PAGES 225–320

ISSN 0003—2654

The Analyst

A monthly international journal dealing with all branches of analytical chemistry

Vol. 110 No. 3
March
1985

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The Analyst

The Analytical Journal of The Royal Society of Chemistry

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The Analyst (ISSN 0003–2654) is published monthly by The Royal Society of Chemistry, Burlington House, London W1V 0BN, England. All orders accompanied with payment should be sent directly to The Royal Society of Chemistry, The Distribution Centre, Blackhorse Road, Letchworth, Herts. SG6 1HN, England. 1985 Annual subscription rate UK £134.50 Rest of World £141.00, USA $260.00. Purchased with *Analytical Abstracts* UK £293.00, Rest of World £308.00, USA $566.00. Purchased with *Analytical Abstracts* plus *Analytical Proceedings* UK £330.00, Rest of World £347.00, USA $638.00. Purchased with *Analytical Proceedings* UK £169.00, Rest of World £178.00, USA $327.00. Air freight and mailing in the USA by Publications Expediting Inc., 200 Meacham Avenue, Elmont, NY 11003.
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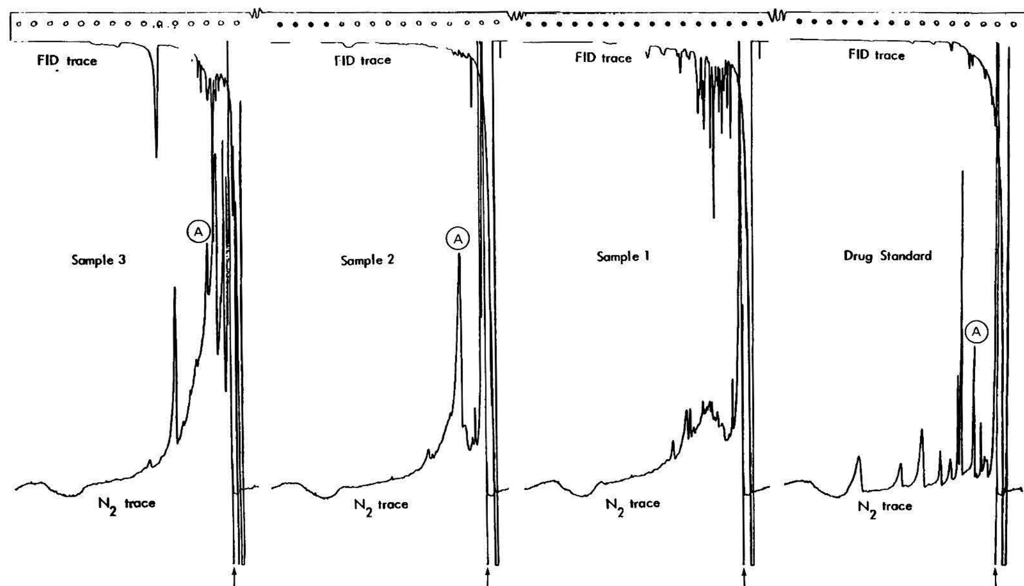
Advertisement Feature

Application of an Improved Nitrogen Detector in Gas Chromatography

Key words: Nitrogen detector, Drugs.



INTRODUCTION

The advantages of a selective detector are two-fold: there is very little response to the solvent, which with the FID can appear as a large trailing peak; it can respond selectively to a particular component in a complex sample with little or no interference from other components.

The alkali flame-ionisation detector was one of the first nitrogen detectors available. The heated bead detector was introduced later, and adopted by a number of manufacturers, although there is recent evidence of a return to the flame type. We at Pye Unicam prefer the flame type because we believe that our detector is easier to set up and to use. Further, this detector responds to all compounds containing nitrogen. Bead detectors do not respond to all compounds, they require very careful setting of low gas flow-rates and very precise control of the current supplied. They are also more easily contaminated, which means a long time may have to be spent in replacing the bead.

A recent comprehensive programme of evaluation of the alkali flame-ionisation detector has shown conclusively that this detector operates reliably, with no problems and with good long term stability, over more than 100 sample analyses using an automatic system.

INSTRUMENTATION

Pye Unicam Series 304 Chromatograph* fitted with outlet splitter to a flame-ionisation detector and to a nitrogen (alkali flame-ionisation) detector:

Pye Unicam PU4700 Autojector
Column: 1.5 m × 4 mm i.d. glass, 3% ON-17 on 100–120 mesh Chromosorb W-HP
Column temperature: 150 to 260 °C at 25 °C min^{-1}
Injector temperature: 250 °C
Detector temperature: 300 °C
N_2detector: Range 1, Attenuation 16
FID: Range 10, Attenuation 16
Carrier: Nitrogen, 30 ml/min
Sample preparation: Extraction into methanol
Sample size: 0.5 µl
Chart speed: 300 mm h^{-1}

RESULTS AND DISCUSSION

The work reported here was the detection and measurement of traces of drugs in samples of urine from horses being tested for doping.

The accompanying chromatograms show the simultaneous traces from the FID and nitrogen detectors for a drug standard containing caffeine, barbitone and phenobarbitone and three samples.

The nitrogen detector chromatograms of samples 2 and 3 show positive evidence of the presence of drug A whereas no conclusion whatever can be drawn from the FID chromatograms.

For further information please contact: D. F. K. Swan, Pye Unicam Ltd., York Street, Cambridge CB1 2PX (telephone 0223 358866).

* The Pye Unicam Series 304 Chromatograph has now been superceeded by the PU4550 which is also suitable for this application.

To obtain further details of this Company's series of Application Notes, please use our Reader Enquiry Service quoting No. A202.

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KONTRON ANALYTICAL

HPLC Reports
Application 182

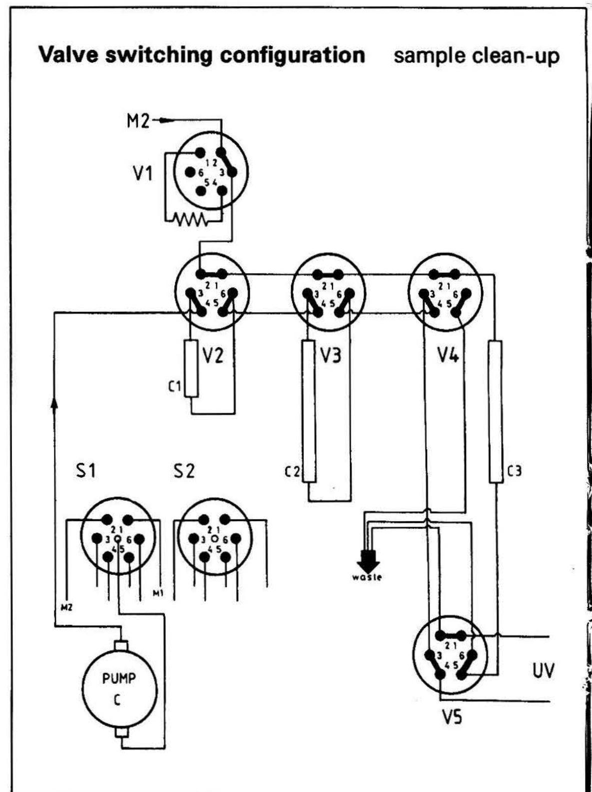
KONTRON TRACER MCS 670: AUTOMATIC SAMPLE CLEAN-UP

Time consuming manual sample preparation is, in many cases, eliminated using the MCS670; samples can be injected directly onto a pre-column which gives partial resolution of the components of interest. The chromatographic zone containing these compounds is transferred to an analytical column where higher resolution is achieved.
Meanwhile, the pre-column is rapidly cleaned and re-equilibrated in readiness for the next injection.

The advantages of this approach are:
(i) In many cases, isocratic elution on the analytical column is possible.
(ii) Analysis time is markedly reduced, thus increasing sample throughput.
(iii) The lifetime of the analytical column is increased.

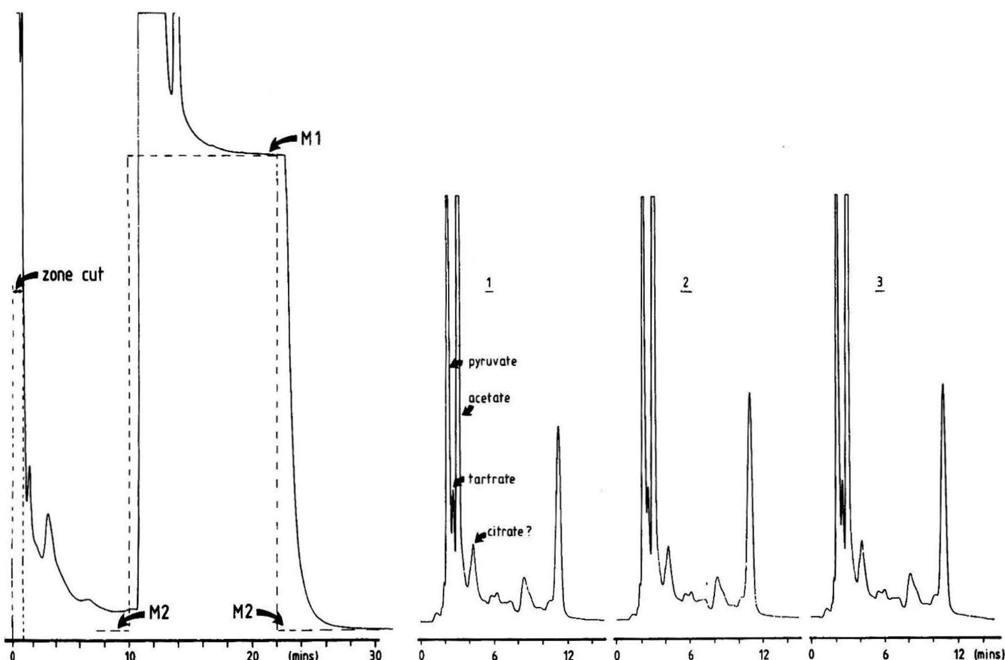
ORGANIC ACIDS IN BEVERAGE

This analysis is important because the acids influence the flavour; they are characteristic of the beverage and they can be used to monitor the fermentation process.
The acids in wine, fortified wine, beers, fruit juices, etc. occur in the zone at the front of the chromatogram. Using a front cutting technique, we can prevent the elution of impurities such as polyphenols and pigmentation products onto the analytical column. The separation can be carried out isocratically on a reversed phase column without the problem of baseline drift. This is very important as the analysis is carried out at 214 nm.
Without column switching, elution from the analytical column, followed by 2 step gradients to clean and re-equilibrate the column would take about 60 minutes. Using the TRACER MCS 670, we can inject undiluted, untreated samples every 15–20 minutes.



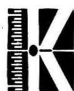
KONTRON MCS 670	**Valve switching schematic** – wine acids								
FUNCTION ACTIVATED	0	6	12	0	6	12	0	6 (mins)	
Inject	↑			↑			↑		
C1	PA M2	PC–M1	PC–M2	PA M2	PC–M1	PC–M2	PA M2	PC–M1	
C3	PA–M2								
Pump A (PA)	1 ml/min								
Pump C (PC)	off	1·5 ml/min		off	1·5 ml/min		off	1·5 ml/min	
Valve V2 position	b	a		b	a		b	a	
Valve S1 position	1 = M1		2 = M2	1 = M1		2 = M2	1 = M1		
Detector	Uvikon 720 LC, 214 nm, 0·5 AUFS								
Recorder	0·5 cm/min								
FUNCTION ACTIVATED	0	6	12	0	6	12	0	6 (mins)	

COLUMNS		**MOBILE PHASES**			
C1	3 cm S 5-ODS, Brownlee	M 1	Methanol	M 7	
C2		M 2	0·01 M NaH_2PO_4 (pH = 2·4)	M 8	
C3	12·5 cm S 5-ODS, Kontron	M 3		M 9	
C4		M 4		M 10	
		M 5		M 11	
		M 6		M 12	



Separation of red wine on C1 showing zone cut, column regeneration & column re-equilibration

Separation of wine acids – sample clean-up on KONTRON TRACER using a zone cutting method – triplicate analyses on reject red wine



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Paar Scientific Ltd.

Application Study

Brewing Industry

Customer: Bass Brewing Limited, Runcorn.

Product and Application: DMA 55 digital density measuring and SP 2 automatic sample systems.

Automated sampling in quality control.

In a recent automation project Bass Brewing at Runcorn have linked a Paar DMA55 digital density measuring system to a gas chromatograph and a Paar temperature controller. Now installed in the Packaging Hall laboratory, the system is used for measuring original gravity of beer samples on alcohol and residue as a quality control operation. The gas chromatograph has eliminated the need for pre-distillation for measurement of percentage alcohol and beer gravity. A sample is split with part injected into the gas chromatograph and the rest into the DMA55. Both are integrated for automatic calculation of the final results.

Automation of a tedious and lengthy procedure but a nonetheless vital one in the laboratory has saved much time and ensured repeatable accuracy and reliability. The DMA55 with the SP2 automatic sample system has its own microcomputer and an accuracy of -1×10^{-5}. It is especially valuable in continuous sampling applications such as the one at Bass Brewing and measuring time is selectable between 0.7 and 5.0 seconds. Special programs can be provided for the minicomputer to suit individual applications throughout the brewing, food, chemical and pharmaceutical industries.

The Paar digital density meters are based on an oscillating principle for measurement with a U-shaped oscillating sample tube. In operation the tube is entirely filled with sample and electromagnetically activated. Density, specific gravity or sample concentration is precisely determined by the automatic measurement of the period of oscillation of the sample tube.

Bass Brewing installed the Paar DMA55 system after highly satisfactory use of a similar Paar instrument in their Analytical Laboratory where the gravity of beer samples is measured in a screening application by refractive index.

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Large Batch Sealed Tube Decomposition of Geochemical Samples by Means of a Layered Heating Block

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John Lovell
Barringer Resources Inc., 1626 Cole Blvd, Golden, Colorado 80401, USA

Decomposition of geochemical samples by an acid mixture in re-usable sealed glass tubes has been shown to give results comparable to standard methods, at a lower cost and with no large-scale evolution of corrosive fumes. Samples are exposed to the acid mixture at a temperature well above its normal boiling point, in a layered block that cools the tops of the tubes to protect the caps. Volatile analytes are retained, and the test solution is suitable for a variety of instrumental methods of analysis.

Keywords: *Sealed tube; decomposition method; geochemistry; heating block*

Applied goechemistry encompasses the use of methods of inorganic elemental analysis that are rapid, sensitive and relatively cheap. The majority of such methods incorporate two stages: (*i*) the preparation from the sample of a test solution containing the analytes; and (*ii*) the instrumental analysis of the test solution. While the instrumental aspect of the analysis has seen marked developments in capability and sophistication during the past three decades, no comparable improvements in sample decomposition have been devised.

Complete solubilisation of rocks, soils and sediments for analysis can be effected by fusion[1] or, for all but the most resistant oxide minerals, by digestion with a hydrofluoric acid mixture.[2] However, a complete dissolution is rarely necessary for the objectives of mineral exploration or environmental studies, and a wide range of partial digestion procedures, some selective for particular minerals, have been adopted.

Probably the method most widely used in routine practice in applied geochemistry involves heating the sample in a borosilicate glass test-tube with a 4 + 1 mixture of nitric acid and perchloric acid, first at 120 °C to expose the sample to near-boiling nitric acid, then at 190 °C to complete the digestion with the fuming perchloric acid.[3] This treatment dissolves organic matter and most secondary oxides, and extracts a high proportion of the total trace element content of most silicate minerals. Large batches (of up to 300 samples) can be treated by this procedure over a 24-h cycle without operator intervention, by using a temperature-programmable aluminium-block bath. When the perchloric acid has completely fumed off, the residue, consisting of metal perchlorates, precipitated silica and resistant silicates, is leached with hydrochloric acid to provide the test solution.

This procedure and its allied variants are undoubtedly efficient, but suffer from the following shortcomings. Analytical-reagent grade perchloric acid is a costly reagent and the method requires special and expensive fume cupboards, to avoid explosive hazards resulting from acid condensates coming into contact with oxidisable construction materials. In addition, the venting of large amounts of acid fume into the atmosphere from laboratory fume cupboards, although currently allowed in many countries, is acknowledged as being undesirable on account of its environmental impact. Lastly, there is a partial or complete loss of some volatile elements, for example, arsenic, mercury, sulphur and selenium, which are often of considerable interest. Some of these problems can be obviated by bomb decompositions; however, the use of cased PTFE bombs is too time consuming and expensive for large-batch analysis. This paper describes equipment for acid decomposition in re-usable sealed glass tubes. The method, termed here pressurised acid leaching, can be used with a variety of acid mixtures to meet specific applications. The reagent used for illustration in this paper is a 4 + 1 mixture of nitric and hydrochloric acids.

Experimental

Equipment

Sealed tubes

Two types of sealed tubes were used, both constructed from thick-walled borosilicate glass, approximately 13 mm i.d., 16 mm o.d. and 160 mm long, with a rounded base and wide neck. One design [Sovirel brand (from V. A. Howe & Co. Ltd., London), Fig. 1(*a*)] had an external screw thread round the neck to take a plastic screw-cap. The other tube, which was designed specifically for this work, had a narrow flange around the top of the neck (as supplied by Wheaton Scientific, distributed in UK by Jencons Scientific Ltd., Leighton Buzzard, Bedfordshire) to hold an aluminium septum cap retainer [Fig. 1(*b*)] attached by a Fermpress crimper. Both tubes were sealed with cap liners designed for this work, consisting of a 3.2 mm thick disc of Esco SR70 silicone rubber faced with a 0.36 mm thick film of chemically resistant Dupont Teflon FEP type A. Both silicone rubber and FEP discs could be washed, dried and re-used a number of times. The plastic caps were re-usable, but the aluminium retainers were destroyed on removal with a Fermpress decapitator.

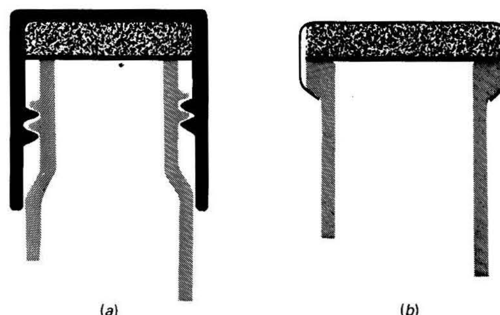

Fig. 1. Closures for (*a*) the Sovirel brand tube and (*b*) the pressurised leach tube, showing the chemically resistant FEP facing disc (black) backed by the silicone-rubber disc (mottled) within retaining caps

Heating block

The layered heating block was constructed from two blocks of aluminium separated by a block of thermal insulation, bolted together to form a unit (Fig. 2). The bottom layer was an aluminium block 100 mm thick. Above this was a block of thermal insulation (Sindanyo) 25 mm thick, and the top layer was a second block of aluminium 25 mm thick. The plan size was 310 × 460 mm, the same size as the commercial laboratory hot-plate on which the heating block stood and which acted as the heating unit. The composite block was drilled vertically to receive 77 pressurised leaching tubes, with a clearance of 2 mm on the diameter. The top layer of the block was also drilled horizontally and plugged in such a way as to provide single continuous bore channels for a continuous flow of cooling water.

In use, a Simmerstat control on the hot-plate provided a temperature stable to within a few degrees over long time periods under steady conditions. Lateral heat loss was reduced by providing a layer of insulation around the bottom part of the block. A dummy sample tube containing silicone oil received a thermometer connected to a Fison's Fi-monitor, and this system acted as a safety cut-out in the event of a temperature excursion beyond the suitable range. The top layer of the block was cooled by a 2 l min^{-1} flow of tap water. Under steady conditions at 175 °C, the vertical temperature gradient in the bottom layer of the block was between 2 and 3 °C over the 100 mm, and the top layer remained at about 20 °C.

Reagents

Leaching acid. Freshly prepared from a mixture of nitric acid (70%, AnalaR) and hydrochloric acid (concentrated, AnalaR), 4 + 1.

Procedure

Weigh the sample (0.250 g of powdered material) into a clean, dry tube. Add the leaching acid (1.00 ml) and, if necessary, set the tube aside to allow any carbon dioxide liberated from carbonate to escape. Seal the tube in the appropriate manner and place it in the block at 175 °C for 1 h, then remove it and allow to cool to ambient temperature. Shake the tube vigorously so that any volatile analyte that has collected in the cool upper part of the tube is redissolved and unseal it. Add water (9.00 ml, purified) and mix thoroughly. Analyse the clear test solution by an appropriate method after the solid residue has either settled out or been separated by centrifugation.

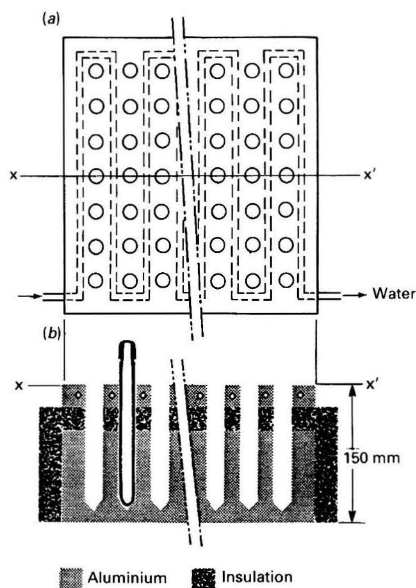

Fig. 2. Layered heating block (*a*) in plan and (*b*) in cross-section

Safety Precautions

The potential hazards of the pressurised leach and their avoidance are discussed in detail below. It is appropriate to emphasise that the procedure must not be undertaken unless the analyst is properly protected at every stage from the results of destructive failure of a pressurised tube.

Results and Discussion

Performance of the Equipment

The critical characteristic of digestion tubes used for pressurised leaching is that, under an internal pressure of perhaps several atmospheres, they do not suffer vapour leakage or destructive failure. Vapour leakage results in loss of acid from the tube. This could bring about a diminished attack on the sample, a loss of liquid volume or loss of volatile analytes. Leakage is not hazardous provided the block is operated in a low-grade fume cupboard.

Of the two types of tubes used, the flanged tubes with the septum caps were found to possess superior sealing characteristics, and rarely suffered leakage. The screw cap Sovirel tubes were more prone to vapour leakage. Insufficient torque in tightening the screw caps led to vapour leakage, whereas excessive torque led to destructive failure of the cap under pressure. The safe margin between these levels was, in some instances, difficult to achieve. Under the conditions specified in this work, the normal PTFE cap liners supplied by Sovirel could not prevent vapour leakage. However, under some other conditions, *i.e.*, at lower temperatures or with less volatile acid mixtures, the Sovirel caps were completely satisfactory.

The bottoms of the tubes were maintained at 175 °C, well above the boiling-points of either nitric or hydrochloric acid, and the top layer of the block remained at about 20 °C. The effect was apparently a smooth refluxing of the acid within the tubes, producing a protracted attack on the samples at a much higher temperature than would normally be possible with the reagent in open test-tubes. The purpose of the cooled upper layer on the block was to prevent heating and consequent softening of the cap seals, a factor that tended to cause vapour leakage.

The layered block maintained a constant temperature over long periods, so long as the flow of coolant water was above a critical minimum. After protracted use, however, an accumulation of scale, deposited from the cooling water, partly occluded the channels and reduced the water flow. This gave rise to a temperature excursion in the bottom of the block. Thus either regular de-scaling of the channels is needed, or soft or recirculated water should be used for cooling.

Safety

Fault conditions in the block

Destructive failure (*i.e.*, explosion) of a sealed tube containing a hot acid mixture under pressure is a hazard that must be evaluated and guarded against. Likewise, operations with pressurised tubes must be managed in a way such that explosions do not result in personal injury. Under the conditions described above, no destructive failure has been

observed. Failure could ensue, however, if either layer of the block were to overheat and cause excessive pressure in the tubes, or if defective tubes were used.

Overheating of the lower layer of the block could occur if the hot-plate controls were set incorrectly or the supply of coolant water to the top layer failed. In a study of the first problem, surplus power was supplied to the hot-plate and the normal water supply was maintained. As a result, the bottom layer of the block reached a steady maximum temperature of 235 °C, while the upper layer rose to 30 °C. Some previously secure screw caps began to leak and some septum caps began to balloon. However, there were no explosions.

Loss of coolant, however, led to a more hazardous situation. During a trial of this condition, initiated from the normal working conditions (*i.e.*, 175 °C in the lower layer), the temperature of both layers of the block rapidly increased to 250 °C, at which point destructive failure of the tubes was almost invariable.

In practice, both of these conditions would be averted by the independent cut-out device, inserted into the lower block, which permanently disconnected the power if the block temperature rose more than 10 °C above the required working temperature. Loss of coolant flow was quickly followed by automatic disconnection of power to the hot-plate and this prevented a large temperature excursion. The top layer of the block reached a maximum temperature of 80 °C about 1 h after loss of coolant and power, by which time the temperature in the bottom layer had drifted downwards to 140 °C (Fig. 3). This condition caused no problem to the tubes or seals. As a further safeguard in practice, the water flow was visually monitored by means of a paddle wheel flow indicator.

Personal safety

Additional precautions were essential to guard against injury from unforeseen destructive failure of the tubes. In use, the loaded block was completely contained, but visible from the front through a 6-mm screen of transparent Perspex. Loading and removal of tubes was accomplished with the analyst protected by this screen, manipulating the tubes by means of a 60-cm steel rod with a spring clip at the end. Only the analyst's hand, protected by a stout leather gauntlet, was ever inside the enclosure. These precautions were considered mandatory for these operations. A completely foolproof system would be required for routine use. It is not known whether the tubes become prone to bursting through "ageing" after prolonged use. Account of this possibility should be taken when the method is used.

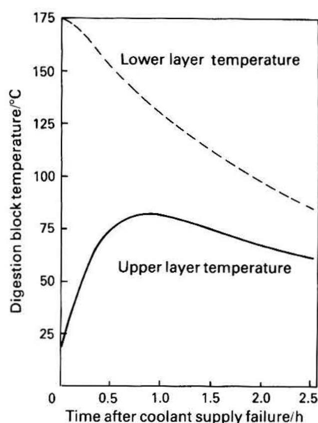

Fig. 3. Effect of loss of coolant with automatic power cut-off on the temperatures of the upper and lower layers of the block

Effectiveness of Decomposition

Choice of reagent and conditions

The reagent used to illustrate the use of sealed tubes was designed for general purposes in applied geochemistry to replace the 4 + 1 nitric and perchloric acid mixture. Choice of reagents was limited to acids that: do not significantly attack borosilicate glass; do not render any important elements insoluble by virtue of their anions; and do not cause interference effects in subsequent determinations. The obvious possibilities were, therefore, nitric acid, hydrochloric acid and perchloric acid. Perchloric acid was eliminated because of the possibility of explosion when it is used undiluted and because it has no special virtues in the presence of diluents. Trial digestions carried out on soil samples, by means of nitric acid, hydrochloric acid and various mixtures of the two, showed the respective 4 + 1 mixture to be the most vigorous. This is attributed to the combined oxidising power of nitric acid and the complexing power of the chloride ion.

The optimum duration for exposure of the samples to this mixture at 175 °C was established by treating a variety of soil and sediment samples for periods ranging from 15 min to 16 h. The resulting test solutions were analysed for copper, lead, zinc, cadmium, iron and manganese by atomic-absorption spectrophotometry. In almost all instances a 15-min leach extracted as much metal as more protracted leaches. Consequently, the period of 1 h that is recommended is more than sufficient.

Effectiveness of the Extraction

The effectiveness of the pressurised leaching was judged largely by comparison with that of standard methods of decomposition. Comparative methods used included the previously described nitric and perchloric acid method and an attack involving a hydrofluoric acid - nitric acid - perchloric acid mixture that was used to determine the "total" content of some elements. Several suites of soil samples and a number of individual silicate and sulphide minerals were used as test materials. Test solutions were analysed by atomic-absorption spectrophotometry (employing conventional nebulisation into a flame, the cold-vapour technique for mercury and hydride generation) and by inductively coupled plasma atomic-emission spectrometry.

As judged by the initial results, the performance of the pressurised leach method was satisfactorily close to that achieved by the nitric and perchloric acid method for the elements Cu, Pb, Zn, Mn, Fe, Co and Ni, both on soil samples and the individual minerals. Mercury results compared closely with those obtained by the standard method for mercury.[4] Sulphide minerals, when present as a minor constituent of the sample (*i.e.*, up to 10% *m/m*) were completely dissolved, and good results for sulphur and arsenic were obtained on the solutions. Whether other elements that form volatile hydrides (*e.g.*, Se, Te, Sb and Bi) can be conveniently and accurately determined after pressurised leaching with this reagent is currently under investigation. A comprehensive survey of the efficacy of this reagent is in the course of preparation and will be published in due course.

Conclusions

The combination of re-usable sealed tubes and a layered heating block has been demonstrated for the decomposition of soil and sediment samples for applied geochemistry. Results have been obtained that compare closely to those of standard methods, and the range of elements covered has been extended to include some normally regarded as volatile and liable to loss in open tube attacks.

ห้องสมุดกรมวิทยาศาสตร์บริการ

Both capital and recurrent costs for pressurised leaching are considered to be less than half of those for conventional methods of decomposition. Expensive perchloric acid is not used and a high-grade fume cupboard is not required. A further saving stems from the avoidance of separate decomposition procedures for various analytes.

While the value of the method is evident, the design of the block, which should be considered essentially as a prototype, needs improvement. The principal shortcomings at present are the inconvenience of the safety precautions required and the understandable reluctance of analysts to use pressurised glassware. This would be overcome by incorporating devices that enable the tubes to be inserted or withdrawn as a batch by remote handling, with interlocks preventing any access to the tubes until they were cooled to ambient temperatures. Other refinements could be incorporated into a more sophisticated device and would make it suitable for rapid routine decomposition of large batches of samples.

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Paper A4/242
Received July 19th, 1984
Accepted November 15th, 1984

Rapid Determination of Molybdenum in Soils, Sediments and Rocks by Solvent Extraction with Inductively Coupled Plasma Atomic-emission Spectrometry

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This paper describes the development of a high-throughput method for the determination of molybdenum in soils, sediments and rocks, providing a detection limit lower than can be readily obtained by other methods. The molybdenum is solubilised by treatment of the sample with 6 M hydrochloric acid in capped tubes at 120 °C. It is then extracted from the same medium into heptan-2-one, in which it is determined directly by inductively coupled plasma atomic-emission spectrometry (ICP-AES). Extraction of iron, which can otherwise interfere with the determination, is minimised by the presence of a reducing agent. A number of general problems relating to solvent extraction of metals for ICP-AES are also studied.

Keywords: *Molybdenum determination; inductively coupled plasma atomic-emission spectrometry; solvent extraction; pre-concentration*

Molybdenum occurs to the extent of about 1 µg g^{-1} in igneous rocks and to about the same extent in most sedimentary rocks and soils, although it may be present at considerably higher levels in marine black shales and in mineralised zones, especially around porphyry copper deposits. In igneous rocks the element is found mostly as molybdenite, MoS_2. In residual soils it is completely oxidised and largely associated as the MoO_4^{2-} ion with iron(III) oxide minerals, although it may also be associated with organic matter. In marine black shales the molybdenum is correlated with organic matter, but no organic complexes of the metal have been demonstrated, and the metal probably occurs in sulphide phases, especially pyrite.[1]

Molybdenum is an important element with respect to its biological response. An excess of molybdenum in forage is toxic to livestock.[2] Sub-clinical effects are often observed when soil Mo levels exceed 3 µg g^{-1}. It is also an essential element, being a constituent of several mammalian enzyme systems and in nitrogen-fixing bacteria. Deficiency diseases in livestock have been recorded when soil molybdenum levels were below 0.5 µg g^{-1}.[3]

For these reasons, the capability for determining molybdenum in geological materials quickly and accurately at low concentrations is important in geochemical and agricultural laboratories. The analytical method normally employed is spectrophotometry, making use either of the toluenedithiol complex or of the thiocyanate complex of molybdenum. The practical detection limit, which can be obtained by either of these methods, is about 1 µg g^{-1} unless time-consuming refinements are incorporated into the methods. Atomic-absorption spectrophotometry with nebulisation into a flame provides a detection limit of no better than about 10 µg g^{-1} for molybdenum, while direct spectrography gives about 3 µg g^{-1}.

Inductively coupled plasma atomic-emission spectrometry (ICP-AES) provides several lines of high sensitivity for molybdenum with detection limits of about 0.01 µg ml^{-1} in pure aqueous solutions. These lines are affected by line overlaps, mostly from iron and aluminium, which degrade the detection limits obtainable. The line used in this study (Mo 281.62 nm) has an instrumental detection limit in aqueous solutions equivalent to 0.6 µg g^{-1} in solid samples. However, spectral interferences from various elements normally present in solutions derived from geological materials (Mg, Ca, Fe and especially Al) adversely affect the practical limit in most sample types, raising it to 5–10 µg g^{-1}.[4]

The method described in this paper employs solvent extraction to separate molybdenum almost completely from spectral interferents and to perform a pre-concentration before the ICP-AES determination. Practical detection limits obtained were better than 0.1 µg g^{-1} and a stable drift-free operation was achieved.

Experimental

Equipment

Layered heating block. This was used as previously described,[5] and consisted of a lower heated section and a thermally insulated upper layer, which was cooled by a flow of water. The upper layer was required to prevent the tube caps being heated.

Capped tubes. These were of Sovirel brand, thick walled 160 × 16 mm, with screw caps lined with the proprietary cap linings faced with PTFE.

ICP-AES. An ARL 34000 ICP instrument equipped with lines for 36 elements including Mo (281.62 nm) and Fe (259.94 nm), both in the second order was used. Heptan-2-one solutions were introduced into the plasma by means of a Meinhard (Type TR-30-A3) nebuliser modified by a platinum-iridium capillary sealed into the rear end with Araldite epoxy resin.[6] The uptake rate to the nebuliser was restricted to 0.3 ml min^{-1} by means of a PTFE tube, 310 mm long and 0.31 mm i.d. No pump was used. The nebuliser discharged into a small (*ca.* 100 cm^3) Scott-type double-pass spray chamber connected directly to the plasma torch. A stabilisation time of 20 s was followed by three 5-s integration periods, the output being the arithmetic mean of these three readings. The flow-rates of argon gas to the plasma torch were as follows: coolant gas, 12 l min^{-1}; injector gas (humidified by means of a bubbler system) 1 l min^{-1}; and auxiliary gas, 0.5 l min^{-1}. Viewing height was 14 mm above the load coil and forward power was set at 1.70 kW.

Reagents

Hydrochloric acid, 6 M. AnalaR grade.

Heptan-2-one. Spectrosol grade.

Ammonium iodide solution, 10% *m/V*. Prepared from

AnalaR grade reagent in 6 M hydrochloric acid.
L-*Ascorbic acid.* AnalaR grade.
Bromine. AnalaR grade.
Phenol.
Ammonium molybdate standard solution, 1000 μg ml^{-1} *Mo.*

Safety Precautions

Although no instance of a tube bursting has occurred in the operation of this procedure, the possibility must be guarded against. The analyst must be properly protected against injury from flying glass and fumes at all times while the tubes are pressurised, and fail-safe devices must be used to prevent the temperature of the block exceeding the desired level.

Procedure

Weigh each sample (0.250 g of dry powder) into a clean, dry tube. Add 6 M hydrochloric acid (5.00 ml) from a dispenser. (For samples containing carbonate minerals, the acid should be added cautiously in small portions, and the tubes set aside until evolution of gas has ceased.) For rock samples or others suspected of containing a small proportion of sulphide minerals, add two drops of bromine. Cap the tubes firmly and place them in the block at 120 °C. After the decomposition period (2 h for soils, 3.5 h for rocks) remove the tubes from the block and, when they are cool, break the seal momentarily to equalise the pressure. Shake the tubes vigorously to mix the contents. If bromine has been used, add 2 drops of phenol solution to precipitate the bromine as tribromophenol. Centrifuge the tubes and transfer 4.00 ml of the centrifugate into a test-tube equipped with a clear silicone-rubber stopper. Add concentrated hydrochloric acid (0.50 ml), ammonium iodide solution (0.5 ml) and ascorbic acid (0.2 g from a graduated scoop) to each tube, stopper the tubes and shake them until the ascorbic acid has almost dissolved. Set the tubes aside for 2 h or more. Add to each tube heptan-2-one (3.00 ml, by dispenser) and shake well for 6 min on a mechanical shaker. Centrifuge the tubes briefly (or allow them to stand for 20 min) to separate the phases, and remove some of the top layer for nebulisation into the ICP-AES.

Calibration

Calibration solutions were prepared by following the solvent extraction procedure on tubes containing 4.00 ml of pure 6 M hydrochloric acid and on similar tubes to which had also been added 10 μg of Mo as a 0.1-ml spike of ammonium molybdate solution. The calibration was linear for all ranges likely to be encountered in practical analysis of soils, sediments and rocks. The normal computer correction was made for the spectral interference caused by the small concentration of iron also extracted.

Results and Discussion

Solvent Extraction of Molybdenum from Hydrochloric Acid Solutions

A large number of systems have been described for the solvent extraction of molybdenum for determination by atomic-absorption spectrophotometry.[7] Of these methods, the extraction of Mo(VI) from strong solutions of hydrochloric acid into 4-methylpentan-2-one[8] seemed most attractive for use with ICP-AES, as it avoids complications resulting from the use of organic reagents, and is easy to combine with the decomposition of samples by hydrochloric acid.[9] In this study, heptan-2-one was substituted for 4-methylpentan-2-one. The higher ketone has a smaller mutual solubility with water, and volume variations are therefore minimised. In addition, being less volatile, less of this solvent is carried to the plasma torch in the

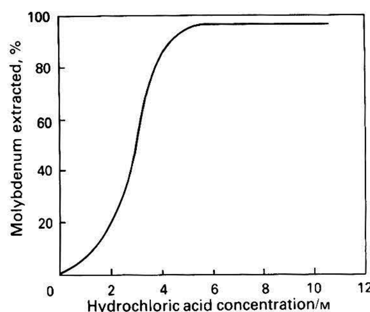


Fig. 1. Effect of hydrochloric acid molarity on the extraction of Mo(VI) from 5 ml of aqeuous phase into 3 ml of heptan-2-one

gas phase after passage through the nebuliser. This in turn requires less power to be coupled into the plasma to achieve the same degree of atomisation and excitation of the analyte. Investigation of a higher ketone was of interest also in the wider context of multi-element solvent extraction, as 4-methylpentan-2-one is commonly used for this purpose in combination with atomic-absorption work.

As no data were available for heptan-2-one, it was necessary to study the extraction of the system for Mo(VI) as a function of hydrochloric acid concentration. This was carried out by extracting 10 μg of Mo(VI) from hydrochloric acid solutions of various concentrations, but otherwise under the conditions described for calibration in the experimental section. After separation, both phases were analysed for molybdenum by ICP-AES. The results are shown in Fig. 1. It can be seen that the extraction is essentially complete at acid concentrations above 6 M. As constant-boiling hydrochloric acid (the least volatile mixture of HCl and H_2O) has a composition of about 6.1 M and a boiling-point of 108.6 °C at 1 atm pressure a concentration of 6 M was selected for use in the method. This mixture would give the least pressure in sealed tubes at a given temperature and the resulting solution could be used directly for the extraction.

Selection of ICP-AES Operating Conditions

While it was important to select ICP-AES operating conditions that gave good signal to background and signal to noise characteristics for the determination of molybdenum in the extracts, other factors were also important. One such factor was that sensitivity drift should be acceptably low. Another important criterion was that in a busy analytical laboratory the system for nebulising organic liquids should be rapidly interchangeable with that for aqueous solutions. For this reason the observation height, the injector gas flow-rate, the coolant gas flow-rate and the tuning of the impedance matching network were kept at the same levels as used for aqueous solutions. The remaining parameters (*viz.*, forward power, auxiliary gas flow-rate and solution uptake rate) were allowed to vary, within limits set to suit other practical requirements. No multivariate optimisation was attempted, but after several iterations of univariate trials, satisfactory working conditions were obtained.

Forward power

With the other parameters set as given above, forward power was varied in the range 1.2–1.85 kW. At each power the sensitivity, signal to background, background signal and background noise were recorded. The results obtained are shown in Fig. 2.

Net sensitivity increased steadily with power, as did background intensity. However, the signal to background

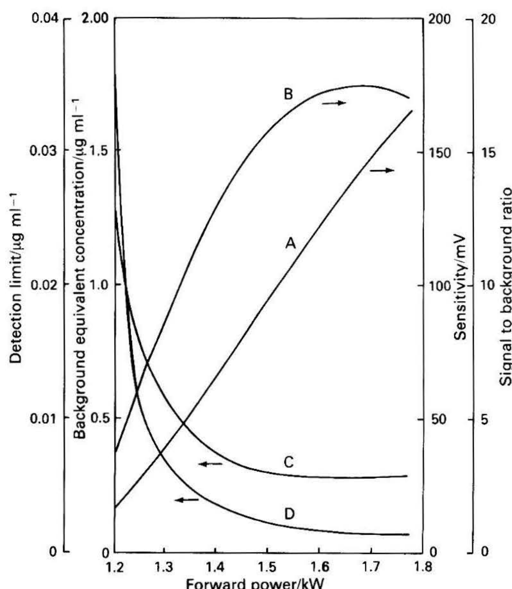
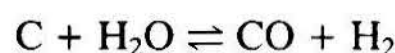


Fig. 2. Effect of varying the forward power on figures of merit for determining molybdenum in heptan-2-one extracts, showing: A, net sensitivity; B, signal to background ratio; C, background equivalent concentration; and D, detection limit

ratio levelled off (or possibly maximised) at 1.65–1.7 kW, and neither instrumental detection limit nor background-equivalent concentration showed any improvement at powers greater than about 1.70 kW. On this basis, a power of 1.70 kW was selected for routine use.

Prevention of soot formation on the plasma torch

Initial trials with the nebulisation of heptan-2-one resulted in the formation of heavy deposits of carbon on the plasma torch, which gave rise to rapid changes in sensitivity. This deposition was completely obviated over long time intervals by the simultaneous application of two preventive measures. Firstly, an auxiliary gas flow-rate (*i.e.*, through the intermediate tube of the plasma torch) of 0.5 l min^{-1} was employed. The main effect of this was to lift the plasma a few millimetres with respect to the tip of the injector. As a second measure (the first was not completely effective) the injector gas was saturated with water by means of the bubbler system used for aqueous solutions. This further inhibited sooting-up, as at temperatures higher than about 1000 K, the Gibb's free energy change for the reaction



$$C + H_2O \rightleftharpoons CO + H_2$$

favours the formation of carbon monoxide. This combined strategy, together with the limited uptake of solution, completely eliminated carbon deposition and sensitivity was stable to within 5% relative over 6-h periods.

Use of the bubbler to humidify the injector gas had an unexpected but helpful side-effect. In comparison with the use of dry argon, but with all other conditions unchanged, the sensitivity for molybdenum was increased by a factor of 1.6, signal to noise was improved by a factor of greater than 2.0 and blank noise was unaffected.

Uptake rate of the test solution

The use of a peristaltic pump to control the uptake rate was found to be impossible with heptan-2-one. No pump tubing was identified that could withstand the effects of this aggressive solvent for more than short periods without serious deterioration in performance. Because of this, liquid uptake was controlled by varying the length and i.d. of the flexible capillary tube. This arrangement had previously been found to be perfectly satisfactory with aqueous solutions, giving a signal stability comparable or even superior to the best peristaltic pumps.[10] With heptan-2-one, however, sensitivity drifting was observed when polyethylene uptake capillary was used. This effect may have been due to a swelling of the polyethylene in contact with the solvent. The effect could be obviated by the use of PTFE uptake capillary.

It was found that sensitivity could be substantially improved, with no corresponding increase in noise, if the uptake of test solution was restricted to levels well below normal uptake rates. These effects have been reported previously for aqueous solutions,[10] but the effects are relatively much larger with the heptanone system and constitute an important feature of the method. The uptake rates were measured accurately by continuous gravimetry, by placing the vessel containing the test solution on a top-pan balance. By replacing tubing of diameter 0.58 mm by the same length of 0.31 mm tubing, the uptake rate was reduced from 1.2 to 0.35 ml min^{-1} and the net signal intensity on the molybdenum channel was increased by a factor of 1.6. All other conditions were as specified in the experimental section above.

Extraction of Concomitant Ions

As the rationale of solvent extraction is largely to effect a separation of the analyte from interferent elements, it was necessary to check that this was in fact achieved so that the analyte could be determined without bias. Accordingly, the extraction of molybdenum from aqueous 6 M hydrochloric acid containing various ions was compared with the extraction of pure molybdenum solutions. The following major and minor ions were found to cause no measurable effect on the extraction of molybdenum: 2% Na^+ (as NaCl); 2% K^+ (as KCl); 2% Ca^{2+} (as $CaCl_2$); 2% Mg^{2+} (as $MgCl_2$); 1% Al^{3+} [as $Al(NO_3)_3$]; 2000 µg ml^{-1} Mn^{2+}; 400 µg ml^{-1} Ti; and 4000 µg ml^{-1} PO_4^{3-}. In addition, the absence of these elements in the heptan-2-one extract was confirmed by direct ICP-AES analysis. Iron (Fe^{3+}) was extracted to a marked extent, and is considered separately below. Other elements usually present at trace levels (*i.e.*, Cr, V, Sn, W, Cu and Zn) were found to cause no effect at concentrations well above normal, and their presence was not detected in heptan-2-one extracts, except for low concentrations of tin.

The selective and virtually complete exclusion of the matrix elements (other than iron) is an important feature of the method.

Effect of the Extraction of Iron

Unless it is masked, iron is strongly extracted into the heptan-2-one as the ketonium tetrachloroferrate(III), and was therefore a potential interferent. The extraction of Fe^{3+} from 6 M hydrochloric acid into the ketone is shown in Fig. 3. Two types of interference were observed, a spectral overlap and a matrix effect that depressed the molybdenum signal. No decrease in the extraction efficiency of molybdenum was observed.

For the line overlap the selectivity ratio in heptan-2-one is 3.5×10^{-5}, comparable to that found for aqueous solution (3.0×10^{-5}). As concentrations of iron in the aqueous phase after the dissolution of a typical sample could sometimes exceed 5000 µg ml^{-1} the correction term in the organic phase could be equivalent to as much as 0.17 µg ml^{-1} of molybdenum. The uncertainty in this term (*ca.* 0.02 µg ml^{-1}) would substantially degrade the instrumental detection limit obtained for pure solutions of molybdenum (*ca.* 0.002

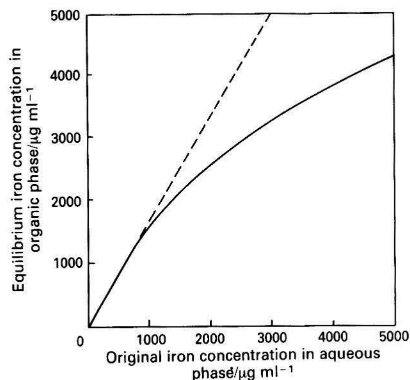


Fig. 3. Extraction of Fe^{3+} from 5 ml of 6 M hydrochloric acid into 3 ml of heptan-2-one. The broken line shows the theoretical concentration in the ketone for 100% iron extraction and no volume change in the phases

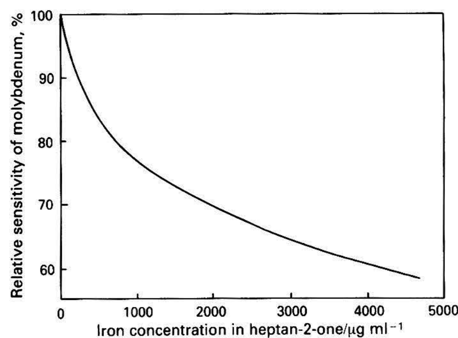


Fig. 4. Suppression of the molybdenum signal caused by the co-extraction of iron into the organic phase. The molybdenum data have been corrected for the enhancement owing to the spectral interference

µg ml^{-1}). It was therefore important to prevent the extraction of iron by masking, to the largest possible degree.

While the translational interference effect of the line overlap is more important at low levels of the analyte, iron causes a substantial rotational interference effect, *i.e.*, a factorial bias at all levels of molybdenum. Fig. 4 illustrates the suppression of the molybdenum signal when iron is present in the organic phase at levels easily attainable during the analysis of geological samples. Effects of this magnitude cannot be tolerated as they cannot be readily corrected, and it is clearly of paramount importance to minimise the extraction of iron.

Masking agents that are the anions of weak acids are ineffective in strong acid solutions because of the formation of the protonated form of the reagent, which competes with the formation of a metal complex. Thus even diaminoethanetetra-acetic acid and related reagents were unable to prevent the extraction of iron into the ketone. Anions of stronger acids (*e.g.*, phosphoric acid) should be more successful, but again did not have any significant success in sequestering the iron. This was no doubt due to the formation of the complex $FeCl_4^-$, with a high stability constant reinforced by its high partition coefficient into the organic phase.

Attempts were made, therefore, to reduce the iron to iron(II), which is not extractable as a chloride complex. However, again because of the stability of $FeCl_4^-$ and its strong extraction into the ketone, many reducing agents, which normally bring about the reduction of Fe(III) to Fe(II) were largely or completely ineffective. Reagents in this class included sulphur dioxide, hydroxylammonium chloride, hydrazinium chloride, potassium iodide and hypophosphorous acid. Ascorbic acid gave a partial reduction of the iron, but the reaction was inconveniently slow. Tin(II) chloride gave a rapid reaction but was less complete than the ascorbic acid.The iodide ion was found to have a catalytic effect on the reducing power of ascorbic acid, however, and this combination proved to be the best system of those investigated. Ammonium iodide was used rather than the potassium salt, to avoid the precipitation of potassium chloride from the 6 M hydrochloric acid solution. The reduction reaction was virtually complete in a 2-h period. The effect of reducing systems on the extraction of iron is shown in Fig. 5.

Table 1. Correction terms for iron in aqueous solution

Reducing system	Fe in organic phase/ µg ml⁻¹	Correction term (≡ µg ml⁻¹ of Mo)
None	4300	0.150
Ascorbic acid (5 h)	283	0.01
Ascorbic acid + I^- (2 h) . .	179	0.006

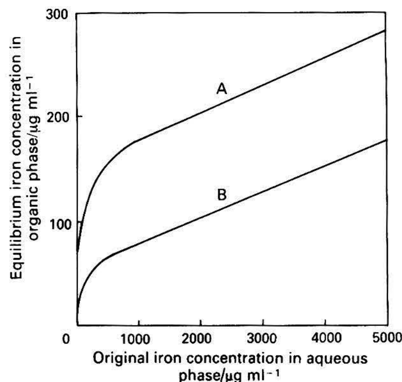


Fig. 5. Efficiency of A, ascorbic acid alone and B, ascorbic acid with ammonium iodide, in reducing the extraction of iron into heptan-2-one (3 ml) from 6 M hydrochloric acid (5 ml)

The suitability of these measures for preventing translational interference can now be assessed. For the given experimental conditions, the translational interference correction terms for iron initially present at an exemplary level of 5000 µg ml^{-1} in the aqueous solution (equivalent to 10% *m/m* of Fe extracted from the solid sample) are shown in Table 1.

It is clear that the uncertainties in the correction term, when the ascorbic acid plus potassium iodide reducing system is employed, would be of the order of 0.0006 µg ml^{-1} (*i.e.*, about 10% of the term), which would not adversely affect the detection limit of about 0.002 µg ml^{-1}, or cause perceptible bias at higher levels of molybdenum.

For rotational interference, the exclusion of iron is more critical, as the rate of change of signal suppression is greatest at low levels of iron (Fig. 4). Even the relatively low levels of iron extracted from the reducing system can cause a 5–10% attenuation of the molybdenum signal. While the problem is not regarded as solved, it is felt that an interference of that order of magnitude is tolerable in low trace analysis.

Effect of Nitrate and Perchlorate Ions

The initial conception of the method for molybdenum was to combine the solvent extraction with a standard method of decomposition involving evaporation to dryness with hydro-

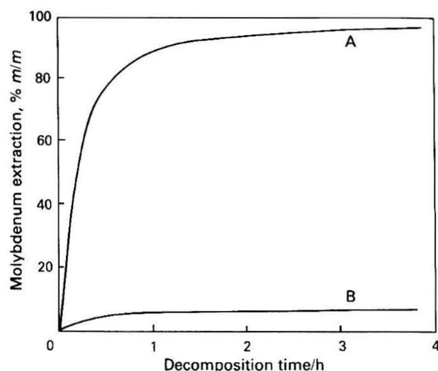


Fig. 6. Decomposition of a sample containing 50 µg g^{-1} of molybdenum as MoS_2 using A, 6 M hydrochloric acid with added bromine and B, 6 M hydrochloric acid alone, at 120 °C for various periods of time

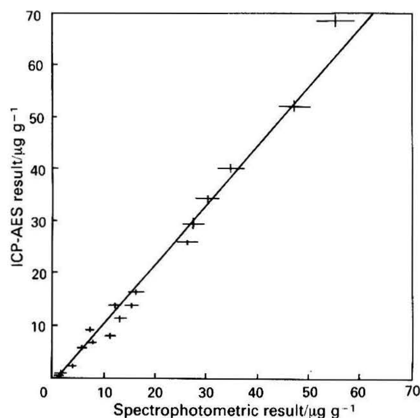


Fig. 7. Results obtained by the analysis of a set of soil samples for molybdenum by the proposed method and by a standard spectrophotometric method. Error bars show ±1 standard error on the results

fluoric, nitric and perchloric acids. The residue, consisting of metal perchlorates, is dissolved in hydrochloric acid for direct determination by ICP-AES of a suite of about 25 elements. The bulk of the solution would, however, be used for the solvent extraction and molybdenum determination.

This projection could not be put into effect, however, because of an unexpected interference problem. It was found that perchloric acid was being extracted from the aqueous solution into the ketone. This raised the viscosity of the extract to such an extent that the uptake rate was reduced to about half of its value in the absence of perchlorate. This produced a comparable suppression in the molybdenum signal. In addition, it was unacceptable practice to allow a solution of perchloric acid in an organic liquid to form because of the possible explosion risk. For this reason, the standard decomposition was abandoned in favour of the specially devised method given here. Nitric acid was found also to be extracted into the ketone to produce a similar, although smaller, decrease in uptake and sensitivity.

Optimum Conditions for Decomposition

The optimum conditions for the decomposition of samples by the pressured leach with 6 M hydrochloric acid was studied by subjecting different kinds of sample to the attack at 120 °C for periods of time ranging from 15 min to 4 h. Molybdenum was then determined by the proposed method.

Soil samples gave maximum extractions of molybdenum after periods of not greater than 1 h and reached 95% of this value after only 15 min. The addition of bromine gave no significant increase in the concentration of molybdenum extracted.

Further trials were carried out on a synthetic mixture of molybdenum disulphide and a silica sand, containing 50 µg g^{-1} of Mo. In this instance extraction of molybdenum was very low, even after 3 h of treatment. The addition of bromine, however, greatly improved the extraction of the analyte, which approached completion after 3 h of treatment (Fig. 6). Treatment of a marine black shale sample under the same conditions gave similar results, although in this instance the true Mo concentration was not known.

Validation of Method

Comparison with standard method

A set of soil samples containing a wide range of molybdenum concentrations was analysed by the proposed ICP-AES method (without the addition of bromine) and by a commonly used method based on the solvent extraction and atomic-absorption spectrophotometry of the toluenedithiol complex. Results plotted with realistic errors in Fig. 7 show a distinct rotational bias towards higher results by the proposed method, which is also clearly more precise.

A statistical analysis of the data by weighted regression confirmed the tendency of the atomic-absorption spectrophotometric method to give a significantly low rotational bias, mainly affecting higher results, and a small translational bias at low levels of the analyte. The biases estimated by the regression were as follows: $Mo_{Spec} = (1.0 \pm 0.3) + (0.87 \pm 0.03)Mo_{ICP\text{-}AES}$ with the standard errors given 17 degrees of freedom. The translational bias may be due to a haze in the solvent extract used for atomic-absorption spectrophotometry, the rotational bias to incomplete extraction of molybdenum.

Analysis of reference materials

In view of the uncertainties in the atomic-absorption spectrophotometric method used for comparison, further attempts were made to validate the proposed method by the analysis of reference materials that had been widely distributed among appropriate laboratories. Data obtained are shown in Table 1. Interpretation of the data was not simple, however, because (*i*) there are no certified results available for low levels of molybdenum and consensus values obtained from compendia have large margins of error; (*ii*) there are few reference materials containing high levels of molybdenum; (*iii*) there is a negligible amount of information provided on molybdenum speciation in the available materials; and (*iv*) until recently few workers had reported reliable determinations of low levels of molybdenum, *i.e.*, with standard deviations better than 0.1 µg g^{-1}. Especially difficult in the current context is the lack of soil reference materials with well established levels of molybdenum.

The data in Table 2 show that, for samples analysed both with and without the addition of bromine, the higher results were obtainable with the bromine treatment if there was any significant difference between the two methods. As most of the materials were igneous rocks, this finding is consistent with the molybdenum being present in trace minerals such as sulphides, which are decomposed by bromine, rather than in the lattices of acid-resistant silicates. Where high-quality analyses by other workers are available, the values agree reasonably well with results obtained by the bromine-added method. As these other workers had used methods employing complete decomposition of the sample before the determination, the inference is that the bromine - hydrochloric acid

Table 2. Comparison of molybdenum results obtained by the proposed method and those reported by other workers, for a number of soil and rock reference materials. All determinations carried out are included in the table

Sample	Results obtained by proposed method/μg g^{-1}		Results reported by other workers in references 11–15†/μg g^{-1}				
	With bromine	Without bromine	11	12	13	14	15
SO-1*	—	0.42, 0.44, 0.40	—	2 ± 1	—	—	—
SO-2*	—	1.54, 1.56, 1.48	—	2 ± 1	—	—	2?
SO-3*	—	0.74, 0.72, 0.67	—	2 ± 1	—	—	—
SO-4*	—	0.53, 0.52, 0.53	—	1 ± 1	—	—	1?
SY-2	0.57	0.26	0.53	—	—	—	—
GXR1	22.0, 21.3	19.0, 18.6, 19.7	20.1	—	—	—	—
GXR2*	1.00, 1,02	0.95, 0.95, 1.14	1.30	—	—	—	—
GXR3	5.8, 5.8	6.2, 5.9, 5.9	6.8	—	—	—	—
GXR4	437, 410	299, 334, 337	340	—	—	—	310
GXR5*	40.7, 39.6	39.6, 38.5, 38.5	32.3	—	—	—	30
GXR6*	1.93, 2.02	1.79, 2.42, 2.38	2.27	—	—	—	—
BCR-1	1.36	1.38	1.38	—	—	1.51	1.5
DTS-1	0.00	0.00	—	—	—	0.05	—
PCC-1	0.00	0.00	—	—	—	0.04	—
NIM-D	0.69	0.69	0.77	—	—	—	—
NIM-L	1.20, 1.40	—	1.21	—	—	—	—
NIM-N	0.93, 0.93	—	0.89	—	—	—	—
GS-N	1.29	0.74	—	—	1.21	—	—
DR-N	0.53, 0.55	—	—	—	0.84	—	—
UB-N	0.25, 0.18	—	—	—	0.37	—	—
GH	1.36	0.21	1.44	—	1.93	—	—
GA	0.43	—	0.47	—	0.50	—	—
BR	2.19, 1.90	—	2.12	—	2.21	—	—
AN-G	0.15, 0.10	—	—	—	0.19	—	—
BE-N	2.28, 2.25	—	—	—	2.51	—	—
ST-1A	0.80, 0.79	—	—	—	—	—	1.8
SGD-1A	1.05, 1.05	—	—	—	—	—	1.5
SG-1A	0.45, 0.45	—	—	—	—	—	1.3

* Soil samples.
† Question marks indicate uncertain results.

attack extracts nearly all of the molybdenum from igneous rocks, despite the incomplete attack on the silicates.

Accuracy

Although not in themselves conclusive (because of the paucity of reference data), the foregoing data show that the proposed method with the addition of bromine should give accurate results for low levels of molybdenum in most rocks, unless the molybdenum is partly encompassed in the lattice of a resistant silicate. For residual soils, where the molybdenum is present as readily soluble molybdates of iron, calcium, etc., the hydrochloric acid treatment alone should prove sufficient. In areas where soils may contain non-residual material (*e.g.*, mill tailings or other contamination) the addition of bromine is advisable to secure the dissolution of any sulphide minerals that may also be present. The use of bromine when black shale materials are being examined is also beneficial.

Although the efficiency of the decomposition is imperfectly characterised, there is no doubt about the efficiency of the solvent extraction system. Spikes of molybdenum (as ammonium molybdate) in appropriate amounts, when added to typical soil samples both before and after the digestion, were extracted with efficiencies exceeding 97%. Thus the system for solvent extraction and ICP-AES determination could be coupled with decomposition procedures other than the method given here, such as the "available metal" procedures favoured for environmental and agricultural studies, so long as the interfering nitrate and perchlorate anions were absent.

Precision and detection limit

An informal estimate of the precision that was produced by the proposed method can be culled from Table 2 where replicate determinations on a number of reference materials have been recorded. Most of the replication was executed in separate batches.

More formally, triplicate analyses of a series of 20 soil samples shows that the standard deviation (s) can be expressed approximately at a concentration c as $s = 0.03 \pm 0.03c$ within the concentration range 0–100 μg g^{-1}. This result is confirmed by two further replicate analyses of soil samples, giving, respectively: $m = 0.32$, $s = 0.02$ ($n = 11$); and $m = 33.7$, $s = 0.83$ ($n = 7$). All of these results were obtained by performing the complete procedure on separate sub-samples, with no exclusion of outlying data.

Thus the procedure seems capable of providing, under practical conditions, a detection limit of about 0.06 μg g^{-1} of molybdenum, and a coefficient of variation of 3% at concentrations well above the detection limit. The coefficient of variation of 3% is satisfactory for low trace analyses, and its magnitude seems to be due mainly to the uncontrolled final volume of the extract rather than variations in ICP-AES sensitivity. An internal standard method of correcting for the final volume could be envisaged, by the addition of an element that occurs at low concentrations in geochemical samples and is quantitatively extracted by the ketone from 6 M hydrochloric acid. Elements that could fulfil this role include gallium, germanium and gold. No such attempt was made in this study, because the spectrophotometer used was not equipped with lines for these elements.

Instrumental detection limit

Detection limits, quantified as twice the standard deviation of 11 contiguous measurements of blank intensity converted into concentration units (instrumental detection limit), were measured at intervals over several months and consistently fell within the range 0.001–0.0025 μg ml^{-1}. This compares with the value of 0.004 μg ml^{-1} obtained (under different operating

conditions) at the same line for aqueous solutions. If the instrumental detection limit is multiplied by 15, the dilution factor for the sample dissolution and extraction procedure, the result, a hypothetical best possible detection limit for the solid samples, would be in the range 0.02–0.03 $\mu g\ g^{-1}$. The estimated practical detection limit (*ca.* 0.06 $\mu g\ g^{-1}$) is about double this value, showing the extra variance introduced by sample manipulations, instrumental drift and various correction terms.

Conclusions

A procedure has been devised that provides a satisfactory method for the determination of molybdenum in soils and rocks down to low detection limits. In addition, the general problems of solvent extraction for ICP-AES determinations have been studied. ICP-AES operating conditions that give rise to stable instrumental performance over long periods have been established. A method for solvent extraction with small volumes of liquid in ordinary test-tubes has been outlined.

In addition, solvent extraction problems that are likely to arise in the further development of pre-concentration procedures, especially multi-element methods, have been examined. These comprise the necessity for a selective exclusion of matrix elements that give rise to spectral interferences and the likely sensitivity of the system to rotational (matrix) effects in the organic phase, owing to either concomitant elements (such as iron) coextracted from the sample, or reagents (such as perchloric acid), both hitherto unrecognised difficulties. While it may prove relatively easy to retain most matrix elements (*i.e.*, Na, K, Ca, Mg and Al) in the aqueous phase, iron is likely to remain a problem, because of its tendency to form complexes with high stability constants with a large range of reagents useful in solvent extraction, and its general high levels in geological samples. These solvent extraction problems have been overcome for the specific instance of the extraction of molybdenum for 6 M hydrochloric acid, except, perhaps, for samples such as lateritic soils that are very high in iron content. In the general case for multi-element extraction, the problems remain unsolved.

We would like to express our debt to M. H. Ramsey, B. J. Coles and S. J. Walton for their helpful suggestions during the course of this study.

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Paper A4/302
Received August 23rd, 1984
Accepted November 20th, 1984

Comparison of Digestion Methods for Total Elemental Analysis of Peat and Separation of its Organic and Inorganic Components

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In order to find the most efficient digestion method for the total elemental recovery in peat, ten samples were subjected to different techniques and analysed for Ca, Mg, Fe, Al, Na, K, Mn, P, Zn, Cu, Li, Cd, Co, Ni, Pb and Si using atomic-absorption spectrophotometry. The most satisfactory procedures were dry ashing followed by hydrofluoric acid treatment and wet digestion using a mixture of hot nitric, perchloric and hydrofluoric acids. The wet digestion offers the advantage of a single decomposition method for the determination of Ca, Mg, Fe, Al, K, Na, Mn, Cu, Li, Zn and P. An alkaline fusion technique was required for the determination of Si. Hydrogen peroxide was used to separate the peat into its organic and inorganic components, leading to the total recovery of the elements for both fractions.

Keywords: *Total elemental analysis; digestion methods; atomic-absorption spectrophotometry; peat*

The elemental analysis of peat is important for several reasons, including ecological studies of peatlands and geochemical prospecting. Such studies often emphasise the total elemental content of peat. Total recovery of the elements requires the destruction of the organic matter and the dissolution of the siliceous material. Digestion of the sample is a crucial part of the analysis. In the first part of our study, we compared digestion techniques to determine which yields the highest recovery of the major and trace elements. We used conventional dry- and wet-ashing procedures with some modifications suitable for the digestion of peat. Fusion techniques were compared for the determination of Si.

The understanding of the geochemical cycling in peatlands involves the elemental composition of the organic and inorganic fractions of peat. Hydrogen peroxide has often been used to oxidise organic matter prior to analyses of soils.[1] In the second part of our investigation, we used it to separate the peat into these fractions, attempted to determine the number of extractions necessary for the removal of most of the organic matter and studied the distribution of selected major and trace elements between fractions.

Experimental

Equipment and Reagents

A Perkin-Elmer 306 atomic-absorption spectrophotometer was used for most determinations. A Beckman B spectrophotometer was used for the determination of P. Other equipment included a muffle furnace for the dry ashing of the samples, centrifuge, balance, hot-plates, pH meter, Vycor crucibles, calibrated flasks, Savillex PFA PTFE jars and test-tubes.

All chemicals were of certified reagent grade: concentrated nitric, hydrofluoric, hydrochloric and 70–72% perchloric acids, hydrogen peroxide, sodium carbonate, potassium nitrate, sodium hydroxide and lanthanum oxide. For the preparation of standards, 1000 μg ml^{-1} stock solutions were used. The acid concentration of the dilute standard solutions matched that of the samples.

Sample Preparation

The samples were dried in an oven at 50 °C. They were ground in a Waring Blendor but were not sieved. Because they were mixtures of fine-grained matter and plant material, sometimes fibrous, sieving would have separated essential parts of the sample. Extra precautions had to be taken to mix the samples before weighing for analysis in order to have a representative sub-sample.

Digestion Techniques

Wet digestion methods

Weigh 0.5 g of dry ground peat sample into a 60-ml pressure-resistant PTFE jar. Add 15 ml of concentrated nitric acid and heat on a hot-plate set at 130–135 °C for 30 min with the jar open. Remove the jar from the hot-plate, allow to cool and add 10 ml of concentrated nitric acid, 1 ml of perchloric acid and 20 ml of hydrofluoric acid. Close the jar and digest the sample overnight at 110–120 °C. A blank must be carried through the digestion procedure to detect and correct occasional contamination for Zn and Cu. Use the same source and volume of acids as for the samples. After completing the digestion (at this point the solution is pale yellow), remove the lid from the jar and evaporate the acids to dryness at 130–135 °C. Add 2 ml of 25% nitric acid solution. Replace the lid to keep the acid from evaporating and warm the jar for 10 min to dissolve the sample residue. Transfer the sample into a 16 × 50 mm 10-ml graduated test-tube using small portions of water to rinse the jar. Make the solution up to 10 ml with water, mix and centrifuge. Transfer 1 ml of this solution into a second 10-ml graduated test-tube and make up to volume with a solution containing 1% of La and 2% of hydrochloric acid.

Use the HCl - La solution for the atomic-absorption determinations of Ca, Mg, Fe, Al, K and Na; use the nitric acid solution for those of Zn, Cu, Li and Mn. Determine P spectrophotometrically from an aliquot of the nitric acid solution as yellow molybdovanadophosphoric acid. The HCl - La and nitric acid solutions can be used interchangeably depending on the concentration of the elements. Only Ca and Mg have to be determined from the HCl - La solution to compensate for interferences. Aluminium requires a dinitrogen oxide - acetylene flame for atomic-absorption determination.

Cd, Co, Ni and Pb were determined with a separate digestion using 1 g of sample because of their low concentrations in peat. After digestion and evaporation of the acids, dissolve the residue in 3 ml of 4 N hydrochloric acid and 1 ml of concentrated nitric acid. Adjust the pH of the sample to 2 with ammonia solution. Complex the elements with diethylammonium diethyldithiocarbamate and extract into 5 ml of isobutyl methyl ketone.[2] Taking a blank and mixtures of the four standards through the digestion procedure is recommended.

* The use of trade names is for descriptive purposes only and does not imply endorsement by the US Geological Survey.

This same wet digestion was performed without adding hydrofluoric acid, and the recovery of the elements was compared in both digestions.

Dry-ashing methods

Place 4 g of dry ground peat in a crucible and ash it in a muffle furnace at 550 °C overnight. Heat aliquots of this dry-ashed sample in a boiling water-bath for 30 min with dilute hydrochloric acid for one set of standard atomic-absorption determinations. For a second set of determinations, weigh aliquots of the dry-ashed sample into PTFE jars and heat with hydrofluoric acid to destroy any silicates present. Slowly evaporate the solutions to dryness. Add dilute hydrochloric acid and heat for 10 min to dissolve the residues. Atomic-absorption results were compared by these two procedures. Ash concentration varied from 25 or 50 mg in 5 or 10 ml of acid for the determination of most elements. For the determinations of Co, Cd, Ni and Pb weigh 0.25 g of ash into 16 × 150 mm test-tubes. Add 2 ml of concentrated nitric acid and 4 ml of 4 N hydrochloric acid. Place the test-tubes in a heating block and boil the samples for 30 min. If the dry ashed samples were treated with hydrofluoric acid first, dissolve the residues in the same acids by heating them on a hot-plate. Adjust the pH of the solutions to 2 prior to extracting in 5 ml of isobutyl methyl ketone.

Fusion methods

Fuse over a Bunsen burner 100 mg of dry ground peat or 25 mg of ashed sample in nickel crucibles with 1 g of sodium hydroxide pellets or a mixture of equal parts of sodium carbonate and potassium nitrate powder until the melt is clean. Dissolve the fused samples in hot water and transfer into 100-ml calibrated flasks. Wash the crucibles with two 3-ml portions of concentrated hydrochloric acid that are added to the flasks. Adjust the volumes with 10% hydrochloric acid solution containing 2% sodium hydroxide and 0.24% potassium chloride solutions. Determinations for Al and Si were made from these solutions with atomic-absorption spectrophotometry using a dinitrogen oxide - acetylene flame.

Table 1. Comparison of dry ashing and wet digestion methods. Results given are for dry mass and are the means of duplicate or triplicate determinations

Digestion	Sample No.	Ash, %	Ca, %	Fe, %	P, p.p.m.	Mg, %	Al, %	Mn, p.p.m.	Zn, p.p.m.	Cu, p.p.m.	Cd, p.p.m.	Co, p.p.m.	Ni, p.p.m.	Pb, p.p.m.	K, %	Na, %	Li, p.p.m.
Dry ashing + acid leach	b–3	36	1.2	0.58	600	0.21	1.30	58	11	22	0.07	0.60	3.0	5.8	0.08	0.014	1.5
Dry ashing + HF + acid leach . .	b–3		1.1	0.58	620	0.26	3.00	79	18	72	0.14	0.58	5.0	5.8	0.86	0.47	9.0
HNO_3 - $HClO_4$ (unashed peat) . .	b–3		1.2	0.64	600	0.26	2.80*	80	16	76	0.40	0.70	5.2	7.0	0.17	0.012	6.0
HNO_3 - $HClO_4$, HF (unashed peat) . .	b–3		1.2	0.63	680	0.25	2.70	84	16	70	0.40	0.75	5.8	9.0	0.67	0.45	8.0
Dry ashing + acid leach	c–6	14.2	1.3	0.60	540	0.16	0.50	71	12	36	0.085	0.54	2.5	4.0	0.05	0.009	0.85
Dry ashing + HF + acid leach . .	c–6		1.3	0.62	570	0.16	0.93	91	18	71	0.14	1.10	6.2	4.3	0.23	0.13	3.8
HNO_3 - $HClO_4$ (unashed peat) . .	c–6		1.2	0.58	520	0.16	0.96*	84	14	62	0.20	0.90	6.7	4.0	0.055	0.009	2.0
HNO_3 - $HClO_4$, HF (unashed peat) . .	c–6		1.3	0.58	560	0.16	0.96	86	20	63	0.30	1.20	8.0	4.3	0.18	0.11	2.8

* Al values determined by fusion techniques.

Table 2. Three replicate determinations for the nitric - perchloric - hydrofluoric acid digestion of the unashed peat

Sample No.	Ca, %	Mg, %	Fe, %	Al, %	K, %	Na, %	Mn, p.p.m.	P, p.p.m.
a	1.6, 1.6, 1.6	0.18, 0.20, 0.19	0.54, 0.6, 0.57	0.96, 1.1, 1.03	0.2, 0.26, 0.25	0.12, 0.17, 0.16	64, 84, 68	720, 720, 720
b–1	1.0, 1.1, 1.0	0.14, 0.16, 0.15	0.41, 0.45, 0.43	0.78, 0.82, 0.80	0.12, 0.16, 0.14	0.08, 0.11, 0.11	68, 68, 68	520, 600, 560
b–2	0.86, 0.9, 0.86	0.20, 0.22, 0.21	0.46, 0.49, 0.48	1.3, 1.5, 1.40	0.4, 0.4, 0.40	0.26, 0.3, 0.29	64, 72, 68	700, 680, 640
b–3	1.0, 1.3, 1.2	0.24, 0.26, 0.25	0.6, 0.65, 0.63	2.6, 2.8, 2.70	0.64, 0.7, 0.67	0.44, 0.46, 0.45	76, 88, 88	680, 680, 680
c–1	1.0, 1.2, 1.1	0.14, 0.16, 0.15	0.64, 0.7, 0.67	1.1, 1.2, 1.15	0.24, 0.26, 0.25	0.19, 0.21, 0.20	112, 136, 124	720, 760, 730
c–2	1.1, 1.1, 1.1	0.18, 0.20, 0.19	0.57, 0.64, 0.57	1.5, 1.6, 1.55	0.2, 0.22, 0.21	0.14, 0.18, 0.16	64, 84, 73	880, 1040, 960
c–3	1.0, 1.1, 1.1	0.14, 0.16, 0.15	0.48, 0.49, 0.48	0.94, 1.0, 0.97	0.16, 0.19, 0.18	0.09, 0.13, 0.13	56, 64, 60	560, 600, 580
c–4	1.3, 1.4, 1.3	0.23, 0.25, 0.24	0.72, 0.72, 0.72	1.7, 2.1, 1.90	0.46, 0.54, 0.50	0.28, 0.3, 0.29	68, 88, 78	780, 820, 800
c–5	0.76, 0.9, 0.83	0.34, 0.39, 0.36	0.82, 0.86, 0.84	3.4, 4.0, 3.70	0.72, 0.82, 0.77	0.46, 0.48, 0.47	72, 76, 74	1100, 1300, 1200
c–6	1.2, 1.3, 1.3	0.16, 0.16, 0.16	0.58, 0.58, 0.58	0.94, 0.97, 0.96	0.16, 0.19, 0.18	0.08, 0.11, 0.09	72, 88, 80	520, 560, 540

Sample No.	Zn, p.p.m.	Cu, p.p.m.	Li, p.p.m.	Cd, p.p.m.	Co, p.p.m.	Ni, p.p.m.	Pb, p.p.m.	Ash, %
a	13, 15, 14	20, 24, 22	2.8, 3.2, 3.0	0.15, 0.2, 0.18	1.0, 1.2, 1.1	5.2, 7.7, 6.5	4.0, 5.0, 4.5	14.4
b–1	9, 12, 11	56, 62, 59	2.4, 2.8, 2.6	0.4, 0.4, 0.40	1.0, 1.2, 1.1	5.3, 5.3, 5.3	2.5, 4.0, 3.2	16.7
b–2	11, 12, 11	55, 57, 56	6.2, 6.4, 6.3	0.3, 0.5, 0.40	1.1, 1.3, 1.2	4.7, 6.0, 5.4	4.5, 7.0, 5.8	36.0
b–3	15, 17, 16	64, 71, 68	7.6, 8.4, 8.0	0.4, 0.4, 0.40	0.6, 0.9, 0.8	5.8, 6.2, 6.0	8.0, 10.0, 9.0	36.0
c–1	17, 19, 18	23, 28, 26	3.6, 3.8, 3.7	0.3, 0.3, 0.30	1.3, 1.6, 1.4	4.0, 6.0, 5.0	4.0, 7.0, 5.5	19.8
c–2	14, 14, 14	53, 55, 54	5.4, 5.2, 4.4	0.2, 0.35, 0.28	1.2, 1.4, 1.3	5.3, 5.8, 5.5	6.5, 8.0, 7.3	20.0
c–3	13, 15, 14	27, 32, 30	2.8, 2.8, 2.8	0.2, 0.3, 0.25	1.4, 1.6, 1.5	4.2, 6.3, 5.2	4.0, 5.0, 4.5	13.9
c–4	18, 20, 19	120, 140, 130	9.2, 9.0, 8.6	0.3, 0.3, 0.30	1.2, 1.5, 1.4	6.4, 7.4, 6.9	9.0, 11.0, 10.0	34.4
c–5	22, 27, 25	58, 76, 67	10.0, 14.0, 12.0	—	—	—	—	58.4
c–6	18, 22, 20	58, 64, 61	2.6, 3.0, 2.8	0.3, 0.3, 0.30	1.1, 1.5, 1.2	7.8, 8.1, 8.0	4.0, 4.5, 4.3	14.2

Separation of peat into its organic and inorganic fractions by dilute hydrogen peroxide

Weigh 0.5 g of dry ground peat sample into a 25 × 150 mm test-tube. Add 4 ml of dilute hydrogen peroxide solution (dilute 10 ml of 50% H_2O_2 to 100 ml with water) and heat the suspension for 15 min in a 70 °C water-bath. Centrifuge the sample and decant the supernatant liquid into a PTFE jar. Repeat this treatment three times, and transfer the supernatant liquid into the same jar. Evaporate the solution to dryness on a hot-plate. This procedure removes easily soluble and organically bound elements from the peat. Transfer the inorganic residue from the 25 × 150 mm test-tube into a second PTFE jar using small portions of concentrated nitric acid. Add nitric, perchloric and hydrofluoric acids to both PTFE jars and proceed with the wet digestion described earlier. Determine the elemental content of the organic and inorganic fractions of peat from these digestions. Again Cd, Co, Ni and Pb were determined in a separate digestion using 1 g of sample.

Results and Discussion

The ten samples used in our study came from three different cores and from different depths within the cores. The Carex bogs from which these cores were extracted are typical of the peatlands of the Rocky Mountains of Colorado. The samples were selected to represent a wide range of ash content (Table 2). The diverse compositions of different peat samples, reflected by their ash content, require different dissolution techniques for total elemental analysis. In the first part of our study we compared two dry-ashing methods and two wet digestions, which have been described under Experimental. (A nitric acid - sulphuric acid - hydrogen peroxide treatment of the samples was discarded because the recovery of some of the elements was poor, especially Ca.) Because the dissolution of the samples is a lengthy procedure, the closed jars left on the hot-plates overnight were fairly efficient tools for this type of digestion. The rest of the procedure was not time consuming. It consisted of some dilutions, followed by atomic-absorption determinations for the different elements. All the elements were recovered simultaneously from a single digestion with the exception of Si, Cd, Co, Pb and Ni as shown under Digestion Techniques. Sometimes these digests were yellow, interfering with the determination of P as yellow molybdovanadophosphoric acid. In this instance P can be determined in the ash treated with hydrofluoric acid.

Table 1 illustrates the results obtained from two samples with different ash contents by the four preparation methods. The following patterns were observed in the samples. The differences in the results for Ca, Fe and P were insignificant in all digestions. The results for Mg, Zn, Cu, Mn and Al were comparable for the ash treated with hydrofluoric acid and for both wet digestions; analysis of the ash without hydrofluoric acid led to significantly lower values. Co, Cd, Pb and Ni had the highest results from the wet digestions. K, Na and Li values were the highest in the ash that had been treated with hydrofluoric acid. They were 2–20 times higher when hydrofluoric acid treatment followed the dry ashing or was part of the wet digestion. The differences were the greatest in the samples that yielded the highest percentage of ash. We adopted the nitric - perchloric - hydrofluoric acid digestion of dry ground peat as our standard procedure for the total elemental analysis of peat. Table 2 illustrates our results from three replicate analyses. All the results are reported on a dry mass basis.

For the determination of Si and Al, fusion techniques were tried using NaOH or Na_2CO_3 + KNO_3. This made it possible to compare fusion techniques for Si and Al, and to compare Al values from fusions with those obtained from the nitric -

Table 3. Comparison of fusion techniques for Al and Si. All results given are for dry mass

	NaOH fusion of unashed peat		NaOH fusion of ash		Na_2CO_3 + KNO_3 fusion of unashed peat	
Sample	Al, %	Si, %	Al, %	Si, %	Al, %	Si, %
a	1.2	3.7	1.0	3.4	1.2	4.0
b–1	0.84	4.8	1.0	4.6	0.82	4.8
b–2	2.3	11	2.4	12	2.2	11
b–3	3.1	9.9	2.8	9.8	2.6	8.9
c–1	1.2	6.0	1.1	5.0	1.2	5.4
c–2	1.7	6.0	1.5	5.0	1.7	5.4
c–3	0.96	3.6	0.98	3.4	0.96	3.4
c–4	2.6	9.1	2.3	9.1	2.4	7.4
c–6	0.96	2.8	0.92	3.3	0.98	3.2

Table 4. Comparison of data on the three reference peat samples from Minnesota. All results given are for dry mass

		Major elements									
Sample No.	Method*	Ca, %	Mg, %	Fe, %	Al, %	K, %	Na, %	Mn p.p.m.	P, p.p.m.	Method*	Si, %
951	A	0.22	0.08	0.51	0.98	0.28	0.15	38	600	B	5.2
	B	0.24	0.10	0.51	1.00	0.29	0.13	32	620	C	5.3
952	A	1.40	0.20	0.97	0.78	0.23	0.09	238	1500	B	3.3
	B	1.52	0.21	0.98	0.82	0.24	0.08	266	1600	C	3.3
953	A	1.80	0.31	1.40	1.30	0.34	0.16	465	1200	B	5.3
	B	1.90	0.30	1.40	1.30	0.33	0.14	486	1220	C	5.5

		Trace elements							
Sample No.	Method*	Zn, p.p.m.	Cu, p.p.m.	Li, p.p.m.	Cd, p.p.m.	Co, p.p.m.	Ni, p.p.m.	Pb, p.p.m.	Ash, %
951	A	40	5.3	2.7	0.8	0.8	2.9	40	15.8
	D	40	5.3	2.7	1.1	1.4	3.8	33	
952	A	25	6.0	2.2	0.3	2.2	3.0	11	14.9
	D	26	4.0	2.2	0.4	2.5	4.6	8	
953	A	57	8.4	4.0	0.8	3.1	6.2	14	22.1
	D	59	5.4	4.0	0.9	2.9	7.0	10	

* Methods: A, atomic-absorption results from the nitric - perchloric - hydrofluoric acid digestion of the unashed peat (described in this paper); B, results by X-ray fluorescence using lithium tetrahydroborate fusion of the ash; C, atomic-absorption results from NaOH fusion of the unashed peat (described in this paper); and D, ICP results from nitric - perchloric - hydrofluoric acid digestion of the ash.

Table 5. Recovery of elements for the organic and inorganic fractions of peat (H_2O_2 extraction)

Sample No.	Ca, %			Mg, %			Fe, %			Al, %			K, %			Na, %			Mn, p.p.m.			P, p.p.m.		
	T*	T_f†	R,‡ %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %
a	1.60	1.50	93.8	0.19	0.20	105.0	0.57	0.54	94.7	1.03	1.20	117	0.24	0.22	91.7	0.15	0.15	100.0	72	78	108;0	720	730	101.0
b–1	1.03	1.03	100.0	0.15	0.16	107.0	0.43	0.42	99.7	0.80	1.10	138	0.14	0.14	100.0	0.10	0.10	100.0	68	70	103.0	560	580	104.0
b–2	0.87	0.93	107.0	0.21	0.22	105.0	0.48	0.50	104.0	1.40	1.80	129	0.40	0.44	110.0	0.28	0.30	107.0	68	72	106.0	640	620	97.0
b–3	1.17	1.15	98.3	0.25	0.26	104.0	0.63	0.64	102.0	2.70	2.70	100	0.67	0.64	95.5	0.45	0.42	93.3	84	98	117.0	680	660	97.0
c–1	1.10	1.05	95.5	0.15	0.16	107.0	0.67	0.66	98.5	1.15	1.30	113	0.25	0.26	104.0	0.20	0.21	105.0	124	129	104.0	730	740	101.0
c–2	1.10	1.15	105.0	0.19	0.18	95.0	0.59	0.58	98.0	1.55	1.70	110	0.21	0.21	100.0	0.16	0.17	106.0	74	74	100.0	960	980	102.0
c–3	1.07	1.10	103.0	0.15	0.16	107.0	0.48	0.51	106.0	0.97	1.00	103	0.18	0.18	100.0	0.12	0.13	108.0	60	64	107.0	580	590	102.0
c–4	1.33	1.20	90.3	0.24	0.25	104.0	0.72	0.73	101.0	1.90	2.30	121	0.50	0.47	94.0	0.29	0.31	107.0	78	75	96.2	800	840	105.0
c–6	1.27	1.15	90.6	0.16	0.15	94.0	0.58	0.58	100.0	0.96	0.96	100	0.18	0.18	100.0	0.09	0.09	100.0	80	78	92.0	540	580	107.0

Sample No.	Zn, p.p.m.			Cu, p.p.m.			Li, p.p.m.			Cd, p.p.m.			Co, p.p.m.			Ni, p.p.m.			Pb, p.p.m.		
	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %	T	T_f	R, %
a	14	14	100.0	22	21	95.0	3.0	2.7	90.0	0.18	0.20	110.0	1.1	1.1	100.0	6.5	6.0	92.3	4.5	4.5	100.0
b–1	11	11	100.0	59	57	97.0	2.6	2.4	92.0	0.40	0.42	105.0	1.1	1.0	91.0	5.3	5.2	98.0	3.2	3.2	100.0
b–2	11	12	109.0	56	55	98.0	6.3	6.2	98.0	0.40	0.40	100.0	1.2	1.1	92.0	5.4	5.3	98.2	5.8	5.5	95.0
b–3	16	17	106.0	68	64	94.0	8.0	7.3	91.0	0.40	0.40	100.0	0.8	0.8	100.0	6.0	5.6	93.3	9.0	8.2	91.0
c–1	18	19	106.0	26	24	92.0	3.7	3.4	92.0	0.30	0.30	100.0	1.4	1.3	93.0	5.0	4.8	96.0	5.5	5.5	100.0
c–2	14	13	93.0	54	51	94.0	4.7	4.3	91.0	0.28	0.30	107.0	1.3	1.4	108.0	5.5	6.1	111.0	7.3	7.2	99.0
c–3	14	14	100.0	30	31	103.0	2.8	3.0	107.0	0.25	0.25	100.0	1.5	1.4	93.0	5.2	5.6	108.0	4.5	4.7	104.0
c–4	19	19	100.0	130	131	101.0	8.6	8.0	93.0	0.30	0.35	117.0	1.4	1.4	100.0	6.9	7.5	109.0	10.0	9.5	95.0
c–6	20	20	100.0	61	58	95.0	2.8	2.6	93.0	0.30	0.30	100.0	1.2	1.3	108.0	8.0	7.2	90.0	4.3	4.2	98.0

* T, Mean of three replicate determinations of the nitric - perchloric - hydrofluoric acid digestion of the whole peat.
† T_f, Mean of the totals from adding the elemental contents of the organic and inorganic fractions of peat (triplicate determinations).
‡ R, Recovery of the elements from the two fractions.

Table 6. Elemental content of the organic and inorganic fractions of peat. Results are the means of three replicate determinations

Sample No.	Ca, %		Mg, %		Fe, %		Al, %		K, %		Na, %		Mn, p.p.m.		P, p.p.m.	
	O*	I†	O	I	O	I	O	I	O	I	O	I	O	I	O	I
a	0.96	0.56	0.13	0.07	0.36	0.18	0.30	0.90	0.00	0.22	0.010	0.14	55	23	540	190
b–1	0.75	0.26	0.10	0.06	0.27	0.15	0.20	0.90	0.00	0.14	0.006	0.09	52	18	360	220
b–2	0.73	0.20	0.09	0.13	0.22	0.28	0.40	1.40	0.00	0.44	0.009	0.29	44	28	440	180
b–3	0.89	0.26	0.10	0.16	0.29	0.35	0.50	2.20	0.00	0.64	0.020	0.40	58	40	440	220
c–1	0.65	0.40	0.08	0.08	0.44	0.22	0.20	1.10	0.00	0.26	0.010	0.20	95	34	520	220
c–2	0.90	0.25	0.09	0.09	0.32	0.26	0.50	1.20	0.00	0.21	0.017	0.15	52	22	760	220
c–3	0.78	0.32	0.08	0.08	0.31	0.20	0.25	0.80	0.00	0.18	0.010	0.12	45	19	390	200
c–4	0.96	0.24	0.08	0.17	0.37	0.36	0.48	1.80	0.00	0.47	0.012	0.30	47	28	600	240
c–6	0.90	0.25	0.08	0.07	0.40	0.18	0.30	0.65	0.00	0.18	0.007	0.08	62	16	380	200

Sample No.	Zn, p.p.m.		Cu, p.p.m.		Li, p.p.m.		Cd, p.p.m.		Co, p.p.m.		Ni, p.p.m.		Pb, p.p.m.	
	O	I	O	I	O	I	O	I	O	I	O	I	O	I
a	6.0	8.0	14	7	0.0	2.7	0.10	0.10	0.50	0.60	3.5	2.5	1.5	3.0
b–1	5.0	6.0	38	19	0.0	2.4	0.12	0.30	0.50	0.50	3.3	1.9	2.2	1.0
b–2	4.0	8.0	39	16	0.0	6.2	0.25	0.15	0.50	0.60	2.7	2.6	2.5	3.0
b–3	4.0	13.0	40	24	0.0	7.3	0.25	0.15	0.30	0.50	2.9	2.7	4.7	3.5
c–1	8.0	11.0	16	8	0.0	3.4	0.15	0.15	0.70	0.60	2.1	2.7	1.5	4.0
c–2	4.0	9.0	34	17	0.0	4.3	0.20	0.10	0.90	0.50	3.2	2.9	4.2	3.0
c–3	5.0	9.0	18	13	0.0	3.0	0.15	0.10	0.70	0.70	2.6	3.0	1.7	3.0
c–4	5.0	14.0	71	60	0.0	8.0	0.25	0.10	0.65	0.75	3.0	4.5	2.5	7.0
c–6	7.0	13.0	40	18	0.0	2.6	0.15	0.15	0.70	0.60	4.8	2.4	2.2	2.0

* O, Organic fraction.
† I, Inorganic fraction.

perchloric - hydrofluoric acid digestion procedure (Tables 1 and 3). No major differences were observed in the results among the various techniques.

Three in-house standard peat samples from Minnesota were used to compare our analyses with earlier analyses performed by the US Geological Survey using X-ray fluorescence for the major elements[3] and an inductively coupled plasma (ICP) for the trace elements. For the major elements, there was good agreement between the nitric - perchloric - hydrofluoric acid digestion of unashed peat and a fusion technique on the ash using lithium tetrahydroborate and X-ray fluorescence. For the remaining elements, the results from the nitric - perchloric - hydrofluoric acid digestion of the unashed sample followed by atomic-absorption analyses compared favourably with those provided by a similar acid digestion of the ash and ICP analyses (Table 4).[5]

The second part of our investigation concentrated on the separation of peat into its organic and inorganic components. We used hydrogen peroxide for chemical extraction of the organic constituents of the samples. Our extraction is a modification of Jackson's method,[1] in which a pH of 5.8 or lower is recommended for increasing the effectiveness of the organic matter extraction; we did not buffer the samples because their original pH was about 5. Various workers, including Jackson, have pointed out the possibility of dissolving sulphides and Mn oxides with hydrogen peroxide. In our samples this possibility is minimal for two reasons. Firstly, in freshwater peat, organic sulphur is the dominant and pyrite is the minor form. Secondly, the Mn concentration in our peat samples is low (Table 2), suggesting that MnO_2 is minimal. In general, the peat environment is probably too reducing to support Mn oxides.

Several researchers, including Anderson,[7] Lavkulich and Wiens[8] and Hoffman and Fletcher,[9] used sodium hypochlorite for organic matter destruction in soils and stream and lake sediments. They indicated that it was more effective than hydrogen peroxide. We tested their extraction method at pH 9.5 and also the sodium pyrophosphate extraction described by Aleksandrova.[10] They gave comparable results to the hydrogen peroxide extraction but they could not be used for Na and P determinations.

After the organic and inorganic fractions had been separated, they were subjected to atomic-absorption determinations using the nitric - perchloric - hydrofluoric acid digestion. The decomposition of the organic matter is gradual and the first extract is dark. The successive extracts were combined, evaporated and subjected to the acid digestion to decompose any remaining organic material and to have a clear, colourless solution. The extraction and the following digestion were carried out in triplicate for each sample. Recoveries ranged from 90 to 110% for all elements with the exception of Al, which ranged from 100 to 138% (Table 5). Table 6 illustrates the distribution of the elements between fractions: 100% of K and Li, 90–96% of Na, 70–80% of Al, 35–70% of Mg, 55–75% of Zn, 30–55% of Cu and Fe, 30–70% of Cd, Co, Ni and Pb and 20–40% of Ca, Mn and P are contained in the inorganic fraction. For Al, Mg, Fe, Zn and Cu, this percentage increases with increasing ash content of the sample.

In order to determine the number of extractions necessary for the removal of the organic fraction, five aliquots of three samples were weighed and subjected to one, two, three, four and six extractions, respectively. After the respective number of extractions had been completed on each sample, it was analysed for the elemental content of both fractions. After three extractions, the results in both fractions remained constant so we adopted three extractions as our standard procedure.

In conclusion, hydrofluoric acid treatment is essential for the total recovery of K, Na and Li and significantly enhances the recovery of Zn, Cu, Mn, Al, Cd, Co, Ni, Mg and Pb. It can follow the dry ashing or be part of the wet digestion, as both techniques lead to comparable values for most elements. For Cd, Co, Ni and Pb, wet digestion is recommended. An alkaline fusion technique is required for the determination of Si.

We found the use of hydrogen peroxide to be satisfactory

for separating the organic and inorganic fractions of the peat samples. The procedure is simple and, followed by nitric - perchloric - and hydrofluoric acid digestion of both fractions, leads to the total recovery of the elements.

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Paper A4/224
Received July 4th, 1984
Accepted October 2nd, 1984

Determination of Aluminium in Dialysate Fluids by Atomic-absorption Spectrometry With Electrothermal Atomisation

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Matrix effects in the determination of aluminium in dialysate fluids by electrothermal atomic-absorption spectrometry are overcome by the addition of nitric acid (to give a final concentration of 2% *V/V*) to the fluid. Analyses may be made by a rapid two-step heating programme giving a total time per injection of 59 s; in the first step, drying and ashing are combined. A detection limit of 1 μg l^{-1} is achieved and reproducibility is better than 2% in the range 30–120 μg l^{-1}. Concentrations of aluminium of <1–11 μg l^{-1} were determined in various batches of commercially supplied peritoneal dialysis fluid. Haemodialysate fluid concentrations varied from <1 to 31 μg l^{-1} with 62% of the samples examined having concentrations less than 5 μg l^{-1}.

Keywords: *Aluminium determination; atomic-absorption spectrometry with electrothermal atomisation; dialysate fluid; haemodialysis; peritoneal dialysis*

Aluminium toxicity has been shown to be a problem for patients with renal failure on dialysis, leading, in severe cases, to dialysis dementia, bone disease and anaemia.[1] The aluminium principally comes from water supplies used to make up dialysate fluids[2] and from the ingestion of phosphate-binding preparations based on aluminium hydroxide.[3] Accidental contamination of peritoneal dialysis fluids has also been noted.[4]

The measurement of aluminium in dialysate fluid can be used to monitor the exposure of patients on dialysis. The change in concentration of aluminium in the fluid after dialysis can be used to calculate transfer of aluminium to and from a patient and to follow the removal of aluminium with the chelating agent, desferrioxamine.[5]

The fluid used in dialysis contains sodium, potassium, calcium, magnesium and chloride at concentrations designed to control the net transfer of the major inorganic ions from blood during dialysis. In addition, the fluids contain glucose and lactate (or acetate). The similarity to the inorganic composition of blood serum might lead one to expect that dialysate fluid could be analysed by electrothermal atomic-absorption spectrometry with electrothermal atomisation (ETA-AAS) in the same way as serum.[6] In our experience, this gave low recoveries, as has been found by others.[7,8] Parkinson[7] and Brown *et al.*[8] used matrix-matched standards for calibration. An alternative approach based on the colorimetric determination of the ternary complex formed between aluminium, Chrome Azurol S and cetylpyridinium chloride has been described for the measurement of aluminium in dialysate fluid.[9]

The object of this work was to develop a sensitive and accurate method based on ETA-AAS, reducing the analysis time in accordance with principles described previously.[10]

Experimental

Instrumentation

Two atomic-absorption systems were used. The first was a Perkin-Elmer 2280 spectrometer with an HGA 500 furnace, AS1 autosampler and 056 recorder; the second, a Perkin-Elmer 272 spectrometer with an HGA 400 furnace, AS1 autosampler and 056 recorder. Table 1 shows the conditions used. As the HGA 400 and HGA 500 time the change in gas flow during atomisation differently, slightly different programmes are needed for the two furnaces. This difference is discussed later.

Standard uncoated graphite tubes were used throughout.

Table 1. Instrumental conditions for the analysis of aluminium in dialysate fluid

Wavelength	309.3 nm	Background correction	On
Slit width	0.7 nm	Volume injected	20 μl
Lamp current	17 mA	Scale expansion	×2

Furnace programme:

Step	Temp./°C	Ramp time/s	Hold time/s
For HGA 500			
1. Dry ash	1400	10	5
2. Atomise	2700	2	10*
For HGA 400			
1. Dry ash	1400	10	8†
2. Atomise	2700	2	10‡

* Internal flow-rate, 0 ml min^{-1}; Autozero and Record at −2 s.
† Base line at 16 s.
‡ Stop Flow and Record switched on in this step.

Reagents

Standards were prepared from a 1 g l^{-1} solution of aluminium as aluminium nitrate (BDH Spectrosol standard solution). Nitric acid was of BDH Aristar grade and water was de-ionised and then distilled.

Control of Contamination

Autosampler cups were cleaned with 20% *V/V* nitric acid and then rinsed in distilled water. Pipette tips were cleaned before use by first pipetting with 20% *V/V* nitric acid (twice) and then distilled water (twice).

Sample Containers

The choice of sample containers is very important. Many containers release aluminium, giving significant contamination. Caps with cork sealing discs or rubber sealing rings are particularly poor in this respect. Glass containers can also release aluminium in small amounts. In our experience, all-plastic 30-ml universal containers (Sterilin, Teddington, Middlesex) have proved satisfactory and do not require cleaning with acid.

Recommended Analytical Procedure

Standards of 0, 30, 60, 90 and 120 μg l^{-1} of aluminium in distilled water are prepared directly in plastic universal containers. These are stable for at least 1 week. Samples are

acidified with nitric acid to give a final concentration of 2% *V/V*. A blank is prepared by adding 400 µl of nitric acid to 20 ml of distilled water. The standards, blank and samples are transferred into autosampler cups and analysed using the conditions in Table 1. The absorbance of the blank is subtracted from the peak absorbances of the samples and the concentration read from the calibration graph. The blank is normally equivalent to less than 2 µg l^{-1}.

Results and Discussion

Matrix Effect

It was found that low recoveries for aluminium added to dialysate fluid were obtained when the furnace programme used was that described for serum aluminium determination by Gardiner *et al.*[6] Because they found it necessary to use a slow ramp rate to minimise losses of aluminium in the ashing stage, we studied the effect of varying the ramp rate in the ashing stage on the peak absorbance for aluminium in dialysate fluid to see whether this would improve recovery. As seen in Fig. 1, the recovery is low (45%) with a 10-s ramp time and improves only slightly with an increase in ramp time (56% at 40 s).

Effect of Acidification

Fig. 1 shows the effect of acidification with nitric acid on the peak absorbance for 100 µg l^{-1} aluminium in dialysate fluid compared with a 100 µg l^{-1} standard. As can be seen, the recovery can be improved markedly by the addition of nitric acid. Nitric acid (1% *V/V*) removes most of the matrix effect. For complete removal of the matrix effect in all samples studied, 2% *V/V* nitric acid was found necessary. Sulphuric acid would also remove the matrix effect, but for routine determination, nitric acid was preferable as sulphuric acid, owing to its high viscosity, is more difficult to pipette.

Standards prepared in 2% *V/V* nitric acid were found to give no significant difference in absorbance from standards prepared in distilled water. This gives an important practical advantage in that standards prepared in distilled water for serum aluminium measurement[6] may be used also for measurement of aluminium in dialysate fluid.

Effect of Changes in the Furnace Programme

Successive changes were made in the furnace programme to examine how the determination could be made as rapid as possible without sacrificing accuracy and reproducibility. The ash stage was found to be critical and a slow ramp up to 1400 °C of at least 10 s was found to be important.

Reduction in the time in the drying stage could be made according to previous recommendations.[10] Indeed in this instance the drying stage could be omitted completely, the slow ramp up to 1400 °C covering both drying and ashing. The final programme developed is given in Table 1. As in the method for serum,[6] no cleaning stage is used. No problems due to carryover have been observed.

In the method for serum,[6] background correction was not found necessary. With dialysate samples, it was found that with the same programme, certain samples gave a small background. Background correction was, therefore, advisable and was used in the previously described experiments. In the rapid programme with its reduced ashing time it is even more important to use background correction, as can be seen in Fig. 2. Background absorbance is, however, well within the range with which the background correction system can cope.

Slight differences are necessary in programmes for the HGA 400 and 500 furnaces. The HGA 500 switches to the reduced gas flow condition at the beginning of the atomisation step, whereas the HGA 400 automatically switches 5 s before the beginning of the step. As in this method ashing times are kept to a minimum, full gas flow is important to remove the products of ashing as quickly as possible. With the HGA 400, it is, therefore, necessary to increase the hold time in the first stage (dry ash) to overcome this problem.

The cycle time per injection is 56 s for the HGA 500 and 59 s for the HGA 400. Of this, 29 s is taken up by automatic sample injection.[10]

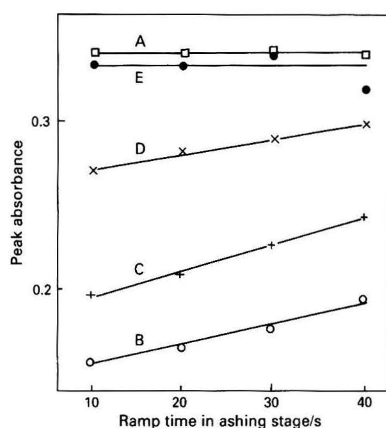

Fig. 1. Effect of acidification and ramp time in the ashing stage on the peak absorbance for aluminium in dialysate fluid. A, Standard solution of 100 µg l^{-1} Al in water; and B, haemodialysate fluid with an endogenous Al concentration of less than 1 µg l^{-1} spiked with B, 100 µg l^{-1} Al; and similarly spiked fluid acidified to C, 0.5%; D, 1%; and E, 2% *V/V* with nitric acid. Conditions: separate drying stage at 120 °C, ramp time 1 s and hold time 15 s; ashing at 1400 °C, hold time 10 s and ramp time as indicated; atomisation stage as step 2 of programme in Table 1

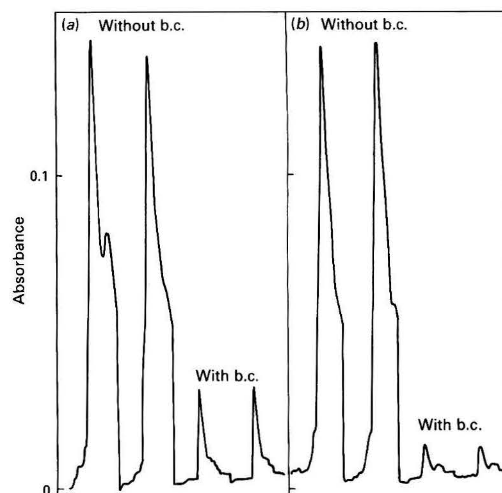

Fig. 2. Signals from (*a*) peritoneal dialysis fluid and (*b*) haemodialysis fluid without and with background correction (b.c.) recorded in duplicate at 4× scale expansion. Measured concentrations in fluids are 4 and <1 µg l^{-1} of aluminium, respectively. The signal from the haemodialysis fluid is equal to that of the blank. The furnace programme used is that given in Table 1

Analytical Performance

Fig. 3 shows the precision profile for the determination. A precision of better than 2% is obtained in the range 30–120 μg l^{-1}. At 10 μg l^{-1}, the precision is about 5%. The detection limit is 1 μg l^{-1}, calculated as twice the standard deviation at 13 μg l^{-1}. Table 2 shows recovery data for both haemodialysate fluid and peritoneal dialysate fluid. A recovery of 99 ± 4% (mean ± s.d.) is obtained for an addition of 100 μg l^{-1}.

It should be pointed out that the method will not work with undiluted haemodialysis concentrate. This requires dilution with aluminium-free distilled water to the working strength (1 + 37), which is then analysed as described.

Concentrations Determined

Peritoneal dialysis fluid is prepared by the manufacturers in sealed bags and aluminium levels are generally low. Table 3 shows the results of the analysis of seven samples from different batches from one manufacturer.

Haemodialysate fluid is prepared by the user from dialysate concentrate and water. The aluminium levels in dialysate concentrate are normally low and the final dialysate concentration depends mainly on the concentration of aluminium in the water used for dilution. With the introduction of reverse osmosis purification equipment for home dialysis in the West of Scotland and the use of such equipment in many hospital dialysis units, aluminium levels in dialysate are now low. Fig. 4 shows the distribution of the aluminium concentration in 50 samples recently submitted for analysis; 62% of the samples had concentrations less than 5 μg l^{-1} and 76% less than 10 μg l^{-1}. To ensure low levels in the dialysate, the purification equipment requires regular maintenance and the aluminium concentration of the purified water supply should be regularly monitored.

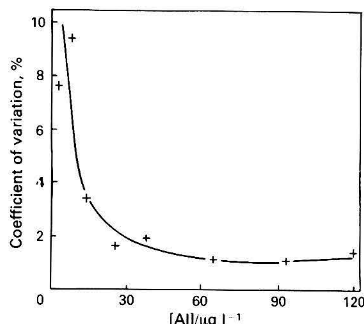

Fig. 3. Precision profile for the determination of aluminium in dialysate fluid. Coefficent of variation at each point calculated from at least 10 readings

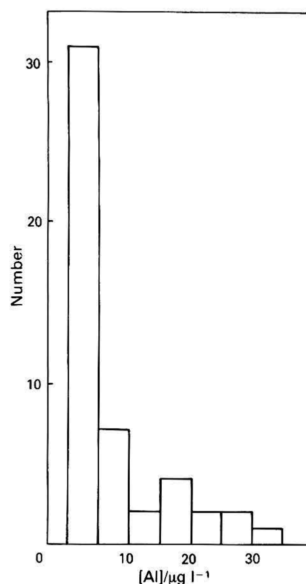

Fig. 4. Histogram of the aluminium concentration of haemodialysate fluid samples received for analysis, principally from patients on home dialysis in the West of Scotland

Conclusions

The beneficial effect of nitric acid in reducing or eliminating interferences in ETA-AAS, particularly from chlorides, has been observed previously.[11–13] Here, it is shown to be effective in eliminating the interference from the dialysate matrix. Reduction of the furnace programme time allows duplicate analysis at a rate of 2 min per sample. The method has been in routine operation for over 1 year and has proved reliable.

The authors are grateful to Dr. A. C. Leung and Dr. I. S. Henderson of the Renal Unit, Glasgow Royal Infirmary, for their advice and for providing samples of dialysis fluid.

Table 2. Recovery of aluminium added to dialysate fluid

Sample	Measured Al concentration/ μg l^{-1}	Al concentration found after addition of 100 μg l^{-1} Al/ μg l^{-1}	Difference/ μg l^{-1} (= % recovery)
Haemodialysate fluid:			
1	7	101	94
2	4	99	95
3	12	108	96
4	7	111	104
5	4	109	105
6	8	111	103
7	9	109	100
8	6	105	99
9	38	138	100
10	49	141	92
CAPD fluid:			
1. 1.36% glucose	10	116	106
2. 1.36% glucose	18	118	100
3. 3.86% glucose	4	102	98
4. 3.86% glucose	15	110	95
5. 1.36% glucose	17	115	98
		Mean ± 1 s.d. (n = 15)	99 ± 4

Table 3. Aluminium concentrations found in peritoneal dialysis fluid in previously unopened bags of batch number indicated, manufactured by Fresenius, Bad Homburg, West Germany

Batch No.	Type*	Al concentration/ μg l^{-1}
HKB29 ..	Intermediate	1
61B1201 ..	Iso	2
1AB51 ..	Hyper	1
1AB11 ..	Iso	<1
1AB08 ..	Hyper	11
1EB13 ..	Iso	1
HDB26 ..	Hyper	1

* Iso, Intermediate and Hyper correspond to glucose concentrations of approximately 1.5, 2.3 and 4.5%, respectively.

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Paper A4/333
Received September 24th, 1984
Accepted October 17th, 1984

Accuracy of Determination of Trace Concentrations of Dissolved Cadmium in River Waters: Analytical Quality Control in the Harmonised Monitoring Scheme

Analytical Quality Control (Harmonised Monitoring) Committee*
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The Department of the Environment in collaboration with the Regional Water Authorities has initiated a Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Waters in England and Wales. The Scottish Development Department has been closely associated with the development of this Scheme, and has introduced a similar scheme in Scotland in collaboration with the River Purification Boards. A programme of Analytical Quality Control (AQC) has been designed to ensure laboratories achieve the required comparability of results. In work on the determination of cadmium, it has not been possible to adhere to the recommended programme of AQC. An assessment of the comparability of laboratories' analytical results for the determination of dissolved cadmium in river water is presented. The accuracy requirements for this determinand (that the total error on a single result should be not larger than 0.1 μg l^{-1} or 20% of sample concentration, whichever is the greater) was not achieved by participating laboratories.

Keywords: *River water analysis; cadmium determination; accuracy of results; inter-laboratory comparability; analytical quality control*

The Scheme for the Harmonised Monitoring of the Quality of Inland Fresh Water has been described in detail.[1] It is intended to provide objective data on river water quality so that accurate assessments can be made of long-term trends in the qualities of rivers and of the amount of materials discharged by them to the sea. The Scheme complements monitoring carried out for regional or local purposes and one of its essential aims is to achieve comparability of the results from all participating laboratories. To that end, special investigations have been made to establish suitable sampling locations and to define the necessary sampling frequency. Sampling procedures have been recommended, and each participating laboratory carries a specially designed programme of tests to ensure that its analytical results are of adequate accuracy for the Scheme.

The Water Research Centre (WRC) acted under contract to the Department of the Environment to advise on and to co-ordinate this analytical quality control (AQC) programme.

The need for, and details of an approach to, a planned AQC system for this and similar monitoring schemes have been discussed elsewhere.[2] In view of the growing interest in achieving comparable results from a number of laboratories,[3–5] it was thought useful to describe the AQC work for the Harmonised Monitoring Scheme and to present the results for different determinands. This paper considers the determination of trace concentrations of cadmium; earlier papers describe the work for chloride,[6] ammoniacal nitrogen,[7] total oxidised nitrogen and nitrite,[8] suspended solids[9] and pH and conductivity[10] and subsequent papers will deal with other determinands of importance in rivers.

Approach to Analytical Quality Control

A Committee was formed to plan the collaborative work and has representatives from the Department of the Environment, the Scottish Development Department, each Regional Water Authority, the Scottish River Purification Boards and the WRC. The laboratories participating in this work (though not all were able to report complete sets of results) were: Anglian Water, Regional Standards Laboratory, Cambridge; Northumbrian Water, Howdon Laboratory, Wallsend; North West Water, Rivers Division Laboratory, Warrington; Severn-Trent Water, Regional Laboratory, Finham; Southern Water, Environmental Laboratory, Otterbourne; South West Water, Rivers and Marine Laboratory, Exeter; Thames Water, Thames Conservancy Division Laboratory, Reading; Welsh Water, Chester Area Laboratory, Chester; Wessex Water, Bristol Avon Division Laboratory, Saltford; Yorkshire Water, Headquarters Laboratory, Leeds; Forth River Purification Board, Headquarters Laboratory, Edinburgh. The sequence of participating laboratories in the above list does not relate to the order of numbering of laboratories in the tables.

In the work described here, it was not fully possible to adhere to the sequential approach to AQC, which was used for determinands studied previously.[2] The establishment of procedures in participating laboratories for the determination of cadmium at low levels, in some instances involving the use of new equipment, required considerable time. Between completion of that stage and the end of the contract under which the AQC work has been coordinated, insufficient time remained for strict adherence to the sequential approach, particularly for the remedial actions that should be taken to rectify problems encountered, before the next stage is entered.

The performance of laboratories in the determination of these low concentrations of cadmium is, therefore, an indication of what might initially be expected from such a group of laboratories rather than a reflection of what might be achieved in terms of compliance with the required accuracy if the sequential programme of testing and control of errors had been rigorously undertaken.

Required Analytical Accuracy

The following requirements were agreed for the determination of total cadmium: maximum tolerable bias, 10% of the determinand concentration of the sample or 0.05 μg l^{-1}, whichever is the greater; maximum tolerable total standard deviation, 5% of the determinand concentration of the sample or 0.025 μg l^{-1}, whichever is the greater. The maximum tolerable total error on a single result is therefore 0.1 μg l^{-1} or 20% of the determinand concentration, whichever is the greater.

These targets, more stringent by a factor of ten than those that had been in force previously in the Harmonised Monitoring Scheme,[11] in terms of tolerable error at low concentration

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Table 1. Results of the precision tests for the determination of cadmium (conducted in 1982)

Standard solution 1

Laboratory No.	Nominal concentration/ $\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$ s_w	s_b	s_t	Relative total standard deviation,† %
1	0.20	0.015	N.s.‡	0.017	8.5
2	N.r.§	—	—	—	—
3	0.50	0.031	N.s.	0.039¶	7.8
4	0.56	0.022	N.s.	0.026	4.6
5	0.50	—	—	0.015	3.0
6	0.375	0.038	N.s.	0.049¶	13.1
7	N.r.	—	—	—	—
8	N.r.	—	—	—	—
9	0.50	0.046	N.s.	0.056¶	11.2
10	0.495	0.048	N.s.	0.078¶	15.7
11	N.r.	—	—	—	—

Standard solution 2

Laboratory No.	Nominal concentration/ $\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$ s_w	s_b	s_t	Relative total standard deviation,† %
1	1.80	0.032	N.s.	0.039	2.2
2	N.r.	—	—	—	—
3	4.50	0.106	N.s.	0.106	2.3
4	5.56	0.064	N.s.	0.069	1.2
5	5.00	—	—	0.134	2.7
6	3.75	0.096	0.197	0.219	5.8‖
7	N.r.	—	—	—	—
8	N.r.	—	—	—	—
9	1.50	0.067	N.s.	0.099	6.7‖
10	4.50	0.072	0.231	0.242	5.5‖
11	4.50	0.125	N.s.	0.125	2.8

River water

Laboratory No.	Concentration found/ $\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$ s_w	s_b	s_t	Relative total standard deviation,† %
1	0.04	0.007	N.s.	0.009	20.9
2	N.r.	—	—	—	—
3	0.51	0.031	N.s.	0.052	10.2¶
4	0.24	0.045	N.s.	0.045¶	18.7
5	0.24	—	—	0.022	9.2
6	0.06	0.022	N.s.	0.031‖	51.7
7	N.r.	—	—	—	—
8	N.r.	—	—	—	—
9	0.093	0.044	N.s.	0.049¶	52.6
10	0.45	0.021	0.134	0.136¶	30.2
11	0.512	0.052	N.s.	0.064	12.5¶

Spiked river water

Laboratory No.	Concentration found/ $\mu g\ l^{-1}$	Standard deviation*/$\mu g\ l^{-1}$ s_w	s_b	s_t	Relative total standard deviation,† %	Spiking recovery,** %
1	1.61	0.044	N.s.	0.044	2.7	98.3 ± 1.2
2	N.r.	—	N.s.	—	—	—
3	2.48	0.144	N.s.	0.174	7.0‖	98.9 ± 6.2
4	2.97	0.036	N.s.	0.049	1.6	98.0 ± 1.1
5	4.44	—	—	0.099	2.2	105.1 ± 2.1
6	2.77	0.139	N.s.	0.225	8.1¶	108.4 ± 7.7
7	N.r.	—	—	—	—	—
8	N.r.	—	—	—	—	—
9	1.12	0.055	N.s.	0.055	4.9	102.9 ± 4.4
10	4.50	0.077	0.188	0.204	4.5	—
11	2.68	0.049	0.137	0.145	5.4‖	108.3 ± 5.3

* s_w, s_b and s_t refer to estimates of within-batch, between-batch and total standard deviation, respectively.
† Target total standard deviation, 5% of determinand concentration or 0.025 $\mu g\ l^{-1}$, whichever is the greater.
‡ N.s., not significant (*F*-test, $p = 0.05$).
§ N.r., Laboratory did not report results for this test.
¶ Exceeds target.
‖ Not significantly (*F*-test, $p = 0.05$) greater than target.
** Spiking recovery is shown as a percentage of the expected recovery with a 90% confidence interval.

and in the implied requirement for limit of detection, made it necessary to develop and implement new analytical methods in participating laboratories. AQC activity relating to the earlier targets has been reported separately.

All tests reported on river samples were conducted after filtration of the sample through a 0.45 μm membrane filter. The performance obtained is therefore that relating to the determination of "dissolved" cadmium. Firstly it is necessary to attain the required degree of accuracy for the measurement stage of the analytical procedure (the analytical finish), thereby establishing the ability to discern important changes in determinand concentration, prior to addressing the problems of sample digestion, which relate to the determination of the total metal.

Analytical Methods

The methods used by participating laboratories were in the following categories: laboratory 1, graphite furnace AAS, lanthanum addition,* platform (pyrolytic carbon) atomisation, duplicate determination; 2, graphite furnace AAS, lanthanum addition; 3, graphite furnace AAS, lanthanum addition, non-pyrolytic furnace, duplicate determination; 4, solvent extraction, ammonium tetramethylene dithiocarbamate - isobutyl methyl ketone (20×), flame AAS; 5, graphite furnace AAS, lanthanum addition, non-pyrolytic furnace, duplicate determination; 6, graphite furnace AAS, lanthanum addition, non-pyrolytic furnace, duplicate determination; 7, graphite furnace AAS, lanthanum addition; 8, graphite furnace AAS, lanthanum addition; 9, graphite furnace AAS, ammonium oxalate (0.125%) addition, standard additions calibration; 10, graphite furnace AAS, lanthanum addition, non-pyrolytic furnace, duplicate determination; and 11, graphite furnace AAS, lanthanum addition, non-pyrolytic furnace, duplicate determination.

Within-laboratory Precision Tests

On each of 10 d, each laboratory made duplicate determinations on each of the following solutions: a blank, two standard solutions, a river water with a cadmium concentration near the lowest routinely reported for the Harmonised Monitoring Scheme and the same river water spiked with a known concentration of cadmium.

The concentrations of the standard solutions used by each laboratory were, respectively, 10 and 90% of the upper concentration limit of the laboratory's method. All results were blank corrected.

* Lanthanum addition as 0.05% *m/V* lanthanum in the sample atomised.

On completion of the tests, each laboratory analysed its results to obtain estimates of within-batch (s_w), between-batch (s_b) and total (s_t) standard deviations, where $s_t^2 = (s_w^2 + s_b^2)$. The values of s_t were compared with the appropriate target value using an *F*-test and were accepted as satisfactory provided s_t was not significantly greater ($p = 0.05$) than the appropriate target. The results of the precision tests are summarised in Table 1. The results for the spiked and unspiked river water samples were used to calculate a "spiking recovery" for each metal. These are also shown in Table 1. A spiking recovery that was not significantly (at the 95% confidence level) less than 95% or greater than 105% was considered acceptable.

Comparison of Standard Solution

At this stage in the sequential scheme of AQC it is considered appropriate to assess and control between-laboratory bias caused by differences in the concentrations of standard solutions used in each laboratory for calibration. Each laboratory was asked to analyse sufficient portions of its own standard solution and one prepared and distributed by WRC so as to allow the detection (95% confidence level) of a difference of 2% in their concentrations. Results were returned for nine laboratories. Of these, there was only one instance (Laboratory 11) of a difference greater than 2% (mean difference, WRC standard − laboratory standard, of −2.57%, maximum possible difference, −4.12%). Owing to the compression in time of AQC work, Laboratory 11 did not take steps to remedy the problem before the inter-laboratory bias test. The over-all mean difference between the standard solution distributed by WRC and those of participating laboratories was −0.08 ± 0.65% ($p = 0.05$). If the result of Laboratory 11 is excluded this difference becomes 0.23 ± 0.31% ($p = 0.05$). The results of this test are shown in Table 2.

Each laboratory then set up a preliminary statistical quality control chart[2] based on the analysis of a standard solution in each batch of analyses. These charts are intended to aid the continuing, long-term assessment of accuracy in each laboratory and are not discussed further here.

Tests for Inter-laboratory Bias

The possibility that matrix interferences affecting graphite furnace AAS methods might be responsible for unacceptably large inter-laboratory bias was reflected in the decision to examine a greater number than the customary two river water samples in the inter-laboratory bias test. Four samples of filtered river water and a standard solution were distributed by the WRC. The solutions were analysed once on each of 4 d. The results were then examined in relation to the agreed accuracy requirements.

It was felt that the mean result of the laboratories could not necessarily be considered an unbiased estimator of the true determinand concentration of the river samples, as almost all laboratories were using graphite furnace AAS, a technique known to be subject to potentially large bias caused by matrix interference. To overcome the need to rely on the mean result of laboratories in evaluating the results of the tests, the river samples were prepared for distribution as follows. The sample was collected from a source thought unlikely to be subject to cadmium pollution. It was then filtered and acidified in bulk. The concentration of cadmium was determined. The sample was then accurately spiked with cadmium and divided for distribution. The nominal concentration used in the interpretation of the test was then the residual cadmium concentration plus the spiked value. The analytical error associated with the determination of cadmium in the unspiked water, whilst potentially high in relation to the observed concentration, is small with respect to the sum of that concentration and the considerably larger concentration of added determinand. This method of sample preparation allowed the determination of a nominal value with a very small associated uncertainty. Details of the preparation and composition of the samples used in the test of inter-laboratory bias are given in Table 3.

To assess whether or not the bias of a laboratory exceeded the target value, the following procedure was adopted. Let the mean result and its 90% confidence interval of Laboratory *i* be denoted by $x_i \pm L_i$. The value of the maximum possible bias of Laboratory *i* (95% confidence level) was then calculated as

$$100(X_i + L_i - X)/X \text{ if } x_i > X$$

or

$$100(x_i - L_i - X)/X \text{ if } x_i < X$$

where X is the prepared concentration of cadmium in the distributed standard solution and the calculated nominal concentration for the river water samples.

For each test of inter-laboratory bias, statistical tests for outlying results were carried out as described elsewhere.[12] None was found. The results of the test of inter-laboratory bias are given in Table 4.

Table 2. Comparison of concentrated standard solutions. The agreed target for this test was that the maximum possible ($p = 0.05$) difference between each laboratory's and the distributed standard solution should not be greater than 2%. The results shown are those finally reported by laboratories. Some laboratories used their current standard solution; others prepared fresh stock standards before the test or after a preliminary check. The sign of the difference is that given by subtraction of the measured concentration of the laboratory's standard from that of the distributed solution

Laboratory No.	Mean difference between distributed and laboratory standards, %	Maximum possible difference ($p = 0.05$), %
1	0.68	1.39
2	N.r.*	
3	0.63	1.20
4	0.28	1.23
5	0.14	0.52
6	0.05	0.38
7	N.r.	
8	0.28	0.53
9	−0.74	−1.55
10	0.52	0.68
11	−2.57	−4.12

* N.r., Laboratory did not report results for this test.

Table 3. Composition of river samples distributed in the test of inter-laboratory bias

Concentration of species present	River A	River B	River C	River D
Calcium/mg l^{-1}	134	136	28	115
Magnesium/mg l^{-1}	51	59	3	5
Ca and Mg hardness/mg l^{-1} $CaCO_3$	547	586	82	308
Sodium/mg l^{-1}	49	39	7	34
Potassium/mg l^{-1}	11	10	1	7
Sulphate/mg l^{-1} SO_4^{2-}	265	116	11	62
Chloride/mg l^{-1}	48	50	10	43
Total organic carbon/mg l^{-1} C	6.7	8.3	3.6	5.4
Cadmium:				
Background concentration (90% confidence limits)/µg l^{-1}	1.44 (±0.04)	0.06 (±0.01)	0.07 (±0.01)	0.13 (±0.01)
Spiked with Cd/µg l^{-1}	—	0.20	0.49	1.68
Nominal value	1.44 (±0.04)	0.26 (±0.01)	0.56 (±0.01)	1.82 (±0.01)

Table 4. Results of inter-laboratory bias test on the determination of cadmium (December 1983). All results in μg l⁻¹ Cd unless otherwise indicated. The target for maximum possible bias is 10% of the concentration of the sample or 0.05 μg l⁻¹ (whichever is the larger)

River water sample A

Laboratory No.	Mean	Standard deviation	Difference from nominal value, %	Maximum possible bias, %
1	1.413	0.042	−2.05	−5.51
2	N.r.†	—	—	—
3	1.337	0.066	−7.32	−12.74‡
4	1.440	0.027	−0.14	−2.32
5	1.328	0.113	−7.94	−17.16‡
6	N.r.	—	—	—
7	1.150	0.063	−20.25‡	−25.41‡
8	1.550	0.050	7.49	11.54‡
9	1.425	0.060	−1.21	−6.10
10	N.r.	—	—	—
11	1.871	0.044	29.55‡	33.36‡
Mean of all laboratories	1.439			
Nominal value	1.442			

River water sample B

Laboratory No.	Mean	Standard deviation	Difference from nominal value	Maximum possible bias*
1	0.264	0.026	0.009	0.040
2	N.r.	—	—	—
3	0.227	0.030	−0.028	−0.064‡
4	0.271	0.021	0.016	0.040
5	0.203	0.046	−0.052‡	−0.106‡
6	N.r.	—	—	—
7	0.193	0.032	−0.062‡	−0.100‡
8	0.450	0.022	0.195‡	0.220‡
9	0.189	0.037	−0.066‡	−0.110‡
10	N.r.	—	—	—
11	0.356	0.056	0.101‡	0.167‡
Mean of all laboratories	0.269			
Nominal value	0.255			

River water sample C

Laboratory No.	Mean	Standard deviation	Difference from nominal value, %	Maximum possible bias,* %
1	0.562	0.009	−0.66	−2.48
2	N.r.	—	—	—
3	0.558	0.005	−1.37	−2.36
4	0.596	0.032	5.34	12.05‡
5	0.493	0.088	−12.99‡	−31.29‡
6	N.r.	—	—	—
7	0.500	0.032	−11.66‡	−18.23‡
8	0.755	0.042	33.39‡	42.13‡
9	0.494	0.036	−12.81‡	−20.26‡
10	N.r.	—	—	—
11	0.619	0.044	9.41	18.62‡
Mean of all laboratories	0.572			
Nominal value	0.566			

River water sample D

Laboratory No.	Mean	Standard deviation	Difference from nominal value, %	Maximum possible bias,* %
1	1.844	0.028	1.49	3.32
2	N.r.	—	—	—
3	1.768	0.069	−2.68	−7.15
4	1.861	0.018	2.44	3.60
5	1.620	0.068	−10.84‡	−15.27‡
6	N.r.	—	—	—
7	1.425	0.044	−21.57‡	−24.45‡
8	1.760	0.050	−3.14	−6.35
9	1.685	0.150	−7.26	−16.96‡
10	N.r.	—	—	—
11	2.234	0.060	22.95‡	26.81‡
Mean of all laboratories	1.775			
Nominal value	1.817			

Standard solution

Laboratory No.	Mean	Standard deviation	Difference from nominal value, %	Maximum possible bias,* %
1	1.006	0.028	0.58	3.92
2	N.r.	—	—	—
3	0.999	0.031	−0.08	−3.76
4	1.049	0.023	4.88	7.52
5	0.953	0.114	−4.75	−18.14‡
6	N.r.	—	—	—
7	0.920	0.041	−8.00	−12.80‡
8	1.058	0.010	5.75	6.88
9	0.866	0.031	−13.40‡	−17.01‡
10	N.r.	—	—	—
11	1.191	0.015	19.08‡	20.82‡
Mean of all laboratories	1.005			
Nominal value	1.000			

* The values given for maximum possible bias are quoted at a confidence level of 95%.
† Laboratory did not report results for this test.
‡ Exceeds target.

Discussion

Eight out of a total of 11 laboratories provided precision data. The results presented in Table 1 indicate that most of the laboratories are not achieving the required precision of analysis. Only two laboratories, 1 and 5, reported observed standard deviations that were, in all instances, less than the appropriate target value. The incidence of failure to meet the precision target was greatest for solutions of low cadmium concentration. For low concentration cadmium standard solution and the unspiked river sample, the number of laboratories for which the standard deviation was significantly greater than the target was four (out of seven) and five (out of eight), respectively. When all laboratory - solution combinations are considered, the incidence of unacceptable precision (*i.e.*, statistically significantly greater than target at 0.05% probability level) is 32%.

Data on spiking recovery met the criterion that recoveries should not be significantly outside the range 95–105% in all instances. This is somewhat surprising in view of the choice of analytical technique made by most laboratories. Graphite furnace AAS is well known for its tendency to be subject to suppressive matrix interferences, which are likely to be detected as low spiking recoveries. It appears, however, that such interferences are relatively well controlled in participating laboratories.

The usual response to such poor precision relative to requirements in the majority of laboratories would be to consider the development and application of the chosen analytical methods and, if such action did not appear likely to improve matters, to explore the possibility of adopting alternative, more precise techniques. For reasons given under Approach to Analytical Quality Control constraints on the time available for the work meant that this was not possible. It was decided, therefore, to proceed with the assessment of inter-laboratory bias.

The comparison of standards test indicated that bias in laboratories' stock standard solutions was not a major contribution to inter-laboratory bias.

The results of the test of inter-laboratory bias show that many laboratories do not achieve the required accuracy. Considering all laboratory and solution combinations, there are 25 (62%) instances of failure to meet the target for maximum possible bias. The seriousness of these failures is reflected in the fact that 16 (40%) are examples where the mean bias is greater than the target. Only Laboratory 1 (no failures), Laboratory 3 (2 marginal failures) and Laboratory 4 (1 marginal failure) can be said to have achieved acceptable performance in relation to the accuracy requirements.

Two further points can be made concerning the results of the bias test. Firstly, poor precision, whilst contributing to the incidence of maximum possible bias greater than target, does not appear to be the dominant cause of inadequate accuracy. Lack of control over bias seems to be the major source of error.

Secondly, the negative bias of graphite furnace AAS methods, caused by matrix interference, is not evident in the over-all mean result for each sample. Bias of the mean result of laboratories with respect to the nominal concentration is, for river waters A–D, −0.2%, +0.14 $\mu g\ l^{-1}$, +1% and −2.3%, respectively. This might be interpreted as confirmation of the results of the tests of spiking recovery that matrix interference is not a problem. Three laboratories show consistent bias (Laboratories 7 and 9 negative bias and Laboratory 11 positive bias) in one direction for all samples, including the standard solution. This is indicative of calibration problems rather than matrix interferences. In any event, these small differences mean that the interpretation of the test is substantially the same if bias is calculated with respect to the mean results of laboratories.

Allowing for the fact that the target accuracy was not attained, it may be of value to consider the monitoring requirements that might be said to have been fulfilled. This is made difficult owing to the tests being designed to monitor analytical performance at the low concentrations of interest. A consequence of this is that there is no information on precision at concentrations greater than 5 $\mu g\ l^{-1}$ and none relating to inter-laboratory bias above 1.8 $\mu g\ l^{-1}$. However, bearing this limitation in mind, the results of the tests suggest that the accuracy achieved would meet a requirement that errors should not be greater than 0.5 $\mu g\ l^{-1}$ or 20% of determinand concentration, a target five times the requirement agreed. When this target is applied, there are no instances of failure on precision tests and relatively few failures on bias—no instances of mean bias greater than target and 6 failures (15%) of a possible 40 on maximum possible bias.

Conclusions

Many participating laboratories did not achieve the required accuracy for the determination of dissolved cadmium. Failure to meet targets is attributable to both random and systematic errors.

The approach to AQC, adopted by the Analytical Quality Control (Harmonised Monitoring) Committee in work on other determinands, has not been possible in work on trace amounts of cadmium. The magnitude of analytical errors does not, therefore, reflect that which might exist if laboratories had undertaken the recommended sequential assessment and control of the various sources of error. Previous work, on determinands for which the recommended approach has been followed, suggests that the continued collaboration of laboratories in a programme of AQC would aid in improving accuracy. Such collaboration would also provide the necessary demonstration that adequate comparability had been achieved and was being maintained. Further work dealing with the important subject of sample collection and handling would then be required.

The standard of accuracy that was apparently achieved was reasonably consistent with a requirement that total error should not be greater than 0.5 $\mu g\ l^{-1}$. The experimental work described here, conducted in relation to the Harmonised Monitoring requirements, which allow errors of one fifth of this value, does not provide evidence that this accuracy was attained, with respect to both precision and bias, for samples of concentration greater than 2 $\mu g\ l^{-1}$.

Although the analytical work reported here was performed by Regional Water Authority and River Purification Board laboratories, the co-ordination of the work was carried out by the Water Research Centre under contract to the Department of the Environment, whose permission to publish is acknowledged.

Appendix

The following are or have been members of the Analytical Quality Control (Harmonised Monitoring) Committee in the period during which this work was performed: Mr. J. G. Flint, Dr. R. J. D. Otter, Mr. R. Donachie, Mr. P. H. Garnett, Mr. L. R. Pittwell and Mr. N. Taylor (Department of the Environment); Dr. D. T. E. Hunt and Mr. M. J. Gardner (Water Research Centre); Mr. M. J. Beard and Mr. N. Loaring (Southern Water); Mr. B. E. P. Clement and Mr. R. Lamb (Welsh Water); Mr. M. G. Firth and Mr. D. Best (Yorkshire Water); Dr. B. T. Croll (Anglian Water); Mr. D. V. Hopkin (Thames Water Authority); Mr. A. G. Poole (Wessex Water); Mr. B. Milford and Mr. B. Dale (South West Water); Mr. T. Hooton and Mr. T. D. Macdonald (Scottish Development Department); Mr. J. B. Allcroft and Mr. A. Hollington (North West Water); Mr. W. Wollers

(Northumbrian Water); Dr. K. C. Wheatstone and Mr. K. Bamford (Severn-Trent Water); Mr. I. R. M. Black (Forth River Purification Board).

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Paper A4/353
Received October 9th, 1984
Accepted November 12th, 1984

Peak Dispersion in a Liquid Chromatography - Atomic-absorption Spectrometry System

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The dispersion characteristics of a flame atomic-absorption spectrophotometer, when used in conjunction with a chromatographic column and with different interfaces, were examined. It was found that the AA unit alone produces excessive peak dispersion (variance 43 µl²) and consequently, when used in conjunction with modern high-performance liquid chromatographic columns, their capabilities cannot be fully realised. The use of low-dispersion serpentine tubing, however, does permit some of the advantages of such columns to be obtained, provided the solutes are eluted at k' values in excess of 3. It is found that the polyethylene tubing commonly used as an interface between the LC and the AA units has a variance contribution of 1.7 µl² cm^{-1}, which, for a practical length of 50 cm, is 83.5 µl² and consequently far too high for the efficient use of the LC - AAS system. Should modern atomic-absorption spectrophotometers be designed to provide sufficiently low dispersion so that they can be used with modern high-performance liquid chromatographic columns, then the major contribution to solute dispersion will reside in the interface and, consequently, the use of low-dispersion serpentine tubing will become essential.

Keywords: *Liquid chromatography - atomic-absorption spectrometry; peak dispersion; serpentine tubing*

The combination of liquid chromatography (LC) and atomic-absorption spectrometry (AAS) can be useful in the determination of metals in both environmental samples and samples of biological interest. The use of LC - AAS systems for identifying metal speciation has been reviewed.[1-3] To utilise successfully any liquid chromatograph - spectrometer combination, the operating parameters of both the LC and the spectrometer system have to be optimised, and this necessary optimisation procedure has been the subject of a number of publications.[4-6] It has been claimed[5] that the poor sensitivity achieved in an LC - AAS combination is due to the dispersion that takes place in the chromatographic column. This fallacy arises from the chromatograph being viewed merely as a sample introduction device and not as a separating system. The point of interfacing a liquid chromatograph with an atomic-absorption spectrophotometer is to achieve a separation prior to detection, for example, to identify two different species of the same metal element in a sample. Consequently, the important dispersion characteristics of the system are not those that occur in the column, but those that occur in the interface and the atomic-absorption spectrophotometer. Dispersion in these two sources does not merely reduce element sensitivity but can destroy the separation originally attained in the column. The magnitude of extra-column dispersion[7] is particularly important if high-speed liquid chromatographic columns packed with small particles are to be employed, as these columns produce very narrow peaks, a few microlitres in volume. High-speed columns appear to be ideally suited for coupling to the AAS unit as they can be operated at the high flow-rates necessary for efficient solvent aspiration into the AAS nebuliser. Moreover, their low dead volumes lead to low solute dilution and also reduced sample size and solvent consumption. However, the benefits of using high-speed liquid chromatographic columns may not be realised if the peak width of the solute is not dominated by the column but by the associated apparatus.

Dispersion characteristics of connecting tubes have been discussed[8] and it has recently been shown[9] that tubes having a serpentine shape introduce very low dispersion so that long connecting tubes can be safely employed without impairing column performance. Modern chromatographic detectors that provide low dispersion characteristics are now commercially available, and their performance has been discussed.[10] To employ the atomic-absorption spectrophotometer successfully as a chromatographic detector, the same requirements of low dispersion have to be met. However, there are no data in the literature on the dispersion that takes place in an AAS unit. It was the aim of this work to investigate sources of extra-column dispersion arising in the LC - AAS system and to determine their magnitude, so that the maximum performance of modern LC columns can be realised.

The Serpentine Tube

Details of the performances and characteristics of the serpentine tube were given earlier.[9] However, as this type of tube is particularly important for the LC - AAS interface, some discussion of its characteristics should be given here. A diagram of a serpentine tube in the form of a complete LC - AAS interface is shown in Fig. 1. It can be seen that it takes the form of semi-circular bends in a cylindrical tube and the ratio of the radius of the semi-circular bends to the inner diameter of the tube is made as small as is practical. It is important that the bends are semi-circular and not zig-zag. The latter has sections of straight portions of tube with sudden bends and in the straight portion of the tubes the parabolic velocity profile of the moving liquid normally existing in a straight tube can re-form. In a serpentine tube the velocity of the liquid is continually changing in direction, which periodically reverses and produces secondary flow inside the tube. This secondary flow results in radial velocities across the tube and disrupts the parabolic velocity profile of the moving liquid. This, in effect, increases the diffusivity of the solute radially across the tube and permits the whole of the band to move approximately at the same velocity along the tube.

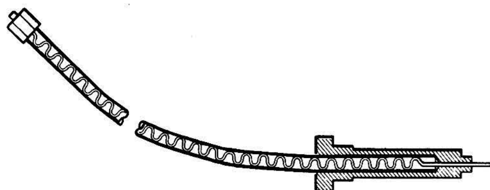

Fig. 1. Serpentine tube interface

Consequently, the band spreading is significantly reduced as there is no static layer of liquid at the walls of the tube and the linear velocity at the centre of the tube approximates to that of the mean.

Experimental

Apparatus and Operating Conditions

A Perkin-Elmer Series 3B liquid chromatographic pump and a Valco valve with a 1-µl injection loop were alternately used with either a Model LC 85B UV detector or a Perkin-Elmer Model 3030 atomic-absorption spectrophotometer. A Bascom - Turner Model 8120 recorder was employed as the recording device. The LC 85B detector had a low dispersion, 1.4-µl flow cell, and the detector response time was set at 20 ms. Three types of tubes were examined as possible interfaces between the LC unit and the flame AAS instrument. One tube was a serpentine tube of 0.25 mm (0.010 in) i.d. and 49 cm long, the ends of which were soldered into a 1.5-cm long straight tube of 1.27 mm (0.050 in) i.d. and 1/16 in o.d. so that the tube could be employed with standard LC connectors. The second was a straight tube, 49 cm × 0.01 in i.d. and 1/16 in o.d. The third tube was a polyethylene tube of 0.58 mm (0.023 in) i.d. and also 49 cm long; for convenience, throughout this paper the polyethylene tube will be referred to as the standard tube, which, in fact, is the tube normally supplied for sample introduction into the AAS instrument. The length of 49 cm was sufficient to interface the chromatograph conveniently to the spectrophotometer. The test solute used was a solution of $Mg(NO_3)_2$ in water, and 35 ng of Mg were injected. The dispersion of $Mg(NO_3)_2$ was studied in methanol at a flow-rate of 2 ml min^{-1} at room temperature. The high-speed liquid chromatographic column was a Perkin-Elmer 3 × 3 column (3.3 cm × 4.6 mm i.d.) packed with octadecyl-bonded silica gel having a particle diameter of 3 µm. All dispersion values were taken as the average of five replicate measurements; any value differing from the mean by more than 5% was rejected and the measurement repeated.

Peak Dispersion Measurements

The dispersion in the LC 85B UV detector and the Valco valve was measured by connecting them directly with a straight tube of 0.18 mm (0.007 in) i.d. and 6 cm long. The dispersion in the three interface tubes was measured by inserting the interface tubes between the straight tube and the sample valve. The dispersion in the flame atomic-absorption spectrophotometer was measured by connecting the same serpentine tube to the AAS unit. To this purpose, one end of the serpentine tube was disconnected from the 1.5-cm straight tube of 1/16 in o.d. and the open end of the tube was tightly joined to a nebuliser capillary tube through a 1-cm polyethylene tube. The dispersion in the polyethylene tube was measured in the same way by placing the polyethylene tube between the injection valve and the AAS unit, connecting the tube to the valve with a 2-cm serpentine tube. The dispersion in each tube and the 3-µm column was determined in terms of the volume peak variance of the test solute eluted from the system. The solute peak width was measured at 0.6065 of the peak height. The response time of the AAS amplifier together with the digital recording device was measured as follows: a thin stainless-steel plate was allowed to pass by the monochromator window under gravity, so that instantaneous interruption of the light passing through the system would result in an impulse response of the AAS electronics that was registered as a peak by a high-speed Bascom - Turner recorder.

Results and Discussion

Dispersion in Different Interface Tubes

The dispersion of a serpentine tube, a straight cylindrical tube of the same length with the same inner diameter, and a polyethylene tube with a larger inner diameter given as the variance in µl^2 is shown in Table 1 for three different flow-rates. The actual solute concentration profile derived from each tube at a flow-rate of 2 ml min^{-1} is shown in Fig. 2. The dramatic effect of the serpentine tube on the shape of the concentration profile is clearly demonstrated. The serpentine tube not only provides the narrowest peak but has completely eliminated the long tail that is caused by the parabolic velocity profile that always exists in simple straight cylindrical tubes. The advantage of the serpentine tube is also clearly demonstrated by the data given in Table 1. However, as the variance is conventionally measured at 0.6065 of the peak height, the effect of the asymmetric tail shown in Fig. 2 is not taken into account. Nevertheless, even when considering only the upper portion of the peak where the elution curve from each interface tube is nearly symmetrical, the variance of the

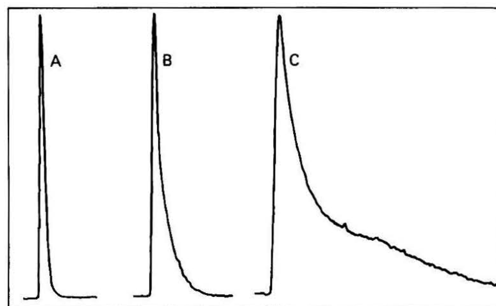

Fig. 2. Peak dispersion in connecting tubes. Mobile phase, methanol; flow-rate, 2 ml min^{-1}; solute, $Mg(NO_3)_2$; data acquisition rate, 10 ms per point (5.06 cm s^{-1}). A, Serpentine tube (0.25 mm i.d., 49 cm long), σ^2 = 4.3 µl^2; B, straight tube (0.25 mm i.d., 49 cm long), σ^2 = 8.5 µl^2; and C, polyethylene tube (0.58 mm i.d., 49 cm long), σ^2 = 86.4 µl^2

Table 1. Measurements of peak dispersion (σ^2/µl^2) in connecting tubes employing an LC system. Mobile phase, methanol; solute, $Mg(NO_3)_2$

	Flow-rate/ml min^{-1}		
Connecting tube	1.0	2.0	4.0
Serpentine tube (49 cm × 0.25 mm i.d.) ..	9.5	4.3	3.2
Straight tube (49 cm × 0.25 mm i.d.) ..	21.1	8.5	7.4
Polyethylene tube (49 cm × 0.58 mm i.d.)	140.7	86.4	46.4

Table 2. Measurements of peak dispersion (σ^2) in the LC - AAS system. Mobile phase, methanol; flow-rate, 2 ml min^{-1}; solute, $Mg(NO_3)_2$

Components of the LC - AAS system	Method of measurement	σ^2/µl^2
Valco valve + UV detector	Straight tube (6 cm × 0.18 mm i.d.)	1.5
Serpentine tube (49 cm × 0.25 mm i.d.)	Valco valve + UV detector	5.8
Serpentine tube (49 cm × 0.25 mm i.d.)	Valco valve + flame AAS	47.1
Polyethylene tube (49 cm × 0.58 mm i.d.)	Valco valve + flame AAS	124.6
Column, 3 µm (3 cm × 4.6 mm i.d.)	Valco valve + serpentine tube + flame AAS	66.0

serpentine tube is still significantly less than that of the straight tube of the same inner diameter and very much less than that of the polyethylene tube of significantly wider diameter.

Band Dispersion in the LC - AAS System

Solute dispersion taking place in the instrument, in both serpentine and polyethylene tubes and in the column is given in Table 2. As it was shown in Fig. 2 and Table 1 that the serpentine tube exhibited less dispersion than a straight cylindrical tube of the same length and inner diameter, the straight tube (0.01 in i.d.) was not examined in conjunction with the AAS instrument. It can be clearly seen that the magnitude of the solute dispersion in the AAS instrument is much greater than that in the UV detector when the serpentine tube is common to both. This indicates that the dispersion in the over-all system is mainly determined by the dispersion in the AAS detector. Moreover, the performance of the 3-μm column appeared to be very poor, largely owing to the extra-column dispersion occurring in the AAS detection system. It has been already mentioned that the width of the Mg peak eluted from the serpentine tube is mainly determined by the dispersion occurring in the AAS detector, the magnitude of which is very significant in comparison with that of the UV detector. However, the contribution of the polyethylene tube to the total dispersion of the system is even greater than the dispersion in the AAS detector.

It has been noted earlier that slow response times of the detector and recording systems may contribute significantly to the over-all extra-column dispersion. Therefore, it was of interest to estimate the magnitude of the dispersion that could be due to the response time of the AAS detection system. The response profile of the amplifier together with the recording device of the flame AAS instrument is shown in Fig. 3. It can be seen that a peak of distorted shape is obtained, and there are eight distinct steps that form this peak. It was determined

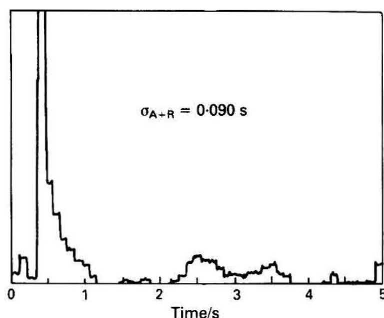

Fig. 3. Measurement of the response time of the amplifier together with the recording device of the flame atomic-absorption spectrophotometer. Recorder: Bascom - Turner. Data acquisition rate: 100 points s^{-1}

Table 3. Peak dispersion in different components of the LC - AAS system

Components of the LC - AAS system	$\sigma^2/\mu l^2$	$\sigma/\mu l$	σ^2/s^2	σ/s
Serpentine tube	4.3	2.1	0.004	0.062
Flame AAS	42.8	6.5	0.039	0.196
Polyethylene tube	81.8	9.0	0.074	0.271
Column, 3 μm (3 cm × 4.6 mm i.d.)	18.9	4.4	0.017	0.131
Amplifier + recorder*	9.0	3.0	0.008	0.090
Burner system	33.6	5.8	0.030	0.174

* Measured independently as described in the text.

that the response time of the recording device together with the amplifier was equal to 0.090 s (which is equivalent to a data acquisition rate of 11 points s^{-1}) by measuring each step, taking an average of eight measurements and expressing the length of each step in time units. It follows that if a column was employed with this system that produced a peak, the time standard deviation of which was lower than or comparable to that of the instrument, the true performance of this column would never be realised.

Having collected all data on band dispersion in terms of the peak variance, the dispersion taking place in each individual component of the LC - AAS system can now be calculated following the law of summation of variance.[11] For example, it can be written that

$$\sigma_I^2 = \sigma_{II}^2 + \sigma_{s.t.}^2$$

where σ_I^2 is the peak variance of the total system (*e.g.*, Valco valve + UV detector + serpentine tube), σ_{II}^2 is the peak variance of the Valco valve and the UV detector (given in Table 1) and $\sigma_{s.t.}^2$ is the peak variance of the serpentine tube, or

$$\sigma_{s.t.}^2 = \sigma_I^2 - \sigma_{II}^2$$

In a like manner, the dispersion of all individual components can be calculated, and the values of the volume and time-related peak variances obtained are summarised in Table 3. It can be seen that the dispersion of the polyethylene tube far exceeds (by a factor of 20) that of the serpentine tube and, moreover, it is significantly greater than the dispersion resulting from the AAS unit itself. The serpentine tube alone would produce a variance contribution of 0.09 μl² cm⁻¹, whereas the polyethylene tube would produce one of 1.7 μl² cm⁻¹. It follows that for a 10-cm length of the connecting tube (which is likely to be the shortest practical length for the interface and would render the apparatus highly inconvenient to use), the variance contribution of the polyethylene tube to the total peak variance of the system will still be 17 μl², constituting 40% of the dispersion in the AAS unit alone. Obviously, the serpentine tube presents an easy and convenient way to minimise the total instrument dispersion. It can also be seen in Table 3 that the dispersion in the burner system constitutes 79% of the total dispersion taking place in the AAS unit. The performance of the 3-μm short column measured in terms of the variance of the unretained Mg peak appeared to be very poor. However, Table 3 shows that the peak standard deviation due to the column itself is equal to only 4.4 μl, which is in close agreement with the theoretical prediction (4.9 μl). It is obvious that this column cannot be used with the system if solutes are eluted from the column at low k' values.

Optimisation of the LC - AAS Parameters

There could be two ways to overcome the incompatibility of the liquid chromatograph and the atomic-absorption spectrophotometer. Firstly, one could modify the AAS unit which, although obviously necessary, was not the purpose of this particular work. Secondly, one could accept a denigrated column performance and thus match the performance of a given column to that of the apparatus. It was, therefore, interesting to examine the performance of columns of different dimensions and packed with particles of a different size, so that the most appropriate columns that could be coupled to the AAS unit could be selected.

The peak variance of the solute eluted from a given column is given by

$$\sigma_C^2 = V_0^2/N \text{ or } \sigma_C^2 = V_R^2/N \quad \ldots \quad (1)$$

where V_0 is the column dead volume, V_R is the retention volume of a solute eluting at a given value of k' and N is the column efficiency. The column dead volume can be calculated from

$$V_0 = \pi r^2 L \varepsilon \quad \ldots \quad (2)$$

where r is the radius of the column, L is the length of the

column and ε is the fraction of the cross-section of the column available for flow and for a well packed column can be taken as 0.7. The retention volume of a solute eluted at a given value of k' can be determined as

$$V_R = V_0(1 + k') \quad \ldots \quad (3)$$

The column efficiency is given by

$$N = L/H \quad \ldots \quad (4)$$

where H, if a column is well packed and operated at its optimum linear velocity, can be taken as equivalent to two particle diameters.

In order to be able to identify those columns that can be satisfactorily employed with a given apparatus, it is necessary to define the magnitude of the instrument dispersion that can be tolerated without impairing the column performance. This is a matter of arbitrary choice, but it is typically assumed[12] that a 10% increase in the column peak variance due to the instrument variance can be acceptable. Thus, it can be written that

$$\sigma_A^2 = 0.1\sigma_C^2 \quad \ldots \quad (5)$$

where σ_A^2 is the peak variance due to the dispersion taking place in the instrument outside the column.

Using these relationships, the peak variance for columns of different dead volumes was calculated. Calculations were performed for solutes having values of k' equal to 0 and 3 for columns packed with 3- and 5-µm particles. The results are summarised in Tables 4 and 5. Values of the peak variance for those columns that could be employed satisfactorily with the serpentine interface - AAS detector are given under solid lines, and values of the peak variance for those columns that could be employed efficiently with the polyethylene interface - AAS detector are given under broken lines. Table 4 clearly demonstrates that for the unretained solutes ($k' = 0$) only one column (50 cm × 4.6 mm i.d.) can be safely used with the AAS detector and then only if a serpentine tube is employed as the interface. It should also be noted, however, that this particular column would have to be packed in short lengths and would be operated only at a linear velocity well below its optimum value, assuming a pressure limitation of 6000 lb in^{-2} (the maximum pressure of most LC pumps). Table 4 shows that short (3 and 5 cm) 3-µm columns of 4.6 mm i.d. can be used for solutes eluted at a k' value of 3 and higher, but again only if fitted with a serpentine tube as the interface. Examining Table 5, where the data on peak variance calculated for columns of different dead volumes packed with 5-µm particles are collated, it can be seen that for unretained solutes three columns can be employed with a serpentine tube interface, and only one column (100 cm × 4.6 mm i.d.) (being impractical to use), can be employed with a polyethylene tube interface. For solutes eluted at a k' of 3, columns of 2 mm i.d. can now be utilised with the serpentine tube, but they have to be long. It is also shown that only wide columns that have a length of 10 cm and longer can be employed if the polyethylene connecting tube is to be used for the interface. It follows that for solutes eluting at low k' values, the benefits of modern high-speed chromatographic columns packed with small particles could not be realised if they are used with the AAS detector. However, for solutes eluting at a k' value of 3 and higher, short columns packed with small particles can be employed successfully provided that low-dispersion serpentine interface tubes are utilised. The use of standard polyethylene tubes would requre not only wide but also long columns, which leads to unnecessarily high solute dilution, greatly decreased mass sensitivity, increased solvent consumption and longer analysis times.

Table 4. Peak variance (σ^2/µl^2) calculated for columns of different dead volumes packed with 3-µm particles

k'	Column length/cm	Column diameter/mm 1.0	2.0	3.0	4.0	4.6
0	3	0.05	0.87	4.38	13.9	24.4
	5	0.09	1.45	7.32	23.2	40.7
	10	0.18	2.90	14.7	46.5	81.2
	15	0.27	4.36	22.0	69.7	122
	25	0.45	7.26	36.7	116.2	203
	50	0.91	14.5	73.4	232	406
3	3	0.87	13.9	70.1	223	390
	5	1.45	23.2	117	371	650
	10	2.90	46.5	235	743	1299
	15	4.36	69.7	352	1115	1949
	25	7.21	116	588	1859	3247
	50	14.5	232	1174	3717	6497

Table 5. Peak variance (σ^2/µl^2) calculated for columns of different dead volumes packed with 5-µm particles

k'	Column length/cm	Column diameter/mm 1.0	2.0	3.0	4.0	4.6
0	3	0.09	1.45	7.3	23.2	40.6
	5	0.15	2.42	12.2	38.7	67.7
	10	0.30	4.84	24.5	77.4	135
	15	0.45	7.26	36.7	116	203
	25	0.75	12.1	61.2	194	338
	50	1.51	24.2	122	387	677
	100	3.03	48.4	245	774	1353
3	3	1.45	23.2	117	372	650
	5	2.42	38.7	195	619	1084
	10	4.84	77.4	392	1239	2164
	15	7.26	116	587	1859	3248
	25	12.0	194	979	3098	5412
	50	24.2	387	1957	6195	10828
	100	48.4	774	3916	12390	21652

It has been pointed out earlier that one of the benefits of using high-speed liquid chromatographic columns is that they can be operated at fast flow-rates without a decrease in column performance. It is known, however, that band dispersion due to the response time of the detection systems can become very significant in fast liquid chromatography. The maximum permissible detector response time that can be tolerated for any given chromatographic column was, therefore, calculated. This can be achieved using the equation given by Scott[13]:

$$\tau = 0.32\, V_0/(N)^{1/2}Q \quad \ldots \quad (6)$$

where τ is the maximum permissible detector response time, Q is the volumetric flow-rate and the other symbols have the meanings previously ascribed to them.

Peak standard deviations expressed in time units were calculated for three typical columns utilising equations (1)–(4), and the maximum permissible detector response times were calculated according to equation (6). The results of the calculations are summarised in Table 6. It should be noted that the linear velocity of 0.2 cm s^{-1} is approximately the optimum linear velocity for both 3- and 5-µm columns. The time standard deviation of the AAS detector electronics was found to be 0.09 s. Table 6 indicates that 5- and 10-µm columns can be employed at their optimum linear velocity. Further, 3-µm columns can be used provided that the k' of the solute exceeds unity. At the higher velocities, however, the AAS electronic response time has a greater impact on the column performance; 10-µm columns can be employed without a detrimental effect but 5-µm columns can be used only for solutes eluting at a k' of unity or greater. With the 3-µm column operating at 0.43 cm s^{-1} the limiting k' value may be as high as 2. However, the dispersion resulting from the response time of the electronics is only part of the spectrophotometer dispersion. If the whole of the AAS dispersion is taken into account and a relatively poor chromatographic performance is accepted, then the AAS unit must be carefully matched to that of the

Table 6. Time-related peak standard deviation (σ) together with corresponding maximum permissible detector response time (τ) calculated for columns packed with particles of different size

	U = 0.2 cm s^{-1}				U = 0.43 cm s^{-1}			
	σ/s		τ/s		σ/s		τ/s	
Column	k' = 0	k' = 3	k' = 0	k' = 3	k' = 0	k' = 3	k' = 0	k' = 3
3 µm (3 cm × 0.46 mm i.d.)	0.21	0.85	0.071	0.29	0.098	0.39	0.033	0.13
5 µm (12.5 cm × 0.46 mm i.d.)	0.28	1.12	0.095	0.38	0.13	0.52	0.044	0.18
10 µm (25 cm × 0.46 mm i.d.)	1.12	4.47	0.38	1.52	0.52	2.08	0.18	0.71

chromatographic column if the LC - AAS system is to be employed satisfactorily in practice. This will be achieved at the expense of a long analysis time, low resolution, high detection limits and high solvent consumption relative to that available in modern LC today.

Determination of Iron in a Blood Sample

One application of the LC - AAS system is its use for metal speciation in blood samples, as the determination of a particular metal bound to a given protein molecule by AAS alone may often give erroneous results. It is known that the determination of blood iron, which is an important element in physiological studies, presents certain difficulties.[14] A chromatogram of a blood sample is shown in Fig. 4 together with the operating conditions used. The conditions for the separation have not been optimised as it was not the purpose of this study, but an interested reader can refer to work[15] where major and minor haemoglobin components were well separated using a cation-exchange LC packing. Fig. 4 demonstrates the benefit of using the LC - AAS detector in comparison with the conventional LC - UV system; the AAS chromatogram clearly shows only the Fe peak. It would appear from Fig. 4 that the peak for iron from the AAS system is much narrower than the apparent complementary peak provided by the UV detector. This anomaly results from the fact that the UV peak is a multi-component peak containing a large number of unresolved substances. If the time scale of the chromatogram was expanded, it would have been found that the peak width from the AAS chromatogram was much wider than would be expected from the column that was employed. The two chromatograms do, however, illustrate the advantage of the AAS instrument acting as a specific detector. The resolution of the multi-component peak shown by the UV detector was not necessary for determining the iron as the AAS instrument conveniently solely monitors the elution profile of the solute containing ions only. Consequently, it may be combined in an elution envelope containing many other solutes.

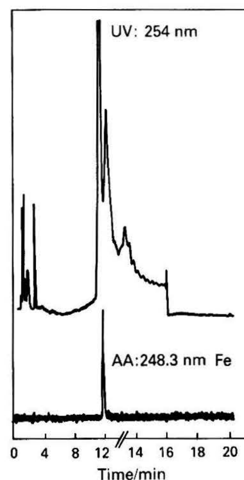

Fig. 4. Chromatograms of a blood sample. Column, HS-3 C18 (10 cm × 0.46 cm i.d.); solvent A, methanol; solvent B, 0.01 M Na_3PO_4 in water; gradient, linear from 0% MeOH to 70% MeOH for 10 min; and flow-rate, 2 ml min^{-1}

Conclusion

Sources of extra-column dispersion arising in the LC - AAS system have been identified. It has been found that the atomic-absorption spectrophotometer produces a variance contribution of 43 µl^2, about 80% of which is exclusive of the response time of the AAS electronics and probably results from dispersion in the burner system. It follows that as the modern atomic-absorption spectrophotometer introduces excessive peak dispersion, the high performance of modern chromatographic columns cannot be realised.

Peak dispersion that takes place in polyethylene tubing employed as the interface has been determined to be equal to 1.7 µl^2 cm^{-1} and, consequently, constitutes a very significant and deleterious effect on chromatographic performance. If the atomic-absorption spectrophotometer is interfaced to the liquid chromatograph by means of low-dispersion serpentine tubing, some of the advantages of modern high-speed columns can be realised, provided that the solutes are eluted at a k' value in excess of 3. Finally, should atomic-absorption spectrophotometers be designed to provide low dispersion so that they can be used with modern liquid chromatographic columns, then the major contribution to solute dispersion will reside in the interface and the use of low-dispersion serpentine tubing will become essential.

The authors thank Mr. David Manning for help with the operation of the atomic-absorption spectrophotometer.

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Paper A4/221
Received July 3rd, 1984
Accepted November 15th, 1984

Determination of Ivermectin in Feeds by High-performance Liquid Chromatography

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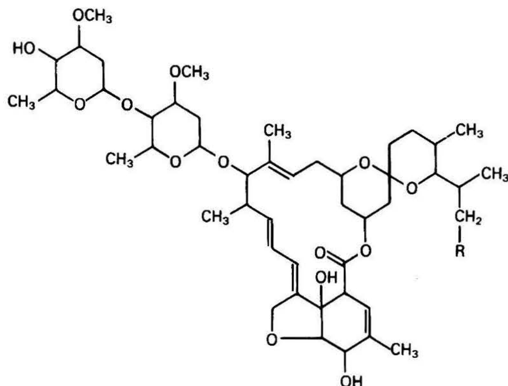
A direct high-performance liquid chromatographic method has been developed for the determination of ivermectin in feeds over the concentration range 50–500 mg kg^{-1}. Sample preparation is based upon extraction into methanol and separation of interferences on a gravity-fed alumina (activity III) column in advance of introduction into a standard reversed-phase chromatographic system. Experiments are described to document the efficiency of this sample preparation scheme and the chemical yield of the analyte through the process. The method has been applied to three types of feed samples with an accuracy of ±3.4% mean relative error and a relative standard deviation (precision) of <3%. It has a lower limit of detection with a signal to noise ratio ≥ 2 : 1 of 6.0 mg kg^{-1} of ivermectin.

Keywords: *Ivermectin determination; high-performance liquid chromatography; medicated feeds*

Ivermectin is an extremely potent antiparasitic drug, which is effective against both internal and external parasites in a wide variety of animals. It is composed of a mixture of two homologous pentacyclic 16-membered lactones[1,2] [5-*O*-demethyl-25-de(1-methylpropyl)-22,23-dihydro-25-(1-methylethyl)avermectin A_{1a} and 5-*O*-demethyl-22,23-dihydroavermectin A_{1a}] having the structures shown in **I**. A recent comprehensive report reviews the efficacy, chemistry, microbiology, isolation, structure determination and metabolic distribution of ivermectin.[3]

Several analytical methods for the determination of ivermectin in biological samples have been reported. This drug has been measured in plasma using high-performance liquid chromatography (HPLC) of a fluorescent derivative formed by an aromatisation reaction run with acetic anhydride in pyridine.[4] An alternative, more rugged, direct analytical method for these samples is based upon ultraviolet absorptiometric detection in the same reversed-phase system using the Δ^2 isomer as an internal standard.[5] The fluorescent derivative has also been applied in tissue analysis (liver, fat, muscle and kidney)[6] via a more rapid derivatisation reaction that uses an *N*-alkylimidazole nucleophilic catalyst (in place of pyridine) as reported by Connors and co-workers.[7–9] The metabolic studies of the drug were based upon NMR and mass spectrometric data as well as reversed-phase HPLC.[10]

Because ivermectin is active by oral administration, one of the pharmaceutical formulations under development is a pre-mix, which will be diluted into feed for administration. This paper describes a simple, rapid and direct analytical method for the determination of ivermectin in the final feed over the concentration range 50–500 mg kg^{-1}. It is based upon HPLC analysis using the reversed-phase conditions previously described.[4–6,10] Sample preparation involves the separation of interferences on alumina to obviate derivatisation over the concentration range examined.



Component a R = CH_3
Component b R = H

Experimental

Principle

Ivermectin is extracted from a feed sample into methanol at room temperature and interferences are separated by gravity-fed column chromatography on alumina. The analyte is then determined using high-performance liquid chromatography on a Zorbax (DuPont) ODS reversed-phase column using a mixed solvent mobile phase of acetonitrile - methanol - water and direct ultraviolet absorptiometric detection. This method is applicable to feeds formulated with ivermectin over the concentration range 50–500 mg kg^{-1}.

Reagents

All reagents were of analytical-reagent or HPLC grade.

Methanol.

Acetonitrile.

Millipore-filtered water.

Chromatographic mobile phase. Prepare by mixing 53 ml of acetonitrile, 35 ml of methanol and 7 ml of water.

Ivermectin analytical standard. Accurately weigh *ca.* 30 mg of ivermectin standard of known composition as declared by the supplier (Merck & Co., Rahway, NJ) and quantitatively transfer it into a 100-ml calibrated flask. Dissolve and dilute to volume with methanol. Dilute 5.0 ml of this stock solution to 50.00 ml in a calibrated flask with the same solvent.

Clean-up column.

Alumina. Woelm neutral alumina (02084) with 6% water added (activity III).

Glass columns. 10–14 mm i.d. × 30–40 cm long with a restricted end for a glass-wool plug.

Preparation of column

Add 5 g of alumina to the column and settle the adsorbent by tapping the sides of the column.

HPLC Apparatus and Conditions

Pump.
Detector.
Column. Zorbax ODS (DuPont), 4.6 × 250 mm.
Wavelength. 245 mm.
Attenuation. 0.16 a.u.f.s.
Flow-rate. 1.2 ml min^{-1}.
Temperature. Ambient.
Injection volume. 100 µl (loop).

Under these conditions the retention times of the a and b components of ivermectin are 11.3 and 9.2 min, respectively.

Procedure

A feed sample of a mash or a pelleted feed is ground in a sample mill and a mass is taken to contain 1.0 mg of ivermectin (*e.g.*, 10.00 g of a feed expected to contain 100 mg kg^{-1} of the drug). The sample is transferred into a 120-ml wide-mouth brown bottle for extraction, and 50.0 ml of methanol are added. For extraction, the bottle is placed in an ultrasonic bath for 20 min and then on a reciprocal shaker for 1 h. A 40-ml aliquot of the extract is transferred into a centrifuge tube and centrifuged at 2000 rev min^{-1} for 5 min. The alumina column is charged with 20 ml of the supernatant. After the first 5 ml of the eluate have been discarded the remainder is collected for introduction into the HPLC system.

Calculations

$$\text{Ivermectin (a or b) (mg kg}^{-1}) = \frac{A_x}{A_s} \times C_s \times \frac{50}{M}$$

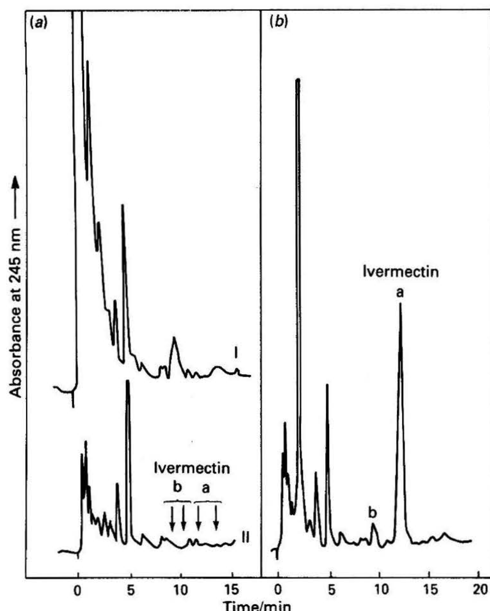


Fig. 1. Chromatograms obtained from representative feed samples. (*a*) The effect of the alumina column sample pre-treatment on drug-free feeds: I, in the absence of column purification; and II, following column separation. Arrows define the range of ivermectin retention times. (*b*) Feed containing 100 mg kg^{-1} of ivermectin. Chromatographic conditions: column, Zorbax ODS (DuPont), 4.6 × 250 mm; flow-rate, 1.2 ml min^{-1}; injection volume, 100 µl; mobile phase, acetonitrile - methanol - water (53 + 35 + 7); and attenuation, 0.16 a.u.f.s.

where A_x is the peak area of the invermectin a or b component from the sample; A_s is the peak area of the ivermectin a or b component from the analytical standard; C_s is the concentration of the ivermectin a or b component in the analytical standard solution (µg ml^{-1}); and M is the feed sample mass (g). The result for ivermectin is the total of the a and b component peaks.

Results

Sample Preparation

Efficiency of separation of interferences

In advance of quantitative HPLC measurement, this analytical procedure uses gravity-fed chromatography on alumina (activity III) for the isolation of ivermectin from unidentified extraneous feed interferences that are coextracted into methanol with the analyte. The solubility of ivermectin is 220 mg ml^{-1} in this solvent at room temperature, and in this environment the drug is stable for several weeks.

To demonstrate the efficiency of the alumina column chromatographic separation, a drug-free feed was examined in the absence of this step, and also following the complete

Table 1. Chemical yield of ivermectin through the column separation

Ivermectin concentration in methanolic solution/ µg ml^{-1}	Ivermectin recovery, %		
	Component a	Component b	Over-all
16.4	98.7	100	98.8
21.9	98.5	93.9	98.1
27.4	99.1	100	99.2
27.4	99.4	101	99.6
32.9	99.4	103	99.7
43.8	99.1	104	99.5
			Mean = 99.2
			S.d. = 0.61

Table 2. Analyses of known feed samples (10 g of feed sample taken)

A. Drug-free feeds

Commercial feed type	Apparent ivermectin found/mg kg^{-1}
A	3.2
A	1.8
A	2.2
B	1.8
B	3.4
C	2.1
	Mean apparent ivermectin 2.4 ± 0.57*

B. Laboratory-supplemented feeds

Pre-mix taken/ mg	Ivermectin† added/ mg kg^{-1}	Ivermectin found/mg kg^{-1}			
		Component a	Component b	Total	Relative error, %
89	52.9	51.2	5.4	56.6	7.0
124	73.7	72.2	4.8	77.0	4.5
160	95.0	90.9	6.6	97.5	2.6
172	102.2	101.3	7.0	108.3	6.0
174	103.4	98.4	7.1	105.5	2.0
196	116.4	110.3	10.9	121.2	4.1
228	135.4	126.9	9.3	136.2	0.6
259	153.8	143.5	10.4	153.9	0.1
				Mean relative error	3.4
				Standard deviation	2.5

* 95% Confidence limits (n = 6).

† The composition of the drug is 93% component a and 7% component b.

procedure. The resulting chromatograms are shown in Fig. 1. Because of the interference appearing at the retention time of the b component of ivermectin, integration over the retention times shown in chromatogram (*a*) I yields an apparent ivermectin concentration ≥20 mg kg^{-1} in this drug-free feed. Chromatogram (*a*) II shows that treatment of the methanolic extract obtained from this feed by the adsorption column removes these interferences: the resulting integrated area represents only 3.2 mg kg^{-1} of ivermectin. Comparison of chromatograms I and II in Fig. 1(*a*) also demonstrates the efficient separation of many extraneous compounds appearing at retention times ≤5 min in this system. In addition, when this feed was supplemented with 103 mg kg^{-1} of ivermectin and carried through the analytical procedure in the absence of the alumina chromatographic step, the result from this treatment was 122 mg kg^{-1} of ivermectin, demonstrating that the interferences were the source of an 18% positive systematic error.

Chemical yield of analyte through the sample preparation process

To confirm that this chromatographic operation does not affect the chemical yield following extraction, six methanolic solutions containing ivermectin in a concentration range 16.4–43.8 µg ml^{-1} were carried through the alumina-column step and measured by HPLC. These solutions, which correspond to the extracts obtained from feed samples formulated with 50–150 mg kg^{-1} ivermectin, were compared with three aliquots of a pure methanolic solution used as a standard and measured in the absence of the chromatographic treatment. The results of the HPLC analyses are shown in Table 1. The average recovery, 99.2%, confirms that ivermectin is not adsorbed on the stationary phase and that it does not undergo any chemical reaction during this chromatographic pretreatment. The over-all recoveries listed in the last column of Table 1 are weighted four-fold by the a component as a result of the composition of this drug, which is 80% component a and 20% component b. In addition, the data in Table 1 also demonstrate the linearity of the chromatographic system over a wide concentration range and the precision of the measurement (relative standard deviation = 0.61%).

Accuracy and Precision

To determine the accuracy and precision of this procedure, three drug-free feeds were supplemented in the laboratory with weighed amounts of a standard ivermectin pre-mix to provide medicated feed samples of known analyte concentrations over the range 50–150 mg kg^{-1}. These feeds were obtained from three different suppliers. They were all based on corn and/or wheat middlings, alfalfa meal and meat meal, bone meal and soybean meal in various proportions as sources of protein in addition to numerous vitamins and minerals. The pre-mix contained 0.594% *m/m* ivermectin and was prepared on a substrate of ground corn cobs. After thoroughly blending this known pre-mix into the feeds, the samples were analysed by the described procedure. A representative chromatogram obtained from a medicated feed sample containing 100 mg kg^{-1} of ivermectin, illustrating the efficiency of this chromatographic process, is shown in Fig. 1(*b*). None of the formulation excipients interfered in the procedure. The results are shown in Table 2. This table includes results from the analyses of six drug-free samples obtained from the three different commercial feed sources. The average apparent ivermectin contributed by unidentified extraneous feed interferences in these feeds was 2.4 mg kg^{-1} with 95% confidence limits (n = 6) of ±0.57 mg kg^{-1} apparent ivermectin. These results, which confirm the selectivity of this procedure, also establish that the lower limit of detection with a signal to noise ratio of ≥ 2:1 is 6.0 mg kg^{-1} of analyte. The results in Table 2*B* demonstrate that this method has an accuracy over the concentration range 50–150 mg kg^{-1} of ivermectin of ±3.4% mean relative error with a relative standard deviation (precision) of <3%.

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Paper A4/295
Received August 22nd, 1984
Accepted October 23rd, 1984

Determination of Chlordane in Human Blood by Gas Chromatography

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A clean-up method for the removal of organochlorine interference prior to the determination of chlordane by gas chromatography was studied. Chlordane, polychlorinated biphenyls and organochlorine pesticides were treated with a cold mixture (5 ml) of concentrated sulphuric acid and fuming nitric acid (1 + 1 *V/V*) at 0 °C for 15 min. The product was mixed with cold water (100 ml) and submitted to continuous distillation (cyclic steam extraction) for 90 min to extract chlordane into 3 ml of heptane. The extract was cleaned up by reduction with iron in acetic acid and the chlordane was determined by gas chromatography with electron capture detection. The acid treatment removed polychlorinated biphenyls and organochlorine pesticides, which gave peaks overlapping those of chlordane. The method was applied to the determination of chlordane in human blood. The blood sample was haemolysed with water and extracted with acetone - hexane (1 + 9 *V/V*), and the extract was treated by the above method. The recovery of chlordane added to human blood was above 88%.

Keywords: *Chlordane determination; gas chromatography; organochlorine compounds; human blood*

Chlordane has been widely used for protecting soil and house foundations against termite infestation. Chlordane consists of a mixture of *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, heptachlor and related compounds.[1] Chlordane, nonachlor and heptachlor are metabolised to oxychlordane and heptachlor epoxide, which accumulate in adipose tissue.[2–5]

Kutz[6] determined *trans*-nonachlor, oxychlordane and heptachlor epoxide in human blood serum and adipose tissue collected from the general population in the USA. Kawano *et al.*[7] determined chlordane and its metabolites in the blood of pest control operators exposed to chlordane.

Chlordane has usually been extracted with organic solvents and separated from other organochlorine compounds by Florisil column chromatography prior to the determination by gas chromatography with electron-capture detection.[8,9] Column chromatography is suitable for the multi-residue analysis of organochlorine compounds, but it requires careful control of adsorbent activity and a large amount of organic solvent as eluent. Therefore, we sought a more convenient separation method.

Musoke *et al.* [10] separated chlordane from other organochlorine compounds by hydrodechlorination with a platinum catalyst. Erro *et al.*[11] applied treatment with a cold mixture of concentrated sulphuric acid and fuming nitric acid (1 + 1 *V/V*) to the determination of toxaphene in the presence of *p,p'*-DDT. Holdrinet[12] used acid treatment at 70 °C to separate mirex and *cis*- and *trans*-chlordane from other organochlorine pesticides and polychlorinated biphenyls, but oxychlordane was removed together with polychlorinated biphenyls, *p,p'*-DDT and related compounds. Matsumoto *et al.*[13] also showed that polychlorinated biphenyls, *p,p'*-DDT and related compounds were removed by acid treatment at 0 °C for 15 min, but heptachlor epoxide and hexachlorocyclohexane (HCH) isomers remained. They used continuous distillation (cyclic steam extraction) as the extraction and clean-up method after the acid treatment. Because oxychlordane has a similar structure to heptachlor epoxide, we thought that the acid treatment at 0 °C might also be applicable to the separation of chlordane containing oxychlordane from other organochlorine compounds, and continuous distillation is simpler than the conventional solvent extraction.[14–17]

This paper describes a method for removing interfering organochlorine compounds without column chromatography. This procedure consists in acid treatment with a cold mixture of concentrated sulphuric acid and fuming nitric acid (1 + 1 *V/V*), continuous distillation and reduction with iron in acetic acid, and is applicable to the determination of chlordane in human blood. The recovery of fortified chlordane was satisfactory.

Experimental

Materials

The reagents were of analytical-reagent or high-purity grade and were used without further purification, unless indicated otherwise.

Chlordane. trans- and *cis*-chlordane (1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), *trans*-nonachlor (1,2,3,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene), oxychlordane (1,2,4,5,6,7,8,8-octachloro-2,3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene) and γ-chlordene (4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) were obtained from Velsicol Chemical Co.

Pesticide standards. α-, β-, γ- and δ-HCH (1,2,3,4,5,6-hexachlorocyclohexane), *p,p'*-DDE [1,1-dichloro-2,2-bis(*p*-chloropheny)ethylene], *p,p'*-TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene), dieldrin and endrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene), heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene) and heptachlor epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene), for pesticide residue analysis, were obtained from Wako Pure Chemical Industries, Tokyo, Japan. Polychlorinated biphenyls were supplied by the National Institute of Hygienic Sciences of Japan. Standard solutions in hexane were prepared as follows: chlordane, 0.05 μg ml^{-1}; organochlorine pesticides, 0.01 μg ml^{-1}, except

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β-BHC, 0.05 μg ml^{-1}; and polychlorinated biphenyls, 0.1 μg ml^{-1}.

Heptane. Re-distilled.

Acetic acid solution, 10%. Washed with hexane.

Sodium hydroxide solution, 0.1 N. Washed with hexane.

Reduced iron powder. Washed with 1 N hydrochloric acid, distilled water, methanol and diethyl ether and dried at 60 °C for 30 min.

McIlvaine's citric acid - phosphate buffer,[18] *pH* 2.2–8.0.

Water. Volatile organic compounds were removed by continuous distillation before use.

Silica gel. Wako gel C-100 (100–200 mesh) was activated by heating at 130 °C overnight and the activity was adjusted with water (5% *m/m*).

Sodium sulphate, anhydrous.

Sulphuric acid, concentrated.

Nitric acid, fuming.

Zinc powder.

Tin powder.

Apparatus

A Shimadzu GC-6A gas - liquid chromatograph, equipped with a ^{63}Ni electron-capture detector, was used, with 2 m × 3 mm i.d. glass columns packed with (a) 2% OV-101 on 100–120-mesh Gas-Chrom Q or (b) 1.5% OV-17 + 1.95% QF-1 on 60–80-mesh Chromosorb W HP. The column temperature was 200 °C, injection port and detector temperature 230 °C and carrier gas (nitrogen) flow-rate 35 ml min^{-1} (OV-101) or 50 ml min^{-1} (OV-17 + QF-1).

A continuous distillation apparatus as described by Matsumoto *et al.*[14] was used. Glassware was washed with acetone before use.

Extraction

To 2 g of whole blood in a 50-ml centrifuge tube, 10 ml of water and 20 ml of acetone - hexane (1 + 9 *V/V*) were added. The tube was stoppered, shaken vigorously for 5 min and the two phases were separated by centrifugation at 2000 rev min^{-1} for 3 min. The upper phase was carefully removed and the lower phase was re-extracted with 20 ml of acetone - hexane. The combined organic phases were dried with anhydrous sodium sulphate.

Silica Gel Column Chromatography

The extract of blood was evaporated to dryness *in vacuo* and the residue was dissolved in a small amount of hexane and poured on to the silica gel column (5 g, 70 × 12 mm i.d.). β-HCH and *p,p'*-DDE were eluted with 50 ml of benzene - hexane (2 + 8 *V/V*), evaporated to dryness *in vacuo* and the residue was dissolved in hexane for gas-chromatographic analysis.

Acid Treatment

The extract of blood was evaporated to dryness *in vacuo* in a 300-ml round-bottomed flask cooled with ice and the residue was treated with a freshly prepared mixture (5 ml) of concentrated sulphuric acid and fuming nitric acid (1 + 1 *V/V*) at 0 °C for 15 min with occasional swirling, and then with cold water (100 ml).

Continuous Distillation

The flask from the acid treatment was cooled with ice and connected to a continuous distillation apparatus containing 3 ml of heptane. A Dimroth condenser was fitted to the top of the apparatus to prevent loss, and the solution was boiled for 90 min. The condenser was washed with a small amount of water and the volume of heptane was adjusted to 3 ml whenever necessary. Heptane was separated through a stopcock and dried with anhydrous sodium sulphate.

Reduction

A 2-ml aliquot of the heptane extract was shaken with 4 ml of 10% acetic acid and about 300 mg of iron powder in a 10-ml glass-stoppered test-tube for 3 min at room temperature, washed with 2 ml of 0.1 N sodium hydroxide solution and dried with anhydrous sodium sulphate.

Determination by Gas Chromatography

A 3-μl aliquot of the heptane extract was injected into the gas chromatograph operated as described under Apparatus. Each isomer of chlordane was determined by comparing the peak height with that of a standard.

Results and Discussion

Gas-chromatographic Conditions

The gas-chromatographic conditions for separating chlordane and heptachlor epoxide were examined by use of OV-101, OV-17 + QF-1, OV-17, OV-225 and Thermon 3000. They were best separated with a column containing OV-101 (methylsilicone) the stationary phase. Although the peaks of *cis*-chlordane and *trans*-nonachlor partly overlapped, both could be determined satisfactorily. A column containing OV-1 + QF-1 was used for the determination of oxychlordane and heptachlor epoxide because their peaks overlapped on the OV-101 columns.

Ratio of Sulphuric and Nitric Acids and the Reaction Temperature

The percentage of the remaining chlordane constituents after treatment with nine different mixtures of concentrated sulphuric acid and fuming nitric acid (10 + 0 to 0 + 10 *V/V*) at 0 °C for 15 min was examined. The percentage of *cis*-chlordane, *trans*-chlordane and *trans*-nonachlor was independent of the proportions of the acids. About 60% of oxychlordane was degraded in concentrated sulphuric acid, but it remained

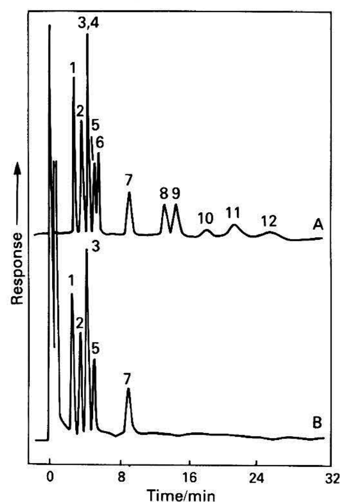

Fig. 1. Gas chromatograms of organochlorine pesticide mixture (using OV-17 + QF-1): A, before and B, after acid treatment. Peaks: 1, α-HCH; 2, γ-HCH; 3, β-HCH; 4, heptachlor; 5, δ-HCH; 6, aldrin; 7, heptachlor epoxide; 8, *p,p'*-DDE; 9, dieldrin; 10, endrin; 11, *p,p'*-TDE; 12, *p,p'*-DDT

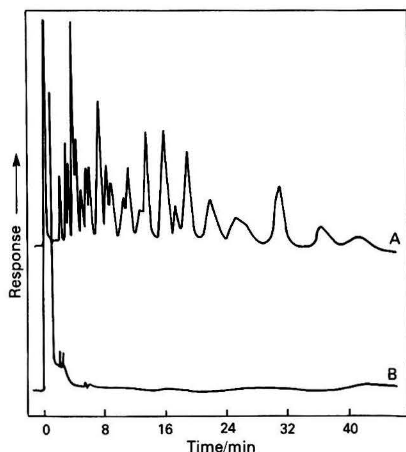

Fig. 2. Gas chromatograms of polychlorinated biphenyls A, before and B, after acid treatment

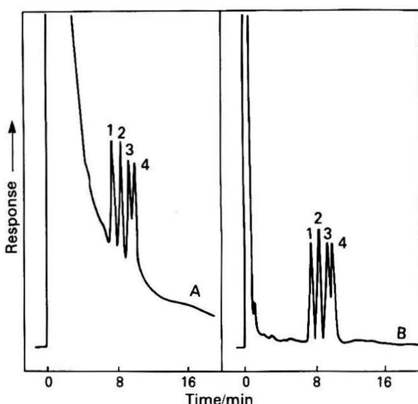

Fig. 3. Gas chromatograms of chlordane A, before and B, after iron - acetic acid reduction. Peaks: 1, oxychlordane; 2, *trans*-chlordane; 3, *cis*-chlordane; and 4, *trans*-nonachlor

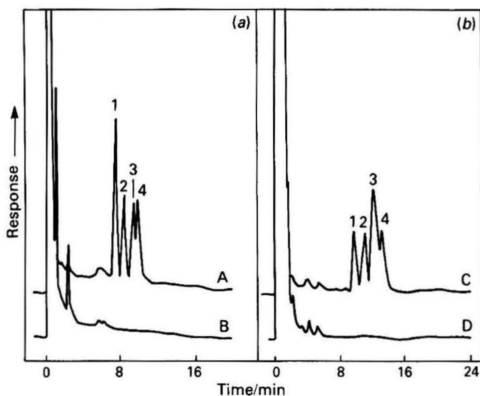

Fig. 4. Gas chromatograms of chlordane in human blood using glass columns packed with (*a*) OV-101; and (*b*) OV-17 + QF-1. A and C, sample with added chlordane; B and D, control. (*a*) Peaks: 1, oxychlordane - heptachlor epoxide; 2, *trans*-chlordane; 3, *cis*-chlordane; 4, *trans*-nonachlor. (*b*) Peaks: 1, heptachlor epoxide; 2, oxychlordane; 3, *trans*-chlordane - *trans*-nonachlor; 4, *cis*-chlordane

unchanged in other solutions. γ-Chlordene was completely degraded in acid mixtures with compositions between 8 + 2 and 2 + 8, but partly remained in other solutions.

The effect of the reaction temperature was examined in 10 °C increments from 0 to 70 °C. It was found that *cis*- and *trans*-chlordane and *trans*-nonachlor were degraded slightly above 40 °C. Oxychlordane was stable below 20 °C, but increasingly degraded with increase in temperature above 30 °C. At 70 °C, more than 90% of oxychlordane was degraded, as described by Holdrinet.[12] Fig. 1 shows gas chromatograms of a mixture of organochlorine pesticides (A) before and (B) after the treatment with the 1 + 1 *V*/*V* acid mixture at 0 °C. Isomers of HCH (α-, β-, γ- and δ-) and heptachlor epoxide remained unchanged. Fig. 2 shows gas chromatograms of polychlorinated biphenyls (A) before and (B) after the acid treatment. The acid treatment removes organochlorine pesticides and polychlorinated biphenyls, which give peaks overlapping those of chlordane and heptachlor epoxide on the gas chromatogram.

Continuous Distillation

More than 90% of chlordane was recovered from both water and aqueous acid mixtures within 30 min by the continuous distillation procedure. The total recovery was constant after 60 min of boiling. Therefore, the distillation time was set at 90 min. No appreciable degradation or loss of chlordane was observed.

Removal of Interfering Compounds by Reduction

When an aliquot of the heptane extract from continuous distillation was injected into the gas chromatograph, tailing of the "solvent" peak was sufficient to interfere with the determination of chlordane (Fig. 3A). The interfering electron-capturing compounds are presumed to be nitro compounds produced by the mixed acid treatment.[19] It was found that this interference could be removed by reduction with a metal in acid. Zinc, tin and iron were each investigated as reducing agents at pHs from 2.2 to 8. Iron in 10% acetic acid (pH 2.4) was found to remove the interfering "solvent" peak tailing without affecting the oxychlordane, *cis*- and *trans*-chlordane or *trans*-nonachlor peaks (Fig. 3B).

Determination of Chlordane in Human Blood

Several methods for the extraction of organochlorine pesticides have been reported. Dale *et al.*[20] showed that there was a difference in *in vitro* and *in vivo* binding and that DDT and related materials present *in vivo* were more difficult to extract from blood. A sample of human blood containing chlordane is desirable for comparing the extraction recovery, but it is difficult to obtain a large amount of such a blood sample. Therefore, the efficiency of extraction of β-HCH and *p,p'*-DDE was compared by several reported methods[9,21–26] and by the present method with treatment of the sample, before extraction, with either water (method 1) or ethanol (method 2), as these two compounds behave in the same manner as chlordane on extraction [8–9,27] and are widely found in human blood. The extract was cleaned up by silca gel column chromatography before the determination by gas chromatography.

Table 1 shows the results. There is no great difference between the methods, but method 1 is simple and of higher efficiency than the others. Therefore, we pre-treated the blood with water and used acetone - hexane (1 + 9 *V*/*V*) as the extraction solvent, and examined the usefulness of the acid treatment, the continuous distillation and the reduction for the clean-up of the blood extract. The recovery of *cis*-chlordane, *trans*-chlordane, *trans*-nonachlor, oxychlordane and heptachlor epoxide shown in Table 2 is satisfactory. Fig. 4

Table 1. Efficiency of extraction of β-HCH and *p,p'*-DDE residues in human blood using different extraction methods

Reference	Pre-treatment before extraction	Extraction solvent	β-HCH* Mean ± s.d./ μg kg^{-1}	β-HCH* Coefficient of variation, %	*p,p'*-DDE* Mean ± s.d./ μg kg^{-1}	*p,p'*-DDE* Coefficient of variation, %
This work (method 1)	Water	Acetone - hexane (1 + 9)	5.1 ± 0.1	2.3	4.5 ± 0.2	2.7
This work (method 2)	Ethanol	Acetone - hexane (1 + 9)	5.1 ± 0.2	3.1	4.4 ± 0.2	4.2
9	Ethanol + acetone	Hexane	5.2 ± 0.1	2.0	4.4 ± 0.3	4.8
21	60% sulphuric acid	Acetone - hexane (1 + 9)	4.8 ± 0.2	4.3	3.8 ± 0.1	1.7
22	Formic acid	Acetone - hexane (1 + 9)	4.8 ± 0.1	1.3	4.6 ± 0.1	2.3
23	None	Acetone - hexane (1 + 9)	4.8 ± 0.1	1.2	3.7 ± 0.1	1.6
24	Methanol	Hexane - diethyl ether (1 + 9)	5.0 ± 0.1	1.2	3.9 ± 0.1	2.7
25	Water	Ethyl acetate	4.6 ± 0.3	4.7	3.9 ± 0.1	1.7
26	Acetonitrile + ethanol	Hexane	3.6 ± 0.1	1.6	4.6 ± 0.1	2.3

* Three determinations.

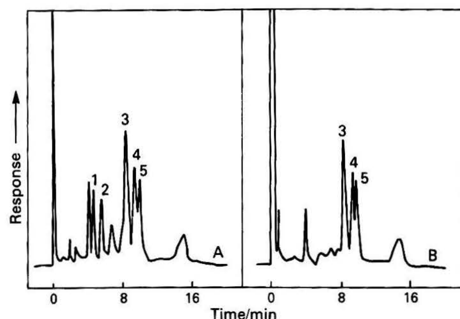

Fig. 5. Gas chromatograms of technical chlordane A, before and B, after the acid treatment. Peaks: 1, heptachlor; 2, γ-chlordene; 3, *trans*-chlordane; 4, *cis*-chlordane; 5, *trans*-nonachlor

shows gas chromatograms of control human blood with and without added chlordane. Interfering peaks are not observed on the gas chromatogram of a blood extract without added chlordane.

Fig. 5 shows the gas chromatograms before and after acid treatment of technical chlordane. *trans*-Nonachlor, *cis*-chlordane and *trans*-chlordane remained unchanged, but heptachlor and γ-chlordene disappeared after acid treatment. Oxychlordane and heptachlor epoxide as metabolites also remained unchanged after acid treatment. Kawano *et al.*[7] determined chlordane and its metabolites in the blood of pest control operators exposed to chlordane, and reported that *trans*-nonachlor, *cis*-chlordane, *trans*-chlordane and the metabolites oxychlordane and heptachlor epoxide were more persistent and, of the component chemicals of chlordane, heptachlor and γ-chlordene did not remain in human blood. It was interesting that the chlordane components that remained unchanged after acid treatment were more persistent than the changed components in human blood.

Conclusion

Acid treatment with a mixture of concentrated sulphuric acid and fuming nitric acid (1 + 1 *V/V*) at 0 °C for 15 min successfully removed compounds that interfere in the gas chromatography of chlordane. Oxychlordane, a metabolite of chlordane, was not reactive under these conditions. Continuous distillation was effective for extracting chlordane and also organochlorine pesticides and polychlorinated biphenyls, into a small amount of heptane. Reduction with iron in acetic acid removed interfering compounds produced by the treatment with mixed acids. Chlordane was not dechlorinated by this reduction. This method was suitable for rapid and efficient clean-up prior to the determination of chlordane in the presence of interfering organochlorine compounds.

This method was applied to the determination of chlordane in human blood. The chlordane in blood was pre-treated with water, chlordane was extracted with acetone - hexane (1 + 9 *V/V*) and the extract was treated by the mentioned method. The recovery of chlordane added to human blood was satisfactory.

Table 2. Recovery of chlordane added to human blood by the proposed method

	Fortification level/ μg kg^{-1}	Recovery* Mean ± s.d., %	Recovery* Coefficient of variation, %
trans-Chlordane ..	10	96.5 ± 1.2	1.1
cis-Chlordane	10	96.3 ± 1.9	1.8
trans-Nonachlor ..	10	94.8 ± 2.4	2.2
Oxychlordane	10	89.0 ± 1.5	1.5
Heptachlor epoxide ..	10	88.5 ± 1.1	1.1

* Five determinations.

The authors thank Dr. H. Inoue, Aichi Prefectural Institute of Public Health, for his support and Dr. Y. Sakabe, Nagoya City Health Research Institute, for supplying the standards.

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Paper A4/252
Received July 30th, 1984
Accepted October 4th, 1984

Determination of Sulphides in Water and Effluents Using Gas Chromatography

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A gas-chromatographic method is described for the determination of sulphides in water and effluents, using a flame photometric detector. The results obtained do not differ significantly from those obtained using the standard acid displacement procedure.

Keywords: *Sulphide determination; gas chromatography; effluents*

The determination of sulphides in sewerage systems is important because of the potential for toxic atmospheres and corrosion of sewer fabrics. In a polluted watercourse, sulphide is a toxic nuisance and is a measure of the prevalence of anaerobic conditions. The problems cited arise from the liberation of hydrogen sulphide, which is odorous, toxic and liable to biochemically mediated oxidation to sulphuric acid.[1] For these reasons, control authorities tend to determine sulphide species in terms of their capacity to liberate hydrogen sulphide. The Department of the Environment's Standing Committee of Analysts has recently endorsed the traditional acid displacement procedure,[2] in which hydrogen sulphide, liberated by acidification, is displaced in a stream of carbon dioxide and titrated after absorption in zinc acetate solution. This procedure probably receives wider application than Pomeroy's methylene blue method or the ion-selective electrode method, both of which are published in reference 2. The procedure described below is based on a field method for the determination of sulphides,[3] in which the concentration of hydrogen sulphide vapour in equilibrium with dissolved sulphide is measured with gas analysis tubes. In the following procedure, solution vapour equilibrium is achieved under conditions of acidity that approximate to those of the standard acid displacement procedure. Thus, the forms of sulphide determined may be described as "total hydrogen sulphide liberated by acidification of the sample." The concentration of hydrogen sulphide vapour is determined by gas chromatography, using the flame photometric detector. The conditions for the GC determination are based on the work of Bruner *et al.*[4]

The procedure presented in this paper is shown to produce results that do not differ significantly from those obtained using the standard acid displacement procedure. The GC procedure is also considerably faster to use, *i.e.*, after preparation of reagents, a four-point calibration graph and ten samples may be processed in 1 h. In the acid displacement procedure, the process time for a single sample is 1.25 h and during this period the apparatus requires frequent attention from the operator.

Experimental

Scope and Field of Application

The procedure specifies a gas-chromatographic method for the determination of total hydrogen sulphide liberated by acidification of the sample. The procedure is applicable to water, sewage and effluents containing 0–2.0 mgl^{-1} of sulphide and samples containing higher concentrations of sulphide may be analysed after dilution with water. A wider working range may be obtained with alternative GC equipment (see Discussion).

Principle

The sample is acidified in a partly filled screw-top bottle and the hydrogen sulphide is brought to solution - vapour equilibrium by shaking the bottle. The hydrogen sulphide in the headspace is analysed by gas chromatography using a flame photometric detector. The concentration of hydrogen sulphide is determined by interpolation on a calibration graph prepared using a standard sulphide suspension.

Materials

Caution—Hydrogen sulphide is extremely toxic; disposal of reagents and cleaning of glassware should be carried out in a fume cupboard.

Carrier gas and auxiliary gases

Nitrogen, hydrogen and air were all of high-purity grade.

Reagents

Analytical-reagent grade chemicals and de-ionised water were used throughout.

Sodium sulphide nonahydrate.

Sodium carbonate solution, 0.75 M. Dissolve 80 ± 5 g of anhydrous sodium carbonate in water and dilute to 1 l.

Zinc acetate solution 0.5 M. Dissolve 110 ± 5 g of zinc acetate dihydrate in water. Add 1.0 ± 0.1 ml of hydrochloric acid (sp. gr. 1.19) and dilute to 1 l with water.

Potassium iodate - iodide solution, 0.00417 M. Dissolve 0.8920 ± 0.0005 g of potassium iodate (previously dried for 1 h at 110 °C), 8.7 ± 0.1 g of potassium iodide and 0.6 ± 0.1 g of sodium hydrogen carbonate in water and dilute to 1 l.

Sodium thiosulphate solution, 0.25 M. Dissolve 62.50 ± 0.01 g of sodium thiosulphate pentahydrate in water and dilute to 1 l. Store in a dark glass bottle and add 1 ml of chloroform as a preservative.

Sodium thiosulphate solution, 0.0125 M. Dilute 50.00 ± 0.05 ml of 0.25 M sodium thiosulphate solution to 1 l. Add 1 ml of chloroform as a preservative and store in a dark glass bottle. Standardise this solution against 0.00417 M potassium iodate - iodide solution using starch glycollate indicator.[2]

Acid chloride solution. Dissolve 10 ± 1 g of sodium chloride in water. Add 415 ± 5 ml of hydrochloric acid (sp. gr. 1.19) and dilute to 1 l.

Standard sulphide suspension, approximately 10 mg l^{-1} *S.* Wash crystals of sodium sulphide nonahydrate with water and dry on a filter-paper. Quickly weigh 0.09 ± 0.005 g and dissolve in water by swirling. When solution is complete, add 20 ± 1 ml of 0.75 M sodium carbonate solution and mix. Add

20 ± 1 ml of 0.5 M zinc acetate solution, mix and dilute to 1 l. This suspension is stable for at least three weeks at ambient temperature.

Apparatus

Gas chromatograph

The following conditions were used: a Pye GCV gas chromatograph with flame photometric detector in the sulphur mode with an injector temperature of 100 °C, column oven temperature of 50 °C, detector temperature of 120 °C and nitrogen flow-rate of 30 ml min^{-1}; a detector air pressure of 1.2 kg cm^{-2}; a detector hydrogen pressure of 0.9 kg cm^{-2} (corresponding to air and hydrogen flow-rates of 28 and 25 ml min^{-1}, respectively); and an amplifier range of 1024×10^2. The steel tubing between the column and detector was replaced with PTFE tubing.

Injection device

An all-PTFE six-port injection valve (Rheodyne Model 5020) was used. The septum injection system was removed from the GC and the outlet from the valve was connected directly to the column, via a 1/16–1/4 in adaptor; 1/16 in PTFE tubing and Altex HPLC fittings were used throughout. A female Luer fitting was connected to the valve inlet. The sample loop was of approximately 100 µl capacity. The exact volume was calculated by filling the loop with methane and comparing the flame-ionisation detector response with that obtained by accurately measured syringe injections of methane. The loop was filled using a 1-ml PTFE gas-tight syringe. The sample bottle septum was pierced with stiff wire and a 6 cm length of 1/16 in o.d. PTFE tubing was inserted into the septum. Connection between the PTFE tubing and the male Luer fitting on the gas-tight syringe was made by means of a silicone-rubber sleeve. The opening in this sleeve was blocked with a PTFE plug after sampling the vapour.

Column and packing

A pre-packed glass column, 6 ft × 2 mm i.d., filled with 60–80 mesh Carbopack B with a 1.5% XE-60 and 1.0% phosphoric acid stationary phase was used. This column is manufactured by Supelco Inc. and is available from R. B. Radley and Co. Ltd., Sawbridgeworth, Hertfordshire. The column was conditioned for 18 h at 100 °C with a carrier gas flow-rate of 20 ml min^{-1} before use.

Recorder

A Servoscribe 1S recorder with a full-scale deflection of 2 V and a chart speed of 300 mm h^{-1} was used.

Analysis bottles

Soda glass Winchester style bottles of 100 ml volume were used.

Shaker

A Stuart flask shaker was used.

Procedure

Stabilisation of samples

The sample bottle must contain 2 ml of 0.75 M sodium carbonate solution per 100 ml of sample volume. Add 2 ml of 0.5 M zinc acetate solution per 100 ml of sample volume, cap the bottle and mix by inversion.

Standardisation of sulphide suspension

Mix the suspension thoroughly and quickly transfer 100 ± 1 ml into a centrifuge tube. Centrifuge for 5 ± 1 min at 2000 rev min^{-1} and discard the supernatant. Place 10.00 ± 0.02 ml of 0.004 17 M potassium iodate - iodide solution in a conical flask and add 2.0 ± 0.2 ml of dilute hydrochloric acid (1 + 1). Break up the zinc sulphide in the centrifuge tube with a glass rod and wash into the conical flask with water. Mix by swirling and titrate the excess of iodine with 0.0125 M sodium thiosulphate solution. Record the titre (T_1). Carry out a blank titration without the sulphide suspension and record the titre (T_2). Then, the sulphide concentration = $F(T_2 - T_1)$ where F is the sodium thiosulphate factor.

Preparation of standards and samples for analysis

Allow the samples and standards to reach ambient temperature before commencing the analysis. Prepare dilute sulphide suspensions with nominal concentrations of 2.0, 1.5, 1.0 and 0.5 mg l^{-1} using the following procedure. Mix the standard sulphide suspension thoroughly* and pipette 10.0, 7.5, 5.0 and 2.5 ± 0.1-ml aliquots into each of five analysis bottles. Dilute each aliquot to 50 ± 1 ml with water. Add 5 ± 0.5 ml of acid chloride reagent to each bottle and immediately cap the bottle tightly after each addition. Secure each bottle in the flask shaker and shake for 2 ± 0.2 min at speed 1. The sulphide concentration of reagents and water is negligible and it is not necessary to prepare a blank solution. Prepare an approximately 5 mg l^{-1} standard, by following the above procedure with 25 ml of sulphide suspension (using a measuring cylinder). This standard is used to condition the apparatus.

Remove the bottles from the shaker and ensure that the ambient temperature does not change by more than 2 °C between shaking and analysis. The concentration of hydrogen sulphide in the headspace is stable for at least 1 h. Samples are prepared for analysis by following the above procedure and using 50 ± 0.1 ml of sample (or an appropriate dilution in water) in place of the standard sulphide suspension.

GC analysis

Set up the GC and allow it to stabilise for 2 h before making injections, which are carried out using the following procedure. Insert the PTFE tube, described above, into the sample bottle septum. Fit the gas-tight syringe to the silicone-rubber sleeve and flush the syringe by filling to the 1-ml mark and emptying three times, without disconnecting the syringe. On the final fill, empty the syringe to 0.6 ± 0.05 ml and immediately connect to the Luer fitting on the gas sampling valve. Fill the sampling loop and immediately turn to the injection position. The retention time of hydrogen sulphide is 0.6 min and a peak height of at least 0.5 full scale should be obtained with 2 V full-scale deflection.

Condition the apparatus by making three injections of the 5 mg l^{-1} S standard headspace. Make duplicate injections of the remaining standards and samples. Make three injections of the 5 mg l^{-1} S standard headspace, before every batch of analyses, to ensure that the sensitivity of the hydrogen sulphide peak is maintained.†

Calculate the mean peak height for standards and samples. Plot a calibration graph of log(peak height) *versus* log (sulphide concentration) and obtain the concentration of hydrogen sulphide liberated by acidification, after calculating the exact concentration of sulphide in the standards.

* The suspension settles rapidly and it is advisable to maintain constant agitation, *e.g.*, by placing the flask on a magnetic stirrer.

† If sulphide injections are discontinued for 30 min, the sensitivity of the hydrogen sulphide peak falls by approximately 10%. Sensitivity is maintained if the 5 mg l^{-1} S headspace is injected before each fresh batch of analyses. This is also important when a batch of low or zero sulphide samples is analysed.

Table 1. Determination of sulphide by the proposed and standard procedures

Sample	Hydrogen sulphide liberated by acidification: Standard procedure (mean of two results)/ mg l^{-1} S	Proposed procedure (mean of two results)/ mg l^{-1} S	Mean bias of proposed procedure/ mg l^{-1} S
Septic tank effluent	13.48	13.85	+0.37
Septic sewage	11.19	10.25	−0.94
Septic sewage	6.41	6.31	−0.10
Tip effluent	2.40	2.23	−0.17
Trade effluent (abattoir)	3.80	3.80	0.0
Tap water spiked with sulphide standard to give 6.36 mg l^{-1} S	5.82 (89% recovery)	6.43 (101% recovery)	+0.61
Tap water spiked with sulphide standard to give 2.52 mg l^{-1} S	2.04 (81% recovery)	2.39 (95% recovery)	+0.35

Performance Characteristics

Precision

The within-batch precision was calculated by making 100-µl injections of the headspace above different portions of each of the nominal 2.0 and 0.5 mg l^{-1} S standards. The relative standard deviations at the former and latter concentrations were 4.8 and 13.8%, respectively, for five degrees of freedom. The precision of the injection technique was calculated from repeated 100-µl injections of the headspace above the 2 mg l^{-1} S standard and found to be 1.1% relative standard deviation for six degrees of freedom.

Bias

A range of samples were analysed in different batches on different days by the proposed procedure and by the standard acid displacement procedure.[2] Samples for comparative analyses were drawn concurrently from the same portions of stabilised samples. All analyses were carried out in duplicate. The recovery of sulphide from the standard sulphide suspension, which was analysed by the standard method with every batch of determinations, was found to fall in the range 92–95%. The relative standard deviation of the determination of sulphide in the standard sulphide suspension was 2.2% for four degrees of freedom. The corresponding statistic for an effluent sample containing 3.80 mg l^{-1} S was 7.6% for five degrees of freedom. The results of bias tests are presented in Table 1.

Effect of Potential Interfering Substances

The following types of potential interferents were investigated: 1, substances that might affect solution vapour equilibrium of hydrogen sulphide, *e.g.*, surfactants; 2, sulphur-containing vapours that might interfere with the GC determination, *e.g.*, SO_2; and 3, substances that are unstable in the presence of sulphide but are known to give interferences in the standard procedure, *e.g.*, thiosulphates and metabisulphites. It should be noted that the effect of sewage constituents on the solution vapour equilibrium of hydrogen sulphide has already been investigated.[3] Further, the acid chloride reagent has been formulated with a high ionic strength to minimise disturbance of solution vapour equilibrium due to variations in the ionic strength of samples. The results of the effect of potential interfering substances are presented in Table 2.

Table 2. Effect of potential interfering substances on the determination of sulphide by the proposed procedure

Substance	Alteration to response of 1 mg l^{-1} sulphide standard at specified concentration of interferent: 10 mg l^{-1}	100 mg l^{-1}	1000 mg l^{-1}
Mineral oil	N.s.*	N.s.	N.s.
Anionic detergent (as Manoxol OT)	N.s.	N.s.	N.s.
Sulphite	N.s.	N.s.	N.s.
Thiosulphate (as sodium thiosulphate)	−50%	−60%	−100%
Metabisulphite (as sodium metabisulphite)	−50%	−60%	−100%

* N.s. = not significant (P = 0.05).

Discussion

Development of the Method

The choice of column was based on obtaining a short retention time for hydrogen sulphide and adequate resolution of sulphur dioxide, and other sulphur-containing gases. The particular combination of column and detector has been applied to the analysis of sulphur compounds at parts per billion levels by introducing a 10-ml sample volume via a gas sampling valve.[4,5] As the detection limit of the standard method is aproximately 0.25 mg l^{-1} S, it was decided to work at as high a range as possible for the GC method in order to minimise sampling and dilution errors. Using a 100-µl sample, the detection limit in aqueous solution corresponds to approximately 0.2 mg l^{-1} S and the detector becomes saturated at 2.5 mg l^{-1} S. These concentrations in aqueous solution correspond to approximately 40 and 500 mg l^{-1} of hydrogen sulphide in the vapour phase.[6] For these reasons, a calibration range of 0–2.0 mg l^{-1} S in aqueous solution was selected. Using a 100-µl injection, the detection limit is determined by loss of hydrogen sulphide in the apparatus when the vapour phase concentration is less than 40 mg l^{-1} S. Calibration and analysis have also been carried out using the Packard 439 GC system. A linear range of 0–50 mg l^{-1} S was obtained.

When the calibration data were plotted as square root (peak area) *versus* sulphide concentration (mg l^{-1}) a curved graph was obtained. At the concentrations studied, the vapour distribution of the hydrogen sulphide is expected to be a linear function of the aqueous sulphide concentration.[6] The curved calibration graph was therefore attributed to a characteristic

of the detector's response to hydrogen sulphide. Mowery and Benningfield[7] have pointed out that a linear calibration graph may be obtained over a limited range by using a log(peak area) *versus* log(hydrogen sulphide concentration) plot. The data obtained during these experiments gave linear calibrations when plotted on log - log graph paper.

The method was originally developed using a syringe injection technique. This procedure proved accurate but was abandoned for the following reasons. The column has a high back pressure and unless the plunger was held down while making injections, the carrier gas would displace the plunger and blow hydrogen sulphide back into the syringe. This problem was aggravated when the plunger moved freely after repeated use and under these circumstances, it was likely that some gas was leaking around the plunger at all times. However, a more serious problem was caused by the rapid corrosion of the needles. Sulphide deposits rapidly blocked the bore and necessitated frequent cleaning of the syringe. The injection device described in this procedure is designed for use in liquid chromatography and has a maximum working pressure of 300 lb in^{-2}. No problems were experienced in its application to GC. The simple but effective means of withdrawing sulphide vapour from the sample bottle was used because of the absence of a suitable commercially available inert fitting.

The need to prime the column by repeated injections of hydrogen sulphide vapour does not appear to have been reported by other workers; however, the importance of using PTFE tubing has been stressed.[5] The need to operate the detector at as low a temperature as possible has also been mentioned.[5,8] The workers all cited loss of hydrogen sulphide at metal surfaces as the reason for taking these precautions. Adsorption and decomposition of low concentrations of sulphide within the column and detector are also mentioned. It was concluded that priming served to saturate the column with sulphide. It was considered that the 10% reduction in sensitivity when injections were discontinued for 30 min would not affect the accuracy of the method. The hydrogen sulphide retention time is approximately 35 s and if a large batch of zero sulphide samples are analysed, it is feasible to maintain sensitivity by regularly injecting the highest standard. By leaving the oven door open, it was possible to maintain a column temperature of 30 °C. At this temperature, the need to prime the apparatus was almost eliminated. However, depending on the ambient temperature, it is probably not feasible to maintain a column temperature of less than 50 °C with the GC used under conditions of normal operation.

Precision and Accuracy

It is considered that a major source of error in both the proposed and standard methods lies in removing an aliquot from the stabilised sample. The precipitated zinc sulphide settles rapidly and, in real samples, there is a wide distribution of particle shape and size. The results presented in Table 1 represent the analysis of typical effluent samples containing several hundred milligrams per litre of suspended solids.

Application of a paired sample t-test to the results in Table 1 indicates that there is no significant difference between the two methods ($P = 0.05$). The results of analysis of the spiked samples, when considered in isolation, suggest that the proposed procedure gives a higher recovery of sulphide than the standard method. This hypothesis is not supported by examination of the data for effluents where the average bias for all samples is -0.17 mg l^{-1} S. This suggests that recovery of hydrogen sulphide from an acidified zinc sulphide suspension may not represent the recovery obtained from real samples.

Inspection of Table 2 confirms that species such as oils or detergents, which affect the surface properties of a liquid, do not interfere significantly in the solution - vapour distribution of hydrogen sulphide. The potential interference of sulphur dioxide in sulphide analysis is well known. The sulphur dioxide peak (retention time 1.1 min) was well separated from hydrogen sulphide and no interference was observed. The results for metabisulphite and thiosulphate confirm that sulphides are unstable when these species are present in acid solution. Tests with pure metabisulphite and thiosulphate solutions confirmed that there is no positive bias in the absence of sulphide.

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Paper A4/310
Received September 3rd, 1984
Accepted October 25th, 1984

Sorption Studies of Chromosorb Porous Polymers and their Potential Use in Passive Monitors*

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The sorptive behaviour of Chromosorb Century series porous polymers has been investigated using nitrogen, octane, benzene and trichloroethylene sorptives. Similar data have been obtained for the carbons used in commercially available passive monitors. The Chromosorbs, in many instances, show a significantly higher surface area with organic sorptives than with nitrogen but the carbons generally exhibit the highest surface area with nitrogen adsorbate. The data are interpreted in terms of a partition mechanism operating in addition to adsorption. It is suggested that this effect could indicate potential advantages of using Chromosorbs rather than carbons in passive monitors; in particular, it may help to reduce any losses by desorption from the surface during sampling.

Keywords: *Sorption studies; Chromosorb porous polymers; passive monitoring*

The use of passive monitors potentially provides a ready means of assessing individual exposure to vapours of organic compounds. The device is entirely self contained and is essentially very simple—indeed this is one of its major attractions. A bed of sorbent is separated from the external atmosphere by an air gap. Diffusion of molecules from the exterior atmosphere across the air gap to the surface of the sorbent can, at equilibrium, be defined by Fick's first law of diffusion:

$$W = -DA\frac{dc}{dx} \quad \quad \ldots \ldots \ldots (1)$$

where W is the rate of mass transfer; D is the diffusion coefficient of the organic compound in air; A is the cross-sectional area of the diffusion path; and dc/dx is the instantaneous rate of change in concentration along the diffusion path (x is the position in the diffusion path).

If $(C_1 - C_0)$ is the change in concentration along the diffusion path length $-L$ (*i.e.*, $x_1 - x_0$) then equation (1) becomes

$$W = D\frac{A}{L}(C_1 - C_0) \quad \quad \ldots \ldots \ldots (2)$$

where L is the diffusion path length; C_1 is the concentration of organic vapour in the exterior atmosphere; and C_0 is the concentration of organic vapour in the air space immediately above the sorbent surface.

If both sides of equation (2) are multiplied by time t and it is assumed that the concentration of organic vapour above the sorbent surface is zero, equation (2) becomes

$$M = D\frac{A}{L}C_1t \quad \quad \ldots \ldots \ldots \ldots (3)$$

where M is the total mass of substance taken up by the sorbent and t is the time for which the monitor is exposed. Thus the mass of substance taken up by the sorbent is directly related to the concentration of the substance in the air sampled and the time of exposure. The factors A and L are determined by the physical construction of the monitor and remain constant. The diffusion coefficient, D, may be obtained from the literature and is constant at constant temperature.

Passive monitors, or diffusive samplers, have been described by various workers.[1–6] Several designs of passive monitor are now commercially available and these generally use carbon or carbon-based materials as sorbent. The measure of exposure to organic vapours is obtained by desorbing the sorbed compounds followed by analysis of the sorbate. The majority of methods use solvent desorption with carbon disulphide and gas-chromatographic analysis of the carbon disulphide solution; however, thermal desorption is an increasingly popular method of treatment,[7] again followed by gas-chromatographic analysis.

Relatively little attention has been given to the precise nature of the sorbent in passive monitors. Active carbon was used by analogy to the active method of drawing air by a pump through a tube containing a bed of the material. Although active carbon has been the preferred adsorbent in active sampling and has been shown to perform well, it may not be the most appropriate adsorbent for passive monitoring. In particular, the justification for supposing that passive monitors are useful samplers makes the assumption that the concentration of organic vapour above the sorbent surface is zero. This implies that the sorbent is ideal and that no vapour pressure of the substance exists at equilibrium. Such an assumption is not justified in absolute terms and the success of a sampler may be related to how close an approximation to the ideal is achievable. Adsorption on to a carbon-based adsorbent is a purely surface phenomenon and the adsorbed species undoubtedly exhibit a finite vapour pressure.

Surface-area measurements are usually obtained by producing a monolayer of adsorbate on the surface of the sorbent being studied. If the mass of adsorbate forming the monolayer is known together with the surface area of the individual adsorbate molecule it is possible to calculate the area covered by the sorbed molecules and hence the sorbent surface area.

Although a nitrogen adsorbate is normally used there is no restriction provided a sufficient vapour pressure of a suitable sorptive can be generated. In this study organic adsorbates, specifically benzene, octane and trichloroethylene, were used. Their monolayer coverage was determined and, with estimates of molecular surface area, the resulting apparent surface area was calculated.

These experiments were carried out on the Chromosorb Century series polymers produced by Johns Manville (Denver, CO) and, for comparison, samples of active carbon were taken from commercially available passive monitors.

* Paper presented at Analyticon 83, London, 12–14th October, 1983.

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Experimental

The adsorption of nitrogen, benzene, octane and trichloroethylene vapour on the porous polymers was studied. A number of carbon samples from commercially available passive monitors were also examined for comparison.

Nitrogen adsorption studies were carried out on a Carlo Erba Sorptomatic Series 1800. Between 0.2 and 0.5 g of the solid was accurately weighed and out-gassed overnight at 150 °C under a vacuum of 2×10^{-4} Torr. After out-gassing the sample was inserted into the liquid nitrogen cooling bath and the determination carried out.

The technique was of the statio-volumetric type where the gas volume adsorbed or desorbed was calculated by measuring the pressure variation resulting from the adsorption or desorption of a known volume of nitrogen.

At the onset of a determination small incremental volumes of nitrogen were added in order to obtain several points in the region of monolayer formation. Differential adsorption pressure, P, was recorded as a function of the nitrogen volume introduced, V_0. P/P_0 was plotted against $[P/(P_0-P)]V_a$ where P_0 is the saturation pressure and V_a is the volume of gas adsorbed per gram of adsorbent.

A straight line was obtained and from the slope and intercept the monolayer capacity, V_m, was calculated from $V_m = 1/(\text{slope} + \text{intercept})$.

Having obtained V_m the BET surface area was calculated using the equation

$$S_{BET} = \frac{V_m N_0 A_m}{M_v}$$

where S_{BET} is the specific surface area; N_0 is Avogadro's constant; A_m is the average area occupied by the adsorbate molecule in the completed monolayer, which was taken to be 16.2 Å^2; and M_v is the gram molecular volume.

Adsorption studies of the organic vapours were carried out using a Cahn RG electromicrobalance. This recorded the uptake of the adsorbate by the sorbent as a function of the generated pressure of adsorptive.

All sample masses were in the range 20–40 mg and were out-gassed overnight at 150 °C under a vacuum of 4×10^{-4} Torr. The sample was then transferred into the instrument. Vapour of the organic adsorbate was passed from a reservoir of the liquid into the adsorption chamber. The mass of vapour taken up by the sorbent was recorded as was the pressure of vapour. Between 10 and 15 readings were taken at 30-min intervals covering a P/P_0 range of 0.02–0.8.

A straight line was obtained by plotting P/P_0 against $(P/P_0)/[W_a(1 - P/P_0)]$ where P/P_0 is the relative pressure; and W_a is the mass adsorbed per gram of sorbent. The monolayer capacity, B_m, was calculated from the equation $V_m = 1/(\text{slope} + \text{intercept})$ and subsequently the BET surface area was obtained from the equation

$$S_{BET} = \frac{V_m N_0 A_m}{M}$$

where M is the relative molecular mass of the adsorbate and A_m is the average molecular area of the adsorbate. This was taken to be 40 Å^2 for trichloroethylene, 61.0 Å^2 for octane and 43 Å^2 for benzene.

Results and Discussion

The surface-area determinations show many interesting comparisons and particularly highlight porous polymers that may be worthy of further study. The results are given in Table 1.

Values of surface area determined by nitrogen adsorption were generally in agreement with the literature values.[8,9] The exception was Chromosorb 105 where the measured surface area of 162 m^2 g^{-1} was significantly less than the literature value of 600–700 m^2 g^{-1}. The experimental value was checked by repeated determinations in the laboratory and by blind determination in another laboratory with the same result. If the sample was representative of Chromosorb 105, and there is no reason to suppose otherwise, it seems that the literature value quoted is in error.

Although the method of measurement was not recorded it must be assumed that the nitrogen-adsorption technique was used. With adsorbents it would be expected that the surface area determined with other sorptives would be less than that measured with nitrogen. The precise values would depend on the size and shape of the sorptive molecule relative to the pore size of the sorbent. As other adsorbate molecules are larger than nitrogen they would certainly find it more difficult to infiltrate the porous structure in the same way. Thus, when the carbon materials from commercial passive monitors were analysed the highest surface area was obtained with nitrogen, as the results given in Table 2 show. This is not so with the Chromosorbs.

Chromosorb 101 has a surface area of 23 m^2 g^{-1} with nitrogen but this increased to 109 m^2 g^{-1} with octane and 231 m^2 g^{-1} with benzene. There may be some errors introduced into the calculation by way of the assumption of values of molecular surface area for these two adsorbates, but the errors cannot be of sufficient magnitude to account for such variations. Even if there were an error of a factor of two in the calculation the surface area would still be several times the surface area with nitrogen. The explanation of these data would be the existence of a partition mechanism in addition to or instead of simple adsorption. This would permit uptake of the condensed vapour into the body of the porous polymer. A characteristic of such a mechanism would be a relatively slow approach to equilibrium and this is what is observed.

Chromosorb 101 has a low surface area and relatively large pores. The large pores mean that larger adsorbates can infiltrate the structure readily to the extent that most if not all

Table 1. Surface area of Chromosorb porous polymers measured with various adsorbates

	Literature value[8,9]		Polymer and surface area/m^2 g^{-1}			
Polymer type	Polymer surface area/m^2 g^{-1}	Average pore diameter/nm	Nitrogen	Octane	Benzene	Trichloroethylene
101	50	300–400	23	103	231	26
102	300–400	8.5	335	275	290	130
103	15–25	300–400	19	100	170	22
104	100–200	60–80	128	121	177	84
105	600–700	40–60	162	251	226	137
Molecular area of adsorbate/Å^2			16.2	61	43	40

Table 2. Surface area of carbons, from passive monitors measured with various adsorbates

Carbon sample	Carbon surface area/$m^2 g^{-1}$			
	Nitrogen	Octane	Benzene	Trichloro-ethylene
A	627	364	673	604
B	1337	744	1215	1180
C	1140	519	1187	1082
D	975	836	855	840

the surface accessible to nitrogen is accessible to larger molecules. The increase in the surface area measured with octane suggests that about three times as much organic material is present in the bulk of the sorbent as is present on the surface. For benzene there appears to be about nine times as much organic material dissolved in the bulk sorbent as is on the surface. The increased uptake of benzene over octane may be explained by the chemical structure of the porous polymer. As a styrene - divinylbenzene copolymer it might be expected to show a greater affinity for the aromatic benzene rather than aliphatic octane.

Comparison may be made with the sorption of trichloroethylene. The result for the surface area, 26 $m^2 g^{-1}$, is effectively the same as for nitrogen adsorption. As the surface area of the trichloroethylene molecule was of the same order as benzene and octane it would be expected to have similar accessibility. Such a relatively low surface area in these circumstances indicates little or no partition in this instance.

Chromosorb 102 has a much larger nitrogen surface area than 101, but much of this area is internal as a result of a great number of narrow pores. As the pores are so small some are inaccessible to larger molecules and thus other adsorbates produce smaller surface areas in comparison with nitrogen. However, there is still indication here that partition is an important mechanism. The surface areas of 275 and 290 $m^2 g^{-1}$ using octane and benzene, respectively, are little reduced from the nitrogen surface area determination. The value of 130 $m^2 g^{-1}$ using trichloroethylene suggests what might be produced in the absence of a partition mode of uptake. There are suggestions that the nature of the organic substances may be important although it cannot be assumed that 275 and 290 $m^2 g^{-1}$ are significantly different and could be a measure of the experimental error of the determinations.

Chromosorb 103 has much in common with Chromosorb 101. Once again there is a low surface area combined with large pores. The surface area obtained by nitrogen adsorption agrees with the literature value and that measured by trichloroethylene adsorption is the same within experimental error. Once again this shows the absence of a partition process for the sorptive. The surface areas using octane and benzene again show the influence of a partition process. There are large amounts of organic compounds present in the bulk of the polymer compared with those present at the surface; about four times as much for octane and eight times as much for benzene. Once again the aromatic benzene has a greater affinity for the aromatic polymer than does octane.

Chromosorb 104 and 105 can be treated in a similar way. Both have some pores that are inaccessible to the larger organic molecules. This is shown by the smaller surface areas determined using trichloroethylene sorptive. Partition results in a rather larger surface area for octane and benzene. For octane on Chromosorb 104 there is insuffient uptake by partition to produce a higher surface area than for nitrogen adsorption. Benzene on Chromosorb 104 and 105 and octane on Chromosorb 105 are sufficiently partitioned to result in higher apparent surface areas than for nitrogen adsorption not withstanding the reduced surface available as a result of narrow pores.

The foregoing discussion shows that for the Chromosorb porous polymers partition into the polymer matrix may be responsible for a large proportion of the total uptake, although the precise amount is a function of the individual sorbed species.

This phenomenon has implications for the use of these sorbents in passive monitors, particularly in comparison with active carbon adsorbents. For active carbon the adsorbed species remain on the surface of the adsorbent and there exhibit a vapour pressure that may, when sampling has proceeded for some time and the amount adsorbed is large, represent a significant fraction of the concentration of vapour being sampled from the ambient atmosphere. In such situations the assumption of zero or negligible concentration at the adsorbent surface could not be justified. In extreme cases there may be the possibility of diffusion in the reverse direction. The only means of amelioration would be the movement of adsorbate through the adsorbent bed away from the region of highest concentration. Equilibrium considerations would dictate that this happens, but under the influence only of a concentration gradient it would be exceedingly slow and of little significance in relation to a sampling or exposure time of a few hours for a typical passive monitor.

If porous polymers are used in passive monitors there may be significant advantage. Adsorption on to the surface of the polymer may be regarded as the first stage in the dissolution of the sorbate into the body of the polymer. When dissolution occurs it would act as a means of reducing the surface concentration of organic substance and so make the concentration in the air space above the sorbent a closer approximation to the assumed value. Thus passive monitors incorporating porous polymer sorbents would be operating more closely in line with the ideal conditions set out in the justification for their operation.

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Paper A4/309
Received September 3rd, 1984
Accepted October 25th, 1984

New Approach to the Simultaneous Determination of Pollutants in Waste Waters by Flow Injection Analysis

Part II.* Cationic Pollutants

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A simultaneous determination for three or more pollutants in waste waters by reversed flow injection analysis is presented. The insertion of several valves with different functions along the manifold allows a simple, fast and versatile photometric determination of pollutants using a single configuration. These features made this type of analysis suitable for monitoring polluting species in waste waters.

Keywords: *Pollutant determination; flow injection analysis; waste waters; simultaneous determination*

The analysis and monitoring of noxious species in waters, either because of their toxic nature or their high concentration, is an aspect of increasing importance owing to the present steady increase of pollution. Therefore, there is an urgent need for every country to have suitable laboratories, equipped with inexpensive instrumentation, which allows the determination of these pollutants to be carried out in a fast and cheap way without sacrificing precision. Flow injection analysis (FIA) is a technique that satisfies these requirements and can be afforded by most laboratories.

The reversed FIA mode (rFIA) is undoubtedly the most convenient for these requirements, because the waste water sample (generally abundant) is used as the carrier and a specific reagent is injected for each substance, thus saving an important amount of reagent. When the simultaneous determination of two or more pollutants is required, this mode attains even more practical interest, because the sequential introduction of several reagents into the water stream is easier than using them as carriers, thus risking mutual interaction.

In this paper, a continuation of an earlier one,[1] we report the simultaneous determination of several cationic pollutants in waste waters employing rFIA and suitably placing several valves (with different functions) at different points along the manifold. A single photometer is used as the detector, a specific reagent that provides the indicator reaction being injected for each species. No treatment or previous separation of the sample is required. Finally, a manifold is proposed that takes into account some of the systems optimised in this research to tackle the simultaneous determination of up to five species in the same water sample, drawing useful conclusions for this uncomplicated multiple determination.

Experimental

Reagents

All reagents were of analytical-reagent grade and solutions were prepared with distilled water.

Oxalic acid bis(cyclohexylidene hydrazide), "cuprizone" reagent. Dissolve 0.5 g in 100 ml of 50% *V/V* ethanol with heating.

Citric acid - ammonia solution. Dissolve 75 g of citric acid in 100 ml of water. Treat the solution with 95 ml of 25% ammonia solution and make up to 250 ml with distilled water.

*4,7-Diphenyl-*1,10*-phenanthroline (bathophenanthroline) reagent.* Dissolve 0.30 g of the reagent in 100 ml of ethanol - water (1 + 1 *V/V*).

Acetate - hydroxylamine mixture. Dissolve 10 g of sodium acetate and 10 g of hydroxylammonium chloride in 100 ml of water.

Eriochrome Cyanine R reagent. Dissolve 0.1 g in a small volume of water in a 100-ml calibrated flask, treat with 0.5 ml of 1 M hydrochloric acid and complete to volume with distilled water.

Acetic acid - sodium acetate buffer - thioglycollic acid mixture. Dissolve 27.5 g of ammonium acetate in 50 ml of distilled water, add 0.5 ml of hydrochloric acid and 0.2 ml of thioglycollic acid and make up to 100 ml with distilled water.

Copper, iron and aluminium standard solutions, each of 1.000 g l^{-1}. Prepared from metallic copper, iron and aluminium.

Apparatus

A Pye Unicam SP6-500 spectrophotometer connected to a Radiometer REC 80 recorder was used as the detector. A Gilson Minipuls-2 peristaltic pump, a Hellma 178.12 flow cell (inner volume 18 µl), a Tecator L 100-1 injection valve, two Omnifit 1103 eight-way valves and a Tecator TM II chemifold were also used.

Results and Discussion

Firstly the determinations of copper, iron and aluminium are considered separately, followed by a discussion of the assembly of the three manifolds into a single one for the simultaneous determination of the cations.

Single Determination of Cationic Pollutants

Copper

The complex formation reaction between Cu(II) and cuprizone [oxalic acid bis(cyclohexylidene hydrazine)][2] is used as an indicator reaction for the determination of copper. In a basic solution (ammonia solution) the reagent forms a blue soluble complex with maximum absorption at 595 nm. In the conventional manual photometric method citric acid is added to the samples to remove a large number of interfering substances.

The manifold shown in Fig. 1 corresponds to the single determination of pollutants by rFIA. The buffer, citric acid - ammonia (pH 8.5–9.0) in this instance, merges with the

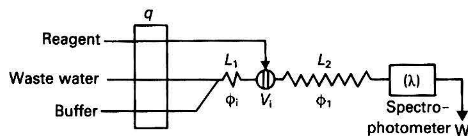

Fig. 1. Manifold for the single determination of cationic pollutants by rFIA

* For Part I of this series, see reference 1.

sample stream prior to the reagent injection. The optimum pH for the complex formation (between 7 and 10) is thus ensured; at the same time, the citrate ion present masks possible interfering species.

The values of the FIA variables optimised are shown in Table 1 and the concentration of the reagents employed is listed under Experimental. Under these conditions, trace amounts of Cu(II) can be determined in the range also shown in Table 1. Both the sensitivity and the precision of the method are good.

Iron

Bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) has been used to induce the indicator reaction with iron.[3] The absorption maximum of the Fe(II) - bathophenanthroline complex appears at 535 nm. Although iron is found in waste waters mainly as Fe(III), the buffer (acetic acid - sodium acetate) also contains hydroxylamine to reduce Fe(III) to Fe(II). The optimum concentration for the different reagents appears under Experimental. The optimum values of the FIA variables are shown in Table 1, which also shows the possibility of determining trace amounts of iron with good sensitivity and precision.

Aluminium

The formation of a complex between Al(III) and Eriochrome Cyanine R in slightly acidic medium[4] has been chosen in this instance as the indicator reaction (maximum absorption wavelength, 535 nm) and the manifold used is shown in Fig. 1. The optimum values for the different variables are listed in Table 1, and the concentration of the reagents appears under Experimental. Thioglycollic acid is added to the buffer to mask other cations, especially Fe(III), which interferes in the determination of aluminium. Note the high sensitivity of this determination (slope of the calibration straight line 0.730), which allows the detection of up to 0.06 µg ml^{-1} of Al(III) with an absorbance of 0.046 after subtracting the blank signal. This high sensitivity is unusual for a non-catalytic photometric method taking into account the dispersion inherent in the FIA technique. Excellent precision is also obtained.

Simultaneous Determination of Copper, Iron and Aluminium

Owing to the similarity in the optimum values of FIA variables for the single determinations of these cations, the assembly of the systems into a single manifold presents no difficulty and the loss in sensitivity relating to the optimum manifold for each single determination is small. The most dispersed variable is the wavelength because, although the same for iron and aluminium (535 nm), it is different from that of copper (595 nm). As the determination of copper is the least sensitive of all three, a wavelength of 575 nm (closer to that of copper) was chosen as a compromise condition for the simultaneous determination.

In order to simplify the final manifold by a method other than the introduction of a configuration with three in-series injection valves, similar to that used for the determination of anionic pollutants (see Part I), the manifold shown in Fig. 2 is suggested as an alternative. It has only one injection valve, but two selecting valves (R and B) for the suitable selection of reagent and buffer for each determination. Thus for the determination of cation A, the selecting valves should adopt a position that allows them to select the confluence of buffer B_1

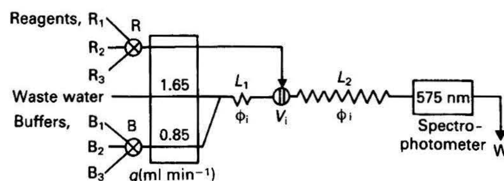

Fig. 2. Manifold for the simultaneous determination of cationic polluting species by rFIA

Table 1. Single and simultaneous optimisation and determination of polluting species

Operation	Copper	Iron	Aluminium	Copper, iron and aluminium
Optimisation:				
q/ml min^{-1}	2.13	2.72	2.67	2.54
V_i/µl	43.9	40.8	40.8	40.8
L_1/cm	35	35	35	35
L_2/cm	850	625	880	800
ϕ_i/mm	0.5	0.5	0.5	0.5
λ/nm	595	535	535	575
Determination:				
Equation*	$A = 0.178[Cu^{2+}] + 0.016$	$A = 0.221[Fe^{3+}] + 0.013$	$A = 0.730[Al^{3+}] + 0.002$	$A = 0.089[Cu^{2+}] - 0.044$ $A = 0.060[Fe^{3+}] + 0.044$ $A = 0.284[Al^{3+}] - 0.002$
Regression coefficient	0.999	0.999	0.999	0.999 0.998 0.999
Range/µg ml^{-1}	0.3–4.0	0.1–3.0	0.06–0.80	0.8–10.0 0.5–8.0 0.15–2.20
R.s.d.,† %	0.51	0.15	0.62	1.02 0.86 0.57
Concn. for r.s.d./µg ml^{-1}	1.5	1.5	0.2	3.0 3.0 0.25
Sampling frequency/h^{-1}	44	34	42	—

* Equations include the blank correction.
† R.s.d. from 11 different samples with triplicate injection.

(valve B) with the water stream (sample) and the loop of the injection valve should be filled with reagent R_1 (valve R). In this way, the consumption of reagent decreases with respect to the in-series configuration of the injection valves, as only the reagent and buffer corresponding to the cation analysed are consumed. The number of channels needed is also diminished, because the selecting valves are placed prior to the peristaltic pump. Finally, the simplification of the manifold makes automation much easier.

The values of the variables for this manifold are listed in Table 1, which also displays the equations of the calibration graphs for the simultaneous determination of copper, iron and aluminium, together with the detection range, regression coefficients and relative standard deviations.

Simulation of the Simultaneous Determination of Copper, Iron and Aluminium in Waste Waters

To simulate the periodical monitoring of these pollutants in a waste water, several synthetic water samples were prepared containing different ratios of these three cations. The recordings obtained are shown in Fig. 3, and the corresponding results are summarised in Table 2. The errors in these determinations are not very large taking into account that it is a simultaneous determination of three cations at low concentrations (trace level).

The data obtained by the method suggested were compared with those found by atomic-absorption spectrophotometry. For that purpose, a simulation to monitor the concentration of these pollutants (Cu, Fe and Al) was carried out on a hypothetical sample of waste water during a working day. The procedure was as follows. A 40-ml volume of tap water was added to a 3-l reservoir. Four burettes containing 1 g l^{-1} Cu(II), Fe(III) and Al(III) solutions and water allowed the discretional addition of each pollutant (or water) to the reservoir, a magnetic stirrer being used to homogenise the solutions. The reservoir solution was continuously aspirated and circulated through the manifold by the pump. At 30-min intervals the concentration of the cations was measured by the proposed method and the waste collected for the analysis of these species by atomic-absorption spectrophotometry, simultaneously.

The results found by both techniques are in agreement as can be observed in Table 3, and in Fig. 4 the concentration of these pollutants throughout the day are shown.

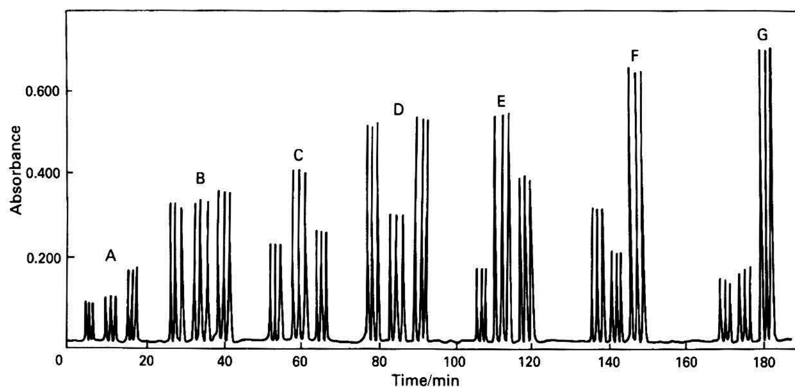

Fig. 3. Simulation of the determination of polluting species in waste water. Cu, first set of triplicate peaks; Fe, second set of triplicate peaks; and Al, third set of triplicate peaks. A–G correspond to the times the analysis were made and the corresponding results are given in Table 2

Table 2. Simultaneous determination of pollutants in synthetic waste-water samples by rFIA

	Concentration added/ µg ml^{-1}			Concentration found/ µg ml^{-1}			Error, %		
Time *	Cu	Fe	Al	Cu	Fe	Al	Cu	Fe	Al
B	3.00	3.00	0.64	3.05	3.11	0.66	+1.6	+3.6	+3.1
C	2.10	4.20	0.37	2.03	4.35	0.34	−3.3	+3.5	−8.1
D	5.10	2.50	1.36	5.28	2.65	1.33	+3.5	+6.0	−2.2
E	1.40	6.50	0.81	1.41	6.76	0.78	+0.7	+4.0	−3.7
F	2.90	1.40	1.74	3.02	1.34	1.71	−4.1	−4.2	−1.7
G	0.90	0.90	2.05	0.86	0.94	2.07	−4.4	+4.4	+0.9

* The instant of the analysis corresponds to that indicated in Fig. 3 ('A' being the blanks).

Table 3. Simulation of a hypothetical monitoring of pollutants in waste water during a working day

	Concentration found by the proposed method/µg ml^{-1}			Concentration found by AAS/µg ml^{-1}		
Time	Cu	Fe	Al	Cu	Fe	Al
8.00 h	0.00	0.00	0.00	—	—	—
8 h 30 min	1.68	0.99	1.59	1.71	1.05	1.52
9.00 h	3.08	2.37	1.02	3.07	2.29	1.06
9 h 30 min	4.33	3.02	1.64	4.23	2.95	1.58
10 h	3.10	3.47	1.32	3.16	3.52	1.39
10 h 30 min	2.27	2.81	1.07	2.14	2.76	1.11
11 h	2.10	2.36	1.27	2.00	2.38	1.29

Interferences

Table 4 summarises the results obtained in the study of interfering species for the determination of copper, iron and aluminium. Species (either polluting or not) generally present in waste waters were tested. The most important interferences are due to masking and precipitating agents such as EDTA, CN^- and S^{2-}. The cations tested are tolerated up to, at least, a 10:1 (foreign ion to analysed cation) ratio. The possibility of the suggested simultaneous determination of the three cations is based on this behaviour.

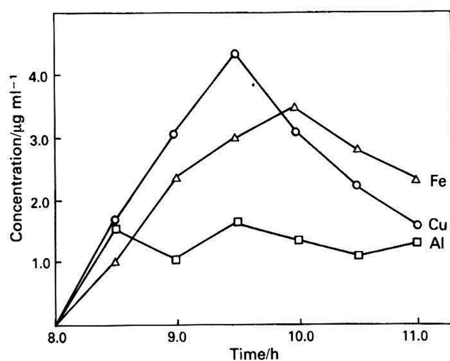

Fig. 4. Concentration of pollutants (Cu, Fe and Al) in a hypothetical sample of waste water during a working day

Joint Determination of Cationic and Anionic Pollutants in Waste Waters by rFIA

To conclude, the possibility of assembling into a single manifold the schemes for the determination of anionic (Part I) and cationic (this paper) pollutants and of simultaneously analysing cationic and anionic species, either reported or not in this research, with a single photometer is suggested. In addition, the possibility of keeping two sets of FIA variables in a simultaneous determination without making it necessary to choose a compromise condition when several single manifolds are assembled is also considered, taking as an example the simultaneous determination of pH, nitrite, copper, iron and aluminium. The manifolds in Fig. 2 (this paper) and Fig. 1(*c*) (Part I), with the sole insertion of a glass - calomel microelectrode for the continuous control of the pH, have been adhered to. It is even possible to work at different flow-rates in each manifold, placing the microelectrode in the manifold with lowest flow-rate in order to obtain a better response. The general manifold shown in Fig. 5 would be employed—the variables of the manifold for the determination of cations being as indicated in Table 1, and those for nitrite and pH being the following: $q' = 1.80$ ml min^{-1}; $V_2 = 44.0$ µl; $L_3 = 50$ cm; $L_4 = 500$ cm; ϕ_i (inner diameter of all components of the manifold) = 0.5 mm; R_1' = sulphanilamide and R_2' = *N*-(1-naphthyl)ethylenediamine. The selected wavelength of the photometer is 575 nm.

The valves play a fundamental role in this type of manifold; they have three functions depending on their location at different points in the manifold. (*i*) The selecting valves, R and B, allow the selection of the appropriate reagents and buffers, respectively, for each determination. They are disposable valves that must be placed prior to the peristaltic

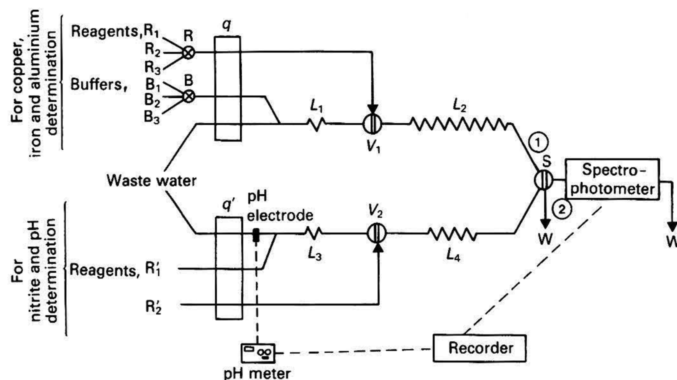

Fig. 5. Manifold for the simultaneous determination of pH, nitrite, copper, iron and aluminium in waste waters by rFIA

Table 4. Interferences in the determination of pollutants in waste waters by rFIA

Tolerated ratio of the foreign species*	Added species		
	Cu determination	Fe determination	Al determination
100	Cl^-, NO_3^-, NO_2^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-} Na^+, K^+, NH_4^+, Ca^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Mg^{2+}	Cl^-, NO_3^-, NO_2^-, SO_4^{2-} Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Al^{3+}	Cl^-, NO_3^-, NO_2^-, SO_4^{2-} Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}
50	Pb^{2+}		CO_3^{2-} Mn^{2+}, Fe^{3+}, Cu^{2+}
10	Fe^{3+}, Al^{3+}	CO_3^{2-}, PO_4^{3-}	PO_4^{3-}, S^{2-}, CN^-
1		CN^-	EDTA
<1	EDTA, S^{2-}, CN^-	EDTA, S^{2-}	

* For 1.5 µg ml^{-1} of copper, 0.5 µg ml^{-1} of iron and 0.2 µg ml^{-1} of aluminium.

pump. *(ii)* The injection valves V_1 and V_2 carry out their normal function. *(iii)* The six-way valve, S, selects the stream reaching the flow cell in the photometer. It is an ordinary injection valve in which one way has been disconnected; a pair of them act as input for streams 1 and 2; another two act as waste for the non-selected channel (one way for each channel) and the remaining one is an output for the selected channel. This valve is the key to the simultaneous determination of nitrite, copper, iron and aluminium with this manifold.

Undoubtedly, the most important limiting factors in these simultaneous photometric determinations with a single manifold are the mutual interferences of the species determined, as well as the difficulty in finding indicator reactions that yield coloured products that show absorption in very close zones of the spectrum. The former factor affects the selectivity of the determinations, whereas the latter influences the sensitivity of the analysis.

Conclusions

The feasibility of determining simultaneously several pollutants in waste waters by rFIA, suitably placing several injection or selection valves between two or more carriers, reagents or buffer channels, has been demonstrated. Both the schemes and the chemical systems are simple; reagent consumption is minimal; instrumentation is available to any analytical laboratory and FIA's features make these configurations extremely versatile, allowing them to be adapted to various analytical problems. In addition, the rapidity and minimum manipulation with which three, four or five parameters can be determined emphasises the great practical interest of this technique in laboratories for controlling pollution in waste waters.[5]

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NOTE—Reference 1 is to Part I of this series.

Paper A4/194
Received June 4th, 1984
Accepted October 17th, 1984

Determination of Trace Amounts of Boron in Geological Samples with Carminic Acid after Extraction with 2-Ethylhexane-1,3-diol

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Trace amounts of boron are determined in geochemical samples spectrophotometrically with carminic acid after prior extraction with 2-ethylhexane-1,3-diol. In this work this method has been tested on 14 international reference samples.

Keywords: *Boron determination; geological material; 2-ethylhexane-1,3-diol; carminic acid; spectrophotometry*

In analytical geochemistry boron is one of the trace elements that are very difficult to determine quantitatively. Hence, there is a scarcity of reference samples with well established boron values. For example, the published boron values of the international reference sample Dunite DTS-1 range from <0.7 p.p.m. B up to 20 p.p.m. B (see Table 1). Although in the last 35 years a great number of analytical methods for determining trace amounts of boron have been described in various papers, the boron content of geological reference materials has been determined previously almost exclusively by optical emission spectroscopic techniques. Accuracy and especially the sensitivity of conventional arc emission spectroscopy are not satisfactory for trace analyses in geochemistry.

Owens *et al.*[4] and Grallath *et al.*[9] described a sensitive emission spectroscopic technique (ICP technique), which they applied to the determination of trace amounts of boron in quartz, silicon and metals; Owens *et al.* performed their analyses on geological reference samples, silicate glass and fly ash reference samples. The detection limit of the reported results is about 5 p.p.m. The importance of the trace element boron in the geochemistry of sediments, especially in marine environment, for instance as an indicator for paleosalinity, justifies renewed efforts to develop a reliable technique for obtaining more accurate measurements of the boron content of silicate materials than is possible by conventional optical emission spectroscopy.

The method used in this work is based on that of Agazzi[10] and Peterson and Zoromsky.[11] Agazzi established that boron in silicate material (glasses) could be extracted from sodium carbonate fusions (after acidification) into a chloroform solution of 2-ethylhexane-1,3-diol. Peterson and Zoromsky modified and extended the method to determine water-soluble boron in fertilisers. They established that boron in the boron - 2-ethylhexane-1,3-diol complex could be recovered from the chloroform solution into a sodium hydroxide solution. Boron could then be determined using carminic acid as the reagent.

Experimental

Apparatus

For absorbance measurements a Perkin-Elmer double-beam grating spectrophotometer was used, equipped with thermostatically controled cells (Haake, FRG). The extractions were carried out in PTFE separating funnels (Nalgene, USA).

Reagents

2-Ethylhexane-1,3-diol solution, 20%. Prepared by dissolving 2-ethylhexane-1,3-diol (No. 820032, Merck, FRG) in chloroform.

Carmine reagent. Prepared by dissolving 100 mg of carminic acid (No. 211, Merck) in 200 ml of concentrated sulphuric acid (95–97%).

Boron stock standard solution. Prepared by dissolving 0.5716 g of boric acid (Suprapur, No. 765, Merck) in distilled water and diluting to 1 l; aliquots of this solution were diluted to produce the calibration standards.

Procedures

Sample preparation

A 1000-mg mass of the sample, dried at 105 °C for 2 h, are placed in a platinum crucible, mixed with 6 g of Na_2CO_3 (anhydrous) and covered with 1 g of Na_2CO_3. A lid is loosely put on the crucible and this is placed in a muffle furnace at 500 °C and the temperature is raised to 1000 °C. After 30 min at this temperature the crucible is taken out and cooled. The contents of the crucible are leached with 4 N HCl in a 250-ml PTFE vessel. The amount of acid used should be such that after dissolving the carbonate a pH of 1–2 is obtained. This solution is then filtered through a filter-paper (No. 589, Merck) into a quartz beaker. The residue on the filter-paper is washed with dilute HCl (0.1 N). The pH of the filtrate is adjusted with HCl to pH 1 (±0.3) and the volume is diluted to 100 ml with water.

Extraction procedure

A 100-ml volume of sample solution containing boron in the range 0–200 µg are extracted twice in a PTFE separating funnel with 20 ml of ethylhexane-1,3-diol solution. The duration of each shaking procedure is 120 s. Boron is re-extracted from the combined extracts (shaking time 120 s) with 20 ml of 0.5 N NaOH solution. The solution obtained by this step is diluted with water to 50 ml (to 25 ml for samples of low boron content). In addition, for each series of measurements four calibration standards and a reagent blank are treated in the same way.

Photometric determination

Aliquots (2 ml) of sample, standard and reagent blank solution are pipetted into quartz beakers. Two drops of concentrated HCl are added. While cooling in a water-bath (10 °C), 10 ml of carmine reagent and 10 ml of H_2SO_4 (concentrated) are added and stirred using a magnetic stirrer. After 90 min the absorbance is measured against a reagent blank solution at λ = 610 nm using cuvettes 5 cm in width.

Evaluation measurements

From the absorbances a calibration graph is established. This graph is linear in the range from 0 up to at least 8 µg of boron in 22 ml of solution. This corresponds to a boron content in the sample from 0 to 200 p.p.m. for 1000 mg of sample. Because

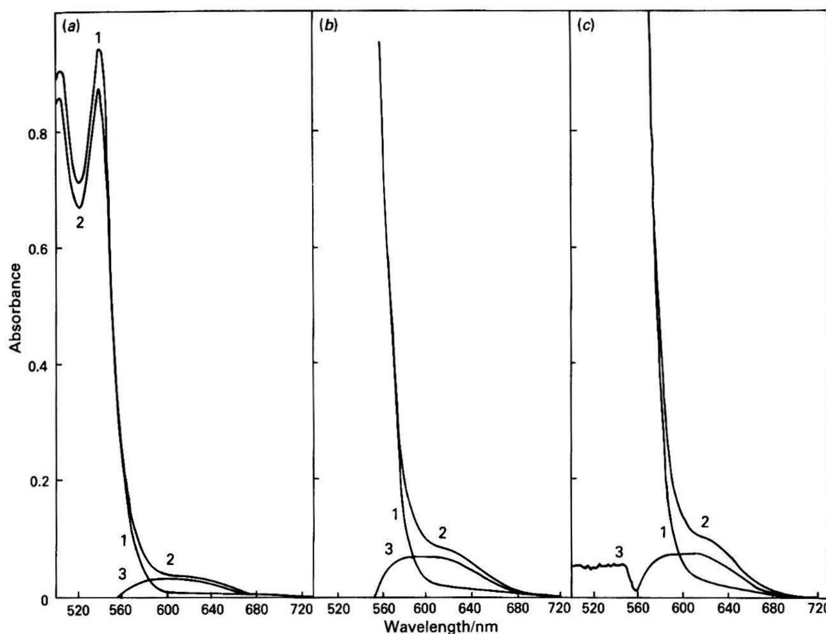

Fig. 1. Absorption spectra of 1, carminic acid solution and 2, B - carminic acid complex *versus* concentrated sulphuric acid; 3 represents the difference between curves 1 and 2. (*a*) Concentration of carminic acid reagent: 10 mg of carminic acid per 200 ml of solution; (*b*) concentration of carminic acid reagent, 50 mg of carminic acid per 200 ml of solution; (*c*) concentration of carminic acid reagent, 100 mg of carminic acid per 200 ml of solution

sodium carbonate used for the sample fusion always contains traces amounts of boron, the boron content of each sodium carbonate lot is determined according to the technique described and subtracted from the boron content of each sample.

Results and Discussion

Effect of Carminic Acid Concentration

The absorption spectra of the boron - carminic acid complex and of the carminic acid reagent are measured against concentrated sulphuric acid. The results for carminic acid concentrations of 10 mg of carminic acid per 200 ml of reagent solution, 50 mg of acid per 200 ml of reagent solution and 100 mg of carminic acid per 200 ml of reagent solution are shown in Fig. 1(*a*)–(*c*). As the absorption of the carminic acid reagent solution is high compared with the absorption of the B - carminic acid complex, it is desirable for optimum precision of the method to mimimise the carminic acid concentration in the reagent used. For boron amounts up to 75 µg in the sample solution a carminic acid concentration of 50 mg per 200 ml of reagent solution will give satisfactory results. For higher boron concentrations in the sample solution we recommend the use of a reagent solution with 100 mg of carminic acid per 200 ml of sulphuric acid.

Effect of Temperature and Standing Time

A boron standard sample containing 75 µg of B in 100 ml of sample solution was treated using the proposed method. A second boron standard sample with the same boron concentration was treated in the same way, but the solutions were cooled at 0 °C during addition of the sulphuric acid and

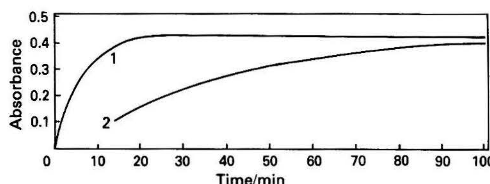

Fig. 2. Absorbance of the B - carminic acid complex as a function of time *versus* reagent blank. 1, Temperature during complex formation at 22 °C; 2, maximum temperature during complex formation at 10 °C

reagent solution and subsequently cooled at 10 °C. The complex formation of the uncooled solution is complete after a standing time of 30 min and the absorbance remains constant for at least 3 h. The formation rate of the complex under cooling conditions is unsatisfactorily low; after a standing time of 100 min the colour of the complex was unstable. The time - absorbance graphs of the cooled and uncooled samples are shown in Fig. 2.

Application to International Reference Samples

As in general various chemical techniques for analysis have different specific sources of error, it is necessary to apply appropriate, but physico-chemically different methods of determining "recommended values." The spectrophotometric technique presented in this paper was applied to the determination of boron in 14 international geochemical reference samples. The results in comparison with literature values are shown in Table 1.

Table 1. Boron content (p.p.m.) of 14 international reference samples compared with literature values

Reference sample	Reported values	Mean	Analytical method*	Reference
ZGI-TB	83, 87, 76, 86	83	FYJ	This work
		92†	OOS, OOJ	1
		118†	OOS	2
ZGI-GM	9, 7, 5, 7, 7	7	FYJ	This work
		13†	OOS, OOJ, OOR	1
		11†	OOS	2
SY-2	92, 92, 93, 95, 104	95	FYJ	This work
	33		ODS	3
	77		OOS	3
	178		OOO	3
	110		OVS	3
	95		FYJ	3
	83		BVS	3
	84		OVS	3
	87		FOS	4
MAG-1	132, 139, 140	137	FYJ	This work
	159, 163, 147, 131, 156, 177	155	OOS	5
		130†	OOS	5
	128		OOR	6
	138		FOS	4
SDC-1	13, 15, 20, 16	16	FYJ	This work
	10.8, 10.0, 12.8, 10.6, 10.1, 10.0	10.7	OOS	5
		30†	OOS	5
DTS-1	9, 12, 9, 9, 10, 10	10	FYJ	This work
	<10, <10, <10, <10, <10, <10	(<10)‡	OOS	5
		5	OOO	1
	<10	<10	OOS	2
		20†	OOO	5
		5†	OOO	5
	<20, <8.0, <0.7		OOS	7
SCo-1	71, 76, 63, 78, 79	73	FYS	This work
	75.7, 102, 89.1, 83.4, 90.1, 88.9	93.2	OOS	5
		70†	OOS	5
		64.4†	OOR	5
	72.1		OOR	6
BHVO-1	2, 3, 2, 1, 3	2	FYJ	This work
	<10, <10, <10, <10, <10, <10	(<10)‡	OOS	5
	0		OOS	5
	2.3		OOR	6
	<5		FOS	4
SGR-1	41, 37, 43	40	FYJ	This work
	65.0, 57.2, 53.6, 67.0, 53.7, 56.2	58.8	OOS	5
		29.8†	OOS	5
	50.4		OOR	6
NIM-G	5, 6, 8, 6	6	FYJ	This work
		10†	OOO	1
ASK-1	12, 19, 12, 15, 13	14	FYJ	This work
	20, 20, 10	17	OOS	8
ASK-2	125, 130, 123, 118, 125	124	FYJ	This work
	160, 150, 150	153	OOS	8
BCS-375	9	—	FYJ	This work
BCS-376	7	—	FYJ	This work

* To describe the analytical methods we have used a three-letter code, as given by Abbey *et al.*[3]:
Sample pre-treatment:
B pelletisation;
F fusion, sintering;
O not used or not specified.
Separations:
D fractional distillation;
V bulk volatilisation;
Y solvent extraction;
O not used or not specified.
Final measurement:
J absorptiometric;
S spectrographic;
O not specified;
R radiometric, neutron activation.
† Only an average value was reported.
‡ These values were given in parentheses in the original paper.

Sources of Error

A possible source of error, caused by forming the BF_4^- complex, is discussed by Agazzi.[10] Agazzi masked the fluoride by adding zirconium nitrate solution prior to extraction. The fluorine present in the analysed rock samples had no effect on the results for the determination of boron. Investigations showed that the fluorine had partly evaporated during fusion. Experimental results showed that the use of plastic apparatus is absolutely necessary. Between 25 and 53 µg of boron were detected after shaking blank solutions with ethylhexane-1,3-diol solution fo 1–2 min in 250-ml glass separating funnels. Tests of chemicals for boron showed that sodium carbonate (No. 6392, Merck) contains boron in the range 2–3 p.p.m. Hence the detection limit of the method depends essentially upon the purity of the sodium carbonate used for the fusion.

Conclusion

Trace amounts of boron can be separated from geological samples by ethylhexane-1,3-diol extraction into chloroform. A combination of the extraction procedure with the carminic acid method provides satisfactory results with samples of various matrix compositions. The sensitivity is sufficient for geochemical applications within a boron concentration range of between 1 and 1000 p.p.m.

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Paper A4/236
Received July 16th, 1984
Accepted November 15th, 1984

Fluorimetric Determination of Aluminium with Morin After Extraction with Isobutyl Methyl Ketone

Part II.* Extraction - Fluorimetric Determination of Aluminium in Natural and Waste Waters

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A fluorimetric study of the extraction of the Al - morin complex into isobutyl methyl ketone (IBMK) was carried out. At pH 5–7 an extraction efficiency of between 90 and 100% was obtained, being a maximum (100%) at pH 6 when the Al content was 10 p.p.b. and 97% at 20 p.p.b.

A 1-ml volume of 0.125% morin solution was added to 50 ml of Al solution adjusted to pH 6 ± 0.5; 10 ml of IBMK were introduced and the mixture was shaken for 5 min, then allowed to settle for 10 min. The organic phase was separated and dispersed with 10 ml of ethanol - methanol - water (86 + 9 + 5); the pH was adjusted to 3.7–3.8 and the solution was diluted with water to 25 ml. The fluorescence intensity was measured after 1 h at 495 nm with excitation at 418 nm, and the results were compared with those obtained with standards of aluminium that did not require extraction.

The fluorescence intensity was linear up to 20 p.p.b. of Al and the limit of detection was 0.1 p.p.b. From a study of interferences it was deduced that, after the extraction, the F^- and PO_4^{3-} interferences were eliminated, and the selectivity towards SiO_3^{2-}, Be, Cu, Mg and NH_4^+ was also increased.

The proposed method was applied to the determination of Al in natural and waste waters, and the results are compared with those obtained by atomic-absorption spectrophotometry by chelation with quinolin-8-ol into IBMK using a heated graphite atomiser and a dinitrogen oxide - acetylene flame.

Keywords: *Aluminium determination; water analysis; fluorescence analysis; extraction; aluminium - morin complex*

Morin (3,5,7,2′,4′-pentahydroxyflavone) has been widely used as a complexing reagent in the extraction of different metal complexes in both spectrophotometric and fluorimetric methods. For the extraction of metal - morin binary complexes, different solvents have been used. For example, in spectrophotometric determinations butanol and isobutyl methyl ketone (IBMK) have been used in the extraction of Mo complexes,[1,2] ethyl methyl ketone for extracting Ga[3] and isoamyl alcohol for extracting the V[4] and Fe[5] complexes. Isoamyl alcohol has also been used by Blank and co-workers[6–8] in a wide study of the extraction of numerous metal complexes of morin. Extraction has also been used in fluorimetric methods for the determination of metals, *e.g.*, chloroform as an extractant in the determination of Ga,[9] butanol in the determination of Ta,[10] isopentyl acetate for lanthanide complexes[11] and isoamyl alcohol for the extraction of Be, Zn, Al, Ga, In, Th, Zr and Sn(IV) complexes.[12]

On the other hand, numerous mixed-ligand complexes have been extracted as ion-association complexes with anions such as ClO_4^-. Chloroform has been used as an extractant of the Lu - morin - diantipyrylmethane - ClO_4^- complex[13] and of Sc,[14] Al[15] and In[16] complexes with morin - antipyrine - ClO_4^- in spectrophotometric (Sc, Al) and fluorimetric determinations (Lu, Sc, In). V was determined spectrophotometrically once the V - morin - antipyrine complex had been extracted into dichloroethane.[17] Blank *et al.*[18] studied the extraction of Zr and Th complexes with morin - ClO_4^- into isoamyl alcohol and proposed a method for the spectrophotometric determination of Zr and Th. Ti can also be determined spectrophotometrically after extraction of the Ti - morin - aniline complex into benzyl alcohol - butanol (3 + 1).[19]

In this work, the optimum conditions for the extraction of the Al - morin complex into IBMK were studied and subsequently the aluminium was determined fluorimetrically by measuring the fluorescence of the organic layer that was dispersed in water with ethanol - methanol - water (86 + 9 + 5). This possibility of dispersion renders unnecessary the preparation of standards by means of extraction, as they can be obtained by direct introduction in the final medium studied (IBMK - ethanol - water).[20]

Moreover, the concentration that takes place in every extraction and the high content of ethanol in the final medium lead to a very low detection limit (0.1 p.p.b. of aluminium), as in other fluorimetric methods that are very sensitive for the determination of aluminium.[21]

Experimental

Apparatus

Fluorescence intensity (I_F) measurements were made on a Perkin-Elmer Model 3000 spectrofluorimeter with a Termotronic S-389 thermostat, which measures temperature to within ±0.5 °C.

A Perkin-Elmer Model 2380 atomic-absorption spectrophotometer equipped with a Model 561 recorder, a deuterium background corrector and a Model HGA 400 programmer heated graphite atomiser was used to measure atomic absorption.

pH was measured with a Crison Model Digilab 517 pH meter (pH ±0.001).

Reagents

All of the reagents used were of analytical-reagent grade (Merck). Distilled, de-ionised water was used.

A stock solution of 10 p.p.m. of aluminium was prepared by dissolving 0.1758 g of $AlK(SO_4)_2.12H_2O$ in water, adding 1 ml of 1 + 1 sulphuric acid and diluting to 1 l with water.

* For Part I of this series, see reference 20.

Morin solutions were prepared by dissolving 0.125 or 0.0125 g of the reagent in 100 ml of ethanol - methanol - water (86 + 9 + 5) (alcohol F-30).

General Procedure

A 50-ml volume of the aqueous solution of aluminium was transferred into a 100-ml separating funnel and the morin solution in alcohol F-30 containing 1.25 mg of the reagent was added. The pH was adjusted to different values using HCl and NaOH solutions and then extracted for 5 min with 10 ml of IBMK. The organic layer was separated and dispersed with 10 ml of alcohol F-30, the pH was adjusted to 3.7–3.8 by means of HCl - KCl and the solution was diluted to 25 ml with water, a dispersion being obtained (I). The I_F was measured after 1 h at 495 nm, with excitation at 418 nm, at 25 °C.

The standards were prepared as follows: 10 ml of IBMK, 10 ml of 0.0125% morin solution in alcohol F-30 and an aliquot of Al solution (between 0 and 1 μg) were placed in a 25-ml beaker, the pH was adjusted to 3.7–3.8 using HCl - KCl and the mixture was diluted to the mark with water, giving a dispersion (II).

The extraction efficiency was deduced by comparing the I_F of dispersions I and II, from the expression

$$\eta = \frac{I_F(I)}{I_F(II)} \times 100$$

Results and Discussion

All the experiments to determine the optimum extraction conditions were carried out using solutions that contained 10 or 20 p.p.b. of Al in the final IBMK - ethanol - water medium, and the fluorescence measurements were carried out under the following conditions[20]: 40% IBMK, 40% alcohol F-30, 0.005% morin, pH 3.7–3.8 (HCl - KCl), $\lambda_{ex.}$ 418 nm, $\lambda_{em.}$ 495 nm and T = 25 °C.

In a previous test we found that in order to complex the Al and to extract it subsequently into IBMK, a minimum volume of morin solution must be added. This is because the morin is dissolved in a chiefly ethanolic medium (86% ethanol) and ethanol increases the solubility of IBMK in water. In order to avoid this possible solubility, 1 ml of 0.125% morin solution in alcohol F-30 was added to 50 ml of the aqueous solution of Al, and so the solubility of IBMK in water was hardly affected.

Effect of pH

The optimum pH of extraction was studied over the range 1–10 (adjusted with HCl and NaOH solutions) for solutions containing 5 and 10 p.p.b. of Al. Taking into account that in every extraction and subsequent dispersion of the organic phase it was concentrated twice, the Al contents will be 10 and 20 p.p.b., respectively, in the final IBMK - ethanol - water medium.

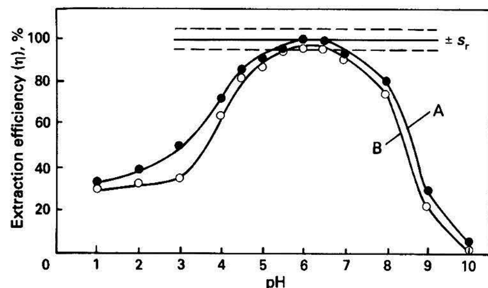

Fig. 1. Effect of pH on the extraction of the Al - morin complex with IBMK. η = (I_F after extraction/I_F value of reference) × 100. Al concentration: A, 10 p.p.b.; and B, 20 p.p.b. The horizontal solid line is 100% extraction efficiency and the broken lines are $+s_r$ (+5%) and $-s_r$ (−5%)

On the other hand, the corresponding standards containing 10 and 20 p.p.b. of Al were prepared without extraction, as in the general procedure. The fluorescence of the organic phase dispersed in ethanol - water was compared with that of the standard mentioned above. The extraction efficiency was maximal when the pH was between 5 and 7; at pH 6 efficiencies of 100 and 96.8% were obtained for Al contents of 10 and 20 p.p.b., respectively (Fig. 1).

The pH chosen for subsequent experiments was 6 ± 0.5, adjusted by means of HCl and NaOH solutions. Finally, the optimum pH value in IBMK - ethanol - water was 3.7–3.8, adjusted using HCl–KCl.[20]

Range of Applicability

The linear range of the method is conditioned by the linearity of the standards in IBMK - ethanol - water, which corresponds to the range between 0 and 40 p.p.b. of Al[20]; therefore, aqueous solutions containing between 0 and 20 p.b.b. of aluminium can be determined after extraction of the Al - morin complex into IBMK and subsequent dispersion in ethanol - water.

Precision

The precision of the method was calculated for 12 replicates of a blank and a solution containing 1 p.p.b. of Al. The relative standard deviation for the blank (morin) was 6.3% and for 1 p.p.b. of Al it was 5%.

Detection Limit

The detection limit was calculated following the recommendations given by IUPAC,[22] and was 0.2 p.p.b. of aluminium in the final IBMK - ethanol - water medium,[20] which is the same as 0.1 p.p.b. of aluminium in the aqueous phase problem. This detection limit is the lowest found so far for the Al - morin system.

Interference Study

A comparative study of the selectivity of the method in relation to another method for the fluorimetric determination of aluminium was carried out. The most important interferences pointed out by Will[23] are due to F^-, PO_4^{3-}, Cr(III),

Table 1. Tolerances to various ions

Ion	Limiting concentration, p.p.b. Al - morin	Extraction	Al - morin - Genapol
SO_4^{2-}	>1000	>10000	>10000
PO_4^{3-}	3	>10000	>10000
Cl^-	—	—	>10000
NO_3^-	—	—	>10000
SiO_3^{2-}	>1000	4000	100
F^-	5	1000	10
Be(II)	—	100	20
Ca(II)	>1000	—	5000
Co(II)	>1000	—	>10000
Cr(III)	30	—	>10000
Cu(II)	20	400	100
Fe(III)	100	70	100
Mg(II)	200	>10000	>10000
NH_4^+	500	>10000	>10000
Ni(II)	>1000	—	>10000
Pb(II)	1000	80	100
Zn(II)	>1000	70	20

Table 2. Comparison of extraction - fluorimetric and atomic-absorption methods for the determination of aluminium in waters

	Flame AAS		HGA AAS		Extraction - fluorimetry		
Water sample*	Al, p.p.b. (*A*)	s_r,† %	Al, p.p.b. (*B*)	s_r,† %	Al, p.p.b. (*C*)	s_r,† %	Relative error, %
N_1	69.2	6.4	—		71.7	8.8	3.6
N_2	—		45.5	8.5	49.2	9.3	8.1
N_3	—		45.9	1.9	42.9	10.0	6.5
W_1	548.7	3.5	522.8	5.5	451.4	13.0	17.7(*A*/*C*) 13.6(*B*/*C*)
W_2	274.6	11.0	284.4	5.9	304.7	6.7	11.0(*A*/*C*) 7.1(*B*/*C*)
W_3	—		38.6	8.6	40.9	22.0	5.9

* N, natural water; W, waste water.
† s_r, relative standard deviation (5 replicates).

Cu(II), Fe(III), Mg(II) and NH_4^+. We have previously shown that when a non-ionic surfactant is used to sensitise the fluorescence of the Al - morin complex, in general the tolerance limits increased, the main interferences being F^-, SiO_3^{2-}, Cu(II), Fe(II), Pb(II), Zn(II) and Be(II)[24] [Be(II) was not studied by Will]. When we previously studied the fluorescence of the Al - morin complex in IBMK - ethanol - water,[20] we found that the interferences were very similar, and NO_3^-, SO_4^{2-}, Ba(II), Ca(II), Co(II), Cr(III), Mg(II), Mn(II) and Ni(II) did not interfere.

In this work, the study of interferences was carried out with elements that have less tolerance. Solutions of 1000 p.p.m. of the sodium salts of SO_4^{2-}, PO_4^{3-}, SiO_3^{2-} and F^- and sulphates or nitrates of Be(II), Cu(II), Fe(III), NH_4^+, Pb(II) and Zn(II) were prepared, and increasing volumes of these solutions were added to a solution of 1 p.p.b. of aluminium, subsequently carrying out the extraction. An interference was tolerable if its effect on the fluorescence signal was less than the relative standard deviation; hence the tolerance limit in the fluorescence measurements was the same as the relative standard deviation for this method (5%).

The limiting concentrations for different ions are shown in Table 1, and the results are compared with those obtained by Will for the Al - morin complex[23] and with those we obtained for the sensitised complex with a non-ionic surfactant, ethylene oxide - propylene oxide condensate (Genapol PF-20; Hoechst).[24]

It can be seen that SiO_3^{2-}, Cu(II), Be(II) and especially F^- can be tolerated in higher concentrations when the extraction method is used. On the other hand, PO_4^{3-}, Mg(II) and NH_4^+ have tolerance limits similar to those for the Al - morin complex sensitised by addition of Genapol PF-20, and much higher than those in the conventional method.

Determination of Aluminium in Water

The proposed method was applied to the determination of Al in natural and waste waters, and the results were compared with those obtained by AAS after extraction of aluminium as the quinolin-8-olate into IBMK.

The samples of water were filtered through a 0.45-μm pore diameter membrane filter, and acidified to 1% with concentrated nitric acid.

The quinolin-8-olate of aluminium was extracted by a recommended method.[25] Concentrations of Al higher than 50 p.p.b. were measured by AAS with a dinitrogen oxide - acetylene flame or with a heated graphite atomiser (HGA); for concentrations lower than 50 p.p.b. only the HGA was used, with drying, charring and atomisation temperatures of 150, 1500 and 2700 °C, respectively. The results, given in p.p.b. of Al, are shown in Table 2.

The precision (expressed as relative standard deviation) for the extraction - fluorimetric method varies between 6.7% for waste water W_2 and 22% for W_3. The average precision for the six waters analysed is 11.6%. The AAS methods have, in general, a better precision; thus, with a flame the relative standard deviation varies between 3.5 and 11%, with a mean of 7%, and with the HGA it varies between 1.9 and 8.6% with a mean of 6.1%.

On the other hand, the results obtained when we applied the extraction - fluorimetric method are comparable to those obtained by AAS. The relative errors with respect to the AAS methods are less than 11%, except for waste water W_1, for which the difference is 17.7% and 13.6% from the flame and HGA methods, respectively.

Taking into account the results obtained, we concluded that there are no important interferents in the waters analysed by the extraction - fluorimetric method; in fact, the prior dilution of the sample (especially with waste waters) that is necessary for the Al content to be in the linear range of the method avoids possible interferences.

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NOTE—Reference 20 is to Part I of this series.

Paper A4/292
Received August 22nd, 1984
Accepted November 5th, 1984

Photometric Titration of Group II Metal Ions with EDTA Using Very Precise Linear Extrapolation End-point Location

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Results from the photometric titration of Group II metal ions with EDTA have been linearised using an approach based on Higuchi's method. Readily available indicators and pH conditions required to give very precise abscissa intersections have been investigated and a coefficient of variation of 0.04% has been achieved.

Keywords: *Photometric titration; linearised titration plots; magnesium, calcium, strontium and barium determination*

The linearisation of indicator weak acid or base photometric titrations was developed by Higuchi *et al.* with excellent results.[1] They implied that such an approach should be applicable to complexometric titrations and this was shown to be so by two other groups of workers who offered a few illustrative examples.[2,3] We developed our own theory, though we later found it to be related to that in reference 3, and applied it to discovering which of the readily available metallochromic indicators gave high quality linearised photometric titration curves with individual Group II ions. These recommended indicators and pH conditions are, in many instances, rather different from those pertaining to visual titrations.

Theory

The following are a list of principle symbols used throughout the paper: $K_{M'Y'}$ = metal ion - titrant conditional stability constant; $K_{M'In'}$ = metal ion - indicator conditional stability constant; [M]' = concentration of metal ion not combined with titrant or indicator in mol dm^{-3}; [Y]' = concentration of metal-free titrant in mol dm^{-3}; [In]' = concentration of metal-free indicator in mol dm^{-3}; [MY] = concentration of metal - titrant complex in mol dm^{-3}; [MIn] = concentration of metal - indicator complex in mol dm^{-3}; C_M = concentration of metal ion solution to be titrated in mol dm^{-3}; C_Y = concentration of titrant solution in mol dm^{-3}; C_{In} = concentration of indicator solution in mol dm^{-3}; V = volume of titrant added in cm^3; V_M = volume of metal ion solution taken in cm^3; V_e = volume of titrant added at the equivalence point in cm^3; V_{In} = volume of indicator solution added in cm^3; V_0 = volume of solution at the start of the titration in cm^3; A = absorbance of titrated solution at any stage; A_{In} = absorbance of free indicator; and A_{MIn} = absorbance of metal - indicator complex.

If a metal ion solution is titrated with a complexing agent in the presence of an indicator and only 1 : 1 metal - titrant and metal - indicator complexes form, the following equations apply:

$$K_{M'Y'} = \frac{[MY]}{[M]'[Y]'} \quad \quad (1)$$

$$K_{M'In'} = \frac{[MIn]}{[M]'[In]'} \quad \quad (2)$$

The mass balances are:

$$[M]' + [MY] + [MIn] = \frac{C_M V_M}{V + V_0} \quad \quad (3)$$

$$[Y]' + [MY] = \frac{C_Y V}{V + V_0} \quad \quad (4)$$

$$[In]' + [MIn] = \frac{C_{In} V_{In}}{V + V_0} \quad \quad (5)$$

From equations (3), (4) and (5),

$$[M]' - [Y]' - [In]' = \frac{C_M V_M}{V + V_0} - \frac{C_Y V}{V + V_0} - \frac{C_{In} V_{In}}{V + V_0} \quad (6)$$

Substituting equation (1) into equation (4) and rearranging gives:

$$[Y]' = \frac{C_Y V}{(V + V_0)(1 + K_{M'Y'}[M]')} \quad \quad (7)$$

Similarly substituting equation (2) into equation (5) gives:

$$[In]' = \frac{C_{In} V_{In}}{(V + V_0)(1 + K_{M'In'}[M]')} \quad \quad (8)$$

Substituting equations (7) and (8) into equation (6) we obtain:

$$[M]' + \frac{C_Y V}{V + V_0}\left\{1 - \frac{1}{(1 + K_{M'Y'}[M]')}\right\} + \left(\frac{C_{In} V_{In}}{V + V_0}\right) \times \left\{1 - \frac{1}{(1 + K_{M'In'}[M]')}\right\} - \frac{C_M V_M}{V + V_0} = 0 \quad \quad (9)$$

If data before the end-point are considered and $K_{M'Y'}$ is large, [Y]' is insignificant compared with [M]' and if $C_{In}V_{In} \ll C_M V_M$ equation (6) becomes

$$[M]' = \frac{C_M V_M}{V + V_0} - \frac{C_Y V}{V + V_0} \quad \quad (10)$$

Substituting $C_Y V_e = C_M V_M$ and (2) into (10) and rearranging,

$$\frac{[MIn]}{[In]'}(V + V_0) = K_{M'In'} C_Y V_e - K_{M'In'} C_Y V \quad (11)$$

Hence a plot of $\frac{[MIn]}{[In]'}(V + V_0)$ against V is linear with slope = $K_{M'In'}C_Y$ and intercept $K_{M'In'}C_Y V_e$.

When

$$\frac{[MIn]}{[In]'}(V + V_0) = 0$$

$$V = V_e$$

Equation (11) is called the "approximate equation."

Let

$$F_1 = \frac{[MIn]}{[In]'}(V + V_0)$$

If $C_{In}V_{In}$ is not negligible compared with $C_M V_M$, *e.g.*, in the titration of a small amount of metal, equation (6) becomes:

$$[M]' + \frac{C_{In} V_{In}}{V + V_0}\left\{1 - \frac{1}{(1 + K_{M'In'}[M]'}\right\} = \frac{C_Y V_e}{V + V_0} - \frac{C_Y V}{V + V_0} \quad \quad (12)$$

Substituting for [M]′ from equation (2) we obtain:

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

Hence a plot of

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

against V is linear with the same slope and intercept as the approximate equation. This is called the "corrected equation."

Let

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

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

and is readily determined experimentally at any point in the titration.

Fig. 1 shows a plot of A and F_2 against V for a symmetrical indication colour change where $pM_{trans} = pM_{eq}$.[4] This is good for visual titration but not for photometric types because the volume range over which reasonable values of [MIn]/[In]′ are found is very limited.

Fig. 2 illustrates the instance where $pM_{trans} < pM_{eq}$; this is an ideal arrangement for photometric titration because [MIn]/[In]′ values lie in the most suitable range. The recommended indicators all behave in this way. Fig. 3 shows two typical experimental graphs: the titration of calcium with EDTA using Eriochrome Black T and Calmagite as indicators. Fig. 4 illustrates the problem that arises when low metal concentrations are titrated and $C_{In}V_{In}/C_MV_M$ becomes large in order to maintain reasonable absorbance values. Note that if F_2 is plotted as ordinate all lines appear as (A).

Fig. 5 shows the degeneration of the Higuchi plot at low metal concentrations when $K_{M'Y'}$ is reduced.

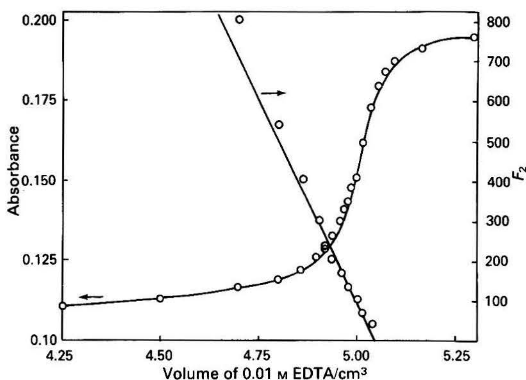

Fig. 1. Unsatisfactory nature of linearisation of a symmetrical photometric titration graph. Titration of 5.014 cm³ of 0.01 M magnesium solution with 0.01 M EDTA solution using 1.00 cm³ of 3 × 10^{-4} M Arsenazo solution as indicator. pH = 10.0; λ = 470 nm. Note the excessive F_2 values

Experimental

Reagents

All reagents were of AnalaR grade and de-ionised water was used throughout.

Standard calcium solutions, 0.01 *and* 0.001 M. Prepared from calcium carbonate, dried at 130 °C for 3 h, by dissolving the appropriate mass in a small excess of dilute hydrochloric acid and diluting to volume.

Various solutions, 0.01 M. Prepared from magnesium sulphate heptahydrate, strontium chloride hexahydrate, barium chloride dihydrate and the disodium salt of EDTA; 0.07 M sodium perchlorate solution was used to fix the ionic strength and 5 M (total) ammonia - ammonium chloride mixtures of pH 9.0, 10.0 and 11.0 were used for pH adjustment.

Indicator stock solutions. The indicators were readily available from BDH Chemicals and stock solutions were prepared at the concentrations indicated under $C_{In}V_{In}$ in Table 1. These concentrations allow for indicator purity as determined by elemental analysis.

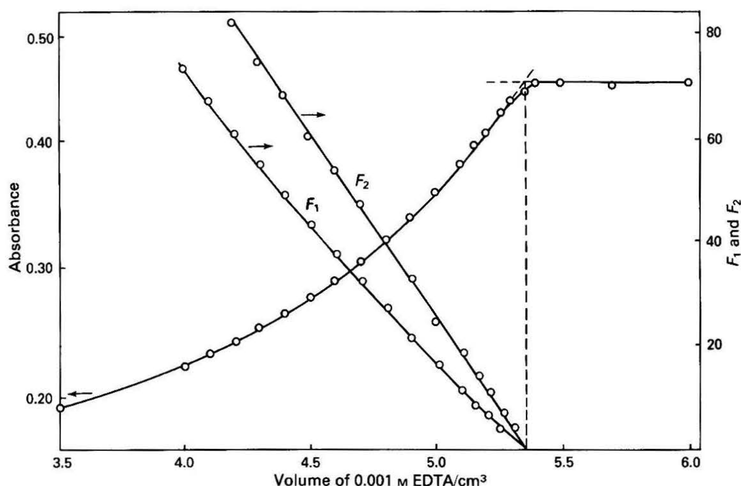

Fig. 2. Value of F_2 plot for dilute titrants. Photometric and "linearised" photometric titrations of 5.014 cm³ of 0.001 M calcium solution with 0.001 M EDTA solution using 1.00 cm³ of 6.8 × 10^{-4} M Eriochrome Blue Black SE solution as indicator. pH = 10.0; λ = 620 nm; $K_{M'In'} = 6 \times 10^4$

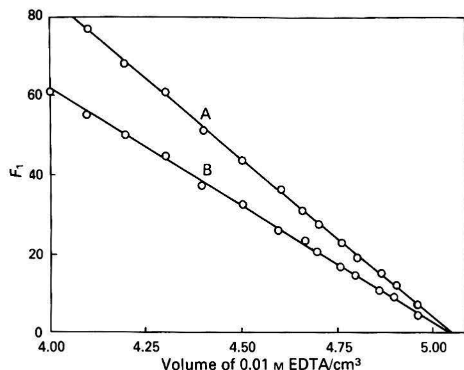

Fig. 3. Linearised photometric titrations of 5.014 cm³ of 0.01 M calcium solution with 0.01 M EDTA solution using A, 6.5×10^{-4} M Eriochrome Black T and B, 7.0×10^{-4} M Calmagite solutions as indicators. pH = 10.0; λ = 620 nm

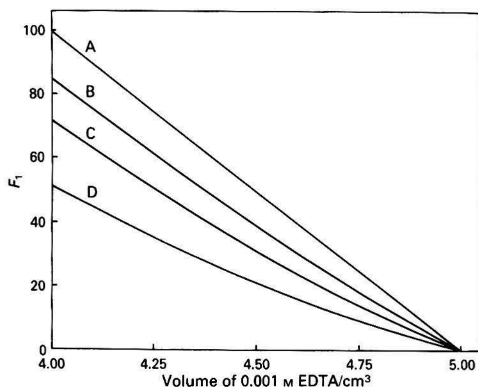

Fig. 4. Effect of a high concentration of indicator. Theoretical linearised F_1-type photometric titration of 5.00 cm³ of 0.001 M metal solution with 0.001 M EDTA solution. Log $K_{M'Y'}$ = 10.0 and log $K_{M'In'}$ = 5.0; $C_{In}V_{In}/C_MV_M$ = A, 0; B, 5%; C, 10%; D, 20%. Note, an F_2 plot would give line A each time

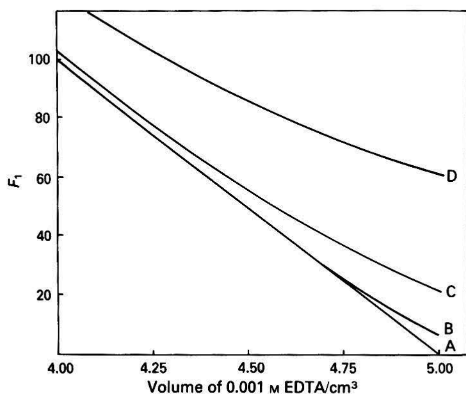

Fig. 5. Requirement for a high value of $K_{M'Y'}$. Theoretical linearised photometric titration of 5.00 cm³ of 0.001 M metal solution with 0.001 M EDTA solution. Log $K_{M'In'}$ = 5.0, $C_{In}V_{In} = 5 \times 10^{-4}$ mmol. Log $K_{M'Y'}$ = A, 9.0; B, 8.0; C, 7.0; and D, 6.0.

Apparatus

A Sybron-Brinkmann Model PC 801 Digital Probe Absorptiometer (ChemLab Instruments Ltd., Hornchurch, Essex) fitted with various interference filters and a 2-cm polycarbonate probe tip was used. A Pye Unicam PW 9415 ion-selective meter was used to check pH values. Results were processed separately using a Sinclair Spectrum microcomputer. A Grade A 10-cm³ microburette fitted with a magnifying reader and Grade B calibrated pipettes and calibrated flasks were also used.

Procedure

Pipette 5.00 cm³ of metal ion solution and 2.0 cm³ of appropriate buffer solution into a 100-cm³ tall-form beaker held in a large crystallising dish containing water at 22 °C and mounted on a magnetic stirrer. Dilute the test solution to 49 cm³ with 0.07 M sodium perchlorate solution. Select the recommended filter then insert the fibre optic probe and a stirring bar, set the absorbance to read zero, add 1.00 cm³ of indicator solution and titrate photometrically with standard 0.01 M EDTA solution. Correct absorbance values for the increase in volume in the usual way.

Estimate A_{In} from the volume-corrected absorbance at 20% overtitration and A_{MIn} by measuring the absorbance of a 0.1 M metal ion solution (0.01 M for Mg^{2+}) containing 2.0 cm³ of buffer, 1.00 cm³ of indicator solution and of total volume 50 cm³. For 0.001 M calcium titration determine A_{MIn} by adding 1.0 cm³ of 1.0 M calcium solution at the end of the titration.

Results

The results are summarised in Tables 1 and 2. The end-point for the 0.01 M titrations was the extrapolated F_1 linear regression best-fit abscissa intersection to give V_e. F_2 was used for the 0.001 M titrations.

Indicator purity was examined by TLC using microcrystalline cellulose as the stationary phase and a 5 + 5 + 3 + 1 mixture of propanol, ethyl acetate, water and acetic acid, respectively, as the mobile phase. All the hydroxyazo-type indicators showed the presence of impurities. These indicators were purified on a modest preparative scale using a Sephadex G-25 column with a 30% ethanol mobile phase but the use of the main-component heart cut in titrations produced no improvement in precision and accuracy, though an approximately 10% increase in $K_{M'In'}$ was noted.

Indicator purities calculated from elemental analyses of carbon and nitrogen were as follows: Eriochrome Blue Black G 25%; Eriochrome Black T 66%; Eriochrome Blue Black R 41%; and Eriochrome Blue Black SE 53%.

Discussion

The theoretical model showed that to obtain linearity with both the approximate and corrected plots, $C_MK_{M'Y'}$ must be $> 10^5$. For approximate plots, to maintain an end-point error of < 0.4%, $C_{In}V_{In}$ must be <3% of C_MV_M. Corrected plots (*i.e.*, F_2 *versus* V) are not affected by indicator concentration; for these plots a value of $K_{M'In'}$ is required but this is readily obtained from the appropriate approximate plot.

The indicators chosen had a pM_{trans} in the range 3.5–4.5,

Table 1. Photometric titration of 5.014 cm³ of 0.0100 M calcium chloride solution with 0.01 M EDTA

Indicator	V_e*/cm³	Standard deviation/ cm³	Filter wavelength/ nm	$C_{In}V_{In}$/ mmol	Log $K_{M'In'}$	pH	Ionic strength
Methylthymol Blue	4.981	0.0022	620	5×10^{-4}	3.95	9.0	0.11
Eriochrome Blue Black B	5.033	0.0026	620	5×10^{-4}	4.31	10.9	0.07
Eriochrome Black T	5.041	0.0042	620	6.5×10^{-4}	3.91	10.0	0.11
Murexide	4.979	0.0045	470	1×10^{-3}	4.17	10.0	0.11
Arsenazo	4.998	0.0045	470	1×10^{-3}	4.07	9.0	0.11
Calmagite	5.038	0.0039	620	7×10^{-4}	3.77	10.0	0.11
Eriochrome Blue Black SE	4.961	0.0031	620	5×10^{-4}	4.69	10.0	0.11

* Mean of four determinations.

Table 2. Photometric titration of 0.01 M magnesium, strontium and barium with 0.01 M EDTA; 0.001 M calcium with 0.001 M EDTA

Metal	Indicator	V_e/cm³	Standard deviation/ cm³	Filter wavelength/ nm	$C_{In}V_{In}$/ mmol	Log $K_{M'In'}$	pH	Ionic strength
Mg^{2+}	Eriochrome Blue Black R	5.097*	0.0041	620	2.5×10^{-4}	4.29	10.0	0.11
Mg^{2+}	Pyrocatechol Violet	5.008*	0.0052	620	5×10^{-4}	4.39	10.0	0.11
Mg^{2+}	HSN†	5.061*	0.0022	620	1×10^{-3}	4.13	10.0	0.11
Mg^{2+}	Hydroxynaphthol Blue	5.056*	0.0041	620	1×10^{-3}	4.50	10.0	0.11
Sr^{2+}	Alizarin Fluorine Blue	5.066*	0.0050	620	1×10^{-3}	4.09	10.0	0.11
Ba^{2+}	Methylthymol Blue	5.021‡	0.0022	620	5×10^{-4}	3.58	10.9	0.07
Ca^{2+}	Eriochrome Blue Black SE§	5.359‡	0.0029	620	6.8×10^{-4}	4.87	10.0	0.09

* Mean of four determinations.
† HSN: 2-hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)-3-naphthoic acid, Patton and Reeder's indicator.
‡ Mean of seven determinations.
§ F_2 plot.

i.e., 0.5–1.5 greater than the starting p*M*; this arrangement yields the longest precise linear region for these titrations.

The best precision of these methods was a standard deviation of 0.002 cm³ (coefficient of variation 0.04%) identical with the precision of dispensing a volume for the burette. A typical linear regression precison of a single end-point intersection was around 0.003 cm³, about the same as the usual precision of a set of titrations.

All the listed indicators are recommended for the given metals and conditions except perhaps for Pyrocatechol Violet, whose unstable nature at pH 10 gives rise to a slowly falling absorbance reading towards the end of the titration.

Indicator purity does not appear to be critical.

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Paper A4/297
Received August 29th, 1984
Accepted 17th October, 1984

Lithium-selective Polymeric Membrane Electrodes Based on Dodecylmethyl-14-crown-4

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Lithium-selective electrodes were prepared with polymeric membranes containing dodecylmethyl-14-crown-4 as a neutral carrier. The effects of the crown ether content in the membrane and the type of membrane solvent on the electrode properties were investigated. Typical Li^+-selective electrodes showed a linear Nernstian response in the activity range 1×10^{-5}– 1 M LiCl, preferring lithium to sodium and hydronium ions by factors of 150 and 6800, respectively. Addition of trioctylphosphine oxide to the membrane enhanced the lithium selectivity over sodium in the electrode, although it had adverse effects on the lithium selectivity over hydronium ion and the detection limit. The determination of lithium in artificial serum samples was also attempted with these lithium-selective electrodes.

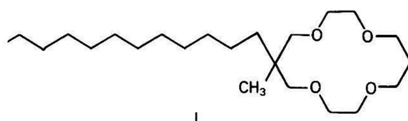
Keywords: *Lithium-selective electrode; PVC membrane; crown ether*

Considerable attention is being focused on the potentiometric determination of the Li^+ activity in biological systems by using Li^+-selective electrodes. In special applications such as the monitoring of Li^+ during therapy of maniacal psychosis,[1–3] a high selectivity of Li^+ electrodes is required, owing to the coexistence of high concentrations of Na^+ in blood.

Several attempts have been made to develop Li^+ electrodes,[4–12] but most are still inadequate for monitoring Li^+ in biological systems, because the selectivity coefficients for Li^+ over Na^+ range from 1 to 1×10^{-2}. Neutral carrier-type electrodes appear particularly promising as ion-selective electrodes; in this type of Li^+-selective electrode, the Li^+ selectivity depends mainly on how selectively the neutral carriers complex Li^+. Simon and co-workers[6,8] have synthesised several lipophilic diamide derivatives as Li^+ neutral carriers. The Li^+ electrode based on one of the derivatives possesses fairly good selectivity, showing a $k^{Pot}_{Li\,Na}$ value of slightly less than 1×10^{-2}; however, the electrode suffered from serious interference by H^+, the $k^{Pot}_{Li\,H}$ value exceeding unity.

Alternative neutral carriers for Li^+-selective electrodes are crown ether derivatives. Very few crown ethers exhibit a great affinity for Li^+, whereas many crown ethers favour Na^+ or K^+ on complexing. Nevertheless, it has been reported that crown ethers containing four oxygen atoms (crown-4 derivatives) possess Li^+ affinity.[13,14] Several crown-4 derivatives have been applied as neutral carriers with Li^+-selective electrodes,[10–12] but only poor Li^+ selectivity has been obtained.

We have prepared lipophilic crown-4 derivatives containing 13- to 16-member rings to obtain highly Li^+-selective neutral carriers[15] and recently found that the corresponding 14-crown-4 derivatives are most selective for Li^+ when they are utilised as neutral carriers of polymeric membranes.[16] In this paper we report details of Li^+-selective polymeric membrane electrodes based on dodecylmethyl-14-crown-4 (**I**). A practical application of the Li^+ electrodes to an artificial serum sample is also described.



I

Experimental

Chemicals

The synthesis of dodecylmethyl-14-crown-4 (6-dodecyl-6-methyl-1,4,8,11-tetraoxacyclotetradecane, **I**) has been reported elsewhere.[15] *o*-Nitrophenyl octyl ether (NPOE),[17] *o*-nitrophenyl phenyl ether (NPPE),[18] dipentyl phthalate (DPP)[19] and tris(2-ethylhexyl) phosphate (TEHP)[20] were prepared according to the literature. Commercially available dioctyl phthalate (DOP) and bis(2-ethylhexyl) sebacate (DOS) were distilled under vacuum. Poly(vinyl chloride) (PVC, average degree of polymerisation 1100) was purified by reprecipitation from tetrahydrofuran (THF) in methanol. Potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB) was produced according to a previous procedure.[21] Trioctylphosphine oxide (TOPO) was used as received. All of the salts and the HCl were of analytical-reagent grade. Water was de-ionised and distilled.

Electrode Preparation

The general procedure for the preparation of the polymeric membrane is as follows. PVC (100 mg), the crown ether **I** (3.6 mg), a plasticiser (250 mg) and KTpClPB (50 mol-% with respect to the crown ether) were dissolved in 3 ml of THF. In some instances, an appropriate amount of TOPO was included in the THF solutions. The solution was then poured into a flat Petri dish of 34 mm inner diameter. Gradual evaporation of the THF at room temperature gave a transparent, flexible membrane of 0.1–0.2 mm thickness. A disc of 7 mm diameter was cut from the PVC membrane by using a cork borer and incorporated into the body of an Orion Model 92 or Philips Model IS561 electrode. After injection of an appropriate concentration of LiCl solution (1 M unless stated otherwise) as the internal solution, the PVC membrane electrode was conditioned by soaking overnight in LiCl solution of the same concentration as the internal solution. The external reference electrode was a double-junction Ag - AgCl electrode, the filling solutions of which for the inner and outer compartments were 4 M KCl and 0.1 M NH_4NO_3 aqueous solution, respectively. The composition of the electrochemical cell is Ag - AgCl | 1 M LiCl || PVC membrane || sample solution | 0.1 M NH_4NO_3 | 4 M KCl | AgCl - Ag.

E.m.f. Measurements

All e.m.f. measurements were made at 25 °C using a Corning Model M-130 or Toko Model TP-1000 pH - millivolt meter. Sample solutions were kept in a double-walled glass container connected with a circulating bath while stirring magnetically. The electrode system and the millivolt meter were contained in a Faraday cage to cut off any electrical noise. The e.m.f. values were corrected by liquid-junction

potentials between the external reference electrode and the sample solution, which were calculated according to Henderson's equation.[22]

The selectivity coefficients were determined by the fixed interference method (FIM) according to IUPAC recommendations.[23] The constant concentrations of interfering ions were 5×10^{-2} M for alkali metal ions and H^+ and 5×10^{-1} M for alkaline-earth metal ions and NH_4^+.

Determination of Lithium in Artificial Serum Samples

The artificial serum samples contained 145 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, 0.8 mM $MgCl_2$, 2.5 mM urea, 4.7 mM glucose and an appropriate amount of LiCl. The samples were subjected to e.m.f. measurements without dilution. The Li^+ determination was carried out using a standard additions method without prior calibration of the electrodes.[24] The volume of the sample solution was 10 ml and the standard additions solution was 0.1 or 1.0 M LiCl, 0.1 or 0.05 ml of which was added each time. A least-squares curve fitting was applied to evaluate the unknown Li^+ concentration. Calculations were made with a microcomputer program modified from the published FORTRAN source program (ADDFIT).[24]

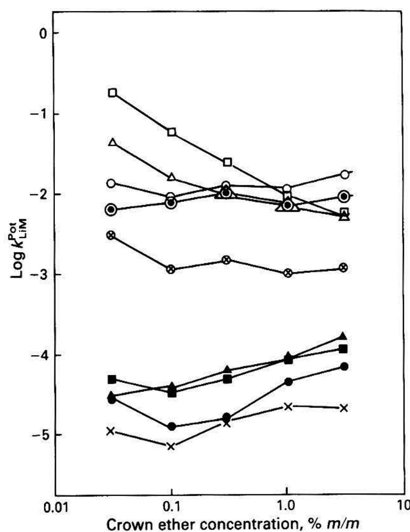


Fig. 1. Dependence of selectivity coefficients (k^{Pot}_{LiM}) on crown ether concentration in PVC membranes. (●) Na^+; (○) K^+; (△) Rb^+; (□) Cs^+; (⊗) NH_4^+; (×) Mg^{2+}; (●) Ca^{2+}; (▲) Sr^{2+}; (■) Ba^{2+}

Results and Discussion

Polymeric membrane Li^+-selective electrodes were constructed by using dodecylmethyl-14-crown-4 (**I**), which is a highly Li^+-selective neutral carrier bearing a lipophilic aliphatic chain. The polymeric membranes contained PVC as the polymer support, an appropriate plasticiser as the solvent, the Li^+ neutral carrier and other additive(s). As one of the additives to the membrane, lipophilic salts such as KTpClPB are often employed to reduce electrical membrane resistance and interferences from lipophilic sample anions.[25] In the PVC membrane without KTpClPB, our electrodes suffered from long response times and severe e.m.f. drift owing to the high membrane resistance. KTpClPB at a concentration of 50 mol-% with respect to the crown ether was therefore added to the PVC membranes in this study. Higher KTpClPB concentrations caused a decrease in the potentiometric Li^+ selectivity over alkaline-earth metal ions.

The Li^+ selectivity of the electrodes may depend on the composition and the kind of membrane components. Fig. 1 illustrates the effect of the concentration of the crown ether in the PVC - NPOE membrane on the electrode selectivity. The Li^+ selectivities over Na^+, K^+, and NH_4^+ in the PVC membranes remained almost unchanged in the concentration range 0.003–3% *m/m*. This means that these monovalent cations form crown ether complexes with the same stoicheiometry (1:1) as the Li^+ complex, the stoicheiometry of which has been determined conductimetrically.[15] The selectivities for Li^+ over Cs^+ and Rb^+ were improved by increasing the crown ether concentration, which indicates a low stability of the crown ether complexes with the metal ions. The selectivity coefficients for Li^+ over alkaline-earth metal ions

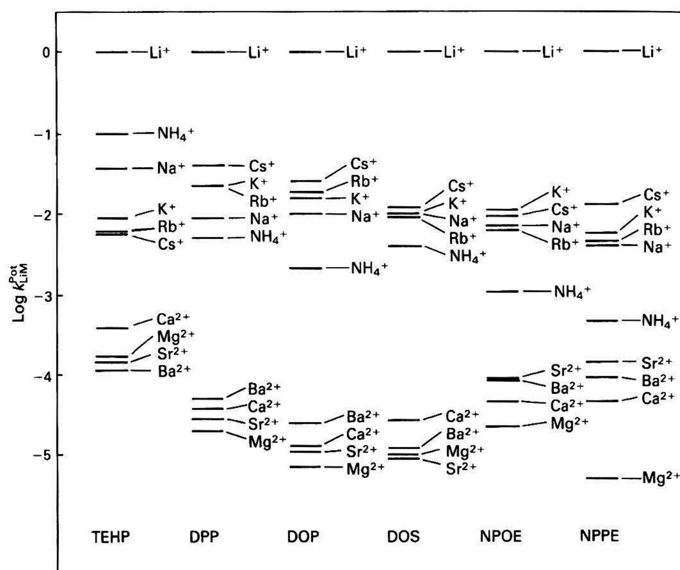


Fig. 2. Selectivity coefficients (k^{Pot}_{LiM}) of PVC membranes containing different plasticisers

increased gradually with increasing the crown ether concentration, but the increases were not substantial. Hence crown ether concentrations around 1% *m/m* proved suitable for the PVC membranes for Li^+-selective electrodes.

Several plasticisers in addition to NPOE were tested as the membrane solvent for the Li^+-selective electrodes based on **I**, and comparisons of the selectivity coefficients are shown in Fig. 2. The PVC membrane containing TEHP is inferior to those with the other plasticisers with respect to potentiometric Li^+ selectivity. In the membranes with the diester-type solvents DPP, DOP and DOS, the Li^+ selectivities of the electrodes are similar. Also, the NPOE membrane resembles the NPPE membrane with respect to electrode selectivity. Considering the Li^+ selectivity over alkali metal ions, the phenyl ether-type solvents are generally superior to the diester-type solvents, but for the Li^+ selectivity over alkaline-earth metal ions the opposite applies. This is partly due to the difference in the dielectric constants of the two types of solvents.

One of the most important parameters for Li^+-selective electrodes is the high preference for Li^+ over Na^+, particularly in the determination of Li^+ in biological and environmental systems. The selectivity coefficient for Li^+ over Na^+ is about 1×10^{-2} for membranes of the diester-type solvents and below 1×10^{-2} for the phenyl ether-type solvents, the interference by Na^+ being small with the Li^+ electrodes. It should be noted that the $k^{Pot}_{Li\,Na}$ values for NPPE (4×10^{-3}) and NPOE (7×10^{-3}) are better than that of the Li^+ electrode of Zhukov *et al.*,[8] which, to our knowledge, had been the most selective for Li^+. Moreover, interference by H^+ is small in comparison with those for the previous electrodes, the $k^{Pot}_{Li\,H}$ value being about 4×10^{-4}. The calibration graphs for the Li^+ electrode of the NPOE membrane exhibited a linear Nernstian response (59 mV decade^{-1}) in a wide activity range from 1×10^{-5} to 1 M Li^+, as shown in Fig. 3. The Li^+ electrodes with the other membrane solvents gave similar calibration graphs to that for the NPOE membrane. The LiCl concentration of the internal filling solution in the electrode hardly affects the Li^+ activity range of the calibration graphs for a linear Nernstian response or the electrode selectivity.

Some attempts were made to enhance the Li^+ selectivity of the electrodes based on **I** by using TOPO[26] as an additive in the PVC membranes containing NPOE, NPPE or DOS. Enhancement of the Li^+ selectivity over alkali metal ions, although not very substantial, was achieved by adding a small amount of TOPO to the PVC membranes (Fig. 4). For example, in the PVC membranes with 1% *m/m* of TOPO, the selectivity coefficients for Li^+ over Na^+ are 2×10^{-3} for the NPOE system and 3×10^{-3} for the NPPE system, compared with from 7×10^{-3} and 4×10^{-3}, respectively, for the membranes without TOPO. The $k^{Pot}_{Li\,Na}$ values are very good for Li^+-selective electrodes. A TOPO content of more than 1% *m/m* did not increase further the Li^+ selectivity over alkali metal ions. On the contrary, some decrease in the Li^+ selectivity over the alkaline-earth metal ions and H^+ was observed on addition of TOPO. The selectivity coefficients for Li^+ over alkaline-earth metal ions increased by a factor of less than 10 on the addition of 1% *m/m* of TOPO, but were still at the 10^{-4} level. The problem, however, may be that the addition of TOPO to the PVC membrane causes a pronounced decrease in the electrode selectivity for Li^+ over H^+. The selectivity coefficient $k^{Pot}_{Li\,H}$ changed drastically, from 4×10^{-4} to 2×10^{-1}, even when 1% *m/m* of TOPO was employed. Further, the addition of TOPO to the PVC membrane increased the lower detection limit of Li^+ activity for the **I**-based electrodes, as demonstrated in Fig. 3. The logarithms of Li^+ activity at the detection limit were −5.5 for the NPOE membrane without TOPO, −5.0 for that with 1% *m/m* of TOPO and −4.5 for that with 4% *m/m* of TOPO. The increase in the lower detection limit is probably due to the marked interference by H^+. Fig. 5 shows pH dependence of the e.m.f. readings for a sample of 1×10^{-2} M LiCl solution, showing that when TOPO is present in the PVC membranes very high e.m.f. values were attained at low pH. However, with the membrane with 1% *m/m* of TOPO, the e.m.f. readings remained constant down to pH 4. Hence the addition of 1% *m/m* of TOPO to the PVC membrane may be accepted in order to obtain a higher preference for Li^+ over Na^+ with the Li^+-selective electrodes based on **I**.

The e.m.f. response of artificial serum samples containing various concentrations of Li^+ was investigated by using two typical Li^+-selective electrodes based on crown ether **I**. The

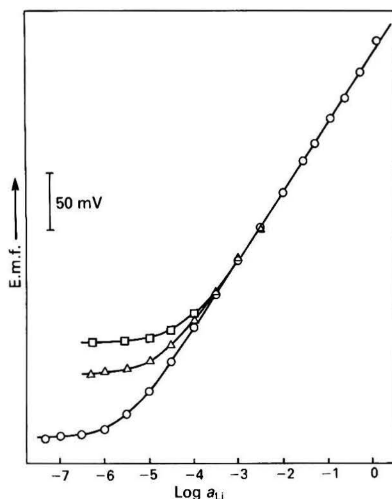


Fig. 3. Calibration graphs for pure LiCl solution using Li^+-selective electrodes with the PVC - NPOE membrane (○) without or (△) with 1% *m/m* and (□) 2% *m/m* of TOPO. Gradient, 59 mV decade^{-1}

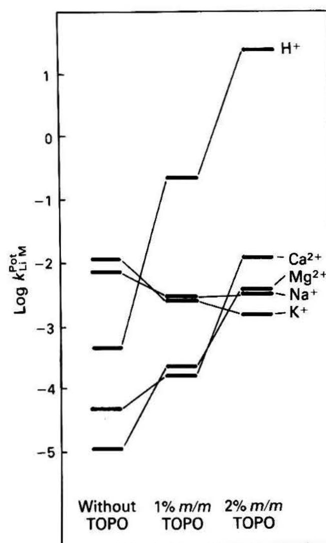


Fig. 4. Selectivity change of Li^+-selective electrodes on addition of TOPO to PVC - NPOE - **I** membrane

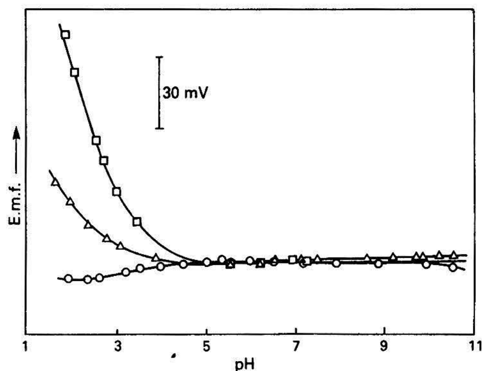


Fig. 5. pH dependence of e.m.f. values for Li^+-selective electrodes with PVC membranes (○) without or (△) with 1% *m/m* and (□) 2% *m/m* of TOPO

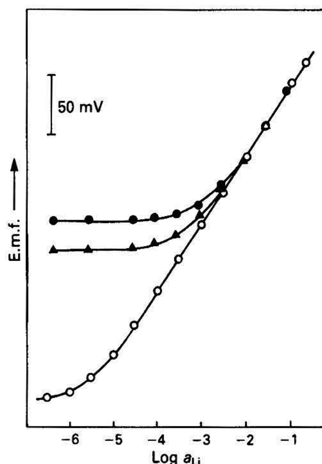


Fig. 6. Calibration graphs for Li^+-selective electrodes with PVC membranes (●) with or (▲) without TOPO for artificial serum samples. (○) Membrane without TOPO, for pure LiCl solutions. Gradient, 59 mV $decade^{-1}$

polymeric membranes of the Li^+ electrodes consisted of PVC (28% *m/m*), NPOE (about 70% *m/m*), the crown ether (1.0% *m/m*), KTpClPB (50 mol-% with respect to the crown ether) and, in one of the membranes, TOPO (1.0% *m/m*). The calibration graphs for Li^+ in the artificial serum samples, obtained with the Li^+ electrodes prepared from PVC membranes with or without TOPO, are shown in Fig. 6, together with those for pure LiCl solutions. With either of the Li^+ electrodes the detection limit of Li^+ activity for the artificial serum samples is high compared with those for pure LiCl solutions, on account of the high background concentration of Na^+ (145 mM) in the samples. The Li^+ electrode with the PVC membrane containing 1% *m/m* of TOPO has a superior detection limit of Li^+ activity to that without TOPO, which reflects the higher Li^+ selectivity over Na^+ of the former electrode.

The determination of Li^+ in the artificial serum samples was also attempted with the Li^+-selective electrode with the PVC - NPOE - **I** - KTpClPB - TOPO membrane system, by using a standard additions method without prior calibration of the electrode. The Li^+-selective electrode responded to the Li^+ activity changes fairly rapidly, the response time being less than 20 s by this incremental method (Fig. 7). A correlation between the found and actual values for the Li^+ concentration in the artificial serum samples is shown in Fig. 8. The values obtained potentiometrically were in good agreement with the actual values for Li^+ concentrations down to 1 mM. It is difficult to detect Li^+ concentrations below 1 mM in artificial serum samples by means of the Li^+-selective electrode.

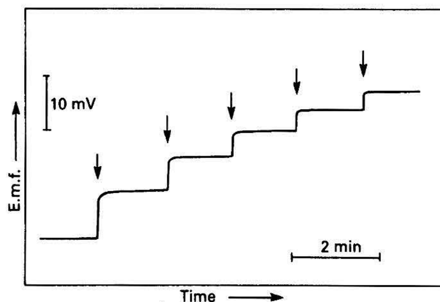


Fig. 7. E.m.f. *versus* time profile for Li^+-selective electrodes based on crown ether **I** using artificial serum samples. Initial Li^+ concentration in the artificial serum sample, 2 × 10^{-3} M. The arrows represent additions of the standard solution (0.1 M LiCl, 0.1 ml)

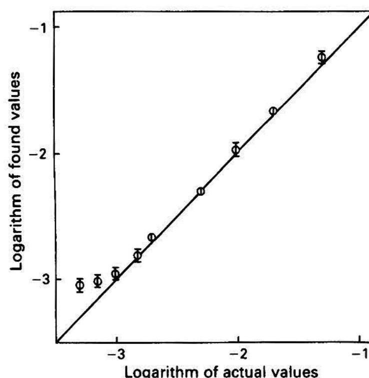


Fig. 8. Correlation of actual values of Li^+ concentration (M) in artificial serum samples and results obtained with Li^+-selective electrodes

In conclusion, the Li^+-selective electrodes based on crown ether **I** enabled us to determine low concentrations of Li^+ even in samples with high Na^+ backgrounds such as blood. Hence the Li^+-selective electrodes described here are very promising for monitoring Li^+ in biological systems.

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Paper A4/282
Received August 13th, 1984
Accepted October 8th, 1984

SHORT PAPERS

Pyridine-2-carbaldehyde Derivative of Girard-P as a Fluorimetric Reagent for the Determination of Gallium in Aluminium

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A fluorimetric method for the determination of gallium, based on the formation of a fluorescent 1 : 1 chelate with PAHP (the pyridine-2-carbaldehyde derivative of Girard-P), is described. In aqueous solution, the fluorescent species has excitation and emission maxima at 388 and 442 nm, respectively, and the calibration graph is linear over the range 10–500 p.p.b. at pH 3.7. The method has been employed for the determination of gallium in aluminium.

Keywords: *Gallium determination; pyridine-2-carbaldehyde derivative of Girard-P; spectrofluorimetry*

Girard's reagents are acetohydrazides of quaternary ammonium salts, which were proposed by Girard and Sandulesco[1] for the isolation of steroids, hormones and other substances of biological significance. In the analytical field, Girard-T hydrazones have been used for the polarographic identification of ketosteroids in samples of urine or blood,[2] Girard-T and -D complexes with copper(II), cobalt(II), nickel(II) and manganese(II)[3,4] have been studied spectrophotometrically, as have Girard-T derivatives of 2-hydroxy-l-naphthaldehyde,[5,6] resacetophenone,[7] isatin and methylisatin.[8]

The pyridine derivative of Girard-P has previously been tested as an analytical spectrophotometric reagent,[9] and this work describes the spectrofluorimetric properties of the reagent and its metal chelates, and its use for the determination of gallium.

Experimental

Apparatus

Fluorescence measurements were performed on a Perkin-Elmer Model LS-5 spectrofluorimeter, equipped with a xenon discharge lamp pulsed at a line frequency of 8.3 W and 1 × 1 cm quartz cells. The fluorescence data are given with spectral correction. An ultrathermostatic water-bath circulator K5 (Colora Messtechnic GmbH) was also used.

Reagents

Salts and solvents of analytical-reagent grade or better were used throughout. All metal ion solutions were standardised.

PAHP solution, 0.1% *m/V*. PAHP was synthesised as previously described[9] from Girard-P reagent and pyridine-2-carbaldehyde (picolinaldehyde).

Gallium stock solution. Prepared by dissolving 6.421 g of $GaCl_3$ in 1000 ml of 1 M hydrochloric acid. The exact gallium content was determined by titration with EDTA. Solutions of lower concentrations were prepared by dilution with de-ionised water.

Buffer solution, pH 3.6, *sodium acetate - acetic acid* (0.2 M).

Titanium chloride stock solution, 15% *m/V*, *containing* 10% *of hydrochloric acid*. Solution obtained from Merck.

Procedures

Recommended procedure for the determination of gallium

Into a 25-ml calibrated flask place an aliquot of sample containing 0.25–2.50 µg of gallium. Add 1 ml of buffer solution (pH 3.6), 5 ml of 0.1% PAHP solution and dilute to the mark with de-ionised water. Allow this to stand for 30 min and measure the fluorescence intensity at 422 nm with excitation at 388 nm, at 20 ± 0.5 °C, against a reagent blank.

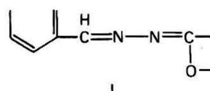
For a greater concentration of gallium (2.50–12.50 µg) an alternative procedure, with a 5-fold concentration of reagent, but maintaining the other conditions identical, must be used.

Determination of gallium in aluminium

Weigh 0.25–0.50 g of aluminium metal sample (BCS No. 195g) and place in a vessel. Add 20–30 ml of 6 M hydrochloric acid and heat moderately to dissolve. Add the titanium chloride solution, drop by drop, until a violet colour develops. Allow the solution to stand for 2–3 min and then transfer into a 100-ml separating funnel. Rinse the beaker with 3–5 ml of 6 M hydrochloric acid. Add an equal volume of diethyl ether to the solution and shake energetically for 1 min. Discard the separated lower layer and wash the extract twice with 6 M hydrochloric acid (2–3 ml portions). Re-extract gallium with 10–15 ml of water for 1 min. Transfer the lower layer into a porcelain dish. Repeat the re-extraction of gallium with water and add the re-extract to the dish contents. Add 0.1 g of NaCl and evaporate the solution on a water-bath to dryness. Dissolve the residue in water and transfer into a 100-ml calibrated flask, adding de-ionised water to the mark. Gallium was determined from an aliquot of this solution as described under *Recommended procedure for the determination of gallium.*

Results and Discussion

PAHP is a chelate-forming agent, but the complexes are colourless with the exception of Cu(I) and Fe(II).[9] Its fluorogenic reactions with metallic ions have been tested, and emission properties have been detected for gallium and aluminium chelates, especially for the former, whose reaction has a remarkable sensitivity at pH 3.7. The higher relative fluorescence intensity of the gallium chelate is surprising, because of the decreasing effect of the heavy atom on the sensitivity of the fluorescence effects; but antecedents of this behaviour have been previously reported for pyridine-2-carbaldehyde 2-furoylhydrazone,[10] which holds the same atom grouping (I) as that of PAHP, and fluorogenic action of this group on gallium may be predicted.



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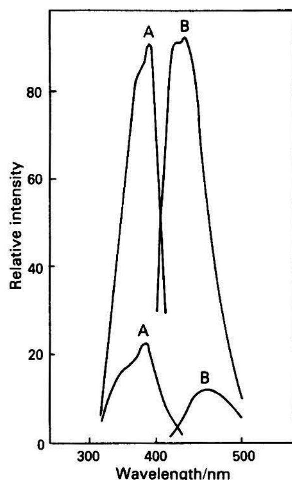


Fig. 1. Uncorrected excitation (A,A′) and emission (B,B′) spectra of complex (A,B) and free ligand (A′, B′), at pH 3.7. [Ga] = 1.4×10^{-5} M and [PAHP] = 7.23×10^{-4} M

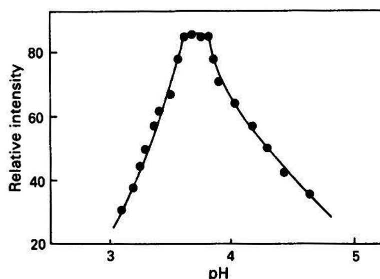


Fig. 2. Effect of pH on the formation of the Ga - PAHP chelate

Spectrofluorimetric Study of the Gallium - PAHP System

The reaction between gallium and PAHP in aqueous solution fluoresces immediately. The excitation and emission spectra are shown in Fig. 1 at pH 3.7 ($\lambda_{exc.}$ = 388 nm, $\lambda_{em.}$ = 442 nm). Fig. 1 also shows the fluorescence spectra of the reagent ($\lambda_{exc.}$ = 392 nm, $\lambda_{em.}$ = 463 nm). The stability of the fluorescence intensity of the reagent and the gallium complex was 3 and 2 h, respectively.

Influence of Experimental Variables

The pH of the medium had a major effect on the fluorescence intensity. Fig. 2 shows that the Ga(III) - PAHP system shows a narrow pH interval (from 3.5 to 3.8) in which the fluorescence is a maximum, descreasing drastically at both lower and higher pH values. For this reason an acetic acid - acetate buffer solution (pH 3.6) was used for further experiments. The concentration of the buffer also decreased the fluorescence intensity; the volume of buffer added, between 0.25 and 2.00 ml, was finally optimised at 1.0 ml.

Whether the presence of ethanol would decrease the fluorescence intensity was also considered, which does question the applicability of the method owing to the high solubility of PAHP in water.

The effect of PAHP concentration on the intensity for a 1.43 $\times 10^{-4}$ M gallium solution was also tested under analogous conditions to those given in the recommended procedure. The intensity of the fluorescence increased with an increase in concentration of PAHP up to 4.3×10^{-4} M, reaching a maximum and constant value at 2.3×10^{-3} M, this behaviour can be attributed to the low stability of the chelate, which makes it necessary to use a large excess of reagent in order to complete the complexation reaction.

The temperature significantly affects the fluorescence intensity, which substantially falls (25%) on increasing the temperature from 20 to 30 °C (if the temperature was increased from 20 to 60 °C the decrease of intensity was about 80%). Probably, the increase in temperature causes a higher internal conversion of the non-radiative deactivation of the excited singlet state; in addition, the carbonyl group of the PAHP may contribute to the higher inter-system crossing rate as the temperature increases. For these reasons the measurements have been carried out at 20 ± 0.5 °C.

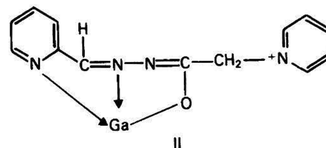
Table 1. Effect of foreign ions on the determination of 60 μg l^{-1} of gallium. For criteria for interference see text

Foreign ion	Tolerance/ μg l^{-1}
Be, Sr, Mg, Ba, Pb, Ca, Cl^-, SCN^-, NO_3^-	6.000
Tl(I), Mn, Sb(III), Cd	3.000
Ni	1.800
Co, Zn, Hg(II), Th, Y	1.200
In, Pd, PO_4^{3-}	600
Fe(III), Cr(III), Hg(I), F^-	300
Al	180
C(II), Sn(II), citrate	60
Fe(II), EDTA	60

Composition and Stability of the Complex

The molar ratio of metal ion to PAHP was determined under the experimental conditions for maximum radiative emission following the methods of Asmus[11] and Román Ceba *et al.*,[12] which are suitable for weak complexes. The composition of the Ga(III) - PAHP chelate was always 1 : 1, and a hypothesis may be established for the co-ordination sights of the ligand; this is shown in (**II**). From evidence in the literature on pyridine-2-carbaldehyde 2-furoylhydrazone,[10] the molecular grouping shown in (**I**) can be presumed to be responsible for the reactivity of the reagent.

The effective stability constant (β) of the complex was 1.3×10^3.



Analytical Characteristics

The calibration graph was linear for two ranges of gallium(III) concentrations, 10–100 and 50–500 ng ml^{-1} with two concentrations of PAHP, 7.2×10^{-4} and 3.6×10^{-3} M, respectively. The relative error from the 11 measurements for the two ranges of metal concentrations assayed were 1.89% (with 60 ng ml^{-1} of gallium) and 1.30% (200 ng ml^{-1} of gallium).

Effect of Foreign Ions

The influence of several ions on the fluorescence intensity of gallium chelate (at a level of 60 μg l^{-1}) was studied. The criterion for interference was fixed at ± 4% variation of the average fluorescence intensity, calculated for the established level of gallium. The results are given in Table 1.

In(III) was tolerated at a 10-fold molar ratio and Al(III) at a 3-fold molar ratio. Moderate interference was experienced from Co(II), Zn(II), Hg(II), Th(IV) and Ni(II).

Application

The proposed method was satisfactorily applied to the determination of gallium in aluminium (BCS No. 195g: Al, 99.85%; SiO_2, 0.03%; Fe, 0.08%; Cu, 0.001%; Mn, 0.001%; Ti, 0.002%, V, 0.004%; Zn, 0.015%; and Ga, 0.009%). The sample was dissolved as described under Procedure for the determination of gallium in aluminium, and gallium separated as described under Experimental, following the method proposed by Busev *et al.*[13] The percentage of gallium calculated as the average of three determinations was 0.0083 ± 0.0004%.

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Paper A4/159
Received April 25th, 1984
Accepted October 4th, 1984

Spectrophotometric Determination of Osmium with 4(5)-D-Arabinotetrahydroxybutylimidazoline-2-thione

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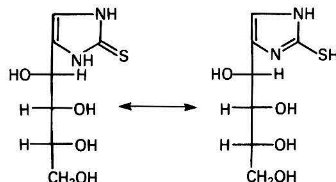
4(5)-D-Arabinotetrahydroxybutylimidazoline-2-thione (THBIT) forms a bluish violet complex in sulphuric, perchloric or hydrochloric acid media that has been utilised for the determination of osmium by spectrophotometry. The colour system has its absorption maximum at 530 nm and obeys Beer's law over the range 1–47 μg ml^{-1} of Os in 0.01–3.6 M H_2SO_4. The optimum range is 7–35 μg ml^{-1}. The molar absorptivity is 4.5×10^3 l mol^{-1} cm^{-1}. The method has a wide range and is simple, rapid and free from interferences from many metal ions, including some platinum metals.

Keywords: *Osmium determination; 4(5)-D-arabinotetrahydroxybutylimidazoline-2-thione (THBIT); spectrophotometry*

4(5)-D-Arabinotetrahydroxybutylimidazoline-2-thione, or THBIT, has previously been included in studies on some imidazole derivatives as spectrophotometric analytical reagents[1] and, more recently,[2] has been proposed as a spectrophotometric reagent for the microdetermination of palladium and used satisfactorily for its determination in various samples. This paper describes the investigation of the colour reaction of THBIT with Os(VIII) for the rapid spectrophotometric determination of osmium.



A large number of organic reagents for the spectrophotometric determination of osmium have been proposed[3] and have been reviewed by Beamish and Van Loon.[4] However, many of the methods need rigid control of acidity, temperature, reagents, etc., or are time consuming. The major advantage of the proposed method is that the maximum colour intensity is obtained in an aqueous medium almost instantaneously at room temperature. The proposed method offers the advantages of simplicity, rapidity, a wide range and good selectivity without the need for an extraction step or heating the solution.

Experimental

Reagents

All chemicals were of analytical-reagent grade unless specified otherwise.

Synthesis of THBIT. Potassium thiocyanate (10 g) was added to an aqueous solution (100 ml) of D-glucosamine hydrochloride (20 g). The mixture, in a porcelain capsule, was evaporated on a boiling water-bath almost to dryness. The crude sample was recrystallised from water using activated charcoal as decolorant, until the melting-point was constant (204–206 °C), then 0.2 or 0.5% *m/V* solutions were prepared in doubly distilled water.

Os(VIII) solution. Prepared by dissolving 1 g of osmium tetraoxide (Merck) in about 100 ml of 0.2 M sodium hydroxide solution and by diluting the solution to 1 l with doubly distilled water.[5] This solution was standardised by the method of Klobbie[6] as follows: to 25 ml of the solution were added 15 ml of 6 M sulphuric acid and 2 g of potassium iodide and the liberated iodine was titrated against 0.025 N sodium thiosulphate solution. The end-point was determined using starch paper externally as indicator. Working solutions were prepared by suitable dilution of this stock solution.

Apparatus

Spectrophotometric measurements were made with a Perkin-Elmer 124 spectrophotometer with matched 10-mm quartz cells. The pH values were determined with an Orion Research Model 701 A digital pH meter using a combined electrode.

Procedure

In a 25-ml calibrated flask place an aliquot of the sample solution containing 25–1175 μg of Os(VIII). Add 5 ml of 4 M sulphuric acid and 5 ml of 0.5% reagent solution and dilute to the mark with doubly distilled water. Mix well and measure the absorbance at 530 and 575 nm against a reagent blank prepared identically but without osmium.

For the concentration range 25–250 μg of Os(VIII) identical results were obtained with 5 ml of 0.2% reagent solution. Because the ligand solution is colourless and does not absorb at 530–580 nm, a water blank is suitable for the absorbance measurements in this instance.

Results and Discussion

Absorption Spectra

The absorption spectra of the osmium complex in acidic media (Fig. 1) showed that the complex absorbed maximally over the range 530–580 nm, depending on the nature and concentration of the acid used. Thus, in 1 M sulphuric, perchloric or hydrochloric acid the spectra showed a maximum at 530 nm and a shoulder at 560–580 nm. The bluish violet complex was not formed in nitric acid medium. The reagent blank, under similar conditions, showed no appreciable absorption over the wavelength range 500–700 nm.

* To whom correspondence should be addressed.

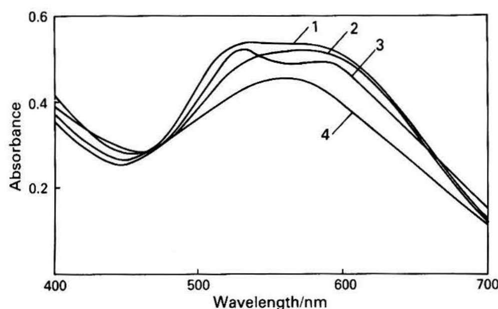


Fig. 1. Absorption spectra of the Os(VIII) - THBIT complex. 1, 0.4 M H_2SO_4; 2, 5 M H_2SO_4; 3, pH 2.6; and 4, 4 M HCl. All solutions contained 550 µg per 25 ml

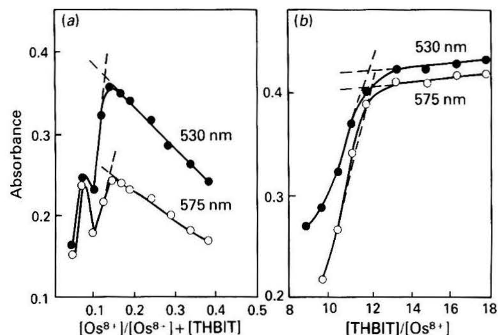


Fig. 2. Stoicheiometry of the Os(VIII) - THBIT complex. (*a*) Continuous variations method; and (*b*) molar ratio method

Effects of Acidity and Reaction Time

The system shows a maximum colour intensity over the acidity range 0.01–3.6 M sulphuric acid and 0.01–1 M hydrochloric acid (with similar absorbance values in both instances). At higher acidities the absorbance decreases slowly and the shoulder becomes a maximum at 560–580 nm. The absorbance at pH 2.60 (chloroacetic acid buffer) also decreases and colour development is slower.

Constant absorbance values were obtained almost immediately after adding THBIT solution to osmium solution in the presence of sulphuric or hydrochloric acid (the acid concentration being higher than 0.1 M) and they remained constant for at least 24 h. Owing to its wider range, sulphuric acid medium was selected for further studies.

Effects of Reagent Concentration and Order of Addition

The effect of THBIT concentration was investigated by measuring the absorbance at 530 and 570 nm of solutions containing 29.8 p.p.m. of Os(VIII) and various amounts of THBIT. A 20-fold molar excess of the reagent over Os(VIII) was required for maximum absorbance. The order of addition of reactants was critical, maximum absorbance readings being attained only if the reagent solution was added after the cation and the acid.

Calibration, Range, Sensitivity and Precision

The osmium - THBIT complex obeys Beer's law at 530 nm over the concentration range 1–47 p.p.m. of Os(VIII) in 0.8 M H_2SO_4. The optimum working range as evaluated by the Ringbom method is 7–35 p.p.m. The molar absorptivities are 4.5×10^3 l mol^{-1} cm^{-1} at 530 nm and 4.3×10^3 l mol^{-1} cm^{-1} at 575 nm and the Sandell sensitivity of the reaction is 41.8 ng cm^{-2} at 530 nm. The standard deviation calculated from 11 determinations on a solution containing 9.2 p.p.m. of Os(VIII) is 0.0020 and the relative error (P = 0.05) of the method is 0.62%.

Composition and Nature of the Complex

The composition of the osmium - THBIT complex was studied by the Job continuous variations and molar ratio methods (see Fig. 2). Job's method showed a maximum that corresponds to a stoicheiometry of the osmium - THBIT complex of 1 : 6. The molar ratio method showed a break at a ratio of 12 : 1 of ligand to metal.

Table 1. Tolerance of the osmium(VIII) - THBIT system to diverse ions. All solutions contained 230 µg per 25 ml

Foreign ion	Molar ratio	Foreign ion	Molar ratio
Ag(I)	19	Fe(II)	15
Pb(II)	10*	Al(III)	76
Tl(I)	10	Cr(III)	40
W(VI)	11*	Cr(VI)	20
Hg(II)	10	Ti(IV)	43
Bi(III)	10	Zr(IV)	23
Cu(II)	32	V(V)	20
Cd(II)	18	In(III)	18
Pd(II)	2	Ni(II)	35
Rh(III)	20†	Co(II)	35
Ru(IV)	4	Mn(II)	38
As(III)	7	Zn(II)	32
Sb(III)	17	NO_3^-	333
Sn(II)	17	Cl^-	583
Mo(VI)	21	Br^-	258
Pt(IV)	10†	I^-	8
Au(III)	10†	SCN^-	9
Fe(III)	37	ClO_4^-	208

* After centrifugation or filtration.
† Measurements were made at 575 nm.

The nature of the complex was studied by passing an aliquot of a solution of the complex through Dowex 50-X8 cation-exchange resin and Dowex 1-X8 anion-exchange resin. The bluish violet complex was retained by Dowex 50-X8 and not by Dowex 1-X8, indicating that the complex is cationic in nature.

The results obtained in the study of the composition and nature of the osmium complex showed an important analogy with those for the osmium - thiourea complex.[7] Hence, a composition of 1 : 6 (metal to ligand) can be regarded as probable. From the break at a molar ratio of 1 : 12 (1 : 11 in the thiourea complex), it is evident, if we assume that the ratio in the complex is 1 : 6, that the remainder of THBIT must have been consumed in the reduction of Os(VIII) to Os(II) [Os(VIII) to Os(III) in the thiourea complex].

Effect of Diverse Ions

In order to study the effect of various ions on the determination of osmium(VIII) with THBIT, a fixed amount (9.2 p.p.m.) of osmium(VIII) was taken with different amounts of foreign ions (the maximum levels tested were 100 and 500 p.p.m. for cations and anions, respectively) and the recommended procedure was followed. An error of 2% in the absorbance readings was considered tolerable. The tolerances for various foreign ions are shown in Table 1.

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Paper A4/205
Received June 18th, 1984
Accepted November 16th, 1984

Reaction of Antimony(III) with Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine Trisodium Salt as a Spectrophotometric Method for the Determination of Sulphide

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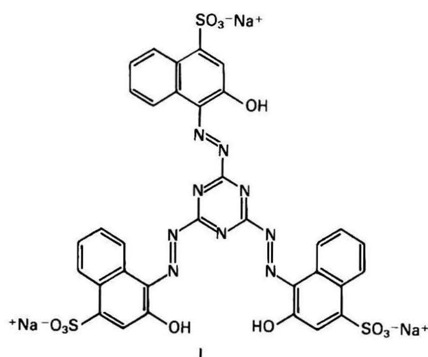
Antimony(III) interacts with tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine trisodium salt (THT) to give a 1 : 1 dark red complex at pH 3.3–7.0. Sandell's sensitivity of the colour reaction at $\lambda_{max.}$ 535 nm is 0.0029 µg cm^{-2} of Sb ($\varepsilon = 4.16 \times 10^4$ l mol^{-1} cm^{-1}). Among the anions, sulphide only decomposes the antimony - THT complex quantitatively replacing THT from the complex and 6–52 µg per 25 ml of sulphide are determined indirectly.

Keywords: *Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine; antimony(III) determination; indirect sulphide determination; spectrophotometry*

Antimony and arsenic occur in small amounts in fission products in nuclear reactors. Antimony is also an important constituent of many lead alloys. A few analytical reagents are available for the determination of antimony based on the ion association complexes of $SbCl_6^-$ with some basic dyes or with Rhodamine B.[1] However, some other methods using potassium iodide,[1] methyl violet,[1] phenylfluorone,[2] sodium diethyl dithiocarbamate,[3] alizarin red-S[3] and thiourea[3] have been reported for its spectrophotometric determination. Of these, the first two are widely used, but suffer from interference by several ions. Another reagent, methylfluorone,[4–7] has been recommended and the complex formed with antimony exists as a sol in the presence of gelatine. Some heterocyclic azo dyes, *viz.*, 1-(2-pyridylazo)-2-naphthol (PAN),[8] thiazolylazo reagents[9] and 6-(quinolylazo)-3,4-dimethylphenol[10] have been reported, but they also involve long extraction procedures. In this paper the recently synthesised[11] azo dye, tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-s-triazine trisodium salt (THT) (I) is reacted with micro amounts of



I

antimony forming a water-soluble dark red complex. The method developed is very simple, rapid and sensitive in comparison with some of the other published methods.[12,13]

Sulphide ions replace the ligand molecules quantitatively from the antimony - THT complex in aqueous solution and the decrease in the colour intensity of the complex is proportional to the concentration of the sulphide ion. No other anion decomposes the complex quantitatively but thiocyanate interferes only when its concentration is more than five times that of sulphide. The method is both highly sensitive and selective.

Experimental

Apparatus

A Bausch and Lomb Spectronic 2000 spectrophotometer with 10-mm matched glass cells was used for recording spectra and a Beckman pH meter was used for pH measurements.

Reagents

Synthesis of trihydrazino-s-triazine. 2,4,6-Trichloro-*s*-triazine (9.25 g, 0.05 mol) was reacted with an excess of hydrazine hydrate (*ca.* 100 ml, 99%) and refluxed for half an hour at 50–60 °C, cooled and filtered. The purity of the compound was checked by thin-layer chromatography. The compound decomposed at 280 °C.[14]

Synthesis of THT. A 3.42-g amount of trihydrazino-*s*-triazine was dissolved in the minimum amount of dilute hydrochloric acid and 15.60 g of the sodium salt of 1,2-naphthaquinone-4-sulphonic acid were dissolved in the minimum amount of water, the two solutions were mixed and heated on a water-bath for half an hour and then cooled and neutralised with dilute ammonia solution to give a deep red precipitate of the dye. The precipitate was filtered, washed with water and dried. The purity was checked by thin-layer chromatography and elemental analysis (calculated values for $Na_3C_{33}H_{18}N_9O_{12}S_3$: C 44.05, H 2.04 and N 14.1%; found values: C 44.15, H 2.0 and N 14.05%).

A 0.18% solution of THT (2×10^{-3} M) was prepared in water. The colour of the solution was red and it was stable for several weeks.

Antimony(III) solution, 1000 µg ml^{-1}. A stock solution of antimony(III) was prepared by dissolving an appropriate amount of antimony potassium tartrate ($KSbO.C_4H_4O_6$) in dilute hydrochloric acid. Working solutions were obtained by suitable dilution with water.

Sulphide solution, 1000 µg ml^{-1}. An appropriate amount of $Na_2S.9H_2O$ was dissolved in water and the solution standardised iodimetrically.[15]

Acetate buffer solution, pH 5.0. An acetate buffer solution was prepared by diluting 300 ml of 0.5 N acetic acid to 1 l with 0.2 N sodium acetate solution. A 2-ml amount of this solution was used to bring the solutions for complexation reaction into the appropriate pH range.

Recommended Procedure

Determination of antimony(III)

To a suitable volume of sample containing 10–80 µg of antimony(III) add 5 ml of 0.18% THT solution followed by 1

ml of 0.05% EDTA solution and 2 ml of acetate buffer solution. Dilute to 25 ml with water and measure the absorbance at 535 nm against a corresponding reagent blank prepared under identical conditions.

Determination of sulphide

To a solution containing 75 μg of antimony(III) add 5 ml of 0.18% THT solution followed by a suitable volume of sulphide solution containing 6–48 p.p.m. of sulphide. Add 2 ml of acetate buffer solution and dilute to 25 ml with water. Measure the absorbance against a reagent blank (5 ml of 0.18% THT + 2.0 ml of acetate buffer diluted to 25 ml). The difference between the absorbance of the complex before and after addition of sulphide and the reagent blank is proportional to the concentration of sulphide ions.

Results and Discussion

THT is a multi-dentate water-soluble heterocyclic azo dye and is very sensitive towards antimony(III). It gives an intense dark red colour with antimony(III) with maximum absorbance at 535 nm (Fig. 1). The colour development is maximum and constant at pH 3.3–7.0 (acetate buffer). Some transition metals also produce colour with this reagent at this pH but they can be masked with EDTA, as at least up to a 100-fold excess of EDTA did not have any effect on the absorbance of the complex. During the anionic interference study, it was found that only sulphide interfered seriously and no other anion interfered except a few at high concentrations.

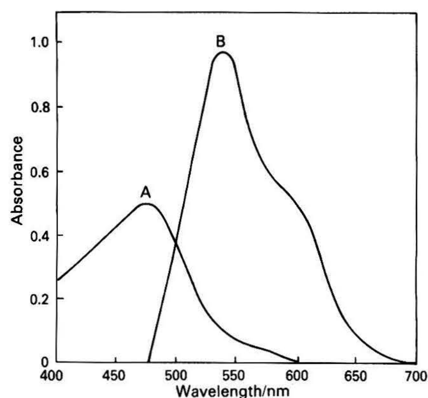


Fig. 1. Absorption spectra of A, 0.0018% THT solution against water; and B, Sb(III) - THT complex in water against THT. [Sb], 2.92 μg ml^{-1}; pH, 5.0; THT, 0.036%

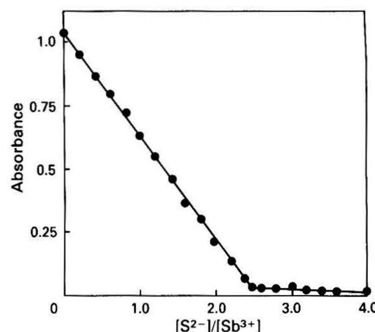


Fig. 2. Effect of sulphide on the Sb(III) - THT complex. [Sb], 3.0 μg ml^{-1}

Physico-chemical Characteristics of the Antimony - THT Complex

Absorbance data recorded for a series of solutions containing 2.4×10^{-5} M antimony(III) and varying amounts of reagent at pH 5.0 showed that a 10-fold molar excess of THT was required for full complexation. The concentration range over which Beer's law was obeyed and the optimum concentration range for the accurate determination of metal ion were 0–4.5 and 0.4–3.2 μg ml^{-1}, respectively. The composition of the complex was 1 : 1 (metal to ligand) as determined by Job's method of continuous variations. The complex was not extracted in non-aqueous organic solvents, thereby showing the ionic character. Sandell's sensitivity of the colour reaction was 0.0029 μg cm^{-2} of Sb [for log I_o/I = 0.001 with a molar absorptivity (ε) of 4.16×10^4 l mol^{-1} cm^{-1}.]

Effect of Diverse Ions on the Determination of Antimony(III)

In the determination of antimony(III) at a level of 3.0 μg ml^{-1}, fluoride, chloride, bromide, iodide, nitrite, nitrate, sulphite, sulphate, thiosulphate, citrate, tartrate, oxalate, borate, phosphate, thiourea, alkaline earths, lanthanides, aluminium(III), molybdenum(VI), tungsten(VI), platinum metals and thorium(IV) did not interfere at all. EDTA, vanadium(V), chromium(III), cobalt(II) and copper(II) did not interfere up to a 100-fold excess. The tolerance limits of other ions after masking with EDTA were as follows: zinc(II), cadmium(II), manganese(II), iron(II), nickel(II), lead(II), 20-fold; and uranyl(II), bismuth(III), indium(III), 5-fold; however, thiocyanate was tolerated up to a 5-fold excess over antimony and sulphide interfered seriously in the determination.

Stoicheiometry of the Antimony(III) - THT Complex and Its Reaction with Sulphide

A molar ratio study carried out on the reaction of sulphide with the antimony(III) - THT complex showed that the decrease in absorbance reached a maximum at a molar ratio of 2.5 : 1 of S^{2-} to Sb - THT and then remained negligible at a higher ratio (Fig. 2), presumably owing to the formation of Sb_2S_5 species.

Determination of Sulphide

The suppression of the colour of the antimony(III) - THT complex by sulphide was studied by taking a known aliquot of antimony(III) in the range 10–80 μg, adding 5 ml of 0.18% THT solution and varying the concentration of sulphide in a total volume of 25 ml. Using the particular conditions adopted for the determination of antimony(III), 6–52 μg per 25 ml of sulphide could be determined. The sensitivity of the method was 0.0019 μg cm^{-2} for sulphide. Sulphide was also determined by reacting it with an excess of antimony(III) and determining the unreacted antimony(III) with THT and the results showed reasonable reproducibility.

The precision results for sulphide at eight known concentration levels (each repeated four times) with 75 μg ml^{-1} of antimony(III) yielded a coefficient of variation of less than 0.65%. The tolerance limit of anions in the determination of sulphide were as follows: fluoride, chloride, bromide, nitrite, nitrate, sulphite, sulphate, citrate, tartrate, borate, phosphate (250-fold), iodide, thiosulphate, oxalate, 100-fold; EDTA, thiourea, 50-fold; and thiocyanate, 5-fold.

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Paper A4/136
Received April 4th, 1984
Accepted October 29th, 1984

Rapid Spectrophotometric Determination of Nitrate in Mineral Waters with Resorcinol

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A spectrophotometric method for the determination of nitrate, using resorcinol as the reagent, is described. The method is rapid with good reproducibility and low interference from common inorganic ions. The method has been applied to the determination of nitrate in mineral waters.

Keywords: *Nitrate determination; spectrophotometry; resorcinol; mineral waters*

A well known spectrophotometric method for the determination of nitrate is based on the nitration of phenolic compounds[1,2]: chromotropic acid,[3] 2,4-xylenol,[4,5] 2,6-xylenol,[6] 3,4-xylenol,[7,8] phenoldisulphonic acid[9] and phenol.[10]

In this study resorcinol is proposed as the reagent. Resorcinol is only slightly toxic and is commercially available at high purity. A further advantage is the high nitration rate, and good solubility, which allows the use of elevated concentrations. It is these properties that make resorcinol promising for the analysis of samples containing compounds that interfere by competitive reaction with nitrate.

The proposed method is performed easily and very rapidly and the reproducibility and the degree of interference of various common inorganic ions compare favourably with those of previous methods.

Experimental

Apparatus

A Shimadzu UV-240 spectrophotometer was used for the determination and recording of absorption spectra.

Reagents

Unless stated otherwise all chemicals were of analytical-reagent grade and de-ionised water was used throughout.

Resorcinol for synthesis. Obtained from Merck and used without further purification.

Resorcinol, aqueous solutions.

Nitrate standard solutions. Prepared from a stock potassium nitrate solution (1000 mg l^{-1} of nitrate-nitrogen).

Procedure

Pipette 1 ml of the sample solution or 1 ml of water (blank) into a 15-ml test-tube. Add 50 µl of 5% resorcinol solution and mix. Add 1.3 ml of 36 N sulphuric acid and mix for 15 s with a vortex mixer. Transfer the test-tube into a water-bath at room temperature. After cooling, measure the absorbance at 360 nm in a 1-cm cuvette.

For samples containing iron a correction should be made for its absorption at 360 nm. For this 1 ml of sample, 50 µl of water and 1.3 ml of 36 N sulphuric acid are measured against 1.05 ml of water and 1.3 ml of 36 N sulphuric acid.

Results and Discussion

Under the proposed experimental conditions, a rapid reaction occurs between resorcinol and nitrate. The nitro compound formed gives an absorption spectrum with a maximum at 347 nm [Fig. 1(*a*)]. Measurement at 360 nm was preferred because of the smaller absorbance of resorcinol [Fig. 1(*b*)].

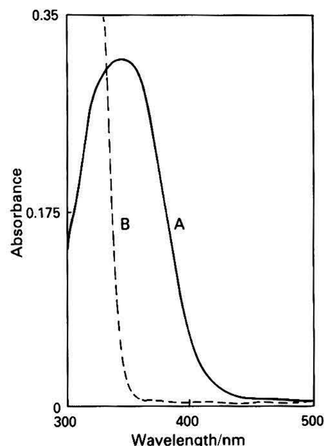

Fig. 1. Absorption spectra obtained A, for nitrate measured against blank; and B, blank measured against sulphuric acid - water (1.3 + 1.05)

Effect of Sulphuric Acid and Resorcinol Concentration

The analysis of samples containing 1 mg l^{-1} of nitrate-nitrogen was carried out using different volumes of sulphuric acid (36 N) and concentrations of resorcinol solution (50 µl). The volume of sulphuric acid was varied between 0.9 and 1.5 ml. From 1.2 to 1.5 ml (20.5 to 22.8 N) the ratio of absorbance to volume of reaction mixture was independent of concentration. For smaller volumes this ratio decreased because of incomplete nitration.

The concentration of resorcinol was varied between 1 and 40%. Maximum absorbance was obtained at 5%. For concentrations below 2%, the absorbance was decreased by incomplete nitration. For concentrations between 2 and 10%, the absorbance varied by only 1%. Increasing the concentration to 40% caused a decrease of absorbance to 94% of the maximum.

Effect of Time Before and After Cooling

Variation of the time before cooling between 10 s and 5 min had no effect. After cooling, the absorption of the solutions remained stable for at least 1 h.

Calibration

A linear calibration graph passing through the origin was obtained for concentrations up to 4 mg l^{-1} of nitrate-nitrogen. The relative standard deviation, calculated by 10-fold analyses of 0.05, 0.2, 1 and 4 mg l^{-1} samples, was 4, 1, 0.2 and 0.2%, respectively.

Interferences

The effect of various inorganic ions was investigated on samples containing 1 mg l^{-1} of nitrate-nitrogen. In the discussion interferences of ≤1% have been neglected. Chloride concentrations up to 500 mg l^{-1} had no effect. At 1000, 2000 and 3000 mg l^{-1} interference of +2.5, +6.3 and +9%, respectively, occurred.

In addition, the influence of other ions was studied in the absence and presence of 100 mg l^{-1} of chloride.

At the 1 mg l^{-1} level only nitrite interfered, giving an interference of +118%. Nitrite can be removed by reduction with hydrazine sulphate as follows: 25 µl of 2% hydrazine sulphate and 25 µl of 12 N sulphuric acid are mixed with 1 ml of sample. After 15 s, reduction is complete and the determination of nitrate is carried out (*cf.*, Procedure). In the absence of chloride, the absorbance obtained was not influenced by hydrazine sulphate; in the presence of chloride an interference of −1.6% was measured.

At the 10 and 100 mg l^{-1} level no interference occurred for ammonium, calcium, magnesium, zinc, manganese(II), phosphate, hydrogen carbonate, sulphite and fluoride. The interference of the other ions studied is summarised in Table 1. These data suggest that the reducing substances thiosulphate, sulphide, iodide and iron(II) have a more pronounced effect in the presence of chloride; this corresponds to the higher noted influence of hydrazine sulphate. Formation of easily reducible nitrosyl chloride[11] is thought to be the cause of this chloride effect.

The interference of mercury(II) ions in the absence of chloride is due to the reaction with resorcinol. In the presence of 100 mg l^{-1} of chloride, this reaction is prevented by the formation of mercury(II) chloride. For samples containing no chloride the interference can be eliminated by addition of an excess of chloride or 3 ml of water to the reaction mixture.

Iron(III) interferes by reaction with resorcinol; iron(III) can be removed prior to analysis by precipitation with ammonia solution.[11]

The interference of lead(II) by precipitation can be eliminated by centrifugation of the reaction mixture 10 min after preparation.

Table 1. Interference of diverse ions

Ion	Concentration/ mg l^{-1}	Interference, %: Without chloride	With chloride
Thiosulphate	10	≤1	−4.4
	100	−3	−7.8
Sulphide	10	−1.7	−5.2
	100	−6.0	−11.8
Bromide	10	≤1	≤1
	100	≤1	+2.5
Iodide	10	≤1	−3.2
	100	−3.7	−10.3
Copper(II)	10	+2.0	+2.1
	100	+2.6	+3.1
Mercury(II)	10	+6.7	≤1
	100	+111	≤1
Iron(II)	10	≤1	−7.8
	100	−2.3	−19
Iron(III)	10	+3.7	+3.8
	100	+28	+27
Lead(II)	10	≤1	≤1
	100	Precipitation	Precipitation

Table 2. Nitrate content of mineral waters

Sample	Concentration of NO_3^-/mg l^{-1}: Label	UV spectrophotometry	Present method
Apollinaris (0.75 l)	8.9	7.57	7.53
Bronwater GB (1 l)	17	26.8	25.6
Bronwater GB (1.5 l)	0.07	19.1	18.7
Contrexéville (1.5 l)	1	3.10	2.74
Evian (1.5 l)	3.8	3.59	3.65
Spa Monopole (1 l)	1.5	1.51	1.45
Spa Reine (1.5 l)	1.9	2.08	2.05
Highland Spring (1.5 l)	1.5	0.89	0.92
Vichy Hôpital (1 l)	0	0.26	0.18

Determination of Nitrate in Mineral Waters

The nitrate content of nine mineral waters was determined by the proposed method and by UV spectrophotometry at 220 nm and the results are shown in Table 2. For Appolinaris (0.75 l), Bronwater GB (1 l), Bronwater GB (1.5 l), Evian (1.5 l), Spa Monopole (1 l), Spa Reine (1.5 l) and Highland Spring (1.5 l) the concentration found by both methods differed by less than 5%. For Contrexéville (1.5 l) and Vichy Hôpital (1 l) the result obtained by UV spectrophotometry was, respectively, 12 and 31% higher than in the resorcinol method. In the last sample the large difference can be attributed to interference of species absorbing at 220 nm.

For seven mineral waters the concentration of nitrate found agreed within 2 mg l^{-1} of the label value; for Bronwater GB (1 l) and Bronwater GB (1.5 l) the label value was, respectively, 8.6 and 18.6 mg l^{-1} smaller than that found by the resorcinol method.

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Paper A4/304
Received September 3rd, 1984
Accepted October 5th, 1984

COMMUNICATION

Material for publication as a communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are usually examined by one referee and inclusion of a Communication is at the Editor's discretion

Zeeman-corrected Graphite Furnace Atomic-absorption Spectrometric Screening Method for the Determination of Lead in Infant Formulas and Powdered Milks

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Keywords: *Lead determination; infant formulas; powdered milk; Zeeman graphite furnace; atomic-absorption spectrometry*

The accurate and precise determination of lead in infant formulas and canned milks by routine procedures is not simple. A recent paper[1] analyses the results of a collaborative study with a graphite furnace atomic-absorption spectrometric method on the determination of lead in infant formulas, and the conclusion is discouraging. Major problems were encountered and poor results were obtained, in particular in terms of accuracy. This was caused primarily by inadequate simultaneous background correction capabilities on the instruments used.

In our laboratory we have recently analysed a limited number of infant formulas and dried milks for lead, and we used a Zeeman atomic-absorption spectrometric method, requiring no digestion of the samples prior to analysis. The procedure used may be of some interest outside this laboratory, especially in the light of the reported problems associated with deuterium arc background correction.[1]

Experimental

Instrumentation

A Perkin-Elmer Zeeman 5000 atomic-absorption spectrometer equipped with a Perkin-Elmer AS-40 autosampler was used. The atomisation signals were displayed on a Perkin-Elmer R 100-A recorder and their areas printed out on a Perkin-Elmer PRS-10 printer. The hollow-cathode lamp current was 10 mA and the 283.3-nm line with a spectral band pass of 0.7 nm was used. The graphite furnace programme is given in Table 1. The internal argon gas flow was stopped and the Zeeman correction was on during atomisation. Pyrolytically coated graphite tubes with solid pyrolytic graphite platforms inserted were used, and so was matrix modification (see below). As temperatures may vary slightly between instruments for a given setting, they should be regarded as approximate values only.

Reagents

$NH_4H_2PO_4$ and $Mg(NO_3)_2.6H_2O$ were of analytical-reagent grade and Triton X-100 was of scintillation grade, all purchased from E. Merck (Darmstadt, FRG). A certified 1 g l^{-1} lead reference solution (Titrisol; E. Merck) was used, and aliquots of this were diluted to yield working standards. Milli-Q water was used throughout, and all laboratory ware, including pipette tips and sample cups, were rinsed with 4 M nitric acid before use.

Procedure

Approximately 0.5 g of infant formula or milk powder was weighed into a 5-ml calibrated flask, 2 ml of water were added and the powder was dissolved by placing the flask on a Whirlimixer (Fisons Scientific, Loughborough, UK) for a few seconds. A 2-ml volume of 0.2% Triton X-100 solution was added, followed by further homogenisation on the Whirlimixer. Finally, water was added to volume and the sample was homogenised again. Subsamples of this mixture were diluted 1 + 1 with 0.2% Triton X-100 solution and 40-µl aliquots were injected on to the graphite platform. A 5-µl portion of matrix modifier solution consisting of 4.0 g of $NH_4H_2PO_4$ and 0.3 g of $Mg(NO_3)_2.6H_2O$ in 100 ml of water was also injected for each sample aliquot. The lead contents were evaluated by the standard additions technique.

Table 1. Graphite furnace programme

Step	Temperature/°C	Ramp/s	Hold/s
Dry I	120	30	30
Dry II	200	20	10
Char	850	30	20
Atomise	2000	0	5
Clean	2500	1	3
Cool	20	1	2

Discussion and Conclusion

The accuracy and precision of the procedure were tested by analysing BCR No. 63 EEC standard reference material, which is a skim milk powder with natural levels of trace elements. The certified value for lead is 0.1045 ± 0.0031 $\mu g\ g^{-1}$ (n = 57), and we found 0.103 ± 0.016 $\mu g\ g^{-1}$ (n = 3).

In conclusion, our procedure is reasonably accurate and precise at an appropriate level and this, in combination with the simple pre-treatment of the samples required, indicates that it may be useful as a screening method.

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Paper A4/443
Received December 21st, 1984

BOOK REVIEWS

Analytical Biochemistry

David J. Holme and Hazel Peck. Pp. xii + 460. Longman. 1983. Price £26. ISBN 0 582 45082 9.

The term analytical biochemistry summarises a very large area of scientific endeavour that has seen tremendous expansion in the last two or three decades. It is indeed arguable that the present importance of analytical science largely derives from its application to fields such as clinical chemistry, biochemistry and pharmacology. To summarise this whole area in a single volume for student use is thus extraordinarily difficult. Holme and Peck must be given fairly high marks for their attempt. They have produced an attractive and readable text that covers, albeit with a light brush, an astonishingly large number of topics.

An obvious problem confronting the authors is whether to classify their subject according to the analytical methods used or according to the molecules studied. In practice they have done both. The first seven chapters cover the methods under mostly broad headings such as "Separation Methods" and "Spectroscopy"; and the last five chapters cover the properties and analysis of (in order) enzymes, carbohydrates, amino acids, proteins and lipids. This order is a little curious, and the host of modern and very important methods for the study of nucleic acids are not mentioned at all. In a book that must summarise colossal fields in a few sentences, criticisms of the choice of material are inevitable. Other curiosities are the inclusion of no less than 20 pages of detailed description of the AutoAnalyzer, and discussions of prism monochromators and the Warburg apparatus. The former section could be drastically shortened, and the latter topics omitted in the cause of modernity. Such changes might have allowed more coverage of enzymatic methods (*i.e.*, with the enzyme as the analytical reagent, not the analyte), more than a one-page discussion of graphical calibration methods, and so on. The choice of material is sometimes inconsistent: for example, methods for the determination of protein relative molecular mass and primary structure are given, and protein secondary structures are described with the aid of diagrams, but the chiroptical methods often used to study such secondary structures are not discussed. It would also be valuable to have additional (and properly dated) references cited at the end of each chapter: sometimes reference is made only to other text-books which are hardly more detailed than the present one.

Most important of all in a student text that aims to summarise so many topics are clarity of writing and factual exactitude. This book sometimes disappoints in these respects. For example, the distinction between sensitivity and limit of detection is not clearly made. On p. 61 it is stated (incorrectly) that the right-angled optics in a fluorescence spectrometer allow only fluorescent radiation to reach the detector. On the next page this statement is corrected by the admission that scattered light is also detected, but the fact that the optical arrangement is *designed* to minimise the detected Rayleigh scattered light is not mentioned. Such ambiguities can be very confusing to student readers. There are several other incorrect statements. For example, TCPO does not emit chemiluminescence on reaction with hydrogen peroxide (p. 34); it excites by energy-transfer emission from any fluorophores that are also present. ANS and DNS (p. 243) do not form covalent conjugates with proteins or other molecules, and so cannot be used directly as labels in fluorescence immunoassays. The absorption spectra of tyrosine and phenylalanine are given on p. 390, but nowhere is it mentioned that it is tryptophan that dominates the near-ultraviolet absorption of many proteins. The areas under the peaks in densitometer traces of dyed proteins are not proportional to the protein concentrations (p. 400), because different proteins bind many dyes to different degrees.

There are a few small misprints, and the index is indifferent (for example there is no reference to that phenylalanine spectrum on p. 390). These errors, apparent in the parts of the book of particular interest to this reviewer, give rise to the fear that other errors may also be present elsewhere. If so, they prevent a good book from being an outstanding one; perhaps a Second Edition will be even more successful.

J. N. Miller

Analysis Using Glass Electrodes

Peter W. Linder, Ralph G. Torrington and David R. Williams. Pp. xii + 148. Open University Press. Price £20. ISBN 0 335 10420 7.

"Analysis Using Glass Electrodes" is about (1) routine pH measurements and (2) the determination of complexation constants from pH titrations. For the latter, pH is defined as $-\log c_{H^+}/c_0$, where c_{H^+} is the hydrogen ion concentration and $c_0 = 1$ mol l^{-1}, whereas for the former it is notionally defined as $-\log a_{H^+}$, where a_{H^+} is relative hydrogen ion activity. On page 1, the authors state that "many scientists are 'bilingual' in that they have a mastery of both concepts." The dichotomy is really between those interested in deriving information about species distribution (speciation and complexation constants) and those to whom pH values only need to be reproducible numbers for quality control and related purposes. Instead of falling between two stools, the authors would have been better advised to concentrate on the second, their personal interest. This would have required only the omission of Chapter 3 (20 pages), which is a summary of much that can be found elsewhere, together with a brief account of the recent international controversy about the number of pH reference standards necessary to define the pH scale. As elsewhere in the book, not enough can be said in a few paragraphs to be very helpful to those not already familiar with the problems.

Chapter 2 deals with the design and maintenance of electrodes (8 pages); the meat of the book is in Chapters 4 and 5, on the determination of c_{H^+} and complexation constants and speciation, with related computer programs given in four appendices (53 pages). The approach is that pioneered by the Scandinavian school and the Rossottis, and it is perhaps useful to have it summarised. Of the four computer programs, three are in the literature (two in FORTRAN, one in BASIC) and the fourth, LIGEZ, is by one of the authors (R.G.T.) for the Hewlett-Packard 41C desk-top calculator. Programs for the BBC, PET or APPLE computers (if they exist) would have been far more widely used.

The book, according to the back cover, "will be of special interest to people who require a fundamental interpretation of glass electrode potentials," "certain honours/degree students . . .," "postgraduate and research workers" and "anyone who uses glass electrodes"—an unfortunate series of overstatements (by the publisher?) for which the authors should have assumed more responsibility.

I would like to give a wholehearted recommendation to this book, but I find it difficult to do so.

A. K. Covington

Magnetic Resonance. Introduction, Advanced Topics and Applications to Fossil Energy. Proceedings of the NATO Advanced Study Institute on Magnetic Resonance Techniques in Fossil Energy Problems, Malema, Crete, July 3–15, 1983

Edited by Leonidas Petrakis and Jacques P. Fraissard. *NATO ASI Series. Series C: Mathematical and Physical Sciences, Volume* 124. Pp. xii + 807. D. Reidel Publishing Company. 1984. Price Dfl255; $98 (hardback). ISBN 90 277 1752 4.

This latest volume in the Nato ASI series is a most useful addition that presents current attitudes and trends in the two significant areas of magnetic resonance and organic geochemistry.

The organisation of the topics is commendable: the longer review articles supported by short papers, relating particularly to the applications of magnetic resonance to fossil fuels, are sub-divided into four sections of unequal length. The first of these four sections consists of seven papers that commence with a discussion of the important parameters of NMR and EPR, followed by a consideration of some of the mathematical techniques used to study magnetic resonance. Line broadening interactions are discussed followed by two-dimensional NMR, Mössbauer, ENDOR and electron spin echo spectroscopy.

The second section contains five papers that provide a general review of fossil carbonaceous matter. Sedimentary organic matter, kerogen, coal and oil shales are described together with coal liquefaction and gasification in terms of structure, reactivity and resource implications, such as geographical distribution and utilisation of reserves. As well as petrographic descriptions, the treatment reviews present conceptions of chemical composition derived from the application of the many other analytical techniques. Examples of the use of magnetic resonance studies, particularly in the characterisation of coal and coal liquefaction products, introduces the third and largest section, which also includes about 20 short papers describing a wide range of both quantitative and instrumental aspects of NMR and ESR applied to fossil fuel materials. It is the inclusion of these latter presentations that provides such up-to-date accounts of not only applications of the resonance methods, including very high field and ^{17}O NMR, but also of the current ideas regarding the structures and reactivity of coal, oil shale and kerogen.

The final section is brief but illuminating: specific recommendations made by sub-committees set up to define the frontiers and opportunities in applying magnetic resonance techniques to fossil energy systems are reported. Some useful conclusions are drawn and strategies identified, the need for a bank of standardised or chemically well defined samples of coal and coal macerals was a common suggestion.

Symposia reports often show wide variations not only in scientific content of each contribution but also in the typographical presentations, with the plenary sessions rarely receiving an adequate space allocation. In the present compilation, however, the adoption of the particular format of long and short contributions has allowed each topic to be discussed in adequate detail and the unique styles to show through. The typographical presentation is superb; the photomicrographs, maps, formulae, diagrams and spectra are reproduced particularly clearly, providing excellent support especially to the descriptions of kerogen, sedimentary organic matter and coal. It is unfortunate that so splendid a presentation contains the juxtapositioned pages 802/803 in the index.

The compilation is highly recommended to both spectroscopists and organic geochemists; as the Editors suggest, it can serve as an introduction to magnetic resonance with discussion of some newer trends regardless of the applications. The tremendous impact of magnetic resonance studies in the elucidations of the structures of sedimentary organic materials is clearly illustrated. The section containing the descriptions of fossil energy systems can equally serve as a most useful and up-to-date review. The volume is worthy of a place in any library, it provides an excellent reference text to researchers, students and anyone with an interest in either of these two challenging areas.

G. V. Garner

Atlas of Polymer and Plastics Analysis. Second, Completely Revised Edition. Volume 2, Plastics, Fibres, Rubbers, Resins; Starting and Auxiliary Materials, Degradation Products. Part a/I, Introduction, Classification, Spectra and Part a/II, Spectra, Indexes

Dieter O. Hummel. Pp. lvi + 456 (Part a/I); 457–1035 (Part a/II). Carl Hanser Verlag/Verlag Chemie. 1984. Price DM770. ISBN 3 446 12563 9 (Carl Hanser Verlag); 3 527 25798 5 (Verlag Chemie); 0 89573 013 8 (Verlag Chemie International). (Bilingual, German/English.)

These two substantial books comprise Volume 2 of the new Second Edition of "Hummel," which is described by the publishers as "completely revised." It is intended to replace "Infrared Analysis of Polymers, Resins and Additives. An Atlas," which was first published more than ten years ago in two volumes: Volume I/Parts 1 and 2 covering plastics, elastomers, fibres and resins, and Volume II covering additives and processing aids. The new edition will consist of three volumes, as follows: Volume 1, "Polymers: Structures and Spectra"; Volume 2, (in two parts), "Plastics, Fibres, Rubber, Resins"; and Volume 3, "Additives and Processing Aids." The entire work is being prepared jointly by Dr. Hummel and Dr. Friedrich Scholl, Dr. Hummel in particular being responsible for Volumes 1 and 2. As in the earlier edition the text is in two languages (German and English), the translator being Dr. F. Hampson.

Each of the two tomes comprising Volume 2 is less bulky than a volume of the earlier edition, and although heavy, they appear stoutly bound and should be sufficiently convenient and serviceable to use. Together they contain more than 2750 infrared or Raman spectra (over 200 of the latter). An introduction in Part a/I covers the preparation and measurement of samples and describes the decimal classification system that has been employed. This Part is concerned particularly with the infrared spectra of macromolecular natural products and raw materials and includes polymeric plastics, natural and synthetic fibres, elastomers, miscellaneous resins and adhesives and related compounds. Part a/II covers infrared spectra of some additives (including crosslinking agents, initiators, accelerators, oils, waxes, tars, asphalts, bitumens and pitches), low relative molecular mass materials and inorganic substances and has sections on Raman spectra of macromolecular and inorganic substances. The indices of course are also bilingual and include: an alphabetical index of trade names; alphabetical index of systematic chemical names; and chemical formulae and molar masses of low molecular mass compounds.

A caption below each of the spectra included gives the name of the manufacturer and of the product (or description, if a mixture), the appearance, type and use where applicable, and the form of specimen prepared for analysis. The first spectrum in Volume 2 Part a/I is numbered 1904 (the system of numbering continuing from Volume 1) and Part a/II concludes with spectrum 4674. It appears that there will then be a gap before the numbers that commence Volume 3, and this could

possibly be used for supplementary spectra, suggesting that, happily, the authors have further plans for the future.

This is an important and indeed indispensable work in its field, and no doubt the new edition will be in constant use, just as its predecessor has been. As indicated, the various spectra given are, for the most part, of materials and products that have been offered in the market and are in commercial use, not (as is sometimes found in such compendia) abstruse compounds not often seen or encountered. The idiomatic English version of Dr. Hummel's introduction expresses thanks to numerous people who helped with the "donkey work"; the efforts that all concerned have made will save others a great deal of "donkey work" in the future.

Comparison with the earlier edition is difficult since the present volumes are arranged differently and much extended. Also, in the intervening years there have been many changes in materials, requirements and techniques. The author mentions that the spectra given are now available in a data bank and no doubt the future holds much further development in the storage and recognition of spectra (just as with other data) by electronic means.

The present work costs quite an appreciable sum of money but contains such a wealth of useful information (of usefulness by no means limited to establishments in the immediate fields of plastics and resin compositions), that it becomes a necessary purchase for the great majority of technical libraries.

D. Simpson

Ultrahigh Resolution Chromatography

Edited by Satinder Ahuja. *ACS Symposium Series* 250. Pp. viii + 231. American Chemical Society. 1984. Price $44.95 (USA and Canada); $53.95 (Rest of World). ISBN 0 8412 0835 2.

To those of us who are still frequently unsure as to what constitutes high-resolution chromatography the term ultrahigh resolution may pose something of a recognition problem. The Preface to this book sets out to clear up any difficulties we may have: high resolution, it seems, is what capillary columns have been delivering whilst ultrahigh resolution is what they can or soon will deliver. To what extent then does this Symposium volume illuminate expectation in this direction?

There are 12 papers covering gas-, liquid- and super-critical fluid-chromatography and one on a small volume thermal conductivity device. They range, in substance, from reviews, through theory both ancient and modern, to the performance of micro-bore columns for gases or liquids, and finally, to "super" (presumably, better than ultra!) resolution via numerical deconvolution. Superficially, a very wide prospectus, but in fact, largely a re-telling of oft-told tales. If it were the case that a crisp listing of those features of forefront technology or understanding that constitute the ultra-springboard were to be found in more than one or two of the articles, the reader would profit very considerably. Unhappily, most of the articles are sufficiently diffuse that the average practitioner will be hard put to it to know what interpretation to place on the information presented and the views expressed.

It probably is true that we are about to enter a new phase in chromatographic practice. It is probably equally true that no one clearly envisages at the moment what current developments will see us through into that phase, and which lead only to a dead end. While this volume does not help us much in this, it does, in fact, have merit; it at least brings together for the first time reference to and discussion of a wide range of recent speculative studies. Whether it is worth almost £50 is quite another matter.

J. H. Purnell

Chemistry for Toxicity Testing

Edited by C. W. Jameson and D. B. Walters. Pp. xiv + 231. Butterworth. 1984. Price £32.50. ISBN 0 250 40547 4.

Increasingly, the examination of chemical substances for their toxic potential plays an important part in the protection of human health and the environment. The purpose of this book is to describe the analytical chemistry aspects of *in vivo* toxicity studies, and the various chapters contributing to this aim have been adapted from presentations to the "Symposium on Chemistry & Safety for Toxicity Testing of Environmental Chemicals," the 183rd meeting of the American Chemical Society in March 1982.

The book is divided into four parts, the first of which gives a general overview of many analytical problems in this work. Part II describes dosage mixing and analysis, while Part III covers chemical inhalation studies, including methods for generating and monitoring test atmospheres. The final part deals with data evaluation and the question of good laboratory practices.

The book is well presented and provides a brief insight of this important area of analysis. However, the high price, limited references and inevitably superficial treatment of certain aspects makes it an unlikely purchase for an individual working in the area.

R. S. Barratt

Asymmetric Synthesis. Volume 1. Analytical Methods

Edited by James D. Morrison. Pp. xiv + 201. Academic Press. 1984. Price $32. ISBN 0 12 507701 7.

Fifteen years ago assymetric synthesis was considered to be an exotic academic research speciality, today it is a prime focus activity for many of the leading academic and industrial organic research groups. This first volume on asymmetric synthesis, "Analytical Methods," begins with a chapter, "A Summary of Ways to Obtain Optically Active Compounds" (J. D. Morrison, 29 refs.), to place asymmetric synthesis in context. The following eight chapters on analytical methods, written in the main by authors who pioneered their development, show how one can evaluate the efficiency of an asymmetric synthesis or other processes that produce chiral products. The chapter on the oldest method of study of chiral systems, "Polarimetry," (G. D. Lyle and R. E. Lyle, 37 refs.) pays particular attention to requirements for the precision of data. This is followed by a discussion of "Competitive Reaction Methods for the Determination of Maximum Specific Rotations" (A. R. Schoofs and J. P. Guetté, 30 refs.). "Isotope-Dilution Techniques" (K. K. Anderson, D. M. Gash and J. D. Robertson, 34 refs.) presents this rather old and at times confusing technique in a fresh and lucid manner.

There are two chapters dealing with separation methods. "Gas Chromatographic Methods" (V. Schurig, 98 refs.) deals with separations of derivatised enantiomers on chiral stationary phases and by chelation of underivatised enantiomers on metal containing chiral stationary phases, including a discussion of precision of analysis and other matters of practical importance. The chapter "Separation of Enantiomers by Liquid Chromatographic Methods (W. H. Pirkle and J. Finn, 86 refs.), contains a discussion of indirect resolutions, using chiral derivatising agents, and direct resolutions, using natural and synthetic chiral phases and predicts rapid expansion and commercialisation of a variety of chiral columns in the next few years.

The last three chapters concern chiral aspects of NMR, namely "Using Chiral Derivatives" (S. Yamagachi, 131 refs.), "Chiral Solvating Agents" (G. R. Weisman, 104 refs.) and lastly "Using Chiral Shift Reagents (R. R. Fraser, 97 refs.), taken in totality they provide an excellent guide and introduction to the field.

This is a scholarly and well produced volume and should be of wide appeal to those teaching modern aspects of organic analysis or concerned with analysis of pharmaceutical products.

D. Thorburn Burns

Analytikum: Methoden der Analytischen Chemie und ihre Theoretischen Grundlagen. 6 durchgesehene Auflage

Edited by K. Doerffel and R. Geyer. Pp. 616. VEB Deutscher Verlag für Grundstoffindustrie, Leipzig. 1984. Price DM55. (In German.)

This Edition is identical with the Fifth Edition in basic text, diagrams, tables, index and pagination, the revision being mainly minor corrections, addition of further references, mainly concerned with IUPAC nomenclature and updating of the text on electrophoresis (p. 483). The remarks in the review of the Fourth Edition (*Analyst*, 1980, **105**, 1135) still apply. I would, however, stress that the authors treat the subject as a coherent whole and have avoided the series of monotechnique approaches so common in books of non-European origin. They discuss the basis and the inter-relations of techniques and methods in a logical precise and mathematical manner with an emphasis on theory and its implications, instrumental aspects are dealt with in terms of basic design patterns and functions rather than "sales descriptions" of specific instruments. This is an important fundamental teaching text, which should be made more readily available by a careful translation.

D. Thorburn Burns

Methods of Enzymatic Analysis. Third Edition. Volume V. Enzymes 3: Peptidases, Proteinases and Their Inhibitors

Edited by Hans Ulrich Bergmeyer. Pp. xxviii + 598. Verlag Chemie. 1984. ISBN 3 527 28045 5 (Verlag Chemie); 0 89573 235 1 (Verlag Chemie International).

Volume Five of Bergmeyer's Third Edition of "Methods of Enzymatic Analysis" documents various methods for the analysis of peptidases, proteinases and their inhibitors. It follows the same style and layout as previous volumes in the series and combines brief accounts of the various enzymes with carefully prepared details for the analysis of the enzymes by one or more selected methods.

Measurement of certain of the peptide hydrolases (E.C. 3.4 . . .) can provide useful information for the diagnosis and management of disease. For example, determination of oxytocinase activity is useful in assessing placental function and angiotensin-converting enzyme (ACE) is useful in sarcoidosis. The enzyme of the human digestive tract, such as chymotrypsin, trypsin and the pepsins, are also members of these general groups of enzymes. Numerous assays for proteinases may have been developed but a particularly interesting analytical procedure is that based on immobilised enzyme-labelled proteins, *e.g.*, beta-glucosidase-labelled casein immobilised on Sepharose beads. In this type of assay, known as LEAP (linked enzyme assay procedure), the proteinase releases soluble peptide fragments carrying the enzyme label. The enzyme activity of the released fragments is related to protease activity and the assay has great sensitivity by virtue of the amplification provided by the enzyme label. Full details of this assay together with instructions for preparation of the LEAP substrate are presented in Section 2.15.

Two of the four chapters are devoted to the blood coagulation factors and the complement enzymes. Jackson reviews the haemostatic process and the protease-based reaction cascade that leads to the formation of fibrin. Subsequent sections cover the assay of blood coagulation factors (*e.g.*, factor X), proteinase inhibitors (*e.g.*, antithrombin III), fibrinogen and heparin. Loos presents a concise account of the complement system and this is accompanied by selected methods for C1$\bar{s}$-esterase, C2 and factors B, D and I.

This is an excellent book and has maintained the high standard of the previous volumes.

L. J. Kricka

Aquametry. Second Edition. Part II. Electrical and Electronic Methods. A Treatise on Methods for the Determination of Water

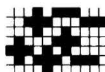
Donald Milton Smith and John Mitchell, Jr. *Chemical Analysis Series, Volume 5.* Pp. xiv + 1352. Wiley-Interscience. 1984. Price £156.75. ISBN 0 471 02265 9.

This, the second part of a three part series on aquametry, deals with electrical and electronic methods for the determination of water, or more appropriately, the water content of a wide variety of materials. The book is divided into three lengthy chapters, Dielometry, Conductometry and Coulometric Hygrometry and a short final chapter on Microcoulometry.

Dielometry as the name implies, deals with the measurement of the dielectric constant of a variety of solids and liquids and how this property varies with their water content. The authors state that the technique compares favourably with ultraspectroscopy for trace analysis and purity control and for these applications is surpassed only by cryoscopy and gas chromatography. This chapter, like others in the book, contains an introduction to the technique, apparatus used for measurement and a thorough discussion of numerous applications. Figures and diagrams are abundant (160 figures in the first 250 pages) and overall these are well presented. In a few instances I thought that photographs of commercial apparatus were superfluous. Conductometry has many applications in common with dielometry but care has been taken in the chapter on this topic to avoid duplication of material already given. Apart from the measurement of conductance in solids and liquids there is a comprehensive section on electrical hygrometry, which could almost merit a separate chapter. The treatment of Coulometric Hygrometry in the third chapter is excellent and I thought this to be the most readable section of the book. It contains a thorough discussion of the technique followed by a large number of applications.

As in many books of this length, the subject index at the back of the book is by no means complete. However, this lack is partly offset by introductory indexes and summary tables included in each chapter. The book has obviously gathered together information from the foremost published works and although one is initially tempted to ignore the quality and feel the width, I am sure that it will become the source book on aquametry for some years to come.

K. Torrance



The Determination of Au^{+} and Au^{3+} in Gold Cyanide Plating Baths

INTRODUCTION

With the high cost of gold, it is increasingly important that gold plating baths maintain optimum plating characteristics. Both total gold and the ratio of Au^{+} to Au^{3+} are of importance as an increase in Au^{3+} content can lower plating efficiencies.

In the presence of excess cyanide, Au^{+} and Au^{3+} exist as the very strong anionic $Au(CN)_2^{-}$ and $Au(CN)_4^{-}$ complexes. Due to the strength of these complexes, previous analysis for total gold involved time consuming distillations to break the complexes prior to end analysis by titration, or precipitation methods or atomic spectroscopy.

Besides being the time consuming and tedious, these methods do not allow speciation of Au^{+} and Au^{3+}. Ion chromatography offers a rapid means of analysis for calculation of total gold as well as speciation betwen Au^{+} and Au^{3+}.

Cobalt is often used in gold plating solutions to impart hardness to the deposit. The cobalt is usually added as a salt or complex in the (+2) oxidation state. In the presence of cyanide (generated during bath use) and oxygen, the cobalt is quickly oxidized to the very stable cyano complex, $Co(CN_6)^{3-}$. Once oxidized, the cobalt no longer can be plated and the quality of the product quickly deteriorates. Thus, a measure of the level of this complex in the bath can be used to maintain the proper cobalt (+2) content. Cobalt (+2) could also be monitored directly using a transition metal system described in Technical Note 10R.

RESULTS AND DISCUSSION

Since the gold and cobalt cyanide complexes exist as stable anions, they are easily separated by Mobile Phase Ion Chromatography (MPIC) and are easily detected by suppressed conductivity. Figure 1 illustrates the determination of $Co(CN)_6^{3-}$ in cobalt hardened gold cyanide bath.

The presence of trace iron contaminants in gold bath can cause brittle deposits. The two iron cyanide complexes can be easily analyzed under approximately the same conditions as shown in Figure 2.

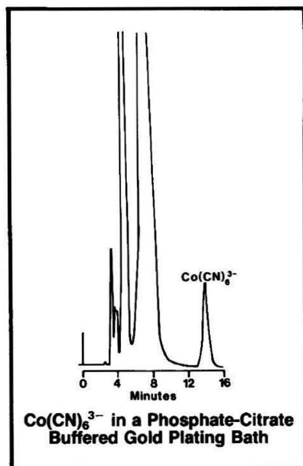
Figure 3 illustrates the separation of Au^{+} and Au^{3+} in a phosphate buffered unalloyed gold plating solution. No iron cyanides were observed as contaminants. Similar results were obtained from citrate buffered baths. This analysis required no sample pretreatment except for dilution in eluent.

INSTRUMENT CONDITIONS—(Figure 3)

Eluent:	2mM TBAOH 40% CH_3CN 0.2 mM Na_2CO_3 1 ml/min	Detector: Injection Volume:	6 × 60 ASC/ conductivity 50 µl loop
		Chart Speed:	0.5 cm/min
Columns:	1 MPIC NG1 1 MPIC NS1	Sample Dilution:	0.3 ml of bath/ 50 ml Di H_2O

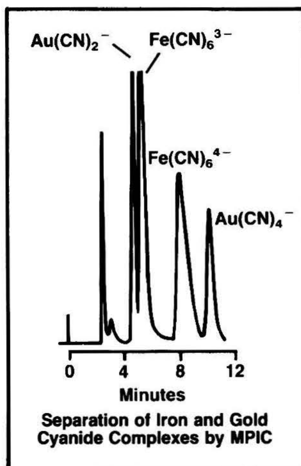
INSTRUMENT CONDITIONS—(Figure 1)

Eluent:	2mM TPAOH 10% CH_3CN, 1 ml/min.	Detection:	6 × 60 ASC/conductivity at 10 µs
Columns:	1 MPIC-NG1	Injection Volume: Sample Dilution:	50 µl 1/400



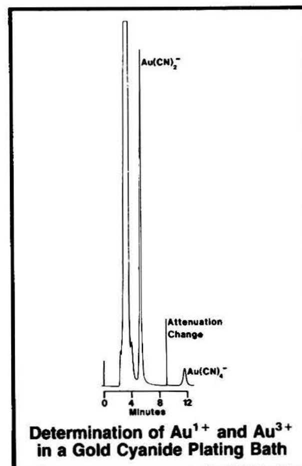
$Co(CN)_6^{3-}$ in a Phosphate-Citrate Buffered Gold Plating Bath

FIGURE 1



Separation of Iron and Gold Cyanide Complexes by MPIC

FIGURE 2



Determination of Au^{1+} and Au^{3+} in a Gold Cyanide Plating Bath

FIGURE 3

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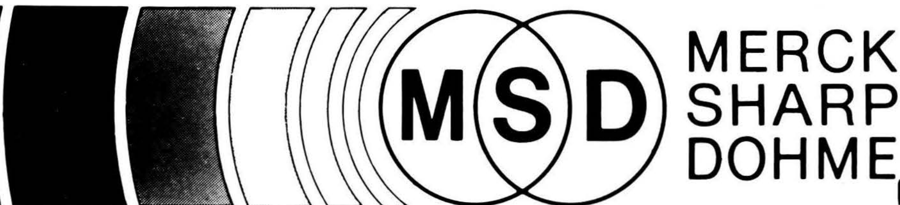

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The Analyst

The Analytical Journal of The Royal Society of Chemistry

CONTENTS

225 **Large Batch Sealed Tube Decomposition of Geochemical Samples by Means of a Layered Heating Block**—Martin Hale, Michael Thompson and John Lovell

229 **Rapid Determination of Molybdenum in Soils, Sediments and Rocks by Solvent Extraction with Inductively Coupled Plasma Atomic-emission Spectrometry**—Michael Thompson and Liang Zao

237 **Comparison of Digestion Methods for Total Elemental Analysis of Peat and Separation of its Organic and Inorganic Components**—Clara Sara E. Papp and Thelma Fern Harms

243 **Determination of Aluminium in Dialysate Fluids by Atomic-absorption Spectrometry with Electrothermal Atomisation**—David J. Halls and Gordon S. Fell

247 **Accuracy of Determination of Trace Concentrations of Dissolved Cadmium in River Waters: Analytical Quality Control in the Harmonised Monitoring Scheme**—Analytical Quality Control (Harmonised Monitoring) Committee

253 **Peak Dispersion in a Liquid Chromatography - Atomic-absorption Spectrometry System**—Elena D. Katz and Raymond P. W. Scott

259 **Determination of Ivermectin in Feeds by High-performance Liquid Chromatography**—Allen Fox and David W. Fink

263 **Determination of Chlordane in Human Blood by Gas Chromatography**—Isao Saito, Norihisa Kawamura, Keiichi Uno and Yasuhiro Takeuchi

269 **Determination of Sulphides in Water and Effluents Using Gas Chromatography**—David J. Hawke, Anthony Lloyd, David M. Martinson, Philip G. Slater and Charles Excell

273 **Sorption Studies of Chromosorb Porous Polymers and their Potential Use in Passive Monitors**—Brian A. Colenutt and Jamal Ara

277 **New Approach to the Simultaneous Determination of Pollutants in Waste Waters by Flow Injection Analysis. Part II. Cationic Pollutants**—Angel Ríos, M. Dolores Luque de Castro and Miguel Valcárcel

283 **Determination of Trace Amounts of Boron in Geological Samples with Carminic Acid after Extraction with 2-Ethylhexane-1,3-diol**—Georg Troll and Alfred Sauerer

287 **Fluorimetric Determination of Aluminium with Morin After Extraction with Isobutyl Methyl Ketone. Part II. Extraction - Fluorimetric Determination of Aluminium in Natural and Waste Waters**—J. Medina Escriche and F. Hernandez Hernandez

291 **Photometric Titration of Group II Metal Ions with EDTA Using Very Precise Linear Extrapolation End-point Location**—Colin G. Halliday and Michael A. Leonard

295 **Lithium-selective Polymeric Membrane Electrodes Based on Dodecylmethyl-14-crown-4**—Sadaya Kitazawa, Keiichi Kimura, Hideki Yano and Toshiyuki Shono

SHORT PAPERS

301 **Pyridine-2-carbaldehyde Derivative of Girard-P as a Fluorimetric Reagent for the Determination of Gallium in Aluminium**—Manuel Callejón Mochón, Jose L. Gómez Ariza and Alfonso Guiraúm Pérez

305 **Spectrophotometric Determination of Osmium with 4(5)-D-Arabinotetrahydroxybutylimidazoline-2-thione**—Constantino Fernández Pereira and Juan Gasch Gómez

309 **Reaction of Antimony(III) with Tris[2,4,6-(2-hydroxy-4-sulpho-1-naphthylazo)]-*s*-triazine Trisodium Salt as a Spectrophotometric Method for the Determination of Sulphide**—Ishwar Singh and Pratap Singh Kadyan

313 **Rapid Spectrophotometric Determination of Nitrate in Mineral Waters with Resorcinol**—Noël Velghe and Albert Claeys

COMMUNICATION

315 **Zeeman-corrected Graphite Furnace Atomic-absorption Spectrometric Screening Method for the Determination of Lead in Infant Formulas and Powdered Milks**—Jan Rud Andersen

317 **BOOK REVIEWS**

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