane filter (Durapore; Millipore).

Drug Standards

Phenytoin, carbamazepine, phenobarbital, 5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH) and 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) (internal standard) were obtained from Sigma Chemical (St. Louis, MO, USA). A chromatographic standard mixture was prepared as follows: 25 mg each of phenytoin, carbamazepine, phenobarbital and 5-(4-hydroxyphenyl)-5-phenylhydantoin were dissolved in 100 ml of methanol. The internal standard was 25 mg of MPPH in 100 ml of acetonitrile. These solutions are stable at 4 °C for at least 6 months.

Procedure

A 50 µl volume of acetonitrile containing 0.5 µg of 5-(4-methylphenyl)-5-phenylhydantoin (internal standard) was

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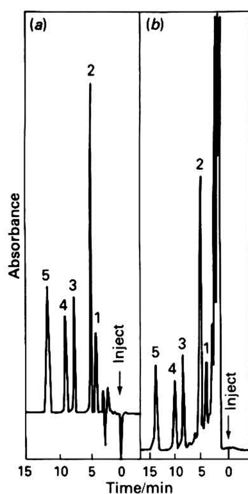

Fig. 1. (*a*) Liquid chromatogram of a 5 µl injection of standard aqueous mixture with the following concentrations of drugs: 1, 5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH), 3 µg ml^{-1}; 2, phenobarbital, 15 µg ml^{-1}; 3, phenytoin, 7.5 µg ml^{-1}; 4, carbamazepine, 3 µg ml^{-1}; and 5, 5-(4-methylphenyl)-5-phenylhydantoin (MPPH), 10 µg ml^{-1} (internal standard). (*b*) Liquid chromatogram of a 5 µl injection of a serum sample supplemented to give the same concentration of drugs as in (*a*)

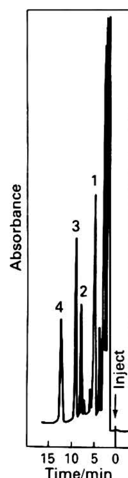

Fig. 2. Chromatogram of serum from a patient with: 1, phenobarbital, 8.9 µg ml^{-1}; 2, phenytoin, 6.8 µg ml^{-1}; 3, carbamazepine 4.7 µg ml^{-1}; and 4, internal standard, 5-(4-methylphenyl)-5-phenylhydantoin (MPPH)

added to 50 µl of serum or plasma to be assayed in an Eppendorf 1.5 ml polypropylene microtube (Fisher Chemical, St. Louis, MO, USA). The mixture was vortex mixed for 10 s, then centrifuged for 2 min at 9500 *g* in a microcentrifuge (No. 9527–15 Abbott Diagnostic Division, Irving, TX, USA). The supernatant was transferred into a similar tube and centrifuged for 30 s to ensure that no particulate matter would be injected into the chromatograph. A 5–10 µl aliquot of the supernatant was injected into the column and eluted with the mobile phase at a flow-rate of 1 ml min^{-1}. Before incorporation into the chromatographic system, the phosphate buffer

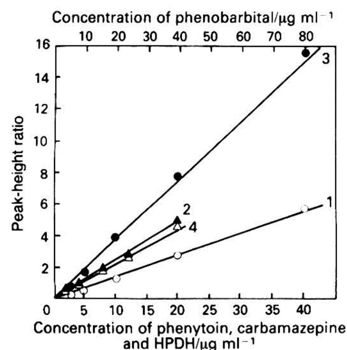

Fig. 3. Peak-height ratios of 1, phenytoin; 2, carbamazepine; 3, phenobarbital and 4, 5-(4-hydroxyphenyl)-5-phenylhydantoin (HPPH) to the internal standard 5-(4-methylphenyl)-5-phenylhydantoin (MPPH) against concentration of each drug. Points are averages of seven replicate samples for each concentration

Table 1. Retention times of some drugs tested

Drug	Retention time/ min*
Salicylate	2.2
Theobromine	2.4
Theophylline	2.6
Caffeine	2.9
N-Phenylethylmalonamide	3.1
Primidone	3.3
Ethosuximide	3.5
5-(4-Hydroxyphenyl)-5-phenylhydantoin	4.1
Phenobarbital	5.0
Carbamazepine-10,11-epoxide	5.5
Phenytoin	8.1
Hexobarbital	8.8
Carbamazepine	9.3
5-(4-Methylphenyl)-5-phenylhydantoin	12.0

* From time of injection into the column.

was filtered through a 0.45 µm pore-size HA filter, acetonitrile and methanol were filtered through a 0.5 µm pore-size FH filter (Millipore). The chart speed was 2 mm min^{-1} and the effluent was monitored at 214 nm, at 0.05 a.u.f.s. The column was run at 40 °C.

Results and Discussion

The composition and the pH of the mobile phase and the column temperature were varied to achieve the optimum chromatographic conditions. A mobile phase consisting of acetonitrile - methanol - phosphate buffer (22 + 28 + 50, *V/V*) gave the optimum resolution of the three antiepileptic drugs and the phenytoin metabolite, HPPH, under study. The effect of the pH of the mobile phase was also studied. Szabo and Brown[9] reported that at pH less than 6.0, phenobarbital co-elutes with HPPH; however, we found that these are well resolved at pH 4.8 and no interference from other components in the serum samples was observed. The optimum flow-rate for the mobile phase, 1 ml min^{-1}, resulted in an analysis time of 12 min. The effect of column temperature was also evaluated over the range 30–50 °C. At lower temperatures there was an increase in retention times but there was no loss of peak resolution. Higher temperatures improved the column efficiency and reduced the back-pressure, but phenobarbital and HPPH were less well resolved at 50 °C. At 40 °C Kabra *et al.*[2] reported that phenytoin and carbamazepine were less well resolved by the use of an acetonitrile - phosphate buffer

Table 2. Precision study of antiepileptic drug assay in serum

	Within-day precision*			Between-day precision†		
Drug	Concentration/ μg ml^{-1}	Mean measured value/ μg ml^{-1} ± s.d.	CV, %	Concentration/ μg ml^{-1}	Mean measured value/ μg ml^{-1} ± s.d.	CV, %
Phenobarbital	15	15.12 ± 0.30	1.98	15	14.79 ± 0.35	2.37
	30	30.65 ± 0.66	2.15	30	30.43 ± 0.88	2.89
	60	60.1 ± 1.30	2.16	60	61.22 ± 1.25	2.04
Phenytoin	7.5	7.79 ± 0.18	2.31	7.5	7.60 ± 0.24	3.16
	15	15.46 ± 0.18	1.16	15	15.38 ± 0.40	2.60
	30	29.70 ± 0.50	1.68	30	30.57 ± 0.66	2.16
Carbamazepine	3	3.17 ± 0.08	2.52	3	2.96 ± 0.10	3.38
	6	6.15 ± 0.12	1.95	6	6.17 ± 0.17	2.76
	16	16.27 ± 0.34	2.10	16	16.10 ± 0.42	2.61
HPPH	3	3.42 ± 0.11	3.22	3	3.21 ± 0.10	3.12
	6	6.26 ± 0.08	1.28	6	6.28 ± 0.19	3.03
	16	16.22 ± 0.22	1.36	16	16.22 ± 0.40	2.47

* Mean values represent 15 different serum samples for each value.
† Mean values represent 15 different serum samples analysed on different days for each value.

Table 3. Absolute and relative recovery of antiepileptic drugs added to serum

		Mean recovery* ± s.d., %	
Drug	Concentration/ μg ml^{-1}	Absolute	Relative
Phenobarbital ..	15	99.40 ± 1.39	100.80 ± 1.15
	30	100.82 ± 4.15	100.82 ± 2.0
	60	94.0 ± 0.55	100.18 ± 2.10
Phenytoin ..	7.5	104.0 ± 0.86	100.34 ± 1.11
	15	109.0 ± 1.10	103.14 ± 2.17
	30	95.0 ± 1.10	99.0 ± 0.42
Carbamazepine	3	99.38 ± 3.41	104.0 ± 3.18
	6	106.0 ± 1.10	102.46 ± 1.88
	16	96.52 ± 2.16	101.74 ± 1.40
HPPH	3	102.6 ± 4.63	104.0 ± 0.93
	6	108.8 ± 0.98	104.34 ± 3.95
	16	100.6 ± 6.12	101.40 ± 1.36
Internal standard ..	10.0	100.0 ± 1.4	

* Five replicate determinations for each concentration.

(21 + 79, *V/V*) mobile phase. We found that at 40 °C complete resolution of all components of the mixture was achieved with an analysis time of 12 min. Standard chromatograms were used to calculate the relative retention times and the response factors for these drugs. A standard chromatogram is shown in Fig. 1(*a*). When the optimum conditions were established with the drug standards, supernatants (5 μl) were prepared from serum-based controls containing all the drugs at concentrations from 3 to 15 μg ml^{-1}. The chromatogram obtained is shown in Fig. 1(*b*). Fig. 2 shows a representative chromatogram of serum from a patient receiving phenytoin, phenobarbital and carbamazepine.

Quantitation

The quantitation of the chromatograms was performed using peak-height ratios of the drugs to the internal standard. To determine the linearity, we injected various standards ranging from 2 to 80 μg ml^{-1}, prepared by adding known amounts of each drug to blank serum. The concentrations and peak-height ratios (standard drug to internal standard) were linearly related over this range. The calibration graphs for all drugs ranged from subtherapeutic and therapeutic to toxic concentrations. Fig. 3 represents peak-height ratios of phenytoin, phenobarbital, carbamazepine and 5-(4-hydroxyphenyl)-5-phenylhydantoin to the internal standard (MPPH) against concentrations of each drug.

Sensitivity

All three drugs and the main phenytoin metabolite (HPPH) were detected and reproducibly measured at a concentration of 1 μg ml^{-1} in serum.

Specificity

We studied other drugs for possible interference, by chromatographing aqueous solutions and analysing samples spiked with known drug concentrations. Table 1 lists the retention times for the drugs tested. Other drugs that were tested but not detected were propranolol, digoxin, gentamicin, valproic acid and amikacin.

Precision

Within-day precision was evaluated by the replicate analysis of a pooled serum sample containing each of these drugs at three different concentrations (subtherapeutic, therapeutic and toxic). Between-day precision was similarly evaluated from several days to 6 weeks. Within-day coefficients of variation (CVs) varied from 1.2 to 3.22% and between-day CVs from 2.0 to 3.4 (Table 2).

Recovery

We measured the absolute analytical recovery from serum for the three drugs, metabolite and the internal standard in the following way. The drugs, metabolite and internal standard were added to drug-free serum to achieve the concentrations shown in Table 3. This serum was then analysed by our method, except that the acetonitrile used to precipitate proteins did not contain any internal standard. Carefully measured aliquots of the supernatants were then injected and their peak heights measured. Absolute recovery was calculated by comparing these peak heights with the peak heights obtained by the direct injection of the pure drug standards. As shown in Table 3, absolute recoveries of the drugs ranged from 94% to 109%.

The relative recovery of these drugs was also calculated by comparing the concentrations obtained from the drug-supplemented serum with the actual added concentrations. The relative recovery ranged from 99 to 104% (Table 3).

Conclusions

We conclude that the proposed HPLC assay for the simultaneous measurement of phenobarbital, carbamazepine,

phenytoin and its main metabolite (HPPH) in serum is both sensitive and precise. The method involves a simple protein precipitation and direct injection of the supernatant. Use of a column temperature of 40 °C resulted in a better reproducibility of retention times and shortened the analysis time to 12 min. As the method is so rapid, it lends itself not only to drug-level analysis but also to the emergency monitoring of toxic levels of these drugs. The procedure can determine each drug over its entire sub-therapeutic, therapeutic and toxic concentration range, not only for adult samples but also for paediatric micro-samples.

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Lithium Ion-selective Electrodes Containing TOPO: Determination of Serum Lithium by Flow Injection Analysis

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Trioctylphosphine oxide (TOPO) acts as a neutral lithium carrier in lithium ion-selective electrode membranes. When TOPO was used with 1% potassium tetrakis(*p*-chlorophenyl) borate in NPOE - PVC membranes, the electrodes exhibited a nearly Nernstian response and had a lithium to sodium selectivity of 1 : 60, which is better than some electrode sensors reported earlier. TOPO was also added to 14-crown-4, ETH 1810 and UWXC 10 electrode membranes, and the lithium to sodium selectivity of the 14-crown-4 electrode was dramatically improved. However, TOPO had a negative effect on the selectivities of the ETH 1810 and UWXC 10 electrodes. Diluted serum samples were analysed for lithium using the 14-crown-4 - TOPO electrode in an FIA system. A statistical analysis of the ISE and corresponding AAS results indicated that these two methods have no statistical difference at the 95% confidence level. Because of the improved selectivity of the electrode and the elimination of the dialysis membrane from our original FIA design, the precision and accuracy of this determination were both improved, and the measurement time decreased.

Keywords: *Lithium ion-selective electrodes; TOPO; lithium to sodium selectivity; serum lithium determination; flow injection analysis*

In the early 1970s, trioctylphosphine oxide (TOPO) and a β-diketone, dibenzoylmethane, in *p*-xylene were suggested as an extraction system for the separation of lithium ions from other alkali metal ions.[1] In 1983, TOPO was incorporated in a PVC membrane with the β-diketone in this laboratory to produce a lithium ion-selective electrode (ISE); however, the performance of this electrode was unsatisfactory. In 1985, Kitazawa *et al.*[2] added 1–2% of TOPO to their 14-crown-4 lithium ion-selective membrane and the lithium to sodium selectivity of this membrane electrode was dramatically improved. In searching for a better lithium ion-selective electrode for the determination of serum lithium, we studied a series of existing lithium ion-selective membranes and the effect of TOPO in these. We found that TOPO, a commercially available and inexpensive compound, when used only with a small percentage of potassium tetrakis(*p*-chlorophenyl) borate (KTpClPB) in a PVC membrane, exhibited a good lithium to sodium selectivity (Table 1). However, comparison showed that the best lithium ion-selective electrode investigated was the 14-crown-4 - TOPO electrode.

Although the determination of serum lithium had been successfully performed in a FIA - dialysis system,[3] a better lithium selectivity was still required. The elimination of the dialysis membrane was preferred because the membrane reduced the over-all lithium to sodium selectivity.[3] A flow design was therefore constructed in which the direct injection of diluted serum samples was performed. The results of this determination were in agreement with atomic absorption spectrometry (AAS) results. The advantages and disadvantages of the direct sample injection and dialysis systems are discussed in this paper.

Experimental

Chemicals

N,N-Dicyclohexyl-*N′,N′*-diisobutyl-*cis*-1,2-cyclohexane-1,2-dicarboxyldiamide (ETH 1810) was obtained from the Department of Organic Chemistry, Swiss Federal Institute of Technology (ETH). *N,N′*-Diethyl-5,5-dimethyl-*N,N′*-bis(3-oxapentyl)3,7-dioxanonanediamine (UWXC 10) and 3-dodecyl-3-methyl-1,5,8,12-tetraoxacyclotetradecane (14-crown-4) were synthesised in our laboratory. The procedures of these syntheses are reported elsewhere.[4,5] TOPO was obtained from Aldrich Chemical and all the other chemicals are the same as those reported in our earlier papers.[3,6]

Apparatus

Fig. 1 illustrates the flow design of the FIA - ISE system. As many as four electrodes of the same or different composition may be mounted in the carrier stream for simultaneous investigations. The 3 M KCl stream forms a flow salt bridge between the sample carrier stream and the reference electrode (channel B–A in Fig. 1). This design reduces the junction potential at point A by 3 mV compared with the 140 mM NaCl

Table 1. Performance of the TOPO lithium ion-selective electrode

	Electrode			
	1	2	3	4
	TOPO, 1%; KTpClPB, 1%	TOPO, 3%; KTpClPB, 1%	TOPO, 6%; KTpClPB, 1%	TOPO, 10%; KTpClPB, 1%
Selectivity*				
$K^{pot}_{Li,Na}$	0.017	0.023	0.027	0.029
$K^{pot}_{Li,K}$	0.0017	0.0015	0.0015	0.0016
$K^{pot}_{Li,Mg}$	0.013	0.038	0.23	0.20
$K^{pot}_{Li,Ca}$	0.15	0.71	1.4	1.0
Slope/mV decade^{-1}	46.4	45.5	53.4	54.4
Correlation coefficient . .	0.999	0.995	0.997	0.996

* Selectivity coefficients were calculated by matched potential method.[9]

liquid junction when diluted serum samples are injected into the system. It also avoids problems such as contamination and liquid junction blocking, which are commonly associated with some conventional salt bridge designs.[7] An attempt at passing 3 M KCl through the reference electrode (channel B–C in Fig. 1) failed as the reference Ag - AgCl electrode was unstable in such a high chloride ion concentration. The flow-rates of the streams were adjusted to the values shown in Fig. 1 in order to obtain a fast response and a stable base line. The flow meters were purchased from Cole Parmer. The other apparatus was the same as reported in our earlier papers,[3,6] in which the construction of the electrodes and microconduits are also described.

The membrane compositions of the electrodes were 33 mg of PVC and 65 mg of *o*-nitrophenyl octyl ether (NPOE), with different mass percentages of neutral carriers and additives as indicated in Tables 1 and 2. (The mass percentages in the tables are equivalent to milligrams of each component added to the above masses of PVC and NPOE.)

Standards and Samples

Lithium-free serum and serum from manic-depressive patients on lithium treatment were obtained from the University of Washington Hospital. Equal volumes of five normal blood sera were mixed (*i.e.*, pooled serum samples) and diluted 10-fold with 2.5 mM $Na_2B_4O_7$ buffer solution as blanks. Serum lithium standards were prepared by 10-fold dilution of the pooled normal serum with a series of LiCl standards in 2.5 mM $Na_2B_4O_7$ solution. The serum samples from manic-depressive patients were also diluted 10-fold with 2.5 mM $Na_2B_4O_7$ before analysis. The buffered standards and diluted sample solutions had a pH of 9.2.

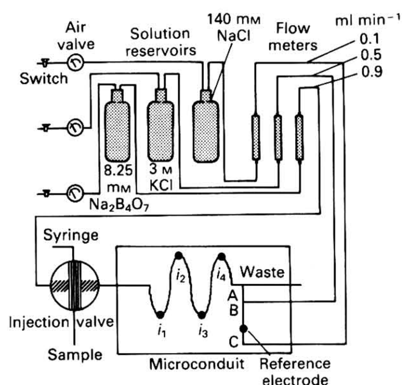

Fig. 1. Design of flow injection analysis ISE system

Selectivity Coefficients

Fixed interference[8] and matched potential[9] methods were used to measure the selectivity coefficients, $k^{pot}_{Li,M}$. A discussion of this subject has been given in our earlier papers.[3,6]

Results and Discussion

TOPO as a Neutral Lithium Carrier

TOPO with dibenzoylmethane has been successfully used for the extraction of lithium from other alkali metal ions and the lithium complex formed is probably $PhCOCH_2COPh–Li^+–(TOPO)_2$.[1] Because liquid membrane ion-selective electrodes are based on the same complexation - extraction phenomenon, our first step was to make lithium ISEs with 2% TOPO

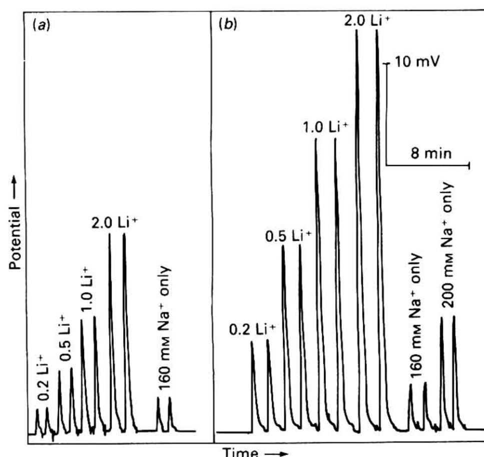

Fig. 2. Response of the 14-crown-4 electrodes with (*b*) and without (*a*) 1% TOPO. Li^+ in 140 mM Na^+, concentration in mM

Table 2. Evaluation of 14-crown-4 and ETH 1810 lithium ISEs. M.P. = matched potential method; F.I. = fixed interference method

	Electrode*				
	1	2	3	4	5
	A, 1%; B, 1%; crown, 3%	A, 3%; B, 0%; crown, 3%	A, 0%; B, 0.5%; crown, 1.5%	A, 0%; B, 0.4%; ETH, 1.4%	A, 1%; B, 1%; ETH, 2%
Selectivity					
$K^{pot}_{Li,Na}$ (M.P.)	0.0042	0.0088	0.012	0.014	0.019
(F.I.)	0.0025	0.0086	0.0080	0.0071	0.011
$K^{pot}_{Li,K}$ (M.P.)	0.0062	0.0013	0.013†	0.0025‡	0.0034
$K^{pot}_{Li,Mg}$ (M.P.)	0.0010	0.0054	0.000016†	0.00010‡	0.0027
$K^{pot}_{Li,Ca}$ (M.P.)	0.0015	0.073	0.000077†	0.0020‡	0.026
Slope/mV dec^{-1}	59.0	59.9	58.9	59.9	45.3
Correlation coefficient	1.000	0.999	0.999	0.999	0.996
Linear range§/mM	2–200	2–200	5–200	5–200	5–200

* A, TOPO; B, KTpClPB.
† Data from reference 2.
‡ Data from reference 12.
§ In the presence of 140 mM Na^+.

and 1% dibenzoylmethane in PVC membranes. Although different plasticisers were used to prepare the electrodes, none of the electrodes responded to lithium ions. However, when TOPO was used with 1% of KTpClPB in NPOE - PVC membranes, the electrodes exhibited a nearly Nernstian response (Table 1) and the lithium to sodium selectivity of the electrode was better than for some of the electrode sensors reported earlier.[10] These results imply that the function of a complex extraction system in an organic solvent cannot be assumed to be completely applicable to electrode membranes, and that the selectivity for metal ion extraction in electrode liquid membranes is a combined effect of all the membrane materials. However, TOPO, like other neutral carriers, plays a key role in the electrode.

Table 1 shows that the best combination in the electrode is 1% of TOPO and 1% of KTpClPB. The lithium selectivity decreases with an increasing percentage of TOPO in the membrane, and calcium and magnesium interferences also increase with increasing TOPO percentage. Potassium selectivity remains about the same. It should be noted that the $k^{pot}_{Li,M}$ values in the tables have uncertainties of approximately 10%. However, these values do indicate trends in the performance of the electrode.

Effect of TOPO in Existing Lithium ISEs

Among the many reported lithium ISEs,[5,10–13] 14-crown-4, ETH 1810 and UWXC 10 are the best three lithium ion-selective electrodes. We constructed 14-crown-4 electrodes with and without TOPO in order to compare the selectivities of the two electrodes in 140 mM NaCl. The results are shown in Fig. 2. The lithium response of the electrode with TOPO was three times more sensitive than that of the electrode without TOPO, whereas the sodium response was nearly the same. Therefore, the lithium to sodium selectivity of the electrode was also tripled ($k^{pot}_{Li,Na}$ = 0.0025). Our selectivity value compares favourably with the results of Kitazawa *et al.*,[2] who prepared the electrodes in a conventional manner (membrane-body type) and performed batch-wise selectivity measurements. It is important to point out that the reported sensitivity loss (higher detection limit) of this electrode in pure lithium solution[2] has no realistic meaning in most electrode applications, as real samples, *e.g.*, serum samples, generally contain a high background of interfering sodium ions. Here, the higher lithium selectivity results in a better detection of lithium in the presence of sodium. Thus, the restriction of sensitivity loss[14] is not applicable when the electrode is designed for application purposes.

A higher percentage of TOPO was then added to the electrode membranes. The selectivity change (Table 2) followed the same trend observed in Table 1. This change also agreed with the previous report.[2] TOPO was also added to the ETH 1810 electrode membranes for comparison purposes. The selectivity and sensitivity of the resulting electrode were somewhat inferior to those of the ETH 1810 electrode without TOPO (Table 2). TOPO added to the UWXC 10 electrode suppressed the lithium response of the electrode and the lithium to sodium selectivity of the electrode was reduced to 40; this is approximately one-third of the original selectivity of the UWXC 10 electrode.[11]

TOPO electrode and TOPO additive studies support the suggestion that the selectivity of liquid membrane electrodes is not governed solely by the neutral carrier itself; the chemical nature of additives and liquid phases also greatly affects the selectivity of the electrodes. At present the best lithium ion-selective electrode for determinations in the presence of sodium is the 4-crown-4 - TOPO electrode. Therefore, we used this electrode for serum lithium determinations.

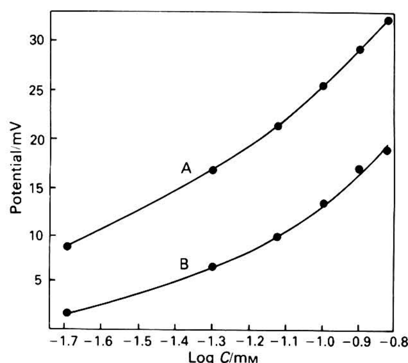

Fig. 3. Calibration graphs of (A) aqueous standards and (B) serum standards using electrode 1 of Table 2

Serum Lithium Determination

The direct injection of diluted serum samples (200 μl) was carried out in the FIA system shown in Fig. 1. Using this system, no significant base-line drift or change in response time was observed and the system was also free from blockages. Calibration graphs for standards in both aqueous solutions and serum solutions are shown in Fig. 3. Because of the matrix effects in serum samples, a significant blank response shifted the background potential in the samples and the lithium responses were slightly suppressed, therefore aqueous standards cannot be used. Even so, in the usual therapeutic lithium concentration range (0.5–1.5 mM), the observed voltage interval of diluted serum standards was 12.4 mV, a sensitivity improvement of about 2.5 times over our previous system.[3] This concentration range is in the non-linear

Table 3. Results of serum lithium determination by AAS and ISE (electrode 1 of Table 2)

Sample No.	AAS Li^+ concentration/mM	ISE Li^+ concentration/mM	Absolute error/mM	Relative error, %	ISE Na^+ concentration/mM
1	0.82	0.88	+0.06	+7	150
2	0.4	0.41	0.0	0	150
3	1.17	1.24	+0.07	+6	151
4	1.00	0.98	−0.02	−2	149
5	0.61	0.57	−0.04	−6	149
6	0.88	0.96	+0.08	+9	145
7	0.76	0.69	−0.07	−9	149
8	0.68	0.60	−0.08	−12	149
9	0.73	0.62	−0.11	−15	149
10	0.75	0.70	−0.05	−7	150
Blank	0.00	0.00	0.00	0	145
Average			−0.016	−2.9	

region of the calibration graph, owing to high background sodium. Linearity is achieved down to 2 mM serum lithium in either diluted or undiluted serum, Table 2. Precise measurements are nevertheless obtained with the system and the lithium selectivity relative to sodium is greater than with any other electrode. Hence, the lithium response in serum is at a maximum.

Ten hospital serum lithium samples were analysed by this electrode and the results are listed in Table 3. Table 3 also lists the AAS results, absolute errors, relative errors and the Na^+ content of samples. A statistical analysis of the ISE results and the AAS results indicated that at the 95% confidence level, these two methods have no statistical difference (t = 0.76).[15] When ISE values were plotted against AAS values, a good linearity, with a correlation coefficient of 0.970, was obtained. As the sodium contents were nearly constant in the sample and the electrode had a high lithium to sodium selectivity, sodium corrections were not needed.

Five normal individual sera blanks (diluted) were injected into the system. The standard deviation of the potential values of these separate blanks was ±0.97 mV. If we assume that the average sodium concentration, C_{Na}, in the serum samples is 140 mM and the lithium concentration, C_{Li}, is 1.0 mM, then according to the equation

$$dE = \frac{S}{C_{Li} + k^{pot}_{Li,Na} C_{Na}} dC_{Li}$$

this potential uncertainty corresponds to a ±0.05 mM lithium concentration fluctuation (S is the slope of the electrode response, which is 59 mV decade^{-1}). Thus, an average ±5% relative error could be observed in this determination (at the 1.0 mM level), owing to uncertainties in the background reading of the prepared serum standards. This essentially agrees with our observations (Table 3).

The precision for six measurements of an individual serum sample at 0.5 mM lithium is ±0.17 mV or ±0.0049 mM lithium. Therefore, the uncertainties in the serum lithium determination are mainly from the sample matrix uncertainties rather than the instrumentation.

Previously we determined serum lithium in a FIA - dialysis system.[3] The advantages of this system are that it is free from serum protein interferences and free from the risk of electrode poisoning. Also, it is possible to use aqueous standards and little sample preparation is needed. The disadvantages are the reduced lithium to sodium selectivity and the slow sampling rate due to the dialysis process. The elimination of the dialysis membrane in this study increased the over-all lithium to sodium selectivity by five-fold and tripled the sampling speed. As a better electrode was employed, the precision and accuracy were also improved.

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Chemically Immobilised Bi-enzyme Electrodes in the Redox Mediated Mode for the Flow Injection Analysis of Glucose and Hypoxanthine

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Flow injection analysis systems incorporating amperometric bi-enzyme electrodes for glucose and hypoxanthine determination are described. The sensors were based on glucose oxidase - peroxidase for the determination of glucose and xanthine oxidase - peroxidase for the determination of xanthine and hypoxanthine and the enzymes were immobilised on nylon mesh and held over a platinum electrode. The hydrogen peroxide product of the enzymatic catalysis was monitored amperometrically in a three-electrode Stelte cell, adapted for flow injection analysis, after its peroxidase-catalysed reaction with hexacyanoferrate(II).

The use of the hexacyanoferrate(II) mediator permitted a low applied potential (−100 mV *vs.* Ag - AgCl) to be used, enabling the determination of glucose in blood serum samples without difficult sample pre-treatment. The xanthine oxidase - peroxidase bi-enzyme electrode was used to determine hypoxanthine in fish meat as an indicator of deterioration during storage and the results compared favourably with an alternative spectrophotometric approach recommended by the Analytical Methods Committee (AMC) according to the equation $[\text{hypoxanthine}]_{\text{Electrode}} = 1.01[\text{hypoxanthine}]_{\text{AMC}} + 4.0 \times 10^{-3}$, with a correlation coefficient of 0.998.

Keywords: *Blood glucose determination; glucose enzyme electrodes; xanthine and hypoxanthine determination; flow injection analysis; fish meat*

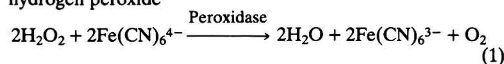
The pioneering work of Clark and Lyons[1] has stimulated progress in the use of immobilised enzymes for analysis in the biochemical and clinical field.[2–4] Present research focuses on combining the specificity of the enzyme reaction with a suitable electrochemical detection system. The electroanalytical approach offers great potential by means of its relative simplicity and ease of interfacing to other equipment, and the method is unaffected by turbidity.

Electrochemical methods based on enzyme electrodes frequently involve monitoring either the consumption of oxygen using a Clark electrode, as demonstrated by Updike and Hicks,[5] or the amperometric monitoring of hydrogen peroxide formed using a platinum electrode.[4,6] Several workers[7–9] have successfully produced flow injection systems with detector electrodes based on monitoring the anodic decomposition of hydrogen peroxide. These electrodes operate at potentials of 0.6–0.7 V *vs.* Ag - AgCl. However, at such high potentials, other electroactive species such as ascorbic acid and uric acid, which are present in physiological samples, will also be oxidised. The effect of such interferences renders this approach to the analysis of clinical and biochemical samples difficult unless there is a tedious pre-treatment. Nevertheless, interferents may be removed, *e.g.*, by treatment with copper(II) dithiocarbamate as demonstrated by Masoom and Townshend[10] in an approach based on a silica gel column stage, incorporating the copper(II) reagent, in the flow injection analysis of glucose in samples of clinical origin.

Although hydrogen peroxide is electroactive, its oxidation and reduction are irreversible and its concentration cannot be determined by zero current potentiometry at a noble metal electrode. The coupling of the reduction of peroxide to a more reversible system, the I_2 - I^- couple for the potentiometric monitoring of glucose using a solid-state iodide ion-selective electrode has been demonstrated.[11,12] For amperometric systems the use of mediators[13,14] and conducting organic salts[15] is currently a very active area of research. The mediator is immobilised on the electrode surface leading to a sensor that is potentially independent of oxygen for substrate oxidation. Thus, the resulting electrode is in principle an electrocatalytic sensor for the substrate, avoiding the direct measurement of peroxide but, although promising, these sensors are at a relatively early stage of development.

This paper describes the use of the iron(II) - iron(III) cyanide couple for the amperometric determination of hydrogen peroxide produced in enzyme reactions. The proposed system also extends the principle of the chemical immobilisation of enzymes on nylon mesh by the agency of glutaraldehyde to the coupling of two enzymes. Thus, the response characteristics of enzyme electrodes for glucose and hypoxanthine, respectively, incorporating glucose oxidase - peroxidase and xanthine oxidase - peroxidase bi-enzyme membranes were studied in a flow injection analysis system. The glucose system served as a model for the xanthine - hypoxanthine studies, which demanded a lower applied potential because of the electroactivity of xanthine and hypoxanthine at the 0.6–0.7 V required for direct hydrogen peroxide monitoring. Also, the uric acid product of enzymic xanthine oxidation interferes at high applied potentials.

Peroxidase catalyses the hexacyanoferrate(II) reaction with hydrogen peroxide



$$2H_2O_2 + 2Fe(CN)_6^{4-} \xrightarrow{\text{Peroxidase}} 2H_2O + 2Fe(CN)_6^{3-} + O_2 \qquad (1)$$

and the generated hexacyanoferrate(III) is reduced at a platinum electrode at a low applied potential, *i.e.*, −100 mV *vs.* Ag - AgCl

$$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-} \quad .. \quad .. \quad (2)$$

The electrodes were tested for the determination of glucose in serum and of hypoxanthine in fish meat.

Experimental

Reagents

Glucose oxidase (E.C. 1.1.3.4, 100 I.U. mg^{-1}, purified from *Aspergillus niger*), xanthine oxidase (E.C. 1.2.3.2, 1–2 I.U. mg^{-1} from buttermilk), peroxidase (E.C. 1.11.1.7, 250 I.U. mg^{-1} from horseradish), lysine monohydrochloride, dimethyl sulphate, glutaraldehyde (25% aqueous solution), β-D(+)-glucose, xanthine (2,6-dihydroxypurine) and hypoxanthine (6-hydroxypurine) were obtained from Sigma Chemical. All the enzymes were stored in a refrigerator. Other chemicals used were of the best available laboratory-reagent grade.

* To whom correspondence should be addressed.

Glucose, xanthine and hypoxanthine standards were prepared from their respective stock solutions (100 mM) in sodium dihydrogen orthophosphate buffer (100 mM) containing potassium hexacyanoferrate(II) (2.5 mM).

Serum samples for glucose determination (already analysed for glucose by a colorimetric procedure) were obtained from the University Hospital of Wales, Cardiff, and the fish for hypoxanthine determination was from the local market. Nylon mesh, obtained from Henry Simon, Cheshire, had the following characteristics: open surfaces 38%, thread thickness 80 μm, mesh count 47.15 cm^{-1} and mesh aperture 132 μm.

Immobilisation of Enzymes

Bi-enzyme membranes based on nylon mesh were prepared by a modification[16] of the method of Hornby and Morris.[17] Prior to enzyme attachment the nylon mesh was activated with dimethyl sulphate and reacted with lysine and glutaraldehyde as previously described.[9]

The enzyme was attached to the nylon by dipping the activated nylon mesh in glucose oxidase or xanthine oxidase (10 mg cm^{-3}), as appropriate, in phosphate buffer (100 mM, pH 7.0) containing peroxidase (5 mg cm^{-3}) for 2 h at room temperature and then overnight at 4 °C. The attachment of the enzyme to nylon mesh is achieved by bifunctional glutaraldehyde, with lysine acting as a spacer between the nylon and the enzyme structure. Hornby and Morris[17] demonstrated a doubling of enzyme activity by incorporating a lysine spacer molecule.

The ratio of the oxidase to peroxidase immobilised was at an optimum at 2:1. Greater proportions of peroxidase (1:1, 1:2) resulted in a poor substrate response and lower proportions (3:1, 4:1) yielded membranes of poor reproducibility.

The bi-enzyme electrode was then fabricated by stretching the membrane over a smooth platinum electrode as previously described.[9] The excellent mechanical strength of the mesh gave a tight fit over the electrode with the aid of an O-ring.

Apparatus

Electrode potentials were controlled and currents monitored with a potentiostat (Metrohm VA-detector E611). A Linear *y* - *t* chart recorder (Model 500) was used to record the flow injection analysis signals. The sample was propelled with a four-channel peristaltic pump (Ismatec Model IP-4) with sample injections being made with an electrically activated valve (Valco Instruments, Texas). All connecting tubing was of PTFE (nominal i.d. 1.27 mm). The pump pulsation was reduced with a suppressor placed immediately after the pump.

The detector was based on a three-electrode assembly incorporating a modified (Metrohm-EA1102) Stelte micro-cell with a platinum enzyme working electrode, a glassy carbon auxiliary electrode and a silver - silver chloride reference electrode. The working electrode of the Stelte micro-cell was modified to produce a "wall jet" type of working electrode chamber as described previously.[9] The apparatus was used in the normal mode for flow injection analysis with standards and sample solutions being introduced through the injection valve into an optimised carrier stream of sodium dihydrogen phosphate (100 mM) buffer with appropriate amounts of hexacyanoferrate(II).

Determination of Glucose in Serum

Blood serum samples were diluted ten-fold in phosphate buffer (100 mM at pH 7.0) containing potassium hexacyanoferrate (2.5 mM). Sample volumes of 500 mm^3 were used for the flow injection analysis of glucose using the glucose oxidase - peroxidase electrode. The data were compared with results obtained with a colorimetric soluble enzyme test kit method[18] carried out at the University Hospital of Wales.

Determination of Hypoxanthine in Fish Meat

Fish meat (5–10 g) was homogenised in perchloric acid (50 cm^3 of 0.6 M acid) according to the procedure of the Analytical Methods Committee[19] for extracting hypoxanthine. After homogenisation, the filtered extract (5 cm^3) was mixed with potassium hydroxide (0.557 M) - phosphate (0.20 M) buffer (5 cm^3). Precipitated perchlorate was removed by centrifugation; the pH of the supernatant was 7.2. For spectrophotometric determination the supernatant extract (1 cm^3) was mixed with enzyme (0.5 cm^3 of 0.05 I.U. cm^{-3} of solution) and phosphate buffer (0.1 M, 3.5 cm^3), mixed well and incubated at 37 °C for 30 min and the absorbance measured at 290 nm in 1-cm silica cells.

For the flow injection analysis of hypoxanthine using the xanthine oxidase - peroxidase electrode the supernatant centrifugate (1 cm) was diluted with carrier solution (4 cm^3) and 500 mm^3 aliquots were used. The data were compared with spectrophotometric results obtained by the method recommended by the Analytical Methods Committee.[19]

Results

The enzymatic and flow parameters were optimised on 500 mm^3 samples to obtain the best response to glucose and hypoxanthine. Xanthine oxidase exhibits twice the activity to xanthine compared with hypoxanthine and consequently the optimum response to xanthine was also investigated with the xanthine oxidase - peroxidase bi-enzyme electrode. The pH, flow-rate and mediator concentration were optimised prior to calibration.

Optimisation of pH

The optimum pH was determined by injecting the 500 mm^3 samples of glucose (2 mM), xanthine (0.02 mM) and hypoxanthine (0.1 mM) into carrier streams passing over their respective electrodes using a buffer flow-rate of 3 cm^3 min^{-1}. The buffer consisted of sodium dihydrogen orthophosphate (100 mM) containing the mediator potassium hexacyanoferrate(II) (21.5 mM). The resulting peak height - pH profile (Fig. 1) for the carrier stream pH adjusted with sodium hydroxide solution exhibits an optimum pH range between 6.75 and 7.25 for both types of electrodes. Hence, all further work was carried out at pH 7.0 for each of the enzyme electrodes.

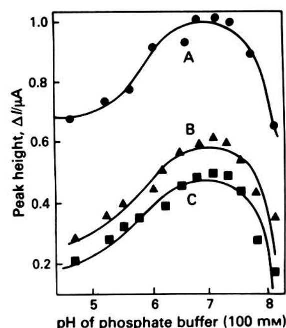


Fig. 1. Effect of pH on the response of the electrodes to: A, glucose (glucose oxidase - peroxidase electrode) 2 mM; B, hypoxanthine (xanthine oxidase - peroxidase electrode) 0.1 mM; and C, xanthine (xanthine oxidase - peroxidase electrode) 0.1 mM

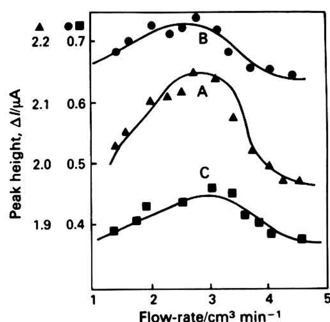


Fig. 2. Optimisation of the flow-rate for the bi-enzyme electrodes with: A, glucose (glucose oxidase - peroxidase electrode) 5 mM; B, hypoxanthine (xanthine oxidase - peroxidase electrode) 0.2 mM; and C, xanthine (xanthine oxidase - peroxidase electrode) 0.02 mM

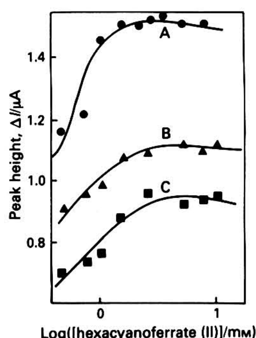


Fig. 3. Effect of hexacyanoferrate(II) mediator concentration on the response of: A, glucose (glucose oxidase - peroxidase electrode) 2 mM; B, hypoxanthine (xanthine oxidase - peroxidase electrode) 0.5 mM; and C, xanthine (xanthine oxidase - peroxidase electrode) 0.05 mM

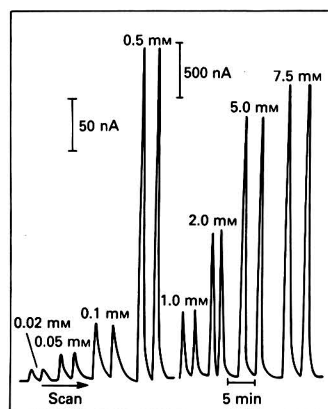


Fig. 4. Typical recorder output for hexacyanoferrate(II) mediated glucose calibration of the glucose oxidase - peroxidase bi-enzyme electrode

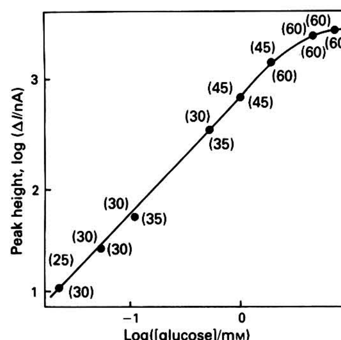


Fig. 5. Glucose calibration graph for the bi-enzyme glucose oxidase - peroxide electrode. Numbers above the line are response times and those below the line indicate wash times, *i.e.*, times between samples during which the electrode is washed by carrier streams

Optimisation of Flow-rate

The effect of carrier stream flow-rate at pH 7.0 was investigated over the range 1–5 cm^3 min^{-1}. Again a redox mediator concentration of 2.5 mM was used. The characteristics of the respective flow-rate - peak height responses for glucose (5 mM), xanthine (0.02 mM) and hypoxanthine (0.2 mM) are similar to each other (Fig. 2). Thus, all three show a maximum substrate response between a flow-rate of 2.75 and 3.25 cm^3 min^{-1}. Hence a flow-rate of 2.75 cm^3 min^{-1} was adopted in each instance.

Optimisation of Potassium Hexacyanoferrate(II) Concentration

The concentration of the potassium hexacyanoferrate(II) mediator was varied between 0.5 and 10 mM to investigate the effect on substrate response. As can be seen from the resulting peak height profile (Fig. 3), all three systems exhibit a limiting type of response, that is, at a 1.5 mM mediator concentration for glucose (2 mM) and 2.5 mM mediator for both xanthine (0.05 mM) and hypoxanthine (0.5 mM). Increasing the mediator concentration further produced no increase in response. Hence, a mediator concentration of 2.5 mM potassium hexacyanoferrate(II) was used in all further work.

Electrode Calibration

The optimised carrier stream and flow parameters determined above, that is, a carrier of phosphate buffer (100 mM) at pH 7.0 containing potassium hexacyanoferrate(II) (2.5 mM) and a flow-rate of 2.75 cm^3 min^{-1}, were used in the FIA analysis studies of the bi-enzyme electrodes as described below.

The glucose oxidase - peroxidase enzyme electrode was calibrated for glucose standards prepared in the phosphate buffer from a 10 mM stock solution. Standards (500 mm^3) were usually injected in duplicate. Fig. 4 shows a typical chart recorder trace and Fig. 5 the corresponding calibration graph, which was linear over the range 0.02–3 mM glucose where log(current/A) = 1.09 log ([glucose]/M) − 2.91 with a correlation coefficient of 0.999.

In a similar fashion, the xanthine oxidase - peroxidase bi-enzyme electrode was calibrated for xanthine, its primary substrate and for hypoxanthine. A typical chart recorder output is shown in Fig. 6 for the duplicate injection of xanthine standards and Fig. 7 is the corresponding calibration graph, which is linear over the range 2–100 μM, that is, for the straight line

$$\text{Log(current/A)} = 0.93 \log([\text{xanthine}]/\text{M}) - 2.08 \quad (3)$$

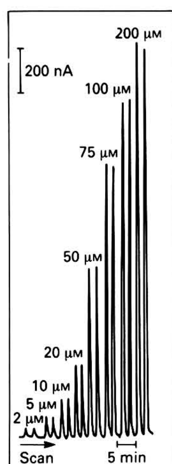


Fig. 6. Typical recorder output for a hexacyanoferrate(II) mediated xanthine calibration of the xanthine oxidase - peroxidase bi-enzyme electrode

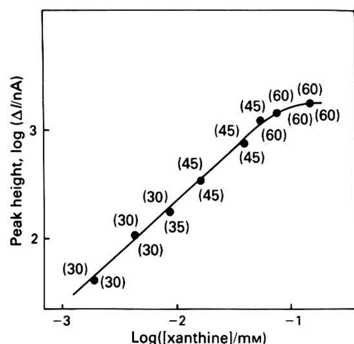


Fig. 7. Xanthine calibration graph for the bi-enzyme xanthine oxidase - peroxidase electrode. Numbers above the line are response times and those below the line indicate wash times

with a correlation coefficient of 0.998. The hypoxanthine calibration profile is almost identical with that illustrated for xanthine and the equation for the straight line is

$$\text{Log(current/A)} = 0.895 \log ([\text{hypoxanthine}]/\text{M}) - 2.62 \quad (4)$$

with a correlation coefficient of 0.999. Linearity was obtained over the range 2–100 μM of hypoxanthine.

Determination of Glucose in Serum

For the determination of glucose in blood serum the glucose oxidase - peroxidase bi-enzyme electrode was calibrated for glucose. Linearity was observed over the range 0.02–3 mM (Fig. 5). Ten-fold diluted serum samples were analysed and the data obtained are summarised in Table 1, alongside those obtained with the soluble enzyme test kit method. For each electrode method the sample was injected in duplicate and for the eight samples (sixteen peaks) a mean variation of 1.5% between each pair of peaks was obtained.

The results obtained with the bi-enzyme electrodes and the soluble enzyme test kit method showed good agreement with each other and gave the line

$$[\text{Glucose}]_{\text{Electrode}} = 1.00\,[\text{glucose}]_{\text{Soluble test kit}} - 0.219 \quad (5)$$

with a correlation coefficient of 0.997.

Table 1. Determination of glucose in blood serum. Mean of two determinations

Sample number	Soluble enzyme test kit result/mM	Glucose oxidase - peroxidase electrode result/mM
1	3.99	3.8
2	5.91	5.2
3	9.76	10.1
4	11.68	12.1
5	13.75	13.0
6	15.64	15.2
7	17.59	17.4
8	19.36	19.2

Determination of Hypoxanthine in Fish Meat

The xanthine oxidase - peroxidase bi-enzyme electrode was calibrated in the range 1–100 μM for hypoxanthine. The deterioration of fish meat quality was reflected by the increases in hypoxanthine levels that occurred during the storage at room temperature (20 °C) for 20 h (Table 2).

The tests were carried out on four different kinds of fish, namely rainbow trout, herring, hake and plaice and the results for the bi-enzyme electrode method with redox mediator were compared with those of the spectrophotometric based method recommended by the Analytical Methods Committee.[19] Thus, for the sets of data relating to fresh fish and stored fish together the line

$$[\text{Hypoxanthine}]_{\text{Electrode}} = 1.01[\text{hypoxanthine}]_{\text{AMC}} + 4.0 \times 10^{-3} \quad (6)$$

with a correlation coefficient of 0.998 was obtained.

With regard to illustrative xanthine oxidase - peroxidase bi-enzyme electrode data for the hexacyanoferrate(II) mediated system, herring and hake gave mean output currents of 0.816 μA with a standard deviation of 0.088 μA and 0.54 μA with a standard deviation of 0.086 μA, respectively.

Discussion

Electrode Response Characteristics

Effect of pH

The rate of an enzyme reaction as a function of pH normally passes through a maximum. This is also characteristic of the bi-enzyme electrode response (Fig. 1). This optimum pH is a direct result of the amphoteric nature of the amino acids that make up the enzyme, specifically those amino acids affecting the binding of the substrate and the effect of protonation on the rates of formation of the enzyme - substrate complex. Further, this concept must also include the effect of pH on the decomposition of the active enzyme - substrate complex. Waley[20] has proposed a general equation encompassing these concepts, which predicts an optimum pH.

Enzyme immobilisation will produce a shift in the optimum pH as a direct result of constitutional changes imposed on the enzyme. Thus, whereas the optimum pH of soluble glucose oxidase is pH 5.5 as shown by Weibel and Bright,[21] the immobilised glucose oxidase - peroxidase nylon mesh immobilised bi-enzyme electrode exhibits a more alkaline optimum at pH 7.0. A similar, more alkaline, pH was also exhibited by the single glucose oxidase enzyme membrane electrodes described previously.[9]

Table 2. Determination of hypoxanthine in fish meat using xanthine oxidase - peroxidase bi-enzyme electrode (BE) and Analytical Methods Committee[19] (AMC) recommended method

	Fresh fish				Fish stored at room temperature for 20 h			
	μmol dm^{-3} (extract)		μmol g^{-1} (tissue)		μmol dm^{-3} (extract)		μmol g^{-1} (tissue)	
	BE*	AMC	BE	AMC	BE*	AMC	BE	AMC
Rainbow trout	22.5 (7.432)	22.1	0.32	0.32	32.1 (89.321)	30.0	0.42	0.39
Herring	44.1 (6.393)	52.2	0.74	0.88	120.1 (6.910)	117.6	1.87	1.84
Hake	57.9 (10.588)	61.7	0.59	0.63	70.0 (6.898)	84.5	2.19	2.40
Plaice	85.0 (9.664)	81.3	0.95	0.91	84.6 (4.852)	79.1	1.89	1.76

* Mass of fish meat sample used given in grams in parentheses.

For the soluble xanthine oxidase the optimum pH is 4.6. As for glucose oxidase a shift to an optimum pH of 7.0 is observed here owing to its immobilisation on nylon mesh.

Effect of flow-rate

With regard to flow-rate the optimum maximum response between 2.75 and 3.25 cm^3 min^{-1} for both electrodes may seem rather unexpected (Fig. 2). As the pumping rate is increased the residence time of the substrate in contact with the electrode decreases and consequently a smaller peak height would be expected. However, the optimum response region may be attributed to less dispersion by faster flow and partly by greater turbulence at the electrode to improve diffusion to the sensor. These effects lead to an optimum peak height in the range 2.75–3.25 cm^3 min^{-1}.

A further increase in the flow-rate results in a reduction of response due to the fast passage of sample substrate counteracting the favourable optimum conditions by reducing the residence time in the vicinity of the electrode.

Effect of redox mediator

The variation of the concentration of the redox mediator potassium hexacyanoferrate(II) on the peak height of the substrate is in effect a matter of fine tuning of the reaction between the hydrogen peroxide generated and the mediator depicted in reaction (1). The resulting profile (Fig. 3) exhibits for each of the bi-enzyme electrodes a limiting region beyond a mediator concentration of 2.5 mM for hypoxanthine and xanthine and 1.5 mM for glucose, respectively. Therefore, 2.5 mM hexacyanoferrate(II) was used for the electrode calibrations and analytical applications.

Lifetime

The glucose bi-enzyme electrodes have a lifetime of three months for intermittent use with storage in buffer at 4 °C when not in use. This is slightly shorter than that for single enzyme glucose oxidase membranes where lifetimes in excess of four months were observed.[9] The shorter lifetimes experienced here may be attributed to the fact that the second enzyme, peroxidase, reduces the surface area taken up by the primary glucose oxidase.

The xanthine oxidase - peroxidase electrode had a working lifetime of two months under intermittent use, with storage in buffer at 4 °C when not in use. The reduced electrode lifetime when compared with the glucose electrode is probably due to the low activity of enzyme for initial immobilisation, namely, 1–2 units mg^{-1} for xanthine oxidase compared with 100 units mg^{-1} for glucose oxidase.

Applications to Analysis of Real Samples

The justification for using bi-enzyme electrodes under conditions of redox modification relates to the need to overcome electrochemical interference and interferences by sample components, such as the ascorbic acid and uric acid cited above for physiological samples. Therefore, it was pertinent to evaluate the nylon mesh chemically immobilised bi-enzyme electrodes for the analysis of real samples. The data obtained for the two electrode systems, as mentioned above and as summarised in Tables 1 and 2, compare favourably with those for the alternative recommended and established methods. The bi-enzyme electrode flow injection analysis approaches are convenient to use and lend themselves to dealing with large numbers of samples. In particular, the xanthine oxidase - peroxidase bi-enzyme electrodes offer possibilities for quality control monitoring in the food industry.

Conclusion

Glucose oxidase - peroxidase and xanthine oxidase - peroxidase can be chemically immobilised on nylon mesh and fitted over a platinum electrode to form bi-enzyme electrodes for glucose and hypoxanthine, respectively. These bi-enzyme electrodes set up for flow injection analysis in a modified Stelte micro-cell for the amperometric detection of the reaction between hydrogen peroxide (a product of enzyme catalysis) and potassium hexacyanoferrate(II) redox mediator permit the use of a low potential with the bi-enzyme electrode in order to reduce electrochemical interference and interferences from electroactive species frequently present in real samples. The appropriate bi-enzyme electrodes can be used for determinations such as glucose in serum samples and hypoxanthine in fish meat.

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Determination of Free Hydrofluoric and Nitric Acids in Pickling Bath Liquors Using a Fluoride-selective Electrode and Alkalimetric Titration

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A method is proposed for the determination of free hydrofluoric and nitric acids in pickling bath liquors by combining direct potentiometry with a fluoride-selective electrode and an alkalimetric titration. The correct choice of bridging solution for the reference electrode eliminates the influence of varying nitric acid concentrations on the determination of fluoride. The results of the methods have been compared with results obtained using the LECO AT-50 acid analyser and the correlations obtained were good. The determinations do not require expensive equipment and can be completed within 10–15 min.

The method outlined has been in use at the Outokumpu Oy Stainless Steel Division since 1978. The events of under- and over-pickling and total acid consumption have decreased in this time.

Keywords: *Fluoride-selective electrode; alkalimetric titration; hydrofluoric and nitric acid determination; pickling bath liquor; stainless steel*

Pickling baths containing nitric and hydrofluoric acids are commonly used for pickling stainless steel and other metals such as aluminium and zirconium. Typically the pickling liquors contain 1–3% hydrofluoric and 10–15% nitric acid. During pickling the metals are dissolved in the pickling liquor and subsequently the pickling strength is decreased. This can be compensated for to a certain extent by increasing the acid concentration, especially the hydrofluoric acid concentration. Inevitably the metal content will eventually reach a value above which there is no economical way of increasing the pickling strength *in situ*. The pickling liquor is then either regenerated or discarded.

Cold-rolled stainless steel is preferably pickled in pickling liquors containing fairly high concentrations of metals, notably iron. The acid contents of such liquors have to be known exactly in order to achieve the best pickling results. For this reason the development of reliable methods of determination has received considerable attention in steel works laboratories over the past decade.

In order to determine the concentrations of the individual acids, especially in metal-containing pickling liquors, it has previously been necessary either to use an indirect method involving the separation of the total fluoride content and subtraction of the fluoride complexed by the metal, or to measure the bleaching action of hydrofluoric acid on coloured metallic complexes.[1] The former is time consuming and the latter is subject to interferences. The use of ion-selective electrodes has also been investigated.[2,3] The LECO AT-50 acid analyser is a result of Swedish studies of ion-selective electrodes and measures the free fluoride and nitric acid concentration by direct potentiometry with two ion-selective electrodes. More recently ion-chromatographic techniques have been employed.[4]

In the methods outlined in this paper, the free hydrofluoric acid concentration is determined directly in pickling bath liquors using an Orion fluoride electrode system and the total acid concentration is determined by titration with 1 M sodium hydroxide solution using phenolphthalein as an indicator after the complexation of the metals with an excess of fluoride. The nitric acid concentration is then calculated by difference. The results were compared with results obtained with the LECO AT-50 acid analyser.

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Experimental

Cell potentials were measured with an Orion 94-09 fluoride-selective electrode and an Orion 90-01-00 single junction reference electrode. The bridge solution in the reference electrode was changed as described under Results and Discussion.

Measurements were made to the nearest 0.1 mV with an Orion 701 A digital pH/mV meter. All measurements were made at 25 °C. The titrimetric determinations of total acid concentration were made with 1 M sodium hydroxide solutions after complexation of the metals with an excess of fluoride (2.5 g of potassium fluoride per sample) using phenolphthalein as indicator. The titrations were carried out in glass burettes of a conventional type; for the test solutions containers of polypropylene plastic ware were used. Series of standard solutions containing 0.5–1.5 M hydrofluoric and 1.5–2.5 M nitric acid, respectively, were prepared.

The investigations were carried out on pickling bath liquor samples directly from the pickling line. The samples contained typically 0.5–1.5 M hydrofluoric and 1.5–2.5 M nitric acid and metals such as iron (0–60 g l^{-1}), chromium (0–7 g l^{-1}) and nickel (0–7 g l^{-1}). All the results obtained were compared with the results from the LECO AT-50 acid analyser.

Results and Discussion

The fluoride-selective electrode potential (E) is given by the equation

$$E = E^0_{F^-} - \frac{RT}{F}\ln a_{F^-} + E_D - E_{Ref} \quad \ldots \ldots (1)$$

where E = cell potential, $E^0_{F^-}$ = standard potential of the fluoride electrode, E_D = diffusion potential, E_{Ref} = reference electrode potential, F = Faraday's constant, R = gas constant, T = absolute temperature (K) and a_{F^-} = fluoride ion activity. $E^0_{F^-}$ and E_{Ref} can be combined to give one constant, E_V. Hence

$$E = E_V - \frac{RT}{F}\ln a_{F^-} + E_D \quad \ldots \ldots \ldots (2)$$

Experimentally it has been shown that linear relationships exist between the fluoride electrode potential and the hydro-

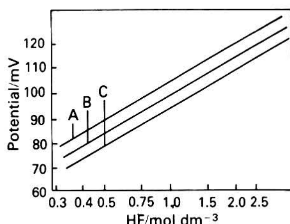

Fig. 1. Dependence of potential of the fluoride electrode system on nitric acid concentration. Bridge solution in the reference electrode is 4 M KCl. A, 1.5 M HNO_3; B, 2.0 M HNO_3; and C, 2.5 M HNO_3

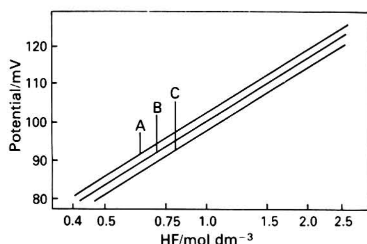

Fig. 2. Dependence of potential of the fluoride electrode system on the nitric acid concentration. Bridge solution in the reference electrode is 2 M KCl. A, 1.5 M HNO_3; B, 2.0 M HNO_3; and C, 2.5 M HNO_3

fluoric acid concentration. As the fluoride electrode is also sensitive to changes in the hydrogen ion concentration, this will lead to several parallel calibration graphs (Fig. 1). This implies that the nitric acid concentration must be known before the hydrofluoric acid concentration can be determined.

The nitric acid concentration can be determined by measuring the hydrogen ion concentration with a membrane electrode.[5] However, during experimental work in our laboratory we found that the fluoride electrode system could be made insensitive to changes in the nitric acid concentration by changing the concentration of potassium chloride in the bridge solution of the reference electrode from 4 to 0.05 M KCl. This can be clearly seen in Figs. 1–3.

Cell Conditions

The dissociation reaction of hydrofluoric acid can be written as

$$HF \rightleftharpoons H^+ + F^- \quad \ldots \quad (3)$$

and the thermodynamic acid constant K_a represented by

$$K_a = \frac{a_{H^+} \cdot a_{F^-}}{a_{HF}} \quad \ldots \quad (4)$$

where a_{H^+}, a_{F^-} and a_{HF} are the activity coefficients of the corresponding ions. Replacing the expression for a_{F^-} in equation (2) by that in equation (4) gives

$$E = E_V - S\log K_a - S\log Y_{HF} + S\log Y_{H^+} + S\log c_{H^+} - E_D - S\log c_{HF} \quad (5)$$

where S = the slope of the calibration graph (for an ideal electrode this is RT/F), Y = the activity coefficient (concentration scale) and c = the molar concentration. According to the experimental results, when c_{H^+} is constant equation (5) is described by a linear graph:

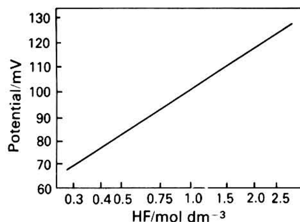

Fig. 3. Dependence of potential of the fluoride electrode system on the nitric acid concentration. Bridge solution in the reference electrode is 0.05 M KCl. 1.5, 2.0 and 2.5 M HNO_3 gave the same line

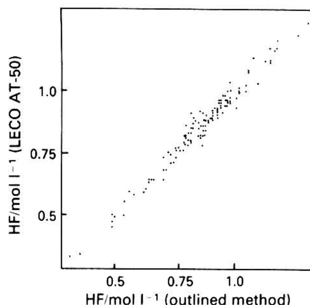

Fig. 4. Correlation between the hydrofluoric acid results of the outlined method and the LECO AT-50 analyser. The correlation factor is 0.98

$$E = \text{constant} - S\log c_{HF} \quad \ldots \quad (6)$$

Because pickling acids contain large amounts of nitric acid, the concentration of F^- can be neglected compared with that of HF. The forms HF_2^- and $(HF)_2$ can also be disregarded.[6] The constant in equation (6) can be expressed by the symbol K, hence

$$K = E_V - S\log K_a - S\log Y_{HF} + S\log Y_{H^+} - S\log c_{H^+} + E_D \quad (7)$$

The constant K is the intercept of the calibration graph with the potential axis and determines the position of the graph in the vertical direction. As the nitric acid concentration changes, K also changes. However, when 0.05 M KCl is used as a bridge solution, K is a real constant remaining unaffected by changes in the nitric acid concentration. It has been suggested[7] that the bridge solution effect may be caused by compensational changes among the terms on the right-hand side of equation (7). Unfortunately, a lack of basic scientific data, for instance the activity coefficients for ions in strong mixed-acid solutions, have made it impossible to verify the theoretical approach experimentally. The discussion will hence continue with experimental evidence for the suitability of the method outlined for pickling process control purposes.

Comparison of Results Obtained with the Outlined Method with Results Obtained with the LECO AT-50 Acid Analyser

The LECO AT-50 acid analyser is, according to the manufacturer, designed to determine simultaneously hydrofluoric and nitric acid concentrations in pickling baths. Because the fluoride electrode is sensitive to changes in the nitric acid concentration, the designers developed a cation-exchange membrane electrode suitable for measuring hydrogen ion concentrations in solutions containing hydrofluoric acid.[6]

The results obtained with the outlined method were compared with results obtained with the LECO AT-50 acid

Table 1. Hydrofluoric acid concentration obtained by the outlined method by measuring the recovery from spiked samples

HF added/mol l^{-1}	HF found/mol l^{-1}
0.05	0.06
0.12	0.12
0.24	0.20
0.36	0.36
0.49	0.40

Table 2. Nitric acid concentration obtained by the outlined method by measuring the recovery from spiked samples

HNO_3 added/mol l^{-1}	HNO_3 found/mol l^{-1}
0.20	0.18
0.40	0.38
0.60	0.59
0.80	0.79
1.00	0.99

analyser during a three-week period, during which three pickling cycles at our cold-rolling mill were terminated. The number of samples analysed was 144. The correlation between the hydrofluoric acid concentrations is shown in Fig. 4. The correlation factor is 0.98 and the standard error of the determination is 0.03 mol of HF (1 σ). The outlined method was studied by measuring the recovery from spiked samples. A sample from the pickling line containing 1.35 mol l^{-1} of nitric acid, 0.86 mol l^{-1} of hydrofluoric acid and 0.82 mol l^{-1} of iron was used. The results can be seen in Table 1.

The results fit reasonably well; the greatest difference between HF added and found is only 1.8 g l^{-1}. The correlation between the nitric acid results is shown in Fig. 5. The correlation factor here is 0.86 and the standard error of the estimate is 0.18 mol of nitric acid (1 σ). The correlation is obviously not as good as with HF and there are evidently several sources of error. The errors relating to the LECO method have been discussed elsewhere[5] and will not be dealt with here. The errors relating to the outlined method originate from the alkalimetric titration and the hydrofluoric acid determination, and have been estimated to be about 7%, although detailed investigations have not been carried out. In order to study the accuracy of the outlined method, a measurement with spiked samples was made. A sample from the pickling line containing 2.03 mol l^{-1} of nitric acid, 1.00 mol l^{-1} of hydrofluoric acid and 0.77 mol l^{-1} of iron was used. The results are given in Table 2.

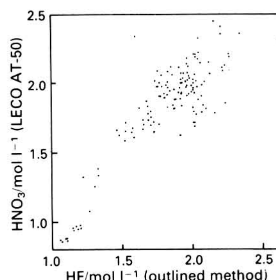

Fig. 5. Correlation between the nitric acid results of the outlined method and the LECO AT-50 analyser. The correlation factor is 0.86

As can be seen from Table 2, there is a tendency towards lower results in this determination. The reason for this has not been investigated, because the accuracy is sufficient for pickling process control. Some systematic error in connection with the titration might be an explanation.

Conclusion

The methods outlined in this paper are convenient and easy to use, particularly because the correct choice of bridging solution for the reference electrode eliminates the influence of the nitric acid concentration on the determination of fluoride. No expensive equipment is required, only a mV/pH meter and glass burettes and polypropylene containers.

Samples can be analysed in 10–15 min and the methods have been used in our laboratory since 1978. During this time, the events of under- and over-pickling have reached a minimum and the pickling acid consumption has decreased.

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Spectrophotometric Determination of Carbaryl and Propoxur Using Aminophenols and Phenylenediamine

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Three spectrophotometric methods are described for the determination of carbaryl and propoxur in insecticidal formulations, water and grains, based on the formation of coloured species with *p*-aminophenol, *p*-*N*,*N*-dimethylphenylenediamine dihydrochloride and 1-amino-2-naphthol-4-sulphonic acid, respectively, under specified experimental conditions. All the methods are simple, sensitive and accurate to within ±1.0%.

Keywords: *Carbaryl determination; propoxur determination; water, grain and insecticide analysis; spectrophotometry*

Carbaryl (1-naphthyl methylcarbamate) and propoxur (*o*-isopropoxyphenyl methylcarbamate) are carbamate insecticides used on a large scale against a broad spectrum of insects in field crops, fruits and vegetables. Spectrophotometric,[1–13] gas chromatographic,[14–16] HPLC[17,18] and TLC[19,20] methods have been reported for their determination.

This paper describes the development of sensitive and rapid spectrophotometric methods using *p*-aminophenol, *p*-dimethylphenylenediamine dihydrochloride and 1-amino-2-naphthol-4-sulphonic acid, which have been found to be satisfactory for the determination of carbaryl and propoxur in insecticidal formulations, water samples and grains.

Experimental

Apparatus

An Elico Model CL-24 spectrophotometer with 1-cm glass cells and a Systronics Model 324 pH meter were used, together with Grade A microburettes and pipettes of 10- and 5-ml volume, respectively, and a Dhona single-pan digital balance.

Reagents

All reagents were of analytical-reagent grade.

Carbaryl. Supplied by Union Carbide, Bhopal, India.

Propoxur. Supplied by Bayer India, Thane, India.

p-*Aminophenol* (p*AP*) *solution*, 0.09%. Dissolve 90 mg of *p*AP in 100 ml of distilled water.

p-*Dimethylphenylenediamine dihydrochloride* (*DMPD*) *solution*, 0.1%. Dissolve 100 mg of DMPD in 100 ml of distilled water.

1-*Amino*-2-*naphthol*-4-*sulphonic acid* (*ANSA*) *solution*, 0.1%. Dissolve 100 mg of ANSA in 1 ml of 1 M methanolic sodium hydroxide and dilute to 100 ml with methanol.

Sodium metaperiodate solution, 0.001 M. Dissolve 107 mg of sodium metaperiodate in 500 ml of distilled water.

Sodium hydroxide solution. Prepare 1 and 0.05 M aqueous solutions. Prepare a 0.25 M methanolic solution of sodium hydroxide by dissolving 1 g of sodium hydroxide initially in 5 ml of distilled water and dilute to 100 ml with methanol.

Butan-1-*ol.*

Preparation of Standard Solutions

Dissolve 100 mg of carbaryl and propoxur separately in methanol and dilute to 100 ml with methanol. Prepare solutions of lower concentrations (20 μg ml^{-1} for Methods A and B; 100 μg ml^{-1} for Method C) by diluting the stock solution 50 times and 10 times, respectively, with methanol.

Preparation of Calibration Graphs

Method A

Transfer aliquots of 1.0–12.5 ml of standard carbaryl solution or 1.0–10.0 ml of standard propoxur solution (20 μg ml^{-1}) and 1.5 ml of 1 M aqueous sodium hydroxide solution into a series of 25-ml calibrated flasks and allow to stand for 5 min for complete hydrolysis. Add 1 ml of 0.09% *p*AP solution, dilute to the mark with distilled water and shake well. Measure the absorbance of the coloured species during the period of stability (1–7 min for carbaryl; 8–15 min for propoxur) at 600 nm against a reagent blank prepared in a similar manner. The plot of absorbance *versus* concentration shows a linear relationship over the range investigated.

Method B

Transfer 0.3–4.0 ml of standard carbaryl solution or 0.5–4.0 ml of standard propoxur solution (20 μg ml^{-1}) and 0.5 ml of 0.05 M aqueous sodium hydroxide solution into a series of 125-ml separating funnels and allow to stand for 5 min. Add 1 ml of 0.1% DMPD solution and 1 ml of 0.001 M sodium metaperiodate solution, dilute to 25 ml with distilled water and shake well. After 5 min, add 10 ml of butan-1-ol and shake. After the two phases have separated, measure the absorbance of the organic phase at 600 nm against a reagent blank within the stability period (30 min for carbaryl and 60 min for propoxur). The calibration graph is obtained by plotting the absorbance *versus* concentration of the compounds.

Method C

To a series of 10-ml graduated test-tubes, add 0.3–3.5 ml of standard carbaryl solution (100 μg ml^{-1}) and 1 ml of 0.25 M methanolic sodium hydroxide solution and leave to stand for 5 min. Add 3 ml of 0.1% ANSA solution, dilute to the mark with methanol and shake well. Measure the absorbance of the green species formed during the stability period (10–90 min) at 700 nm against a reagent blank. Obtain a calibration graph as under *Methods A* and *B*. This method is specific for carbaryl; propoxur does not give any colour.

Determination of Recovery in Grains

Place 50 g of grain (rice or wheat) in a Waring blender and blend for 5 min with 200 ml of chloroform. Spike the samples in the blender with different concentrations of carbaryl or propoxur in 5 ml of methanol and blend the spiked samples for 2 min. Filter the chloroform solution into a 250-ml calibrated flask through a Whatman No. 1 filter-paper and retain the residue in the blender. Repeat the blending and filtering twice with 10 ml of chloroform. Wash the residue on the filter-paper

twice with 10 ml of chloroform. Combine the chloroform extracts and dilute to the mark. Use known aliquots of the chloroform extract for colour development after removing the solvent *in vacuo* in a fume cupboard (chloroform should be handled with care as it is a suspected human carcinogen). Dissolve the residue in methanol as described under Preparation of Calibration Graphs by any of the three proposed methods.

Determination of Recovery in Water Samples

After the collection of water samples (1 l), adjust each sample to a pH <5 with 20% sulphuric acid. Dissolve 10 g of sodium sulphate in each sample. Next, fortify each sample with different concentrations of carbaryl or propoxur in 5 ml of methanol. Extract each sample in a separating funnel with 150 ml of chloroform, shaking the funnel for 2–3 min. Transfer the chloroform extract into a funnel and re-extract the aqueous phase (2–3 min) with 50 ml of chloroform. Combine the extracts and wash the combined extract with 0.1 M potassium carbonate solution. Dry the chloroform solution by passing through 15–20 g of anhydrous sodium sulphate in a filter funnel and collect the extract in a 250-ml calibrated flask and dilute to the mark. Take known aliquots of the chloroform extract, evaporate the solvent *in vacuo* in a fume cupboard and proceed as given under Preparation of Calibration Graphs.

Determination in Insecticidal Formulations

Dissolve the well mixed formulation, equivalent to 50 mg of insecticide, in 25 ml of methanol and centrifuge for 5 min. Decant and filter the supernatant liquid into a 50-ml calibrated flask. Wash the residue with methanol and dilute to the mark. Use known aliquots of this solution for colour development after suitable dilution as outlined under Preparation of Calibration Graphs.

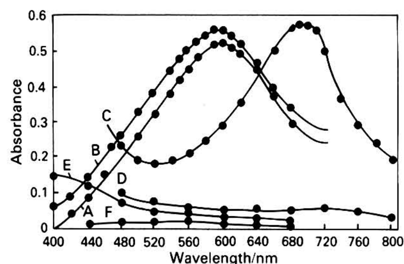


Fig. 1. Absorption spectra of A, carbaryl - (pAP-O_2); B, carbaryl - (DMPD-IO_4^-); C, carbaryl - (ANSA-O_2); D, (pAP-O_2) *vs.* distilled water; E, (DMPD-IO_4^-) *vs.* distilled water; and F, (ANSA-O_2) *vs.* distilled water. Concentration of carbaryl: A, 3.97×10^{-5} M; B, 4.78×10^{-5} M; and C, 1.74×10^{-4} M

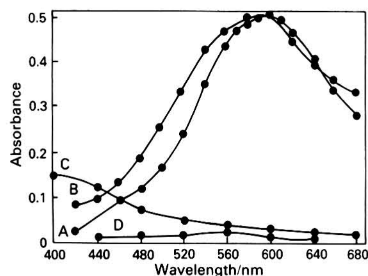


Fig. 2. Absorption spectra of A, propoxur - (pAP-O_2); B, propoxur - (DMPD-IO_4^-); C, pAP-O_2 *vs.* distilled water; and D, DMPD-IO_4^- *vs.* distilled water

Results and Discussion

Absorption Spectra

The absorption spectra of the coloured species are shown in Figs. 1 and 2.

Effect of Alkali Concentration

The optimum over-all sodium hydroxide concentrations for colour development in Methods A, B and C are 0.06 M, 0.001 M and 0.025 M, respectively.

Effect of Reagent Concentration

Volumes of 0.5–1.5 ml of 0.09% pAP, 0.5–1.5 ml of 0.1% DMPD and 2.0–4.0 ml of 0.1% ANSA are necessary for maximum colour development in Methods A, B and C, respectively. *o*-Aminophenol (*o*AP) gives similar coloured species under the proposed conditions for ANSA, but the colour is unstable.

Effect of Solvent

The coloured species formed in Method A can be extracted from aqueous solution into butan-1-ol, causing a bathochromic shift (20 nm) in $\lambda_{max.}$, without a variation in the absorbance. The coloured species resulting from Method B can also be extracted from aqueous solution into butan-1-ol, considerably increasing the absorbance but not affecting $\lambda_{max.}$. Hence butan-1-ol extraction is preferred in Method B as it gives a better sensitivity. As the coloured species formed in Method C is unstable in aqueous media, a methanolic medium is used throughout.

Table 1. Optical characteristics, precison and accuracy of the methods

Reagent	Compound	Time taken for maximum colour development/ min	Stability of colour/ min	$\lambda_{max.}$/ nm	Limits of Beer's law/ µg ml^{-1}	Molar absorptivity/ l mol^{-1} cm^{-1}	Error, %	RSD,* %
pAP . .	. . Carbaryl	Immediate	7	600	0.8–10.0	1.33×10^4	0.833	1.58
	Propoxur	8	15	600	0.8–8.0	1.44×10^4	1.25	1.70
DMPD	. . Carbaryl	2	30	600	0.7–8.0	1.41×10^4	0.98	1.34
	Propoxur	5	60	600	1.0–8.0	1.04×10^4	1.37	1.87
ANSA . .	. . Carbaryl	10	90	700	3.0–35.0	3.35×10^3	1.11	1.53

* Calculated for six samples containing same amount of carbamate.

Table 2. Determination of carbaryl and propoxur in wettable insecticidal formulations

Technical grade sample	Labelled amount, %	Carbaryl or propoxur found,* %			
		Method A	Method B	Method C	Literature method[2]
Carbaryl:					
I	—	98.25	98.14	97.96	97.85
II	85	83.82	83.75	83.68	83.63
III	5	4.19	4.17	4.15	4.14
Propoxur:					
I, Baygon spray ..	1	0.96	0.94		0.89
II, Baygon	4	3.94	3.93		3.90

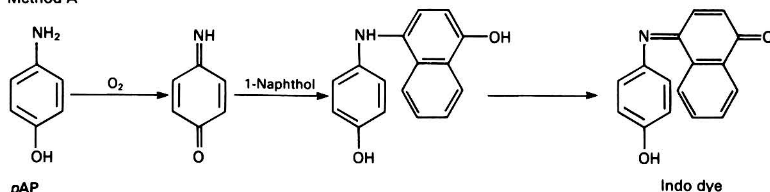
* Each value is an average of three determinations.

Table 3. Recovery of carbaryl and propoxur added to water and grains

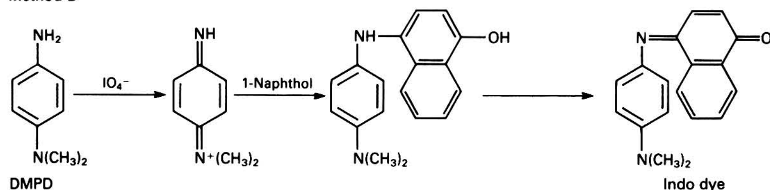
Method	Carbaryl (C) or propoxur (P) added/µg	Recovery, %*		
		Water	Wheat	Rice
A	C_{20}	95.0	97.25	96.25
	C_{40}	96.25	96.75	96.63
	P_{20}	93.0	96.5	95.75
	P_{40}	95.63	96.38	96.15
B	C_{20}	96.4	96.7	96.5
	C_{40}	96.9	97.0	96.5
	P_{20}	92.1	96.0	96.0
	P_{40}	95.6	95.8	95.63
C	C_{30}	93.67	95.5	94.83
	C_{60}	95.83	97.06	96.37

* Each value is an average of three determinations.

Method A



Method B



Method C

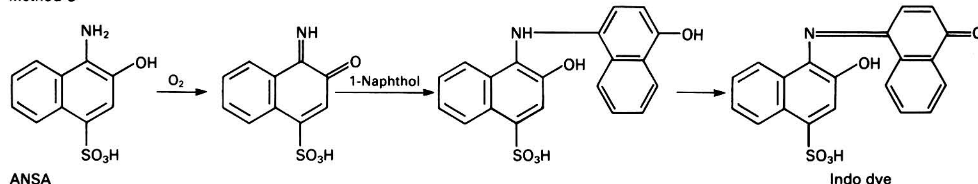


Fig. 3. Proposed reaction mechanism for formation of coloured species using 1-naphthol (hydrolysis product of carabaryl) as an example

Analytical Data

The $\lambda_{max.}$ of the coloured products, the time taken for maximum colour development, the stability of the products, the limits of adherence to Beer's law and the molar absorptivity are given in Table 1.

Precision and Accuracy

The precision and accuracy of the three proposed methods were studied by the analysis of six replicate samples containing known amounts of carbaryl or propoxur (75, 40 and 150 µg of carbaryl in Methods A, B and C or 80 and 40 µg of propoxur in

Methods A and B, respectively). The standard error and relative standard deviation (RSD) are given in Table 1.

Insecticidal formulations containing carbaryl and propoxur were analysed to determine the carbaryl or propoxur content and the results obtained by the proposed and literature methods[2] are presented in Table 2.

Recovery experiments were carried out by adding a known amount of the compounds to different samples of water and grains and determining them by the three proposed methods. Recoveries of carbaryl and propoxur ranged from 92.0 to 97.5%. The data are given in Table 3.

The data incorporated in Tables 2 and 3 suggest that the ingredients present in the formulations in addition to carbaryl and propoxur and other constituents present in water and grains do not interfere in the proposed methods.

The proposed methods are simple, rapid and sensitive and hence can be used for the routine determination of carbaryl or propoxur in formulations. Although the proposed methods are not as sensitive as the gas chromatographic methods reported previously, they are inexpensive and simple. Moreover, the determination of carbamates by all three proposed methods was carried out at higher wavelengths (600, 600 and 700 nm) where the interference of other ingredients is greatly minimised. The methods do not involve the elaborate clean-up procedures required by other methods.

Reaction Mechanism

The course of reaction in the formation of the coloured species in Methods A, B and C may be postulated through analogy. *p*-Aminophenol,[21] *p-N,N*-dimethylphenylenediamine dihydrochloride,[22] *o*-aminophenol,[23] and 1-amino-2-naphthol-4-sulphonic acid undergo oxidation in the presence of molecular oxygen or sodium metaperiodate with a two-electron transfer to the less stable and highly reactive quinoneimine. These species react with the phenolic coupler (in this instance, 1-naphthol or 2-isopropoxyphenol) under alkaline conditions by an electrophilic attack on the most nucleophilic site on the benzene ring of the coupler (*i.e.*, the *p*-position to the phenolic hydroxyl; if the *p*-position is blocked, the *o*-position to the phenolic hydroxyl). The resulting leuco dye is oxidised to the indo dye. The scheme is given in Fig. 3 taking 1-naphthol (hydrolysis product of carbaryl) as an example.

Conclusions

Three spectrophotometric methods for the determination of carbaryl and propoxur using *p*-aminophenol, *p-N,N*-dimethylphenylenediamine dihydrochloride and 1-amino-2-naphthol-4-sulphonic acid have been developed. These methods are simple and accurate to within ±1.0%. They have been applied to the determination of carbaryl and propoxur through their hydrolysis products in formulations, water and grains.

The authors are grateful to the authorities of Andhra University, Waltair, and also to Union Carbide Ltd., Bhopal, India and Bayer India Ltd., Thane, India for the gift samples of carbaryl and propoxur.

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Determination of Lorazepam by Fluorimetric and Photochemical - Fluorimetric Methods

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The photoisomerisation of lorazepam was studied by spectrofluorimetry and ultraviolet spectrophotometry. It was found that lorazepam leads to a strongly fluorescent photoproduct on irradiation in 0.2 M NaOH solution. The effect of pH on the fluorescence intensity of lorazepam and its photoproduct was investigated and their pK_a values were calculated. A fluorimetric method with a detection limit of 16 ng ml^{-1} and a photochemical - fluorimetric method with a detection limit of 0.3 ng ml^{-1} were developed for the determination of lorazepam. The former method was applied to the determination of the drug in tablets; the latter, which is more sensitive, was used for the determination of lorazepam in serum.

Keywords: *Lorazepam determination; fluorimetry; photochemical fluorimetry; 1,4-benzodiazepine*

Of the methods utilised in pharmaceutical analysis, fluorescence techniques are some of the most sensitive. Several reports have appeared concerning the fluorescence properties of 1,4-benzodiazepines and their application in analysis.[1–26] In most of these studies, the fluorescence species were produced thermally in an acidic solvent,[1–3] photochemically,[4,5] by cyclisation to acridines after hydrolysis to benzophenones[6–8] or synthetically by derivatisation with phthalaldehyde[9,10] or fluorescamine.[11–14] Only in some of these methods was the emitting species the compound itself[15–18] and even then it was necessary to work in acidic solution to develop the fluorescence.[17,18]

Fluorescence was also used in several studies as a detection technique for 1,4-benzodiazepines in thin-layer chromatography[6,11,12,19–24] and high-performance liquid chromatography.[25–27]

In this work we have developed two fluorimetric methods for the determination of lorazepam, which has been determined fluorimetrically after phthalaldehyde derivatisation.[10] One method uses the native fluorescence of the drug for its determination in tablets and the other is a photochemical - fluorescence method capable of measuring nanogram levels of lorazepam in serum with good reproducibility and accuracy.

Experimental

Apparatus

Fluorescence measurements were made with a Shimadzu RF-540 spectrofluorimeter equipped with a xenon light source. Absorption spectra were recorded using a Bausch and Lomb Spectronic 2000 spectrophotometer.

For photochemical reactions, a 300-W Osram high-pressure mercury arc lamp was used.

Reagents

Lorazepam was purchased from Llorens (Spain) and was checked for purity by GC - MS. Aqueous solutions of lorazepam were prepared by successive dilutions of a 10^{-3} M stock solution prepared by dissolution of an appropriate amount in methanol and stored under refrigeration protected from light.

All other chemicals were of analytical-reagent grade. A buffer solution of pH 9.5 was prepared by adjusting the pH of a saturated ammonium chloride solution to 9.5 with concentrated ammonia solution.

Photoisomerisation Procedure

Between 1 and 10 ml of 10^{-6} M lorazepam solution in water (10% methanol) and 5 ml of 1.0 M NaOH solution were pipetted into a vessel, diluted to approximately 25 ml with water and irradiated for 20 min. The solution was then diluted to exactly 50 ml and left for 60 min for the fluorescence to develop. The fluorescence intensity of the solution was measured using excitation and emission wavelengths of 252 and 435 nm, respectively.

Extraction Procedure

A Sep-Pak C_{18} cartridge (Waters) was inserted in the Luer tip of a syringe and was pre-washed with 2 ml of methanol, followed by 2 ml of doubly distilled water. Then 1 ml of serum sample was pumped with the syringe through the cartridge. The matrix was washed with 2 ml of 50% methanol solution and the drug was eluted with 2 ml of methanol into a cylindrical quartz tube. The eluent was dried under a stream of nitrogen. The residue was dissolved in 3 ml of 0.2 M NaOH solution and the solution was irradiated. The fluorescence intensity of the solution was measured as above.

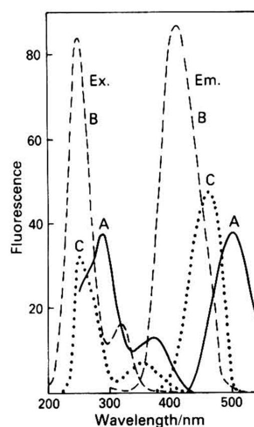


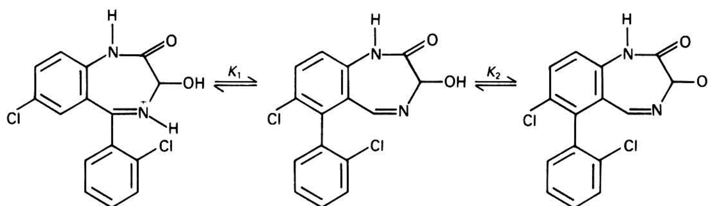
Fig. 1. Corrected excitation (Ex.) and emission (Em.) spectra of lorazepam (concentration 2 × 10^{-5} M) in (A) 1.0 M $HClO_4$, (B) water and (C) 0.1 M NaOH solution

Results and Discussion

Native Fluorescence of Lorazepam

The fluorescence excitation and emission spectra for lorazepam were obtained in aqueous solution at several pH values (Fig. 1). Three different spectra were observed. In acidic media (pH less than 1) the excitation spectrum shows two excitation bands at 250 and 290 nm and the emission spectrum a band at 500 nm. At pH values between 2 and 10, two bands appear in the excitation spectrum, at 250 and 320 nm, and in the emission spectrum a band appears at about 410 nm. At pH values higher than 11 there are two bands with maxima at 250 and 355 nm in the excitation spectrum and a band at 460 nm in emission spectrum.

These results are in agreement with earlier spectrophotometric studies[28] and indicate that three species predominate in the bulk solution in the pH range 0–14, the protonated, neutral and anionic forms, as shown below.



From the decrease in the characteristic relative fluorescence intensity of each species with variation in pH, pK_a values of lorazepam were determined by the Albert and Serjeant method,[29] obtaining values for pK_1 and pK_2 of 0.96 ± 0.07 and 11.15 ± 0.06 (in 1.0 M $NaClO_4$), respectively, which are in agreement with the spectroscopically determined values of 1.3 and 11.5. The pK_a values for the excited state could not be determined owing to the low fluorescence intensity of lorazepam.

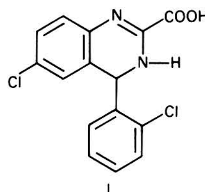
Photochemical Fluorescence

Irradiation of lorazepam in an alkaline medium led to changes in its absorption spectrum and the appearance of fluorescence at concentrations lower than 10^{-7} M. The absorption spectra of lorazepam in 0.1 M NaOH solution after different irradiation times are shown in Fig. 2(*a*). As the irradiation time increases, the lorazepam band at 345 nm decreases and a new absorption band appears at 368 nm.

Otherwise, irradiation of lorazepam produces a broad fluorescence emission band with a maximum at 435 nm, increasing as the irradiation time increases [Fig. 2(*b*)]. In the excitation spectrum two bands appear at 252 and 360 nm (Fig. 3,A).

A study of irradiation time, distance of the solution from the lamp and fluorescence development time was made in order to establish the optimum values for achieving the maximum fluorescence intensity. From the results, a distance of 5 cm and irradiation and development times of 20 and 60 min, respectively, were chosen. It was observed that these parameters were independent of concentration.

The fluorescent photoproduct was studied by IR and NMR spectroscopy and mass spectrometry. The presence of two chloro, an azomethine and a carboxylic acid group in the molecule (from IR and mass spectra) was observed. The compound showed the same molecular mass but higher aromatisation than lorazepam. The photoproduct was identified as 6-chloro-3-(chlorophenyl)-3,4-dihydro-2-quinazoline carboxylic acid (I), an isomer of lorazepam.



A compound with a similar structure has been obtained from oxazepam by refluxing for 2 h in 4 M NaOH solution.[30]

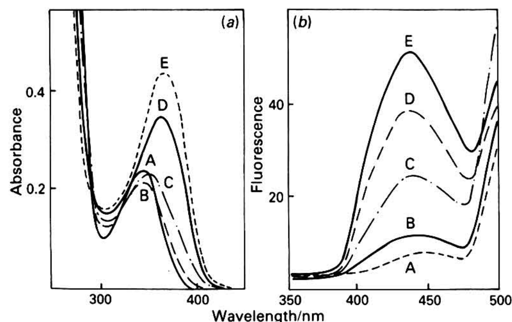


Fig. 2. (*a*) Absorption spectra of lorazepam (concentration 10^{-4} M) and (*b*) uncorrected fluorescence emission spectra of lorazepam (concentration 2×10^{-7} M) in 0.1 M NaOH solution at different irradiation times: (A) 0; (B) 5; (C) 10; (D) 20; and (E) 30 min

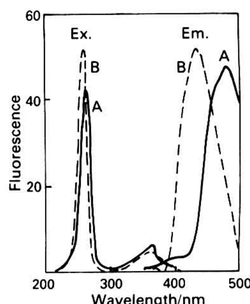


Fig. 3. Corrected fluorescence emission (Em.) and excitation (Ex.) spectra of irradiated lorazepam (concentration 2×10^{-7} M) at (A) pH 3 and (B) pH 13

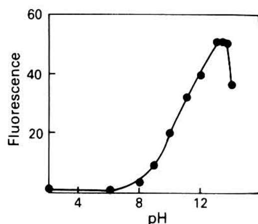


Fig. 4. Effect of pH on photochemical reaction efficiency. Concentration of lorazepam, 2×10^{-7} M. Excitation and emission wavelengths, 252 and 435 nm, respectively

Effect of pH

We have found that pH has important effects on both the synthesis and the absorption and fluorescence spectra of the photochemical - fluorimetric product.

The variation of photochemical reaction efficiency with pH (Fig. 4) showed very weak fluorescence at pH values lower than 8, increasing at higher values and reaching a maximum at pH 13, with a decrease at pH higher than 13.7 owing to lorazepam hydrolysis. This result indicates that the reactive species is the anionic form of lorazepam, rather than lorazepam itself. A concentration of 0.2 M was chosen to obtain the maximum fluorescence intensity.

We also studied the effect of pH on the photochemical product by absorption and fluorimetric spectroscopy. Fig. 5 shows the ultraviolet absorption spectra of the product. Three different spectra were observed, which varied with pH. At pH lower than 1, **I** shows bands at 408, 295, 231 and 204 nm; at pH 2–9, four bands appear at 362, 270, 230 and 206 nm, whereas at pH higher than 10 there are bands with maxima at 369, 234 and 209 nm.

However, **I** shows only two different fluorimetric spectra with variation in pH, one at pH higher than 6 and another at pH less than 5, with a maximum at 460 nm in the emission spectrum and two maxima at 240 and 260 nm in the excitation spectrum (Fig. 3).

pK_a values for **I** were determined by the method described elsewhere.[29] pK_1 and pK_2 values of 0.84 ± 0.04 and 9.47 ± 0.12 (in 1.0 M $NaClO_4$), respectively, were obtained by spectrophotometry, whereas by fluorimetry a pK_a value of 5.53 ± 0.12 was calculated. This value corresponds to the excited state of the anionic form of **I**.

The following scheme is a possible interpretation of the protonation behaviour of **I**.

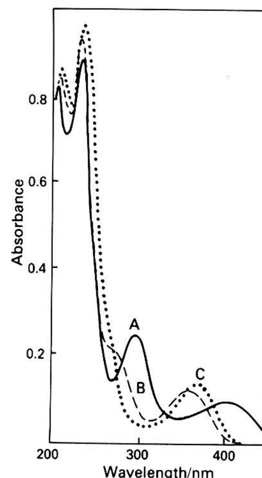
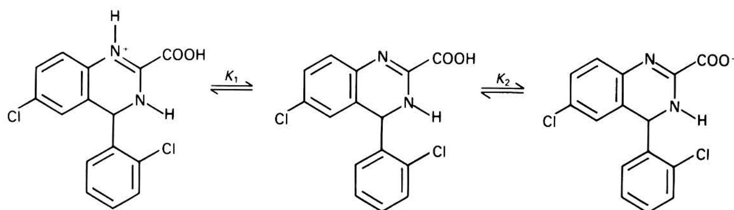


Fig. 5. Ultraviolet absorption spectra of irradiated lorazepam (concentration 3×10^{-5} M) as a function of pH: (A) pH 0.3; (B) pH 6.4; and (C) pH 13



Calibration Graphs and Limits of Detection

In Table 1 the statistical characteristics of the fluorimetric and photochemical - fluorimetric calibration graphs for the determination of lorazepam are compared. The precision, expressed as relative standard deviation, was less than 3% for the fluorimetric method for concentrations between 1.5 and 0.6 μg ml^{-1}, and less than 2% for the photochemical - fluorimetric method for concentrations between 90 and 5 ng ml^{-1}.

In both methods the linearity of the calibration graphs is excellent, as shown by the correlation coefficients, which are very close to unity. The linear dynamic range (ratio of the upper concentration of linearity and the limit of detection) covers two orders of magnitude of concentration. The photochemical - fluorimetric method is an order of magnitude more sensitive than the fluorimetric method.

Analytical Applications

Owing to the limits of quantitation of both methods, the fluorescence method is suitable for the determination of lorazepam in tablets, whereas the photochemical - fluorimetric method is suitable for its determination in body fluids.

A rapid method for the quality control of tablets was attempted by grinding the tablets to a fine powder, dissolving the active constituent in methanol, diluting with water and measuring the relative fluorescence intensity of this solution. The recommended method is as follows. Weigh an amount of finely powdered tablets (Donix) equivalent to about 1 mg of lorazepam into a beaker. Add 20 ml of absolute methanol and stir vigorously with a magnetic stirrer for 10 min, then filter and dilute to 50 ml with methanol. Pipette 1 ml of this methanolic solution into a 50-ml calibrated flask, dilute to the mark with doubly distilled water, then measure its relative fluorescence intensity at 410 nm with excitation at 240 nm. Compare this value with the calibration graph.

The results of five assays of tablets containing 5 mg of lorazepam gave a mean value of 4.96 mg per tablet, with a

Table 1. Statistical treatment of the calibration graphs and limits of detection of lorazepam

Method	Solvent	Upper concentration of linearity/ μg ml^{-1}	Correlation coefficient	Limit of quantitation/ ng ml^{-1}	Limit of detection/ ng ml^{-1}
Fluorimetry	H_2O	1.6	0.9998	54	16
Photochemical-fluorimetry	0.2 M NaOH	0.09	0.9998	1.0	0.3

standard deviation of 0.03 mg. The relative standard deviation was 0.60%.

Lorazepam was quantitatively extracted from serum with Sep-Pak C_{18} cartridges. Initial extraction of lorazepam from five spiked serum samples gave an over-all recovery of 99.7 ± 1.3% for a concentration of 30 ng ml^{-1} of lorazepam added to serum. The results of three assays of a sample of human serum gave individual values of 31.6, 33.2 and 32.1.

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Selective Photometric Titration of Calcium or Magnesium with EDTA Using Various Thiols as Masking Agents

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Calcium or magnesium was titrated photometrically in the individual presence of cobalt, nickel, copper, zinc, cadmium, mercury, silver and lead using a variety of thiol-containing ligand masks. None successfully masked cobalt and cysteine was the most effective masking agent for the remaining metals.

Keywords: *Calcium determination; magnesium determination; photometric EDTA titration; masking agents; thiols*

Many thiol-containing ligands have been used as masking agents for Group B and C metal ions in complexometric titrimetry. Some typical examples of these are given in Table 1.

The work described in this paper examined the suitability of various thiols as masking agents for cobalt, nickel, copper, zinc, cadmium, mercury, silver and lead in the photometric titration of calcium or magnesium with EDTA at pH 10.

Experimental

Apparatus

A Sybron-Brinkmann PC 801 fibre optic absorptiometer fitted with a 2-cm total path-length probe was used for the measurement of solution absorbance values, although any conventional photometric titration arrangement would be feasible. pH values were checked with an EIL Vibron 39A analogue meter using separate glass and calomel electrodes. The results were processed separately on a Sinclair Spectrum microcomputer. A 10-cm^3 microburette (grade A) with a magnifying reader was used to dispense the titrant; calibrated grade-B flasks and pipettes were also employed.

Reagents

All reagents, except the indicators and thiols, were of AnalaR grade. The indicators were obtained from BDH Chemicals and the thiols from Aldrich Chemical and were used as received. De-ionised, distilled water was used for all solutions.

Standard calcium solution, 0.01000 M. Dissolve 1.0009 g of calcium carbonate, dried at 130 °C for 3 h, in a small excess of dilute hydrochloric acid and dilute to 1 l.

pH 10 *buffer*. Dissolve 17.5 g of ammonium chloride in water, add 143 cm^3 of concentrated ammonia solution and dilute to 250 cm^3.

Thiol solutions. All 0.50 M in water; prepare from 3-mercaptopropionic acid (3MPA), 2-mercaptopropionic acid (2MPA), mercaptosuccinic acid (MSA), cysteine, 2-mercaptoethylamine (2MEA) and 2-mercaptoethanol (2ME).

Indicator solutions. Eriochrome Blue Black SE (Erio BBSE), 6 × 10^{-4} M, and Patton and Reeder's indicator (HSN), 8 × 10^{-4} M, both in water with a trace of sodium carbonate to aid solution. Determine the indicator purity by elemental analysis or other suitable means.

Other solutions. Prepare the following solutions in water to 0.010 M: magnesium sulphate heptahydrate, cobalt(II) sulphate heptahydrate, nickel nitrate hexahydrate, zinc sulphate heptahydrate, mercury(II) nitrate hemihydrate (in 0.05 M nitric acid), cadmium chloride.2.5H_2O, lead nitrate, silver nitrate and disodium EDTA dihydrate. Standardise the EDTA solution against the calcium solution and the metal solutions against the EDTA using appropriate conditions.

Procedure

Into a 100-cm^3 tall-form beaker pipette 5 cm^3 of 0.01 M calcium or magnesium solution, 2 cm^3 of pH 10 buffer, 2 cm^3 of 0.5 M thiol masking agent and 5 cm^3 of 0.01 M interferent metal ion solution. Dilute to 49 cm^3 and place the beaker in a small water-bath at 20 °C on a magnetic stirrer. Add the stirrer follower, insert the absorptiometer fibre optic probe tip and set the filter wavelength to 620 nm. Set the absorbance to read zero, add 1 cm^3 of indicator solution (Erio BBSE for calcium, HSN for magnesium) and titrate photometrically with 0.01 M EDTA.

Results and Discussion

Calcium and magnesium were chosen as the metal ions for titration as they were previously investigated at pH 10 and found to give excellent photometric titration curves and linearised plots.[8] They also have little affinity for sulphur ligands.

The end-point of the photometric titration curves was defined as the intersection of two approximately linear portions either side of the break point. The end-point of the linear plots was defined as the intersection of the linear least-squares best fit F_2 plot with the volume axis.[8] Results for each

Table 1. Use of thiol masking agents in visual complexometric titrimetry

Ligand	Metals masked	Determinands	Reference
Thioglycollic acid . .	Zn, Cd, Bi, In, Cu, Pb Hg, Ag	Mn, Ni	1
3-Mercaptopropionic acid	Cu, Hg, Co, Fe, Bi	Ni, Mn, Ca, Mg	2
Mercaptosuccinic acid	Bi, Fe	Th	3
2-Mercaptoethylamine	Co, Ni, Cu, Zn, Cd, Hg	Mn, Ca, Mg	4
Cysteine	Cu, Hg	Pb, Zn, Co, Fe, Ni	5
2,3-Dimercaptopropan-1-ol	Pb, Bi, Zn, Hg	Ca, Mg, Mn	6
2,3-Dimercaptopropane-1-sulphonic acid . .	As, Sb, Bi, Cd, Ge, P Hg, Sn, Zn	Mg, Ca, Ni, Mn	7

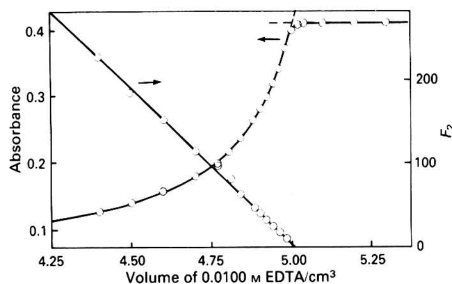

Fig. 1. Photometric titration of 5.008 cm³ of 0.01000 M calcium plus 5.0 cm³ of 0.010 M copper with 0.0100 M EDTA using 3-mercaptopropionic acid as a mask at 620 nm. V_0 = 50 cm³, C_IV_I = 6 × 10^{-4} mmol Erio BBSE, pH = 10.0, [3MPA] = 0.02 M. Photometric end-point = 5.008 ± 0.005 cm³; linear end-point = 5.008 ± 0.0012 cm³

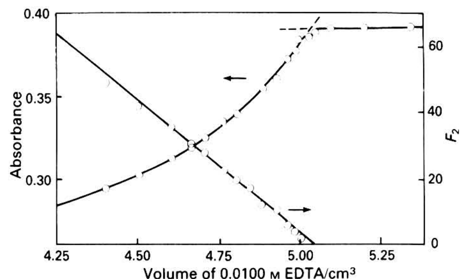

Fig. 2. Photometric titration of 5.008 cm³ of 0.010 M magnesium plus 5.0 cm³ of 0.010 M mercury(II) with 0.0100 M EDTA using 2-mercaptopropionic acid as a mask at 620 nm. V_0 = 50 cm³, C_IV_I = 8 × 10^{-4} mmol HSN, pH = 10.0, [2MPA] = 0.02 M. Photometric end-point = 5.027 ± 0.005 cm³; linear end-point = 5.034 ± 0.005 cm³

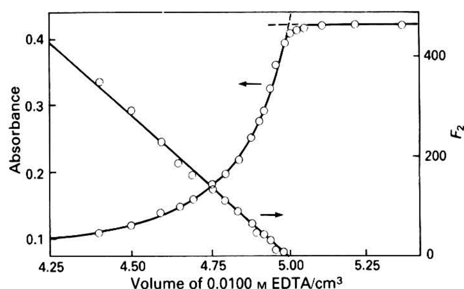

Fig. 3. Photometric titration of 5.008 cm³ of 0.01000 M calcium plus 5.0 cm³ of 0.010 M cadmium with 0.0100 M EDTA using 2-mercaptoethanol as a mask at 620 nm. V_0 = 50 cm³, C_IV_I = 6 × 10^{-4} mmol Erio BBSE, pH = 10.0, [2ME] = 0.02 M. Photometric end-point = 4.993 ± 0.005 cm³; linear end-point = 4.992 ± 0.002 cm³

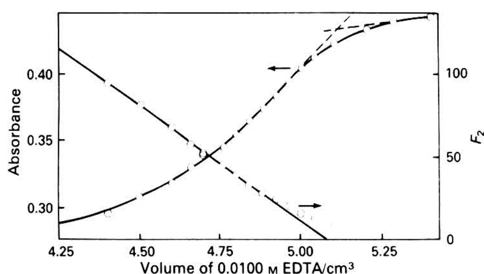

Fig. 4. Photometric titration of 5.008 cm³ of 0.010 M magnesium plus 5.0 cm³ of 0.010 M cadmium with 0.0100 M EDTA using 2-mercaptoethanol as a mask at 620 nm. V_0 = 50 cm³, C_IV_I = 8 × 10^{-4} mmol HSN, pH = 10.0, [2ME] = 0.02 M. Photometric end-point = 5.11 ± 0.02 cm³; linear end-point = 5.083 ± 0.005 cm³

masking ligand are tabulated and Figs. 1–4 show representative examples of these plots.

As derived in our earlier paper[8]

$$F_2 = \frac{[\mathrm{MIn}]}{[\mathrm{In}]'}(V + V_0) + K_{\mathrm{M'In'}}C_{\mathrm{In}}V_{\mathrm{In}}\left(1 - \frac{1}{1 + \frac{[\mathrm{MIn}]}{[\mathrm{In}]'}}\right)$$

and

$$\frac{[\mathrm{MIn}]}{[\mathrm{In}]'} = \frac{A_{\mathrm{In}} - A}{A - A_{\mathrm{MIn}}}$$

Also, from

$$\frac{[\mathrm{MIn}]}{[\mathrm{In}]'}(V + V_0) = K_{\mathrm{M'In'}}C_yV_e - K_{\mathrm{M'In'}}C_yV$$

a plot of $\frac{[\mathrm{M\,In}]}{[\mathrm{In}]'}(V + V_0)$ against V yields a value for $K_{\mathrm{M'In'}}$.

For further details and definition of symbols used, see reference 8.

All the studies involved the photometric titration of 5.008 cm³ of 0.010 M calcium or magnesium solution plus 5 cm³ of 0.010 M interfering metal ions in the presence of 0.02 M masking agent. All volumes are in cm³ and the average of two titrations except when there was no interfering ion present, where the average of four titrations is quoted. The results obtained with the various masking agents and interfering ions are shown in Tables 2–7.

3-Mercaptopropionic Acid

3-Mercaptopropionic acid (Table 2) formed an intense red-brown complex with nickel, which was partially decomposed by EDTA; this completely obscured any indicator colour change and no end-point was found. With silver the mask formed a dense yellow precipitate making photometric titration impossible. Copper(II) and mercury(II) were perfectly masked as colourless complexes but zinc, cadmium and lead were only partially masked. Zinc and cadmium also slowed the kinetics of the titrations.

2-Mercaptopropionic Acid

2-Mercaptopropionic acid (Table 3) formed a pale pink complex with nickel, which was decomposed by EDTA, obscuring the indicator change. Copper, mercury and silver were perfectly masked as colourless complexes. Cadmium, zinc and lead were well masked in the calcium titrations but poorly masked in the magnesium determinations. As with 3MPA, zinc and cadmium caused the kinetics to be slow. This ligand forms 5-membered rings and was a better masking agent than its 6-membered ring structural isomer 3MPA.

Mercaptosuccinic Acid

Mercaptosuccinic acid (Table 4) formed a pink complex with nickel, which did not produce masking in either titration. Copper formed a pale green complex, which quickly faded to almost colourless; masking was effective. Mercury and silver

Table 2. Results obtained using 3-mercaptopropionic acid ($HSCH_2CH_2COOH$) as a masking agent

Interference	Calcium end-point/cm³		Magnesium end-point/cm³	
	Photometric	Linear	Photometric	Linear
—	5.006	5.007	5.025	5.036
Ni^{2+}	—	—	—	—
Cu^{2+}	5.008	5.009	5.040	5.052
Zn^{2+}	—	5.200	—	—
Cd^{2+}	5.053	5.028	—	—
Hg^{2+}	5.017	5.016	5.038	5.046
Ag^{+}	—	—	—	—
Pb^{2+}	—	5.223	—	—

Table 3. Results obtained using 2-mercaptopropionic acid [$CH_3CH(SH)COOH$] as a masking agent

Interference	Calcium end-point/cm³		Magnesium end-point/cm³	
	Photometric	Linear	Photometric	Linear
—	5.006	5.007	5.025	5.036
Ni^{2+}	—	—	—	—
Cu^{2+}	5.012	5.012	5.038	5.047
Zn^{2+}	5.067	5.071	—	—
Cd^{2+}	5.015	5.009	—	5.170
Hg^{2+}	5.013	5.011	5.032	5.034
Ag^{+}	5.011	5.010	5.024	5.028
Pb^{2+}	5.047	5.020	—	—

Table 4. Results obtained using mercaptosuccinic acid [$HOOCCH_2CH(SH)COOH$] as a masking agent

Interference	Calcium end-point/cm³		Magnesium end-point/cm³	
	Photometric	Linear	Photometric	Linear
—	4.994	4.998	5.025	5.036
Ni^{2+}	—	—	—	—
Cu^{2+}	5.000	5.006	5.038	5.047
Zn^{2+}	—	5.093	—	—
Cd^{2+}	5.038	5.029	—	—
Hg^{2+}	4.991	4.993	5.039	5.041
Ag^{+}	4.996	4.997	5.043	5.056
Pb^{2+}	5.070	5.031	—	—

Table 5. Results obtained using cysteine [$HSCH_2CH(NH_2)COOH$] as a masking agent

Interference	Calcium end-point/cm³		Magnesium end-point/cm³	
	Photometric	Linear	Photometric	Linear
—	4.994	4.998	5.025	5.036
Ni^{2+}	5.001	5.001	5.035	5.037
Cu^{2+}	5.014	5.019	5.044	5.060
Zn^{2+}	5.022	5.040	5.058	5.074
Cd^{2+}	5.011	5.010	5.060	5.072
Hg^{2+}	5.002	5.002	5.026	5.020
Ag^{+}	5.000	5.002	5.030	5.042
Pb^{2+}	5.014	5.007	—	5.040

Table 6. Results obtained using 2-mercaptoethylamine ($HSCH_2CH_2NH_2$) as a masking agent

Interference	Calcium end-point/cm³		Magnesium end-point/cm³	
	Photometric	Linear	Photometric	Linear
—	4.996	4.995	5.030	5.032
Ni^{2+}	4.993	4.992	5.035	5.032
Cu^{2+}	5.005	5.007	5.052	5.057
Zn^{2+}	5.036	5.037	5.220	5.139
Cd^{2+}	5.000	4.996	5.225	5.137
Hg^{2+}	5.000	5.001	5.036	5.035
Ag^{+}	5.002	5.002	5.031	5.031
Pb^{2+}	—	5.084	—	—

Table 7. Results obtained using 2-mercaptoethanol ($HSCH_2CH_2OH$) as a masking agent

	Calcium end-point/cm^3		Magnesium end-point/cm^3	
Interference	Photometric	Linear	Photometric	Linear
—	4.996	4.995	5.030	5.032
Ni^{2+}	—	—	—	—
Cu^{2+}	4.990	4.990	5.036	5.034
Zn^{2+}	—	5.156	—	—
Cd^{2+}	4.994	4.991	5.105	5.086
Hg^{2+}	4.998	4.995	5.032	5.031
Ag^{+}	—	—	—	—
Pb^{2+}	—	5.045	—	—

were masked perfectly as colourless complexes. Cadmium and lead were well masked in the calcium titrations but interfered in the magnesium determination. Zinc was only partially masked and no accurate end-points were obtained; zinc and cadmium again caused the kinetics to be slow.

Cysteine

Cysteine (Table 5) formed a pale pink complex with nickel and a pale green complex with copper, which faded rapidly to almost colourless. The colours caused no problem and masking was very effective. Silver, mercury, cadmium and zinc were also well masked through colourless complex formation. Lead was masked well in the calcium titration but poorly in that of magnesium.

2-Mercaptoethylamine

2-Mercaptoethylamine (Table 6) behaved similarly to cysteine with respect to nickel and copper, forming pale coloured chelates that were successful in masking. Silver and mercury were well masked as colourless complexes, whereas zinc and cadmium, although well masked with respect to calcium, gave high results with magnesium. Lead was poorly masked.

2-Mercaptoethanol

2-Mercaptoethanol (Table 7) formed an intense red - brown complex with nickel, which was decomposed by EDTA. It also formed a white precipitate with silver, which interfered badly. Copper and mercury were perfectly masked as colourless complexes, cadmium was effectively masked in the calcium titrations and well masked for magnesium, whereas lead and zinc were poorly masked.

Conclusions

With regard to the calcium determinations, cysteine is an excellent mask for all the interfering metal ions studied, except perhaps for zinc where the results were high. 2-Mercaptoethylamine is almost as good, although in this instance lead gives high results. Nickel, zinc and lead are well masked only by agents containing an amino group in addition to the thiol. Copper and mercury are perfectly masked by all the reagents.

In the magnesium determinations, copper and mercury are perfectly masked by all the reagents, silver by 2MPA, MSA, cysteine and 2MEA. Nickel is masked only by cysteine and 2MEA. Zinc, cadmium and lead are masked fairly well by cysteine. *N*-Methylcarbamodithioic acetic acid [$HOOCCH_2N(CH_3)CSSH$] was synthesised and tested as above but only masked silver and mercury well.

With all the above ligands, cobalt(II) formed a very intense green complex, even in the presence of a large excess of ascorbic acid. This completely obscured all indicator colour transitions. Silver was completely masked by the ammonia buffer and under the conditions used does not require a thiol ligand.

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Turbidimetric Determination of Chlorhexidine Using Flow Injection Analysis

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A study of some chlorhexidine - dye and chlorhexidine - Cu^{II} systems was carried out in order to determine the best precipitate for the turbidimetric determination of chlorhexidine using flow injection analysis (FIA). The reagent selected was thymol blue, which gives an orange solid (1 : 2 stoicheiometry). The chemical and FIA variables were established using the modified simplex method and the calibration graph was linear over the concentration range 10.5–63.0 μg ml^{-1} of chlorhexidine. Some interfering substances were also investigated.

Keywords: *Flow injection analysis; chlorhexidine determination; turbidimetric determination; modified simplex method*

Chlorhexidine, a bactericidal drug, is a member of the biguanide family, several members of which are found in pharmaceutical formulations. Reports of ion-pair compounds between chlorhexidine and dyes have been found in the literature, and methods have been proposed for the spectrophotometric determination of the drug after liquid - liquid extraction of these ion pairs into chloroform.[1,2]

This paper reports a study of several ion pairs, which was carried out in order to develop a direct method for the turbidimetric determination of chlorhexidine that avoided the extraction step. The optimised procedure developed is proposed as a method for the control analysis of chlorhexidine and the chemical and flow variables were optimised using the modified simplex method. The recommended BP[3] and USP method is a titration with $HClO_4$ in an acetic acid medium.

Experimental

Reagents

All reagents were of analytical-reagent grade unless stated otherwise.

Chlorhexidine hydrochloride solutions. The solid was donated by ICI Farma and was titrated potentiometrically against $HClO_4$ in acetic acid medium.[3] The purity was 99.30% (RSD 0.3%, five replicates).

Carrier stream. Aqueous solutions were obtained from a stock solution of thymol blue (Panreac, purity 95%) and buffered with a succinic acid - succinate buffer at various pH values. The ionic strength was 0.01 for any tested solution.

Succinic - succinate buffer solution. Prepared with succinic acid (Probus) and NaOH (Probus). The ionic strength was 0.2 and the pH was determined potentiometrically.

Other compounds. Sucrose (Baker), ascorbic acid (Merck), benzocaine (Riedel) and acetylsalicylic acid [obtained in this laboratory from salicylic acid (Probus) and acetic anhydride (Baker)] were also used.

Apparatus

A Crison digital 501 potentiometer with Radiometer G 20 2 B glass and calomel methanol - saturated KCl electrodes was used together with a Crison potentiometer, Model 517, with an Ingold combined electrode.

A Shimadzu UV - visible spectrophotometer with a 1-cm path-length cell and a Perkin-Elmer spectrophotometer, Coleman 55, with an 18-μl flow cell and a 1-cm path-length cell were used.

The basic FIA apparatus consisted of a Tecator 5020 reaction coil (0.5 mm i.d.) and a Pye Unicam 45 A.R. register.

An Oric-1 microcomputer with cassette and printer was used, with software developed in BASIC MICROSOFT V 1.0.

Procedures

Modified simplex method

The initial simplex was chosen according to Yarbro and Deming[4] with a side length of one and the former vertex on coordinate origin; the region of variables was standardised with the modification proposed by Morgan and Deming[5] and a program that required the ranges of any tested variable to be input into the computer was applied in order to obtain the six vertices.

The modified simplex program for this work was established using the basis of the method and flow-line of Nelder and Mead,[6] which was reported in a review by Cela and Perez Bustamante.[7]

The program was carried out as follows: the data were entered into the microcomputer and another experiment was generated from these data. This process was repeated, the program giving a new set of conditions each time.

The range of variables considered is shown in Table 1; for the FIA variables this range is established on the basis of the apparatus available, whereas for the chemical variables the range was established on the basis of the previous spectrophotometric experiments. The parameter to be optimised was peak height.

When a stable base line was obtained on the chart recorder, a sample was injected, the reaction took place and the resulting peak was recorded and the absorbance determined at 610 nm. This experiment was repeated until a peak height repeatability was obtained with a relative standard deviation of 1% (four or five peaks).

Calibration graph for chlorhexidine

A 140 μl aliquot of chlorhexidine solution, in the concentration range 10.5–63.0 μg ml^{-1}, was injected into the carrier - reagent stream (4.12×10^{-4} M thymol blue buffered with succinic acid - succinate buffer at pH 3.40 and $I = 0.01$ M). The

Table 1. Range of variables examined in the modified simplex method

Variable	Range
Reaction coil length	65–315 cm
Flow-rate	1.34–2.80 ml min^{-1}
pH	3.00–7.00
Thymol blue concentration	1.8×10^{-4}–7.3×10^{-4} M
Sample size	30–200 μl

reaction coil was 142 cm long and the flow-rate was 1.34 ml min^{-1}. A good mix between the sample and carrier - reagent solutions was achieved by inserting a single bed string reactor (SBSR) device near the injection valve. Absorbance values were measured at 610 nm and the peak height was recorded.

Results and Discussion

Reagents were investigated in order to find those that would give a precipitate suitable for the turbidimetric determination of chlorhexidine. Methyl orange, thymol blue, bromocresol green and bromocresol purple gave ion-pair compounds and Cu^{II} gave a chelate. The pH was adjusted prior to any reaction. Turbidimetric spectra were recorded against de-ionised water immediately after the precipitate was formed, and the absorbance was measured at two wavelengths, the first being that at which the spectrum shows only a small slope and the second at which the absorbance value is a maximum. This maximum wavelength is shifted 10–30 nm for the maximum wavelength of the dye or, in the case of the complex with Cu^{II}, 30 nm from the maximum wavelength of chlorhexidine (Table 2).

Any absorbance value obtained at the maximum wavelength for ion-pair compounds is very high, owing to the concentration of dye necessary for the formation of a precipitate with chlorhexidine. This suggests a low accuracy and high photometric errors, and therefore thymol blue (measured at 610 nm), bromocresol purple (750 nm) and bromocresol green (800 nm) were chosen as suitable reagents (Table 2). These reagents were tested with chlorhexidine in the FIA procedure and the thymol blue - chlorhexidine ion pair was selected as it gave the largest peak heights.

Table 2. Turbidimetric study of chlorhexidine - dye (or ion - metallic) precipitates

Reagent	$\lambda_{max.}$/nm	Reagent concentration/M	pH	Calibration graph (l = 1 cm)	Selected λ/nm	Reagent concentration/M	pH	Calibration graph (l = 1 cm)
Cu^{II}	262	1.01×10^{-4}	11.80	$A = 0.200 + 12000\,C$; $r = 0.996$	350	8.1×10^{-5}	11.36	$A = 0.02 + 1300\,C$; $r = 0.9$
Methyl orange	462	9.1×10^{-5}	6.00	$A = 2.00 - 12700\,C$; $r = 0.99$	—	—	—	—
Bromocresol green	620	5.8×10^{-5}	5.70	$A = 2.78 - 35000\,C$; $r = 0.99$	800	8.8×10^{-5}	5.64	$A = -0.07 + 6400\,C$; $r = 0.99$
Thymol blue	—	—	—	—	610	8.6×10^{-5}	4.52	$A = -0.04 + 11000\,C$; $r = 0.993$
Bromocresol purple	424	9.4×10^{-5}	5.06	$A = 2.10 - 19000\,C$; $r = 0.99$	750	9.4×10^{-5}	5.06	$A = -0.08 + 8600\,C$; $r = 0.993$

Table 3. Modified simplex method. R = reflection, C = contraction and E = expansion

Cycle type	Point No.	pH	Flow-rate/ ml min^{-1}	Coil length/cm	Sample size/μl	Reagent concentration/M	Peak height/mm	Best/mm	Current simplex points
	1	3.00	1.34	65	30	1.8	31.3		
	2	3.82	2.67	116	65	2.9	35.2		
	3	3.82	1.64	293	65	2.9	29.1		
	4	3.82	1.64	116	185	2.9	56.5		
	5	6.65	1.64	116	65	2.9	26.7		
	6	3.82	1.64	116	65	6.8	33.9	56.5	1, 2, 3, 4, 5, 6
1/*R*	7	0.66*	1.93	165	100	4.0	0		
C	8	5.15	1.71	129	73	3.2	30.6		1, 2, 3, 4, 8, 6
2/*R*	9	4.02	1.96	−76*	102	4.1	0		
C	10	3.87	1.72	201	74	3.2	34.2		1, 2, 10, 4, 8, 6
3/*R*	11	2.18*	1.89	117	94	3.9	0		
C	12	4.41	1.76	126	79	3.4	39.0		1, 2, 10, 4, 12, 6
4/*R*	13	4.90	2.43	205	157	5.9	27.5		
C	14	3.47	1.61	100	62	2.8	41.3		14, 2, 10, 4, 12, 6
5/*R*	15	3.93	2.12	147	121	−6.7*	0		
C	16	3.85	1.76	124	79	4.9	62.0	62.0	14, 2, 10, 4, 12, 16
6/*R*	17	3.88	2.05	32*	113	3.6	0		
C	18	3.87	1.80	159	84	3.3	50.0		14, 2, 18, 4, 12, 16
7/*R*	19	3.95	0.76*	134	130	4.0	0		
C	20	3.85	2.19	121	81	3.2	56.5		14, 20, 18, 4, 12, 16
8/*R*	21	3.14	1.84	121	118	3.5	95.0		
E	22	2.50*	1.88	120	137	3.6	0	95.0	14, 20, 18, 4, 21, 16
9/*R*	23	3.93	2.08	156	157	4.3	43.0		
C	24	3.82	1.97	142	133	4.0	37.0		14, 20, 18, 4, 21, 16
	25	3.54	1.97	139	137	3.9	57.0		
	26	3.90	2.14	139	119	3.8	57.0		
	27	3.91	1.94	158	120	3.8	67.0		
	28	3.88	1.86	136	171	3.6	73.5		
	29	3.89	1.92	140	118	4.6	57.0	95.0	25, 26, 27, 28, 21, 29
10/*R*	30	3.95	1.92	139	121	3.8	61.7		
C	31	3.84	1.93	139	125	3.8	126.0	126.0	31, 26, 27, 28, 21, 29
11/*R*	32	3.57	1.65	138	142	4.0	155.0		
E	33	3.40	1.41	138	153	4.1	166.5	166.5	31, 33, 27, 28, 21, 29
12/*R*	34	3.38	1.67	137	157	2.9	144.7		31, 33, 27, 28, 21, 34
13/*R*	35	3.15	1.55	112	169	3.4	141.5		31, 33, 35, 28, 21, 34
14/*R*	36	2.88*	1.50	122	118	3.5	0		
C	37	3.63	1.76	133	158	3.6	148.4	166.5	31, 33, 35, 37, 21, 34

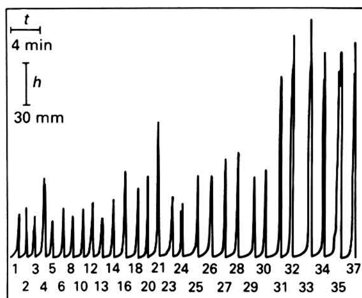

Fig. 1. Peaks corresponding to the modified simplex method

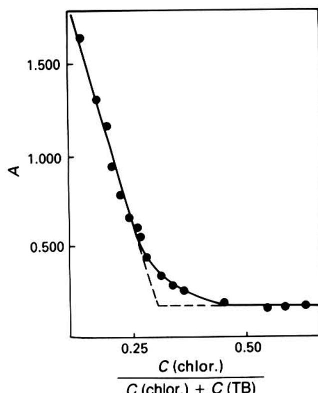

Fig. 2. Stoicheiometry of chlorhexidine - thymol blue precipitate

Copper(II) was not selected as the maximum wavelength of absorption of the chelate formed is in the UV region and many interfering organic ions are found in this region in pharmaceutical samples. Methyl orange gave a precipitate that adhered to the test-tubes walls and was therefore not selected. No better results were observed using dextrin.

The influence of pH on the formation of the precipitate was investigated by adding the reagent to the chlorhexidine solution after adjusting the pH value. Precipitate formation was observed between pH 3.00 and 8.60 and the solid re-dissolved at pH 1.70 and 9.00. This means that the precipitate appears in the pH range at which chlorhexidine and thymol blue are in the di-protonated and mono-anionic forms, respectively.

Modified Simplex Method of Optimisation

The results of this are shown in Table 3 and Fig. 1. The first vertex of the initial simplex gives a 19-mm peak height; in four experiments an improvement from 19 to 57 nm was obtained, and after 15 experiments (point 21) the peak height was 95 mm. After 26 experiments (point 33) the peak height was 167 mm. After 29 experiments it was decided that the system did not merit further experimentation and that point 33 was the optimum that could be obtained. Zero values of peak height were assigned to the entries in Table 3 marked with an asterisk, which were not true or were out of the variable range. The peak corresponding to point 33 is a double peak, which became single when the SBSR apparatus was added to the FIA apparatus after the sample and carrier - reagent solutions had been mixed.

The stoicheiometry of the reaction was established by mixing different volumes of 7.97×10^{-4} M chlorhexidine and 5 ml of 4.24×10^{-4} M thymol blue, previously buffered to pH 3.40 with a succinic acid - succinate buffer (I = 0.2 M), diluted to 10 ml with de-ionised water and centrifuged. The absorbances of aliquots of clear liquid were obtained at 432 nm against de-ionised water. A chlorhexidine : thymol blue stoicheiometry of 1 : 2 (Fig. 2) was obtained.

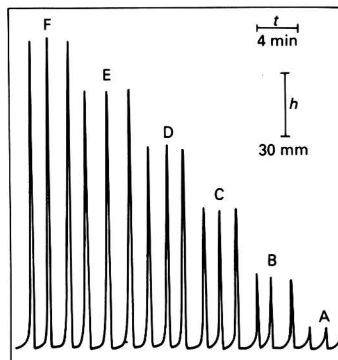

Fig. 3. Peaks corresponding to the linear zone of the calibration graph. Chlorhexidine concentrations: A, 1.8×10^{-5}; B, 3.6×10^{-5}; C, 5.5×10^{-5}; D, 7.4×10^{-5}; E, 9.1×10^{-5}; and F, 10.9×10^{-5} M

Several buffers were tested under the FIA conditions of point 33, namely, tartaric acid - tartrate, citric acid - citrate, phthalic acid - phthalate and succinic acid - succinate. Succinic acid - succinate was selected for further work.

The influence of ionic strength on peak height is important. As the ionic strength increased in the range 0.01–0.14 M, the peak height decreased and double peaks were obtained. Therefore, the ionic strength selected was 0.01 M.

In the concentration range studied (5.0–106.2 µg ml^{-1} of chlorhexidine) the calibration graph ($A = -0.08 + 5252C$) gives a straight line from 10.5 to 63.0 µg ml^{-1} of chlorhexidine, the regression coefficient is 0.9991 and the peaks obtained can be seen in Fig. 3. Twenty different samples were injected into the carrier - reagent stream in order to test the relative standard deviation and sample injection rate, and the results obtained were 1.5% and 53 samples per hour, respectively. The FIA system must be cleaned after 17–20 injections using a dilute NaOH stream.

The influence of foreign compounds found with chlorhexidine in pharmaceutical formulations was also tested. The concentration of chlorhexidine was 7.2×10^{-5} M for any tested solution.

The proposed method can tolerate a 50-fold excess of ascorbic acid, acetylsalicylic acid and sucrose. Benzocaine does not interfere.

Conclusion

A fast and simple method for the determination of chlorhexidine has been described. The procedure is based on the formation of an ion pair between chlorhexidine and thymol blue and uses flow injection analysis (FIA) with turbidimetric detection.

The application ranges for previously published methods using extraction procedures are 2.9–32.2 µg ml^{-1} (ref. 1) and 2–40 µg ml^{-1} (ref. 2), whereas the proposed method can be used to determine 10.5–63.0 µg ml^{-1} of chlorhexidine. FIA techniques usually give higher detection limits than batch procedures, and the lower limit of the calibration graph provided by our method is therefore larger than those found by the extraction procedures,[1,2] or the batch turbidimetric method, which was not fully developed in this work (only the corresponding calibration graph was determined, see Table

2). As the flow injection analysis is precise, fast and easily automated, it is suitable for the determination of chlorhexidine in pharmaceutical formulations.

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A New Type of Argon Ionisation Detector

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A new type of argon ionisation detector is described that is constructed completely of glass and Teflon, and consequently is extremely inert. The production of metastable argon atoms appears to arise from the thermionic emission of electrons from the glass surface. The volume of the detecting cell is less than 5 µl and the applied voltage lies between 1000 and 2000 V, depending on the geometry. The detector can also function with helium as the carrier gas, without the need to subject the helium to excessive purification procedures. The detector in its experimental form appears to have a potential sensitivity at least equivalent to that of the flame-ionisation detector and a linearity of at least three orders of magnitude. Preliminary experiments indicate that the argon detector will detect all substances that the original model will sense and it appears to be extremeley inexpensive to fabricate. As a result of its low dead volume, this detector should be very useful for multi-dimensional separations employing capillary columns.

Keywords: *Argon detector; glass thermal emission; gas chromatography*

The argon detector invented by Lovelock[1,2] is one of the oldest known high-sensitivity detectors used in gas chromatography (GC). It has been shown[3] to have a sensitivity of at least an order of magnitude higher than the flame-ionisation detector and was manufactured commercially by W. G. Pye Ltd. in the late 1950s and early 1960s. However, it proved to be unpopular, mainly owing to the necessary use of a radioactive source, but other contributing factors were its limited linear dynamic range and to a lesser extent its sensitivity to trace impurities in the carrier gas. Despite these disadvantages, further development of the argon detector has been undertaken as a result of a change in the instrument needs of contemporary gas chromatographers.[4] Today there is a need for a high sensitivity, linear, inexpensive chromatographic system with high reliability and a high performance to price ratio. A number of cheaper, reliable instruments are beginning to appear, but because the most universal detector available is the flame-ionisation detector (which requires at least two gas supplies and three flow control systems) a limit is placed on the cost reduction of the chromatographic system. It was therefore decided to investigate further the possibility of constructing an argon detector that did not require a radioactive source, that had an adequate linear dynamic range and, because of the nature of its function, that required only one gas supply system.

The principle of the argon detector is based on the formation of metastable argon atoms in the path of the solute vapour as it is eluted from the column. Argon is a noble gas and, consequently, collisions between argon atoms and electrons are perfectly elastic. However, if the energy of an electron achieves a value greater than 11.6 eV, collision with an argon atom will result in the production of a metastable argon atom. A metastable argon atom is not charged, it merely has one of its electrons moved to an outer orbit. On collision with another molecule, the electron collapses back to its original orbit, releasing an energy equivalent to 11.6 eV. This energy is sufficient to ionise almost all organic compounds (with one or two exceptions) and hence with an appropriate ion-collecting electrode system the formation of metastable atoms can serve as a means of detecting organic vapours. In the original detector, the base electron current was achieved by the use of a radioactive source and the electrons were accelerated across a high potential gradient between two electrodes, producing a cloud of metastable argon atoms in the process. When an organic vapour existed in the detector, collision between the metastable argon atoms and the molecules of the vapour produced electron - ion pairs and the current between the electrodes increased. In fact, there are a number of types of metastable argon atoms that can be found, each having different lifetimes. However, it is sufficient at this time to consider the production of "metastables" in general that permit solute detection.

Initially, to avoid the use of a radioactive source, it was attempted to produce electrons by photoelectric emission from an appropriate metal surface during irradiation by UV light. This procedure functioned satisfactorily,[5] but unfortunately made the device unnecessarily complicated by introducing a UV source, UV-transparent windows and electron-emitting surfaces into the detection system. It was found, however, by complete serendipity, that the production of metastable argon atoms could be initially generated by the thermal emission of electrons from a glass surface. This phenomenon was noted during experiments when a current was observed to pass through an argon detector system, constructed entirely of glass and operated at elevated temperatures. The current could not be accounted for by either photoelectric emission or thermionic emission from a metal surface. As any GC detector must be maintained at elevated temperatures to be used satisfactorily, the process of metastable argon atom production, initiated by the thermal emission from glass, was considered to be an ideal process for use as a detection system. Our preliminary experiments and the results obtained will be discussed in this paper. More detailed discussion of the detection system will be reported in due course.

Detector Designs

The results from four different detector designs are reported. The first design merely established the feasibility of the detection process. The second design established the basic principles on which the detector functions. The third was a practical design that would function with packed columns, and the fourth was miniaturised to allow it to be used successfully with capillary columns. Diagrams of the four detectors are shown in Fig. 1.

Detector A consisted of a glass tube, terminated by Swagelok nuts. In one nut, a 1.59 mm o.d., 0.51 mm i.d. tube, the anode, sleeved with Teflon, penetrated and projected about 20 mm into the glass tube, having 1 mm of metal exposed beyond the Teflon sleeve; through this tube the argon entered the detector. It was necessary to employ Teflon as an insulating material between the glass and the detector anode as glass conducts electricity at elevated temperatures. The nut at the other end contained a Teflon exit tube. The metal argon inlet tube was connected to a positive voltage from the power

supply and the other connection was made to the glass wall of the detector by means of an alligator clip, which, in turn, was connected to an electronic amplifier. The outlet of the detector was connected directly to a flame-ionisation detector (FID) to provide data on comparative performance.

The second detector (B) was fabricated completely from Pyrex glass and consisted of two tubes clamped in Teflon sleeves such that the small tube penetrated 2–3 cm inside the other. The smaller tube was about 0.38 mm i.d. and 1.52 mm o.d. and the larger tube about 3.43 mm i.d. and 7.24 mm o.d. The arrangement was such that the two tubes did not touch. Electrical connections were made to the two tubes by alligator clips, as the glass was conducting at the temperatures at which the detector was to be used. The whole detector could be enclosed in a large container through which a purge gas of argon could flow.

The third detector (C) was designed for practical use with a packed column and was extremely simple. It consisted of a 3.18 mm Teflon Swagelok union carrying two Pyrex pipette tubes of 3.00 mm o.d., 1.90 mm i.d. and 30 mm long separated by a spacer, also made of Teflon. The spacer was 2 mm long and 3.00 mm o.d., the aperture about 1.5 mm i.d. In a similar manner to the second detector, electrical connections were made by means of alligator clips to the glass. It should be pointed out that the detector was connected to the column by another Teflon union to electrically isolate the column from the detector. The outlet of the detector was also connected directly to an FID to allow comparative data to be obtained.

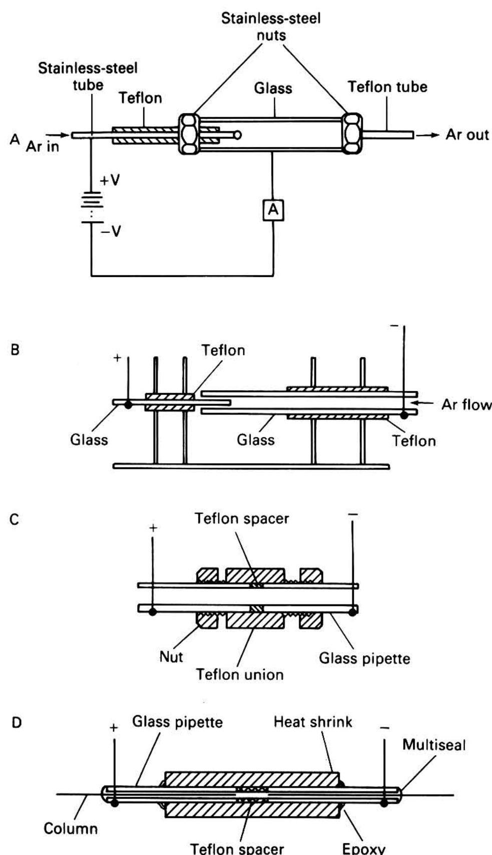

Fig. 1. Different detector designs

The fourth detector (D) was designed to determine if the detection system could be fabricated with a sufficiently small detecting volume to be suitable for use with capillary columns. The capillary column connecting tube and outlet capillary were sealed into a 30 mm length of Pyrex pipette tube of about 1.5 mm o.d. and 0.40 mm i.d. employing Multiseal sealing compound. The outlet capillary was connected to an FID. The two tubes were again separated by an appropriate Teflon spacer and the whole detector was covered by a piece of heat-shrink Teflon, the final seal being made with high temperature epoxy glue. The actual detecting volume was the cylindrical hole in the Teflon spacer. In the model investigated, the cylindical hole was 0.6 mm long, 1.0 mm diameter and had a volume of 0.40 µl. The actual capillary column was connected to the detector-connecting tube by an insulating union to isolate the column electrically from the detector. Electrical connections to the two detector tubes were made with alligator clips, as with the two previous detectors.

Apparatus

The electrical circuit employed is shown in Fig. 2(*a*). A positive voltage is applied to the electrode of the detector from a Power Designs Model 2K-10 power supply that provided potentials ranging from 0 to 2000 V. The other detector electrode was connected, either directly or via a load resistance of 10 or 40 GΩ to the input of an FID amplifier (Perkin-Elmer Part No. 0330–0541). The amplifier was modified to provide a "backing off" current of up to 10^{-7} A. The output of the amplifer was connected to an appropriate potentiometric recorder. The amplifier had a sensitivity range from 10^{-12} to 10^{-5} A full-scale deflection. The negative terminal of the power supply was earthed, as was the other input to the amplifier.

The detector under examination and the testing apparatus were placed in a gas chromatograph (Perkin-Elmer Model Sigma 2000) fitted with an FID and amplifier. The oven had a temperature range of 40–400 °C. The test apparatus was also situated in the oven and is shown in Fig. 2(*b*). It consisted of an argon supply, a septum injection device that could be replaced by a split flow injector for use with a capillary column, if needed, and the packed column. The packed column could be replaced with a capillary column when required. For certain tests the argon supply could be replaced with nitrogen or helium.

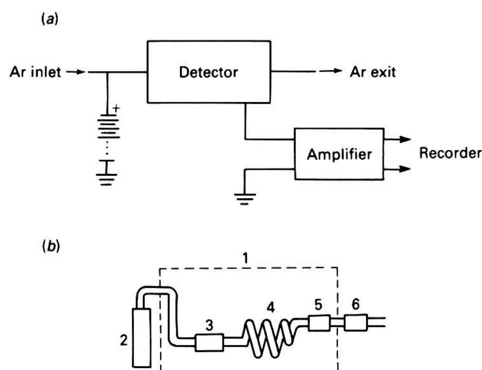

Fig. 2. (*a*) Block diagram of the apparatus. (*b*) Diagram of the apparatus for testing the argon detector. 1, GC oven; 2, argon gas supply; 3, injector; 4, column; 5, argon detector; and 6, flame-ionisation detector

Detector A

Detector A was employed to identify the basic performance characteristics of the system in order to determine its suitability as a GC detector. Base current *versus* applied voltage graphs were obtained for three different temperatures, namely 150, 200 and 250 °C. The results are shown in Fig. 3. It is seen that at low voltages very little current is produced but, as the voltage is increased, the current suddenly increases rapidly and then flattens off to a nearly constant value. This maximum current is very sensitive to temperature; the higher the temperature, the higher is the current. It is also seen that the higher the temperature, the lower is the voltage necessary for the thermionic current to initiate. A potential of 600 V is sufficient to initiate the detector base current at 250 °C, whereas at 150 °C a potential of 800 V is necessary.

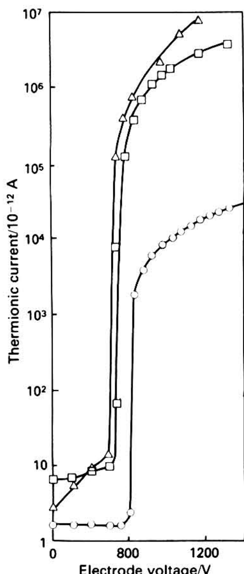

Fig. 3. Graph of detector thermionic current against electrode voltage. Detector body, glass tube. Detector temperature: ○, 150 °C; □, 200 °C; and △, 250 °C

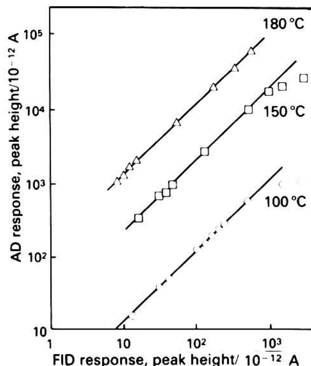

Fig. 4. Graph of the response of the argon detector relative to that of the FID at different temperatures. Detector body, Pyrex tube; electrode voltage, 900 V; and solute, benzene

Such results are to be expected if the electrons constituting the current originated by the thermal emission of the electrons from the glass surface.

The sensitivity of the detector to organic vapours was investigated employing benzene as the test solute. Various masses of benzene were injected on to the column and the eluted peak was monitored by both the argon detector and the FID, which was connected in series with it. Graphs relating peak height from the argon detector to peak height recorded by the FID are shown in Fig. 4. Graphs were obtained for three operating temperatures, 100, 150 and 180 °C, and at an electrode potential of 900 V. It is seen that, within the bounds of the experiment, good linearity is obtained at all three temperatures, the slopes of all three graphs being very close to unity. The higher relative peak heights from the argon detector reflect the higher thermal emission and consequently increased abundance of argon metastable atoms at the higher temperature. In Fig. 5 chromatograms of a hydrocarbon mixture monitored on both the argon detector and FID are shown. It is seen that, indeed, the argon detector (without a radioactive source and without a UV light source) shows considerable promise as a GC detector.

Detector B

Detector B was designed solely to establish the mode of operation of the detection system. There was no physical connection between the electrodes, even insulation was eliminated and therefore the base current produced had to pass across the argon gap. The current was maintained even when the total detector was situated in an atmosphere of argon, eliminating the possibility of any vapours diffusing into the detection cavity and providing a source for the electrons. The free passage of electrons through argon gas has already been discussed and supports the idea that the current-carrying moieties are indeed likely to be free electrons. This was further confirmed by the fact that no current was observed when nitrogen was used as the carrier gas. The base current was not changed when the detector environment was in complete darkness or when strongly illuminated with white light and so the source of the electrons could not be photoelectric. Finally, the current increased with both the electrode potential and detector temperature in the manner expected from the phenomena of thermal emission. There appears to be no alternative source of electrons to consider

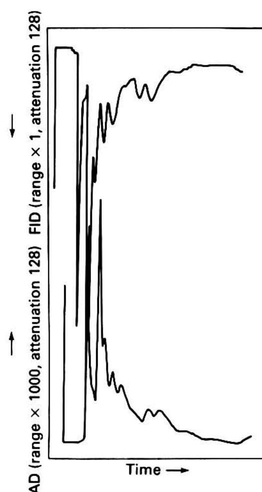

Fig. 5. Chromatogram of a hydrocarbon mixture

and it can therefore be concluded from the evidence obtained so far that the argon detector (as described here) generates the necessary electronic current by the thermal emission of electrons from the surface of the glass.

Detector C

Detector C was the first practical argon detector to be fabricated based on the thermal emission of glass. It was designed to be inert (all parts of glass or Teflon), simple and relatively small in volume. The glass conduit tubes were used as electrodes and so the detector had to be electrically isolated from the column and the FID by another pair of appropriate unions. An example of some results obtained from this detector is shown in Fig. 6. In this instance known masses of benzene were injected on to the column and the concentration at the peak maximum was taken as twice the average concentration; the peak volume was calculated from the peak width at the base, the chart speed and the column flow-rate. The detector was operated at an electrode potential of 1900 V and a temperature of 230 °C. It is seen that the log - log graphs indicate good linearity with a response index[6] or slope of 0.97. It is also seen that the detector exhibits a linear dynamic range of about three orders of magnitude with a noise level of 10^{-10} A. This noise level is significantly greater than that of the FID but, on the other hand, so is the response. The noise level is commensurate with, or perhaps slightly better, than that of the conventional argon detector operating with a radioactive source. The minimum detectable concentration appears to be 1–2 orders of magnitude worse than that of the FID but no attempts to reduce or to identify the source of the noise were made at this stage.

Detector D

Detector D was designed for use with capillary columns. The detector volume could be adjusted by changing the geometry of the Teflon spacer. The detector was very simple to fabricate in the form described but, of course, from a commercial point of view, could not be manufactured from heat-shrink Teflon. The detector was sensitive and linear. An absolute calibration graph of log(peak height) against log(solute concentration) is shown in Fig. 7. Because the quantitative precision obtained from the split injection system was poor, the calibration graph was obtained using on-column injection with a packed column. The detector was operated at 230 °C and at an electrode voltage of 1900 V. The response index or slope of the curve was 0.97, indicating good linearity. The linear dynamic range was over 3 orders of magnitude but, again, the noise level was fairly high. It was thought that the noise level of all the detectors described here could be reduced very significantly if the detectors were placed outside the GC oven with electrical and air screening.

This detector was also operated with helium as the carrier gas. Metastable helium atoms have an energy far in excess of that of metastable argon atoms and thus ionise virtually all compounds and consequently would provide universal detection. Graphs relating base current to electrode potential for the two gases, argon and helium, are shown in Fig. 8. The operating temperature was 200 °C. It is seen that the graphs for the two gases are very similar in shape but the current through the helium is greater than that in argon at the same electrode potential. This probably reflects the higher conductivity of helium over that of argon at elevated temperatures. These graphs were the first indication of the practicability of a helium ionisation detector based on this principle. It should be pointed out that the helium was taken straight from the cylinder with no special purification. Very stringent purification procedures are necessary if the conventional detector employing a radioactive source is to be used satisfactorily with helium.

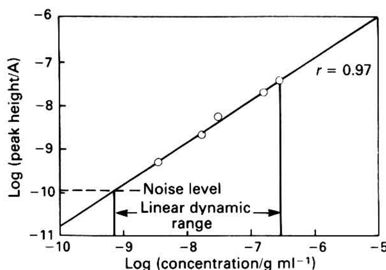

Fig. 6. Linearity graph for the argon detector. Detector temperature, 230 °C; electrode voltage, 1900 V; solute, benzene

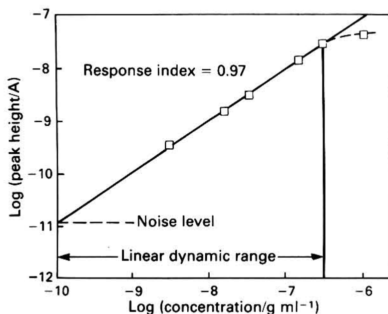

Fig. 7. Graph of response of the argon detector against solute concentration. Conditions as in Fig. 6

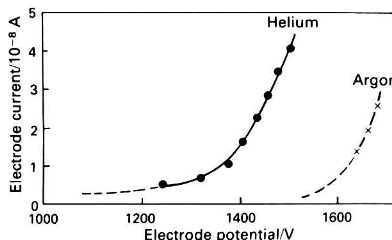

Fig. 8. Graph of electrode current against electrode potential. Dashed portions of the curves are extrapolated; measurement difficult owing to noise from arcing. Cell temperature, 200 °C. Cell annular shape: length 1.3 mm; o.d. 0.96 mm; i.d. 0.41 mm; volume, 0.76 µl

Fig. 9. Chromatograms from ionisation detectors. Carrier gas, helium (99.999%); detector temperature, 250 °C; electrode voltage, 1600 V; external resistance, 40 GΩ

Employing a capillary column, 10 m long, 0.25 mm i.d. and 0.40 mm o.d., coated with a 0.25-μm film thickness of methylsilicone, a mixture of hydrocarbons (C_8, C_{13} and C_{16}) and dibutylphenol was separated and detected by the helium detector. The detector was operated at 250 °C with an electrode voltage of 1600 V and an external resistance of 40 GΩ. The results obtained are shown in Fig. 9. It seems that the detector functioned with helium, but it should be pointed out that the identification of the correct combination of electrical voltage and detector geometry was critical in order to obtain good performance and was difficult to establish. When further experience with the detector has been obtained, it is hoped that optimum conditions will be arrived at more easily.

Conclusions

The thermionic emission of electrons from glass at elevated temperatures has been confirmed, and this effect has been used to operate an argon detector. At first sight, the thermal emission of electrons from glass seems to be unlikely. As a result, the authors discussed the matter in some detail with the original inventor of the argon detector, Dr. James Lovelock,[7] who had a number of very useful suggestions. Nevertheless, it was mutually agreed that from the evidence, the initiation of the current by the thermal emission of electrons (or even positive ions from the surface) was the most likely explanation at this time. The detector, under the appropriate combination of temperature and applied voltage, provides a linear response over at least three orders of magnitude and exhibits a sensitivity within one to two orders of magnitude of that of the FID. The optimum geometry of the detector has not been ascertained and a number of interesting possibilities remain to be investigated. An optimised detector would probably have a sensitivity at least equivalent to that of its radioactivity counterpart, *viz.*, at least one order of magnitude greater than that of the FID. An argon detector, utilising the emission of electrons from glass, can exhibit adequate sensitivity and linearity for general use in gas chromatography and at the same time utilise a simplified pneumatic gas supply. This further provides a basis for additional reduction in instrument cost. In contrast to soda or Pyrex glass, fused silica is neither a conductor nor an electron emitter at elevated temperatures, or at least very much less so. It follows that fused silica has possible use as a high-temperature insulator in the construction of such detectors.

The authors thank Dr. J. E. Lovelock for very helpful discussions.

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SHORT PAPERS

Diazotised 4-Nitroaniline as a Chromogenic Reagent for the Determination of Trace Amounts of Pyrrole in Aqueous Solution

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A sensitive spectrophotometric method for the determination of trace amounts of pyrrole in aqueous solution is described. The method is based on the reaction of pyrrole with diazotised 4-nitroaniline to produce, in the presence of sodium acetate, an intense yellow, water-soluble, stable monoazo dye, which shows maximum absorption at 420 nm. A graph of absorbance *versus* concentration indicates that Beer's law is obeyed over the concentration range 5–100 μg of pyrrole in a final volume of 25 ml, *i.e.*, 0.2–4 p.p.m., with a molar absorptivity of 2.32×10^4 l mol^{-1} cm^{-1}, a Sandell sensitivity of 0.0029 μg cm^{-2}, a relative error of +0.9 to −1.7% and a relative standard deviation of 0.6–2.0%, depending on the concentration. A study has been made to determine the optimum conditions of the colour reaction. Interferences from foreign organic compounds have been examined and the structure of the monoazo dye is suggested.

Keywords: *Pyrrole determination; diazotised 4-nitroaniline reagent; spectrophotometry*

Pyrrole is an important five-membered heterocyclic ring because its nucleus occurs in many natural compounds, *e.g.*, alkaloids, chlorophyll and haematin. Pyrrole also occurs in coal tar and bone oil.[1] The presence of the pyrrole group in hydrocarbons is undesirable because it promotes gum formation, sedimentation and discoloration.[2] Among the various methods available for trace analysis, spectrophotometry continues to be one of the most popular because it is simple and economical.[3] The spectrophotometric methods available for the determination of pyrrole are very few and are not completely satisfactory.The method based on the reaction with 4-dimethylaminobenzaldehyde[4] to give a red colour seems to be slow unless the reaction mixture is heated to boiling and the reagent is in alcohol. Other methods[5,6] based on the reaction with isatin in hydrochloric acid to yield a blue colour are affected by the concentration of acid. Therefore, a new spectrophotometric method for the determination of pyrrole seems desirable. This paper describes a spectrophotometric method for the determination of pyrrole, which relies on the coupling reaction of pyrrole with diazotised 4-nitroaniline reagent to form, in the presence of sodium acetate, an intense yellow azo dye suitable for the quantitative determination of trace amounts of pyrrole in aqueous solution. The intense azo dye is water soluble and does not require prior extraction.

Experimental

Apparatus

Spectral measurements were carried out on a Unicam SP 1800 UV double-beam recording spectrophotometer and absorbance readings were made on a Bausch and Lomb Spectronic 710 single-beam digital spectrophotometer using 1-cm silica matched cells.

Reagents

All chemicals used were of analytical-reagent grade.

Stock pyrrole solution, 10 *mg ml*$^{-1}$. Dissolve 0.25 g of pyrrole in ethanol and adjust the volume to 25 ml in a calibrated flask with the same solvent. Store in a refrigerator.

Working pyrrole solution, 50 μ*g ml*$^{-1}$. Dilute 0.5 ml of the stock pyrrole solution to 100 ml with distilled water in a calibrated flask. Store this solution in a refrigerator when not in use.

Diazotised 4*-nitroaniline reagent solution,* 2 mM. This solution is prepared in the same manner as described elsewhere[7] except that the standing time before the volume is adjusted to 100 ml is 30 min instead of 5 min. This reagent solution is stable for one week when kept in a refrigerator at 0 °C.

Sodium acetate solution, 1 M. Dissolve the appropriate amount of sodium acetate trihydrate in and dilute to volume with distilled water. Prepare the 0.08 M solution by dilution.

Foreign compound solution, 0.1 *mg ml*$^{-1}$. Dissolve the compound in 10% *V*/*V* ethanol in a calibrated flask, except for carbazole, for which 65% ethanol should be used.

Procedure

Transfer increasing volumes of working pyrrole solution, covering the range 5–100 μg, into a series of 25-ml calibrated flasks. Add 10 ml of distilled water, 0.5 ml of 0.08 M sodium acetate solution, 2 ml of 2 mM diazotised reagent and dilute to volume with distilled water. Mix and allow the reaction mixture to stand for 10 min to attain full colour formation. Measure the absorbance against a reagent blank, prepared in the same manner but containing no pyrrole, at 420 nm using 1-cm cells. The colour is stable for at least 1 h. A straight-line calibration graph is obtained, indicating that Beer's law is obeyed over a concentration range of 0.2–4 p.p.m. The apparent molar absorptivity (referred to pyrrole), calculated by the formula given in reference 8, is found to be 2.32×10^4 l mol^{-1} cm^{-1} and the Sandell sensitivity is 0.0029 μg cm^{-2}.

For subsequent experiments, 50 μg of pyrrole were taken and the final volumes were 25 ml.

Results and Discussion

Absorption Spectra

When very dilute aqueous solutions of pyrrole and diazotised 4-nitroaniline reagent solution are mixed in the presence of sodium acetate, an intense yellow monoazo dye forms immediately. The intense azo dye formed shows a maximum absorption at 420–422 nm, in contrast to the reagent blank, which shows a maximum absorption in the ultraviolet region

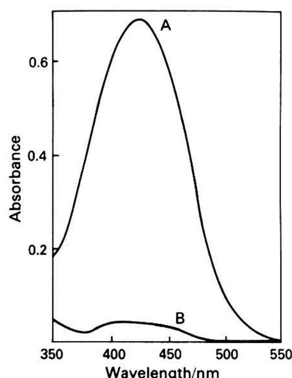


Fig. 1. Absorption spectra of: A, 50 μg of pyrrole, treated as described under Procedure and measured against a reagent blank; and B, reagent blank measured against distilled water

near 325 nm and almost zero absorption in the visible region (380–550 nm). Fig. 1 shows the spectra of the azo dye and of the reagent blank. A wavelength of 420 nm, characteristic of the azo dye, was therefore used in all subsequent determinations.

Table 1. Standard deviation and precision of the proposed method

Amount of pyrrole taken/μg	Relative error,* %	Relative standard deviation,* %
25	−1.7	1.7
50	−1.6	0.6
100	+0.9	2.0

* Four determinations.

Table 2. Effect of foreign compounds on the determination of 50 μg of pyrrole. The amount of interferent added in each instance was 100 μg

Interferent	Interference, %
4-Aminopyridine	+1.2
2-Aminopyrimidine	+0.2
Carbazole	+2.7
4-Diphenylamine-4-sodium sulphonate	+0.3
Furan-2-carboxylic acid	−4.0
Histidine	−0.3
Indole-3-propionic acid	+4.9
Nicotinic acid	−4.2
1,10-Phenanthroline.H_2O	−1.6
Phenol	+3.9
Pyrazole	−3.3
Thiophene-2-carboxylic acid	+2.7
Tryptamine	−2.1
Tryptophan	−3.7
Uracil	−1.2

Study of the Optimum Reaction Conditions

The effects of various parameters on the absorption intensity of the azo dye were studied and the reaction conditions were optimised.

Effect of Diazotised Reagent

In order to achieve the optimum spectrophotometric conditions,[7] three diazotised reagents were tested. These reagents were diazotised orthanilic acid, diazotised sulphanilic acid and diazotised 4-nitroaniline. The latter reagent showed the most useful results for the determination of pyrrole and was therfore studied further with respect to the effect of its concentration on colour formation.

The azo dye formation reached a maximum with about 1 ml of the 2 mM diazotised 4-nitroaniline reagent solution and remained at this maximum when 2–5 ml of the prescribed reagent concentration were added. A 2-ml volume of 2 mM diazotised reagent solution was therefore adopted in the procedure.

Effect of Alkaline Solution

The addition of mineral acid to the azo dye solution resulted in a decreased intensity, whereas the addition of alkali caused a bathochromic shift. Sodium acetate, sodium hydrogen carbonate, sodium carbonate and sodium hydroxide were examined. The experimental investigations revealed that the resultant colour, although less intense, becomes more stable with decreasing base strength, and sodium acetate was chosen for further studies. The use of 0.5 ml of 0.08 M sodium acetate solution gave a reasonable sensitivity and the colour became stable for at least 1 h. Increasing the amount of sodium acetate led to an increased colour intensity but limited stability.

Effect of Light and Temperature

Light had no appreciable effect on the absorbance of either the sample or blank solution. The same absorbance was obtained whether the reaction was performed in daylight or in the dark. Temperatures in the range 5–15 °C had no effect on the colour intensity. However, increasing the temperature to 45 °C resulted in a 10% loss of intensity. Therefore, it is recommended that the determination is carried out at room temperature (15 °C).

Order of Addition of Reagents

The order of addition cited in the procedure should be followed. The colour becomes less stable when sodium acetate is added after the diazotised reagent.

Effect of Time on Colour Development

A study of the effect of time on colour development showed that the reaction mixture should stand for 10 min in order to achieve an absorbance that is stable for at least 1 h.

Accuracy and Precision

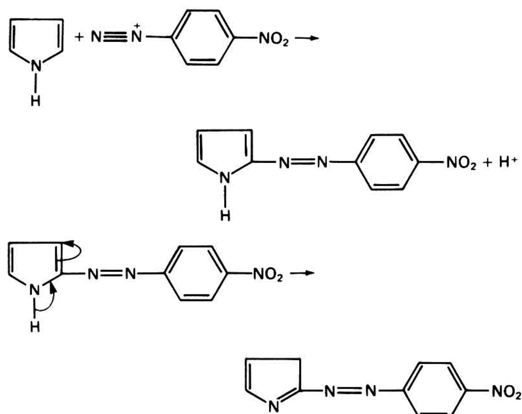
In order to check the accuracy and precision of the method, pyrrole was determined at three different concentrations. The results are shown in Table 1 and indicate that the method is satisfactory.

Interferences

In order to illustrate the selectivity of the method, the interference effects of various organic compounds were examined by carrying out the determination of 50 μg of pyrrole in the presence of each of the interferents using the recommended procedure. The results obtained are given in Table 2.

Nature of the Dye

Pyrrole couples with diazonium salts predominantly at the 2-position.[9] Job's method of continuous variations showed that the dye has the composition 1 : 1 (pyrrole : diazotised 4-nitroaniline reagent), indicating a monoazo dye. The mechanism of azo dye formation may be written as follows:



The azo dye is soluble in water, acetone, dioxane, ethanol, ethoxyethanol, methanol, propanol and tetrahydrofuran. The dye is also quantitatively extracted into benzene, carbon tetrachloride, chloroform and diethyl ether.

Conclusion

A simple and sensitive spectrophotometric method for the determination of trace amounts of pyrrole in aqueous solution has been developed, based on the formation of an azo dye following the coupling of pyrrole with diazotised 4-nitroaniline reagent in the presence of sodium acetate.

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Spectrophotometric Determination of Dobutamine Hydrochloride Using 3-Methylbenzothiazolin-2-one Hydrazone

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A spectrophotometric procedure is described for the determination of dobutamine hydrochloride. The proposed method uses 3-methylbenzothiazolin-2-one hydrazone as the chromogenic reagent. A mixture of aqueous solutions of the drug and reagent is treated with cerium(IV) ammonium sulphate in an acidic medium. Dobutamine reacts to give a pink colour with a $\lambda_{max.}$ at 510 nm ($\varepsilon_{max.}$ = 1.5 × 10^4 l mol^{-1} cm^{-1}). Beer's law is obeyed in the concentration range 4–20 μg ml^{-1} of dobutamine in the final assay solution. The procedure described was successfully applied to the determination of the bulk drug and its dosage form (Dobutrex vials).

Keywords: *Spectrophotometry; dobutamine hydrochloride determination; 3-methylbenzothiazolin-2-one hydrazone*

Dobutamine hydrochloride {(±)-4-[2-3-*p*-hydroxyphenyl-1-methylpropylamino)ethyl]-benzene-1,2-diol hydrochloride} is a sympathomimetic agent which has direct effects on beta-adrenergic receptors. These effects mean that the drug has a prominent inotropic action on the heart.[1] It is widely used in the form of Dobutrex vials in the management of heart failure associated with organic heart disease, myocardial infarction and cardiac surgery.

The USP XXI describes a gas chromatographic assay for both the pure drug and the injection after silylation with 1-trimethysilylimidazole, using a flame ionisation detector and n-triacontane as an internal standard.[2] Other spectrophotometric,[3] GLC[4] and HPLC[5] methods have been reported. Although the official USP procedure is very reliable, the instrumentation and effort required for the assay are much greater than those required for a spectrophotometric assay.

In this paper the development of a procedure based on the formation of a coloured reaction product with 3-methylbenzothiazolin-2-one hydrazone (MBTH) is reported. The colour produced is stable and reproducible and is proportional to the concentration of dobutamine. This highly sensitive procedure is simple, rapid and readily adaptable to both the bulk drug and unit dose determinations.

Experimental

Apparatus

A Pye Unicam SP 1750 spectrophotometer (Pye Unicam, Cambridge, UK) with an AR 55 linear recorder and SP 1805 programme controller was used.

Samples

Dobutamine hydrochloride, pharmaceutical grade. Obtained as a gift from Eli Lilly (Windlesham, Surrey, UK), and used as a working standard.

Dobutamine hydrochloride injection. Dobutrex vials (Eli Lilly) containing dobutamine hydrochloride equivalent to 250 mg of dobutamine were used.

Preparation of sample solutions

An accurately weighed amount of dobutamine hydrochloride, or the contents of the Dobutrex vials, was dissolved in water and diluted stepwise to obtain a concentration of 30 μg ml^{-1} of dobutamine hydrochloride.

Reagents

MBTH solution, 0.2% *m/V.* Freshly prepared in distilled water.

Cerium(IV) ammonium sulphate solution, 0.075% *m/V in* 5% H_2SO_4.

Assay Procedure

A 2.0 ml aliquot of the assay solution was transferred into a stoppered test-tube and 2.0 ml of MBTH solution were added. After 5 min, 2.0 ml of cerium(IV) ammonium sulphate solution were added and the contents were mixed thoroughly. After 30 min, the absorbance of the solution was measured at 510 nm against a blank similarly prepared but using 2.0 ml of distilled water instead of the sample solution.

Construction of Calibration Graph

About 30 mg of dobutamine hydrochloride were accurately weighed, dissolved in water and diluted to volume in a 100-ml calibrated flask. This solution was diluted stepwise to give a series of concentrations suitable for the construction of the calibration graph in the range 12–60 μg ml^{-1}; 2.0 ml of each solution were used for the colour formation with MBTH as described under Assay Procedure.

Results and Discussion

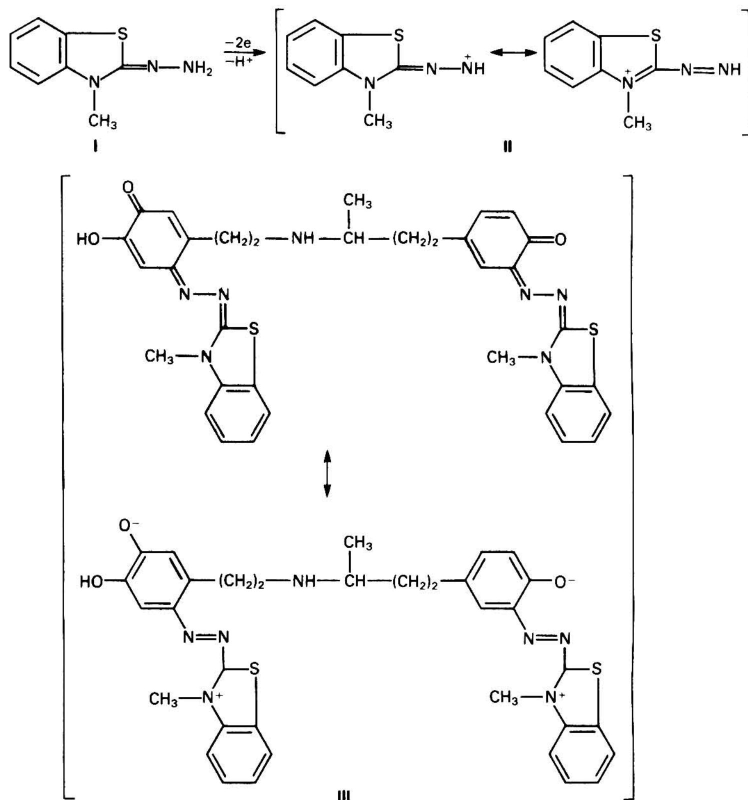
Reaction Mechanism

Dobutamine hydrochloride forms a pink product ($\lambda_{max.}$ = 510 nm) with MBTH in the presence of cerium(IV) ammonium sulphate in acidic media. The molar absorptivity of the chromogen was found to be 1.5 × 10^4 l mol^{-1} cm^{-1}. Under the reaction conditions, MBTH (I), on oxidation with Ce^{4+} ions, loses two electrons and one proton forming an electrophilic intermediate (II), which is the active coupling species.[6–10] Two moles of this intermediate undergo electrophilic substitution with the two phenolic moieties of 1 mol of dobutamine to form a coloured product (III) according to the scheme shown overleaf.

Optimisation of Variables

Effect of MBTH concentration

The optimum concentration of MBTH leading to maximum colour stability was found to be 2 ml of 0.2% reagent per 6 ml of the reaction mixture. Lower reagent concentrations gave



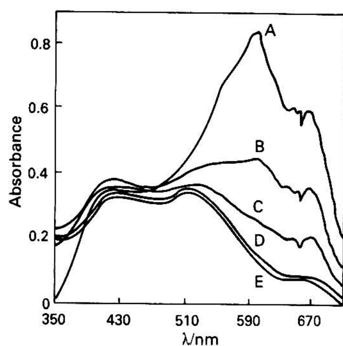


Fig. 1. Spectra of dobutamine - MBTH prepared with A, 0.05%, B, 0.10%, C, 0.15%, D, 0.20% and E, 0.25% MBTH solution. Concentration of dobutamine hydrochloride is 7.3 μg ml^{-1} in the final assay solution

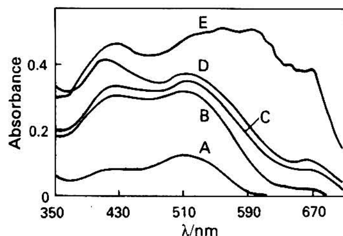


Fig. 2. Spectra of dobutamine - MBTH prepared with A, 0.025%, B, 0.050%, C, 0.075%, D, 0.100% and E, 0.125% cerium(IV) ammonium sulphate solution. Concentration of dobutamine hydrochloride is 7.3 μg ml^{-1} in the final assay solution

Table 1. Effect of H_2SO_4 concentration on colour intensity

H_2SO_4 concentration, %	Absorbance at 510 nm		
	4 μg ml^{-1}	8 μg ml^{-1}	12 μg ml^{-1}
1.0	0.172	0.283	0.399
2.0	0.190	0.312	0.438
3.0	0.204	0.337	0.473
4.0	0.226	0.372	0.520
5.0	0.225	0.370	0.520

Table 2. Effect of reaction time on colour intensity. The concentration of dobutamine hydrochloride in the final assay solution was 16 μg ml^{-1}

Time/min	A_{510}	Time/min	A_{510}
5	0.607	40	0.649
10	0.621	45	0.649
15	0.630	60	0.648
20	0.638	90	0.647
25	0.643	120	0.631
30	0.648	150	0.605
35	0.648	180	0.575

colours of higher intensities (Fig. 1) but these faded very quickly with time, probably owing to the formation of a mixture of monosubstituted dobutamine derivatives.

Effect of Ce^{4+} concentration

The optimum concentration of cerium(IV) ammonium sulphate solution leading to maximum colour stability was found to be 2 ml of 0.075% solution per 6 ml of the reaction mixture. Higher concentrations of the Ce^{4+} ion gave colours of higher

Table 3. Assay of dobutamine hydrochloride in bulk drug and Dobutrex vials by the MBTH method. All determinations were in triplicate

Sample	Amount taken/ mg	Recovery ± SD, %	Amount added/ mg	Recovery ± SD, %
Raw material	30.0	99.7 ± 1.20	—	—
Raw material	60.0	99.4 ± 0.94	—	—
Raw material	90.0	100.1 ± 0.86	—	—
Dobutrex vials	150.0	99.2 ± 1.03	60.0	100.1 ± 0.97
Dobutrex vials	180.0	99.6 ± 0.98	60.0	99.3 ± 1.16

intensities (Fig. 2) but these faded very rapidly with time, probably owing to the formation of several oxidation products or the oxidation of the chromogen itself.

Effect of H_2SO_4 concentration

The optimum concentration of H_2SO_4 solution in which cerium(IV) ammonium sulphate was dissolved was found to be 5% (Table 1). Higher concentrations did not affect the colour intensity.

Effect of reaction time

The maximum colour intensity was obtained after 30 min at 20 ± 5 °C. The colour was stable for a further hour (Table 2).

Quantification, Adherence to Beer's Law, Sensitivity, Accuracy and Precision

A linear correlation (r = 0.9996) was found between the absorbance at 510 nm and the concentration of dobutamine hydrochloride in the range 4–20 μg ml^{-1} in the final assay solution. The minimum detectable amount was found to be 1.0 μg of dobutamine hydrochloride. The reaction can therefore be used for the identification of the drug in bulk or in dosage forms. The precision of the proposed method was determined by analysing ten replicate samples, each containing 10 μg ml^{-1} in the final test solution. At this concentration level the standard deviation was 0.80%.

Application to Bulk Drug and Dosage Form Analysis

The suggested method was applied to the quantitative determination of dobutamine hydrochloride in the raw material and in Dobutrex vials (Table 3). The results obtained indicate that the method is suitable for routine quality control analysis. The proposed method has the added advantages of simplicity and rapidity over the recent USP derivatisation gas chromatographic method. The proposed method has also two advantages over the previously reported spectrophotometric method using thiosemicarbazide.[3] Firstly, MBTH is stable in the solid state whereas thiosemicarbazide is hygroscopic. Secondly, in the proposed method, aqueous solutions are used throughout the whole procedure, whereas in the thiosemicarbazide method acetone, which is volatile and less suitable for spectroscopic measurements, is used as a diluting solvent.

The author thanks Miss V. Mason, Lilly Research Centre, Ltd., UK, for the samples supplied.

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SOFTWARE REVIEW

From time to time, *The Analyst* will publish reviews of computer software packages designed for analytical applications. Commercial and other suppliers of such packages are invited to submit analytical software for review; full technical specifications should be included.

CLUE

A. Thielemans, M. P. Derde and D. L. Massart. Elsevier Scientific Software, 1985. Price $405; Dfl975; £105; Manual only $41.50; Dfl100; £25. Technical specifications:

Computer:	Apple II, II+, IIe, IIc
Language:	Applesoft BASIC (interpreter and compiled)
Operating system:	DOS 3.3
Required peripherals:	140 K 5¼-in diskette drive
Minimum memory:	48K RAM (interpreter) 64K (compiled)
Storage medium:	5¼-in diskette
Order Ref. No.:	ISBN 0 444 42335 4
Computer:	IBM-PC, -XT, -AT
Language:	PC-BASIC (interpreter and compiled)
Operating system:	PC-DOS 2.x, 3.0
Required peripherals:	180K/360K 5¼-inch diskette drive
Minimum memory:	128K RAM
Storage medium:	5¼-in diskette
Order Ref. No.:	ISBN 0 444 42336 2

CLUE is a program that performs cluster analysis on a microcomputer. It is based on a hierarchical divisive method and can be used to detect similarities and differences between objects that are characterised by several measurement variables. This pattern recognition technique is useful in many branches of science; in analytical chemistry it is most often applied to investigate whether complex samples have a common or a different origin.

The maximum size of the data matrix that the program can handle is 50 objects by 50 variables, so real-world problems of somewhat more than moderate size can be attacked with the program. The documentation describes how the source code can be modified to trade off a number of variables for more objects. If the program is used to its limits the availability of the compiled-BASIC version (64K needed) helps to keep the run-time within practical limits.

Much attention has been paid to the user interface of the program, which is menu driven. Together with the excellent documentation this guides the user through the steps necessary to perform a complete cluster analysis. Data input to the program can come from the keyboard or from disk. Keyboard data can be stored on disk and edited for later use. A minor nuisance is the strict formatting rules that demand decimal notation in one particular range and scientific notation outside this range. A nice feature is that data can be appended to a data matrix that is already stored on disk.

To obtain meaningful results in cluster analysis, transformation of the original input data should be performed if the range of values is quite different for different variables. The program offers two options: a logarithmic transformation and the *Z*-transform. The results of these transformations can also be saved to disk.

The clustering can be done on the objects, but also on the variables, using either the Euclidian distance or the correlation as the measure of similarity. In the Euclidian distance option the program can take care of the frequently encountered problem of missing values. If the clustering is to be based on another dissimilarity measure, the required dissimilarity matrix can be entered directly from keyboard or disk on the condition that the values increase with increasing dissimilarity.

The actual clustering takes some time, but its progress can be followed on the screen. The end results are presented in a kind of graphical output that needs some polishing to become recognisable as the dendrograms that are normally used for this purpose.

The documentation of the program is, as stated before, excellent. The manual consists of a 77-page users' guide with a completely worked out example and clear instructions for the installation of the program. The scientific background is given in a 25-page section with references to the relevant literature. The availability of the source code on disk and its listing in the manual enable the user to combine (parts) of the program with data acquisition routines.

In conclusion, it can be said that CLUE is a well written, well documented and complete package for cluster analysis on data sets of moderate to medium size. Its price does not exactly make it a bargain, but the amount and quality of the code certainly are worth it.

M. Bos

BOOK REVIEWS

Mass Spectrometry. Volume 8. A Specialist Periodical Report.
Senior Reporter M. E. Rose. Pp. xvi + 360. Royal Society of Chemistry. 1985. £70; $126. ISBN 0 85186 328 0.

This volume, under the Editorship of a new Senior Reporter, M. E. Rose, maintains the high standards set by his predecessors. In contrast to Volume 7, where an attempt was made to use modern printing methods with some disastrous consequences, this volume has been produced by conventional methods, resulting in a high quality product, but at a high price. It is to be hoped that the price of Volume 9 will be more reasonable, as the book is to be produced from camera-ready copy.

As with previous volumes, contributions consist of a combination of reviews of the latest developments (1982–84) of key areas in mass spectrometry together with specialist reviews of areas of current topical interest. Chapters that fall into the former category are those by I. Powis on "Ionization Processes and Ion Dynamics"; M. A. Baldwin on "Structures and Reactions of Gas-phase Organic Ions"; T. R. Kemp on "Devlopments and Trends in Instrumentation"; J. H. Bowie on "Reactions of Organic Negative Ions in the Gas Phase"; M. E. Rose on "Gas Chromatography/Mass Spectrometry and High-performance Liquid Chromatography/Mass Spectrometry"; D. J. Harvey on "Drug Metabolism, Pharmacokinetics and Toxicity"; and J. Charalambous on "Metal-containing and Inorganic Compounds Investigated by Mass Spectrometry." J. R. Chapman contributes on "Applications of Computers and Microprocessors in Mass Spectrometry," an area not covered in Volume 7, but present in previous volumes. In all instances the authors have done a thorough job, delineating recent developments in these areas and the current status of these topics. The remaining chapters are of a topical nature with J. Dannacher and J.-P. Stadelmann providing an excellent review of the fundamentals of photoelectron - photoion coincidence spectroscopy and its impact on our understanding of ionic decomposition phenomena. N. M. M. Nibbering in his chapter on "Fourier-transform Ion Cyclotron Resonance" provides a useful description of the basics of the technique and its utility in furthering our understanding of gas-phase ion chemistry. Unfortunately, some of the most important developments in this area, from the point of view of the analytical mass spectrometrist, *i.e.*, improvements in sample introduction methodology and high mass applications, have been too recent for inclusion in this chapter. Fast atom bombardment mass spectrometry has revolutionised the ability of mass spectrometrists to provide useful structural information from mass spectrometrically difficult molecules. Much has been written about the qualitative aspects of this technique. R. M. Caprioli provides an insight into the ability of the technique to provide quantitative data and a systematic review of the various factors involved in the production of useful spectra.

The volume is recommended to all practising mass spectrometrists, as it provides a readily accessible overview of recent developments in the area and is of particular value as a source for developments in areas which they do not specialise. It will also be of considerble value to scientists interested in applying mass spectral techniques to the solution of problems in their discipline, because an insight can be rapidly gained into the utility of mass spectral techniques in a wide range of areas. The only reservation I have about the volume is cost, as £70 represents a major investment for the individual purchaser.

D. E. Games

BASIC Molecular Spectroscopy
P. A. Gorry. Pp. x + 144. Butterworths. 1985. Price £8.95. ISBN 0 408 01553 5.

This book, according to the author, has the aim of providing an introduction to molecular spectroscopy for first- and second-year undergraduates in chemistry and physics and covers the physical chemistry theory of the interaction of the electric field component of light with molecular or electronic motions. Following introductory chapters on BASIC and the quantum treatment of molecules, the main topics dealt with are rotational, vibrational, Raman and electronic spectra. Each chapter consists of two parts, essentially theory and three or four programs written in BASIC (hence the title of the book) to provide practical examples and illustrations of the topics under consideration. There are suggestions for further reading and some problems (but no solutions).

Despite the modest aims claimed by the author, only a first-class graduate planning a PhD in physical chemistry will be able to read this book. At best it could provide a summary of the topic to be used in conjunction with a lecture course. The average undergraduate chemistry student could not cope with the maths, and the brevity of the book takes all the fascination out of the subject. The result is boring. Only rarely does the author permit a qualitative view or model of the processes in question. The rest is equations.

It is not clear for whom the computer programs are provided. I cannot see the average student eagerly modifying the programs so that they run on the particular micro that is accessible and then tapping them in, far less modifying them to suit his or her own particular needs as the author suggests. The lecturers running the spectroscopy course might be more interested. It is really at these persons that the book is aimed (and jolly useful they will find it).

No practical aspects of any kind are mentioned, so there is not even the merest hint that most of the spectroscopies mentioned are actually used by practising chemists to provide analytical information.

J. F. Tyson

Handbook of Polycyclic Aromatic Hydrocarbons. Volume 2. Emission Sources and Recent Progress in Analytical Chemistry.
Edited by Alf Bjørseth and Thomas Ramdahl. Pp. x + 416. Marcel Dekker. 1985. Price $95 (USA and Canada); $114 (all other countries). ISBN 0. 8247 7442 6.

Widespread interest and the rapidity of recent developments in the whole field of polycyclic aromatic hydrocarbons (PAHs) and related compounds (PACs) have encouraged the Editors to assemble a second volume to update and add new information to their original Handbook published in 1983. The book is organised into 11 chapters contributed by 13 well known experts in the field. As before, the contents are nicely balanced between emission sources, recent progress in analytical chemistry and exposure, uptake, metabolism and detection of PAHs in the human body.

The scale of the problem is put into perspective in Chapter 1, where the reader learns that it is estimated that 6000 metric

tons of PAHs per year are emitted in the USA alone. The next three chapters discuss specific emissions from coal-fired plants, combustion of biomass and automobiles, respectively. The two main analytical chapters are updates on HPLC and GC. The continued growing importance of HPLC in this field is reflected by the fact that it is the longest chapter in the book and that most of the 104 references cited (1980–84) were not in the original Handbook.

Nitrogen-containing PACs have generated particular interest over the past few years and the second longest chapter in the book reviews the nomenclature, chemical, physical and biological properties and the analytical chemistry of these compounds. Finally, of particular analytical concern, is an account of the work of the Joint Research Centre of the Commission of the European Communities to produce certified reference compounds for calibration of analytical methods and apparatus.

This is an excellent book. A wealth of data is clearly presented in the form of tables, figures and chromatograms. Moreover, the text is readable and the book should therefore appeal to all who have more than a cursory interest in the control of these hazardous and ubiquitous pollutants of the human environment.

R. Amos

Cell Components

Edited by H. F. Linskens and J. F. Jackson. *Modern Methods of Plant Analysis. New Series, Volume* 1. Pp. xx + 399. Springer-Verlag. 1986. DM238. ISBN 3 540 15822 7; 0 387 15822 7.

This new series on methods of plant analysis begins with a volume on cell components and organelles. It is designed to replace the original series, under the same title and by the same publishers, which was published in seven volumes between 1956 and 1964. That series is now no longer modern and, in view of the ever expanding literature in this area, the use of "modern" in any title must be treated with some caution. However, a scan of the references in each chapter gives numerous papers of 1983–84 vintage as well as the occasional 1985 contribution. It is, therefore, as up-to-date as one could expect.

The Editors of the new series are H. F. Linskens and J. F. Jackson, who will attempt to amalgamate the styles and views of each contributor into a coherent unit. The Editors state that they have not tried to interfere too much but in the reviewer's opinion this has led to some chapters not attaining the high quality set by most.

This volume contains chapters on cell wall isolation, cell wall chemistry, protoplasts, the marker concept in cell fractionation, plasma membranes, vacuoles, protein bodies, lipid bodies, chloroplasts as a whole, chloroplast envelope membranes, chloroplast thylakoid membranes, ribulose-biphosphate carboxylase, non-green plastids, mitochondria, endoplasmic reticulum, polyribosomes, the nucleus and microtubules. The normal format is an introduction followed by detailed methodology for the particular component, which not only gives the different methods for isolating the different components but also gives details of purity procedures. Many of the chapters give useful information on places where extra care is required. This is to be recommended for the inexperienced worker. The chapters all contain a wide range of photographs and figures which should help the novice to see what he is looking for and assist in setting him off in the right direction. Inevitably in such a volume, all chapters are not of the same standard. The chapter on cell wall chemistry, structure and components is neither sufficiently up-to-date nor does it consider many of the essential modern methods of analysis.

The authors and Editors, in addition, should pay more attention to their proof-reading. There are too many irritating spelling mistakes in some chapters.

Nevertheless, this book is a most welcome addition to any library that supports laboratories concerned with plant studies. It will be well used by young and old alike. It is a pity that the cost of the whole series will limit the purchase to libraries; a set in the laboratory or the office would be ideal. The reviewer looks forward to seeing the future volumes in the series.

Ian M. Morrison

Developments in Polymer Characterisation, Volume 5

Edited by J. V. Dawkins. *Developments Series.* Pp. x + 343. Elsevier Applied Science. 1986. Price £45. ISBN 0 85334 401 9.

In a brief Preface, Dr. Dawkins summarises the contributions and also their relationship (if any) to papers in the earlier collections. Most of the authors are members of universities, in Canada, Denmark, England, Scotland, Sweden, USA and Wales. There may be one (or perhaps two) contributors who are employed with major US manufacturers of polymers and products in this field (and for which the development of new and improved materials would be expected to be a primary concern).

The nine authors provide us with six chapters. The first topic is small-angle neutron scattering (SANS) with particular reference to the conformation of polymer chains and the determination of dimensions of molecules. The new material is stated to include recent results for amorphous and semi-crystalline polymers, together with examples for deformed networks, block copolymers, blends of polymers and polymers in solution. Theoretical aspects are discussed and it is fair to comment that the subject itself is no longer new and that (as it employs neutron beams) it is very costly in practice.

Other topics are: the examination of polymer solutions using photon correlation spectroscopy; the measurement (on-line) of relative molecular mass and long-chain branching by means of size exclusion chromatography with scattering of low-angle laser light; gel permeation chromatography in the analysis of hydrophilic polymers and polyelectrolytes; dynamic mechanical thermal analysis of polymers; and the application of fluorescence techniques.

Theoretical considerations play a prominent part throughout the text and this militates against its usefulness from the point of view of the working analyst. In a review for *The Analyst* (and bearing in mind also the current cost of volumes of this nature), one can only place emphasis on the aspects that are likely to be of value to the majority of readers.

The most practical chapter (and the one which perhaps makes the most interesting reading) is on the application of gel permeation chromatography to hydrophilic polymers and polyelectrolytes. It contains a good deal of useful information about, for example, the use of standard polymers in calibrating many types of column packings and their properties in relation to this use.

The volume includes a total of about 1000 references, and has a good index. It is thought likely to appeal to the theoretician and the research worker, and that it would be purchased by specialised libraries.

D. Simpson

Applications of NMR Spectroscopy to Problems in Stereochemistry and Conformational Analysis
Edited by Yoshito Takeuchi and Alan R. Marchand. *Methods in Stereochemical Analysis, Volume* 6. Pp. x + 221. VCH. 1986. Price DM135. ISBN 0 89573 118 5 (VCH Publishers); 3 527 26145 1 (VCH Verlagsgesellschaft).

NMR spectroscopy has evolved over 40 years from a phenomenon of interest only to physicists into a technique that embraces just about all the physical, chemical, biochemical, analytical and medical sciences. The title of this volume suggests that a fairly narrow part of this field is being reviewed. In practice, however, a very wide and diverse field is covered, perhaps too wide to be of total interest to any one reader.

The first chapter, by I. O. Sutherland, provides a comprehensive survey of the complexes formed by crown ethers and related compounds. The rate processes and energy profiles of this unique guest - host chemistry are discussed in depth, providing some very interesting data. Unfortunately, this chapter appears to have been completed well before the others in the book, as it is fully referenced only up to the end of 1981. The application of liquid crystal NMR to the determination of the geometry of oriented molecules is reviewed by P. Diehl and J. Jokisaari. Although the complexity of the spectra limit the method to molecules of similar size to *p*-chlorotoluene, the accuracy of the geometry obtained is phenomenal, stated to be at least 100 times better than electron diffraction or IR spectroscopy. In Chapter 3, R. Kitamaru provides a very substantial survey of ^{13}C relaxation theory applied to the conformation and dynamics of macromolecules. For analytical chemists, Chapter 4 by Terao and Imashiro may be of more interest as it deals with solid-state NMR at high resolution using ^{13}C cross-polarisation and magic angle spinning (CPMAS), providing sharp spectra devoid of the dipolar coupling effects normally observed with solids. The ^{13}C spectra obtained are similar to those obtained in solution, although sometimes perturbed slightly by crystal packing and other environmental effects. Thus a new and valuable technique for sampling solids is available with further applications, for instance, in studying polymorphism, intermolecular interactions, molecular motion in solids and chemical shift anisotropy effects. Considering the importance of 2D NMR in structure determination, Chapter 5, by Nagayama, is a rather brief review of the subject, which has been reviewed better elsewhere in my opinion. However, it is complemented by Chapter 6, where Kessler and Bermel provide a thorough and authoritative review of 2D NMR applied to peptide conformation in solution.

For all laboratories where NMR spectroscopy research is carried out, this volume, like others before it, will be received with interest, although the organic chemist in the field of stereochemistry and conformational analysis may be disappointed, in that only *ca.* one third of the book is devoted to what he understands from the title. The amount of information packed into this volume suggests overall that it is probably good value for money, despite the relatively high price.

W. A. Thomas

Modern Techniques of Surface Science
D. P. Woodruff and T. A. Delchar. Pp. x + 453. Cambridge University Press. 1986. ISBN 0 521 30602 7.

That the classical divisions of science into chemistry, physics, etc., have broken down is nowhere more clearly demonstrated than in the vitally important study of surfaces. A would-be researcher in this field must be analyst, electrician, vacuum specialist and mathematician as well as both physicist and chemist. Whatever his or her background, today's surface scientist has great need of a comprehensive introduction to the many and various techniques that are now used both separately and in bizarre and bewildering combinations. Each method, each combination of methods has its own acronym. He who does not know a RHEED from a LEED, who cannot tell a SEXAFS from an EXAFS, who thinks that IRAS is something to do with taxation or that HREELS maybe a dance, has grave and urgent need of this excellent introduction to modern techniques of surface science, where all is revealed.

The rapid development of surface science, especially over the last two decades, has by now generated enough experience and data for a critical review to be made of the many techniques that are now available. Such a review is given in this book. A chapter is devoted to each of the important methods for the study of surfaces: electron diffraction, beam scattering, ion bombardment, desorption, work function measurements as well as infrared, photoelectron and Auger spectroscopy. For each subject the basic physics is outlined, the experimental method described and examples of the results are discussed. In this way, the potential of each technique and its advantages and disadvantages can readily be understood and appreciated. A further important aspect of modern surface studies that is emphasised is the complementary nature of much of the data produced by these individual techniques, nicely reflecting the experimental reality that most equipment is designed to permit a single sample to be investigated in many different ways. There is also a particularly valuable introductory chapter in which the differences to be expected, especially in diffraction, between two-dimensional (as in surfaces) and three-dimensional (as in solids) arrays of atoms, are carefully examined.

This book reveals, in a most dramatic way, just what "analysis," at least for a surface, means today. To analyse a surface is not only to identify the elements present but also to determine their juxtaposition, their order or their lack of it, their state of chemical combination, to identify any chemical reactions that they may or may not have indulged in whilst on the surface, to find out whether the adsorbate atoms have formed a monolayer, or have congregated into islands or are hopping around all over the place, etc. Such are the challenges of surface analysis. This book clearly shows how these challenges are to be met.

The book is well produced, up-to-date and introduces the reader, through its wide choice of examples and numerous references, to all major aspects of the current literature. This invaluable introduction to the techniques of modern surface science will, because of the breadth of topics covered, be equally indispensable to the novice and experienced researcher alike.

D. S. Urch

INSTRUCTIONS TO AUTHORS

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. Papers may be submitted for publication by members of The Royal Society of Chemistry or by non-members. There is no page charge for papers published in *The Analyst*.

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. Communications will normally be examined by one referee.

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.

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Manuscripts. Papers should be typewritten in double spacing on one side *only* of the paper. Three copies of text and illustrations should be sent to the **Editor, The Analyst, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN**, and a further copy retained by the author.

Proofs. The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned immediately (by Air Mail from outside Europe).

Reprints. Fifty reprints of each paper are supplied free on request. Additional reprints can be purchased if ordered at the time of publication. Details are sent to authors with the proofs.

Notes on the Writing of Papers for *The Analyst*

Manuscripts should be in accordance with the style and usage shown in recent copies of *The Analyst.* Conciseness of expression should be aimed at: clarity is increased by adopting a logical order of presentation, with suitable paragraph or section headings.

To facilitate abstracting and indexing by Chemical Abstracts Service, and other abstracting organisations, it would be helpful if at least one forename could be included with each author's family name.

Descriptions of new methods should be supported by experimental results showing accuracy, precision and selectivity.

The recommended order of presentation is as indicated below:

(*a*) *Title.* This should be as brief as is consistent with an adequate indication of the original features of the work. The analytical method used in the work should be mentioned in the title.

(*b*) *Synopsis.* A synopsis of about 100 words, giving the salient features and drawing attention to the novel aspects, should be provided for all papers.

(*c*) *Keywords.* Up to 5 keywords or key phrases, indicating the topics of importance in the work described, should be included after the synopsis.

(*d*) *Aim of investigation.* An introductory statement of the object of the investigation with any essential historical background, followed, if necessary, by a *brief* account of preliminary experimental work.

(*e*) *Description of the experimental procedures.* Working details must be given concisely. Analytical procedures should preferably be given in the form of instructions; well known operations should not be described in detail.

(*f*) *Results.* These are best presented in tabular form, followed by any statistical evaluation, which should be in accordance with accepted practice.

(*g*) *Discussion of results.* This section will comment on the scope of the method and its validity, followed by a statement of any conclusions drawn from the work.

Nomenclature. Current internationally recognised (IUPAC) chemical nomenclature should be used. Common trivial names may be used, but should first be defined in terms of IUPAC nomenclature.

SI units. The SI system of units should be used. These units are summarised in the Appendix. The effect on current style of papers for *The Analyst* includes the following:

(*a*) dimensions should preferably be given in metres (m) or in millimetres (mm);

(*b*) temperatures should be expressed in K or °C (not °F);

(*c*) wavelengths should be expressed in nanometres (nm) (not mμ);

(*d*) frequency should be expressed in Hz (or kHz, etc.), not in c/s or c.p.s.; rotational frequency can be denoted by use of s^{-1}; in mass spectrometry, signal intensity should be expressed in counts s^{-1} and not in Hz;

(*e*) radionuclide activity will be expressed in becquerels (Bq) or curies (Ci); 1 Ci = 3.7×10^{10} Bq;

(*f*) the micron (μ) will not be used; 10^{-6} m will be 1 μm.

Abbreviations. SI units should be used. Molarity is generally expressed as a decimal fraction (*e.g.*, 0.375 M). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and other units represented by symbols. Abbreviations other than those of recognised units should be avoided in the text.

Percentage concentrations of solutions should be stated in internationally recognised terms. Thus the symbols "*m*" for mass and "*V*" for volume are to be used instead of "w" for weight and "v" for volume. The following show the manner of expressing these percentages together with an acceptable alternative given in parentheses: % *m*/*m* (g per 100 g); % *m*/*V* (g per 100 ml); % *V*/*V*. Further implications of the use of the term "mass" are that "relative atomic mass" of an element (A_r) replaces atomic weight, and "relative molecular mass" of a substance (M_r) replaces molecular weight.

Concentrations of solutions of the common acids are often conveniently given as dilutions of the concentrated acids, such as "dilute hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might represent either 1 + 4 or 1 + 3. Dilutions of other solutions can be expressed in a similar manner.

Tables and diagrams. The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns can often be arranged horizontally. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

Either tables or graphs may be used but not both for the same set of results, unless important additional information is given by so doing. The information given by a straight-line calibration graph can usually be conveyed adequately as an equation or statement in the text.

The style used in headings to tables and in labels on the axes of graphs, where the numbers represent numerical values, is, for example: Volume/ml. The diagonal line (solidus) will not be used to represent "per". In accordance with the SI system, units such as grams per millilitre are already expressed in the form g ml^{-1}. For a table (or graph), this would appear as: Concentration of solution/g ml^{-1}. It should be noted that the "combined" unit, g ml^{-1}, must not have any "intrusive" numbers. To express concentration in grams per 100 millilitres, the word "per" will still be required: Concentration/g per 100 ml. It may be preferable for an author to express concentrations in grams per litre (g l^{-1}) rather than grams per 100 ml.

Most diagrams will be retraced and lettered in order to achieve uniform line thicknesses and lettering size and style, so it is not essential to prepare specially traced drawings. However, all diagrams should be carefully and clearly drawn on good quality paper and should be clearly lettered. If possible, complicated flow charts, circuit diagrams, etc., should be supplied as artwork for direct reproduction in order to avoid time-consuming and expensive redrawing.

Three sets of illustrations should be provided, two sets of which may be made by any convenient copying process for transmission to the referees.

All diagrams should be accompanied by a separately typed set of captions. Wherever possible, extensive identifying lettering should be placed in the caption rather than on lines on graphs, etc.

Photographs. Photographs should be submitted only if they convey essential information that cannot be shown in any other way. They should be submitted as glossy or matt prints made to give the maximum detail. Colour photographs will be accepted only when a black-and-white photograph fails to show some vital feature and can be supplied either as prints or transparencies.

References. References should be numbered serially in the text by means of superscript figures, *e.g.*, Foote and Delves,[1] Burns *et al.*[2] or Hirozawa,[3] and collected in numerical order under "References" at the end of the paper. They should be listed, with the authors' initials, in the following form (double-spaced typing):

1. Foote, J. W., and Delves, H. T., *Analyst*, 1983, **108**, 492.
2. Burns, D. T., Glockling, F., and Harriott, M., *J. Chromatogr.*, 1980, **200**, 305.
3. Hirozawa, S. T., *in* Kolthoff, I. M., and Elving, P. J., *Editors*, "Treatise on Analytical Chemistry, Part II," Volume 14, Wiley, New York, 1971, p. 23.

Journal titles should be abbreviated according to the *Chemical Abstracts Service Source Index* (*CASSI*).

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the page number.

Authors must, in their own interest, check their lists of references against the original papers; second-hand references are a frequent source of error. The number of references must be kept to a minimum.

Appendix

The SI System of Units

In the SI system there are seven base units—

Physical quantity	*Name of unit*	*Symbol for unit*
length	metre	m
mass	kilogram	kg
time	second	s
electric current	ampere	A
thermodynamic temperature	kelvin	K
amount of substance	mole	mol
luminous intensity	candela	cd

There are two supplementary dimensionless units for plane angle (radian, rad) and solid angle (steradian, sr). Some derived SI units that have special names are as follows—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
energy	joule	J	$kg\,m^2\,s^{-2}$
force	newton	N	$kg\,m\,s^{-2} = J\,m^{-1}$
power	watt	W	$kg\,m^2\,s^{-3} = J\,s^{-1}$
electric charge	coulomb	C	A s
electric potential difference	volt	V	$kg\,m^2\,s^{-3}\,A^{-1} = J\,A^{-1}\,s^{-1}$
electric resistance	ohm	Ω	$kg\,m^2\,s^{-3}\,A^{-2} = V\,A^{-1}$
electric capacitance	farad	F	$A^2\,s^4\,kg^{-1}\,m^{-2} = A\,s\,V^{-1}$
frequency	hertz	Hz	s^{-1}
magnetic flux density (magnetic induction)	tesla	T	$kg\,s^{-2}\,A^{-1} = V\,s\,m^{-2}$
radionuclide activity	becquerel	Bq	s^{-1}

Examples of other derived SI units are—

Physical quantity	*SI unit*	*Symbol for unit*
area	square metre	m^2
volume	cubic metre	m^3
density	kilogram per cubic metre	$kg\,m^{-3}$
velocity	metre per second	$m\,s^{-1}$
angular velocity	radian per second	$rad\,s^{-1}$
acceleration	metre per second squared	$m\,s^{-2}$
magnetic field strength	ampere per metre	$A\,m^{-1}$

Certain units will be allowed in conjunction with the SI system, *e.g.*—

Physical quantity	*Name of unit*	*Symbol for unit*	*Definition of unit*
volume	litre	l	$10^{-3}\,m^3 = dm^3$
magnetic flux density (magnetic induction)	gauss	G	$10^{-4}\,T$
temperature, t	degree Celsius	°C	$t/°C = T/K - 273.16$
radionuclide activity	curie	Ci	$3.7 \times 10^{10}\,Bq$
energy	electronvolt	eV	$1.6021 \times 10^{-19}\,J$

The common units of time (*e.g.*, minute, hour, day) and the angular degree (°) will continue to be used in appropriate contexts.

Decimal multiples and submultiples have the following names and symbols (for use as prefixes)—

10^{-3}	milli	m	10^3	kilo	k
10^{-6}	micro	μ	10^6	mega	M
10^{-9}	nano	n	10^9	giga	G
10^{-12}	pico	p	10^{12}	tera	T
			10^{15}	peta	P
			10^{18}	exa	E

Compound prefixes (*e.g.*, mμm) should not be used; 10^{-9} m = 1 nm.

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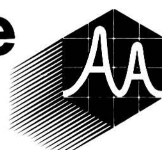

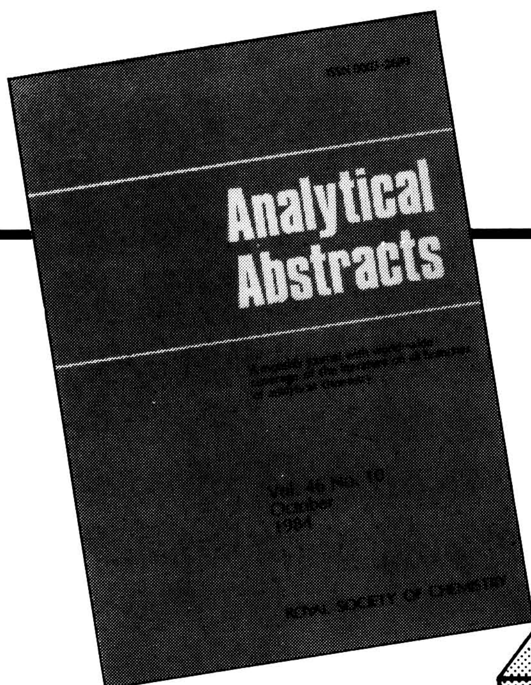

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