The Analyst

The Analytical Journal
of The Royal Society
of Chemistry

A monthly international publication dealing
with all branches of analytical chemistry

Volume 106 No 1260 Pages 257–384 March 1981

THE ANALYST

THE ANALYTICAL JOURNAL OF THE ROYAL SOCIETY OF CHEMISTRY

Published by The Royal Society of Chemistry

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

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

Subscriptions (non-members): The Royal Society of Chemistry, Distribution Centre, Blackhorse Road, Letchworth, Herts., SG6 1HN

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Summaries of Papers in this Issue

The Impact of Microprocessors on Analytical Instrumentation

A Review

Summary of Contents

Keywords: *Review*; *microprocessors*; *analytical instrumentation*; *automated titrator*

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Analyst, 1981, **106**, 257–282.

Computer-linked Atomic-absorption Spectroscopy: a Streamlined Approach to Environmental Analysis

An analytical system using two single-beam atomic-absorption spectrophotometers linked to a microcomputer has been developed to process efficiently large numbers of plant and soil samples for various metal analyses. The rationale underlying the development of the system and details of the interfacing and data handling techniques are given.

Keywords: *Computer-linked atomic-absorption spectroscopy*; *environmental analysis*; *plant and soil analysis*; *metal determination*

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Analyst, 1981, **106**, 283–287.

A Novel Method of Wavelength Modulation for Atomic Spectrometry—Some Preliminary Experiments

The method of wavelength modulation most commonly used in atomic spectrometry employs an oscillating refractor plate driven by a scanning motor. A new mechanical arrangement is described, which involves a rotating quartz mechanical chopper. This quartz chopper is composed of four quadrants of different thickness, which refract radiation by different degrees. The rotating quartz chopper is placed inside the monochromator in a position similar to that used for oscillating refractor plates. Efficient wavelength modulation is achieved. However, the rotating quartz chopper has the advantage that a good square-wave modulation function is obtained.

Keywords: *Square-wave wavelength modulation*; *atomic spectrometry*

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Analyst, 1981, **106**, 288–298.

Determination of Lead in Urine by Atomic-absorption Spectroscopy with Electrothermal Atomisation

A method is described for the determination of lead in urine by atomic-absorption spectroscopy with electrothermal atomisation. The determination is rapid and minimum pre-treatment of the sample is required. Matrix interference is minimised by the addition of orthophosphoric acid and also by pre-coating the graphite tubes with molybdenum. Consideration is given to possible losses of organically bound lead during the drying and ashing cycle and steps to prevent these are incorporated in the procedure. The range of the method is 5–200 $\mu g\ l^{-1}$ of lead.

Keywords: *Lead determination*; *urine*; *electrothermal atomic-absorption spectroscopy*; *matrix interference*

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Analyst, 1981, **106**, 299–304.

Determination of Tin in the Presence of Lead by Stripping Voltammetry with Collection at a Rotating Ring-disc Electrode

Stripping voltammetry with collection at a glassy carbon rotating ring-disc electrode is used for the determination of tin in the presence of lead. Tin can be readily determined at a level of 1 μM (0.12 $\mu g\ ml^{-1}$) in the presence of a 50-fold concentration of lead. Determinations down to 0.25 μM (0.03 $\mu g\ ml^{-1}$) are possible with prolonged deposition times (about 30 min).

Keywords: *Stripping voltammetry*; *rotating ring-disc electrode*; *tin determination*; *interference by lead*

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Analyst, 1981, **106**, 305–310.

Direct Spectrophotometric Method for the Determination of Hydrochloric Acid-releasable Arsenic in Sediments and Soils

A spectrophotometric method for the direct determination of hydrochloric acid-releasable inorganic arsenic has been developed and applied successfully to the quantitative evaluation of arsenic in soil and sediment samples. The method provides reliable data on the quantitative recovery of 2.0 μg of arsenic(V) added to 5.0 g (0.4 mg kg^{-1}) of soil, clay, sand and sediment samples. The method is simple, reliable and relatively rapid; 24 samples can be analysed in about 1 h. It does not require elaborate equipment and can be routinely used for the quantitative determination of arsenic in soil and soil-like material. The detection limit has been established as 0.5 μg of arsenic. The extent of ionic interference in the use of this method for arsenic determination in soil has also been quantitatively evaluated.

Keywords: *Arsenic determination*; *soils*; *sediments*; *spectrophotometry*

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Analyst, 1981, **106**, 311–315.

Anodic Generation of Cerium(IV). Charge-transfer Kinetic Parameters and Conditional Potentials at Platinum, Gold and Glassy Carbon

The anodic oxidation of cerium(III) at platinum, gold and glassy carbon electrodes was investigated by rotating disc voltammetry in sulphuric and perchloric acid solutions. Conditional potentials, limiting currents and charge-transfer kinetic parameters were obtained and the results are discussed. The absence of chemical corrosion and mixed potentials indicated that glassy carbon is the best electrode material. Current efficiencies were calculated.

Keywords: *Cerium(IV)*; *rotating disc electrode*; *charge-transfer kinetic parameters*

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Analyst, 1981, **106**, 316–322.

Polycrystalline Ion-selective Electrode Based on $Ag_2[HgI_4]$ - Ag_2S

The preparation and analytical behaviour of polycrystalline solid-state ion-selective electrodes based on $Ag_2[HgI_4]$ and $Ag_2[HgI_4]$ - Ag_2S membranes was studied. The membranes consist of solid-state pellets made by compressing prepared precipitates. The performance characteristics of the electrodes are described in terms of potential - activity graphs, stabilities, precision, response times, effect of pH and redox potential. The electrode responses towards Ag^+, Hg^+, Hg^{2+}, I^- and CN^- ions are evaluated. It was found that the $Ag_2[HgI_4]$ - Ag_2S type is superior to the $Ag_2[HgI_4]$ type with respect to mechanical properties and lifetime and is more suitable for practical application.

Keywords: *$Ag_2[HgI_4]$ - Ag_2S electrode*; *polycrystalline membrane preparation*; *response to Ag^+, Hg^+, Hg^{2+}, I^- and CN^-*

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Analyst, 1981, **106**, 323–327.

Destructive Neutron-activation Analysis of Toxic Elements in Suspended Materials Released from Refuse Incinerators

Trace element concentrations in the fly-ash collected from a municipal solid waste refuse incinerator have been determined using both instrumental and radiochemical neutron-activation analysis. A method for sample dissolution and subsequent selective and quantitative radiochemical separation for the determination of mercury, selenium, arsenic, chromium and cadmium are presented and discussed. A series of results obtained in the determination of iron, zinc, bromine, cobalt, caesium, scandium, vanadium, selenium and chromium by instrumental neutron-activation analysis are also reported.

Keywords: *Incinerator fly-ash*; *trace element determination*; *radiochemical separation*; *neutron-activation analysis*; *sample dissolution*

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Analyst, 1981, **106**, 328–334.

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The Analyst

The Impact of Microprocessors on Analytical Instrumentation

A Review

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Summary of Contents

Keywords: *Review*; *microprocessors*; *analytical instrumentation*; *automated titrator*

Introduction

In the last 6 years, *microprocessors** have progressed from being a novel, exciting development of potential interest to analytical chemists into part of the everyday experience of the populace. Technical advances are still being made at a phenomenal rate, but it is possible now to survey the scene from the point of view of the analytical chemist to assess the impact of microprocessors on analytical instrumentation, to make some sense of the technical aspects and the jargon used to describe them, and to discuss approaches to the application of the microcomputer.

The microprocessor-based microcomputer is compact, cheap and robust. It is therefore practical and economic to use them in small-scale instrumentation such as titrators, polarographs and balances. Two years ago, it was less profitable to use microcomputers for large instruments such as mass spectrometers because of their relatively small memory and speed of operation. However, improvements have been so rapid that these considerations are now less relevant. In analytical instrumentation they can fulfil a control function, which may range from a simple replacement of mechanical or electromechanical parts with a *chip*, to execution of a program of sequential operations that comprise the total analytical determination. In addition, they may be used with advantage for data acquisition and for data processing, great advances having been made in respect of the latter over the last year. These aspects are discussed in detail in this paper, but first the development of microprocessors and the current availability of *microcomputers* will be reviewed.

Development of Microcomputers

Until the 1940s, the principal devices for digital computation remained the traditional fingers and abacus. Babbage's calculating engine provides a reminder of the practical difficulties of constructing a mechanical computer. During the 1940s, several valve-based electronic digital computers were constructed, and these provided a sound base for the developments that have led to the microprocessor.

* Words that are italicised are defined in the Glossary at the end of this paper.

The valve-based computer, on account of the valves and associated circuitry, was large, dissipated a lot of heat and had many wires and connections. As a consequence it was difficult to accommodate, uncomfortable to operate and subject to frequent breakdown. It was also very expensive to construct and maintain. The replacement of the thermionic valve by the transistor in the late 1950s led to a reduction in size and consumption of power. The concept of putting electronic circuitry on boards and assembling these into large units greatly increased the reliability of the large unit and made it easier to maintain. At this point the large computer was dominant, the opportunities for reducing the size of the computer having been traded off against an increase in computing capacity. The computer was being widely used, but in a *hierarchical* manner. The experimenter had to link his apparatus to the company's *mainframe* computer, possibly via a *minicomputer*, and suffer all the inconveniences of *time sharing*. Further, because of the inherent cost and its capacity, it could only be justified for massive calculations and large-scale analytical operations.

In 1965 Digital Equipment Corporation introduced the PDP-8 minicomputer at a price of $18000, accessories such as printers and *backing store* being extras. However, minicomputers, because they were mainly associated with large instruments such as nuclear magnetic resonance (NMR) and mass spectrometers, made only a small impact compared with the microcomputers that came later. Nevertheless, between 1965 and 1975, 28000 PDP-8s were installed.

Thus in the late 1960s the basic computing rationale was a legacy of the valve computer, its large size and the magnitude of the breakthrough in computations. However, the construction of a shoe-box sized computer, with the calculative capacity of the early mainframe devices, with minimal power consumption and maintenance problems and costing less than $5000, wrought a change in computer usage as profound in its long-term effect as the technological development that led to the microprocessor.

In the mid-1960s the electronics industry, which had successfully developed the concept of circuit boards and solid-state devices, devised a process whereby integrated circuits could be etched on to a *silicon chip*. The resultant product can be made cheaply for a mass market, it has low power requirements and is robust and reliable.

Then, in 1971, a development equivalent in size and consequences to that of integrated circuits brought the computer well and truly into the laboratory. Intel Corporation, a semiconductor manufacturer that supplied integrated circuits used in many minicomputers, devised the first microprocessor chip, the Intel 4004. It was originally intended as a "controller" chip, incorporating a complete central processing unit on a single chip. It required a number of other chips to make it into a complete computer control system and only four binary digits could be processed at a time. In the following year Intel brought out another microprocessor chip, the 8008, which was faster and more powerful than the 4004; it had a 48 *instruction set* and could handle data in units of 8 binary digits. The 8008 became competition for those minicomputers that were dedicated to simpler tasks, because although the cost of the chip was at first high, it soon dropped to the $100 range. Intel's next microprocessor chip was the 8080, again a great advancement over the previous generation. However, by this time other semiconductor manufacturers had started to produce microprocessor chips, some offering new architecture designs and instruction sets, others duplicating other manufacturers' designs. Motorola Semiconductor's MC6800 microprocessor chip and MOS Technology's MCS6502 chip became two of the most popular competitors to the 8080. With an increase in the range of chips available came a decrease in price not only of the microprocessor chips but also of the support chips, *i.e.*, memories and *input/output* devices.

The first successful, low-cost "home computer" was introduced by Micro Instrumentation and Telemetry System (MITS) in early 1975 and was called the Altair 8800. Based on the 8080 microprocessor chip it had a limited memory, a control panel, power supply and a complete packaging. It was an instant success, selling for under $500. MITS soon followed the Altair 8800 with a 6800 based model. On the basis of the success by MITS, other manufacturers started to produce microcomputers, some very similar in design to the Altair, even to the extent of using the same *bus* structure for their plug-in boards. Others were totally different designs based on the 8080, 6800, 6502 or other microprocessors available. Currently there are many products being offered, ranging from the bare essentials of the kits to the

complete microcomputer system with accessories. Table I lists those available at the time of going to press. A basic system consisting of microprocessor, memory, visual display unit and keyboard can be obtained for as little as £500 with accessories being added as the budget allows. For around £2 500 a complete computer system can be obtained for use as a business system or in a control situation, which is equivalent to and in some instances surpasses the typical minicomputers of 8 years ago.

With the advent of the microcomputer, the analyst in possession of a willingness to learn basic programming technique and a modicum of patience can develop many useful programs without recourse to an outsider.

Applications of Microprocessors to Analytical Instrumentation

Table II surveys many of the past and current chemical applications that involve microprocessors. In general, the mode of operation for a microprocessor can be split into two categories, firstly as a "controller" and secondly to accumulate and process data. The choice of which mode to use reflects individual preferences; some of the applications contained in Table II involve both modes, others only one.

The subject of microprocessors in analytical instrumentation is such a rapidly growing area that Table II can in no way be considered complete, but as many references as possible have been included.

As guidelines to the details in Table II, we note the following general trends in the analytical applications of microprocessors:

1. *Spectroscopy.* A number of ultraviolet - visible and infrared instruments are appearing in which the settings are altered and optimised by microprocessors, and in which the peaks are identified and quantified. Similar developments are being made in other branches of spectroscopy, and the matching of image-sensing devices with microcomputers will make a big impact over the next decade.
2. *Chromatography.* A number of commercially available chromatographic integrators make use of a program for peak detection and measurement. The qualitative and quantitative results are conveniently displayed and doubtful assignments are indicated so that the operator may check the results and use his own judgement. The control of the chromatograph may also be carried out by microprocessors.
3. *Electroanalytical chemistry.* Output from electroanalytical determinations such as polarography or potentiometry with ion-selective electrodes can be readily processed by microcomputers, with resulting improvements in quality.

Most manufacturers and many authors discuss only very briefly, if at all, the options open to the chemist who wishes to apply microprocessors to his instrumentation and give little account of the practical difficulties that may be encountered. These practicalities are well illustrated by considering the case history of the design and construction of a microprocessor-controlled automatic titrator.

History of the Development of an Automated Titrator

Between 1975 and 1979, several groups devised microcomputer-controlled titrators.[7,8,32,35,41,53,72] Because of differences in their approach and the available state of microprocessor development, the instruments proposed differed in several respects.

The first were carried out simultaneously but independently in our own laboratories[7] and in those of Radiometer[8] and Mettler. Ours was basically a collaboration between an electrical engineer (E. L. Dagless), an industrial chemist (D. R. Deans) and an academic chemist (D. Betteridge), with a research student (P. David) working in whichever laboratory was appropriate at the time. The problem as posed was to see whether a mixture of organic acids and boric acid from the ICI nylon process plant could be titrated sequentially without addition of mannitol, and with an accuracy of 0.5% or better. The existing procedure was manual and consisted in first titrating potentiometrically the organic acids, and then the boric acid after addition of mannitol, to a pre-determined potential.

Laboratory Prototype

The first stage was to find a solution to the chemical problem, given the capabilities of the microprocessor. Three possibilities were considered: (*i*) to mimic a conventional automatic

Manufacturers of the various microprocessors

Manufacturer	Unit	Hardware description of basic system							
		Configuration	Micro-processor*	Minimum memory	Visual display	Keyboard	Interfaces and I/O ports	Type of backing memory	Other
Acorn Computers	Acorn Micro-computer System I	Consists of two Eurocards —MPU card and keyboard card. Buy in kit form or assembled	6502	1KB RAM	8 digit LED display, VDU interface on extra card	25 key (16 hex, 9 control) keyboard	16 way I/O with 128B of RAM on processor card. CUTS cassette interface on keyboard card		
Apple Computers	Apple II (reviewed in *Practical Computing*, July 1978)	Home computer	6502	16KB RAM 8KB ROM	Extra	Keyboard	8 I/O slots		
Attache	Attache	Chassis system based on the S100 bus	8080	16KB RAM	Video screen extra	Built-in keyboard	Parallel printer interface is extra. 10 slot S100 bus		
Bytronix Micro-computers	Megamicro		8080A/Z80	64KB RAM	12-in, 20 × 80, black and white VDU		2 parallel ports and 2 serial ports	Dual 8-in floppy disks (1MB)	120 cps printer
Commodore Systems Division	KIM1 (reviewed in *Practical Computing*, October 1978)		6502	1KB RAM 2KB ROM	6-digit LED display	Small calculator type keyboard	Built-in interfaces for audio cassette and teletype		
Commodore Systems Division	PET (reviewed in *Practical Computing*, October 1978	Home computer	6502			Full alpha-numerics and graphics, keys are:	IEEE-488 interface and a parallel user port		
	2001–8			8KB RAM	9-in, 25 × 40, integral VDU	Calculator size		Built-in tape cassette	
	2008N			8KB RAM					
	3016N			16KB RAM					
	3032N			32KB RAM		Typewriter size		Extra	
	8032			32KB RAM	25 × 80 column VDU				
Compelec Electronics	Compelec Series		Z80	32KB RAM			Two RS232 serial ports, 1 parallel port	Dual 8-in floppy disks (512KB)	
Compucolor	Compucolour II (reviewed in *Practical Computing*, June 1979)	Packaged system	8086	8KB RAM	13-in, 32 × 64 eight-colour display with alpha-numerics + graphics	72 key detachable keyboard	RS232 port	Built-in single mini floppy disk drive (51KB)	
Compucorp	625	In the 600 series of desk-top computers	Z80	60KB RAM	Built-in 9-in 16 × 64, black and white VDU	Full keyboard with 40 user definable keys	RS232 serial I/O port	Dual 5¼-in floppy disk (700KB)	40 cps printer
Computer Centre	Mini Kit	Kit	Z80	16KB RAM			Serial and parallel I/O, S100 mother board	Minifloppy disk drive	
Computer Workshop	System I	Modular system		32KB RAM	9-in, 16 × 64, black and white VDU			Dual 5¼-in floppy disks (170KB)	
Cromemco	Z–2		Z80	16KB RAM					
Cromemco	Z–2H		Z80A	64KB RAM			RS232 serial, printer interface	11MB hard disk drive, 2 floppy disk drives	
Cromemco	System II	Factory assembled	Z80	32KB RAM			Dual printer interface, serial interface	Dual mini-floppy disks	
	(System 11/64)	(New configuration of System II)		(64KB)					
Cromemco	System III		Z80	32KB RAM			Dual printer interface, RS232 serial (console and printer interface)	Dual 265 KB floppy disks (dual 8-in disk drives)	
	(System III/64)	(New configuration)	(Z80A)	(64KB)					
Equinox Computer Systems	Equinox 300		Z80	32KB RAM 48KB RAM 48KB RAM			S100 bus	Dual mini-floppy disks Dual 8-in floppy disks	

are given at the end of the table.

Approximate price of basic system (excluding VAT)	Maximum memory expansion	Accessories available	Software	Software support and comments on system
Kit £65, assembled £79	Up to 8KB RAM and 8KB EPROM on a fully buffered memory card		Monitor and machine code programming. BASIC and DOS may be contained on the memory card	Aimed as a low-cost 6502 development system for industry, as a control system for electronic engineers
£695	48KB RAM	Single minifloppy disk (115KB), printers, colour graphics	BASIC, operating system, PASCAL	
From £1737 (full business system approx. £5000)	64KB RAM	Two single or double density 8-in floppy disk. VDU screen and parallel printer are extra to basic system	Disk BASIC available	
From £6080			BASIC, FORTRAN, COBOL, PASCAL, all run under CP/M	Aimed at business and university users
From £100	64KB RAM			No software available
£425 £450 £550 £695 £895	8KB PETS may be expanded to 32KB with expansion board	Tape cassette drive, dual minifloppy disk—user storage 950KB; dot matrix	8KB BASIC in ROM, operating system, ASSEMBLER	Plenty of software from user groups and software houses, *e.g.*, PETSOFT
£2400	64KB RAM	Double density floppy disk (1MB), 1KB EPROM	CP/M, BASIC, COBOL, PASCAL, FORTRAN, ASSEMBLER	
From £1390	32KB RAM		Extended BASIC in ROM, ASSEMBLER	System now ranks 4th behind PET, TRS-80 and Apple in "personal" computer sales
£6000	60KB RAM		ASSEMBLER, BASIC	Also a 655 model with 320KB floppy disks and 12-in, 20 × 80 VDU
£786			ALGOL, Microsoft BASIC, COBOL, FORTRAN	Also maxi-kit similar to mini-kit but with larger, 8-in floppy disks
£1600		Printers	ASSEMBLER, BASIC, FORTRAN, FLEX, PASCAL, PILOT	From an extensive range of fully compatible modular systems
From £372 (kit) to £4000+	512KB	3 minifloppies or 4 8-in floppies	BASIC, FORTRAN, COBOL, ASSEMBLERS	Aimed at OEM, educational and industrial/scientific users
£4998				Software available
From £1995	512KB including two additional floppies	Up to 7 terminals	FORTRAN IV, COBOL, Extended BASIC, ASSEMBLERS, word processor	Multi-user system for software development
From £2995 to £8000+		2 additional disks, 12KB, multichannel A/D and D/A interface, PROM programmer, up to 7 terminals	Software as for System II	
£1450 £2495			CP/M, BASIC; also available are FORTRAN, COBOL, PASCAL macro-ASSEMBLER, text processor	With expansion it is a multiuser system for 2–12 users, with time sharing, £40000+

		Hardware description of basic system							
Manufacturer	Unit	Configuration	Micro-processor*	Minimum memory	Visual display	Keyboard	Interfaces and I/O ports	Type of backing memory	Other
Exidy	Sorcerer (reviewed in *Practical Computing*, March 1974)		Z80	Either 16KB or 32KB RAM	12-in video monitor	79 key keyboard 256 character set (128 graphics)	Cartridge and cassette interface		
Heath Data System	WH–89	All-in-one computer	Two Z80s	16KB RAM	"Intelligent" video terminal			Floppy disk	
Heath Kit	H8	Kit	8080A	4KB RAM	"Intelligent" front panel, VDU extra	Octal keyboard	Benton Harbor 50-pin bus		Built-in speaker + power supply
Heath Kit	H11	Fully wired and tested KD11F board with 16-bit LSI CPU	16-bit LSI CPU	12KB RAM				Built-in paper tape reader	
Hewart Micro-electronics	Mini 6800 Mk II			1KB user RAM, 1KB monitor, 1KB VDU RAM	Upper and lower case VDU with graphics option		CUTS interface		
Hewart Micro-electronics	6800S			16KB RAM	Upper and lower case, graphics		Printer and high-speed tape interfaces		
Hewlett-Packard	HP85	Compact portable unit		16KB RAM	5-in, 16 × 32, black and white VDU		RS232 port, 4 parallel ports	Cassette (200K)	64 cps printer
Imsai	VDP 40			32KB RAM	9-in display screen	Standard keyboard	(Serial I/O)	Two 5¼-in floppy disk drives.	
	VDP 42			32KB RAM	9-in display screen	Standard keyboard	(Serial I/O)	Larger disk capacity	
Intertec	Super Brain	Everything contained in one unit	Two Z80As	64KB RAM, 1KB 2708 PROM	Full upper and lower case ASCII. 128 set with limited graphics. 64 user definable keys, program marker cursor and reverse video. 25 rows × 80 characters, 8 × 8 in	Numeric and control keyboard separate	S100 bus	Two double density 5-in floppy disks (160KB each, 320KB total)	
ITT	ITT 2020	Single unit	6502	16KB RAM			Extra	Extra	
Luxor	ABC80	Personal computer	Z80A	16KB RAM, 1KB picture, 2KB monitor ROM, 16KB Basic in ROM	12-in black and white TV screen displays upper and lower case, digits. Built-in graphic mode giving 64 different graphic symbols. Size: 24 rows of 40 characters	Professional type keyboard	46/80 bus to connect peripherals, *i.e.*, floppy disk units, printers plotters, measuring instruments, etc. V24 jack for connecting to a telephone modem, to communicate with other computer systems via the public telephone network. IEEE-488 interface and an RS232 port	Cassette memory	Real-time clock, loudspeaker
Micronics	Micros	Kit or assembled	Z80	2KB RAM		47 key solid state ASCII keyboard	Interfaces for video (48 × 16 character video matrix), cassettes, printer and UHF TV. (RS232 serial I/O and 2 dual parallel I/O ports)		

Approximate price of basic system (excluding VAT)	Maximum memory expansion	Accessories available	Software	Software support and comments on system
From £760 without VDU		Micropolis floppy disks. EPROM pack for your own programs on cartridge	BASIC, ASSEMBLER, EDITOR, word processor	High-resolution graphics capability
	Expandable			
£322 (including VAT)	65KB	Disc drive	Benton Harbor BASIC, extended BASIC, text EDITOR, ASSEMBLER, console debugger	Aimed at education and applications
£1183	32KB	Many	PDP-11 software, including BASIC and FOCAL, EDITOR, relocatable ASSEMBLER, linker, absolute loader	
From £127			BASIC in ROM	Aimed at education and small business
From £275			Room for 8KB BASIC in ROM	Software available
£2240	32KB		BASIC	Full dot matrix graphics
£4507 £4950	64KB			Also available: VDP 44 with floppy disks (780K)
£1995		Printer, S100 bus allows expansion with 2, DSDD 8-in floppy disk—2.4MB, 8–120MB hard disk, colour VDU, plotter, modem main-frame interface, etc.	CP/M disk operating system	Disk and processor use separate Z80 microprocessor, hence computing can continue during disk operations
£867	48KB	Single minifloppy disk (116KB), RS232 port	MONITOR, ASSEMBLER, extended BASIC (ROM)	
£795	40KB	printer, plotter	BASIC with resident EDITOR, ASSEMBLER	Aimed at small business and education markets
Kit £360, assembled £399. System with acoustic coupler and VDU £1020	64KB		2KB BASIC	Aimed at small businesses and process controllers

Manufacturer	Unit	Hardware description of basic system: Configuration	Micro-processor*	Minimum memory	Visual display	Keyboard	Interfaces and I/O ports	Type of backing memory	Other
Micro V	Microstar	Single box		64KB RAM			Three RS232 serial inputs	Twin 8-in floppy disks	
Midwest Scientific Instruments	MSI 6800	Kit or assembled		16 KB		ACT 1 terminal	Cassette interface		
Nascom Micro-computers	Nascom 1 (reviewed in *Practical Computing*, January 1978)	Kit	Z80	4KB RAM		Full alpha-numeric keyboard	Parallel I/O, serial data interface		
National Multiplex	Pegasus		Z80	48KB RAM	12-in CRT	58 key keyboard	S100 bus, two serial interfaces, one parallel interface	Double density floppies (320KB)	
Netronics	Elf II	Single board computer	1802	256KB RAM	(Video output option)	Hex keyboard (ASCII keyboard as an option)	(Cassette interface + RS232 I/O is an option)		
Netronics	Explorer 85		8085A	4KB RAM	VDU board	ASCII keyboard	S100 expansion cassette. RS232, TT7 interface, on board I/O ports		
Newbear	7768			4KB RAM			cassette + VDU interfaces		
North Star	Horizon	Integrated disk hardware + computer in one chassis	Z80A	16KB RAM	Does not include a terminal, instead uses I/O port to a conventional VDU, similarly for a printer			Single mini-floppy disk drive (180KB)	
Ohio Scientific	Challenger C2-4P	Supplied as two boards with open slots for expansion							
Ohio Scientific	Challenger C2-8P	Similar to C2-4P							
Ohio Scientific	Challenger C3	Triple processor architecture	6502A Z80A 6800	32KB RAM				Dual 8-in floppy disks	
Ohio Scientific	Superboard II	Everything on a single board with a built-in keyboard (*not* a kit)	6502	4KB RAM, 2KB monitor in ROM, 8KB Basic in ROM	32 × 32 video interface allowing upper and lower case + graphics	Full 53 key keyboard with upper and lower case	Kansas City standard audio cassette interface for use with an ordinary cassette recorder		
Pertec	System 1300			32KB RAM			Serial interface		
Powerhouse Micro-computers	Powerhouse 2		Z80	16KB RAM	5-in built-in VDU	Full keyboard	RS232 interface		
Processor Technology Corp.	Sol 20/16		8080	16KB RAM	Video interface (including graphics)	Integral keyboard with numeric pad	S100, cassette interface, serial and parallel interface		
Rair	Black Box	Basic system in one unit					2 programmable serial I/O interfaces (can expand to 8 serial interfaces)	Dual mini-floppy disks, 80KB each	Real-time clock
RCA	Cosmac	Kit or assembled	1802			Hex keypad	Output to TV screen		
Research Machines	380-Z		Z80A	16KB RAM			RS232 port	Cassette	

Approximate price of basic system (excluding VAT)	Maximum memory expansion	Accessories available	Software	Software support and comments on system
£4950 (includes a reporter generator language)			STARDOS operating system enables system to have 3 VDUs plus a fourth job running simultaneously	Word processing software available
Basic £1100 (£815 as kit)	3 disk systems: 32KB memory with mini-floppy, 56KB memory with large floppy or hard disk system	Minifloppy system with triple driver of 8 bytes each, large floppy system with 4 312KB disks, hard disk system with 10MB		
£165	64KB	Up to 64 parallel I/O ports		Available as Nascom 2 with 8KB RAM and 8KB BASIC in ROM
£2700 (includes a bi-directional printer)		8-in drives, 1–2MB additional drives, digital recorder		
Basic kit £80, assembled £100, I/O board £35	64KB		(Machine code or Tiny BASIC option)	
From £297 (includes programmable timer)	64KB		Disk software, microsoft BASIC on cassette	8080 and Z80 software can be used
From £45	64KB (only in kit form)		range of BASICS	
£995–£2500	56KB		4 minifloppy disk drives (180KB each). 12 slot S-100 bus enables one to plug in many types of peripheral boards including a hardware floating point board	Extended BASIC, DOS
£343–£1204				
£434–£1900				
£2450–13000		Multiple terminals, printers	Can run virtually all 6502, 6800, 8080 and Z80 code	Software available including word processing + data base management
From £298	Expander board with 24KB static RAM including an additional minifloppy interface, port adaptor for a printer and OS1488 line expansion interface		8KB microsoft BASIC in ROM	
£3000–5500	64KB	4 serial ports		
£1480 (including real-time clock) to £1760	32KB		14KB Basic, X–Y graphics, 2KB monitor	
£1750 excluding monitor and cassette	6KB	Complete floppy disk system with word processing (£5000)	Extended BASIC, FORTRAN, FOCAL, ASSEMBLER, EDITOR	
From £2300	64KB	IMB disk storage (or 10MB hard disk), range of peripherals	Extended BASIC, interpreter, FORTRAN IV and COBOL	
Kit £80, assembled £100			ASSEMBLER and machine code programming, Tiny BASIC extra	
£1048	56KB	Dual 5¼-in or dual 8-in mini-floppy disks (168KB and 1MB, respectively)	Tiny BASIC, extended BASIC, COBOL, APL, FORTRAN, ALGOL CP/M	High-resolution colour graphics board available

TABLE I—

Manufacturer	Unit	Hardware description of basic system							
		Configuration	Micro-processor*	Minimum memory	Visual display	Keyboard	Interfaces and I/O ports	Type of backing memory	Other
Rockwell	Aim-65 (reviewed in *Practical Computing*, July 1979)	Single board unit		1KB RAM	20 characters, 16 segment display	Full alpha-numeric on-board keyboard			On-board thermal printer
Science of Cambridge	Sinclair ZX80 (reviewed in *Personal Computer World*, April 1980)	Single unit (available as kit) personal computer	780–1	1KB RAM	Use domestic TV	Built-in touch-sensitive keyboard. Graphic facilities	Cassette interface, television interface	Use audio cassette recorder	
SDS	SDS100	Single unit		32KB RAM	12-in video display	Keyboard	Serial and parallel inter-facing		
Semel	Semel 1		Z80	16KB RAM	12-in, 24 × 80, black and white VDU	Keyboard	RS232 port	Single floppy disk unit (250KB)	
Sord	M100		Z80	16KB RAM 4KB monitor	VDU	Full key-board and function pad	Dual cassette interface, RS232 port, S100 bus		Two-channel joystick, 24-h clock
Sord	M222		Z80	64KB RAM	VDU, graphics	Full key-board and numeric keypad	Real-time clock, audio cassette inter-face, two serial parts, three S100 slots, interface for two external minifloppy drives	70KB mini-floppy disk drive	
Synertek	Sym 1	Fully assembled and tested	6502	Memory available in 4KB blocks up to 64KB. 4KB ROM monitor		Keypad	High-speed cassette inter-face (2400 baud)		
Tandy	TRS80 Level 1		Z80	4KB RAM	12-in, 16 × 64, black and white VDU			Cassette	
Tandy	TRS80 Level 2		Z80	16KB RAM	12-in, 16 × 64, black and white VDU		RS232 interface, 1 parallel port	Cassette	
Vector Graphics	MZ		Z80	56KB RAM			3 serial ports, 2 parallel ports	Dual mini-floppy disk (630KB)	PROM burner
Zilog	MCZ 1/05		Z80	64KB RAM			RS232 port	Dual 8-in floppy disk (600KB)	Debug in 3KB PROM

* The following are the manufacturers of the microprocessors given in the table:

Intel	8080, 8080A, 8085A, 8086
MOS Technology	MCS6502, MCS6502A
Motorola	MC6800
NEC	780–1
RCA Cosmac	1802
Zilog	Z80, Z80A

continued

Approximate price of basic system (excluding VAT)	Maximum memory expansion	Accessories available	Software	Software support and comments on system
1KB £250 4KB £315	4KB		ASSEMBLER, EDITOR, BASIC	Compatible with Commodore KIM
£100, kit £80, £300 for full expansion	16KB	Requires a mains adapter	4KB BASIC in ROM, EDITOR, operating system	No facilities for trignometric, logarithmic or floating point arithmetic functions, at time of press. Possibly included at a future date
From £3750	46KB, up to 8KB PROM	Line printer	SD monitor program	
From £2900	64KB	Printer, light pen, maximum of 8 × 250KB floppy disk units	BASIC, COBOL, FORTRAN	
£726	48KB			
From £3450 to £4123 (includes desk and printer)		Bar code reader. TMS-1000 development system, two external Minifloppy disk drives		Also M223 model—same hardware as M222 plus single or double 350KB minifloppy disk
From £160	64KB	Port expansion kit, TV interface card, teletype interface		Compatible with KIM1, *i.e.*, can use same software
£385	16KB		BASIC, ASSEMBLER	BASIC in 4KB ROM. Level 1 is upgradable to level 2
£560	48KB	Minifloppy disk unit, numeric pad, line printer	BASIC, macro-ASSEMBLER, FORTRAN	
£2595			DOS, BASIC, ASSEMBLER, ALGOL, C BASIC, COBOL, FORTRAN, PASCAL	System B has graphics and numeric pad
£4200			R10 O/S macro-ASSEMBLER, BASIC, COBOL, FORTRAN, PASCAL	

TABLE II

SUMMARY OF ANALYTICAL APPLICATIONS OF MICROPROCESSORS

Reference	Application	Description of application
1	Description of the hardware of a microprocessor and an application in electrochemistry	Two papers. The first deals briefly with the hardware principles involved in a general-purpose microprocessor. The second describes the use of a microprocessor in the construction of differential stripping electrochemical apparatus
2	Microcomputer for data handling in analytical chemistry	1. On-line filtering of an analogue signal at the output of a liquid chromatograph. 2. Use as a buffer memory to store rapidly data from a gas chromatograph - mass spectrometer and then output it slowly to an inexpensive recorder. 3. Analyse noise from a new liquid chromatograph detector
3	Data processing in an analytical laboratory	Microprocessor performs the data acquisition and reduction whilst the final evaluation and co-ordination are left to a larger processor
4	First microcomputer-controlled infrared spectrometers (Perkin-Elmer, Models 281 and 283)	Microcomputer directly controls the recording and optical systems, *i.e.*, gratings and filters are changed on computer command and both the monochromator and chart positions are tracked precisely
5	Perkin-Elmer, Model 460, atomic-absorption spectrometer with microcomputer	Integration times of 0.2–60 s and scale expansions of 0.01–100-fold can be selected. It contains an automatic calibration graph correction feature
6	Differential corrected spectra unit for the Perkin-Elmer, Model MPF-44A, fluorescence spectrophotometer	Corrected differential excitation, emission or excitation - emission spectra can be recorded
7	Microprocessor-controlled automatic titrator	The Intel 8008 microprocessor controls the titration and determines the equivalence volumes by locating the maximum in the differential $\Delta pH/\Delta V$ *versus* V graph
8	Microcomputer-controlled single-task potentiometric titration system	Successive determinations of calcium and magnesium in drinking water by complexometric, potentiometric digital titration to two equivalence points. The functions of the microcomputer are: (*a*) to check the stability of the electrode signal and ultimately accept it; (*b*) to calculate and control the titrant delivery volume; (*c*) to establish whether inflection points occur and determine the exact location of those detected
9	Real-time monitoring and control of gas chromatographs	Intel 8008 and 8080 microprocessor-based systems were developed to control sampling, operation of the gas chromatograph and perform real-time on-line data acquisition and analysis
10	Microprocessor for data handling in gas or liquid chromatography	Microprocessor-based system consisting of an 8KB memory, a display panel, a cassette tape memory and an ADC is used for data processing. Tangential skimming method and the perpendicular method are used to process unresolved peaks in a tail of a main peak. Concentration of each component is calculated automatically
11	Infrared spectrometer (Wilks Scientific Corp., Model 80, gas analyser)	Microcomputer-controlled single-beam spectrometer can automatically analyse up to 11 compounds. Used to analyse quantitatively atmospheric pollutants
12	General-purpose microcomputer in analytical chemistry	Based on the Intel 8008 microprocessor, its applications include: 1. Use as a buffer memory to store rapidly data from a mass spectrometer and then output it slowly to an inexpensive recorder. 2. Analysis of noise using a conventional recorder as an oscilloscope. 3. Normalisation and linear output of mass spectrometric data

Reference	Application	Description of application
13	Perkin-Elmer Sigma Series interactive gas chromatograph instrumentation	The series consists of a GC data system (minicomputer based) and four gas chromatographs that incorporate microprocessors. The microprocessors provide data processing, control and monitoring, and fault diagnosis facilities
14	Microprocessor applications in the process industry	Brief account of two applications involving microprocessors which were carried out at Warren Spring Laboratory, Stevenage, Hertfordshire
15	Single-cell protein production via a fermentor and a microprocessor	Fermentor coupled to a microprocessor to control single-cell protein production. It regulates pH, temperature, dissolved O_2 and can take into account the O_2 and CO_2 in the exhaust gas. The microprocessor also logs and processes data
16	Hierarchical approach to computers in the handling of time-critical problems in process control of experiments	Microcomputer shared between different experiments and plotting tasks, under the control of a multiprogrammed RC4000 computer, resulting in a cut in resource demands in an on-line plotting system
17	Multi-dimensional gas chromatograph (Packard-Becker, Model 429)	Apparatus consists of a basic gas chromatograph, modular flow system, an analysis module and a controller unit based on an 8-bit microprocessor. The multi-dimensional approach allows analysis of systems that cannot be separated on a single column
18	Microprocessor-controlled photodiode array measurement system for analytical spectrometers	The Intel 8080A microprocessor with its associated hardware controls the functions of the 256 linearly spaced photodiode array system. It controls the scanning operation of the photodiode array, selects a segment of the ultraviolet, visible or near-infrared spectrum that is to be the active area of the photodiodes, collects and processes the data, correcting for fixed pattern noise and background
19	Microprocessor-assisted calibration for a remote working level monitor	The microprocessor calculates β-efficiencies and solves systems of linear equations with several unknowns.
20	Microcomputer for controlling chemical analysis instruments	A review with no references
21	Microprocessor - Ionalyser	A description of the Orion, Model 901, Ionalyser and the ion-selective electrodes available for use with it
22	Automatic degassing of liquid samples for NMR and ESR spectroscopy	Use of a microcomputer and apparatus for the automatic degassing of liquid samples by the cool and thaw technique before analysis by ESR or NMR spectroscopy are described
23	Microprocessor-controlled scanning dye laser for spectrometric analytical systems	The microprocessor controls the wavelength selection (from 360 to 650 nm) by adjusting the angle of a diffraction grating and moves one of several dyes into the laser cavity. It can also collect data directly or be incorporated into a larger instrumental system and run by a minicomputer
24	Application of microcomputers in chemical production plants	Microcomputer use in production plants is discussed on the basis of actual and planned applications
25	Infrared spectrometer equipped with a microcomputer	Wilks, Model 180, infrared spectrometer is used for calculating the amount of various components present in a multi-component mixture
26	Portable neutron spectrometer/ Kerma rate meter	An 8-bit microprocessor system was developed for use in a portable instrument capable of measuring and displaying energy spectra and Kerma rate. On-site analysis of data is possible and thus eliminates the need for a large-scale computer
27	Microprocessor-based multi-wavelength detector system for liquid chromatography	The microprocessor performs control, data acquisition and data manipulation in order to optimise separation. It digitises the analogue output of a linear silicon photodiode array
28	Radiation monitoring system for use in a nuclear power station	A digital radiation monitoring system employing distributed microprocessors is described

TABLE II—continued

Reference	Application	Description of application
29	Instrument - computer interface based on the SDK-80 microcomputer	NMR spectrometers were interfaced through a Fabritex 1074 computer to a DEC System-10 computer by an SDK-80 microcomputer. The system has minimum hardware design, is easy to operate and has LED program state indicators
30	Microprocessor in a pulsed NMR spectrometer	A low-cost pulsed NMR system was built, designed around the Intel 8080 microprocessor. The microprocessor supervises instrument control and data acquisition. Data are stored in RAM and can be transferred over a telephone line to a remote time-shared computer for further processing
31	Potentiometric stripping analysis	Microcomputer controls the equipment for automation of potentiometric stripping analysis. The higher resolution of time compared with manual equipment increases the sensitivity of the method
32	Microcomputer-controlled automatic titrator	An Intel 80/10 microcomputer, programmed in BASIC, controls an automatic titrator. Either fixed or variable titrant additions can be performed and a stable pH reading is ensured. The titrant can be added in volume increment as small as 5×10^{-4} ml. The time taken to collect each point is recorded
33	Applications of microprocessors to analytical spectrometry	Three systems have been designed and integrated together into a hierarchical approach to demonstrate the diverse roles that microprocessors can play in analytical instrumentation: The systems are: 1. a data acquisition module to perform frequency ratio measurements; 2. a monochromator controller; and 3. supervisory system, programmed to link the first two units to it and form a hierarchical system for controlling a spectrometric system
34	Microprocessor-assisted high-precision viscometry	Microprocessor is coupled to a high-precision, high-temperature torsion pendulum viscometer. Photodetector signal level transition times are stored in the microcomputer and after some calculation transferred to a PDP-11 minicomputer where the main viscosity computations take place
35	Microprocessor-controlled differential titrator	Control of titration procedure and recording of data is performed by the microcomputer (Intel MCS-80)
36	Microprocessor-controlled potentiostat for electrochemical measurements	The system consists of a CPU, 16KB RAM, peripheral interfacing, a timer, an ADC and a DAC. It is intended for the control and unattended operation of a standard laboratory potentiostat
37	Microprocessor to solve pH control problems	Development of a microprocessor-based pH control system for batch processing is described
38	Microcomputer-assisted, single beam, photoacoustic spectrometer system for the study of solids	Expensive commercial data acquisition units have been replaced by commonly available, inexpensive and easily constructed components. The use of the microcomputer for data acquisition and reduction has eliminated the problems associated with the single-beam mode of operation, *i.e.*, source output correction, background compensation, etc.
39	A microprocessor on-line to general analytical instruments	Hewlett-Packard 9825A microprocessor was used to collect and process data from smaller, commonplace analytical instruments, *e.g.*, electronic balances, atomic-absorption spectrometers and chloride meters, which are generally characterised by their slow (operator-limited) data rate.
40	Microprocessor controlled HPLC with CRT display	The incorporation of a microprocessor, keyboard and a CRT in the Varian, Model 5020, liquid chromatograph offers the user a simplified approach to instrument operation. The CRT permits continual display of instrument status, which eliminates the need for status lights and indicators

Reference	Application	Description of application
41	Microcomputer-controlled potentiometric titration system for equilibrium studies	The microcomputer is based on an Intel 8080A microprocessor, with the control programme being stored in PROM. The system delivers an optimised volume of base that corresponds to uniform changes in electrode potential throughout the entire titration
42	Microprocessors and the chemist	A review on microprocessors with current and possible future applications for the chemist
43	Microprocessor-based liquid chromatograph with CRT display (Varian, Model 5000, high-performance liquid chromatograph	The use of the 3-solvent capabilities of the Model 5020 chromatograph for the separation of polystyrene oligomers and the advantage of an automatic loop valve of the Model 5000 chromatograph in monitoring the course of a chemical reaction are discussed
44	Applications of microcomputers to analytical chemistry	A review with 34 references
45	Microprocessor-based, linear response time low-pass filter	The filter has a response time half that of an equivalent RC filter and is therefore particularly suitable for application in analytical instrumentation (*e.g.*, atomic absorption) where reduced response time means reduced sample consumption, shorter analysis time and reduced effects from drift
46	Microprocessor-based system for measuring low-frequency (100 Hz) electrical noise spectra	The microprocessor determines the noise autocorrelation function by using simple one-bit autocorrelation arithmetic; subsequent Fourier transformations to find the power spectra are performed on a larger computer. Current noise measurements in insulating polymers are described
47	Microprocessor Mössbauer spectrometer	A Mössbauer spectrometer based on the Motorola MEK 6800D1 microprocessor kit is described; it provides a simple, cheap and flexible alternative to conventional multi-channel analyser systems
48	Microprocessor-controlled ultraviolet - visible spectrometer with incorporated recorder (Perkin-Elmer, Model 554)	The apparatus contains a background compensator to provide extremely straight base lines, a built-in calculating system to control the automatic recorder, automatic standardisation process, a keyboard to input appropriate parameters and an automatic cuvette charger. First and second derivatives of spectra may be used to solve difficult spectroscopic problems and, with integration, measured values can be determined accurately and recorded under unfavourable conditions
49	Microprocessor-based process gas chromatograph	A data processor unit controls from 1 to 6 chromatograph analysers. Each analyser contains a microprocessor that controls all of the functions associated with the analysis, performs all measurements and integrates the area or finds the peak height. Only final data for selected components are transmitted to the data processor
50	Microcomputer-based gas chromatography system	The gas chromatography system has an interlocking microcomputer for regulating temperature, timing integrator and recorder
51	A review of microprocessors in laboratory instrumentation	A small table consisting of five typical microcomputing systems with their respective price, memory, interfaces, etc. Also includes four references, three on hardware aspects and one on microprocessors in instruments
52	General-purpose microcomputer in laboratory automation	A general-purpose microcomputer data system was developed to bring on-line individual chemical instruments. The design features include modularity, optimised I/O structure for real-time experimental control and data acquisition, flexible video display generation for operator intervention and data presentation, etc.

TABLE II—continued

Reference	Application	Description of application
53	Versatile microcomputer-controlled titrator	The titrator is capable of handling ultraviolet - visible spectrophotometric, potentiometric and amperometric end-point detection and acid - base, redox, complexometric and precipitation reactions. The ADD 8080 microcomputer system is used, which is based on the Intel 8080A microprocessor chip. It controls titrant delivery, pre-titration adjustments, end-point measurement, equivalence point determinations, calculations and display of results in the derived units. All titration programs were written in BASIC and stored in paper-tape form
54	Microprocessor applications in the analysis of natural gas and cement kiln gas	Determination of the octane number, the dew and frost points, H_2S, the natural gas calorific value and combustion control
55	Microprocessor-controlled electrochemical system	Based on the Motorola M6800, it is designed as a software orientated system with a minimum of hardware parts. The system performs cyclic voltammetry but can easily be adapted for controlled-potential electrolysis, chronopotentiometry or other electrochemical techniques
56	Automatic analysis of organic pollutants in water via a microcomputer-controlled GC - MS system	The system prepares the sample and processes the analysis data. The sensitivity is 60 p.p.t. of organic compounds
57	Quality control of polymers using a microprocessor-based thermal analyser	A Du Pont R90 modular microprocessor-based programmer was used for quality control testing using differential scanning calorimetry thermograms
58	Microprocessor system to monitor distillation plate efficiencies	An optical probe and microprocessor monitor were used for determining bubble size and velocity in a froth formed on a sieve plate and for evaluating plate efficiency on-line. The technique was used to study the behaviour of surface tension, positive and negative binary systems and the effect of liquid viscosity in gas absorption
59	Thermal analysis of plastics via microprocessor control	Three techniques involving microprocessor control are: measurement of total volatiles in compound resins; separation of extenders or plasticiser, carbon black and inert filler from polymer and elastomer formulations; and separation of fillers
60	Microcomputer-controlled buffer gradient for ion-exchange chromatography	This system was developed for switching between several buffer solutions to produce a mixture with independent elution gradients for two ions. It was used as a gradient programmer for the separation of amino acids by ion-exchange chromatography. The microcomputer also supervises the automatic facilities of an amino acid analyser
61	Nuclear criticality evacuation with telemonitoring and microprocessors	The criticality system at Argonne National Laboratory is described. The site microprocessor maintains a current data table, detects faults, drives a printer and communicates with the central telemonitoring station
62	Microcomputer control of systems in chemical engineering	A review with six references
63	Uses of a desk-top computer with graphics in automated geochemical analysis	Tektronix 4050 series graphics microcomputer was used as an on-line data processor in X-ray fluorescence spectrometry. During calibration, plots of X-ray intensity *versus* elemental abundance in the calibration standard samples were available for on-screen display less than 1 s after depressing a user-designated key. Ten-element iterative matrix correction calculations require less than 3 s per sample
64	The implementation and operation of microcomputers in analytical laboratories are discussed	A review

Reference	Application	Description of application
65	Microprocessor-assisted quality control of the external CPS 24 GeV/c beams	To minimise beam loss and to retain beam quality, a fine control of the CERN ρ synchroton beam positions is required, which is achieved by a microprocessor in the CAMAC system
66	A general purpose electroanalytical system based on a microcomputer-controlled coulostatic generator	This consists of a multimicrocomputer system as the control logic, to control the charge generation (which adds charge to the working electrode) and the voltage measurement system (which monitors the potential). The multi-microcomputer system consists of three independent microcomputers, each responsible for a specific task. One co-ordinates the operation of the other two so that the system works as a functional unit. The electrochemical system was applied to the polarographic determinations of cadmium. The sensitivity of the system was limited to 0.5 μM of cadmium.
67	Microcomputers in laboratory automation	Block diagrams of microcomputers used as communication controllers, device controllers and terminal adapters for 37 pilot plants are given
68	Use of a sequence controller in process control	A review with no references
69	A microcomputer-compatible method of resolving rate constants in mixed first and second order kinetic rate laws	Both the first- and second-order rate constants and $t = 0$ are calculated from absorbance *versus* time data. The method is, except for one minimisation, a one-pass process and is therefore easily used on a microcomputer or programmable calculator
70	Practical microcomputer technology	A review with four references
71	A lightweight microprocessor-controlled solar neutron detector	The microprocessor controls the data acquisition, accumulation of histograms and the encoding of data for the telemetry systems
72	Microcomputer-controlled potentiometric analysis system	The use of CONVERS (a language based on an interpretative compiler) in microcomputer instrumentation combines the high-level software capabilities of a minicomputer, whilst retaining the ease of construction and cheapness of a microcomputer-based system. Application of this system to end-point determination in potentiometric titrations and fully automated generation of calibration graph data for multiple ion-selective electrodes are described
73	Microprocessor-based millivoltmeter in applications of ion-selective electrodes	A commercially available microprocessor-based millivoltmeter was used as a simple method for converting appropriate millivolt readings to sample concentrations
74	Microcomputers interfaced to a high-resolution NMR spectrometer, equipped with a digital integrator	Multi-scan averaging is used to improve signal to noise characteristics and automatic calculation of the percentage purity of the sample is performed. It was successfully used to determine organic compounds quantitatively
75	Microcomputers in the wet-chemistry laboratory	A microcomputer with an analogue to digital convertor is interfaced with an analytical balance, a device for amplifying potentials, a photometer and a motor-driven burette. The microprocessor performs its tasks via eight programs: controlled titration, incremental equilibrium titration, titration at pre-selected end-point, pH titration, dosing, sample dissolution with definite concentration, dilution and conditioning
76	Microprocessor-controlled photon counter for pulsed optically detected magnetic resonance	The multi-accumulator photon counter is capable of sequential counting over as many as four intervals. The microprocessor, which controls the counter, manipulates the photon counts during each of the duty cycles. The instrument is inexpensive yet versatile and is suited for other applications in which sequential counting is necessary

TABLE II—continued

Reference	Application	Description of application
77	Microcomputer on-line to a Mössbauer diffractometer	This was devised to separate the elastic scattered intensity and the inelastic one in the vicinity of Bragg reflections. A selective modulator to move a transducer at a resonant velocity and at a non-resonant one alternatively is used for automatic separation
78	Device for demonstrating microprocessor interfacing	The design of a prototyper for use in interfacing between a microprocessor or minicomputer and an external device is described. Detailed diagrams and a component list are included
79	Basic concepts and terminology of microprocessors	A review with no references
80	New application possibilities for microprocessors in rapid kinetics in clinical chemistry	A review with references of the clinical use of kinetic methods in combustion with built-in microprocessors
81	Development of a microprocessor-based system for simultaneous data processing of different automatic elemental analysers	The microcomputer allows users already in possession of multimeters or integrators, an electronic balance and elemental analysers to combine their equipment into an automated system. After the analysers have been started, the system processes all data and calculates the blanks, calibration factors and percentages successively
82	Microprocessor-controlled liquid chromatograph - atomic-absorption sampling system	Description of a system for trace analysis of metal-containing compounds. It consists of a Motorola 6800-based Heath Kit microprocessor trainer, an external clock, an electronic interface to the dispenser and to the atomic-absorption spectrophotometer, and the dispenser system
83	Pulsed-gradient NMR diffusion measurements with a microcomputer	A microcomputer was adapted for NMR diffusion measurements using the pulsed field-gradient method. This made possible the use of samples with faint spin echoes
84	Microcomputer-controlled titrations	The regulation of the titration process and the detection of the end point by means of a microcomputer, and the construction and software of a process autotitrator are described
85	Practical aspects of microprocessor-based digital controller systems	A review with no references
86	Microcomputer-aided high-speed potentiometric titration system	Titrant additions and time intervals were chosen and controlled by the microcomputer, which also evaluated the equivalence volume based upon linear titration plots by using four titration points before the equivalence point. The apparatus was capable of determining the concentration of strong and weak monoprotic acids in less than 22 s, with relative standard deviations of 0.1–0.2%

titrator, by acquiring feedback to the microprocessor, which would then control the rate of addition of titrant until a pre-set potential was reached and taken as the equivalence point; (*ii*) to use a linear plot of the titration data to determine the equivalence point (Gran's method); (*iii*) to use a differential method to locate the equivalence point.

The first of these is straightforward and can lead to a practical improvement in existing instrumentation. However, we rejected it on the grounds that there was little novelty in such an adaptation and that it did not make full use of the potential of the microprocessor.

The use of Gran plots seemed to have many attractions.[87] They have been advocated for several years as an accurate way of determining the equivalence point, but the transformation of titration data into the functions plotted, *e.g.*, volume $\times$ 10^{-pH} *vs.* volume, are tedious to perform by hand. The microprocessor can perform the necessary conversions on the run and calculate the least-squares fit for the linear plot that should result. Further, most of the points used in the calculation are taken from the mid-part of the titration curve, thus minimising the problems arising from fluctuations of signal that occur near the equivalence point in a potentiometric titration.

In practice, it was found that the curves deviated from linearity near the equivalence point and even refinements of Gran's equations such as that due to Johansson[88] [equation (1)] and Pehrsson *et al.*[89,90] [equation (2)] were not much more successful.

$$V_e - V = K\left([H]V + \frac{V_0 + V}{C}\{H\}[H] = \frac{V_0 + V}{C}\{H\}[OH] + \frac{V_0 + V}{C}\{[H] - [OH]\}\right) \quad (1)$$

$$-V_e + rK + S = 0 \quad (2)$$

where

$$r = V\{H\} + \frac{V_0 + V}{C}\{H\}[H] = \frac{V_0 + V}{C}\{H\}[OH]$$

and

$$S = \frac{V_0 + V}{C}\left([H] - [OH]\right) + V$$

V_e is the equivalence volume, V_0 is the original volume of acid, V is the volume of base of concentration C added, K is the acid dissociation constant and the brackets round species distinguish activity and concentration terms in the usual way.

The key to the difficulty is seen in the need for an accurate value for the dissociation constant and in the need to sort out the activity and concentration terms. This can be done by further computation, but it is going away from the simple relationships that are more suitable for the limited computational capability of a microprocessor.

The third approach, the use of a differential of the titration curve, was then investigated. It had the disadvantages that the peak in the differential curve concides with the equivalence point only when the titration curve is symmetric and it makes most use of the data close to the equivalence point. These disadvantages were offset by the ease of computation and the applicability of the algorithm to many systems, no knowledge of K being required. It was found that the deviations from the equivalence point with asymmetric titration curves was not significant (or could be corrected empirically with a titration factor) and that a simple smoothing routine could reduce the effects of erratic signals close to the equivalence point. (A more effective solution to noise was to reverse the direction of stirring, so that the titrant was better mixed before it reached the electrodes.)

All of these calculations were performed via a terminal on a mainframe computer, with a program written in a *high-level language* such as BASIC. Consequently, the time involved in the development of the best solution to the problem was used more economically than if it had been written directly in *machine language*. The program had (in 1976) to be converted into *assembly code*,[91,92] a good reason for using the simplest adequate program. As it was to be used for a specific application, the algorithm in essence specified the addition of a fixed volume of titrant. During the addition, which was to be made in fixed increments at a fixed rate (controlled and counted by the microprocessor), the titration data were collected and smoothed and the difference between successive points was calculated and stored. At the end of the run the two largest differences were found and associated with the two equivalence points of the sequential titration. A more exact value was calculated by interpolation of the five points around each peak. The values were checked to ensure that they fell within reasonable bounds and were then displayed.

The full titration assembly is shown schematically in Fig. 1.

The automatic burette, pH meter and electrode assembly were taken from a conventional autotitrator. The microprocessor was an Intel 8008. The control functions were exerted as a pulse to a relay, and the analogue to digital conversions and *vice versa* were carried out by cheap solid-state devices. Programming the microprocessor in assembly code took some time and was facilitated by the availability of a development system that included fast punched-tape readers, program *debugging* facilities and *PROM simulators*. Typically, a complete microcomputer development system, MDS, costs approximately £10000.

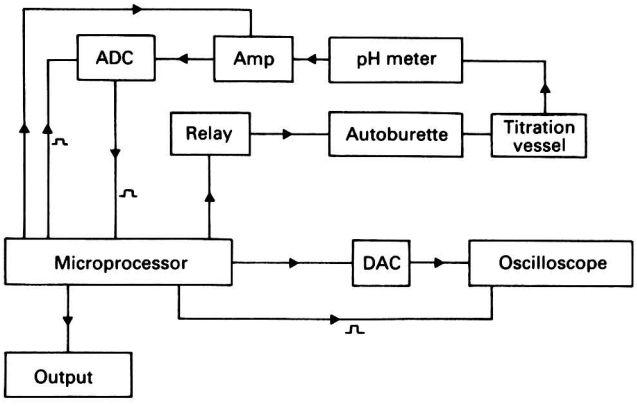

Fig. 1. Arrangements of apparatus for titrations controlled by the microcomputer.

As a laboratory instrument it proved very satisfactory. Two titrations could be performed in sequence in 1 min with a coefficient of variation of 1%. If 4 min were taken the precision improved to 0.25%. At a higher rate there was a slight systematic error, but at the slower rate the accuracy was as good as with a manual titration. The elimination of mannitol from the procedure resulted in sufficient savings in the course of 1 year to pay for the material cost of the apparatus.

The approach of Christiansen *et al.*[8] was similar, except that they used the titration data to slow the rate of addition of titrant as the equivalence point is approached.

If one were to carry out a similar exercise now, the major change in procedure would be to do the program development work on a PET or similar microcomputer, with a suitable *interface*. This would automatically carry out the tedious step of converting an acceptable high-level computer language (BASIC) to machine code and effectively eliminate the development system that is needed to support the microprocessor. However, even a PET linked to the basic components of an automatic titrator represents a laboratory "lash-up."

Control Laboratory Model

The conversion of the laboratory prototype to a robust control laboratory instrument required a further definition of objectives. We held the following to be important:

(*i*) There should be as few control knobs or switches as possible; ideally the operator should only have to press a run button and read off the result;
(*ii*) it should be as compact and robust as possible, by taking full advantage of solid-state electronics;
(*iii*) it should be applicable to more than one titration;
(*iv*) it should have error-detecting routines that indicate common malfunctions such as broken connections and defective electrodes.

At this point, additional advantages of the differential routine for the location of the equivalence point became apparent: it was applicable to all non-linear titrations and it enabled one to exchange the expensive, calibrated pH meter for a voltmeter chip, costing about £25 and occupying very little space. The original program had to be modified to detect the equivalence point straight away instead of assuming a fixed increment of titrant. This was done by a moving window in which the signs of the difference between successive points on the differential titration curve were noted. As the equivalence point is approached, the window shows every difference as positive (*i.e.*, an increment in $\Delta pH/\Delta V$), but as the equivalence point is passed the differences become negative (*i.e.*, a decrease in $\Delta pH/\Delta V$). Some care is needed to discriminate noise spikes from a sharp end-point and to ensure that a poor end-point is detected.

Additional programs were needed to provide the error checks. Economy suggests the use of only one readout and this is used to display the equivalence point, the voltage if the

appropriate button is pressed and an error-identifying number if the apparatus is malfunctioning. An additional control loop is used to re-fill the burette and switch on a "ready" light when the titrator is primed to re-start. When tested the program is placed in *ROM* where it remains fixed, unalterable and immune from erasure by power failure or other electronic crises.

These alterations to the laboratory prototype and the program listing took almost as long as the initial development.

Other Approaches

The strategy outlined above in both stages of development places emphasis on simplicity and implies a willingness to dedicate a microprocessor to a single function. We believe that this emphasises the quality of a microcomputer that distinguishes it from a minicomputer, *i.e.*, its smallness. At the outset of the work there was a severe limit on the available memory of a microprocessor (1–2KB) and this, in a practical way, endorsed the value of a simple approach.

However, this is no longer true. Large-scale integrated circuits (LSI) have become so available that with a PET or similar microcomputer one can obtain many of the facilities of a minicomputer and have, by the standards of Intel 8008, immense computing power. Even with more advanced microcomputer kits, such as the SDK 80, more complicated programs can be used, and interaction between the operator and the computer is possible. One such system has been described by Leggett[41] for the determination of equilibrium constants, and others by Avdeef and Bucher[32] and Martin and Freiser.[72] The latter authors' program allows the user to check on and correct for electrode drift. However, the ultimate in interactive systems must surely be that of Wu and Malmstadt,[53] the block diagram for which is shown in Fig. 2.

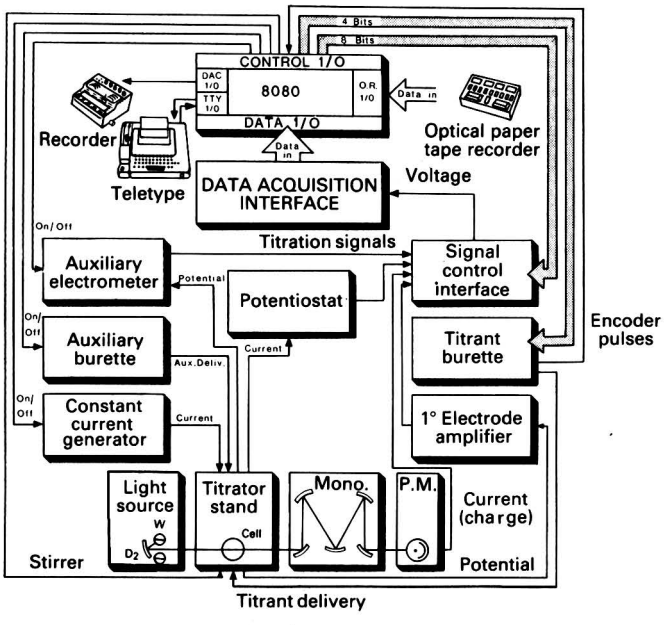

Fig. 2. Block diagram of the Wu and Malmstadt system.[53]

This system permits the operator to select virtually any type of titration and any appropriate program for processing the titration data. Both linear and logarithmic titrations are permitted. However, although the choice of procedure is left completely to the user, the implementation is computer controlled and all of the results reported are satisfactory for most purposes. The flexibility is gained at the expense of simplicity, and cost.

In conclusion, the only thing certain about microprocessor-controlled titrations is that most workers prefer a differential procedure for the determination of the end-point. There are completely different viewpoints as to whether it is preferable to have fixed or *interactive* programs. To some extent this depends on whether one is opting for a versatile laboratory instrument or one for process control. By now most of the possibilities have been tried and the consumer can feel confident that any commercial microprocessor-based titrator is reliable. In view of the development cost if he were to make it himself, he may also feel it is good value for money. As in all computer applications, the really difficult part is getting the chemistry right.

Conclusion

In such a rapidly growing area it is difficult to predict the future, but a report of the University of Sussex Science Policy Research Unit[93] is of interest in that it takes a broad view of the future development of microelectronics.

It predicted that technological advances will bring the cost of a microprocessor down to $1 by 1980 and 10–20 cents by 1985. It notes that "the reductions in cost have been made possible by the increased complexity of integrated circuits"; however, "the technology is now reaching a level where the majority of current and forseeably high-volume applications could be satisfied by single-component systems, so that the demand for further increases in complexity is expected to decline, leading to a stabilisation of technology. The future pattern of microelectronics is expected to show an increasing divergence between very complex circuits offering high performance at high cost and high-volume circuits where the emphasis will be on minimum cost."

The laboratory computer market is very small compared with the automobile industry, which is a major consumer of the microprocessors currently produced. However, it seems reasonable to assume that easily programmable microcomputers for control, computation and report writing will become a standard feature in most analytical laboratories.

The pace of these developments has resulted in such a proliferation of books on microprocessors that it is impossible to list them all. Consequently, we limit our recommendations to a very good general introduction to the field[94] and a recent book written specifically for the chemist by one who has been concerned with using microprocessors in commercial chemical instrumentation, especially mass spectrometers.[95]

Glossary

Assembly code	Instructions written in a machine orientated language.
Backing store	A device for storing programs or data externally from the main memory of the computer. Usually magnetic disks or tapes; access to the stored programs or data may take seconds, as in the case of disk, or minutes, as with tape.
Bus	1. A circuit over which data or power is transmitted. 2. A path over which information is transferred, from any of several sources to any of several destinations.
Cassette	A magnetic tape in a plastic case that can be loaded and unloaded without manual threading of tape. Like a music cassette tape but holding programs or data, which are accessed sequentially.
Central processor	The part of a computer system that performs computations, oversees the use of main memory and monitors input and output operations.
Central processing unit (CPU)	See Central processor.
Chip	See Silicon chip.

Daisy wheel	Circular typing element with spokes around the circumference, each with at least one raised character. Available in a range of type styles. Vying for popularity with the IBM "golf-ball" element.
Debugging	The process of isolating or correcting all malfunctions and/or mistakes in a piece of equipment or a program of operations.
Disk	A recording medium originally developed for mainframe computers when it holds many millions of characters. A random access device in which the access time for any given location on the disk is constant.
Floppy disk	A flexible disk recording storage medium developed for minicomputors and often used with microcomputers.
Hard copy	A machine printed document that can be handled and read by people without using special devices; usually program or data printed on paper.
Hierarchy	A specified rank or order of items.
High-level language	A computer programming language that is less dependent on the limitations of a specific computer; languages common to most computer systems, *e.g.*, ALGOL, FORTRAN, COBOL.
Image-sensing devices	For example, a detector that can collect 250 points in milliseconds.
Input/output	Commonly called I/O. A general term for equipment used to communicate with a computer. The process of transmitting information from an external source to the computer or from the computer to an external destination.
Instruction set	The set of instructions that a computing or data-processing system is capable of performing.
Interactive display	Any display that allows the user to input data in response to the information displayed.
Interface	A common boundary between two systems (or devices) or parts of a single system.
Mainframe	Large computer system (typically costing more than £200000).
Main memory	Another name for the internal storage of a computer.
Microcomputer	A simple silicon chip containing a microprocessor as well as memory and some input/output communicating facilities.
Microprocessor	The central processing unit of a computer, implemented on a single silicon chip.
Minicomputer	Originally a computer significantly smaller in size, capacity, cost and software capability than the larger mainframe computers with which it is contrasted. The differences have been blurred by technical advances.
Processing unit	See Central processor.

PROM	Acronym for programmable read-only memory. It is permanent, non-volatile memory; a program stored in PROM is not lost after power is removed, and is ready for running as soon as the microcomputer is powered on, thus eliminating the need for program loading.
RAM	Although an acronym for random access memory (*i.e.*, any location in this type of memory may be interrogated without prior access to previous locations), this usually refers to read/write memory where memory locations may be read or altered by the user. This memory requires power to retain its contents.
ROM	Acronym for read-only memory. As the name suggests, the contents of this memory may not be altered by the user, nor is it dependent upon power being supplied to retain its contents. It has, however, random access of memory locations.
Shared facility	A series of semi-autonomous workstations sharing some common facilities, normally a large disk store and/or printing capabilities.
Shared logic	A configuration where all the workstations in a system are connected to a minicomputer that provides the processing power, storage and printing facilities.
Silicon chip	A number of wafers of silicon fused together, each containing hundreds or thousands of electronic circuits.
Simulate	To represent the functioning of one system by another, *e.g.*, to represent one computer by another, to represent a physical system by the execution of a computer program.
Software	The collection of programs, *i.e.*, sets of instructions, associated with a computer.
Stand alone	A single independent workstation.
Store	See Memory.
Terminal	A device designed to send data to or receive data from a computer. Normally a typewriter-type keyboard and VDU.
Time sharing	A computing technique in which numerous terminal devices can utilise a central computer concurrently for input, processing and output functions.
Visual display unit (VDU)	Contains a screen similar to a television, which displays text or data as it is entered on the keyboard or recalled from store.

We are grateful to the SRC and AERE, Harwell, for the award of a Studentship to T.B.G.

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Received *June 27th*, 1980
Accepted *October 31st*, 1980

Analyst, March, 1981, Vol. 106, pp. 283–287

Computer-linked Atomic-absorption Spectroscopy: a Streamlined Approach to Environmental Analysis

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An analytical system using two single-beam atomic-absorption spectrophotometers linked to a microcomputer has been developed to process efficiently large numbers of plant and soil samples for various metal analyses. The rationale underlying the development of the system and details of the interfacing and data handling techniques are given.

Keywords: *Computer-linked atomic-absorption spectroscopy*; *environmental analysis*; *plant and soil analysis*; *metal determination*

At present, particularly within the field of environmental monitoring of potentially toxic metals, atomic-absorption spectroscopy is widely applied, largely owing to its flexibility, ease of operation, reliability and relative cheapness.[1] Modern instruments are characterised by their high degree of automation and simplicity in terms of calibration and data output. However, even with these considerable advantages, the determination of some metals, frequently at low levels for routine flame analysis, in large numbers of samples, can be extremely time consuming.

In this laboratory, a 6-year research programme has been initiated to evaulate the effectiveness of different soil covering techniques in reducing trace metal uptake into soft fruit and vegetables from underlying metal-contaminated soil.[2] Over this period and as a monitoring exercise, large numbers of samples will be generated and single-season workloads allow for 30 000 individual metal analyses alone. Considered in the context of a biological exercise, where numerous other plant and soil parameters require examination, it will be appreciated that the analytical system utilised must be extremely efficient and relatively operator independent during bulk sample processing.

Dual-channel atomic-absorption spectrophotometers are available which, under certain circumstances, effectively double the efficiency of traditional single-element systems. An alternative system is described here that utilises two individual instruments, both of which transmit concentration data to a microcomputer for data storage and processing.

Experimental and Results

The analytical system comprises two Pye Unicam SP9 single-beam atomic-absorption spectrophotometers. Each is served by its own SP4-01 automatic sample changer (capable of supplying 100 samples in a single run and of limitless capacity if continuously provided with sample racks) and its own SP9 computer. Both instruments have a four-lamp turret accessory and one instrument is fitted with a device to give alpha-numeric printout.

Both SP9s are linked, by means of teletype cables, to a single input port on an ITT 20/20 (48KB) microcomputer which is served by a VDU and Centronics 779 printer in addition to a disk operating system consisting of two disk drive units. The instrument - microcomputer interface is made by means of a communications interface board utilising an RS232 standard.

In order to permit the use of a BASIC language program, as opposed to one operating in machine code, to classify and store incoming data from the two atomic-absorption spectrophotometers, the software of the SP9 computers (ADP1s) was modified. The two ADP1s are linked by a two-part cable. One lead goes from an output port of the "master" ADP1 to an input port of the "slave" ADP1; this is the master's busy line and the slave's status line. An identical line goes from slave to master.

During operation, if the master wishes to transmit a line of data to the teletype output, it sets its busy line high and checks its status line. This has to be set low before the master may proceed and ensures that the slave is not busy. Once the master has transmitted and set its busy line low, following the same logic the slave may proceed. The result is that the machines carry out each determination and transmit data in a sequential fashion. To avoid

the event of both ADP1s wishing to set their busy lines simultaneously, the slave, if it finds the master busy, will lower its own busy line for approximately 0.2 s to allow the master to gain control.

Fig. 1 illustrates the relationship between the two ADP1s and the procedures they must follow in order to transmit a line of data.

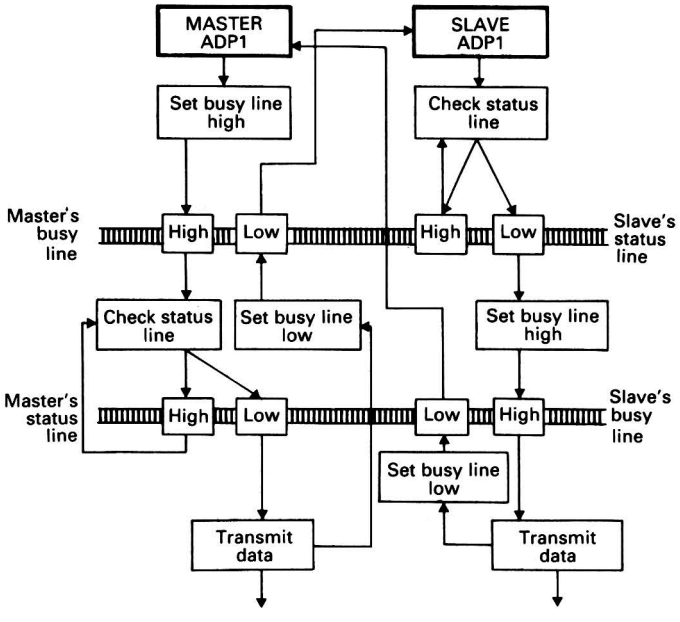

Fig. 1. Flow chart showing procedures followed by master and slave ADP1s before data transmission to the microcomputer can occur.

An additional modification to the software of both the master and slave has the result that any line of data arriving at the microcomputer from the master has the sample number prefixed by the digit 1 and similarly from the slave by a 2. It is immediately obvious, therefore, on inspection of the data displayed on the VDU, which data refer to which instrument and hence to a particular element. As lead and cadmium, copper and zinc and nickel and chromium are treated as pairs, with the first always carried out by the master, no confusion over the determination in hand ever arises.

The normal functions carried out by the SP9 computer, for example, in terms of automatic calibration, curve correction, presentation of data and its control over the operation of accessories such as the automatic sample changer and printer, are unchanged.

During normal operation of the system for the determination of two elements in, for example, lettuce leaf digests, the following procedures are followed:

(*a*) Each instrument is adjusted manually to carry out the determination of one of the pairs of elements. These operations include the selection and alignment of the appropriate hollow-cathode lamp, selection of wavelength, lamp current, band pass, burner height and fuel rates. The SP9 computer is programmed to accommodate the analysis that is to follow. The values of various parameters are keyed in: integration time, number and concentration of standards and initial sample number.

(*b*) Blank, standard and sample solutions are loaded on to each autosampler according to a specific format. With plant digests, a modification of the wet-oxidation method described by Williams[3] is used and the resulting 25 ml of digestate is shared between the two autosamplers, which use 12.8-ml Pyrex tubes.

(*c*) The two atomic-absorption spectrophotometers are set for automatic calibration and continuous operation.

(*d*) The ITT 20/20 microcomputer is switched on and programmed to run in floating point BASIC language. A simple, two-step, immediate command sets the baud rate of the microcomputer to 110 (as opposed to 300), thus making compatible the outputting (atomic-absorption spectrophotometers) and receiving (ITT) devices.

(*e*) A data loading program is selected from disc and run. The program initially asks for the number of data lines that are expected to arrive. Once this information has been typed in, the two atomic-absorption spectrophotometers may be set to run. This stage marks the end of the manual handling of the system. From this point the system operates entirely independently of laboratory personnel.

(*f*) As each line of data is transmitted to the microcomputer, it is stored within the monitor as a string variable of the form A$ (I). On completion of the batch analysis, routines within the data loading program submit the stored data to a floppy disk under a specific file name. This file forms the basis of all subsequent data analysis.

(*g*) At this stage, the various instrumental parameters appropriate to the next element pair may be manually selected. The data loading program is re-run to store information under a new file name and the procedures outlined above are simply repeated until the concentration of all elements of interest has been determined for the particular batch of samples.

Once stored on disk, manipulation of sample concentration data can be carried out using various computer routines. In the present case, an initial visual inspection of the file is made, using the hard-copy printout, to check for curvature, calibration and the presence of spurious sample readouts. Using a second calculator program, all readouts pertaining to sample concentrations only are assigned to a new string array from which, using conventional commands for the conversion of strings to numerical values, concentration data can be corrected for blank values, dilution and sample dry mass parameters. A formating section presents individual sample concentrations ($\mu g\ g^{-1}$ dry mass) in two columns relating to the element pair represented. A third and final program is used to collect all concentration data from each element pair file, first to store a comprehensive record of concentration data in numerical form (for statistical work) and finally to format the results as a complete table of coded samples together with the concentration of each element determined. Tables I–III give examples of the type of readout that can be obtained, if required, at each stage of the proceedings.

Conclusion

The system described has a number of important advantages:

(*a*) Efficiency of operation for the detection of, routinely, six elements for large numbers (typically of the order of 100–150 per batch) of soil extracts and plant digests.

(*b*) Flexibility in pairing of elements to be analysed and in the numbers of pairs (and, if required, single-element operation) that may be included. An important consideration is that analyses are often carried out at low concentrations (typically in the range for lead and cadmium of 1.00–0.05, nickel and chromium < 0.50 and copper and zinc $< 2.00\ \mu g\ ml^{-1}$), and as the operating conditions of each instrument are set independently to optimise sensitivity and reduce noise for the determination in hand, no compromise in these conditions is required as may occur with dual-channel systems.

(*c*) Real savings in analytical and data handling time. Under ideal conditions a typical batch of 100 samples can be analysed for six elements in approximately 2.5 h. This estimate is based on a 10-min interval for the manual setting of instrument parameters together with a 40-min autocalibration and analytical cycle using integration times of 10 s. An additional 0.5 h may be required for loading samples on to racks for each autosampler. On a purely manual basis, it is estimated that with a 5-min period for parameter settings, 5-min calibration time, 5-s handling and 10-s integration time per sample, the processing and analysis time would be 3.5 h. An additional 1 h would be required for loading the resulting 600 data points into a microprocessor for data processing.

TABLE I

FORMAT OF INITIAL DATA FILE FOR CU (SAMPLE NO. PRECEDED BY 1) AND ZN (SAMPLE NO. PRECEDED BY 2) DETERMINATIONS IN LETTUCE LEAF DIGESTS DURING AUTOMATIC PROCESSING

Key to right identifies solution type. AAS denotes instruments are in flame absorption rather than flame emission mode.

Sample No.	Sample code	Instrument mode	Element concentration/ $\mu g\ ml^{-1}$	Key
1997	BL	AAS	0.0000	blank value
2997	BL	AAS	0.0000	
1998	S1	AAS	0.084	standard 1 absorbance
2998	S1	AAS	0.049	
1998	CON	AAS	1.00	standard 1 concentration ($\mu g\ ml^{-1}$)
2998	CON	AAS	2.00	
1999	S2	AAS	0.164	standard 2 absorbance
2999	S2	AAS	0.121	
1999	CON	AAS	2.00	standard 2 concentration ($\mu g\ ml^{-1}$)
2999	CON	AAS	5.00	
1000	CON	AAS	0.01	"real" sample concentrations ($\mu g\ ml^{-1}$)
2000	CON	AAS	0.02	
1001	CON	AAS	0.62	
2001	CON	AAS	3.26	
1002	CON	AAS	0.52	
2002	CON	AAS	2.65	
1003	CON	AAS	0.63	
2003	CON	AAS	2.79	
.	.	.	.	
.	.	.	.	
.	.	.	.	
1100	CON	AAS	0.74	
2100	CON	AAS	3.59	

TABLE II

FORMAT OF SECOND DATA FILE SHOWING CORRECTED CONCENTRATIONS OF CU AND ZN IN SAMPLES

Sample No.	Cu concentration/ $\mu g\ g^{-1}$ dry mass	Sample No.	Zn concentration/ $\mu g\ g^{-1}$ dry mass
1001	15.25	2001	81.00
1002	12.75	2002	65.25
1003	15.50	2003	69.25
1004	15.75	2004	69.00
1005	15.25	2005	65.25
1006	14.25	2006	77.75
1007	15.75	2007	69.50
.	.	.	.
.	.	.	.
.	.	.	.
1100	18.25	2100	89.25

Two of the most significant advantages, however, are the release of often highly skilled personnel from repetitive tasks and the avoidance of transcript errors during data handling.

(*d*) General applicability to computer systems that possess an RS232 standard interface and to personnel without machine language expertise.

(*e*) Cost: although the system described above (two atomic-absorption spectrophotometers, two sample changers, 48KB computer, VDU, dual disk drives and printer) cost 12% more (1979 basis) than a comparable dual-channel atomic-absorption spectrophotometer with sample changer alone, the inherent flexibility of this system justifies

TABLE III

FINAL RESULTS FOR CODED SAMPLES

Plot	Element concentration/μg g^{-1} dry mass					
	Pb	Cd	Cu	Zn	Ni	Cr
A01–1	3.75	2.63	15.25	81.00	1.13	1.75
A01–2	4.75	1.88	12.75	65.25	0.63	1.50
A09–1	3.50	1.62	15.50	69.25	1.62	1.50
A09–2	2.25	1.63	15.75	69.00	1.63	1.50
A23–1	2.25	0.88	15.25	65.25	1.38	1.00
A23–2	2.00	2.38	14.25	77.75	0.88	2.00
A32–1	2.50	2.13	15.75	69.50	1.63	1.25
A32–2	1.25	1.12	15.50	68.00	0.87	1.25
.	.	.	.	.	.	.
.	.	.	.	.	.	.
.	.	.	.	.	.	.
P31–1	26.53	26.69	17.00	87.00	7.56	5.86
P31–2	22.98	24.10	18.25	89.25	7.62	5.99

its adoption. In addition, in order to obtain the reductions in data processing time a microcomputer would also be required for a dual-channel instrument, which would produce a reversal in the cost estimates.

In conclusion, experience gained with this system to date establishes the concept of computer-linked atomic-absorption spectroscopy as a major advance in automating many of the time-consuming aspects of analytical procedures, with concurrent savings in time and cost.

This work was carried out on behalf of, and funded by, the Department of the Environment. The authors thank the staff of Pye Unicam (UK), particularly those in the Development and Technical Departments.

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Received *August 8th*, 1980
Accepted *October 13th*, 1980

A Novel Method of Wavelength Modulation for Atomic Spectrometry—Some Preliminary Experiments

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The method of wavelength modulation most commonly used in atomic spectrometry employs an oscillating refractor plate driven by a scanning motor. A new mechanical arrangement is described, which involves a rotating quartz mechanical chopper. This quartz chopper is composed of four quadrants of different thickness, which refract radiation by different degrees. The rotating quartz chopper is placed inside the monochromator in a position similar to that used for oscillating refractor plates. Efficient wavelength modulation is achieved. However, the rotating quartz chopper has the advantage that a good square-wave modulation function is obtained.

Keywords: *Square-wave wavelength modulation*; *atomic spectrometry*

Wavelength modulation is now well established as a means of background correction in atomic spectrometry. It has been applied particularly successfully to atomic emission (AE) in electrothermal atomisers,[1,2] flames[3–6] and plasmas,[7,8] to continuum source excited atomic absorption (AAC)[9,10] and, to a lesser extent, to continuum source excited atomic fluorescence (AFC).[11,12] The primary advantage of wavelength modulation lies in its ability to discriminate against a continuum whilst measuring atomic line spectra. This leads to improvements in accuracy of atomic spectrometric measurements. The continuum may be atom cell background emission (AE, AAC, AFC), scatter of excitation source radiation (AFC, AAC), matrix emission (AE, AAC, AFC), excitation source continuum viewed directly (AAC) and non-analyte, broad-band, absorption (AAC) or fluorescence (AFC). The discrimination against continuum background can lead to improvements in signal to noise ratio owing to concomitant discrimination against low-frequency fluctuation noise.[13] Wavelength modulation using the zero-crossing technique[5] can also assist in correction for spectral interferences.

The method of wavelength modulation most commonly used in atomic spectrometry employs an oscillating refractor plate. The principle of this was probably first described by McWilliam.[14] It is current practice to cause the refractor plate to oscillate about the vertical axis using commercially available scanning motors. Most researchers have used a sinusoidal waveform to drive the scanning motor because this is the most straightforward to set up experimentally. It results in the wavelength being scanned sinusoidally. In 1977 Koirtyohann *et al.*[15] and O'Haver *et al.*[13] presented experimental and theoretical results, respectively, that demonstrate that square-wave oscillation of the refractor plate improves the ultimate signal to noise ratio of the measurement by a factor of about 1.8. With scanning motors, square-wave oscillation is more difficult to achieve than sine-wave oscillation at frequencies acceptable for atomic spectrometry. However, commercial equipment is available[15] that incorporates sufficient control over the position of the plate to achieve good square-wave modulation at about 17 Hz.

In this paper we describe a mechanical arrangement that achieves wavelength modulation and that results in the wavelength being scanned with a square waveform at about 20 Hz, with potential for higher frequencies. The arrangement involves a rotating quartz mechanical chopper composed of four quadrants of different thickness, which refract radiation by different degrees. The rotating quartz chopper is placed inside the monochromator in a position similar to that used for an oscillating refractor plate and is a more straightforward and cheaper means

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of wavelength modulation than an oscillating refractor plate. This is a result of the mechanical arrangement being no more complex than traditional rotating glass choppers used for intensity modulation in spectrometric instruments. Moreover, no complex electronic control is required to drive the chopper. The experiments described here were involved with flame atomic-fluorescence spectrometry. However, there is no reason why the system should not be equally successfully applied to atomic-emission and atomic-absorption measurements.

Experimental

Instrumentation

The rotating quartz mechanical chopper was incorporated into a flame atomic-fluorescence instrument built at the University of Strathclyde and which has been described elsewhere.[16,17] The line sources discussed in references 16 and 17 were not used. The 300-W xenon arc, normally used at low power for background correction, was operated here at almost maximum power to excite atomic-fluorescence signals in the flame. A schematic diagram of the relevant parts of the instrument is shown in Fig. 1.

Fig. 2 shows the quartz mechanical chopper. The circular quartz chopper blade was constructed of four quadrants of optically polished quartz (Vitreosil, supplied by Jencons Scientific Ltd., Hemel Hempstead, Hertfordshire, UK). Each quadrant was cut from one of three circular discs 50 mm in diameter and 1, 2.5 or 4 mm thick. Two quadrants of the resulting chopper were 2.5 mm thick. The third and fourth quadrants were 1 and 4 mm thick and they were arranged as indicated in Fig. 2. This configuration allows primarily for wavelength modulation in the "2F" mode, although a "1F" mode is possible. The 2F mode allows for background measurement/correction on both sides of the atomic line and is achieved by passing the atomic line wavelength through the exit slit when the 2.5-mm quadrants are in the optical beam. The 1F mode allows for background measurement/correction on one side of the atomic line only and is obtained with the above system by using either the 1- or 4-mm quadrants for the atomic line. In each instance the three other quadrants will correspond to wavelengths on the same side of the atomic line, at either higher or lower wavelengths. All of the possible forms of modulation are discussed later.

Each of the four quadrants was drilled in the position indicated in Fig. 2 and bolted and cemented to the hub of a 33 mm diameter gear wheel (hub diameter 20 mm). The bolts served primarily to locate the quartz accurately during assembly.

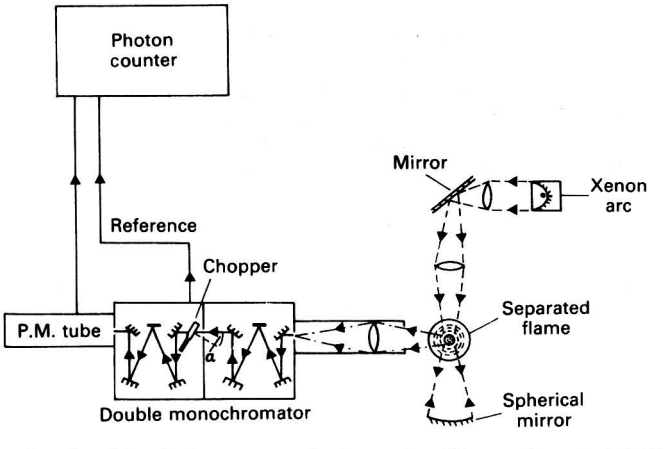


Fig. 1. Atomic-fluorescence instrument with continuum source excitation and wavelength modulation. For details of instrumentation not described in the text, see reference 16.

A second identical gear wheel, driven by a synchronous motor, was meshed with the chopper assembly. This arrangement rotated the chopper at 10 rev s^{-1} to give a modulation frequency of 20 Hz in the 2F mode. Direct drive by the motor was not possible because the bulk of the

motor would have prevented passage of the light beam through the chopper blades. In the double monochromator used here the image of the middle slit was passed through the top half of the chopper blade and aligned along the vertical diameter.

The second gear wheel had slots cut in its body as indicated in Fig. 2. A miniature infrared light-emitting diode (LED, 306–077, R.S. Components Ltd., London) was mounted in the main support bracket at the back of the gear and slots. The infrared radiation was detected by a phototransistor (306–083, R.S. Components Ltd.), which was placed on a post in front of the slots. This arrangement served to provide a reference signal for the synchronous detection system of the photon counter (Fig. 1) that was used to process the signals from the photomultiplier tube. An infrared reference system was preferable to a conventional tungsten bulb system in order to exclude stray light from the monochromator. The photomultiplier tube (9789QB, EMI, Hayes, Middlesex) had a response that fell rapidly at wavelengths longer than 650 nm.

I rovision was necessary to allow variation of the angle of incidence of the light beam at the chopper. This was achieved by pivoting the assembly about the vertical diameter of the chopper blade. After the angle of incidence had been set, the assembly was tightened down to the base of the monochromator with a screw running in a radial slot located as indicated in Fig. 2. Fig. 1 shows the location of the chopper assembly inside the double monochromator of the instrument. It is placed just after the middle slit, which is the entrance slit of the second monochromator in the double monochromator.

Reagents

All reagents were of the highest purity available. High-purity de-ionised water was used for the preparation of all solutions. Metal stock solutions were prepared by dissolving a known amount of spectrographically pure metal usually in 10 ml of 11 M hydrochloric acid. All solutions for analysis were adjusted to be 0.04 M in hydrochloric acid for maximum stability of low concentrations of metal in solution.

The preparation of blood samples for copper determination was carried out as described by Peaston[18] for both atomic-fluorescence and atomic-absorption measurements.

Instrumental Conditions

All instrument settings and operating conditions were the same as those reported in previous publications,[16,17] unless stated otherwise.

Design Principles of the Rotating Quartz Chopper

Elsewhere,[3,19] the basic principles of the use of a refractor plate to obtain a rapid scan over a small wavelength interval have been described. Until the present, a wavelength scan has normally been achieved by placing an oscillating refractor plate in the light path, inside the monochromator, either just after the entrance slit or just before the exit slit. The light beam, in passing through the plate, is displaced laterally by an amount, d, given by the relationship[3]

$$d \approx t\alpha\left(\frac{n-1}{n}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

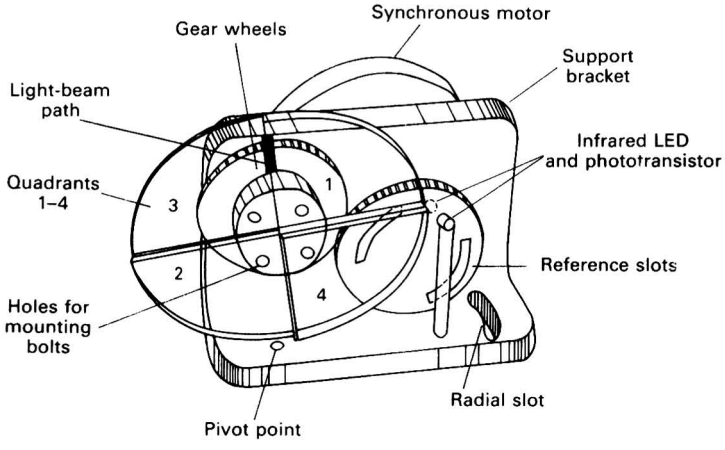
where t mm = thickness of the plate, α rad = angle of incidence of the light beam at the refractor plate (see Fig. 1) and n = refractive index of the quartz plate.

The rotating quartz chopper operates on the same principle as the oscillating refractor plate except that, in order to vary d and hence the wavelength, the thickness, t, is varied rather than the angle of incidence, α.

Square wave

As the quartz chopper rotates, each plate in turn refracts the light beam to an extent depending on the thickness of each plate. The boundary between each plate is sharply defined and provides the basis for the resulting square-wave variation of wavelength with respect to time. However, the change in direction of the refracted beam, which begins to take place as the beam begins to irradiate the next plate in the sequence, occurs gradually in a time related to the width of the light beam and the rotational velocity of the chopper blade. At a constant

(*a*)



(*b*)

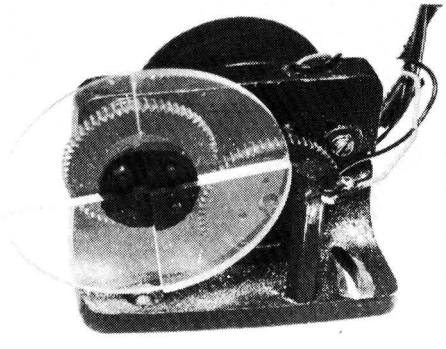
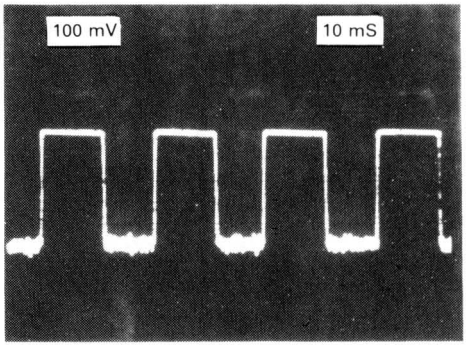
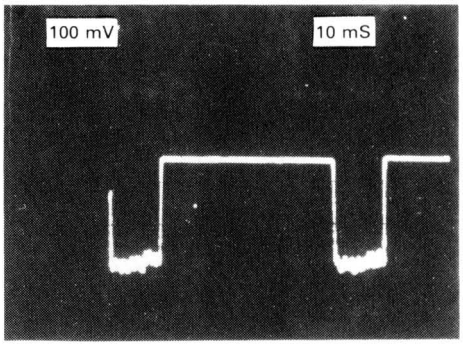


Fig. 2. Quartz mechanical chopper assembly. Quadrant thicknesses were as follows: 1 and 2, 2.5 mm [t_1 and t_2 in equation (2)]; 3, 1 mm [t_3 in equation (2)]; and 4, 4 mm [t_4 in equation (2)].

[*to face page 290*



(*a*) 2F mode



(*b*) 1F mode (case 2, see text)

Fig. 3. Photographs of square wave using rotating quartz chopper. The light source was a lead hollow-cathode lamp observed directly. The monochromator was an échelle (Spectrametrics Inc., Andover, Mass., USA). The signals were taken directly from the photomultiplier tube and displayed on an oscilloscope.[20] The oscilloscope axes are indicated at 100 mV cm^{-1} vertical and 10 ms cm^{-1} horizontal.

rotational velocity the ratio of one quarter of the circumference of the chopper to the width of the light beam determines the proportion of the square wave taken up by the rise and fall times, *i.e.*, for a chopper of fixed size the width of the light beam determines the rise and fall times.

The width of the light beam is initially the width of the entrance slit of the monochromator. By the time it reaches the chopper it has diverged by about 2–3 mm, depending on the distance between the entrance slit and the chopper. It is therefore desirable to place the chopper as close as possible to the entrance slit in order to obtain a well defined square wave. The size of the chopper assembly must be minimised to facilitate a close approach to the entrance slit.

Some spurious reflections of light at the junctions between plates were noticeable when observing the square wave on an oscilloscope. These were rejected by reducing the duty cycle of the detection system by about 20%. This facility was available on the synchronous detector of the photon counter employed and it allows the observation of the first and last 10% of each chopper quadrant to be rejected. Recent trials[20] have demonstrated that most of the spurious reflections can be reduced by applying matt black paint to the edges of each plate at each junction. Fig. 3 shows photographs of the square-wave modulation in the 2F mode (*a*) and in a pseudo-1F mode (*b*) (see case 2 below). These photographs were obtained from a more recent version of the rotating optical chopper, which was not used for the remaining results in this paper but which will be described at a later date.[20] Use of a hollow-cathode lamp indicates clearly the observation of the atomic line (noisy portions) and background on either side of the line (lower noise). The modulation frequency in Fig. 3(*a*) was 40 Hz. The photographs in Fig. 3 demonstrate the excellent square wave obtainable after reduction of spurious reflections by application of optically black paint to plate edges.

Modulation interval

The arrangement of thicknesses of the four plates shown in Fig. 2 was designed to give wavelength modulation in the 2F mode. The two 2.5-mm plates allow the atomic line wavelength, $\bar{\lambda}$, determined by the grating position, to be directed at the exit slit of the monochromator. The 1.0-mm plate refracts light less than the 2.5-mm plate and therefore irradiates the exit slit with radiation of wavelengths displaced to one side of the atomic line. The 4-mm plate refracts light more than the 2.5-mm plates and light from the opposite side of the atomic line is detected. The magnitude of the displacements is determined by the difference in thickness between the 1.0 and the 4.0-mm plates and by the angle of incidence of radiation falling on the plates. This means that for plates of fixed thickness the total displacement (modulation interval, $\Delta\lambda$), can be varied by varying the angle of incidence. The displacement of the light beam can be determined from equation (1), *i.e.*, for the thickest plate (t_4 mm) and the thinnest plate (t_3 mm) the displacement, d, is given by

$$\Delta\lambda = d = (t_4 - t_3)\,\alpha\left(\frac{n-1}{n}\right) \quad .. \quad .. \quad .. \quad .. \quad (2)$$

For the rotating quartz chopper described in Fig. 2, t_3 was 1.0 mm. This was the minimum thickness possible that had acceptable mechanical strength. The plate with $t_4 = 4$ mm was chosen on the basis of the displacement required for the monochromator[17] on our instrument. This was a 220-mm focal length, *f*/4, double monochromator, which, for atomic-fluorescence measurements, was used with a spectral band pass, $\delta\lambda$, of 0.5 nm at slit widths of 0.25 mm (the entrance and exit slits were 0.25 mm, the middle slit was 1.25 mm wide). This corresponds to a minimum total displacement of 0.5 mm for a peak-to-peak modulation interval,[13] $\Delta\lambda = 2\delta\lambda$, of 1 nm for measurements in the 2F mode.

From equation (2), $t_4 - t_3$ is 2.86 mm at an angle of incidence, α, of 30°, displacement, d, of 0.5 mm and refractive index, n, of 1.5. A convenient plate thickness, t_3, of 4 mm was chosen for a $t_4 - t_3$ of 3 mm. At $\alpha = 30°$ this gives a slightly greater modulation interval than $2\delta\lambda$ and larger values of $\Delta\lambda$ are possible by increasing the angle of incidence. This was considered necessary because reports[13] have indicated that $\Delta\lambda$ may need to be up to $2.5\delta\lambda$ for maximum signal to noise ratio.

2F mode

The square modulation waveform in the 2F mode using the rotating quartz chopper can be

defined in terms similar to the three-step waveform described by O'Haver *et al.*[13] for the oscillating refractor plate. When the chopper rotates through one complete revolution from 0 to 2π radians the wavelength changes are shown in Fig. 4, A, and are as follows: the atomic line at $\bar{\lambda}$ is observed through the two 2.5-mm plates ($\lambda = \bar{\lambda}$ at 0 to $\pi/2$ and π to $3\pi/2$) and background measurements are made using the 1-mm plate ($\lambda = \bar{\lambda} - \Delta\lambda/2$ at $\pi/2$ to π) and the 4-mm plate ($\lambda = \bar{\lambda} + \Delta\lambda/2$ at $3\pi/2$ to 2π). This is the normal form of modulation for which the system described was designed and allows correction at each side of the atomic line wavelength and maximum signal to noise ratio.

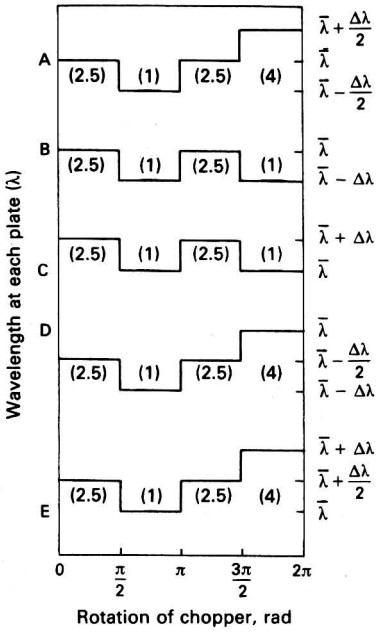


Fig. 4. Change in wavelength with plate thickness. Chopper rotating through 2π rad. For further explanation, see text. Numbers in parentheses refer to thickness of plate (mm) during the relevant part of the modulation cycle. A, 3-step, 2F modulation; B and C, 2-step, 1F modulation; and D and E, 3-step, 1F modulation.

1F mode—case 1

The 1F mode can be obtained by constructing a new chopper blade with only two plate thicknesses. Fig. 4, B, illustrates this for such a two-step chopper constructed of four quadrants alternating between 1- and 2.5-mm thickness and the grating tuned so that $\bar{\lambda}$ is observed through the 2.5-mm plates ($\lambda = \bar{\lambda}$ at 0 to $\pi/2$ and π to $3\pi/2$) and background measurements are made using the 1-mm plates ($\lambda = \bar{\lambda} - \Delta\lambda$ at $\pi/2$ to π and $3\pi/2$ to 2π). Note that $\Delta\lambda$ in Fig. 4, B and C, is only half the modulation interval seen in Fig. 4, A. Fig. 4, C, shows the same two-step chopper with the grating tuned so that $\bar{\lambda}$ is detected through the 1-mm plates ($\lambda = \bar{\lambda}$, at $\pi/2$ to π and $3\pi/2$ to 2π) and background measurements are made using the 2.5-mm plates ($\lambda = \bar{\lambda} + \Delta\lambda$ at 0 to $\pi/2$ and π to $3\pi/2$).

1F mode—case 2

It is inconvenient to change chopper blades in order to change from the 2F to the true 1F mode. However, it is possible to make pseudo-1F measurements using the 2F (three-step) chopper. This is illustrated in Fig. 4, D, where the atomic line is observed through the 4-mm plate ($\lambda = \bar{\lambda}$ at $3\pi/2$ to 2π) and background measurements made using the 1-mm plate ($\lambda = \bar{\lambda} - \Delta\lambda$ at $\pi/2$ to π) and the 2.5-mm plates ($\lambda = \bar{\lambda} - \Delta\lambda/2$ at 0 to $\pi/2$ and π to $3\pi/2$).

In Fig. 4, E, the atomic line is observed through the 1-mm plate ($\lambda = \bar{\lambda}$ at $\pi/2$ to π) and background measurements are made at the opposite side of the line to 4, D, using the 2.5-mm plates ($\lambda = \bar{\lambda} + \Delta\lambda/2$ at 0 to $\pi/2$ and π to $3\pi/2$) and the 4-mm plate ($\lambda = \bar{\lambda} + \Delta\lambda$ at $3\pi/2$ to 2π).

It is clear, from Fig. 4, D and E, and Fig. 3(*b*), that the atomic line is only observed for half of the time that it is observed in the 2F mode of Fig. 4, A. This implies a decrease in signal to noise ratio, at the detection limit, of a factor of 2 compared with the 2F mode. This would not occur if a true two-step chopper were to be used as indicated in Fig. 4, B and C. However, in most circumstances the pseudo-1F mode may be satisfactory relative to the inconvenience of changing chopper blades. The synchronous detector must be sensitive to a signal at half the chopping frequency, or the reference signal could be electronically halved in frequency in order to detect the pseudo-1F signal. The photon counter with synchronous detector used in our experiments registered half the count rate in the 1F mode compared with the 2F mode because the signal channel was not tuned or filtered as would be the case when using an analogue lock-in amplifier. The photon counter therefore presented no problems for detecting 1F signals by retuning the monochromator when using the 2F (three-step) chopper blade.

Angle of incidence

It is interesting that the angle of incidence of the light beam on the rotating quartz chopper is constant throughout the modulation cycle. This minimises changes in reflection losses during the modulation cycle and contrasts with an oscillating refractor plate where the reflection losses change because the angle of incidence is changing during the modulation cycle. However, this is not a real advantage for the rotating glass chopper because the change in reflection losses with angle of incidence is negligible[21] during the conventional maximum angle of oscillation of between 0 and $\pm 15°$.

Results and Discussion

The instrument used for these experiments was designed primarily for atomic-fluorescence measurements using line sources and background correction with a continuum source. The light sources were intensity modulated in the normal manner for a two-source background correction system.[16] Here the continuum source was used alone and at maximum power to excite atomic fluorescence. Ideally, double modulation (source intensity and wavelength modulation) is required to correct for both atomic emission and scatter of source radiation in addition to flame background.[11] If wavelength modulation is used without source intensity modulation then both atomic emission and atomic fluorescence of the analyte metal contribute to the total signal. A disadvantage of this approach is the likely incidence of spectral interferences from structure in the flame background and from structure in matrix thermal emission in the flame. However, prior to assembling an instrument incorporating double modulation, the rotating quartz chopper was used for combined atomic-fluorescence/atomic-emission measurements. This provided an initial evaluation of the utility of the quartz chopper for background correction.

Detection Limits

A comparison was made between the detection limits (1 s count time, signal to noise ratio = 2) obtained using source intensity modulation alone, with detection limits obtained using wavelength modulation alone. These results are shown in Table I. In column 2 under *Detection limit*, atomic emission from the analyte does not contribute to the total signal as the source was intensity modulated. Hence, the detection limits for those elements which are sensitively determined by atomic emission (resonance wavelengths longer than 300 nm) show an improvement in detection limit in going from source intensity modulation (column 2), to wavelength modulation (column 1). At wavelengths shorter than 300 nm, the detection limits are similar for both methods of modulation.

At wavelengths shorter than 250 nm the detection limits appear worse for wavelength modulation, probably because of a loss of ultraviolet output of the xenon arc owing to lamp ageing between the two sets of results, which were obtained about 1 year apart. There is probably also a slight contribution to the poorer detection limits for wavelength modulation due to transmission losses of around 10% at the refractor plates.

TABLE I

ATOMIC-FLUORESCENCE DETECTION LIMITS*

Excitation, 300-W continuum source; nitrogen-separated air - acetylene flame.

Element†	Wavelength/nm	Detection limit/μg l⁻¹	
		1‡	2‡
Zinc	213.9	195	51
Cadmium	228.8	44	19
Nickel	232.0	106	99
Cobalt	240.1	148	49
Iron	248.3	98	19
Gold	267.5	390	390
Manganese	279.5	7	4
Lead	283.3	360	350
Magnesium	285.2	1.3	0.7
Copper	324.7	7	20
Silver	328.1	3	4
Chromium	357.9	6	24
Strontium	460.7	5	7.6

* For definition see text—count time was 1 s.
† Aqueous solution 0.04 M in hydrochloric acid.
‡ 1, Wavelength modulation, rotating chopper; 2, source intensity modulation.

The detection limits were obtained using noise figures calculated by taking the square root of the total background measured in counts per second. This assumed that the background shot noise was dominant, which has been shown previously[22] to be true for source intensity modulated, continuum source excited flame atomic fluorescence. No attempt was made to determine whether there was a low frequency, $1/f$, noise component in the wavelength modulation situation described here. This is at present the subject of further measurements. O'Haver *et al.*[13] have presented a discussion of the predicted effects of wavelength modulation on signal to noise ratio in various atomic spectrometric situations.

Second Derivative

Wavelength Scan of Atomic Line

Wavelength scans of atomic lines with the rotating chopper in operation were essentially analogous to those obtained by Snelleman *et al.*[3] for the oscillating refractor plate. An example of the spectrum for the cadmium atomic fluorescence line at 221.8 nm is shown in Fig. 5. The apparent steps in the peaks are a result of the digital-to-analogue conversion at the output of the photon counter.

Inter-relationship Between Refractive Index, Angle of Incidence and Modulation Interval

The refractive index of the Vitreosil quartz used in the rotating quartz chopper varies with wavelength from 1.52 at 200 nm to 1.44 at 600 nm, and this affects the modulation interval in a manner dictated by equation (2). The modulation interval decreases with decreasing refractive index. From equation (2) it can be seen that it is possible to compensate for the change in modulation interval by varying the angle of incidence. This is equivalent to varying the amplitude of the oscillation when using an oscillating refractor plate[3] and both achieve the same purpose of controlling the modulation interval.

The variation of modulation interval with wavelength dictates that the angle of incidence be optimised for maximum signal whenever measurements are to be made at different wavelengths. This is illustrated in Table II, which lists the optimum angle of incidence that was found to be

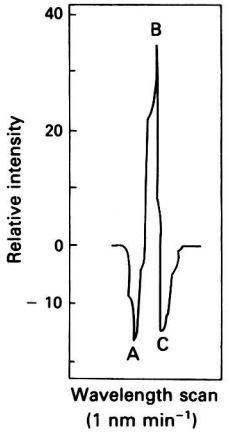


Fig. 5. Atomic-fluorescence spectrum of cadmium at 228.8 nm. Scan rate, 1 nm mm^{-1}; nitrogen-separated air - acetylene flame; solution concentration, 500 μg ml^{-1} in 0.04 M hydrochloric acid; and spectral band pass, nominally 0.5 nm. A, Cadmium atomic fluorescence observed through 4-mm plate; B, sum of cadmium atomic fluorescence observed through each 2.5-mm plate; and C, cadmium atomic fluorescence observed through 1-mm plate.

required to achieve the optimum detection limits for each metal listed in Table I. If the angle of incidence was not optimised at each wavelength but was kept constant at a value optimised in the ultraviolet range, say for zinc, then detection limits deteriorated by up to a factor of two or three for the remaining metals. The results in Table II were obtained by aspirating a solution of each metal of interest into the flame and then adjusting the angle of incidence to maximise the atomic fluorescence signal to (background)$^{\frac{1}{2}}$ ratio. The angle of incidence was then measured directly in the monochromator using a protractor (error about $\pm 1°$). The modulation intervals which resulted were calculated from the experimentally determined angles of incidence and the known refractive index at each wavelength. These results, shown in the last column of Table II, revealed that the modulation interval had remained essentially constant (average displacement of 0.685 mm) throughout the wavelength range studied (213–461 nm).

The 0.685-mm displacement corresponds to a modulation interval of 2.74 $\delta\lambda$ for the 0.25-mm slit width used (spectral band pass 0.5 nm, *i.e.*, $\Delta\lambda = 1.37$ nm). The expected modulation interval for maximum signal is 2.0 $\delta\lambda$ or greater.[13] For sinusoidal modulation the maximum signal to noise ratio is predicted to be at 2.5 $\delta\lambda$.[13] The figure of 2.74 $\delta\lambda$ found here was probably a result of two factors. Firstly, with the rotating refractor plates in position there will be some defocusing of the image of the exit slit.[3] This will tend to increase the spectral band pass, $\delta\lambda$, and therefore the required modulation interval. Secondly, there was some departure from the ideal square wave, which was a result of the finite width of the light beam incident on the

refractor plates. This implies that the modulation waveform had some sine wave character, which would tend to increase the required modulation interval for accurate discrimination between signal and background.

Discrimination Against Flame Background

A wavelength scan between 200 and 600 nm revealed that the wavelength modulation was effectively discriminating against flame background when using the rotating quartz chopper (Fig. 6). The scan was taken with the angle of incidence at the chopper set at 40°, which is optimum only for wavelengths below 230 nm (Table II).

TABLE II

ANGLE OF INCIDENCE REQUIRED TO ACHIEVE OPTIMUM DETECTION LIMITS LISTED IN TABLE I

Element	Wavelength/ nm	Angle of incidence, degrees	Modulation interval/ μm of displacement
Zinc	213.9	40	685
Cadmium	228.8	40	681
Nickel	232.0	40	674
Cobalt	240.1	41	686
Iron	248.3	41	684
Gold	267.5	41	676
Manganese	279.5	41	673
Lead	283.3	41	672
Magnesium	285.2	43	704
Copper	324.7	42	682
Silver	328.1	43	697
Strontium	460.7	44	703
			Average: 685

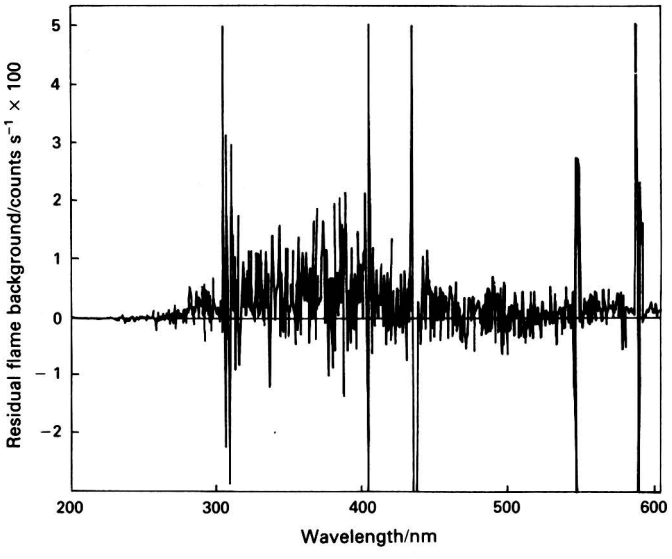


Fig. 6. Residual flame background after wavelength modulation. Spectrum obtained by scanning monochromator wavelength and aspirating water into a nitrogen-separated air - acetylene flame whilst the rotating quartz chopper was in operation. The angle of incidence was 40°.

The magnitude of the flame background, when measured using d.c. detection, varies between about 200 counts s^{-1} at 230 nm and about 10000 counts s^{-1} at around 350 nm.[17] The maximum offset in Fig. 6 is about 40 counts s^{-1} at 350 nm, *i.e.*, less than 0.5% of the total flame background and, therefore, will cause negligible inaccuracies in measurements with real samples. This small offset may have been due to disproportionate light losses in the 4-mm refractor plate relative to the 1-mm plate, leading to a slightly inaccurate average measurement of the flame background.

The small offset was reproducible and was not, therefore, due to low-frequency drift. The large noise peaks in the spectrum occur in regions of the flame background which contain line structure such as the OH bands at around 310 nm.

Determination of Copper in Blood Serum

Table III shows the results for the determination of copper in blood serum using both the wavelength-modulated atomic-fluorescence/atomic-emission instrument described here and a standard flame atomic-absorption instrument. The method used to prepare samples, standards, etc., was identical with that described by Peaston.[18] The blood samples were obtained from the Royal Infirmary Glasgow, and were from a non-normal sample of people (hospital patients).

The results of the analyses using both methods gave a correlation coefficient between the two sets of results that showed good agreement (Table III). Further comparisons with more diverse methods of copper determination are required in order to characterise fully the accuracy of the atomic-fluorescence/atomic-emission, wavelength-modulation method, relative to other techniques. However, these results do indicate that the wavelength-modulation technique described here is giving satisfactory corrections for flame background and scatter of excitation source radiation as otherwise a positive bias would have been obtained for the atomic-fluorescence/atomic emission method. Research is continuing to obtain further support for these results.

TABLE III

DETERMINATION OF COPPER IN BLOOD SERUM USING TWO TECHNIQUES

Flame atomic-fluorescence spectrometry (AFS) with wavelength modulation using a nitrogen-separated air - acetylene flame; flame atomic-absorption spectrometry (AAS) on a Perkin-Elmer 403 instrument with a standard air - acetylene flame. Methodology as in reference 18.

	Copper content/μg ml^{-1}			Copper content/μg ml^{-1}	
Sample No.	AAS	AFS	Sample No.	AAS	AFS
1	1.57	1.61	15	1.89	1.72
2	1.32	1.32	16	1.20	1.19
3	1.14	1.21	17	1.41	1.30
4	0.88	0.86	18	1.32	1.28
5	1.27	1.25	19	1.91	1.95
6	1.22	1.10	20	1.21	1.19
7	1.67	1.72	21	0.98	0.96
8	1.04	0.97	22	0.95	0.91
9	1.67	1.81	23	1.09	1.15
10	1.05	1.04	24	1.49	1.56
11	1.26	1.31	25	1.56	1.59
12	1.18	1.18	26	1.27	1.23
13	1.11	1.12	27	1.95	2.02
14	1.45	1.42	28	1.46	1.46

Mean copper concentration/μg ml^{-1}	= 1.340(AAS); 1.337(AFS).
Standard deviation/μg ml^{-1}	= 0.291(AAS); 0.310(AFS).
Range/μg ml^{-1}	= 0.88–2.02.
Correlation coefficient between the results of each technique	= 0.979, $P < 0.05$ at 95% confidence.
Regression equation	= $A = mF + C$ (A = AAS result; F = AFS result).
Gradient of regression equation	= 1.041.
Intercept of regression equation/μg ml^{-1}	= −0.058.

Conclusions

The rotating quartz chopper described here gives excellent square-wave modulation, and background correction with acceptable accuracy, both for the determination of a metal in a real sample (copper in blood serum) and for the discrimination against flame background. Further research is in progress to resolve the small inaccuracies (less than 0.5%) that still exist after background correction.

The potential of this rotating quartz chopper is in its possible development for higher frequencies of square-wave modulation. This is limited only by the maximum possible safe rotational speed of such a glass chopper and the physical size of the chopper blades. Larger blades will allow a better square wave to be obtained in a manner comparable to the use of conventional mechanical choppers. These considerations are relatively easy to resolve and it appears feasible that modulation frequencies of around 200 Hz could be obtained by careful design of the chopper and its physical location in the monochromator.

The mechanical arrangement is no more complex than traditional rotating glass choppers used for intensity modulation in spectrometric measurements and no electronic control is required. (An oscillating refractor plate does require electronic control of its scanning motor in order to achieve square-wave modulation.)

The rotating optical chopper is equally applicable to atomic emission, fluorescence and absorption and is, in this respect, comparable to an oscillating refractor plate.

The authors acknowledge the support of the Scottish Home and Health Department for the purchase of the major items of equipment used in this project and for the award of a Post-doctoral Fellowship (to support R.G.M.). They also thank the Eastern District, Glasgow Health Board, for a maintenance grant in support of J.S.

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Received *September 17th*, 1979
Accepted *November 13th*, 1980

Analyst, March, 1981, Vol. 106, pp. 299–304

Determination of Lead in Urine by Atomic-absorption Spectroscopy with Electrothermal Atomisation

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A method is described for the determination of lead in urine by atomic-absorption spectroscopy with electrothermal atomisation. The determination is rapid and minimum pre-treatment of the sample is required. Matrix interference is minimised by the addition of orthophosphoric acid and also by pre-coating the graphite tubes with molybdenum. Consideration is given to possible losses of organically bound lead during the drying and ashing cycle and steps to prevent these are incorporated in the procedure. The range of the method is 5–200 $\mu g\ l^{-1}$ of lead.

Keywords: Lead determination; urine; electrothermal atomic-absorption spectroscopy; matrix interference

A method for determining lead in urine was sought that was easy to operate, adaptable to large numbers of samples and of an accuracy adequate for use as a screening test for likely exposure.

The procedure used in these laboratories for many years was that described by Bambach and Burkey,[1] which was superseded by the semi-automatic method of Browett and Moss.[2] Both of these procedures included time- and labour-intensive wet- and dry-ashing stages. There was also the problem of the large sample volume (50–100 ml) required in these methods, which with "snap" samples precluded a second aliquot being taken if necessary owing to accidental loss or contamination of the original aliquot.

Procedures available for the determination of small concentrations of lead in urine include spectrophotometric, polarographic and flame atomic-absorption techniques. All methods for the determination of lead in urine by flame atomic-absorption spectroscopy are based on solvent extraction after complexing the lead with reagents containing the dithiocarbamate group.[3] However, incomplete extraction of endogenous lead has been observed[4] unless some method of pre-ashing the sample is undertaken. The analysis time required by these methods of treatment would not be acceptable for our purpose.

The use of carbon furnace atomic-absorption spectroscopy for the determination of lead in urine offered the possibility of a minimum of sample pre-treatment provided that the following difficulties could be overcome: interference from anions and cations, non-specific absorption and loss of lead by volatilisation either as halide or as organically combined lead. Other workers have had varying degrees of success in eliminating these problems when dealing with natural water samples. Alkaline earth metal interference has been minimised by the addition of ascorbic acid.[5] Matrix modification achieved by the addition of ammonium nitrate[6] has been used to remove the interference of high chloride concentrations. However, none of these methods was effective when applied to urine, which exhibits a combination of these interference effects.

A matrix modification technique involving the addition of orthophosphoric acid and pre-treatment of the carbon tube with ammonium molybdate has been found[7] to be effective in minimising the interferences encountered when analysing urine.

Experimental

Apparatus

A Varian 175B atomic-absorption spectrometer was used together with a CRA-90 atomiser fitted with an ASD-53 automatic injection system. The diluted samples were mixed using a Denley Spiramix 5. The warming plate was constructed from an aluminium block with 48 indents to hold the cups. Electric heating elements were placed in the base of the block and the temperature was controlled using a Pye Ether Mini. The block was maintained at a

surface temperature of 40 °C. Gilson adjustable-volume and Eppendorf fixed-volume disposable-tip pipettes were used for transfer of samples and reagents. Borosilicate glass cups fitted with polythene caps were used for sample preparation.

Reagents

All reagents were of at least AnalaR grade. The orthophosphoric acid was of Aristar grade (BDH Chemicals).

Ammonium molybdate solution, 5% m/V. Dissolve 5 g of ammonium molybdate in approximately 60 ml of de-ionised water, add 5 ml of orthophosphoric acid, mix, transfer into a 100-ml calibrated flask and make up to volume with de-ionised water.

Ammonium molybdate solution, 1% m/V. Dissolve 1 g of ammonium molybdate in about 60 ml of de-ionised water, add 1 ml of orthophosphoric acid, mix and make up to 100 ml in a calibrated flask.

Ascorbic acid solution, 25% m/V. Dissolve 6.25 g of ascorbic acid in de-ionised water and make up to 25 ml in a calibrated flask.

"Urine diluent." To approximately 60 ml of de-ionised water contained in a 100-ml calibrated flask add 5 ml of 1% *m/V* ammonium molybdate solution and 2 ml of orthophosphoric acid. Swirl to mix, then add 1 ml of ascorbic acid solution. Dilute to volume with de-ionised water and shake to mix. The solution will turn a royal blue colour on standing.

Iodine solution, 1.0 N. Dissolve 16.6 g of potassium iodide in approximately 80 ml of de-ionised water contained in a 100-ml calibrated flask; add 12.7 g of iodine and shake to dissolve. Make up to 100 ml with de-ionised water and shake well to mix.

Standard lead solution. Dissolve 0.1599 g of lead(II) nitrate in approximately 80 ml of de-ionised water; add 1 ml of distilled concentrated nitric acid. Transfer the solution quantitatively into a 100-ml calibrated flask, make up to volume with de-ionised water and shake to mix. This stock solution contains 1 mg ml^{-1} of lead.

Dilute standard lead solution. Transfer 125 µl of the stock solution into a 25-ml calibrated flask containing approximately 20 ml of de-ionised water. Make up to volume with de-ionised water and shake thoroughly to mix. This solution contains 5 µg ml^{-1} of lead.

Procedure

Tube conditioning

Fit a standard (pyrolytically coated) carbon tube to the CRA90 work head. Set up the instrument with the following parameters:

Varian 175B spectrometer—	
Wavelength	283.3 nm
Lead hollow-cathode lamp	5.0 mA
Hydrogen lamp	Intensity to balance
Expansion	Low 5
Mode	Peak concentration
CRA-90 atomiser—	
Dry	100 °C, 60 s
Ash	900 °C, 40 s
Atomiser	2100 °C, hold time 0.5 s, ramp rate 50 °C s^{-1}
ASD-53 auto sample dispenser—	
Cam	5 µl
No. of injections	5

Transfer into a clean glass cup approximately 1.5 ml of 5% *m/V* ammonium molybdate solution, place in position 24 of the carousel and initiate the programme start. Set the instrument to read background absorbance. In the course of replicate injections the background signal will stabilise at a value close to zero. Re-set the instrument to read background-corrected absorbance.

Calibration

Change the following instrument parameters:

CRA-90 atomiser—

Atomiser .. .. 2100 °C, hold time 2.0 s, ramp rate 300 °C s^{-1}

ASD-53 auto sample dispenser—

No. of injections .. .. .. 3 (see Note 1)

Into five clean glass cups pipette 0, 5, 10, 15 and 20 μl of the dilute standard lead solution, which represents additions of 0, 50, 100, 150 and 200 μg of lead per litre of urine, respectively. Into each cup pipette 20 μl of iodine solution and 500 μl of a normal low lead content urine sample. Place the cups in the indents on the warming block and allow to stand for 5–10 min. Remove the cups from the block and add 1000 μl of urine diluent to each cup. Stopper the cups with polythene overcaps and place on the Denley mixer for 10 min. Remove the caps, place sequentially in the carousel of the auto sample dispenser and initiate the programme start. Carry out reagent blanks by substituting de-ionised water for the urine.

Plot the readings obtained against micrograms of lead per litre added and extrapolate the line to obtain the lead content of the urine sample. Using this value, construct a calibration graph so that the line obtained passes through the origin.

Analysis of the sample

Into clean glass cups pipette 20 μl of iodine solution and 500 μl of each of the samples. Place the clearly marked cups in the indents on the warming block and allow to stand for 5–10 min. Remove the cups from the block and add 1000 μl of diluent to each cup. Stopper the cups with the polythene overcaps and place on the Denley mixer for 10 min. Remove the caps, place in the carousel of the auto sample dispenser and initiate the programme. Read off the lead content of the sample from the calibration graph.

Samples more than 24 h old and samples containing precipitate must be acidified by adding 10% *V*/*V* of distilled concentrated nitric acid. Transfer 500 μl of the acidified urine into a clean glass cup and add 65 μl of ammonia solution (sp. gr. 0.88) to neutralise the nitric acid, then add 20 μl of iodine and proceed as above.

This dilution must be taken into consideration when calculating the lead content by multiplying the number of micrograms of lead per litre (read off the calibration graph) by 1.16.

NOTES—

1. Better replication can be achieved by making a large number of injections (nine) of the urine blank prior to the remainder of the calibration.
2. When large numbers of samples are to be analysed the calibration should be repeated at frequent intervals; this is conveniently carried out after each 25 samples.
3. To obtain the full benefit of the orthophosphoric acid treatment it is essential that an ashing temperature of at least 800 °C is used.

Instrumentation

The earlier work[7] was carried out using a carbon tube furnace contained in a totally enclosed cell with silica windows, which, over a number of determinations, tended to fog owing to the volatile products formed during the various stages of the analysis. The effect of this was not only a gradual drift in the base reading (which could be compensated for to some extent) but more seriously large and unpredictable jumps in the base-line absorbance reading arising from the thermally unstable nature of the deposit. The presence of the deposit also reduced the effectiveness of the deuterium background correction. It was therefore necessary to clean the silica windows, which involved dismantling the cell after a comparatively small number of sample analyses.

As it was intended that the final instrumentation should incorporate an automatic injection system, the formation of the deposit on the windows could not be tolerated. Various methods of overcoming this problem were tried without success.

It was decided, therefore, to change the instrumentation to incorporate an open-type carbon furnace with a laminar-flow inert gas shield and an automatic sample injector. All of the present work was carried out using the Varian equipment.

However, later versions of both Instrumentation Laboratory and Perkin-Elmer carbon furnaces incorporate modifications to reduce fogging of the side windows so that the analytical scheme is not necessarily restricted to any particular instrument.

Calibration

Calibration graphs were constructed by adding lead nitrate to urine and to distilled water using the method given but without the addition of iodine or ascorbic acid. After subtracting the reagent blank and lead already present in the urine the calibration graphs shown in Fig. 1 were obtained. These show large differences in slope and demonstrate the necessity of using a calibration of standard additions to a urine sample.

In the earlier study[7] where lead calibration data were based on peak-area measurements, little difference in slope was observed. Because of its low surface tension the diluted urine spreads over a comparatively large area when applied to the graphite tube. Consequently, on atomisation the concentration of atoms within the light beam are more diffuse than when urine is omitted. Errors due to this effect are less when peak-area measurements are taken. With the instrument used in this work it was only practical to measure peak heights. However, other factors in design (as mentioned above under Instrumentation) were more important when considering the choice of instrument.

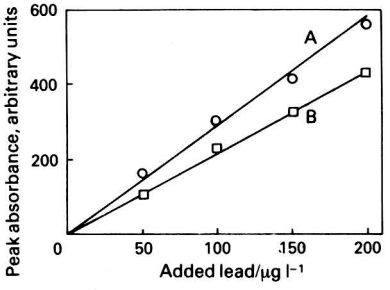

Fig. 1. Calibration graphs for lead in urine. A, Pb^{2+} added to distilled water; and B, Pb^{2+} added to low lead content urine sample.

Recovery of Organically Bound Lead

The form in which lead is excreted in urine has been studied by several workers[8,9] and it is likely that at least part of the lead is present as a complex with organic materials normally present as waste products. Where there has been exposure to alkyllead the lead may be excreted partly in an organic form.[10,11] Further, treatment of lead poisoning by chemotherapy invariably results in elevated levels of lead in urine and it is almost certain that in these instances the lead is excreted as a complex with the agent used.

As the proposed method includes digestion and finally ashing with orthophosphoric acid, any organically bound lead complex in the sample will be decomposed before atomisation. However, it is possible that some lead could be lost as a relatively volatile organic compound during the heating cycle before sufficient destruction of the complex has taken place.

Most organic complexes of lead can be decomposed by oxidation with potassium permanganate, nitric acid, bromine, iodine, etc. Of these, potassium permanganate reacts slowly and it was thought that it might have some lasting effect in the carbon tube furnace; nitric acid normally requires standing at room temperature for some time followed by evaporation to dryness; bromine is very effective, reacting quickly in the cold, but it requires strict safety measures in handling.

Woessner and Cholak[10] used iodine to decompose organically bound lead in their rapid screening method. Because of the detrimental effects of free halogen on the carbon tube furnace, it was considered necessary to reduce any excess of iodine before injection of the samples. The choice of reagents for achieving this was fairly wide and included sodium sulphite,[10] hydroxylammonium or hydrazinium chloride and ascorbic acid. Sodium sulphite was rejected because it would introduce increased salt levels into the solutions and the carbon

furnace. Hydroxylammonium and hydrazinium chlorides were slow in action and also could not be incorporated easily into the diluent. Ascorbic acid, on the other hand, effects a rapid reduction of excess of iodine and the reaction products present no problems.

Ascorbic acid can be incorporated in the diluent make-up. On standing, the diluent turns dark blue, presumably owing to the formation of a molybdenum oxide complex, but this does not interfere with its action.

In order to assess the effectiveness of the iodine treatment in preventing loss of volatile lead complexes, it was decided to determine the recovery of inorganic lead added to urine in the presence of EDTA and D-penicillamine, which are used in the chemotherapy of lead poisoning and are possible excretion products during this treatment. Also, the recovery of lead added to urine as trimethyllead chloride was determined.

In the presence of EDTA and D-penicillamine, lead nitrate was added to a low lead content urine to give lead concentrations in the range 50–200 $\mu g\ l^{-1}$ together with at least a 4-fold excess of the possible interferent. The recovery of trimethyllead was determined over the same range. The procedure was carried out first using the conditions given in the method and then omitting the iodine and ascorbic acid from the treatment.

The results are shown in Table I and the general conclusion is that oxidation with iodine is necessary in order to obtain complete recovery of organically bound lead.

TABLE I

RECOVERY OF ORGANICALLY BOUND LEAD

Conditions	Mean recovery over the range 50–200 $\mu g\ l^{-1}$ ± standard deviation, %		
	Pb^{2+} + EDTA	Pb^{2+} + D-penicillamine	Me_3PbCl
Iodine treatment at 40 °C	99 ± 1.6	102 ± 1.1	96 ± 4.7
No iodine at ambient temperature ..	96 ± 4.0	103 ± 2.4	15 ± 2.2

The recovery of trimethyllead was lowest at 200 $\mu g\ l^{-1}$ of lead (91%), and this was further examined by lengthening the heating period and increasing the temperature of the oxidation stage in steps to 30 min at 60 °C without improving the recovery at this level. Because these lead concentrations are higher than normally experienced the recovery was deemed to be adequate.

Results

The proposed method was compared with that currently in use,[2] which involves nitric acid digestion and furnace ashing of the sample followed by determination of the lead by dithizone automatic spectrophotometric procedure. The comparative study extended over a period of 3 months and involved 2559 samples taken in the course of routine medical examination of workers engaged in the manufacture of lead alkyls. The correlation coefficients and regression line equations are given in Table II.

TABLE II

STATISTICAL COMPARISON OF PROPOSED ATOMIC-ABSORPTION METHOD WITH CURRENT SEMI-AUTOMATIC DITHIZONE METHOD[2]

Month	No. of samples	Correlation coefficient	Regression*
1	759	0.9613	AAS = 0.948D + 1.873
2	830	0.9736	AAS = 0.971D + 1.274
3	970	0.9861	AAS = 0.972D + 0.405

* AAS = result by atomic-absorption method; D = result by dithizone method.

The repeatability of the method was tested by the analysis of six urine samples using ten separate aliquots of each. The range of lead content was chosen to cover the normal calibration levels. The results are given in Table III.

TABLE III

METHOD REPEATABILITY DATA

	Sample No.					
	1	2	3	4	5	6
Mean lead content/$\mu g\ l^{-1}$	108.5	61.3	168.1	79.9	19.2	118.8
Standard deviation/$\mu g\ l^{-1}$ (n = 10) ..	6.29	4.03	3.38	5.80	2.04	6.07

The data given in Fig. 2 typically indicate the range of lead in urine levels for which the method was designed.

Conclusion

The proposed method for the determination of lead in urine is rapid and convenient to use, whilst being economic as regards time and reagent costs. On a routine basis 50–60 samples can be processed, together with calibration standards, in 4 h. The accuracy and precision of the analytical result is comparable to that obtained by more established procedures.

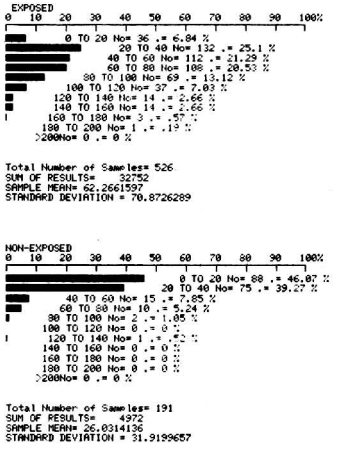

Fig. 2. Histograms showing levels of lead in urine of exposed and non-exposed workers during a period of 1 month.

Acknowledgements are made to The Associated Octel Company for permission to publish this paper.

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Received *May 30th*, 1980
Accepted *September 19th*, 1980

Analyst, March, 1981, Vol. 106, pp. 305–310

Determination of Tin in the Presence of Lead by Stripping Voltammetry with Collection at a Rotating Ring-disc Electrode

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Stripping voltammetry with collection at a glassy carbon rotating ring-disc electrode is used for the determination of tin in the presence of lead. Tin can be readily determined at a level of 1 μM (0.12 μg ml^{-1}) in the presence of a 50-fold concentration of lead. Determinations down to 0.25 μM (0.03 μg ml^{-1}) are possible with prolonged deposition times (about 30 min).

Keywords: Stripping voltammetry; rotating ring-disc electrode; tin determination; interference by lead

A major difficulty in determining tin by anodic-stripping voltammetry is the serious interference by lead in most electrolytes. Various methods have been suggested to solve this problem. A preliminary separation can be performed by extraction,[1] distillation,[2] or co-precipitation of lead[3]; also less common supporting electrolytes may be used.[4,5] In this study the possibility was examined of determining tin in the presence of lead by stripping voltammetry with collection at a rotating ring-disc electrode constructed of glassy carbon. This application was introduced by Johnson and Allen,[6,7] who determined silver and mercury down to 10^{-10} and 5×10^{-8} M, respectively. Its possible use in trace metal determination (of copper, lead and zinc) has also been demonstrated by Laser and Ariel.[8] The method consists of electro-deposition of the species to be determined at the disc of a rotating ring-disc electrode (RRDE) followed by stripping of the deposit using a linearly varying potential. A fraction of the oxidised species is transported to the surrounding ring electrode where a selective detection is possible at a fixed potential. In the acidic medium used it was found that the oxidation product at the disc, tin(II), can be further oxidised to tin(IV) at an appropriate positive ring potential. No reaction and thus no interference by lead(II) occurs at this potential. As the potential of the ring is constant, almost no capacitive current is flowing and the base line is essentially flat. This results in an improved accuracy and sensitivity.[6] Moreover the latter can be enhanced by the use of a high scan rate (v) at the disc, resulting in a higher dissolution current without influencing the base line at the ring.

The charge equivalent with the oxidation current of the tin(II) species collected at the ring is given by the following equation,[6] which can be derived from the Levich equation[9]

$$Q_r = 0.62NAnF\nu^{-\frac{1}{6}}\omega^{\frac{1}{2}}D^{\frac{2}{3}}c_b T_{dep} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

where A is the surface area of the electrode (cm^2), ν the kinematic viscosity (cm^2 s^{-1}), n the number of electrons involved, D the diffusion coefficient (cm^2 s^{-1}), ω the rotation speed (radians s^{-1}), c_b the bulk concentration of the species determined (mol ml^{-1}), T_{dep} the accumulation time (s) and N the collection efficiency.[10] The collection efficiency, N, represents the fraction of the electroactive species produced at the disc that reacts at the ring, part of the reaction product at the disc escaping into the bulk of the solution during the crossing of the gap betw ing and disc. The value of N can be calculated from the dimensions of the ring-dis trode.

Experimental

Reagents

Experiments were performed in 1 M hydrochloric acid. All solutions were prepared from analytical-grade reagents and from water freshly generated by a Milli-Q system (Millipore Inc.). Stock solutions of tin(II) were prepared by dissolving tin(II) chloride dihydrate in

concentrated hydrochloric acid to avoid hydrolysis. Lead(II) solutions were prepared by dissolving lead(II) chloride in 1 M hydrochloric acid. Solutions were carefully de-aerated with nitrogen containing less than 1 p.p.m. of oxygen.

Instrumentation

A home-made ring-disc electrode of glassy carbon (Tokai Electrode Co., Tokyo) was used, with a disc radius of 0.262 cm and inner and outer ring radii of 0.273 and 0.330 cm, respectively. This gives $\alpha = 0.131$ and $\beta = 0.867$.[10] The collection efficiency determined with the copper(II) - copper(I) system in 0.5 M potassium chloride solution[11] was 0.33.

The electrolysis cell was a perspex vessel of about 150-ml capacity in which the glassy carbon ring-disc electrode was centrally placed. The auxiliary electrode (platinum) was placed in a compartment separated from the cell by a fritted glass disc. A saturated calomel electrode (SCE) served as reference electrode and was connected to the cell via a salt bridge and a U-tube containing 1 M hydrochloric acid and saturated potassium chloride solution, respectively. A water-bath allowed the temperature to be held constant at 20 °C.

Electrode rotation was achieved by a motor from Brion-Leroux (Birotax, Type I). Continuous selection of speeds from zero to 315 radians s^{-1} was possible. The rate was strictly controlled using a servo control unit (Tacussel Asservitex). The rotation rate was measured by means of a proximity probe (Philips PR 9373) and frequency meter (Philips PM 6601).

Potential control was maintained by means of a Tacussel sweep generator GSATP and potentiostat Bi-pad. Curves were recorded on a Hewlett-Packard X - YY' recorder, Model HY 7046A.

The areas of the recorded peaks were determined with a planimeter.

Electrode Pre-treatment

The glassy carbon ring-disc electrode was polished by standard metallographic techniques. Final polishing used 0.05 μm alumina on Buehler microcloth to a mirror-like smoothness. After being rinsed with water the polished electrode was activated in 1 M hydrochloric acid by cycling the potential between 1.0 and −1.2 V against the SCE at a scan rate of 0.030 V s^{-1} until a reproducible and very low background was obtained. The electrode was then held successively at −0.5 and 0.0 V against the SCE for 5 min to remove any oxide film that might have formed and metallic impurities that might have been deposited at −0.5 V, respectively. Nitrogen was passed through the solution during this pre-treatment, after which the activated electrode was transferred into the already de-aerated test solution. Between successive recordings the disc electrode was cleaned by anodic polarisation at a potential of 0.6 V for 2 min. No special treatment of the ring was necessary.

Analytical Procedure for Electrolysis

The initial disc potential (E_i) was 0.6 V against the SCE. Pre-electrolysis was started by switching the potential of the disc to the desired E_{dep}. During deposition the potential of the ring, E_r, was held at 0.0 V. One minute before the expiration of T_{dep}, E_r was switched to the appropriate collection potential. After deposition the disc potential was scanned in the anodic direction to 0.6 V. The electrode was kept at this potential for about 2 min during which time nitrogen was passed through the solution. The electrode was then ready for another run. After collection E_r was returned to 0.0 V. The blank was considered to be the charge or peak current obtained for T_{dep} equal to zero, immediately after a scan with finite deposition time.

Results and Discussion

Current - potential graphs for the oxidation of tin and lead in hydrochloric acid with the simultaneous collection of tin(II) at the ring are presented in Fig. 1. When the current at the disc varies with time, the ring current reflects the fraction of the tin(II) ions produced at the disc that is collected at the ring (given by the collection efficiency N). The ring to disc current ratio is no longer equal to N when lead is present and is oxidised at the disc but not collected at the ring. The anodic peak at the disc is seen at approximately −0.45 V. The shift of the ring collection peak to a more positive value can be explained by the high scan

rate and the finite time necessary for the tin(II) species leaving the disc to cross the gap between ring and disc.[6] The unequivocally definable base line for the ring collection peak is notable. The supporting electrolyte was always 1 M because smaller peaks were obtained both at lower and higher hydrochloric acid concentrations. The latter also causes an increased screening effect at the electrode surface because of the enhanced hydrogen evolution.

Fig. 2 shows a plot of Q_r as a function of E_{dep} for $E_r = 1$ V against the SCE. Fig. 3 represents the same for various E_r values at constant E_{dep}.

From the shapes of the curves in Figs. 2 and 3, it appears that the oxidation at the ring proceeds more irreversibly compared with the reduction (deposition) at the disc. A suitable collection potential at the ring and deposition potential at the disc are, respectively, +1 and −1 V against the SCE. Accumulation potentials that are too negative cause lower peaks, probably due to screening of the electrode surface by hydrogen evolution.

The oxidation state of the tin species responsible for the oxidation current observed at the ring was determined with a solution containing only tin(II) by comparing the ring currents obtained at collection potentials of +1 and −1 V against the SCE. The values of Q_r for both oxidation and reduction were nearly the same, indicating that tin(II) is mainly formed during the stripping process.

Equation (1) predicts that Q_r is proportional to the tin(II) bulk concentration, to the deposition time, T_{dep}, and to the square root of the rotation speed of the electrode. Fig. 4 shows the linear dependence found between Q_r and $\omega^{1/2}$ for a solution 10 μM in tin(II) and

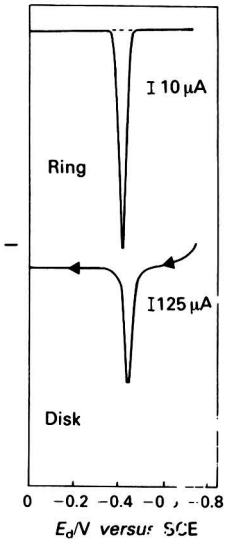

Fig. 1. Current-potential curves for stripping voltammetry with collection in 1 M hydrochloric acid at the glassy carbon electrode. Concentration of tin(II) = 10 μM; concentration of lead(II) = 2 μM; $E_{dep} = -1$ V *versus* SCE; $E_r = 1$ V *versus* SCE; $T_{dep} = 5$ min; $v = 0.1$ V s^{-1}; $\omega = 188$ rad s^{-1}. Dotted line represents constructed baseline.

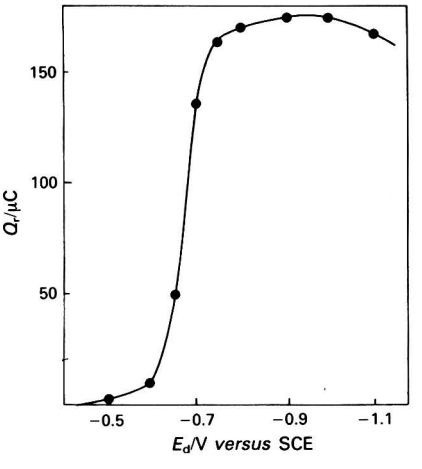

Fig. 2. Dependence of Q_r on E_{dep}. Concentration of tin(II) = 20 μM; $E_r = 1$ V *versus* SCE; $T_{dep} = 5$ min; $v = 0.1$ V s^{-1}; $\omega = 157$ rad s^{-1}.

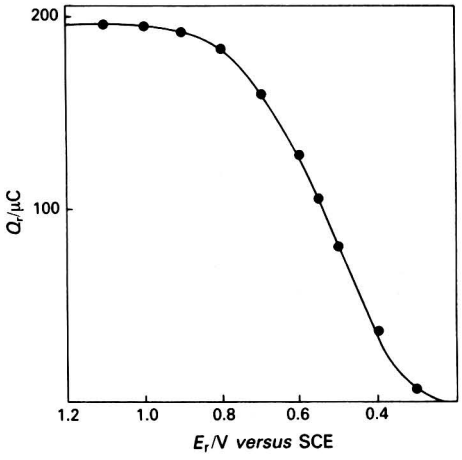

Fig. 3. Dependence of Q_r stripped from the glassy carbon disc electrode on E_r. Concentration of tin(II) = 20 μM; E_{dep} = −1 V *versus* SCE; T_{dep} = 5 min; v = 0.1 V s^{-1}; ω = 188 rad s^{-1}.

5 μM in lead(II). The dependence of Q_r on T_{dep} is shown in Fig. 5. For curve A the solution was also made 2 μM in lead(II) whereas for curve B only tin(II) was added. It is clear that the presence of lead improves tin deposition, probably by providing precipitation centres for the tin. Fig. 6 shows Q_r and I_r as functions of the tin(II) concentration in solution. Neither curve passes through the origin. Small deviations from linearity are also

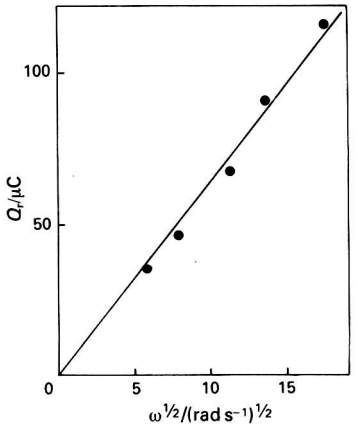

Fig. 4. Dependence of Q_r on $\omega^{1/2}$. Concentration of tin(II) = 10 μM; concentration of lead(II) = 5 μM; E_{dep} = −1 V against SCE; E_r = 1 V; T_{dep} = 5 min; v = 0.1 V s^{-1}.

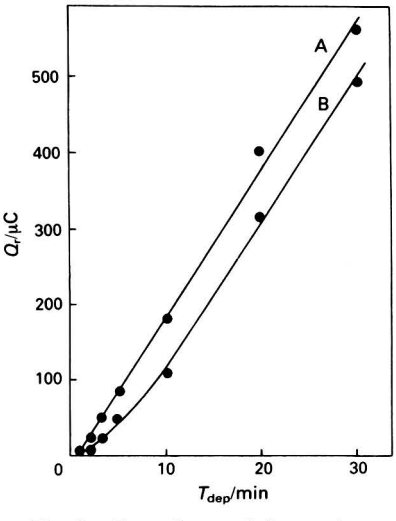

Fig. 5. Dependence of Q_r on electrolysis time. E_{dep} = −1 V *versus* SCE; E_r = 1 V; v = 0.1 V s^{-1}; and ω = 188 rad s^{-1}. A, Concentration of tin(II) = 10 μM and concentration of lead(II) = 2 μM. B, Concentration of tin(II) = 10 μM.

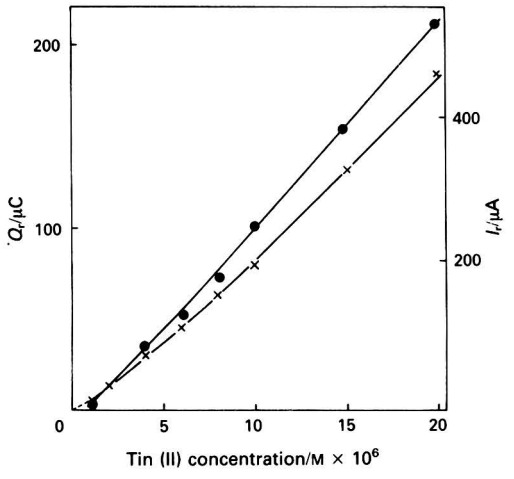

Fig. 6. Dependence of Q_r (×) and I_r (●) on the concentration of tin(II). Concentration of lead(II) = 5 μM; E_{dep} = −1 V *versus* SCE; E_r = 1 V. T_{dep} = 5 min; v = 0.1 V s^{-1}; ω = 157 rad s^{-1}.

observed at low concentrations of tin(II) and short deposition times. Ring and disc peak areas were independent of scan rate while the collection peak heights showed a linear dependence on the scan rate, v, between 0.010 and 0.100 V s^{-1}. Higher scan rates caused deviations, such as broadening of the peaks.

As lead is often present at concentrations higher than those of tin, the influence of adding an excess of lead was also investigated. Fig. 7 shows the dependence of Q_r on lead(II) concentration at constant tin(II) concentration. Lead does not interfere when the ratio of the lead(II) to tin(II) concentrations does not exceed about 50. At higher ratios results become irreproducible and also split collection peaks are observed.

It is also possible to evaluate the amount of excess of lead. At E_r = 1 V against the SCE, tin is determined; at E_r = −1 V both lead and tin re-deposit on the ring and the concentration of lead(II) can be evaluated from the difference between the Q_r values.

At tin(II) concentrations below 1 μM increased deposition times are necessary and down to 0.25 μM of tin(II) can be determined. In this concentration range determinations are less reproducible and accurate, and more time consuming. Fig. 8 shows the dependence of I_r on the concentration of tin(II) at concentrations lower than 1 μM. Satisfactory linearity is found down to tin(II) concentrations of 2.5 × 10^{-7} M when long deposition times are used.

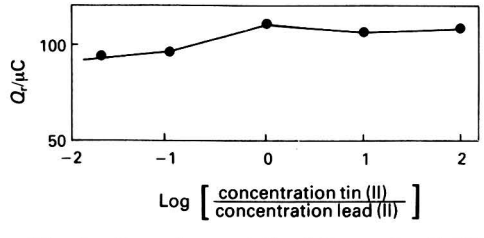

Fig. 7. Dependence of Q_r of tin on the tin(II) to lead(II) concentration ratio for 10 μM tin(II). E_{dep} = −1 V *versus* SCE; E_r = 1 V; T_{dep} = 5 min; v = 0.1 V s^{-1}; ω = 188 rad s^{-1}.

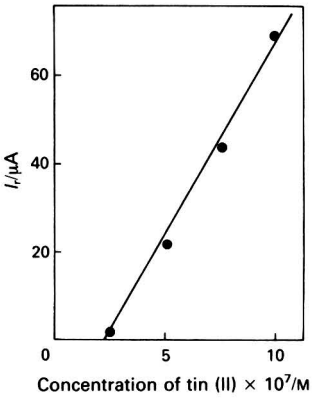

Fig. 8. Dependence of I_r on bulk concentration of tin(II). $E_{dep} = -1$ V *versus* SCE; $E_r = 1$ V; $T_{dep} = 30$ min; $v = 0.1$ V s^{-1}; $\omega = 315$ rad s^{-1}.

Below this concentration no tin deposition could be detected, *e.g.*, for 0.1 μM of tin(II) a dissolution peak could not be obtained even after accumulation times of several hours.

For tin(II) concentrations higher than 1 μM, the relative standard deviation of the results was about 6%. This rather high value is, in our opinion, mainly due to the integration method using a planimeter. For experimental work it is advisable to use ring peak heights, the relative standard deviation of which never exceeded about 4%. The high relative errors encountered for tin determinations, especially at concentrations below 1 μM, indicate the difficulties inherent in depositions at solid electrodes. Better results may be expected using a mercury film electrode[2,5,12] in combination with the ring-disc technique, which is presently under study.

The authors thank the N.F.W.O. for financial support.

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Received *July 14th*, 1980
Accepted *September 15th*, 1980

Analyst, March, 1981, Vol. 106, pp. 311–315

Direct Spectrophotometric Method for the Determination of Hydrochloric Acid-releasable Arsenic in Sediments and Soils

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A spectrophotometric method for the direct determination of hydrochloric acid-releasable inorganic arsenic has been developed and applied successfully to the quantitative evaluation of arsenic in soil and sediment samples. The method provides reliable data on the quantitative recovery of 2.0 μg of arsenic(V) added to 5.0 g (0.4 mg kg^{-1}) of soil, clay, sand and sediment samples. The method is simple, reliable and relatively rapid; 24 samples can be analysed in about 1 h. It does not require elaborate equipment and can be routinely used for the quantitative determination of arsenic in soil and soil-like material. The detection limit has been established as 0.5 μg of arsenic. The extent of ionic interference in the use of this method for arsenic determination in soil has also been quantitatively evaluated.

Keywords: *Arsenic determination*; *soils*; *sediments*; *spectrophotometry*

Arsenic occurs naturally in the earth's crust, but a considerable amount of arsenic is added to the human environment through its uses in wood preservatives, sheep dips, fly paper, arsenical soaps, rat poison, glass additives, dye pigment for calico prints, wall paper, lead shot and pesticides.[1] During 1971, the estimated production of organoarsenical herbicides such as monosodium methanearsenate, disodium methanearsenate and hydroxydimethylarsine oxide (cacodylic acid) in the USA was 10.7×10^8 kg.[2] Generally, soils contain about 5.0 p.p.m. of arsenic, but soils with a known history of arsenic application average about 165 p.p.m.[3] In some places such as Buns, Switzerland, and Wiatapu Valley, New Zealand, the arsenic level in the soil may reach 10^4 p.p.m.[4]; a substantial portion of arsenic in soil and soil-like material (sediment, clay, sand, etc.) is expected to be found in soluble form and probably can be dislodged easily by the action of water moving through the soil. Soluble forms of arsenic are relatively more mobile in the environment[5,6] and pose a greater potential for contaminating both ground water and surface water. Soluble forms of arsenic from soil and soil-like material are likely to enter a bioconversion chain through their initial uptake by vegetation. In order to understand the pollution potential of arsenic, it is desirable that a simple method should be made available for the quantitative evaluation of easily soluble forms of arsenic in soil and soil-like material.

Various methods exist for the determination of arsenic in solutions, including spectrophotometry,[7–9] gas - liquid chromatography,[10–14] ion-exchange chromatography,[15] coulometry,[16–18] activation analysis,[19] atomic-absorption spectrophotometry,[20–22] polarography[23] and distillation.[24,25] The spectrophotometric method of Small and McCant,[9] as described by Woolson *et al.*,[26] is widely used for the determination of total arsenic in soils. This method is tedious and time consuming because it requires 2 h after the soil sample has been digested. This method does not appear to be very precise at low levels of arsenic in soil, because the relative standard deviation for the recovery of 7.5 μg of arsenic added to 1 g of soil (7.5 p.p.m. of arsenic) is approximately 6.4%. The arsenomolybdate spectrophotometric method has never been applied to the determination of easily soluble arsenic in soil prior to its digestion. This paper presents a simple, direct and rapid method for the quantitative determination of easily soluble forms of arsenic in soil, clay, sand and sediment samples at parts per billion (10^9) levels. This technique differs from the spectrophotometric method of Small and McCant[9,26] in that the colour for photometric measurement is developed directly by passing the arsine from the generator through the silver diethyldithiocarbamate reagent. This eliminates three tedious steps involved in the previous method[9,26]: (*a*) absorbing the arsine in an ice-cold iodine trap, (*b*) treating it with colour-developing reagents and (*c*) heating the mixture for 1 h for colour development prior to photometric measurements. This results in a considerable saving of time.

Experimental

Apparatus

The arsine generator, absorber assembly and spectrophotometer have been described previously.[27]

Samples

The soil samples were collected from the locally exposed soil profiles of Dothan and Goldsboro series and were dried and ground to pass through a 2.0-mm sieve. Bentonite (Clay Spur, Wyoming), kaolinite (Silver Peak, Nevada), and sand (Ottawa flint, silica sand) samples were procured from Wards Natural Science Establishment, Rochester, N.Y. Sediment samples were collected from the Edisto River and the Caw Caw Swamp, Orangeburg, South Carolina. These samples were also dried and ground to pass through a 2.0-mm sieve.

Solutions

Stock solutions of arsenic containing an ionic concentration of 1 g l^{-1} were prepared from analytical-reagent grade sodium arsenate ($Na_2HAsO_4.7H_2O$) and sodium cacodylate [$(CH_3)_2AsO_2Na$]. Intermediate solutions were prepared by diluting the stock solutions 1 + 10. Working solutions containing requisite concentrations of arsenic were obtained by diluting the intermediate solutions.

Procedure

The native forms of hydrochloric acid-releasable arsenic in soil, clay, sand and sediment were determined in the untreated samples. Ten grams of each of these materials, followed by 50.0 ml of de-ionised water, 7.5 ml of concentrated (12 M) hydrochloric acid (the volume of acid used varied slightly with the type of soil analysed), 2.0 ml of potassium iodide solution (150 g l^{-1}) and 0.7 ml of tin(II) chloride solution (400 g l^{-1} of $SnCl_2.2H_2O$ in concentrated hydrochloric acid) were transferred into an arsine generator and allowed to stand for about 30 min, with occasional manual shaking, in order to reduce the arsenic to the trivalent state (the hydrochloric acid concentration of the mixture in the generator is about 1.50 M). Three grams of 20–30-mesh zinc were added to the arsine generator, and the generator was immediately connected to the absorber assembly, which was equipped with a lead acetate-impregnated glass-wool scrubber and contained 4.0 ml of silver diethyldithiocarbamate (SDDC) reagent [1 g of $AgSCSN(C_2H_5)_2$ in 200 ml of pyridine]. The arsine reacts with the SDDC and produces a red complex, which is measured at 535 nm against a reagent blank (SDDC solution treated in the absorber tube according to the experimental procedure, but without soil or arsenic). An absorbance calibration graph using 0.0, 1.0, 2.0, 4.0 and 5.0 μg of arsenic was prepared.

A 100-g amount of each of the soil, clay, sand and sediment samples (triplicate determinations were performed) was transferred into 250-ml Pyrex glass evaporating dishes. A 4-ml volume of a 10.0 mg l^{-1} arsenic solution prepared from sodium arsenate and sodium cacodylate was added, a drop at a time, to each sample, the sample being constantly stirred with a plastic spatula during the addition of solution. The stirring was continued until a homogeneous mixture was obtained. The samples, treated with arsenic solution, were spread on clean filter-papers, which were placed on the laboratory bench for uniform drying overnight. The following day (about 24 h later), the samples were transferred into the original evaporating dishes and ground with small pestles. Every possible effort was made to achieve a homogeneous mixture. The samples were transferred into 4-oz bottles for storage and future use. Five grams of each of these samples (triplicate determinations were performed) were transferred to the arsine generator for the determination of total arsenic in accordance with the procedure described previously. The arsenic recovery was obtained by subtracting the amount of native arsenic from the total amount.

Two hundred grams of $Goldsboro_{AP}$ soil was also treated with arsenic(V), chromium(VI) ($K_2Cr_2O_7$), copper(II) [$Cu(NO_3)_2.3H_2O$], nickel(II) ($NiCl_2.6H_2O$), antimony(III) ($SbCl_3$) and mercury(II) ($HgCl_2$) solutions so that 5.0 g of soil contained 6.0 μg of arsenic(V) and various concentrations of the individual metal ions as interferents.

Results and Discussion

The method described here is a modification of the standard method[28] recommended for the determination of arsenic in water and waste water. The detection limit for water samples has been established as 0.5 μg of arsenic.[27] Only the inorganic arsenic in water samples is reduced to arsine (AsH_3) during the zinc - acid reaction.[28] Organically bonded arsenic is expected not to be released unless the water samples are oxidised. Consequently, the spectrophotometric method reported here does not determine the total arsenic in soil, clay, sand and sediment samples; instead, it provides information on hydrochloric acid-releasable inorganic arsenic.

TABLE I

NATIVE ARSENIC AND ARSENIC RECOVERED FROM SOIL, CLAY, SAND AND SEDIMENT SAMPLES

Sample	Arsenic concentration/μg per 5 g				Recovery, %	Standard deviation, %
	Native*	Added	Total*	Recovered†		
$Dothan_{AP}$	1.09	2.0	3.10	2.01	100.00	4.6
$Dothan_{A_2}$	0.28	2.0	2.25	1.97	98.5	5.3
$Dothan_{B_{21}}$	0.36	2.0	2.33	1.97	98.5	4.5
$Dothan_{B_{22}}$	1.50	2.0	3.45	1.95	97.5	4.8
$Goldsboro_{AP}$	0.38	2.0	2.33	1.95	97.5	4.6
$Goldsboro_{B_{21}}$	0.50	2.0	2.40	1.90	95.0	5.0
$Goldsboro_{B_{22}}$	0.59	2.0	2.63	2.04	102.0	4.7
$Goldsboro_{B_{23}}$	0.98	2.0	2.90	1.92	96.0	4.2
Bentonite	6.60	2.0	8.60	2.00	100.0	4.7
Kaolinite	0.92	2.0	3.00	2.08	104.0	4.4
Sand	D‡	2.0	1.96	1.96	98.0	4.3
Sediment (North Edisto River)	1.02	2.0	3.05	2.03	101.5	4.9
Sediment (South Edisto River)	1.27	2.0	3.18	1.91	95.5	4.2
Sediment (Caw Caw Swamp)	3.16	2.0	5.13	1.97	98.5	4.3

* Mean of ten determinations.
† Recovered = Total − Native.
‡ Detected but not quantified.

The amounts of native arsenic found in soil, clay, sand and sediment samples are given in Table I. It appears that the proposed spectrophotometric method is able to detect and quantify trace amounts of arsenic contaminations in soil and soil-like materials (0.056 $\mu g\ g^{-1}$). Bentonite appears to be relatively high in hydrochloric acid-releasable arsenic, whereas the sand samples show very small, non-quantifiable arsenic contamination. The evaluation of acid-releasable arsenic in sediment also appears to be quantitative.

The amount of arsenic(V) added to an individual sample and the total arsenic determined by the proposed method are also given in Table I. The amount of arsenic recovered was determined by subtracting the amount of native arsenic from the total hydrochloric acid-releasable arsenic in the sample. All calculations were based on 5.0 g of sample used for assay. The recovery of arsenic added to soil, clay, sand and sediment samples varied from 104% for kaolinite to 95% for $Goldsboro_{B_{21}}$. It appears that the proposed spectrophotometric method provides reliable information on the recovery of inorganic arsenic(V) added to these samples.

The recovery of 10.0 μg of arsenic added as cacodylic acid to 50.0 ml of de-ionised water (0.2 mg l^{-1}) was 2.6% (Table II). There appeared to be no significant change in the recovery of arsenic when the same sample was analysed 1 week later. There was no discernible recovery of arsenic achieved from 5.0 or 2.0 μg of organoarsenical that was added to 50.0 ml of de-ionised water. This suggests that, although methylarsines may have been generated by cacodylic acid in de-ionised water during the zinc - acid reaction in the generator,[29] they do not appear to produce, under the conditions used, a red complex photometrically measurable at 535 nm. A small amount of arsenic that was recovered from 10.0 μg of organoarsenical added to 50.0 ml of de-ionised water possibly was present as inorganic arsenic in the solution prepared from cacodylic acid.

The recovery of arsenic added to various soils was negligible when the samples were analysed, after treatment for 24 h with cacodylic acid. However, the recovery of arsenic from the same

TABLE II

RECOVERY OF CACODYLIC ACID ARSENIC FROM WATER AND SOIL SAMPLES

Sample	Arsenic added	Arsenic recovered* 24 h μg	24 h %	24 h Standard deviation, %	1 week μg	1 week %	1 week Standard deviation, %
Water	10 μg per 50 ml	0.26	2.60	2.7	0.28	2.8	2.4
$Dothan_{AP}$	2 μg per 5 g	0.03	1.50	3.0	1.96	98.0	2.8
$Dothan_{A_2}$	2 μg per 5 g	0.08	4.00	2.9	0.64	32.0	2.6
$Dothan_{B_{22}}$	2 μg per 5 g	0.05	2.50	3.4	0.23	11.5	3.2
$Goldsboro_{AP}$	2 μg per 5 g	0.10	5.00	2.6	0.59	29.5	2.8
$Goldsboro_{B_{21}}$	2 μg per 5 g	0.13	6.50	2.9	0.52	26.0	3.1

* Recovered = Total − Native (Native from Table I).

set of samples improved significantly when they were analysed 1 week later. The recovery of arsenic from the soil samples is highly variable (Table II), and appears to depend on the soil characteristics. If methylarsines do not react with SDDC, as seems apparent from the behaviour of cacodylic acid in de-ionised water, then the organoarsenical that was added to the soil must be mineralised for the generation of SDDC-intractable arsine. The recovery of organoarsenical added to various soils varied from 98.0% (in $Dothan_{AP}$) to 11.5% ($Dothan_{B22}$) and suggests that some soils are more effective than others in their potential to mineralise cacodylic acid. It must be mentioned that some of the inorganic arsenicals (arsenic sulphide, aluminium arsenate, etc.) are either insoluble or only sparingly soluble in 1.50 M hydrochloric acid.[30] Consequently, the technique described here determines various forms of inorganic arsenic released by 1.50 M hydrochloric acid in soil-like materials.

Although the interference of certain metal ions in the recovery of arsenic added to demineralised water has been reported previously,[27] a further study on the effect of interferences on the recovery of arsenic from the soil system was conducted. No significant change in the recovery of 6 μg of arsenic(V) added to 5.0 g of soil was observed when the soil samples were also spiked with either 200 μg (40 mg kg^{-1}) of chromium(VI), 300 μg (60 mg kg^{-1}) of copper(II), 300 μg (60 mg kg^{-1}) of nickel(II), 15 μg (3 mg kg^{-1}) of mercury(II) or 2 μg (0.4 mg kg^{-1}) of antimony-(III). Interference by combinations of various metal ions was also studied. It appears that the recovery of arsenic generally is not affected up to a combined metal ion concentration of 300 μg per 5 g of soil that had also been spiked with 6.0 μg of arsenic(V). Whereas higher concentrations of antimony(III) and mercury(II) enhance the apparent recovery of arsenic, the other metal ions at levels greater than the concentrations listed above decrease the recovery of arsenic.

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Received *May 12th*, 1980
Accepted *September 17th*, 1980

Anodic Generation of Cerium(IV). Charge-transfer Kinetic Parameters and Conditional Potentials at Platinum, Gold and Glassy Carbon

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The anodic oxidation of cerium(III) at platinum, gold and glassy carbon electrodes was investigated by rotating disc voltammetry in sulphuric and perchloric acid solutions. Conditional potentials, limiting currents and charge-transfer kinetic parameters were obtained and the results are discussed. The absence of chemical corrosion and mixed potentials indicated that glassy carbon is the best electrode material. Current efficiencies were calculated.

Keywords: *Cerium(IV)*; *rotating disc electrode*; *charge-transfer kinetic parameters*

Cerium(IV) is a popular oxidising agent and has been used in coulometric titrations with success.[1–18] Most of the work has been carried out at platinum, but gold[3] and carbon paste[19] have also been used. Vetter,[20] Fronaeus[21] and Fronaeus and Ostman[22] studied the reduction of cerium(IV) in sulphuric and perchloric acids. The rate-determining step is the electron transfer reaction

$$Ce(IV) + e \longrightarrow Ce(III)$$

A survey of the literature indicated that every group who had studied this system and measured kinetic parameters had approached the problem by studying the reduction of cerium(IV) rather than the oxidation of cerium(III). As it is the anodic reaction that is involved in coulometric titrations with cerium(IV) we decided to study the anodic reaction directly.

The purpose of this work was to study the effect of experimental parameters on the charge-transfer kinetic parameters. Both background and cerium(IV) generation reactions were studied for the purpose of evaluating generation current efficiencies.

Experimental

Apparatus

A conventional three-electrode potentiostat was built by using general-purpose FET operational amplifiers. A separate control unit provided the initial and ramp potentials, which, when fed into the potentiostat bridge circuit, allowed control of the working electrode potential.

The rotating disc electrode with interchangeable tips has been described elsewhere.[23]

Voltammograms were recorded on a Hewlett-Packard Moseley 7035 X - Y recorder.

Potentials of the working electrode with respect to a specially designed calomel electrode[24] were measured on an S.E. Laboratories (Engineering) Ltd. digital voltmeter, Model SM 215.

Rotation frequencies of the working electrode were measured on an S.E. Laboratories (Engineering) Ltd. crystal timer, Model SM 200.

Reagents and Materials

All of the reagents used were of AnalaR grade (Hopkin and Williams), except for mineral acids, which were of Aristar grade (BDH Chemicals).

Specially distilled water[25] was used.

Certified-grade volumetric glassware was cleaned with an alcoholic potassium hydroxide solution.

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Procedure

A conventional procedure was used to record voltammograms, using a jacketed cell maintained thermostatically at 20 °C.

Before each set of scans, the working electrode was conditioned as follows: (*i*) immersed in warm aqua regia for 1 min (for platinum and gold) or polished with alumina and grade E Cerirouge (for carbon only); (*ii*) rinsed thoroughly with distilled water; (*iii*) cathodised and anodised for 30 s at 30 mA cm^{-2}, three cycles in 0.5 M Aristar sulphuric acid (for platinum only), or cathodised for 1 min (for gold only) or immersed in chromic acid - sulphuric acid mixture for 1 min (for carbon only); (*iv*) rinsed thoroughly with distilled water; and (*v*) successively scanned in the test solution, between the initial and final voltammogram potentials, until reproducibility was obtained (for platinum and gold only).

Generation current efficiencies (% Eff) were calculated from the relationships

$$\% \text{ Eff} = \frac{i_I}{i_T} \times 100$$

and

$$i_T = i_I + i_B + i_{IMP}$$

where i_I is the current due to cerium(III) oxidation, i_B is the background reaction current and i_{IMP} is the residual current.

The different currents involved were computed as described in the Appendix.

Graphs of current efficiency against total current, i_T, were calculated by the following procedure: (*i*) a working electrode potential, E, was chosen; (*ii*) the cerium(III) oxidation current was calculated; (*iii*) the background current was calculated; (*iv*) the residual current was calculated from experimental current - potential graphs; and (*v*) the total current and current efficiency were calculated.

A Fortran IV program was written for this purpose. This will compute charge-transfer kinetic parameters from experimental current - potential graphs and calculate current efficiencies under different experimental conditions. It is available from the authors on request.

Results and Discussion

Treatment of Experimental Results

Charge-transfer rate constants were expressed as conditional parameters, using conditional potentials ($E^{\circ\prime}$) as the reference point. These were determined by measurement of zero-current potentials (E) throughout a constant-current coulometric oxidation of cerium(III).

Extrapolated conditional potential values were obtained from the relationship

$$E = E^{\circ\prime} + \frac{RT}{nF} \times \ln\left(\frac{1}{FCV/it - 1}\right)$$

where V l represents the volume of cerium(III), C mol l^{-1} is the concentration, i A is the current used and t s is the time of electrolysis.

The charge-transfer conditional rate constant ($k^{\circ\prime}$) and the charge-transfer coefficient (α cathodic; β anodic) were determined by using the relationship

$$\frac{1}{i} = \frac{1}{I} + \frac{K}{\omega^{\frac{1}{2}}}$$

where i A is the current for a particular electrode potential, ω rad S^{-1} is the electrode rotational frequency, K is a potential-dependent constant and I A is the current corrected for diffusion. The intercept of the graph of $1/i$ *versus* $1/\omega^{\frac{1}{2}}$ gives I, which, when measured as a function of potential, gives a Tafel plot:

$$\text{Log } I = \text{Log } nFACk^{\circ\prime} + \frac{\beta nF}{RT}(E - E^{\circ\prime})$$

Experimental currents were corrected for the background contribution.

Conditional Potentials

A typical experiment for the determination of conditional potentials is shown in Fig. 1. Platinum and gold exhibited anomalous behaviour, whereas glassy carbon showed a perfect fit to the Nernst equation.

The conditional potentials found follow the sequence carbon > platinum > gold and steady potential readings were attained much faster at glassy carbon. Corrosion of gold was also observed. This indicates that potentials measured at glassy carbon were true equilibrium potentials and the values measured at platinum and gold were "mixed potentials."

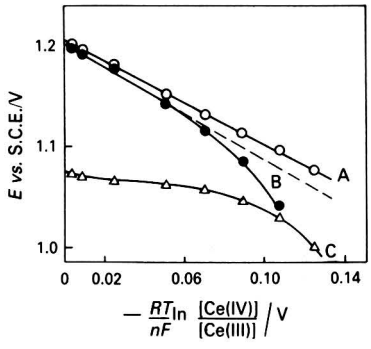

Fig. 1. Zero current potentials measured at (A) glassy carbon, (B) platinum and (C) gold electrodes, throughout a coulometric oxidation of a 0.02 M cerium(III) solution in 1.5 M sulphuric acid. Conditional potentials are obtained by extrapolation to [Ce(IV)]/[Ce(III)] = 1.

Table I summarises values determined in sulphuric and perchloric acid. Higher values were obtained in the latter, as expected, and no mixed potentials were observed. As the sulphuric acid concentration was increased, potentials approached true equilibrium values.

TABLE I

CONDITIONAL POTENTIALS FOR Ce(IV)/Ce(III) *vs.* S.C.E.

	Potential/V				
Electrode	0.5 M H_2SO_4	1.5 M H_2SO_4	4.5 M H_2SO_4	1 M $HClO_4$	3 M $HClO_4$
Glassy carbon ..	1.2277	1.2039	1.1570	1.3925	1.4072
Platinum	1.2166	1.2006	1.1556	1.3732	1.3888
Gold	1.1158	1.0731	1.1531	1.3921	1.4072

Voltammograms

Fig. 2 shows voltammograms obtained in sulphuric acid. These are typical of a mixed charge-transfer, mass-transfer controlled process. Ill-defined limiting currents are observed.

The mass-transport rate constants determined indicate that an increase in sulphuric acid concentration decreases the mass transport, as shown in Table II. This is due to an increase in solution viscosity.

Fig. 3 shows voltammograms obtained in perchloric acid. No limiting current and considerable wave overlapping with background reaction were observed.

Fig. 4 shows the current - potential graphs for the background reaction. The highest overpotential decreased in the order gold, glassy carbon and platinum. This might suggest that gold is a better electrode material than glassy carbon if were not for its corrosion and mixed potentials, as already mentioned.

TABLE II

MASS-TRANSPORT RATE CONSTANTS FOR 0.01 M CERIUM(III) IN SULPHURIC ACID

Rotation frequency, 2 Hz.

Concentration/M	0.5	1.5	4.5
k_m/cm s^{-1}	14.5 × 10^{-4}	11.1 × 10^{-4}	6.1 × 10^{-4}

Conditional Kinetic Parameters

Table III shows the values found for the cerium and background reactions.

Effect of supporting electrolyte

Changing from sulphuric to perchloric acid has no profound effect on the kinetic parameters for cerium. There is a slight decrease in the anodic charge-transfer coefficient and charge-transfer rate constant. This may be attributed to the higher conditional potentials of this couple [redox pair Ce(IV) - Ce(III)] in perchloric acid, which makes the anodic reaction overlap with the background reaction. The effect on background kinetic parameters is not relevant except at gold, where higher rate constants were found.

Effect of electrolyte concentration

An increase in electrolyte concentration has no significant effect in perchloric acid. Changes in both charge-transfer kinetic parameters for cerium are random and within experimental error.

In sulphuric acid, an increase in concentration increases the rate constant. This may be due to the fact that the conditional potential is decreased. The charge-transfer reaction then occurs on a less oxidised surface at a higher rate. The overlap of the reactions is also less. Only the effect on background kinetic parameters, which is to slow down the background reaction by decreasing the rate constant, is apparent. This is due to a considerable decrease in the water bulk concentration.

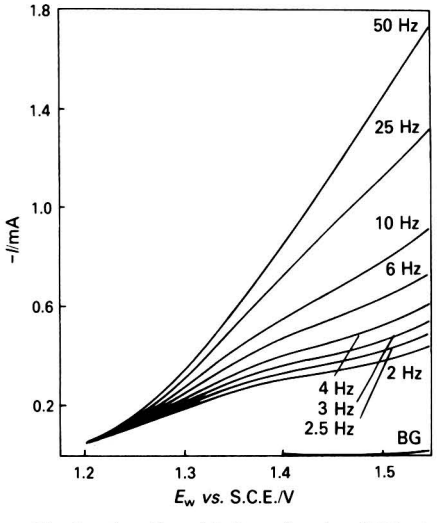

Fig. 2. Anodic oxidation of cerium(III) at platinum. [Ce(III)] = 0.01 M in 4.5 M sulphuric acid. Rotating disc electrode area: 0.5 cm^2. Supporting electrolyte oxidation wave is denoted by BG.

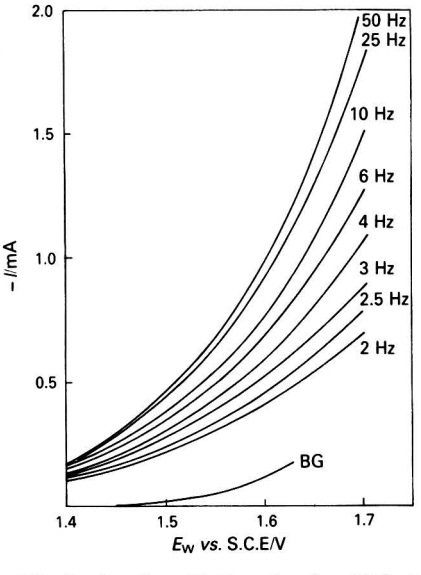

Fig. 3. Anodic oxidation of cerium(III) at platinum. [Ce(III)] = 0.01 M in 1.0 M perchloric acid. Rotating disc electrode area: 0.5 cm^2. Supporting electrolyte oxidation wave is denoted by BG.

TABLE III

CONDITIONAL CHARGE-TRANSFER KINETIC PARAMETERS FOR THE OXIDATION OF CERIUM(III) AND WATER

Supporting electrolyte	Species	Platinum β	Platinum $k^{o\prime}$/cm s^{-1}	Gold β	Gold $k^{o\prime}$/cm s^{-1}	Glassy carbon β	Glassy carbon $k^{o\prime}$/cm s^{-1}
0.5 M H_2SO_4 ..	Ce(III)	0.30	3.5×10^{-5}	0.16	7.3×10^{-5}	0.49	8.7×10^{-4}
	Water	0.11	1.7×10^{-22}	0.17	1.2×10^{-27}	0.09	1.4×10^{-23}
1.5 M H_2SO_4 ..	Ce(III)	0.26	2.9×10^{-5}	0.19	1.4×10^{-4}	0.34	1.4×10^{-4}
	Water	0.14	5.7×10^{-23}	0.15	1.6×10^{-26}	0.09	7.2×10^{-24}
1.0 M $HClO_4$ ..	Ce(III)	0.21	4.8×10^{-5}	0.16	3.5×10^{-5}	0.30	2.4×10^{-4}
	Water	0.11	1.1×10^{-23}	0.13	2.4×10^{-26}	0.09	7.9×10^{-24}
3.0 M $HClO_4$ ..	Ce(III)	0.22	5.6×10^{-5}	0.16	1.6×10^{-5}	0.21	3.5×10^{-4}
	Water	0.13	3.1×10^{-24}	0.08	2.2×10^{-23}	0.09	1.5×10^{-23}

Effect of electrode material

The electrode material has a significant effect on the cerium charge-transfer rate constant. The fastest rate is obtained at glassy carbon, which makes it the best material for cerium(IV) generation. The reaction rate sequence is carbon > platinum > gold in perchloric acid and carbon > gold > platinum in sulphuric acid. The apparent increase in reaction rate at gold on changing to sulphuric acid is due to the higher roughness factor. The electrode area is increased by chemical corrosion of gold. The effect on the background reaction is to give the highest rate constant at platinum, followed by glassy carbon and gold. This sequence is reasonable as the platinum electrode is actually platinum - platinum oxide at these high potentials. It is known to give rise to catalytic effects and to participate in the oxygen evolution reaction.[26] Glassy carbon is less reactive than gold at these high potentials, giving an oxide-free surface.

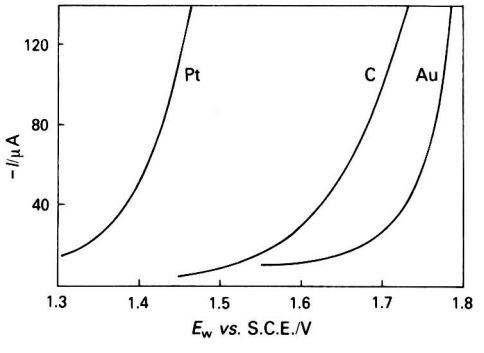

Fig. 4. Background reaction at different electrodes. Oxidation of water in 0.5 M sulphuric acid. Rotating disc electrode area: 0.5 cm^2. Rotating speed: 50 Hz.

Current efficiencies

A summary of the maximum computed current efficiencies, and the corresponding generating current densities, in constant-current coulometry is given in Table IV.

Fig. 5 shows the effect of electrode material. Gold and glassy carbon give similar results. Slightly higher current efficiencies are obtained at gold but a wider practical generating current range can be achieved at glassy carbon. Unfortunately, gold is corroded by cerium(IV) solutions and cannot be used. Glassy carbon proved to be inert. The current efficiencies obtained are not only higher than at platinum, but also a wider practical generating current range can be used.

TABLE IV

MAXIMUM CURRENT EFFICIENCIES FOR CERIUM(IV) GENERATION

[Ce(III)] = 0.15 M; $k_m = 10^{-3}$ cm s^{-1}.

Supporting electrolyte	Glassy carbon		Gold		Platinum	
	Efficiency, %	Current density/ mA cm^{-2}	Efficiency, %	Current density/ mA cm^{-2}	Efficiency, %	Current density/ mA cm^{-2}
0.5 M H_2SO_4	99.89	13.4	99.70	9.54	99.63	5.63
1.5 M H_2SO_4	99.87	12.0	99.91	11.8	99.43	6.49
4.5 M H_2SO_4	99.95	12.1	99.86	12.8	99.94	11.4
1.0 M $HClO_4$	99.82	5.31	99.90	1.14	98.91	0.64
3.0 M $HClO_4$	99.33	7.27	94.08	0.65	97.71	1.90

Conclusions

The determined charge-transfer kinetic parameters, when compared with reported values obtained from cathodic polarisations,[19,20] show good agreement of the conditional rate constants. However, the charge-transfer coefficients do not fit the relationship $\alpha + \beta = 1$.

This supports once more the fact that the charge-transfer coefficient is not a potential-independent parameter.

Medium speed or slow systems may exhibit activation energy - reaction co-ordinate profiles that differ substantially when scanning potential from the cathodic to the anodic region. Therefore, kinetic parameters determined anodically should be used to obtain cerium(IV) generation current efficiencies.

Glassy carbon proved to be by far the best electrode material for cerium(IV) generation.

Conditional potentials are true equilibrium potentials at glassy carbon. Platinum and gold give mixed potentials, and gold is severely corroded by cerium(IV).

As far as current efficiency is concerned, the generation of cerium(IV) can be performed in sulphuric acid with better results than in perchloric acid. The traditional platinum should be replaced with glassy carbon as electrode material. This may improve current efficiencies from a reported 99.8% to 99.95%.

Appendix

Computation of Partial Currents

The total generating current in constant-current coulometry can be decomposed into at least three partial currents: the current due to the oxidation of cerium(III), the current due to the

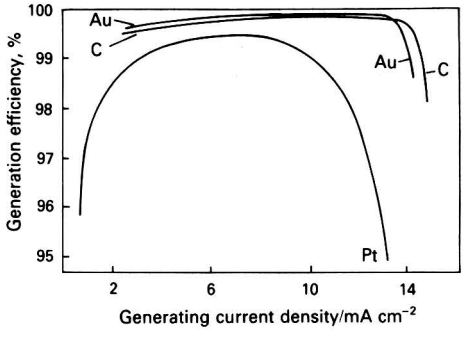

Fig. 5. Cerium(IV) generation efficiencies at three different electrode materials. Cerium(III) concentration: 0.15 M. Supporting electrolyte: 1.5 M sulphuric acid. Mass-transport rate constant: $k_m = 1 \times 10^{-3}$ cm s^{-1}. Electrode area: 1.0 cm^2.

oxidation of water and the residual current. The last partial current was determined experimentally.

The cerium(III) oxidation current, i_I, can be calculated for each working electrode potential by means of the equation

$$-i_I = \frac{FAk^{\circ\prime}[\mathrm{Ce(III)}]}{\frac{k^{\circ\prime}}{k_m} + \exp\left[-\frac{\beta F}{RT}(E-E^{\circ\prime})\right]}$$

where β is the anodic charge-transfer coefficient, $k^{\circ\prime}$ cm s^{-1} is the conditional charge-transfer rate constant, k_m cm s^{-1} is the mass-transport rate constant, $E^{\circ\prime}$ V is the conditional potential, E V is the working electrode potential, A cm^2 is the electrode area, $R = 8.3147$ J K^{-1} mol^{-1}, $F = 96486.7$ A s $equiv^{-1}$ and $T = 293$ K.

The current due to oxidation of water, i_{BG}, was calculated from the equation

$$-i_{BG} = 4FA[H_2O]^6\, k^{\circ\prime} \exp\left[\frac{4\beta F}{RT}(E-E^{\circ\prime})\right]$$

The conditional potential was not measured but calculated as suggested by Bishop.[27]

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Received *April 23rd*, 1979
Accepted *August 13th*, 1980

Analyst, March, 1981, Vol. 106, pp. 323–327

Polycrystalline Ion-selective Electrode Based on $Ag_2[HgI_4]$ - Ag_2S

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The preparation and analytical behaviour of polycrystalline solid-state ion-selective electrodes based on $Ag_2[HgI_4]$ and $Ag_2[HgI_4]$ - Ag_2S membranes was studied. The membranes consist of solid-state pellets made by compressing prepared precipitates. The performance characteristics of the electrodes are described in terms of potential - activity graphs, stabilities, precision, response times, effect of pH and redox potential. The electrode responses towards Ag^+, Hg^+, Hg^{2+}, I^- and CN^- ions are evaluated. It was found that the $Ag_2[HgI_4]$ - Ag_2S type is superior to the $Ag_2[HgI_4]$ type with respect to mechanical properties and lifetime and is more suitable for practical application.

Keywords: $Ag_2[HgI_4]$ - Ag_2S electrode; polycrystalline membrane preparation; response to Ag^+, Hg^+, Hg^{2+}, I^- and CN^-

Sparingly soluble inorganic compounds that exhibit ionic conductivity are of great importance for the development of ion-selective electrodes.[1] It has been established[2] that the silver and mercury ions in silver iodomercurate(II) ($Ag_2[HgI_4]$) show considerable mobility, and this resulted in the application of this compound for the development of both homogeneous[3,4] and heterogeneous[5] ion-selective electrodes. The homogeneous type showed satisfactory performance whereas the heterogeneous type did not fulfill expectations. The homogeneous electrode produced reproducible responses towards Ag^+, Hg^{2+} and I^- ions and a model describing the function of the electrode was suggested.[4] The response to mercury is of great practical interest. Attempts have been made to use the silver iodide electrode for the determination of mercury,[6] but the electrode is not adequately reversible to mercury and the deterioration of the electrode function during the exposure to mercury ion limits its practical application. This paper describes the preparation and evaluation of homogeneous solid-state electrodes based on $Ag_2[HgI_4]$ and $Ag_2[HgI_4]$ - Ag_2S precipitates.

Experimental

Precipitates

$Ag_2[HgI_4]$ was prepared by precipitation from solutions containing stoicheiometric amounts of silver nitrate, mercury(II) nitrate and potassium iodide.[7]

Silver sulphide was precipitated by slow addition of silver nitrate solution to sodium sulphide ($Na_2S.9H_2O$) solution.

All precipitated compounds were washed by decantation with water, rinsed twice with ethanol, filtered and dried.

All chemicals used in the measurements and preparation of the electrodes were of analytical-reagent grade. Stock solutions were analysed by appropriate analytical methods. Standard solutions were prepared by serial dilution of the stock solutions.

Electrodes

The electrodes were prepared by a modified procedure described previously.[8,9] The desired proportions of the membrane components were weighed out to give a total mass of 1.0 g. The precipitates were thoroughly ground and mixed. All membranes were formed at room temperature in a 6 mm diameter die using an applied pressure of 8000 kg cm^{-2} for 10 min. The pellets were polished on both parallel sides and end-mounted in 8-mm polyolefin heat-shrinkable tubes. Internal electrical contact to a stainless-steel rod was achieved with silver epoxy cement. X-ray diffraction analysis confirmed that $Ag_2[HgI_4]$ was present in the β-modification.[10]

The sensitivities, response times, stabilities and precision of the electrodes and the effects of the pH and redox potential of the solution were tested under identical conditions, *viz.*,

constant temperature (25 °C) and constant ionic strength (0.1 M). Potentiometric measurements were carried out in the conventional manner by using an Orion 92–02–000 double-junction reference electrode with a Fisher Accumet Mo 750 millivoltmeter and a Radiometer REC61 servo recorder.

Results

Five $Ag_2[HgI_4]$ and five $Ag_2[HgI_4]$ - Ag_2S (1 : 1 *m/m*) membranes were prepared and tested. Preliminary experiments showed that pure $Ag_2[HgI_4]$ membranes had a tendency to crack and difficulties were experienced during the polishing process. The surface of the membrane deteriorated rapidly during immersion in solution and frequent re-polishing was required. Attempts to limit this problem by fabricating the membranes at different pressures and temperatures were not successful. These membranes required extremely careful handling.

It was found that the incorporation of Ag_2S improved significantly the mechanical properties of the membranes. Membranes prepared from a mixture of $Ag_2[HgI_4]$ and Ag_2S in a 1 : 1 mass ratio were mechanically strong, hard and easy to polish, and no deterioration of the surface was observed even during the long-term measurements.

Sensitivity

The responses of both types of electrodes towards each individual component, namely Ag^+, Hg^+, Hg^{2+}, I^- and CN^-, were studied. Graphs of e.m.f. *versus* p*A* showed Nernstian responses towards all ions of interest. Calibration graphs illustrating the responses of the electrodes to Ag^+, Hg^+, and Hg^{2+} ions are shown in Fig. 1. The electrodes exhibited Nernstian responses down to about 1×10^{-7} M for all cations tested.

The responses of the electrodes to I^- and CN^- are shown in Fig. 2. The response of the electrodes to I^- deviated from Nernstian in dilute solutions. Similar responses were previously observed with common Ag_2S - AgI electrodes and were explained either by the activity of silver defects in the membrane,[11] by oxidation effects[12,13] or by the effect of surface heterogeneity.[14] The electrodes gave near-Nernstian responses to 1×10^{-6} M CN^- solutions.

The performance of the pure $Ag_2[HgI_4]$ electrode deteriorated rapidly, whereas no problems with either mechanical properties or deterioration of the membrane surface was experienced with the $Ag_2[HgI_4]$ - Ag_2S electrode.

Response Time, Stability and Precision

Practical response times of the electrodes were measured in constantly stirred solutions of varying concentrations.[15] The values of t_{95} in Table I indicated relatively fast time responses.

The stability of the potential over a long time period was measured in 1×10^{-5} and 1×10^{-3} M solutions of ions of interest during a 120-min period. The values of the mean

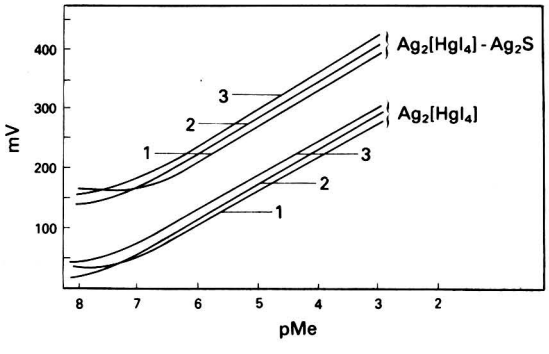

Fig. 1. Calibration graphs for $Ag_2[HgI_4]$ and $Ag_2[HgI_4]$ - Ag_2S electrodes. 1, Response to Hg^{2+}; 2, response to Ag^+; and 3, response to Hg^+.

($\bar{X}$) and standard deviation (S_x), given in Table I, show the superior performance of the $Ag_2[HgI_4]$ - Ag_2S over the $Ag_2[HgI_4]$ electrode.

The precision of the electrode response was determined by recording the responses during ten cycles of immersion (10 min) of the electrodes in and withdrawal (10 min) from 1×10^{-5} and 1×10^{-3} M solutions of ions of interest. The results for S_x (Table I) demonstrate the deterioration of the $Ag_2[HgI_4]$ electrode function.

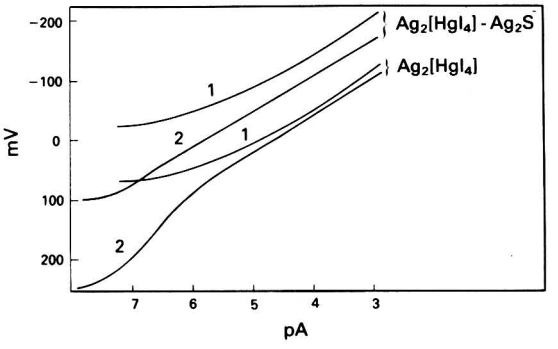

Fig. 2. Calibration graphs for $Ag_2[HgI_4]$ and $Ag_2[HgI_4]$ - Ag_2S electrodes. 1, Response to I^-; 2, response to CN^-.

Effect of pH and Redox Potential

The effect of pH on the electrode potential was tested by measuring the potential at ten pH values in the range 1–14 in a solution containing 1×10^{-5} M I^- and 1×10^{-1} M sodium nitrate. As shown in Fig. 3, the electrodes are not sensitive to pH changes in the range 1–11.

The effect of the redox potential of the solution on the electrode potential was determined by monitoring the potentials of a platinum electrode and the ion-selective electrode in solutions of varying redox potential and of constant activity of the ion of interest. The potential of the ion-selective electrode in 1×10^{-4} M Ag^+ solution was not affected by the addition of persulphate ion up to 1×10^{-1} M. Similarly, the electrode potential of 1×10^{-4} M I^- solution did not change on the addition of ascorbic acid or sodium sulphite. The results comparing the potentials of the platinum and ion-selective electrodes are summarised in Table II.

TABLE I

RESPONSE TIME, STABILITY AND PRECISION OF ELECTRODE POTENTIALS

Electrode	Ion of interest	Time response, $10^{-3} \rightarrow 10^{-5}$ M, t_{95}/s	Stability: $\bar{X}$/mV		Stability: S_x (n = 5)/mV		Precision, S_x (n = 10)/mV	
			10^{-5} M	10^{-3} M	10^{-5} M	10^{-3} M	10^{-5} M	10^{-3} M
$Ag_2[HgI_4]$	Ag^+	7	+385	+500	4.9	4.1	4.5	3.2
	Hg^+	12	+372	+480	6.5	5.2	7.4	4.1
	Hg^{2+}	6	+395	+510	5.3	4.9	3.8	3.2
	I^-	5	−10	−120	5.2	4.9	2.7	2.8
	CN^-	10	+2	−120	6.5	10.8	7.6	10.2
$Ag_2[HgI_4]$ - Ag_2S	Ag^+	5	+275	+391	1.4	1.1	1.3	1.1
	Hg^+	10	+265	+380	1.5	1.2	1.6	1.0
	Hg^{2+}	5	+290	+405	1.3	0.9	0.8	0.6
	I^-	5	−55	−172	1.2	0.9	0.9	0.6
	CN^-	8	−90	−205	1.6	1.2	1.4	1.2

TABLE II

EFFECT OF REDOX POTENTIAL ON THE POTENTIAL OF $Ag_2[HgI_4]$ - Ag_2S ELECTRODE

Redox agent	Solution	E.m.f./mV: Pt electrode	E.m.f./mV: $Ag_2[HgI_4]$ - Ag_2S
—	1×10^{-4} M Ag^+	+405	+335
1×10^{-3} M $K_2S_2O_8$		+560	+335
1×10^{-2} M $K_2S_2O_8$		+825	+335
1×10^{-1} M $K_2S_2O_8$		+880	+337
—	1×10^{-4} M I^-	+400	−110
1×10^{-3} M ascorbate		+167	−110
1×10^{-2} M ascorbate		+131	−110
1×10^{-1} M ascorbate		+122	−109
—	1×10^{-4} M I^-	+395	−112
1×10^{-3} M Na_2SO_3		+267	−112
1×10^{-2} M Na_2SO_3		+95	−111
1×10^{-1} M Na_2SO_3		−28	−108

Interferences

The interfering effect of S^{2-}, CN^-, Br^-, SCN^- and Cl^- ions was studied by recording the electrode potential during consecutive additions of interfering ions. Fig. 4 shows the course of the potential changes in 1×10^{-5} M iodide solution at pH 2 in the presence of interfering ions. No interfering effect of Cl^- and SCN^- ions was observed. Br^- and CN^- interfered when present in a 100-fold excess over iodide. Serious interference occurred in a 1×10^{-6} M solution of S^{2-}. Relatively low interference of S^{2-} and CN^- was caused by the acidic pH of the solution, where both species exist in non-ionic form (H_2S, HCN) and do not contribute fully to the formation of the electrode potential. It is obvious that these anions, and any other anions that form complexes or precipitates with silver or mercury, interfere in the determination of silver or mercury and must be either absent or removed.

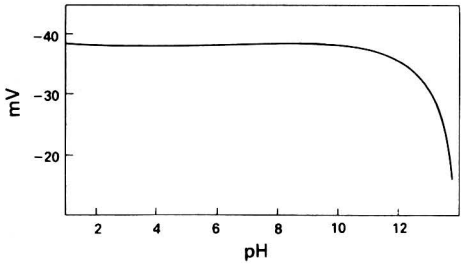

Fig. 3. Effect of pH on the potential of $Ag_2[HgI_4]$ - Ag_2S electrode in 1×10^{-5} M I^- solution.

Conclusion

It can be concluded that the electrode based on the $Ag_2[HgI_4]$ - Ag_2S membrane is superior to that based on the $Ag_2[HgI_4]$ membrane. It has the advantages of better mechanical properties, longer lifetime and better stability and is a good candidate for practical application in routine and automated analysis and monitoring. The low sensitivity of the hybrid electrode to the redox potential of the solution is a promising asset with respect to the possibility of applying this electrode to the determination of organomercury compounds after their decomposition with a strong oxidising agent.

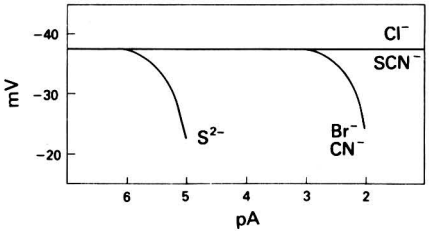

Fig. 4. Interference of S^{2-}, CN^-, Br^-, Cl^- and SCN^- ions in 1×10^{-5} M I^- solution.

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Received *July 14th*, 1980
Accepted *October 6th*, 1980

Destructive Neutron-activation Analysis of Toxic Elements in Suspended Materials Released from Refuse Incinerators*

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Trace element concentrations in the fly-ash collected from a municipal solid waste refuse incinerator have been determined using both instrumental and radiochemical neutron-activation analysis. A method for sample dissolution and subsequent selective and quantitative radiochemical separation for the determination of mercury, selenium, arsenic, chromium and cadmium is presented. Results obtained in the determination of iron, zinc, bromine, cobalt, caesium, scandium, vanadium, selenium and chromium by instrumental neutron-activation analysis are also reported.

Keywords: *Incinerator fly-ash*; *trace element determination*; *radiochemical separation*; *neutron-activation analysis*; *sample dissolution*

In recent years there has been increasing concern about the contamination produced by toxic substances in the urban atmosphere. In addition to pollutant sources such as coal- or fossil fuel-fired plants, heating units and automobile exhausts, the contribution of solid-waste refuse incinerators to urban aerosols has attracted the attention of ecologists, especially with respect to toxic volatile organic compounds.[1] In addition there is increasing interest by many communities in the use of refuse-derived fuel as an energy source for heating or electric power generation.

However, very few data are available on the trace element concentrations involved in these emissions. A study by Greenberg *et al.*[2] estimated that the contribution from refuse incineration is up to 3% of the 80 $\mu g\ m^{-3}$ particulate loading typically found in many urban areas of the USA.

Analytical monitoring of the concentrations of trace elements in fly-ash and suspended particles may contribute to our knowledge of the possible environmental impact of these emissions. Usually, instrumental neutron-activation analysis (INAA) is used to determine many elements in these matrices because of its multi-elemental capability and precision. However, in the determination of elements such as mercury, arsenic and cadmium, radiochemical separation from the neutron-irradiated matrices is necessary if maximum accuracy and precision are required. In fact, despite the relatively high concentrations of these elements, the high activity of the irradiated matrix interferes very strongly in the regions of the gamma-energies of these radioisotopes, thus requiring their selective chemical separation.

In this paper a radiochemical separation method for the determination of mercury, arsenic, selenium, chromium and cadmium in the fly-ash collected from the solid waste refuse incinerator of Bologna, Italy, is presented. In addition, the concentrations of iron, bromine, zinc, scandium, cobalt, caesium, manganese, vanadium, selenium and chromium determined by INAA are reported.

Experimental

Incinerator Plant

The Bologna municipal incinerator has three identical furnace trains, each capable of incinerating 200 metric tons of refuse daily. The refuse is burned over a series of rocking grates into combustion chambers, where the combustion gases and suspended particles reach

* Presented at the 5th SAC International Conference on Analytical Chemistry, Lancaster, July 20–25th, 1980.

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temperatures between 900 and 1050 °C. The effluent passes through a heat exchanger system that reduces the temperature to 300–330 °C and then through electrostatic precipitators to remove the fly-ash particles. The emitted smokes leave the electrostatic chambers via an 80-m stack at about 250 °C.

Reagents and Apparatus

Hydrated manganese dioxide (HMD) inorganic ion exchanger was obtained from Carlo Erba, Milan, Italy.

Zinc diethyldithiocarbamate was prepared by mixing aqueous solutions of sodium diethyldithiocarbamate with zinc nitrate.[3]

The inorganic ion-exchange separation procedure utilised 100 × 10 mm i.d. plastic chromatographic columns packed to a length of 50 mm with HMD.

The fusion - distillation apparatus shown in Fig. 1 was made of quartz tubes of 20 mm i.d. The nickel boats used were 80 mm long and 15 mm deep.

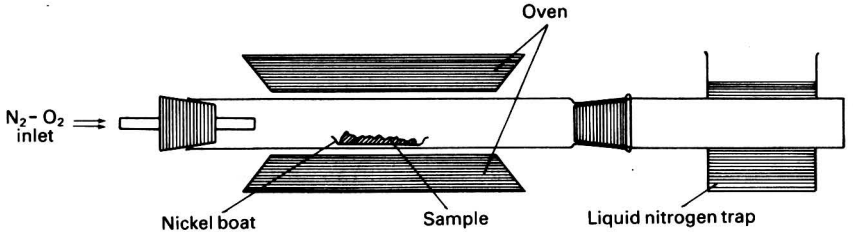

Fig. 1. Na_2O_2 - NaOH fusion and mercury distillation apparatus.

Sampling

Fly-ash samples were collected from the fly-ash settling tanks of two electrostatic chamber precipitators (A and B). Samples of about 5 kg each were homogenised to 170–180 mesh and dried over phosphorus pentoxide for 2 weeks.

Matrices Analysed

In addition to the Bologna incinerator fly-ash, the standard reference materials National Bureau of Standards (NBS) Coal Fly-ash 1633 and European Community Bureau of Reference (BCR) Coal Fly-ash 38 were also analysed in order to check the accuracy of the technique.[4]

Standards and Carriers

A multi-element primary standard was prepared from solutions of high-purity metals or compounds dissolved in electronic-grade concentrated nitric acid and/or sulphuric acid. For carrier additions, a multi-element carrier mixture prepared by mixing mercury, arsenic and chromium oxides and cadmium and selenium powders was used.

Irradiation and Nuclear Data

All samples and standards, weighed and sealed in ultra-pure quartz vials (Specpure), were neutron irradiated in the Triga Mark II Research Reactor of the University of Pavia. Three different irradiation conditions were used: (*a*) short irradiation in the pneumatic system for the instrumental determination of vanadium and manganese; irradiation for 1 min at a neutron flux of 5×10^{12} neutrons $cm^{-2}\ s^{-1}$; (*b*) irradiation in the central thimble facility for the instrumental determination of iron, zinc, bromine, cobalt, caesium, scandium, chromium and selenium; irradiation for 40 h at a neutron flux of 9×10^{12} neutrons $cm^{-2}\ s^{-1}$; and (*c*) irradiation in the central thimble facility for the radiochemical determination of mercury, cadmium, arsenic, selenium and chromium; irradiation for 16 h at a neutron flux of 9×10^{12} neutrons $cm^{-2}\ s^{-1}$.

Nuclear data for the radioisotopes used in both instrumental and radiochemical activation analysis are reported in Table I.

Sample Dissolution

One of the most important problems in the analysis of trace elements using radiochemical neutron-activation analysis is to dissolve the irradiated matrix easily, completely and without losses of volatile elements. Typically, different matrices may require different dissolution methods such as different acid mixtures, a PTFE-lined digestion bomb and fusion with appropriate media. A double problem arises when volatile elements have to be determined in highly insoluble matrices whose complete dissolution often requires high-temperature treatment. The complete dissolution of environmental materials such as fly-ash or materials derived from high-temperature combustion procedures suffers from several difficulties. These samples, and other refractory and siliceous materials, contain large amount of silica, oxides (and their mixtures), incompletely oxidised carbon and fused aluminosilicates, reflecting a typical composition characterised by high insolubility. Further, in determining elements such as mercury, arsenic and cadmium by using their radioisotopes with relatively short half-lives, a dissolution technique that requires prolonged treatment is not suitable.

The method described here consists in a rapid fusion with sodium hydroxide - sodium peroxide carried out in a closed system that allows simultaneous destruction of the sample and quantitative separation of mercury. The molten mass is then easily dissolved and the solution obtained is used for the separation of arsenic, selenium, chromium and cadmium.

TABLE I

NUCLEAR DATA USED FOR INSTRUMENTAL AND RADIOCHEMICAL NEUTRON-ACTIVATION ANALYSIS

Element	Isotope measured	Half-life	γ-Energies used for evaluation/MeV
Hg	^{197}Hg	64 h	0.077
	^{203}Hg	47 d	0.279
	^{76}As	26.3 h	0.559
Se	^{75}Se	121 d	0.136, 0.265
Cr	^{51}Cr	27.8 d	0.320
Cd	^{115}In^{m}	4.5 h	0.336
	^{115}Cd	2.3 d	0.527
Fe	^{59}Fe	45.1 d	1.099
Zn	^{65}Zn	245 d	1.115
Br	^{82}Br	35.34 h	1.317
Co	^{60}Co	5.24 yr	1.332, 1.173
Cs	^{134}Cs	2.07 yr	0.796
Sc	^{46}Sc	83.9 d	0.889
V	^{52}V	3.76 m	1.434
Mn	^{56}Mn	2.58 h	0.847

Fusion and Mercury Separation

After irradiation the quartz vials were opened and the samples (about 100 mg), together with a few milligrams of the multi-element carrier, were placed in the nickel boat and carefully mixed with about 900 mg of sodium peroxide and 150 mg of sodium hydroxide. The boat was then placed in a quartz tube connected to a liquid nitrogen trap. A stream of transporting gas (air) was passed through the apparatus at a flow-rate of 40 ml min^{-1} (see Fig. 1). The nickel boat was first heated gently with a Bunsen burner flame in order to achieve slow fusion of the sample; then the apparatus was placed in the tube furnace and the temperature was gradually increased to 850 °C. When the fusion was completed, the mercury fraction, quantitatively distilled and trapped in the cooled quartz tube, was transferred into a polyethylene counting vial using concentrated nitric acid and distilled water. Table II gives the operating conditions used in the fusion - distillation procedure.

The molten mass was then dissolved in distilled water and 3 M nitric acid; a few drops of concentrated nitric acid and distilled water were used for the final washing of the nickel boat. The resulting solution was saved for the subsequent radiochemical separation.

TABLE II

FUSION AND MERCURY DISTILLATION OPERATING CONDITIONS

Sample/mg	Carriers/mg	Na_2O_2/mg	NaOH/mg	Oven temperature/°C	Rate of temperature increase/°C min^{-1}	Transporting gas flow-rate/ml min^{-1}
100–130	HgO, 10 Se, 2 Cr_2O_3, 5 Cd, 2 As_2O_3, 3	900–1000	200–250	From 750 to 850	3.33	~40

Radiochemical Separation

Fig. 2 shows the scheme for the radiochemical separation followed by the simultaneous determination of mercury, selenium, chromium, arsenic and cadmium. A similar radiochemical separation has previously been used for the determination of arsenic, antimony, selenium, chromium, cadmium and copper in other matrices.[5] In this work, as the dissolution conditions were completely different, possible interferences produced by the high concentrations of Na^+ and Ni^{2+} ions were investigated. However, a series of experiments using radiotracers showed that even with high Na^+ and Ni^{2+} concentrations (30 and 10 mg ml^{-1}, respectively) no appreciable interferences in the HMD absorption and in the cadmium solvent extraction were noticed.

The approximately 3 M nitric acid solutions obtained by dissolving the molten mass were heated at about 80 °C to give a clear green solution, then cooled and passed through HMD columns that had been pre-conditioned with 3 M nitric acid. The flow-rate was adjusted to 0.5 ml min^{-1}. Arsenic, selenium and chromium were quantitatively retained on the HMD beds, and the eluted fractions were collected for the cadmium extraction.

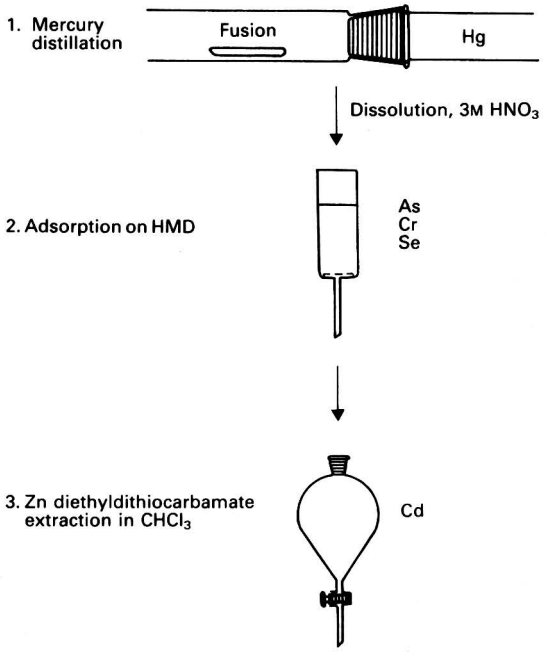

Fig. 2. Radiochemical separation scheme adopted for Hg, Se, As, Cd and Cr determination.

The pH of the eluted fractions was adjusted to 1.5 with ammonia solution and the cadmium extraction was carried out by shaking with 20 ml of a 0.005 M solution of zinc diethyldithiocarbamate in chloroform.[3]

Arsenic, selenium and chromium were determined by counting the gamma activities of the dismantled HMD columns.

The organic fractions containing the cadmium were allowed to stand for 24 h to establish equilibrium between ^{115}Cd and $^{115}In^{m}$ and the gamma-rays from the decay of both of these isotopes were used for cadmium analysis.

Treatment of Standard Solutions

Standard solutions for analysis were prepared in two ways. A first series of standard solutions, irradiated together with the samples, were treated in the same way as the samples by pipetting a known amount on to non-irradiated fly-ash solution. The second series of standards was prepared by pipetting known amounts of irradiated standards directly on to HMD in the polyethylene counting vials for the determination of arsenic, chromium and selenium. The cadmium standards were pipetted into 3 M nitric acid and extracted in the same manner as the samples.

TABLE III

RESULTS FOR A SERIES OF SIX RADIOCHEMICAL SEPARATIONS USING RADIOTRACERS

Element	γ-Activity, %				
	Nickel boat	Liquid nitrogen trap	HMD column	Eluted fractions	Organic phases
Hg	n.d.*	99.12 ± 4.79	<0.005	<0.005	n.d.
Se	<0.005	<0.005	98.90 ± 4.00	<0.005	n.d.
Cr	0.01 ± 0.002	n.d.	99.98 ± 3.08	n.d.	n.d.
As	n.d.	n.d.	98.00 ± 4.25	n.d.	n.d.
Cd	n.d.	n.d.	n.d.	99.02	98.72 ± 3.31

* n.d. = not detected.

Results and Discussion

The over-all radiochemical procedure was tested by using mercury, selenium, arsenic, chromium and cadmium radiotracers. Table III gives the results obtained in a series of six different radiochemical separations.

The method was then applied to the analysis of standard reference materials for which the contents of the elements of interest are certified or suggested. Tables IV and V give the results obtained for the analysis of NBS Coal Fly-ash 1633 and BCR Coal Fly-ash 38. The results indicate very good agreement between the certified and found values, demonstrating that the procedure is accurate. The recovery of mercury was quantitative and its selective isolation from the matrix allowed the use for its determination of the more sensitive ^{197}Hg isotope (77 keV).

Arsenic, selenium and chromium were completely retained on the HMD from 3 M nitric acid, whereas the elution of cadmium was complete. The decontamination of the elements

TABLE IV

RADIOCHEMICAL DETERMINATION OF HG, AS, SE, CR AND CD IN NBS COAL FLY-ASH 1633

Element	Concentrations found/μg g^{-1}	Mean ± standard deviation/μg g^{-1}	Certified values/ μg g^{-1}
Hg	0.16, 0.12, 0.13, 0.12, 0.13, 0.15, 0.15	0.137 ± 0.015	0.14 ± 0.01
Se	10.71, 11.10, 11.02, 10.75, 9.65, 9.22, 9.77	10.3 ± 0.7	9.4 ± 0.5
Cr	128.9, 127.3, 129.2, 136.2, 137.1	131.7 ± 4.6	131 ± 2
As	56.9, 58.1, 56.2, 57.8, 60.3, 59.8	58.1 ± 1.6	61 ± 6
Cd	1.36, 1.37, 1.39, 1.50, 1.46, 1.51	1.43 ± 0.07	1.45 ± 0.06

TABLE V

RADIOCHEMICAL DETERMINATION OF Hg, As, Se, Cr AND Cd IN BCR COAL FLY-ASH 38

Element	Concentrations found/μg g^{-1}	Mean $\pm$ standard deviation/μg g^{-1}	Suggested values/ μg g^{-1}
Hg	2.01, 1.95, 1.87, 2.12, 2.05, 1.85	1.98 $\pm$ 0.10	2.10
Se	8.25, 7.98, 8.53, 7.85, 8.81	8.28 $\pm$ 0.50	—
Ag	47.8, 45.3, 49.6, 44.8, 50.0	48.20 $\pm$ 2.70	48.10
Cr	171, 165, 188, 191, 176	178 $\pm$ 11	171
Cd	3.98, 3.21, 3.65, 3.47, 3.91	3.64 $\pm$ 0.32	4.74

TABLE VI

RADIOCHEMICAL DETERMINATION OF Hg, As, Se, Cr AND Cd IN THE FLY-ASH FROM ELECTROSTATIC PRECIPITATOR A

Element	Concentrations found/μg g^{-1}	Mean $\pm$ standard deviation/μg g^{-1}
Hg	4.81, 5.00, 4.05, 4.6, 3.97	4.48 $\pm$ 0.45
Ag	37.9, 31.1, 29.4, 29.8, 33.5	32.3 $\pm$ 3.5
Se	6.92, 7.86, 6.74, 8.06, 8.21	7.55 $\pm$ 0.67
Cr	1501, 1479, 1639, 1662, 1689	1594 $\pm$ 97
Cd	104, 160, 116, 110, 173, 125	131 $\pm$ 28

of interest from ^{24}Na and other radioactive matrix interferents can be estimated to be a factor of 10^7.

Tables VI and VII give the results obtained in the analysis of the Bologna fly-ash collected in electrostatic precipitators A and B, respectively. The concentrations found in the two different series of samples lay in the same range, but the relatively high standard deviations for cadmium and mercury in both fly-ashes suggest a possible inhomogeneity with regard to these two elements. The high chromium concentration compared with those of the other elements is related to their different volatilities. Consequently, the concentrations of mercury and cadmium should be considered fairly high, especially when their toxicity is considered.

The INAA determination of iron, zinc, bromine, cobalt, scandium, manganese, caesium, vanadium, chromium and selenium in the fly-ash collected at electrostatic precipitators A and B are given in Tables VIII and IX, respectively. The results for selenium and chromium, compared with those obtained by radiochemical separation, are in relatively good agreement even though a better precision is obtained in the determination of selenium by the radiochemical method. The concentrations of vanadium and bromine differ over a wide range of values between the two series of samples, probably because of changes in the composition of the refuse burned in the two different combustion chambers.

Conclusion

As this work is concerned mainly with the development of a radioanalytical technique, no discussion of the possible environmental impact of the incinerators is included. For this purpose more data and more parameters would have to be considered, such as sampling of the suspended particles released from the stack, size of the particles, seasonal variations and

TABLE VII

RADIOCHEMICAL DETERMINATION OF Hg, As, Se, Cr AND Cd IN THE FLY-ASH FROM ELECTROSTATIC PRECIPITATOR B

Element	Concentrations found/μg g^{-1}	Mean $\pm$ standard deviation/μg g^{-1}
Hg	4.04, 4.33, 2.97, 3.21, 3.02	3.51 $\pm$ 0.65
As	21.5, 23.9, 22.7, 20.6, 24.0	22.5 $\pm$ 1.5
Se	8.29, 8.82, 10.02, 9.51, 8.65	9.05 $\pm$ 0.70
Cr	1307, 1298, 1247, 1215, 1275	1268 $\pm$ 34
Cd	135, 107, 124, 138, 125, 109	123 $\pm$ 12.8

TABLE VIII

INSTRUMENTAL NEUTRON-ACTIVATION ANALYSIS OF FE, ZN, CO, SC, MN, V, CR, CS, BR AND SE IN THE FLY-ASH FROM ELECTROSTATIC PRECIPITATOR A

Element	Concentrations found/μg g^{-1}	Mean $\pm$ standard deviation/μg g^{-1}
Fe*	2.85, 3.04, 3.25, 3.16%	3.07 $\pm$ 0.17%
Zn*	2.50, 2.55, 2.87%	2.64 $\pm$ 0.20%
Br	380, 330, 341, 325	344 $\pm$ 25
Co	39.5, 38.0, 40.7, 35.0	38.3 $\pm$ 2.4
Cs	5.47, 5.96, 6.03, 6.14	5.9 $\pm$ 0.29
Sc	3.23, 3.17, 3.57, 3.30	3.31 $\pm$ 0.17
Mn	1284, 1351, 1328	1321 $\pm$ 34
V	48.7, 39.5, 37.6, 43.5	42.3 $\pm$ 4.9
Se	6.87, 6.90, 5.37, 4.98	6.03 $\pm$ 1.00
Cr	1549, 1568, 1728	1615 $\pm$ 98

* Concentration in %.

TABLE IX

INSTRUMENTAL NEUTRON-ACTIVATION ANALYSIS OF FE, ZN, CO, SC, MN, BR, CS, V, CR AND SE IN THE FLY-ASH FROM ELECTROSTATIC PRECIPITATOR B

Element	Concentrations found/μg g^{-1}	Mean $\pm$ standard deviation/μg g^{-1}
Fe*	2.76, 2.55, 3.32, 3.0%	2.90 $\pm$ 0.35%
Zn*	2.16, 2.87, 2.85, 2.5%	2.60 $\pm$ 0.33%
Br	123, 172, 135, 165	148 $\pm$ 23
Co	26.8, 34.7, 32.7, 30.0	31.0 $\pm$ 3.5
Cs	5.05, 4.76, 5.22, 4.91	4.98 $\pm$ 0.20
Sc	3.28, 3.91, 4.16, 3.62	3.74 $\pm$ 0.37
Mn	1054, 1221, 1178	1151 $\pm$ 86
V	125, 120, 103	116 $\pm$ 11
Se	11.04, 6.14, 6.91, 10.12	8.55 $\pm$ 2.39
Cr	1306, 1337, 1319	1320 $\pm$ 15

* Concentration in %.

different types of incinerators.[6,7] However, the proposed procedure meets all the requirements for this type of analysis. The complete dissolution of the matrix is obtainable in a relatively short time and the high selectivity in separating the elements of interest improves the accuracy and sensitivity.

Further, the method allows the quantitative recovery of the elements being analysed, thus avoiding the need for the calculation of chemical yields, which can increase the analytical error.

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Received *July 25th*, 1980
Accepted *October 6th*, 1980

Analyst, March, 1981, Vol. 106, pp. 335–343

Fluorimetric Assay of Polyanions in Complex Fluids: Carrageenan Stabilisers in Dairy Products and Heparin in Hog Mucosa Extracts

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The spectrofluorimetric assay of several polyanions, particularly κ-carrageenan and heparin, is discussed. The presence of lipid and protein significantly affects the assay and techniques are described to overcome these interactions. These methods are then extended to complex real systems, in particular to carrageenan-stabilised milk products and heparin-containing hog mucosa extracts. The significance of the findings in terms of a general assay for biological fluids is discussed, as well as a consideration of the standards and conditions required for complete characterisation.

Keywords: *Spectrofluorimetry*; *carrageenan*; *heparin*; *biological fluids*; *polyanions*

There are many areas in which a rapid and accurate assay procedure for polyanions in complex fluids would be of great advantage. The present work is concerned with the assay of polyanions in general. Particular attention is given to the elucidation of those aspects of the solution environment which present obstacles to the fluorimetric measurement of polyanion concentrations. The basis of the fluorescence technique used has been described elsewhere.[1,2] Using this method it has been found possible to assay accurately dilute aqueous solutions of the sulphated polysaccharide carrageenan, which is used extensively as a stabiliser in a variety of dairy products. Several elaborate methods exist for the assay of carrageenan in such systems[3] and for plant sulphated polysaccharides in general.[4] The fluorescence technique has been extended to provide a systematic assay for carrageenan and other stabilisers (such as carboxymethylcellulose) and, where possible, to ascertain the presence of mixtures of stabilisers. Similarly, the assay of porcine intestinal mucosa is important since this raw material is the major source of pure heparin.[5] The objective in this instance is to monitor the total heparin concentration at various stages of the commercial isolation procedure. Previous assays[3] have encountered difficulties with pigmented materials. Further problems are anticipated because dye binding to polyanions is sensitive to the concentration of metal ions,[6,7] basic amino acids[8] and other monomeric ligands,[9,10] while proteins such as the caseins are known to bind to various carrageenan fractions in the presence of calcium ions.[11]

Experimental

Fluorescence measurements were made with an instrument previously described.[12] A brief description of the dye binding technique is given here, but a more complete account can be found elsewhere.[1,2] Emission from a 1.0×10^{-5} M acridine orange solution was excited at 450 nm and monitored at 540 nm in the right-angled viewing mode. Under these circumstances the 540-nm emission intensity is proportional to the free dye concentration in the solution. Addition of polyanion quenches the free dye emission as the dye binds to the polymer. Addition of a series of aliquots of the polyanion allows the direct recording of a titration curve such as that shown in Fig. 1 for κ-carrageenan. Extrapolation of the initial quenching region to zero emission gives an accurate measure of the concentration of anionic sites in the carrageenan solution as it is found experimentally that under these conditions one dye molecule binds to each ionic site. If we define the relative molecular mass of a polyanion as the average mass of polymer containing one anionic site, then from Fig. 1 it is observed that 4 ml of a 10^{-5} M solution of dye will be completely quenched by 40 μl of a 10^{-3} M

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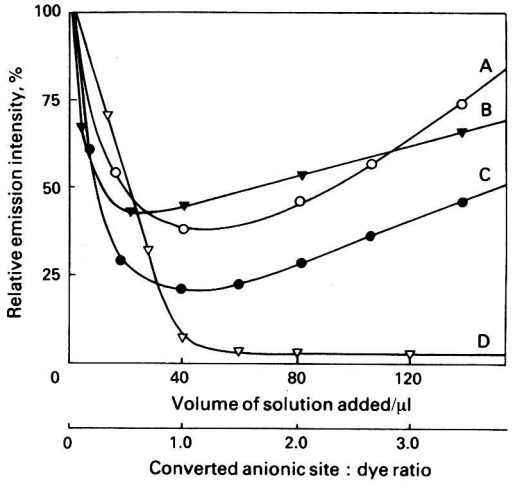

Fig. 1. Interference in the metachromatic activity of carrageenan upon incorporation into milk products. Titration graphs for: A, control, the addition of undoped babyfood to the dye; B, bovine pasteurised milk doped with carrageenan, 10^{-3} M; C, a commercial babyfood doped with carrageenan, 10^{-3} M; D, pure carrageenan, 1.0×10^{-3} M. The concentration of acridine orange was 10^{-5} M in all instances.

polyanion solution, which corresponds to a polyanion site to dye ratio of unity. The titration end-point E, in microlitres, for an unknown polyanion solution is then related to the anionic site concentration, M, by

$$E \times M = V \times 10^{-2}$$

where V is the volume (ml) of the 10^{-5} M dye solution. Titration end-points can be conveniently converted into concentrations using a calibration graph (Fig. 2), which in this instance has $V = 4$ and 5 ml.

The advantages of emission spectroscopy over spectrophotometry are sensitivity and resolution. The latter arises because the absorption spectrum of acridine orange, when free or bound to polyanions, consists of many overlapping bands[1,13] while the fluorescence simplifies into two peaks, corresponding to free dye ($\lambda_{max.} \approx 530$ nm) and bound dye ($\lambda_{max.} \approx 640$ nm), the latter being of much reduced intensity. Monitoring the emission at 540 nm therefore eliminates interference from bound species which distort the binding profile considerably.

Absorbance measurements were made on a Pye Unicam SP1800 spectrophotometer. Acridine orange was purified as described elsewhere.[6]

Interaction Studies

The procedures to be applied to complex systems were devised by looking in turn at the effects of various representative additives on the shape of the standard titration curve (Fig. 1). Bovine serum albumin (BSA) (Serva, Heidelberg) was selected as a model for protein interactions. To elucidate any interference from lipid material, commercially available animal fats (*e.g.*, lard) were dispersed in water by ultrasonic mixing before the addition of carrageenan. The effect of other neutral polysaccharides has already been investigated and found to be minimal.[2]

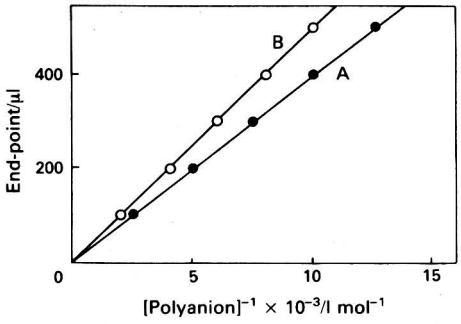

Fig. 2. Calibration graphs for relating measured microlitre end-points to the anionic site concentration in an unknown solution. Dye concentration, $C^{\circ} = 10^{-5}$ M, total volume of dye = A, 4 ml and B, 5 ml.

Carrageenan and Dairy Produce

The sodium salt of κ-carrageenan (Copenhagen Pectin Co.) gave an exactly stoicheiometric end-point upon acridine orange titration (Fig. 1), establishing its suitability for this study. This graph serves as a reference for all subsequent doping experiments. Doping was effected by adding carrageenan to a fresh, polyanion-free dairy product (bovine pasteurised whole milk, babyfoods, flavoured milks, etc.) to give a final carrageenan content of 10^{-3} M. The mixtures were stirred gently overnight at 2 °C to allow equilibration of the stabiliser - product interactions. Metachromatic activity in such doped samples was greatly impaired (Fig. 1) in most instances and virtually eliminated in others. The objective of the assay procedure was to reduce this interference to a level where the full metachromatic activity of the stabiliser was returned. Many of the initial stages of the procedure parallel an earlier study by Graham[3] which used papain digestion at 70 °C to release carrageenan from protein complexes.

Heparin and mucosa extracts were supplied by Abbott Laboratories, North Chicago, Ill., USA. Various crude heparin samples representing stages in the commercial isolation of heparin from mucosa were from the same source.

Samples of babyfood were supplied by Ross Laboratories, Columbus, Ohio, USA. Whole milk was homogenised by shaking prior to doping. Chocolate milk (Satia, Carentan, France) had an unknown level of carrageenan stabiliser. Enzyme digestions were performed in an incubator thermostated at 37 °C. Digestion was self-indicating and was deemed complete upon clarification of the vessel contents as the hydrolysis products are water-soluble. The proteolytic enzymes used were trypsin (Calbiochem, Bishop's Stortford), α-chymotrypsin (Seravac Labs., Maidenhead) and Carlsberg subtilisin (Novo Industries, Copenhagen). These three enzymes exhibit maximum activity in the pH ranges 7–9, 7–9 and 10–11, respectively. They were selected to give a wide range, non-specific hydrolysis.[14] Optimum conditions for the digestion were determined for the trypsin - casein system in particular using the assay described by Laskowski.[15] A ratio of 10 mg of casein to 0.01 mg of trypsin gave almost complete digestion after 20 min. Addition of carrageenan at concentrations between 10^{-5} and 10^{-3} M had little effect on the rate of hydrolysis, in agreement with the observations of Vaughan *et al.*[16] Addition of sodium chloride up to 1.0 M, however, resulted in about 50% reduction of efficiency. These results are presented more fully elsewhere.[17] In all digestions 5 mg of each enzyme were used in 20 ml of solution.

Celite 545 was activated, as described by Graham,[3] immediately prior to use. Celite filtrations were performed by adding a small amount[3] of the activated material to the digestate, shaking gently for 30 min and filtering through glass-wool which had been washed in diethyl ether and water before drying at 130 °C.

Solid de-fatted chocolate milk samples were homogenised in a Potter-Elvehjem tissue grinder (Fisher Scientific Co., Pittsburgh, Pa., USA) prior to extraction.

Results

Polyanion Solutions and Models for Complex Interactions

The effect of a typical protein, BSA, on the uptake of acridine orange by κ-carrageenan is indicated in Fig. 3. BSA and polyanion were pre-mixed and equilibrated together for about 12 h before titration. Even at protein concentrations as low as 0.15 mg ml^{-1} the metachromatic activity of the polyanion was severely impaired, while 0.5 mg ml^{-1} of BSA virtually destroyed dye binding. Various standard methods for the removal of proteins from solution were performed on a test solution (10^{-3} M in carrageenan containing 1.0 mg ml^{-1} of BSA). The results of these operations on the dye binding titration graph are shown in Fig. 4. Ammonium sulphate precipitation in acidic, neutral or basic conditions resulted in reduced metachromatic activity. Other standard precipitants (caesium chloride and trichloroacetic acid) behaved similarly and have been omitted for clarity. It appears that most of the polyanion is co-precipitated with the protein fraction. It is unlikely that this is due entirely to electrostatic interactions because residual activity was not increased by precipitation from media of high pH and ionic strength which would preclude such binding. These conditions are known to dissociate polyanion - protein complexes.[18] Digestion with the three enzyme system at neutral or acid pH gave titration graphs (Fig. 4) that showed little improvement, while centrifugation of the hydrolysate indicated a supernatant of very low activity. Digestion at pH 9.0 in Tris - hydrochloric acid buffer,[19] however, resulted in dye binding graphs that were relatively free from interaction effects and, moreover, accurately reflected the initial doping concentration of carrageenan (Fig. 4).

The addition of lipids (about 1.0 mg ml^{-1}) had a pronounced effect on the shape of the dyebinding curve, similar to that produced by BSA (Fig. 3), even a qualitative estimate of the carrageenan concentration being impossible with the curve increasing monotonically after an initial minimum. Lipids were best extracted by the Folch method (see for example ref. 20) which involves partitioning the aqueous system with chloroform - methanol in the ratio 20 + 30 + 40 V/V. A small amount (about 100 mg) of sodium chloride improves the separation of the phases. Removal of methanol from the aqueous phase by extensive dialysis again produced titration graphs free from interference.

Non-pigmented Dairy Produce Doped with Stabiliser

Attempts to digest doped milk samples directly, at pH 9 and 37 °C, were unsuccessful. Preliminary treatment of the starting material with chloroform - methanol as described above resulted in the separation of the mixture into three phases after centrifugation. The

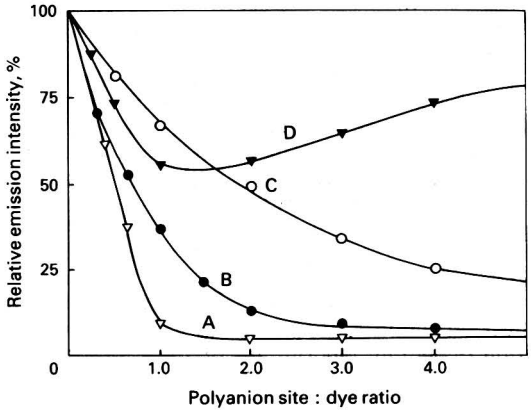

Fig. 3. Dyebinding titration graphs for 10^{-3} M carrageenan (A); for carrageenan solution with an added Bovine Serum Albumin concentration of 0.15 mg ml^{-1} (B) and 0.5 mg ml^{-1} (C); and for carrageenan solution containing 1 mg ml^{-1} of dispersed animal fats (D). The acridine orange concentration was 1.0 × 10^{-5} M in each instance.

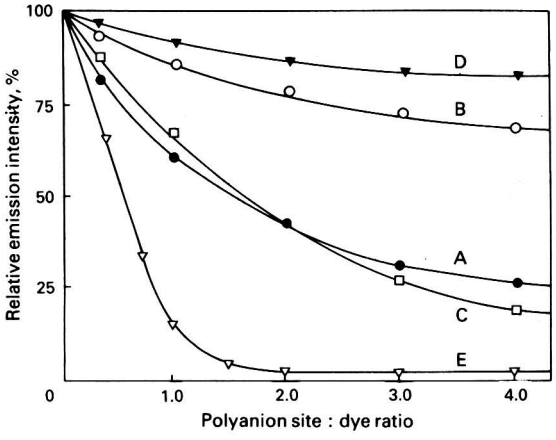

Fig. 4. Dyebinding titration graphs for a solution containing carrageenan (10^{-3} M) and bovine serum albumin 1 mg ml^{-1} (A), and for this same solution after various operations to reduce the protein concentration had been performed: B, treatment by saturation with ammonium sulphate and filtration of the precipitate formed after 6 h; C, digestion at neutral or acid pH; D, after centrifugation of the neutral hydrolysate above; and E, digestion at pH 9.0 in Tris - hydrochloric acid buffer.

lower organic phase was discarded. The upper aqueous phase was found to have no metachromatic activity after extensive dialysis and was also discarded. The third phase, a solid white layer of predominantly denatured protein, collected at the interface between the two solvent phases and could be collected intact after centrifuging. Freeze-drying and digestion resulted in complete clarification of the mixture. Subsequent Celite filtration and dialysis of the hydrolysate produced a highly metachromatically active dialysate, giving a titration graph, which, although not giving an end-point as sharp as that for the standard carrageenan solution, indicated stabiliser recoveries of 95–98% of the initial doping value. The over-all procedure is outlined in Fig. 5. Identical treatment of a doped babyfood sample according to this scheme (Fig. 5) also produced highly active extracts which indicated total carrageenan recoveries in the same range as for the milk samples (*i.e.*, 90–98%).

It is possible to confirm that such quenching curves are indeed due solely to the polyanion by observing the reversal of metachromasia which has been reported upon the addition of polycation to a dye - polyanion complex.[8] The test is particularly useful in systems such as those under study here, where spurious quenching processes are possible (see Fig. 1, control graph).

Pigmented Milk Produce

Special problems were encountered with chocolate milks because the protein phase was highly pigmented and could not be digested directly, apparently owing to inactivation of the enzyme system. An alternative procedure, based on the solubility of the stabiliser fraction under conditions where any interaction of the latter with the insoluble denatured protein fraction was prevented (*i.e.*, high ionic strength and pH[1,18]), is outlined in Fig. 5. The carrageenan was extracted from the homogenised solid phase into pH 9 buffer containing sodium chloride (2.0 M) with agitation for periods of up to 12 h at elevated temperatures (60–100 °C). Under these conditions a small amount of pigment was inevitably co-extracted and although the aqueous phase, after filtration and dialysis, was highly active, the end-point was often ill-defined. Treatment of the extract with activated charcoal or the

proteases had only a minor effect. Furthermore, the period of extraction and the temperature required for maximum recovery varied from sample to sample. Table I shows how recoveries varied for doping of a sample at various stages during the extraction.

TABLE I

TITRATION END-POINTS AND MEASURED STABILISER CONCENTRATIONS FOR CHOCOLATE MILK SAMPLES DOPED WITH CARRAGEENAN IN ADDITION TO THE COMMERCIAL STABILISER CONTENT

Samples were doped with 1 mg per 50 ml of carrageenan in addition to the commercial stabiliser content. Doping was effected at various points in the assay scheme (see Fig. 5).

Point at which doping was effected	End-point/μl	Approximate stabiliser concentration/mg per 50 ml
No doping	158	2.1
Beginning	130	3.0
After freeze-drying	119	3.2
End	115	3.2
Blank (1 mg of stabiliser in 50 ml of water)	300	1.0

Heparin and Heparin-containing Extracts

The assay of heparin in solution poses problems peculiar to this polyanion. One of these difficulties is illustrated by the data shown in Table II. Various powdered heparin samples of differing purity, when assayed by the USP method[21] and by dye binding,[2] indicated little correlation between anticoagulant and metachromatic activity. The latter is generally higher and this suggests that small concentrations of an impurity such as a bound protein may have a profound effect on the bio-activity of a particular sample (and may lead to totally variable results depending on the aspect of the anticoagulant process that is being investigated[22]). The ability of a heparin molecule to bind acridine orange is not usually affected so dramatically. The activities given in Table II were calculated by assigning a value of 150 units mg^{-1} to the purest sample of the drug available.

It is not necessary to extract the pure, totally active form of the drug before assay. Instead, the final scheme should reduce the level of interfering interactions to such a level that dye binding can occur and therefore allow an estimation of the total available heparin in a mucosa extract or crude preparation. Even the assay of such crude fractions has some diagnostic value in assessing purity. For instance, that fraction (Table II) having a USP activity of 119.8 units mg^{-1} is known from metachromatic activity to be at least 95% pure, this small impurity level causing some 20% loss of bio-activity. With this in mind, the general assay scheme outlined in Fig. 5 was applied directly to a hog mucosa extract of USP activity 25–35 units ml^{-1}. The yellow - brown fluid extract was obtained from crude mucosa by sodium sulphate extraction at 50–60 °C and pH 10. After chloroform - methanol treatment the situation was greatly simplified as all pigmentation remained in the solvent phases, which showed no metachromatic activity, leaving two colourless solid phases the

TABLE II

ANTICOAGULANT AND METACHROMATIC ACTIVITIES OF SOME PURE AND LOW-POTENCY HEPARIN SAMPLES

Metachromatic activities are relative to the value 150 units mg^{-1} assigned to pure heparin.

Anticoagulant activity/ USP units mg^{-1}	Metachromatic activity/ units mg^{-1}	Source
150	150	Hog mucosa
147	102	
119.8	142.5	
78.2	133	
36.6	135.5	
40.8	125	
65.0	109	Beef lung

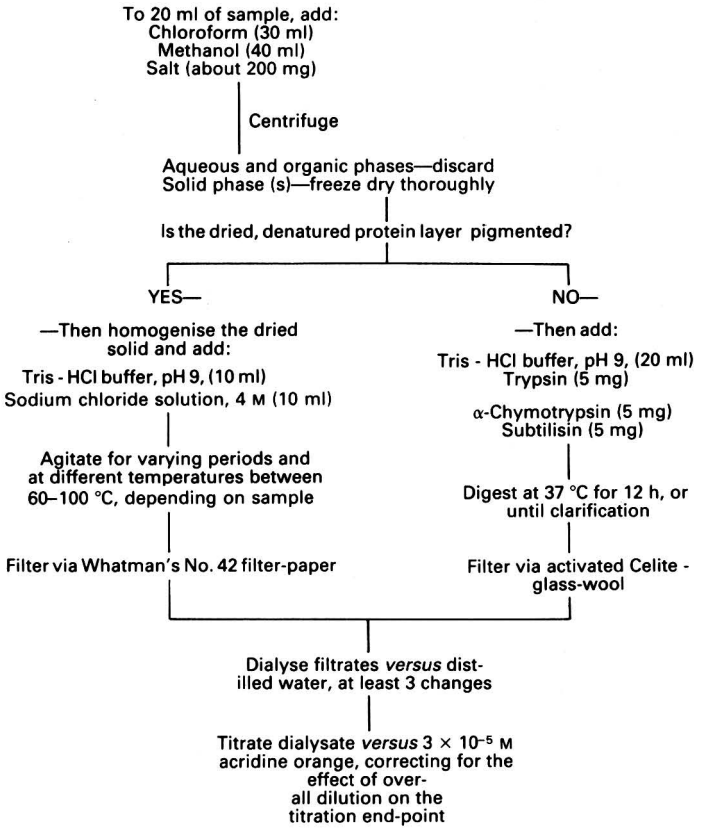

Fig. 5. General scheme for the analysis of carrageenan-stabilised milk products, heparin-containing mucosal extracts and polyanion-containing fluids.

second of which formed a sediment at the bottom of the tube. Both solid phases showed pronounced activity after drying, digestion, Celite filtration and dialysis. In subsequent work the two phases were combined prior to drying. Delipidation with an acetone - diethyl ether mixture (1 + 1) precipitated a single solid phase, but this was considerably pigmented. Analysis of six such mucosa extracts, according to Fig. 5, indicated polyanion site concentrations of between 9×10^{-4} and 1.6×10^{-3} M.

Discussion

Fluorescence spectroscopy provides a powerful technique for the analysis of polyanions in general, the method having the important advantages of speed, sensitivity and accuracy. Problems occur particularly with respect to standardisation of the conversion from anionic site concentration to a mass concentration, each polyanion having peculiarities due to variations in structure. With the carrageenans, problems arise owing to the presence of varying proportions of the major fractions (κ, λ, ι) in most common stabiliser mixtures.[23,24] Using data previously measured for the different dye binding capacities of these major fractions[2] it is evident that failure to account for this variation could lead to errors as large as 30% in extreme cases. Therefore, it is important that the standard carrageenan used in constructing calibration graphs is of as similar composition as possible to that of the stabiliser.

Variations in carrageenan to carboxymethylcellulose (CMC) ratios in stabiliser mixtures can be measured by using the greatly differing pK_a values of these polyanions.[17] Titration at pH 7 allows assay of the total polyanion site concentration, while titration at pH 3 enables an independent determination of the carrageenan, the CMC being calculated from the difference. Determination of the CMC in terms of mass, which can easily be achieved by the methods described above, also requires suitable standardisation, as a wide variation in degree of carboxylation exists among commercial stabilisers.

The problems involved in the standardisation of heparin preparations are different. As with other glycosaminoglycans, the average degree of sulphation and/or carboxylation is fairly well defined,[25] although variations in carboxyl to sulphate ion ratios are known to exist in materials from different sources.[22] Of greater significance with respect to pharmaceutical preparations is the apparent disparity between the many chemical and biological assays, which exists when they are applied to heparins even from the same source. Many explanations have been proposed to account for this. Some authors relate discrepancies to the amount of "bound" impurities, others to the extent of degradation of the polymer due to the method of extraction, either by depolymerisation or by loss or exchange of particularly labile functions. While no firm conclusions can be made on the basis of our data, results included in Table II favour the former explanation. Differences may also result from the relative molecular mass polydispersity of heparins,[26] particularly if partial degradation has occured. A hexasaccharide degradation fragment produced enzymatically[27] showed no anticoagulant activity but had significant metachromatic activity towards toluidine blue. Considering such effects, it would be surprising to find total agreement between the various assays, particularly between chemical and biological methods.[28,29]

The same considerations apply to the analysis of polyanions in more complex fluids but with additional complications since both lipids and proteins interfere with the dye binding of the polyanion (Fig. 3). BSA proved a useful model in this respect as the degrees of interaction of both BSA and caseins with κ-carrageenan are known to be similar.[10,17] This is also illustrated (Fig. 1) in that the titration graph for doped milk is equivalent to simple additivity of the component curves (*i.e.*, milk plus carrageenan), the metachromatic activity of the polyanion being obscured by the other components. The removal of protein was generally best achieved by digestion under conditions where the protein - polyanion interaction was disrupted, otherwise digestion produced titration curves which still showed the effect of protein interference. The use of model lipid systems again indicated suitable conditions for the removal of interference, using chloroform - methanol treatment. In the complex systems studied it was necessary to remove lipid prior to protein digestion to achieve satisfactory assays.

It is interesting to consider the effect of lipids on the titration (Fig. 3). In addition to apparently reducing proteolytic activity, the presence of lipid in the fluid characteristically resulted in titration curves which increased monotonically (after an initial minimum) as the concentration of lipid increased. This may be due to the dye assuming an increasingly hydrophobic environment, at least initially, although the dispersions rapidly become turbid with consequent large contributions to the results because of light scattering.

There are many facets of the procedure which have not been considered in this work. For example,[1] variation of pH allows a determination of the carboxyl to sulphate ion ratio in a particular polymer such as heparin or in a simple mixture of stabilisers such as CMC, carrageenan or alginate commonly used commercially. These results will be reported separately. Of particular relevance to the present scheme is the fact that the pH of the solutions for the determination of polyanions such as heparin must be carefully controlled because at pH 3–5 the metachromatic activity is a continuous function of pH. Dye solutions for such titrations should consequently be buffered or adjusted to a pH close to neutral.

A condition deserving comment concerns the optimum dye concentration (C°) which should be used for a particular assay. Ideally, $C^\circ = 10^{-5}$ M should be adequate; higher concentrations give problems owing to self-aggregation of the dye, while lower concentrations result in reduced sharpness and non-stoicheiometric binding at the theoretical end-point.[6] In several of the systems studied the final extract showed reduced affinity for acridine orange which could be compensated for, in part, by increasing the concentration to 3×10^{-5} M. This was particularly true for chocolate milks where the final dialysate retained some coloration and titration *versus* 10^{-5} M dye gave a rather indistinct end-point. The errors in such

titration graphs are about 5%, compared with 2% for the standard carrageenan preparation. Superimposed on this is the error due to loss and/or degradation of the stabiliser during the extraction. Recoveries from doped samples indicated up to 10% loss on average after doping at the start of the assay procedure but by taking the maximum commonly occuring value for a number of readings the accuracy can be improved considerably. This maximisation procedure applies to any material assayed by the general scheme (Fig. 5) as it should be impossible to measure a polyanion concentration higher than the actual value once spurious quenching has been eliminated.

Conclusions

In conclusion, a general assay scheme, applicable to a variety of polyanion-containing fluids, has been at least partly realised in the scheme outlined in Fig. 5. Acceptable recoveries, relative to doping values, suggest that the determination of indogenous polyanion contents of biological fluids can be undertaken, but it must be stressed that this scheme requires flexibility on the part of the analyst to deal with systems of differing compositions. This must be achieved under conditions, such as extremes of pH and temperature, where degradation of the polyanion is avoided (particularly for the glycosaminoglycans with labile substituents) so that the validity of the initial reference standard is maintained.

We thank Abbott Laboratories, North Chicago, Ill., USA, for financial support and samples.

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Received *June 3rd*, 1980
Accepted *October 14th*, 1980

Use of Thiaminase I in the Determination of Thiamine in Biological Materials

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The usefulness of thiaminase I enzyme in preparing "thiamine-free" blanks for the determination of non-thiochrome fluorescence in thiamine determinations has been evaluated. The level of thiaminase I has been optimised and an improved thiochrome procedure for the assay of thiamine developed. The modified procedure involves the direct measurement of thiamine in crude extracts and thus is much simpler and more rapid than other procedures that involve ion-exchange purification of the thiamine. Unlike the chemical procedures used for the preparation of thiamine-free blanks, thiaminase I is highly specific.

Keywords: *Thiamine determination*; *thiaminase I*; *thiochrome*; *animal tissues*; *cereal products*

Several techniques have been devised for the determination of thiamine (vitamin B_1) in biological materials. The most commonly used chemical method, first described by Jansen,[1] involves the oxidation of thiamine to thiochrome, which is then measured fluorimetrically. A major improvement in this procedure was introduced by Hennessy and Cerecedo,[2] who used the synthetic cation-exchange resin Decalso for the purification of the thiamine and removal of non-specific interfering substances. More recently, a rapid cation-exchange purification procedure was introduced by Edwin *et al.*,[3] in which the thiamine was adsorbed to the resin in a test-tube, washed by decantation, oxidised while still on the resin and extracted directly into 2-methylpropan-1-ol.

To avoid the use of a resin, various chemical techniques[4,5] have been devised to destroy thiamine to produce "thiamine-free" blanks and thus to obtain some estimate of the level of non-thiochrome fluorescence in the sample extract. However, these chemical techniques are non-specific. Specific destruction of thiamine can be achieved by using the enzyme thiaminase I[6] (or thiaminase II). This was first suggested by Melnick *et al.*[7] in 1945 for the preparation of thiamine-free blanks. However, to date there has been no serious attempt to incorporate thiaminase I in the routine assay of thiamine levels in biological materials.

The aim of this work was to perform a detailed study to determine the usefulness of thiaminase I in thiamine assays. These studies have led to the development of a rapid, simple and precise procedure for the determination of thiamine in animal tissues and plant materials.

Experimental

Apparatus

A Foci Mark I spectrofluorimeter fitted with a xenon-arc stabiliser assembly was used. The optimum excitation and analytical wavelengths were 365 and 425 nm, respectively.

Reagents

All reagents were of analytical-reagent grade.

Hydrochloric acid, 0.1 M.

Sodium acetate buffer solution, 2 M, *pH* 5.5.

Clarase. Available from Miles Laboratories Inc., Elkhart, Ind., USA. Dissolve 7.5 g in 100 ml of 2 M sodium acetate buffer (pH 5.5).

Mercury(II) chloride solutions. Dissolve 1 or 5 g of the solid in warm water and dilute to 100 ml.

Sodium hydroxide solution. Dissolve 30 g in water and dilute to 100 ml.

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2-Methylpropan-1-ol. Either re-distil 2-methylpropan-1-ol (b.p. 105–108 °C) or treat it with activated carbon (5 g per 100 ml) and filter it through a glass-fibre filter-paper.

Repelcote. A solution of 2% dimethyldichlorosilane in carbon tetrachloride.

Pyridine. Add pyridine (79.1 g) to distilled water and dilute to 1 l to give a final concentration of 1 M.

Thiaminase I. Prepare thiaminase I as previously described[6] from nardoo fern and purify it by chromatography on DEAE-cellulose and Ultrogel AcA 34. The preparation used had a specific activity of 0.2 μkat per milligram of protein. A solution of thiaminase I with an activity of 1.0 nkat ml^{-1} was routinely employed and this was stored frozen between use. One nanokatal (1 nkat) of enzyme activity is defined as that amount which hydrolyses 1 nmol of thiamine in 1 s at 30 °C, pH 6.5, in the presence of saturating levels of the co-substrate base pyridine.

Standards

Thiamine hydrochloride. Thiamine hydrochloride (Sigma Chemical Co., St. Louis, Mo., USA) was dried over phosphorus pentoxide in a desiccator for several days.[3] A sample (100 mg) was dissolved in 0.1 M hydrochloric acid (100 ml) and then further diluted with 0.1 M hydrochloric acid to obtain standard solutions containing 2.5–50.0 μg ml^{-1}. These solutions were stored in dark-glass bottles at 4 °C. The bottles were first treated with Repelcote to prevent adsorption of thiamine on to the glass.

Quinine sulphate. Quinine sulphate (dried over phosphorus pentoxide) was dissolved (3 mg l^{-1}) in 0.1 M hydrochloric acid and stored at 4 °C in a dark-glass bottle between use.

Assay of Thiaminase I

Thiaminase I was assayed as previously described[6] by incubating the enzyme solution (0.2 ml) with substrate solutions (0.2 ml) at 30 °C for 10 min. Substrate solutions contained 0.1 μCi ml^{-1} of [thiazole-2-^{14}C]thiamine hydrochloride, 100 mM pyridine and unlabelled thiamine (2 or 20 mM), and were prepared in phosphate buffer (0.1 M, pH 6.5). The enzymic reaction was terminated and the released [2-^{14}C]thiazole was extracted by adding ethyl acetate (2 ml) and mixing at maximum speed on a vortex mixer for 10 s. Aliquots (0.5 ml) of the ethyl acetate layer were assayed for the presence of [2-^{14}C]thiazole.[6,8]

Optimising the Amount of Thiaminase I Required to Produce Thiamine-free Blanks

Thiaminase I enzyme (0.1 ml, 0.015 nkat) was added to aliquots (2 ml) of tissue extract containing pyridine (0.10 ml, 1 M), [thiazole-2-^{14}C]thiamine hydrochloride (0.02 μCi) and unlabelled thiamine (0–20 μg), and the mixtures were incubated at 45 °C. After 0, 5, 10 and 20 min an aliquot (2 ml) of ethyl acetate was added to duplicate tubes and the tubes were stirred vigorously and centrifuged (3000 *g*, 5 min). Hydrolysis of thiamine was estimated by the release of [2-^{14}C]thiazole into the ethyl acetate phase.

Optimum Level of Oxidant

Aliquots (0.2 ml) of thiamine stock solution (5.0 μg ml^{-1}) were added to 2.0 ml of either 0.2 M hydrochloric acid or thiamine-free tissue extracts. Thiamine was oxidised by the addition of mercury(II) chloride solution (5%, 0.02–1 ml) followed by 2 ml of sodium hydroxide solution (30% *m/V*).

Recovery of Added Thiamine

An aliquot (0.2 ml) of thiamine stock solution (5, 10 or 50 μg ml^{-1} in 0.1 M hydrochloric acid) was added to samples of brain or liver or to finely ground cereal grains or pasture samples, before extraction. These samples and parallel samples to which no thiamine had been added were extracted and the thiochrome was measured fluorimetrically.

Assay of Thiamine Using Other Techniques

Brain and liver samples were assayed by the method of Edwin *et al.*[3] Wheat, flour and bread samples analysed by the AOAC procedure[9] were kindly provided by the Bread Research Institute, N.S.W., Australia.

General Procedure

Extraction of Thiamine and Dephosphorylation of Thiamine Phosphates

Liver or brain tissue was finely diced, samples (about 2 g) were transferred into pre-weighed Quickfit tubes (30 ml) and the mass of the tissue was measured accurately. To each tube an aliquot (5 ml) of 0.1 M hydrochloric acid was added with mixing and the tubes were incubated in a steam-bath for 15 min. On cooling, the samples were blended with a micro-Ultraturrax, the Ultraturrax shaft was rinsed with the minimum volume of water (approximately 2 ml), 7.5% *m*/*V* Clarase solution (2 ml) in 2 M sodium acetate buffer solution (pH 5.5) was added (to give a final pH of 4.9–5.0) and the mixtures were incubated at 45 °C for 3 h with occasional stirring and then centrifuged (3000 *g*, 10 min). The supernatants were transferred into calibrated flasks (25 ml) and the pellets re-extracted twice with 0.1 M sodium acetate buffer solution (pH 5.5). The extracts were combined and the volume was adjusted to 25 ml with the same buffer solution. Aliquots (2 ml) were taken directly for thiamine determination.

Dry samples such as finely milled cereal grains or dried pasture materials (2 g) were weighed accurately into calibrated flasks (25 ml) and an aliquot (15 ml) of 0.1 M hydrochloric acid was added. The flasks were mixed vigorously and incubated in a steam-bath for 15 min.[3] On cooling, 4 ml of sodium acetate buffer solution (2 M, pH 5.5) and 2 ml of Clarase solution (7.5% *m*/*V*) in the same buffer solution were added. The mixtures were incubated with occasional stirring at 45 °C for 3 h, cooled to room temperature and adjusted to volume with 0.1 M sodium acetate buffer solution (pH 5.5). This solution was centrifuged (3000 *g* for 10 min) before removal of aliquots for thiamine determination.

Formation of Thiochrome

To an aliquot (2 ml) of tissue extract was added mercury(II) chloride solution (0.4 ml, 5% *m*/*V*). The solution was stirred and then sodium hydroxide solution (2 ml, 30% *m*/*V*) was immediately added with stirring using a vortex mixer. For standard thiamine in 0.1 M hydrochloric acid, 0.4 ml of 1% mercury(II) chloride solution was employed.

Extraction into 2-Methylpropan-1-ol

2-Methylpropan-1-ol (5 ml) was added to each tube approximately 5 min after the addition of sodium hydroxide solution, and the tubes were capped and shaken vigorously for 30 s. After centrifugation at 3000 *g* for 5 min using a Clements GS200 bench centrifuge, the clear 2-methylpropan-1-ol extract was transferred to clean cuvettes for measurement of fluorescence. Following centrifugation, the fluorescence in the 2-methylpropan-1-ol layer remained constant for several hours.

Measurement of Fluorescence

A set of thiamine standards (0, 0.25, 0.5 and 1.0 μg of thiamine hydrochloride) were included in all determinations. The fluorimeter was set at zero with the reagent blank and set to the maximum reading (*i.e.*, 30) with the highest standard. Fluorescence was measured at 425 nm with excitation at 365 nm.

Thiamine-free Blanks

Aliquots (2 ml) of tissue extract were treated with pyridine solution (0.10 ml, 1 M) and thiaminase I (0.1 nkat) at 45 °C for 30 min. These samples were then oxidised by the addition of mercury(II) chloride and sodium hydroxide solutions as described above.

Thiamine Calibration Graphs

An aliquot (0.1 ml) of standard thiamine solution (2.5, 5 and 10 μg ml^{-1}) in 0.1 M hydrochloric acid was added to 2 ml of either 0.1 M hydrochloric acid or thiamine-free tissue extract. The thiamine was then oxidised to thiochrome as described previously.

Preparation of Thiamine-free Tissue Extracts

Brain or liver tissue, finely ground cereal grains or dried pasture material (16 g) were extracted with 0.1 M hydrochloric acid and treated with Clarase preparation using the same

ratio of reagents as described previously. Before volume adjustment, the thiamine was degraded by incubation with thiaminase I (10 nkat) and pyridine (4 ml, 1 M) at 45 °C for 30 min. Thiaminase I was then inactivated by incubation of the extract in a steam-bath for 20 min. The volume was adjusted to 200 ml and the solution stored frozen between use.

Results

The level of thiaminase I required to give complete destruction of thiamine in the preparation of thiamine-free blanks was determined using [thiazole-2-^{14}C]thiamine hydrochloride. Hydrolysis of this substrate, in the presence of various amounts of unlabelled thiamine, by thiaminase I is shown in Fig. 1. Under the incubation conditions employed (45 °C, pH 5), 0.015 nkat of thiaminase I gave complete hydrolysis of the thiamine present in the extract (approximately 0.1 μg) as well as added [thiazole-2-^{14}C]thiamine hydrochloride (about 0.5 μg, 0.02 μCi) plus 2.5 μg of unlabelled thiamine, in 20 min. The activity of the thiaminase I in these extracts was greatly enhanced by the addition of the co-substrate base, pyridine. This was particularly true for extracts of plant tissues where the natural level of such compounds is low. In such instances the addition of pyridine increased the rate of destruction of thiamine by thiaminase I by more than 10-fold. Liver and brain extracts contain high levels of co-substrate amines, so that the addition of pyridine was not essential. However, in the routine preparation of thiamine-free blanks, pyridine was always added, together with a large excess of thiaminase I (0.10 nkat). The reaction product, namely pyrimidinylpyridine, gave a fluorescence reading of less than 0.1% of that of thiamine at the wavelengths employed. A similar value was found when either nicotinic acid or nicotinamide was used as the co-substrate base.

The procedures used for the extraction of thiamine from plant materials and animal tissues were essentially as described by Edwin *et al.* and were found to be reliable and reproducible. The recovery of exogenous thiamine added to wheat, flour, bread or tissue material was 100 $\pm$ 5%. Dephosphorylation was performed at pH 5 by adding a solution of Clarase preparation in 2 M sodium acetate buffer solution (pH 5.5) to an extract containing 0.1 M hydrochloric acid (5 ml). A balance between the optimum pH for dephosphorylation and the subsequent optimum pH for thiaminase I activity (in the preparation of thiamine-free blanks) had to be achieved. A pH of 4.9–5.0 was found to be satisfactory for both. To allow for the different pH used for dephosphorylation,[3] a 50% increase in the amount of Clarase over that recommended by Edwin *et al.*[3] was used.

The level of mercury(II) chloride required to oxidise thiamine in crude extracts is considerably greater than that required to oxidise standard thiamine in 0.1 M hydrochloric acid

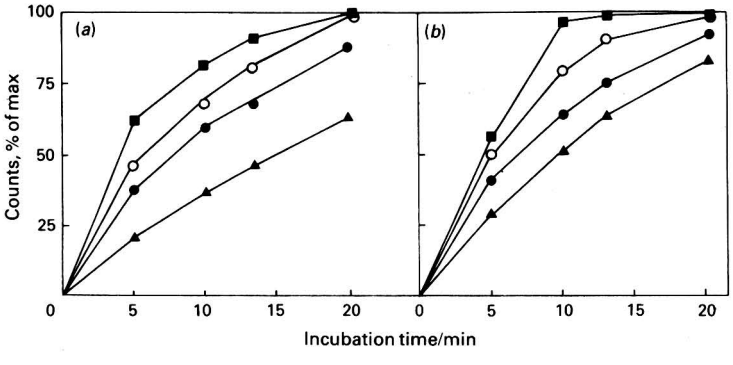

Fig. 1. Hydrolysis of thiamine in tissue extracts by thiaminase I: (*a*) wheat; and (*b*) liver. Assay conditions: thiaminase I enzyme (0.1 ml, 0.015 nkat) was added to 2.0 ml of tissue extract to which had been added [thiazole-2-^{14}C]thiamine.HCl (0.2 ml, 0.02 μCi), thiamine [0.2 ml, 0 (■), 25 (○), 50 (●) or 100 (▲) μg ml^{-1}] and pyridine (0.10 ml, 1 M).

(Fig. 2). The optimum level for the oxidation of thiamine in 0.1 M hydrochloric acid is 3–10 mg per 2-ml aliquot, whereas the optimum level for thiamine in either wheat or liver extract is approximately 20 mg per 2 ml of extract. To standardise the procedure an aliquot of mercury(II) chloride solution (0.4 ml) of either 1% (for standard thiamine in 0.1 M hydrochloric acid) or 5% (for thiamine in crude extracts) was used. However, the procedure routinely adopted in this laboratory involved the preparation of thiamine calibration graphs in thiamine-free tissue extracts so that the same level of mercury(II) chloride was used in both the standards and the unknowns. The reason for the decrease in thiochrome fluorescence when levels of mercury(II) chloride in excess of 10 mg (Fig. 2) were used to oxidise thiamine in 0.1 M hydrochloric acid is not clear, but a similar phenomenon was reported by Myint and Houser[10] for the oxidation of thiamine by alkaline potassium hexacyanoferrate(III).

Calibration graphs for thiamine in 0.1 M hydrochloric acid and for thiamine added to thiamine-free liver and wheat extracts are shown in Fig. 3. The fluorimeter reading for thiamine in tissue extracts was consistently 88% of the value for thiamine in 0.1 M hydrochloric acid. However, this is not always the case when certain baked products are being analysed. Such materials (*e.g.*, bread) contain various amounts of compounds that can cause different degrees of quenching of the thiochrome fluorescence. To overcome such problems, thiochrome fluorescence "response factors" can be determined for each unknown by preparing an extra pair of thiamine-free blanks for each sample, denaturing the thiaminase I enzyme by heat treatment (100 °C, 2 min) and adding a known amount of thiamine before oxidation with mercury(II) chloride.

In Tables I and II the results obtained using the current technique are compared with those obtained with the AOAC method[9] (wheat, flour and bread) and the method of Edwin *et al.*[3] (brain and liver tissues). The thiamine values obtained with the proposed technique agreed reasonably well with those obtained by using the AOAC procedure; for almost all of the samples analysed there was a deviation of less than 10% between the results obtained with the two techniques. In contrast, the values obtained for thiamine in some of the brain and liver samples by using the method of Edwin *et al.*[3] varied markedly from those obtained by using the thiaminase I method. Both techniques clearly show a major difference in the thiamine levels in tissues from normal sheep compared with that from thiamine-deficient sheep (*i.e.*, sheep showing clinical polioencephalomalacia), but the values obtained by using the two techniques varied by as much as 50% for some samples.

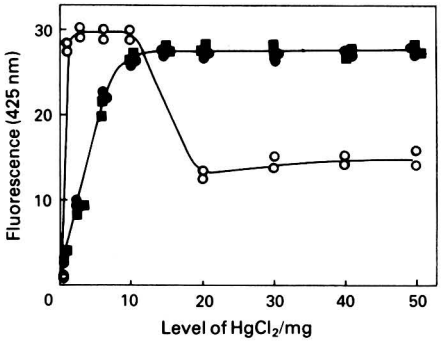

Fig. 2. Optimum levels of mercury(II) chloride for the oxidation of thiamine. ThiamineHCl (1.0 μg) in: ○, 0.1 N HCl (2 ml); ●, thiamine-free liver extract (2 ml); and ■, thiamine-free wheat extract (2 ml).

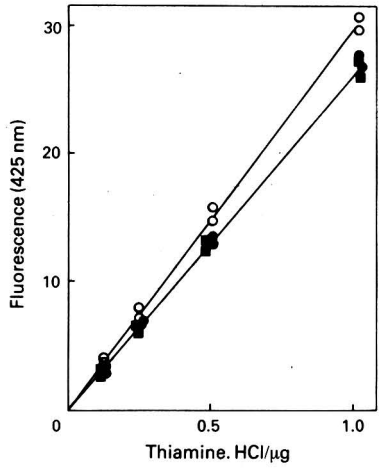

Fig. 3. Thiamine calibration graphs in 0.1 N HCl (○), thiamine-free wheat extract (■) or thiamine-free liver extract (●).

TABLE I

MEASUREMENT OF THIAMINE IN LIVER AND BRAIN TISSUES

Sample		Thiaminase I procedure			Procedure of Edwin *et al.*[3]: thiamine/ $\mu g\ g^{-1}$
		Fluorescence			
Source	Code No.	Sample	Blank	Thiamine/$\mu g\ g^{-1}$	
Brain	79/384*	3.9 ± 0.10	1.4 ± 0.10	0.2 ± 0.002	0.3
	79/357*	3.0 ± 0.10	1.4 ± 0.10	0.2 ± 0.002	0.3
	79/122*	2.6 ± 0.06	1.5 ± 0.10	0.10 ± 0.002	0.1
	79/233	11.3 ± 0.30	1.5 ± 0.10	0.90 ± 0.02	1.2
	79/201	11.4 ± 0.20	1.8 ± 0.10	0.80 ± 0.01	1.0
Liver	79/357*	2.1 ± 0.05	1.4 ± 0.05	0.10 ± 0.002	0.2
	79/122*	1.4 ± 0.05	1.2 ± 0.05	0.03 ± 0.001	0.1
	79/545*	2.8 ± 0.05	1.2 ± 0.05	0.20 ± 0.003	0.2
	79/233	18.7 ± 0.20	2.0 ± 0.10	1.6 ± 0.02	1.2
	79/201	16.8 ± 0.30	1.8 ± 0.10	1.6 ± 0.03	1.5

* Samples collected from sheep with clinical polioencephalomalacia (thiamine deficiency).

The precision of the thiaminase I technique was assessed by performing quadruplicate assays on the extracts of each of the samples shown in Tables I and II. Standard deviations for all samples were less than 5% of the thiamine values. We consider that a significant proportion of the deviation in thiamine values obtained by using the thiaminase I procedure compared with those obtained by using the AOAC procedure originate in the ion-exchange purification step in the latter procedure. The problems associated with the use of the Decalso ion-exchange purification step have been highlighted by Freed[11] and Pippen and Potter.[12]

The standard deviation between replicate thiamine determinations on brain and liver extracts using the current technique is 2% or less and is independent of the level of thiamine in the tissue. In contrast, the standard deviation between thiamine determinations using the method of Edwin *et al.*[3] varies from 60% in tissues deficient in thiamine to about 3% in tissue extracts where exogenous thiamine is added in about a 7-fold excess over likely normal values in healthy sheep. It is concluded that the major deviations in the values for thiamine obtained using the current technique compared with the technique of Edwin *et al.*[3] arise in the latter technique, particularly if thiamine-deficient tissue is being analysed. We have found that the recovery of thiochrome from resin using the technique of Edwin *et al.*[3] was generally in the range 70–80%. This was not a problem if, as recommended by these authors, thiamine standards were run concurrently through the same procedure. However, we did observe that the recovery of thiochrome was dependent on the amount and type of resin used. With Decalso F resin levels of 0.5, 1.0 and 1.5 g, the recoveries of thiochrome were 79, 70 and 66%, respectively, indicating that care needs to be exercised in ensuring that the same level of resin is used with each sample.

Discussion

The most sensitive chemical technique for the measurement of thiamine involves its oxidation to thiochrome followed by fluorimetric quantitation. Oxidation can be achieved with alkaline potassium hexacyanoferrate(III),[1] cyanogen bromide[13] or mercury(II) chloride.[14] As found by several workers and detailed by Edwin *et al.*,[3] mercury(II) chloride has several advantages over alkaline potassium hexacyanoferrate(III) and cyanogen bromide and consequently was used in the development of the current technique.

The oxidation of thiamine using the above reagents is not specific; consequently, other fluorescent, non-thiamine compounds are produced and must be accounted for. Hennessy and Cerecedo[2] overcame this problem by purifying the thiamine on Decalso, a synthetic cation-exchange material. However, this technique is tedious and time consuming and the problems associated with its use were summarised by Freed.[11] These problems include variations in the binding efficiencies of different Decalso preparations and interference with thiamine binding by other compounds such as amino acids. Although the acceptable binding

TABLE II

MEASUREMENT OF THIAMINE IN WHEAT, FLOUR AND BREAD

Sample*		Thiaminase I procedure			
		Fluorescence			
Source	Code No.	Sample†	Blank	Thiamine/μg g^{-1}	AOAC method*: thiamine/μg g^{-1}
Wheat	W5133	20.1 ± 0.3	1.97 ± 0.02	4.1 ± 0.06	3.7
	W5137	21.8 ± 0.8	1.85 ± 0.01	4.4 ± 0.16	4.8
	W5234	21.3 ± 0.8	1.90 ± 0.01	4.4 ± 0.16	4.1
	X9132	31.0 ± 0.5	1.95 ± 0.01	6.0 ± 0.09	6.0
Flour	W5009	13.5 ± 0.5	1.70 ± 0.02	2.7 ± 0.10	2.1
	W5013	16.5 ± 0.8	1.50 ± 0.02	2.1 ± 0.15	2.7
	W5082	8.6 ± 0.5	1.65 ± 0.02	1.6 ± 0.09	1.5
	W6260	6.4 ± 0.3	2.20 ± 0.01	1.0 ± 0.04	1.0
Bread	A361	15.5 ± 0.5	2.8 ± 0.02	2.8 ± 0.09	3.2
	A467	60.0 ± 0.9	3.4 ± 0.02	10.6 ± 0.19	10.6
	70	8.5 ± 0.2	2.45 ± 0.10	1.3 ± 0.04	1.7
	74	10.4 ± 0.3	2.24 ± 0.04	1.8 ± 0.05	1.9

* Samples were kindly supplied by the Bread Research Institute, N.S.W. Thiamine determinations using the AOAC procedure were performed at that Institute.

† All samples were assayed in quadruplicate.

efficiency is quoted as 92–96%, several workers have found that it is frequently as low as 85%.[12] Pippen and Potter[12] were able to obtain consistent thiamine recoveries of approximately 97% by doubling the recommended elution volume to 50 ml and by ensuring that hot acidic potassium chloride solution is used for elution. Edwin *et al.*[3] have recently modified this procedure by adsorbing the thiamine on resin in a test-tube, washing by decantation and then oxidising the thiamine while still on the resin and directly extracting into 2-methylpropan-1-ol. This procedure is rapid and reasonably accurate for samples containing high thiamine levels but is not very accurate or reproducible for samples containing lower levels of thiamine (0.2 μg per gram of tissue).[3]

Numerous other techniques have been devised for measuring the level of non-thiochrome fluorescence. These usually involve the chemical destruction of thiamine to produce thiamine-free blanks. However, the chemicals used, namely sulphite[4] or benzenesulphonyl chloride,[5] are non-specific. Burch *et al.*[15] used ultraviolet light to destroy the thiochrome but this technique has not been widely adopted.

As suggested by Melnick *et al.*,[7] an alternative procedure for destroying thiamine as a means of obtaining a true thiamine blank is to use thiaminase I enzyme. Unlike the other procedures, thiaminase I is highly specific. Defibaugh *et al.*[16] have recently used clam thiaminase I to produce thiamine-free food extracts for use in thiamine recovery experiments. However, they used a very crude preparation of thiaminase I and neither attempted to optimise the conditions to allow maximum activity of the thiaminase I nor accurately determined the level of thiaminase I used.

In this paper we have shown that thiamine can be accurately measured in crude tissue extracts, without the need for resin purification, by employing thiaminase I in the determination of non-thiochrome fluorescence. The level of thiaminase I required in the assay was carefully determined and then a 5-fold excess used. Thiaminase I from nardoo fern has maximum activity at pH values above 7–8 but in the current incubations the enzyme is used at pH 5, to avoid the necessity for a change in pH between pyrophosphatase (Clarase) and thiaminase I treatment. At pH 5 the enzyme was found to have about 50% of its maximum activity. The activity of the enzyme was greatly enhanced by the addition of saturating levels of the co-substrate base, pyridine. Addition of this to wheat extracts decreased the requirement for enzyme by over 10-fold.

In the current procedure, after the dephosphorylation of thiamine phosphates the tissue extract was maintained at pH 5 up to the oxidation stage. In our experience, thiamine is very stable at this pH over short periods of time, and if extracts are to be analysed at a later date they can be stored frozen at this pH for several months with no apparent loss of thiamine. The extracts were maintained at pH 5 to avoid the necessity for a change in pH between the pyrophosphatase and thiaminase I treatments.

The procedure described here is precise and reproducible for samples that contain thiamine at levels in excess of 0.1 $\mu g\ g^{-1}$, but is not applicable to samples that contain less than this amount of thiamine. In such instances microbiological or enzymic methods are superior. With the current technique as many as 40 samples can be extracted and analysed in duplicate in less than 2 days. Thiaminase I* can be obtained from several sources, including a range of ferns, the viscera of fresh water fish and from fresh-water mussels or clams, and is readily purified by column chromatographic techniques.[6]

The authors express their thanks to Prof. R. Dunlop and Dr. E. Thornber, School of Veterinary Studies, Murdoch University, Western Australia, for useful discussions and to Mr. D. Mugford of the Bread Research Institute, N.S.W., for providing wheat, flour and bread samples that had been analysed for thiamine by the Official AOAC method. This work was supported by a grant from the Wool Research Trust Fund on the recommendation of the Australian Wool Corporation.

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Received *June 20th*, 1980
Accepted *September 25th*, 1980

* Samples available from the authors on request.

Profiles of Organic Volatiles in Biological Fluids as an Aid to the Diagnosis of Disease*

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The transevaporator sampling technique is described for the solvent stripping of the organic volatile fraction from 5–500-μl samples of biological fluids prior to analysis by capillary column gas chromatography. The reproducible nature of the isolation and separation technique enables quantitative comparisons to be made between normal and pathological samples and marker substances of use for the diagnosis of the diseased state to be identified. Electron-capture detector profiles provide complementary information to the flame-ionisation detector that may prove to be very useful in developing biological correlations.

Keywords: *Organic volatiles*; *biological fluids*; *capillary column gas chromatography*; *electron-capture detection*; *clinical diagnosis*

To maintain a healthy population with a fully active life-span, it is important that methods be developed for the early detection of disease. The recognition of the complexity of the diagnosis of disease has led to a demand from the physician for more detailed information on the chemical composition of essential fluids and body tissues. The concept of profiling as a diagnostic aid extends from the assumption of Garrod,[1] made in 1908, that pathological states could possibly be reflected in characteristic changes in the concentration of certain constituents in biological fluids. This assumption also forms the cornerstone of Pauling's concept of "orthomolecular medicine."[2,3]

Of particular interest to this laboratory has been the development of techniques for the analysis of the organic volatile fraction from biological fluids. The volatile profile cannot be defined as a single class of substances, rather it is a broad spectrum of materials of different polarities characterised by having a boiling-point in the low to medium range (up to approximately 300 °C) and the fact that the compounds are suitable for gas chromatography without derivatisation. The organic volatile profiles are very complex mixtures of metabolic by-products, intermediates and terminal products of enzymatic degradations composed mainly of alcohols, ketones, aldehydes, pyrazines, sulphides, isothiocyanates, pyrroles and furans.

The concentration of organic volatiles in biological fluids covers a wide range with many important components present at trace levels. The complexity of the organic volatile fraction requires the use of capillary columns for their separation. The nature of biological fluids prevents the use of direct chromatographic injection techniques as, apart from the sensitivity problem, the large amounts of high relative molecular mass biological molecules and inorganic salts destroy the capillary columns used for the separation. The low sample capacity of capillary columns limits the volume injected to a few tenths of a microlitre, which is a problem in trace analysis unless isolation of the organic volatile fraction from the biological matrix can be performed in a selective manner.

No single sampling procedure is ideal for the isolation of the organic volatile fraction from all biological fluids. The headspace sampling technique with condensation of the organic volatiles in a cryogenic trap[3–7] or by collection on a porous polymer[8–21] is well suited to the analysis of 24-h human urine samples where sample volume is not a problem. The minimum sample volume for profile analysis is 25.0 ml of urine because of the low efficiency of removing the organic volatiles from the matrix arising from the slow equilibration between organic molecules in the urine sample and the gas phase above the fluid. Sampling times are long and the profile obtained is representative of the concentration of the volatiles in the sample rather than a complete purging of the volatiles. Headspace techniques have also been used for the

* Presented at the 5th SAC International Conference on Analytical Chemistry, Lancaster, July 20–26th, 1980.

analysis of serum,[22–24] tissue homogenates[22,25] and spinal fluid[12] where sample volume limitations are a problem in developing adequate profiles for diagnostic purposes. For the analysis of small sample volumes (1–5 ml) gas-phase stripping techniques can be employed,[26] but the same observations as applied to the dynamic headspace methods are also true of this technique. When applied to serum, gas-phase stripping techniques failed to reveal the complete complexity of the organic volatile fraction and the sample requirement of the order of a few millilitres was too high for some problems, *e.g.*, animal studies with small rodents.[22,26] This prompted the investigation of solvent extraction techniques as a means of isolating the organic volatile fraction from urine,[27–29] plasma,[26,29,30] tissue homogenates,[26] blood,[31] breast milk and amniotic fluid.[29] The profiles obtained by solvent extraction are selective as the extraction efficiency is dependent on the partition coefficient of the solutes and the area of contact between the solvent and sample. The limitations of the solvent extraction technique are the need for very pure solvents, evaporation of the solvent is usually accompanied by a loss of compounds of low boiling-point and the fact that no solvent will extract to equal extents the complex range of volatile compounds present in biological fluids. The transevaporator sampling apparatus has been used for the analysis of urine, serum, amniotic fluid, breast milk and saliva with a sample volume requirement of 5–500 μl.[32–36]

Experimental

Reagents and Materials

All solvents were of the highest purity obtainable and their purity was checked by gas chromatography before use. Pentane and diethyl ether were of pesticide grade. 2-Chloropropane (Eastman-Kodak, Rochester, N.Y., USA) was distilled from phosphorus(V) oxide in an all-glass apparatus.

Helium. Helium (Iweco, Houston, Texas, USA) of high-purity grade for use in the sampling apparatus was further purified by passage through a U-tube filled with a mixture of molecular sieve and Carbosieve and immersed in a liquid nitrogen Dewar flask.

Glass-wool. Pyrex brand glass-wool (Corning Glass Works, Corning, N.Y., USA) was heated in a muffle furnace at 500 °C for at least 24 h before use.

Porasil E. Porasil E (80–100 mesh, Analabs, North Haven, Conn., USA) was conditioned prior to use by heating to 300 °C in a stream of helium for a minimum of 10 min and stored in a tightly stoppered container. The Porasil E (0.3 cm^3) was packed into a 70 × 2 mm i.d. microextraction column and retained by two glass-wool plugs.

Glass beads. Untreated glass beads (80–100 mesh, Analabs) were washed with Micro solution (International Products Corp., Trenton, N.J., USA), nitric acid (10% *V/V*), distilled water and acetone and then packed into a glass column (11 cm × 8 mm i.d.), retained by two glass-wool plugs and conditioned for 24 h at 280 °C in a stream of helium.

Tenax GC. Tenax GC (60–80 mesh, Applied Science Laboratories, State College, Pa., USA), was packed into 11 cm × 8 mm i.d. glass tubes (1.8 cm^3 of material) and retained by two glass-wool plugs. The Tenax traps were conditioned prior to use by heating at 280 °C for 24 h in a stream of helium.

Serum. Serum samples (obtained without heparin) were separated into 0.5-ml volumes and stored at −10 °C in 2.0-ml glass bottles with PTFE-lined screw-caps. This transfer process was used to eliminate any problems associated with the re-freezing and re-thawing of the samples as they were used.

Tissue samples. Animal samples were taken from mature Sprague-Dawley rats. Liver and kidney were removed intact from killed animals and were immediately frozen using dry-ice. The tissues were kept frozen until prepared for analysis, at which time they were thawed, weighed and rinsed twice with distilled water to remove any excess of blood. The tissues were then minced in a tissue micro-grinder (Fisher Scientific, Pittsburgh, Pa., USA) consisting of a glass tube and pestle. A volume of glass-distilled water equal in mass to the tissue was added and the tissue was homogenised by hand for 1 min. The homogenates were transferred into glass vials and stored at −10 °C until analysed.

Transevaporator Sampling Apparatus

The apparatus is shown in Fig. 1 and can be used to provide in two steps both a modified headspace and an extraction profile from a single sample of 5–500 μl of biological fluid.

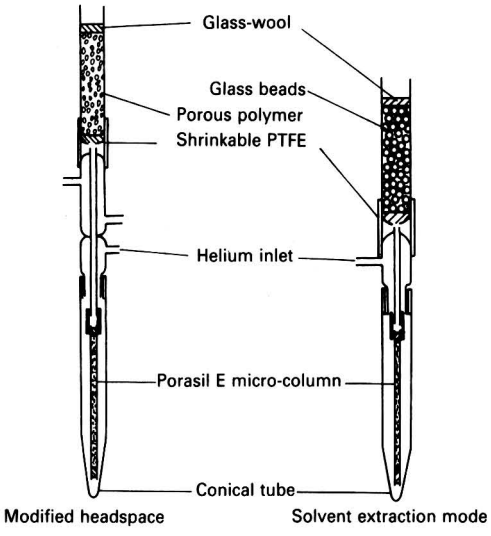

Fig. 1. Transevaporator sampling apparatus.

The sample in a syringe was slowly injected on to the Porasil E micro-column, which was then attached to the transevaporator by a pre-shrunk PTFE sleeve (sample end down). The remainder of the apparatus was assembled with a water-cooled micro-condenser included between the transevaporator and the Porasil E micro-column. The modified headspace sample was then collected by attaching a Tenax trap to the transevaporator using either a ground-glass joint or a shrinkable PTFE sleeve. The sample transfer was effected by passing helium at a flow-rate of 16 ml min^{-1} through the apparatus for 5–10 mins. The Tenax tube was then removed and stored at −10 °C in a screw-capped PTFE-lined culture tube until analysed. The elution micro-column was removed and attached to a second transevaporator without a condenser. The extraction profile was collected on a glass bead column attached to the transevaporator. For extraction, 0.5–1.0 ml of solvent (pentane, diethyl ether, 2-chloropropane) was added by syringe to the conical tip of the centrifuge tube, and the apparatus re-assembled. The helium carrier gas was switched on and adjusted to a flow-rate of 0.5 ml min^{-1} or less so that the solvent travelled slowly up the Porasil E column (1–2 min) and was eventually transferred entirely to the glass bead column. The elution micro-column was removed, the apparatus re-assembled and the helium flow-rate increased to 16 ml min^{-1} to remove excess of solvent (5–10 min).

Desorption of Volatiles

The organic volatiles trapped on either Tenax or glass beads were thermally desorbed in a desorption chamber (Fig. 2) maintained at 280 °C by purging with helium at 7 ml min^{-1} for 10 min. The volatiles were trapped in a U-shaped stainless-steel pre-column (30 cm × 1.0 mm i.d.) coated with the same stationary phase as used for the analytical column and immersed in a liquid nitrogen Dewar flask.

Gas Chromatography

The pre-column was attached to the analytical column (first) via a Swagelok fitting, connected to the carrier gas inlet (injector) by a second fitting, a carrier gas flow-rate of 2–5 ml min^{-1} established and the liquid nitrogen trap removed. When the inside diameter of the pre-column was greater than that of the analytical column the sample was transferred to a second

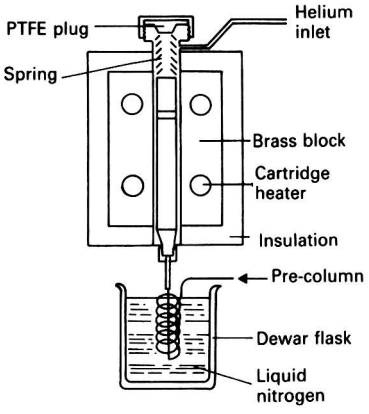

Fig. 2. Desorption chamber.

15-cm pre-column of the same inside diameter as the analytical column by immersing the second pre-column in a liquid nitrogen Dewar flask and heating the first to 200 °C with a small electrical current from a d.c. power supply or by use of a heat gun. When the analytical column was made of metal, the first 15 cm of it can be wound into a spiral and used as the second pre-column. To start the analysis, the liquid nitrogen Dewar flask was removed and the temperature programme initiated.

The organic volatile fraction was separated on either a nickel capillary column (100 m × 0.25 mm i.d.) coated with Witconol LA-23[35,37] or with a 106 m × 0.25 mm i.d. glass column coated with Silar 10C (Quadrex Corp., New Haven, Conn., USA). Further analytical conditions are given in the figure legends. The sampling procedure is designed to be independent of the gas chromatograph used and any gas chromatograph that is compatible with the use of capillary columns can be used for the analysis. An integrator is required for the calculation of peak areas.

Results and Discussion

The transevaporator sampling technique was developed out of a series of experimental improvements in the micro-solvent extraction method.[32,33,35] Removal of the extraction solvent by syringe was inconvenient and the first transevaporator was designed for this purpose. The base was the extraction tube and a narrow-bore tube extended from the glass-wool trap to just above the ether - sample interface.[32] Helium carrier gas was used to transfer the ether layer to the glass-wool trap and then to strip away the ether solvent. At room temperature, ether extracts about 7% *m/m* of water, which can cause problems at the desorption stage by blocking the pre-column. To retain the water and also to distribute the sample uniformly over a large surface area, the biological fluid was injected into a micro-column packed with Porasil E and the extraction solvent forced through the column by gas pressure. The micro-column retains most of the water and the high relative molecular mass polar organics and inorganic salts present in the biological fluids.

The transevaporator is able to provide two profiles, a modified headspace and a solvent elution profile in a sequential manner from the same sample. In Fig. 3 are compared the two profiles of a 60-μl diabetic serum sample. The modified headspace profile contains very few peaks and is of little value for diagnostic purposes compared with the solvent elution profile. The modified headspace profile is useful in air pollution studies and for the characterisation of flavours and fragrances.[33]

As an analytical tool, the transevaporator sampling apparatus is a semi-quantitative technique capable of providing reproducible organic volatile profiles from biological fluids. In absolute terms, the percentage recovery of test compounds depends on both the volatility and

the chemical nature of the compounds being studied. For aqueous standards containing propan-1-ol, butan-1-ol, decan-2-one, heptan-2-one and benzaldehyde prepared to cover the range 0.5–5.0 p.p.m., the percentage recovery was consistent for each compound throughout the concentration range and averaged 20, 40, 50, 55 and 70%, respectively, for the test compounds. The reproducibility of the solvent extraction profile obtained from 50 μl of a pooled normal serum sample is illustrated in Fig. 4 for three separate replicate analyses.

The chromatograms in Fig. 4 also indicate the complexity of the organic volatile fraction from serum. The inter-comparison of chromatograms requires the use of pattern recognition techniques and computer interpretation to handle the immense amount of information generated in a screening programme. The methods used in our laboratory for this purpose have been described recently and will not be dealt with here in detail.[34] The data base for the pattern recognition techniques uses the peak retention time to identify a particular component and its area as a measure of its relative concentration. To make the data compatible between chromatograms, a fixed volume of sample is extracted for each analysis and the peak areas are normalised after elimination of background peaks and of odd peaks that appear in only a few chromatograms. The success of the sampling technique as a diagnostic aid is very dependent on the reproducibility of the profile obtained. For the pooled normal serum (10 replicate analyses), the median average relative standard deviation was 18.3% and the range 6.5–51.1%. Retention times can be reproduced to better than 1% relative standard deviation in the chromatographic system. These results are typical of other biological fluids and illustrate the reproducibility that can be expected when using the transevaporator sampling technique. Another important parameter in profiling techniques is the biochemical individuality of the human population. For 15 serum samples obtained from healthy individuals, the median

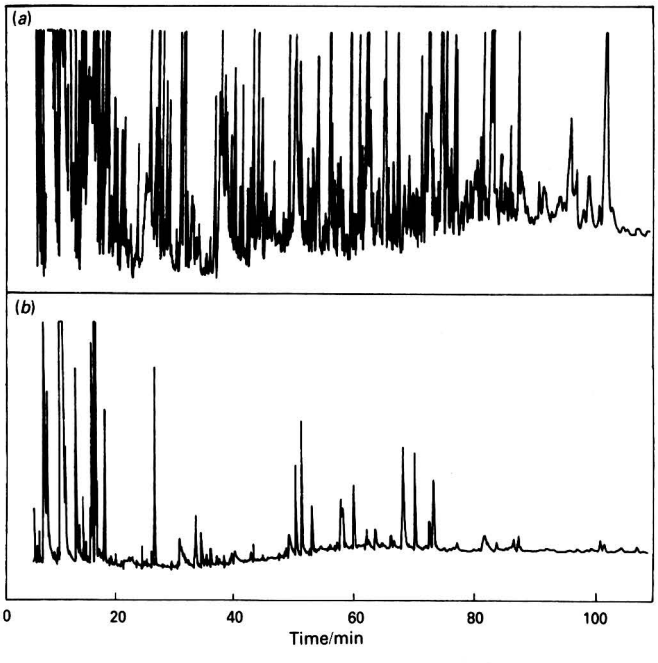

Fig. 3. The organic volatile profile from 60 μl of diabetic serum with the transevaporator used in (*a*) the extraction mode and (*b*) in the modified headspace mode. Both profiles were obtained in the sequence (*b*), (*a*) from one sample. The separation was performed on a 100 m × 0.25 mm i.d. nickel capillary column coated with Witconol LA-23. The column was programmed with an initial isothermal period of 50 °C for 10 min, an increase at 1.5 °C min^{-1} to 160 °C and then an isothermal period at 160 °C for 80 min. The carrier gas was helium at 1.5 ml min^{-1}.

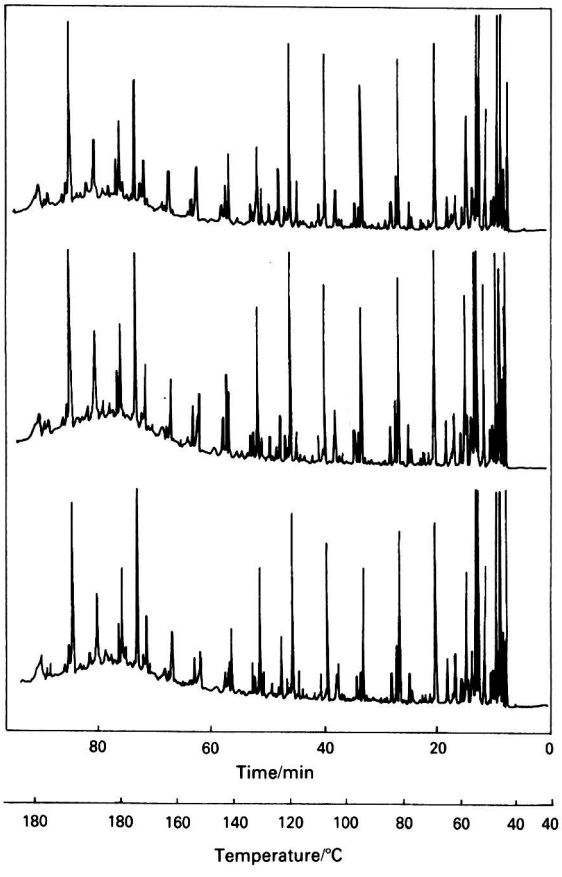

Fig. 4. Repeatability of the transevaporator sampling technique in the solvent elution mode for three 50-μl samples of a pooled normal serum. The three replicate separations were performed on a 106 m × 0.25 mm i.d. glass capillary column coated with Silar 10C. The column was programmed with an initial isothermal period at 40 °C for 6 min, then programmed at 2 °C min^{-1} to 180 °C and held at this temperature for 30 min. Helium carrier gas flow-rate, 1.5 ml min^{-1}.

average relative standard deviation was 32.9% and the range 17.1–87.5% for the selected chromatographic peaks used to provide the data base for comparison with patient's serum. The data base consisted of approximately 150 detected peaks, of which about 75 were detected in all 15 serum samples and used for the calculation. The biochemical individuality is much more suppressed in serum than in urine, making the comparison of different profiles less troublesome. For diagnostic purposes, the consistent change in concentration of a peak or peaks greater than one relative standard deviation of the value obtained for the normal profiles is considered significant and used for the detection of the disease.

The transevaporator sampling technique is suitable for the analysis of organic volatiles in biological fluids other than serum. The use of saliva as a non-invasive sample for biomedical studies is currently receiving much attention. The solvent elution profile for 500 μl of saliva is shown in Fig. 5. The over-all concentration of organic volatiles is relatively low but the

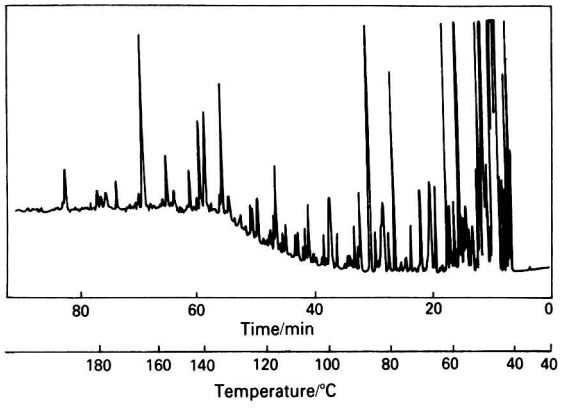

Fig. 5. Organic volatile profile from 200 μl of saliva using the transevaporator in the solvent elution mode. Separation conditions as in Fig. 4.

number of peaks detected indicates the potential use of saliva for profiling studies. The transevaporator can also be used to obtain an organic volatile profile from small tissue samples and this is illustrated in Fig. 6 for a solvent elution profile of rat kidney homogenate.

Not many organic compounds show a significant response to the electron-capture detector (ECD). With only a few exceptions, those compounds with a significant detector response are either important intermediates or coenzymes in the energy transfer process of the living cell or else are substances toxic to these biological processes.[38] Because of the unique possibilities of correlating the detector response with biological activity, we have made a preliminary investigation of the use of ECD profiles of organic volatiles in biological fluids for the detection of disease. In Fig. 7 is shown a solvent elution profile of 20 μl of (*a*) normal serum and (*b*) the corresponding system blank. Compared with the chromatograms obtained using a flame-ionisation detector (FID) there are fewer peaks detected and the profile is essentially different with respect to both the identity of the peaks and their relative peak areas, as might be expected. The reproducibility of the selected peaks in the profile of a pooled normal serum sample is similar to that obtained with the FID, with a mean average relative standard deviation of 24.1% and a range of 8.5–76.6%. Fig. 8 shows the ECD profiles of 50 μl of serum sampled by the solvent elution mode for a patient with (*a*) leukaemia and (*b*) a multiple

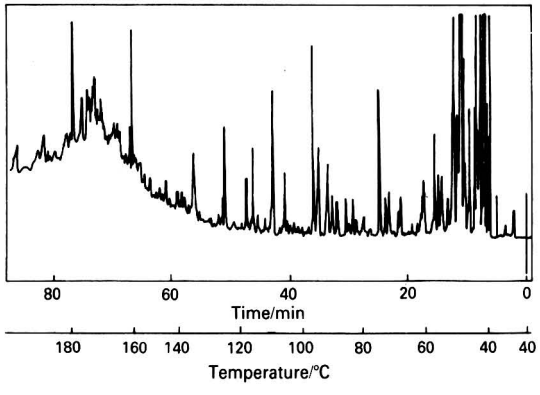

Fig. 6. Organic volatile profile from a rat kidney homogenate using the transevaporator in the solvent elution mode. Separation conditions as in Fig. 4.

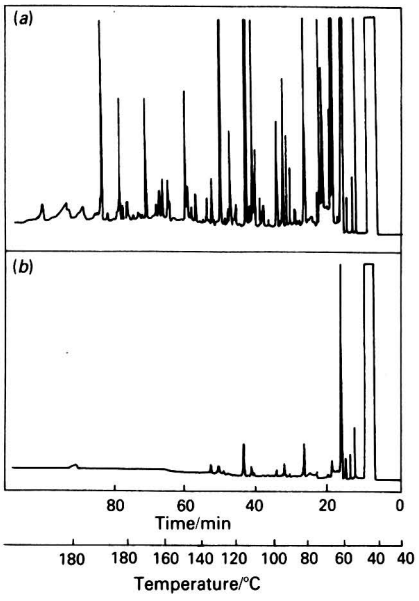

Fig. 7. Electron-capture detector organic volatile profile of 20 μl of (*a*) normal serum and (*b*) system blank obtained using the transevaporator in the solvent elution mode. Separation conditions as in Fig. 4.

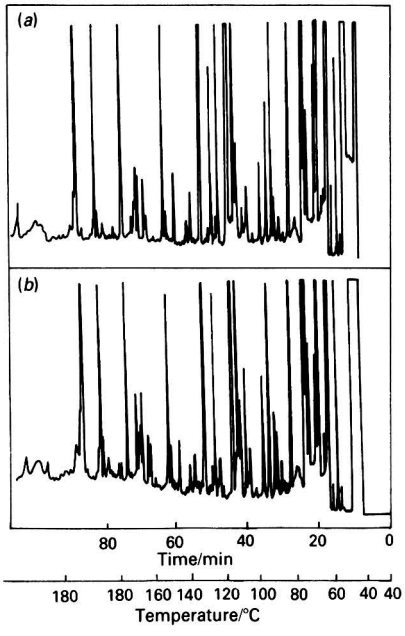

Fig. 8. Electron-capture detector organic volatile profiles of 50 μl of (*a*) leukaemia serum and (*b*) multiple myoloma serum obtained using the transevaporator in the solvent elution mode. Separation conditions as in Fig. 4.

myoloma. Other serum samples investigated with the ECD include breast cancer and lymphoma patients, but in all cases studied the differences between the peak areas for normal and pathological samples were less than or equal to the variation between uninfected individuals.

Conclusions

The transevaporator sampling apparatus described for the isolation of the organic volatile fraction of biological fluids prior to their separation by high-resolution gas chromatography has a small sample requirement of 5–500 μl and is ideally suited to profiling studies of biological fluids for the identification of chemical markers of value as a diagnostic aid for the recognition of metabolic disorders. The sampling technique can be used in conjunction with the electron-capture detector to provide selective profiles of the electron-capturing organic volatiles in biological fluids.

The authors gratefully acknowledge the support of the National Aeronautics and Space Administration, Life Science Directorate, Johnson Space Center, Houston, Texas (Contract NAS 9-15882).

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Received *May* 19*th*, 1980
Accepted *September* 2*nd*, 1980

Analyst, March, 1981, Vol. 106, pp. 361–364

Characterisation of Candicidin and Levorin by Means of High-performance Size-exclusion Liquid Chromatography*

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A bimodal high-performance size-exclusion liquid chromatographic method for the comparison of aromatic heptaene macrolide antibiotics has been developed. Candicidin from different sources and levorin were found to contain components of high relative molecular mass but with different elution patterns. Ultraviolet - visible spectrophotometry of fractions collected during the size-exclusion chromatography demonstrated that both the components of high relative molecular mass and the constituents that were retained by adsorption to the packing material contain a heptaene moiety. The latter was found to be identical with the free aromatic heptaene macrolides previously characterised by reversed-phase high-performance liquid chromatography.

Keywords: Candicidin; levorin; heptaene macrolides; high-performance size-exclusion liquid chromatography; high-performance liquid chromatography

Among antibiotics with antifungal activity the aromatic heptaenes constitute a well classified subgroup of the polyene macrolides.[1] The aromaticity of these compounds is due to the presence of a *p*-aminoacetophenone moiety, and cancidicin, levorin, hachimycin (trichomycin) and hamycin are the most important. The aromatic heptaene macrolides are widely used as topical drug substances but only candicidin is as yet the subject of a pharmacopoeial monograph.[2]

The commercially available substances are complex mixtures containing not only several related polyenes but also fermentation by-products. The characterisation of the mixtures, the isolation of individual polyenes and studies of their structures are complicated by the instability of these molecules and by their poor solubility. So far, structures have been elucidated only for candicidin D and levorin A_2,[3] which are the main components of the candicidin and levorin complexes, respectively, and which were shown to be identical. Several attempts have been made to separate the mixtures extracted from fermentation broths. The favoured method was formerly counter-current distribution as described by Borowski *et al.*[4] and Lightbown *et al.*[5] Pyrolysis gas chromatography has been used by Burrows and Calam[6] and Calam.[7] The use of high-performance liquid chromatography (HPLC) for the separation of polyene antifungal antibiotics was introduced by Mechlinski and Schaffner.[8] An HPLC method utilising a gradient elution technique was subsequently applied to the comparison of candicidin, levorin and hachimycin by Hansen and Thomsen.[9] Recently a rapid isocratic reversed-phase HPLC procedure for the comparison of aromatic heptaene macrolides has been developed by Helboe *et al.*[10]

This paper describes a further method for the characterisation of candicidin from different sources and of levorin based on high-performance size-exclusion liquid chromatography (HPSEC).

Experimental

Apparatus

HPSEC

A liquid chromatograph consisting of a Kontron 410 LC pump equipped with an Altex 110 pulse damper, a Rheodyne 7125 injection valve with a 20-μl loop and equipped with a

* Presented at the 5th SAC International Conference on Analytical Chemistry, Lancaster, July 20–25th, 1980.

Pye Unicam LC-UV detector connected in series with an Optilab Multiref 902 differential refractometer was used. Chromatograms including the traces of a 1-ml siphon counter were recorded on a Kipp and Zonen BD-8 recorder.

HPLC

A liquid chromatograph consisting of a Kontron 410 LC pump, a Rheodyne 7125 injection valve with an 80-μl loop and a Du Pont 837 UV spectrophotometer detector was used. Chromatograms were recorded on a Kipp and Zonen BD-8 recorder.

Ultraviolet - visible spectra

These were recorded on a Beckman Acta III spectrophotometer.

Chemicals

Acetonitrile (HPLC S grade) was obtained from Rathburn Chemicals (Walkerburn, Great Britain). All other solvents and reagents were of analytical-reagent grade and were obtained from E. Merck (Darmstadt, West Germany). The suppliers of the aromatic heptaene macrolides investigated are listed in Table I.

TABLE I

p-AMINOACETOPHENONE-CONTAINING HEPTAENE MACROLIDE ANTIBIOTICS INVESTIGATED

Sample	Antibiotic	Source
A	Candicidin	1st British Standard of candicidin. National Institute for Biological Standards and Control (London)
B	Candicidin	Dumex Ltd. (Copenhagen)
C	Candicidin	S. B. Penick & Co. (Lyndhurst, N.J., USA)
D	Levorin	All-Union Research and Technological Institute of Antibiotics and Enzymes (Leningrad, USSR)

Chromatography

HPSEC conditions

A bimodal column set with a 3-decade linearity (10^3–10^6) when calibrated with dextrans and eluted with dimethyl sulphoxide was used. The columns (250 × 8 mm i.d.) were packed with LiChrospher SI 500, 10 μm (E. Merck) and with Nucleosil 50–10, 10 μm (Macherey, Nagel & Co., Düren, West Germany), respectively. Both columns were packed as described earlier.[11] The efficiency of the columns, expressed as the number of theoretical plates (N) measured for dextrose when eluted with water containing 0.05% m/V sodium azide at a flow-rate of 1 ml min^{-1}, was $N = 6000$ for both the columns with 5- and the 50-nm pore size. The retention volume of dextrose was taken to represent the total permeation volume, V_e, of the system. During the separations dimethyl sulphoxide was used as the eluent and the columns were operated at room temperature. Test solutions were 1% m/V solutions of the sample in question in dimethyl sulphoxide and 20-μl volumes were injected.

HPLC conditions

The conditions for the analytical separations were identical with those recently developed.[10] The HPSEC fractions were used directly as test solutions and 80-μl volumes were injected.

Results and Discussion

The bimodal column set packed with silica was chosen for the HPSEC in order to obtain a system exhibiting a reasonably wide linear calibration range. No problems were encountered in utilising dimethyl sulphoxide, which is the only solvent capable of dissolving the test substances completely, as the eluent.

The chromatograms obtained by HPSEC demonstrate that all of the aromatic heptaene macrolides investigated contain some components of high relative molecular mass, as can be seen in Fig. 1. Their elution patterns are not exactly the same for candicidin from the three

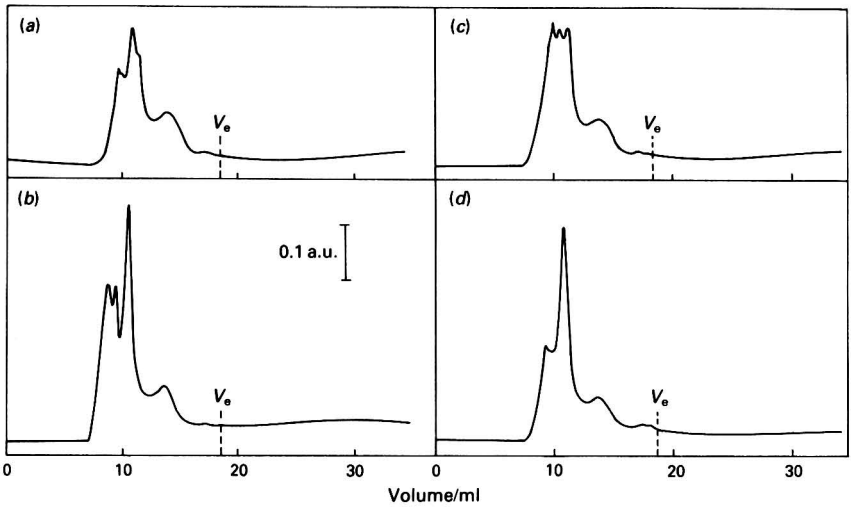

Fig. 1. Chromatograms of four aromatic heptaene macrolide antibiotics (for identification see Table I). Supports, LiChrospher SI 500 (10 μm) and Nucleosil 50 (10 μm); columns, 250 × 8 mm i.d.; eluent, dimethyl sulphoxide; flow-rate, 0.8 ml min^{-1}; detection, 340 nm; and sample, 200 μg.

different sources, nor for levorin. Ultraviolet - visible spectrophotometry of fractions collected during size-exclusion chromatography of sample B (candicidin) indicates that all of the fractions of high relative molecular mass contain a heptaene moiety.

Some sample constituents are retained by adsorption to the column packing material and are eluted with a retention volume substantially greater than V_e, as illustrated in the ultraviolet - visible trace in Fig. 2. Ultraviolet - visible spectrophotometry of a fraction containing the late-eluting constituents at their maximum concentration also showed the presence of a heptaene moiety. The fractions containing components of high relative molecular mass when investigated by means of the previously developed HPLC method[10]

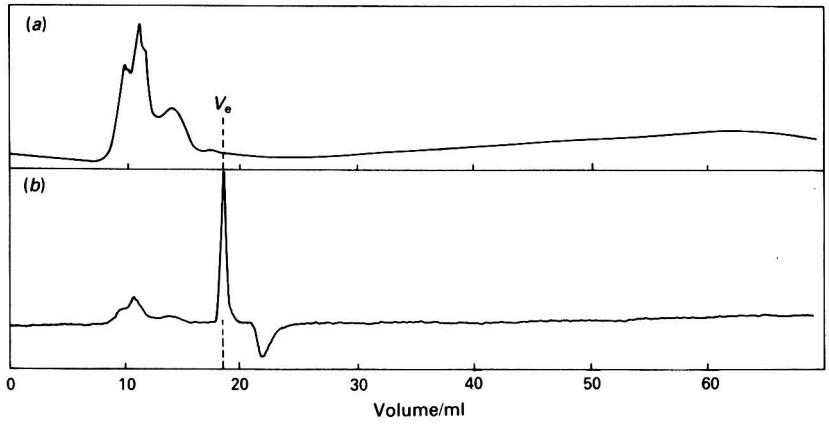

Fig. 2. Chromatograms of freshly dissolved A (candicidin) detected by (*a*) ultraviolet absorption (340 nm) and (*b*) refractive index. Conditions as in Fig. 1.

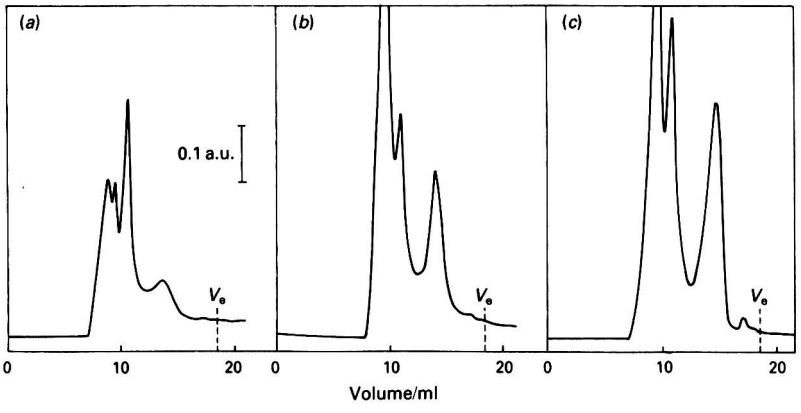

Fig. 3. Repeated chromatography of B (candicidin) as a function of the age of the test solution: (*a*) 0 h; (*b*) 4 h; and (*c*) 24 h. Conditions as in Fig. 1.

were found to contain only minor amounts of candicidin in comparison with the ultraviolet - visible absorbance measured. Free aromatic heptaene macrolides were essentially present only in the late-eluting fractions and in compositions corresponding to those previously found.

The compositions of the test solutions were found to change with time. Repeated chromatography of a test solution that had stood for up to 24 h showed an increase in its content of components of high relative molecular mass, as can be seen from Fig. 3, accompanied by a corresponding decrease in the amounts of late-eluting constituents. This indicates that the free aromatic heptaene macrolides, rather than undergoing polymerisation in solution, couple with some high relative molecular mass by-products present.

The authors thank Professor Igor M. Tereshin, All-Union Research and Technological Institute of Antibiotics, Leningrad, USSR, for the gift of a sample of levorin, and Dumex Ltd., Copenhagen, Denmark, for free samples of candicidin.

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Received *September 2nd*, 1980
Accepted *October 1st*, 1980

SHORT PAPERS

Determination of Allopurinol in Tablets by Differential-pulse Polarography

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Keywords: Allopurinol assay; dosage forms; differential-pulse polarography

Allopurinol (1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol) is widely used in the treatment of gout. Both the BP[1] and the USP[2] use an ultraviolet spectrophotometric method at 250 nm for the determination of the drug in tablets, although the BP[1] uses non-aqueous titrimetry in dimethylformamide for the assay of the raw material. In 1977, Rao *et al.*[3] reported a colorimetric method which employed a Folin - Ciocalteu reagent to form the coloured adduct. The major disadvantage of this procedure is the 1-h refluxing period that is required.

In 1972, De and Dryhurst[4,5] published two reports that dealt with the electrochemical reduction of allopurinol. The first was concerned with the mechanism of the electrochemical reduction while the second involved a direct electrochemical method for the assay of allopurinol and uric acid mixtures in body fluids.

The purpose of this investigation was to establish the experimental conditions that will permit the polarographic determination of allopurinol in tablets.

Experimental

Apparatus and Conditions for Polarographic Analysis

A Fisher, Model 320, pH meter fitted with a glass - calomel electrode system was employed to measure the pH of the solutions.

A PAR, Model 174, polarograph equipped with a drop-timer (Model 172A), and a Houston Omnigraphic Recorder, Model 2000, were used in the investigations. A three-electrode combination was used consisting of a saturated calomel electrode, a dropping-mercury electrode and a platinum wire as the auxiliary electrode. A PAR, Model 9301, cell was maintained at 24 $\pm$ 1 °C and all sweeps utilised a drop-time of 2 s and a scan rate of 2 mV s^{-1}. When dimethylformamide and Sorensen's citrate buffer (pH 4.75) were combined as the electrolysis system, the instrumental parameters were: applied potential range -1.20 to -1.95 V, current 50 μA, height of mercury column 70 cm and flow-rate 1.018 mg s^{-1}.

In 1.0 N sulphuric acid the parameters were: applied potential range -0.75 to -1.50 V, current 100 μA, height of mercury column 75 cm and flow-rate 1.160 mg s^{-1}.

In both instances, the modulation amplitude was set at 50 mV and the low-pass filter at a time constant of 1 s.

Controlled Potential Coulometry

A PAR, Model 379, digital coulometer was equipped with a PAR, Model 377A, three-compartment coulometric cell system and connected to a PAR, Model 173, potentiostat - galvanostat.

Sorensen's citrate buffer (pH 4.75) (19 ml) was placed in the coulometer working electrode compartment, on top of a layer of mercury, and purged with oxygen-free nitrogen for 10 min. The applied potential was set at -1.50 V and the supporting electrolyte was electrolysed until the digital readout indicated a constant but small count (usually about 30 min). The remaining background count was offset by the instrument's compensator. The electrolysis was then stopped, 1 ml of a 10^{-2} M solution of allopurinol in dimethylformamide was added to the working compartment and the system was again purged for 10 min. Electrolysis was then re-initiated at the same potential, stirring rate and flow-rate of nitrogen. About 30 min were required to complete the electrolysis of the sample.

Cyclic Voltammetry

Cyclic voltammetric experiments at a hanging mercury drop electrode were performed with a HI-TDK, Model DT 2101, 100-V, 1-A potentiostat with an *IR* compensator and a HI-TEK PPR1 waveform generator. Current - potential responses at sweep rates greater than 1 V s^{-1} were recorded on a HI-TEK AA1 256-point digital signal averager. Experiments were performed in the same cell described under Apparatus and Conditions for Polarographic Analysis.

Reagents

The following reagents were of analytical-reagent grade: citric acid; 0.1 and 1.0 N sodium hydroxide solutions; 0.1 N hydrochloric acid; 1.0 N sulphuric acid; and dimethylformamide. Sorensen's citrate buffer was prepared at eleven selected pH values over the pH range 4.0–6.5.

pH Dependence Studies

These studies were carried out in 1.0 N sulphuric acid at about pH 0 as well as in dimethylformamide with Sorensen's citrate buffer over the pH range 4.0–6.5.

Preparation of Calibration Graphs

Allopurinol (99.5%) was obtained from Burroughs Wellcome Ltd., Canada and used without further purification. Two calibration graphs were prepared.

Calibration graph 1

A stock solution of allopurinol (10^{-2} M) was prepared in dimethylformamide. Solutions containing various concentrations of allopurinol were obtained by dilution of the stock solution with Sorensen's citrate buffer at pH 4.75. In a total sample volume of exactly 20 ml, the amount of dimethylformamide was always maintained at 1 ml and the final pH of the system at 4.85. Sample concentrations for the preparation of the calibration graph by differential-pulse polarography ranged from 10^{-4} to 5×10^{-4} M.

All samples were purged with oxygen-free nitrogen for 10 min prior to each run and a stream of nitrogen was allowed to flow gently over the surface of the solution during the electroreduction. Samples of each of five concentrations were run five times and resulted in a correlation coefficient for the graph of > 0.9999.

Calibration graph 2

When 1.0 N sulphuric acid was used as the supporting electrolyte a stock solution of allopurinol (10^{-3} M) was prepared and sample concentrations ranging from 10^{-4} to 5×10^{-4} M were made by appropriate dilution of the stock solution with 1.0 N sulphuric acid. A similar procedure to that used for the preparation of calibration graph 1 was followed. The correlation coefficient for the resulting graph was > 0.9999.

Analysis of Pharmaceutical Dosage Forms

Two methods were employed. The dimethylformamide - Sorensen citrate buffer was used for 100-mg tablets. For tablets of higher potency 1.0 N sulphuric acid was generally the system of choice.

100-mg *tablets*

Twenty tablets were weighed and finely powdered. An amount of tablet mass was taken that, according to the label, would result in an approximately 10^{-2} M solution of allopurinol in 50 ml of dimethylformamide. The accurately weighed sample was stirred magnetically for 15 min in 30 ml of dimethylformamide. The solution was then quantitatively transferred into a 50-ml calibrated flask and diluted to volume with dimethylformamide. The solution was filtered by gravity and the first 10 ml were discarded. From the remainder of the filtrate a solution of approximately 2.5×10^{-4} M was prepared by diluting 0.5 ml with 19 ml of Sorensen's citrate buffer and 0.5 ml of dimethylformamide. All samples were purged with oxygen-free nitrogen as previously described.

200- and 300-mg tablets

The same procedure was followed as for the 100-mg tablets except that 1.0 N sulphuric acid was used as the solvent system and the amount of powdered tablet mass taken resulted in a 10^{-3} M solution of allopurinol in a 100-ml calibrated flask. The same filtering process was used and a solution of approximately 2.5×10^{-4} M was prepared by diluting 5.0 ml of filtrate with 15 ml of 1.0 N sulphuric acid. All samples were purged with oxygen-free nitrogen as previously described.

Results and Discussion

De and Dryhurst[4,5] have claimed to have obtained well resolved d.c. polarographic waves with allopurinol over the pH range 0–6. In the present work the d.c. wave was found to be poorly resolved over the pH range 0–6.5.

Fig. 1 shows a comparison between the d.c. and differential-pulse waves for allopurinol in dimethylformamide - Sorensen's citrate buffer at a final pH of 4.85. The d.c. wave was obscured by the background at a current setting of 50 μA. In 1.0 N sulphuric acid the d.c. wave was even more poorly resolved and consequently the differential-pulse mode was used in this investigation.

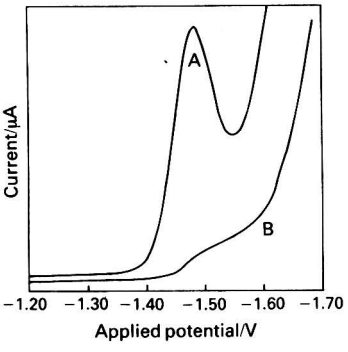

Fig. 1. Differential-pulse and d.c. polarographic waves for allopurinol (5×10^{-4} M) in dimethylformamide - Sorensen's citrate buffer system. A, Differential-pulse wave, current 50 μA; and B, d.c. wave, current 100 μA.

The effect of pH was studied and it was found that a combination of dimethylformamide and Sorensen's citrate buffer (pH 4.75) gave the most satisfactory resolution of the differential-pulse wave. However, when the 200- and 300-mg tablets of manufacturer B and the 100-mg tablets of manufacturer C (Table I) were assayed using this combination of solvents consistently low results were obtained. This situation was corrected by the use of 1.0 N sulphuric acid although this system produced slightly high results with the 100-mg tablets of manufacturers A and B. As the formulations appear to be essentially the same for all of the tablets with only the ratio of drug to excipient changing, it is difficult to offer a satisfactory explanation for these phenomena. These results are particularly unexpected as the tablet formulations were provided by the manufactures and each ingredient had been examined for possible interference in the method and none had been found.

Table I gives the results of assays of the pharmaceutical dosage forms by differential-pulse polarography. The standard deviations of the method together with the values obtained by the manufacturers' quality control laboratories are also given. It should be noted that the 300-mg tablets of manufacturer A gave equally satisfactory results with either procedure.

Controlled potential coulometry confirmed the results of De and Dryhurst,[4] showing that the reduction of allopurinol at pH 4.85 involves a 2-electron process. We were unable to confirm the 4-electron process that they reported in 1.0 N sulphuric acid.

TABLE I

ASSAY OF ALLOPURINOL TABLETS BY DIFFERENTIAL-PULSE POLAROGRAPHY

	Allopurinol content*			
			Differential-pulse polarography	
Manufacturer	Label claim/mg	Manufacturer's result, %	Method 1†, %	Method 2‡, %
A	100	98.5	99.2 ± 0.5	
	300	102.5	101.6 ± 1.0	103.4 ± 0.7
B	100	99.3	98.5 ± 0.8	
	200	101.6		100.8 ± 1.2
	300	100.6		100.4 ± 0.3
C	100	97.2		97.1 ± 0.9

* Each value is the average of five determinations.
† Dimethylformamide - Sorensen's citrate buffer.
‡ Sulphuric acid, 1.0 N.

The primary reduction peak in the cyclic voltammetric experiment occurred at −0.975 V in 1 N sulphuric acid and −1.500 V in pH 4.75 Sorensen's citrate buffer. In the cyclic voltammetric experiment, two waves were observed only in the sulphuric acid solution. For the first peaks, in both 1 N sulphuric acid and pH 4.75 Sorensen's citrate buffer, simple irreversible 2-electron transfer behaviour was observed. Detailed analysis of the responses indicated a peak potential shift of 64 and 66 mV as the sweep rate ν was changed from 50 to 500 mV s^{-1} and then to 5 V s^{-1}, respectively. No reverse anodic current was observed under the ν range studied. The peak current i_p also varied linearly with $\nu^{1/2}$ over this range, indicating the absence of any complicating homogeneous reactions on the time scale of the experiment. The separation between the peak and half-peak potentials was 51 mV at ν = 100 mV s^{-1}.

The second peak obtained in the 1 N sulphuric acid solution was at least nine times more intense and was not reproducible. This wave occurs at approximately −1.275 V against the saturated calomel electrode.

These results were substantiated by noting the absence of anodic faradaic current in a double potential step experiment from 0 to −1.100 V in the 1 N sulphuric acid solution and from 0 to −1.550 V in the pH 4.85 solution. The second pulse was from −1.550 V back to 0 V.

Grateful acknowledgement for financial assistance is made to the University of Alberta General Research Fund and to Burroughs Wellcome Ltd., LaSalle, P.Q., Canada.

The authors acknowledge with thanks the supply of pure allopurinol and the dosage forms as well as the kind co-operation and assistance provided by Burroughs Wellcome.

In addition, they thank Frank W. Horner, Inc., Mount Royal, Quebec, Canada, for a supply of pure allopurinol and ICN Canada Ltd., St. Laurent, Quebec, Canada, for tablets.

One of us (B.S.P.) acknowledges a grant from the Office of Naval Research, Washington, D.C., USA, for partial support of part of this work.

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Received *August 27th*, 1980
Received *October 21st*, 1980

Anodic Voltammetry of 12-Molybdophosphate and Molybdenum Blue at a Stationary Glassy Carbon Electrode

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Keywords: *Orthophosphate determination*; *12-molybdophosphate*; *molybdenum blue*; *anodic voltammetry*

Previously silicate was determined as 12-molybdosilicate by differential-pulse polarography at a dropping-mercury electrode after masking excess of molybdate with tartrate.[1,2] The procedure has been applied to the determination of silicon in iron and steel.[2,3] The polarographic determination of orthophosphate in the presence of excess of molybdate was not possible, but it has been determined by reduction of 12-molybdophosphate to molybdenum blue, which was then extracted into tartrate buffer of pH 3 and polarographed.[4,5]

The aim of this work was to develop a voltammetric method of determining orthophosphate as molybdenum blue at a stationary glassy carbon electrode. Molybdenum blue was expected to give an anodic wave with no interference from excess of molybdate, which cannot be oxidised further. Molybdenum blue was shown to give an anodic peak at +0.30 V *vs.* S.C.E. but unexpectedly 12-molybdophosphate also gave an anodic peak at +0.14 V *vs.* S.C.E. Optimised procedures are described for determining orthophosphate using these peaks. For most samples, however, the use of a spectrophotometric finish would be simpler and more convenient. Nevertheless, the procedures given may be used as a basis for developing analytical methods for more intractable samples for which voltammetry may be more suitable.

Experimental

Voltammograms were obtained using a PAR 174 polarographic analyser (Princeton Applied Research) with three-electrode operation (glassy carbon electrode, platinum counter electrode and calomel reference electrode). The glassy carbon electrode was manufactured by EDT Research. For both linear-sweep voltammetry (LSV) and differential-pulse voltammetry (DPV) a sweep rate of 5 mV s^{-1} was employed: for the latter technique a pulse height of 50 mV and a pulse frequency of 0.5 s were used.

Voltammetric Determination of Orthophosphate as 12-Molybdophosphate

Reagents

Standard orthophosphate solution, 3×10^{-3} M (285 μg ml^{-1} of PO_4^{3-}). Dissolve 0.408 g of analytical-reagent grade potassium dihydrogen orthophosphate in water and dilute to 1 l in a calibrated flask. This solution is 3×10^{-3} M in orthophosphate. Less concentrated standard solutions were prepared by dilution.

Acidic molybdate solution, 2% *m/V*. Add 35 ml of analytical-reagent grade concentrated sulphuric acid to 200 ml of water. Dissolve 5 g of ammonium molybdate in the resulting solution and dilute when cold to 250 ml with water.

Procedure

Transfer by pipette aliquots of standard orthophosphate solution containing 5–1500 μg of orthophosphate into a series of 50-ml calibrated flasks. To each add 5 ml of acidic molybdate solution and dilute to about 20 ml with water. After allowing to stand for 15 min, dilute the solution to 50 ml with water.

Transfer each solution in turn into a voltammetric cell and deoxygenate for 5 min with nitrogen gas without putting the glassy carbon electrode into the solution. Place the clean, dry glassy carbon electrode in the solution. After 10 s close the cell circuit with the initial

potential at 0 V in either the d.c. or d.p. mode. After a further 20 s, when the current has stabilised, obtain a linear-sweep or differential-pulse voltammogram between 0 and +0.3 V.

Clean the glassy carbon electrode between scans by washing it with 1 M sodium hydroxide solution and then water. Occasionally, when the surface of the electrode becomes tarnished, polish it with alumina and ethanol on a polishing cloth.

Voltammetric Determination of Orthophosphate as Molybdenum Blue

Satisfactory conditions for complete formation of molybdenum blue had been found previously.[4] As in the procedure described above, however, twice the amount of molybdate was used here in order to give a maximum excess of molybdate at the high concentrations of orthophosphate determined. When even higher concentrations of molybdate were used, the blank gave a new anodic wave at +0.4 V. The standard orthophosphate solution and the acidic molybdate solution were prepared as described above. It should be noted that the blank is normally blue but gives no anodic wave.

Procedure

Transfer by pipette aliquots of the standard orthophosphate solution containing 0.25–1.5 mg of orthophosphate into a series of 50-ml calibrated flasks. To each add 5 ml of acidic molybdate solution and 2.5 ml of 0.15% hydrazinium sulphate solution. Dilute to about 20 ml with water and gently mix the solutions by swirling. Heat the flasks in a boiling water-bath for 15 min, cool to room temperature, dilute the solutions to 50 ml and mix. Obtain a linear-sweep or differential-pulse voltammogram as described above but scanning between 0 and +0.6 V.

Results

Typical linear-sweep and differential-pulse voltammograms for 12-molybdophosphate and molybdenum blue in dilute sulphuric acid (pH 2) are shown in Fig. 1. The voltammetric signal for 12-molybdophosphate increases with the length of time the glassy carbon electrode is in the sample solution on open circuit before making the scan (Table I); this does not occur with the first (main) peak of molybdenum blue.

TABLE I

EFFECT OF THE LENGTH OF TIME THE GLASSY CARBON ELECTRODE IS IN CONTACT WITH A 2×10^{-4} M SAMPLE SOLUTION ON OPEN CIRCUIT BEFORE SCANNING ON THE DIFFERENTIAL-PULSE VOLTAMMETRIC PEAK CURRENT

Time/min	0.5	7	15	25	40
12-Molybdophosphate peak (+0.14 V)/μA	70	78	89	99	108
Molybdenum blue peak (+0.30 V)/μA	76	77	81	74	—

The coefficient of variation for 10 scans (DPV) with a 2×10^{-4} M solution of 12-molybdophosphate was 2.5% when the strictly timed procedure was used and the electrode was cleaned between scans. The calibration graphs were not rectilinear: this is clearly seen from the differential-pulse voltammetric currents obtained over two orders of magnitude of concentration at 1×10^{-6}, 1×10^{-5} and 1×10^{-4} M, which were 1.4, 8.0 and 40 μA, respectively.

The procedure for the determination of orthophosphate as molybdenum blue has been recommended only for amounts of orthophosphate greater than 0.25 mg because with lower amounts than this the blank value becomes unacceptably high. The use of smaller amounts of molybdate did not improve the blanks significantly. For the recommended procedure the coefficient of variation was 2% at the 2×10^{-4} M level (10 determinations).

Cyclic voltammograms, shown in Fig. 2, indicate that with both 12-molybdophosphate and molybdenum blue the anodic waves exhibit some measure of reversibility.

Brief deoxygenation with nitrogen gas was included in both recommended procedures, as it seemed to improve the shape of the voltammograms slightly. This step may prove to be unnecessary in some applications.

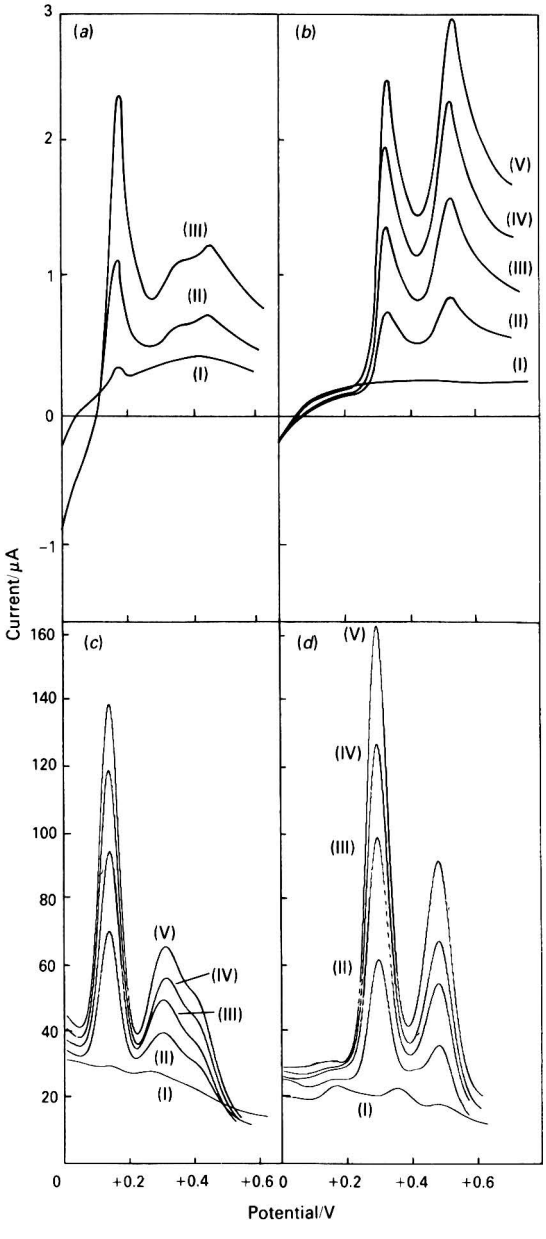

Fig. 1. Anodic voltammograms of 12-molybdophosphate and molybdenum blue in sulphuric acid solution (pH 2). (*a*) LSV of 12-molybdophosphate; (*b*) LSV of molybdenum blue; (*c*) DPV of 12-molybdophosphate; and (*d*) DPV of molybdenum blue. Equivalent orthophosphate concentration in measured solutions: (I) 0; (II) 1; (III) 2; (IV) 3 and (V) 4×10^{-4} M.

Discussion

The origin of the anodic voltammetric wave of 12-molybdophosphate appears to be the re-oxidation of material reduced early in the scan. This was confirmed by cyclic voltammetry [Fig. 2(*a*)] and by negative direction linear-sweep voltammetry (+0.6 to 0 V) which is completely cathodic. Clearly, 12-molybdophosphate is adsorbed in some form at the glassy carbon electrode on open circuit, and increased adsorption produces an increased anodic signal. The high ratio of the differential-pulse current relative to the linear-sweep current would be expected for an adsorption process.

The determination of orthophosphate as 12-molybdophosphate directly at a glassy carbon electrode is very simple but is complicated by adsorption, which causes non-rectilinear calibration graphs to be obtained. Considerable further work is required in order to realise the full potential of the method and this work is being undertaken. This includes the adaptation to flow systems, a study of interferences and their masking, the determination of other phosphate species, the determination of silicate and the development of application procedures. Preliminary studies indicate that masking procedures used in spectrophotometric methods will prove to be adaptable readily to the voltammetric method. No interference is observed in the above procedure based on 12-molybdophosphate from similar amounts of silicate or arsenate, or from 100 times the amount of nickel(II), aluminium(III) or zinc(II). Slight interference is noticeable with ten times the amount of either copper(II) or iron (III).

Orthophosphate can be determined spectrophotometrically as 12-molybdophosphate, molybdenum blue or molybdophosphovanadate at the same levels that can be determined voltammetrically by the recommended procedures. The sensitivity of the voltammetric method, however, should be capable of being increased considerably by the use of a flow cell, provided that the adsorption problems can be overcome.

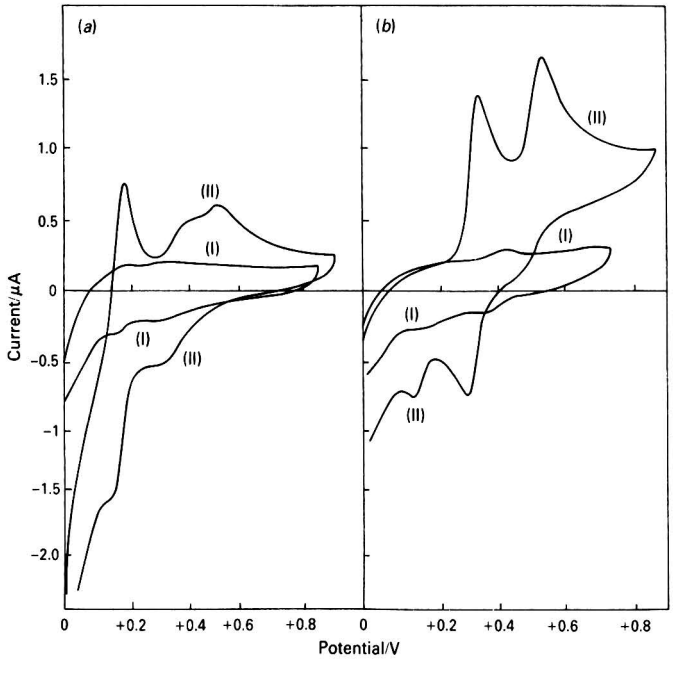

Fig. 2. Cyclic voltammograms of (*a*) 12-molybdophosphate and (*b*) molybdenum blue in sulphuric acid solution (pH 2). Equivalent orthophosphate concentration: (I) 0; and (II) 2×10^{-4} M.

One of us (N.K.B.) thanks the people of the Socialist People's Libyan Arab Jamahiriya for financial support and leave of absence from El-Fateh University, Tripoli.

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Received *May 22nd*, 1980
Accepted *August 22nd*, 1980

Simple Method for the Differentiation of Fuel Oil and Weathered Crude Oil Pollutants

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Keywords: *Weathered crude oil*; *fuel oil*; *differentiation*; *pollutants*

It is well known that a major cause of oil pollution at sea is the spillage of crude oil and fuel oil. Of the 350 pollution samples submitted to the Laboratory of the Government Chemist during the period January 1978–July 1979 (from a variety of sources), 82% were identified as either fuel oil or crude oil. Although tanker washings (crude oil cargo tank residues) and some fuel oils can be differentiated by gas - liquid chromatography, many weathered and unweathered residual fuel oils exhibit a similar profile to weathered crude oil. With very weathered samples of crude and fuel oil there is often no difference at all.

Previously the only means available for distinguishing between fuel oil and weathered crude oil was on the basis of the total asphaltene content,[1–4] but our findings are that this criterion is not always conclusive. It has been found that fuel oils often contain fluorescent compounds not found in crude oil and that these persist through the weathering process and provide a means of differentiation from crude oil.

Although gas - liquid chromatography can differentiate a fuel oil in some instances, in particular unweathered blended fuel oils, the proposed method enables a weathered fuel oil and a weathered crude oil to be distinguished.

The proposed method uses solvent extraction of a cyclohexane solution of the sample with dimethylformamide - water followed by thin-layer chromatography with detection visualisation under ultraviolet light (350 nm).

Experimental

Reagents

All reagents were of analytical-reagent grade.
Cyclohexane.
Dimethylformamide. Dilute 9 + 1 with distilled water.
Toluene.

Materials

Silica gel thin-layer chromatographic plates without fluorescent indicator, size 20 × 20 cm with a 0.25-mm layer.

Procedure

The sample must be separated from extraneous material, *e.g.*, bird feathers or sand, by extraction with cyclohexane. After removal of solvent, approximately 0.4 g of the oil under examination is dissolved in 5 cm^3 of cyclohexane. The solution is shaken for 30 s

with 10 cm^3 of the dimethylformamide (DMF) - water (9 + 1) and allowed to separate, a process facilitated by heating at 75 °C or centrifuging.

The lower dimethylformamide - water phase is transferred into a small separating funnel and diluted with 10 cm^3 of distilled water; 10 cm^3 of cyclohexane are then added and the mixture is shaken briefly. The lower phase is discarded and the upper cyclohexane phase washed twice with 5 cm^3 of water and dried by filtering through anhydrous sodium sulphate. The cyclohexane is evaporated on a steam-bath, the residue dissolved in about 0.2 cm^3 of cyclohexane and 5 μl of the solution are spotted on to the silica gel plate. The plate is developed with toluene (a 10-cm movement of the solvent front is adequate). The fluorescent components appear at $R_F \approx 0.1$–0. 2 and are green - yellow in appearance when viewed under ultraviolet light (350 nm).

It is advisable to run an extract of a known fuel oil and a distillation residue that has not been heated above 300 °C in order to provide positive and negative reference materials, respectively, rather than measure R_F values. Some samples give a faint indication of a fluorescent spot and, when the size of sample permits, a more conclusive result can often be obtained by using a larger amount of sample. It has also been shown to be feasible to test very small samples, for example extracts of bird feathers, by scaling down the amounts of sample and reagents.

Discussion and Conclusion

The fluorescent compounds characteristic of fuel oils can be produced rapidly by heating a crude oil distillation residue to temperatures above 350 °C. At temperatures above 400 °C the fluorescent compounds so produced were collected with the gaseous products. This indicates that the compounds have a boiling-point below 400 °C but they are persistent enough not to be lost in the weathering process. These findings are consistent with fuel oil production methods that include techniques such as "vis-breaking," which involves high-temperature treatment of heavy crude oil residues.

The method has been in routine use for over 4 years and the results have always been consistent with authoritative reference samples. Samples of crude oil from various parts of the world gave a negative test, while fuel oils of both European and American origin gave a positive test.

At least six compounds are known to contribute to the fluorescence, but mass spectrometry and nuclear magnetic resonance spectroscopy have so far failed to identify the individual compounds. However, it is known that they are aromatic or heterocyclic in nature and do not contain sulphur.

Thanks are due to D. M. Green and B. Askew for helpful discussions and to the Government Chemist for permission to publish this paper.

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Received *May 15th*, 1980
Accepted *October 3rd*, 1980

A Novel Filtration Technique for Soil Extracts

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Keywords: *Filtration*; *soil extracts*

A simple method of filtering soil extracts has been developed that eliminates the need for filter-funnels and receivers. It therefore reduces the risk of contamination and speeds up the procedure. It also offers a convenient means of obtaining filtrates in the field for subsequent analysis.

After shaking the soil suspension in the extraction bottle, a tube of filter-paper folded about the centre to form a V with the open ends uppermost is inserted into the bottle (Fig. 1B). Clear filtrate collects inside the paper tube and aliquots are removed with a pipette.

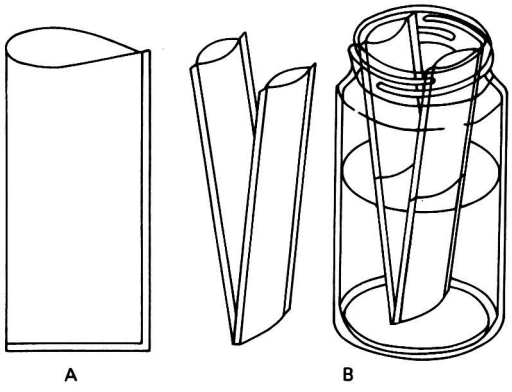

Fig. 1. Filtration apparatus.

Fig. 1 shows two types of tube that were satisfactory. Type B is preferred because it is easier to produce. Type A is formed from a piece of filter-paper of dimensions 92 × 85 mm with two edges glued together with clear impact adhesive. Type B is made from a piece of filter-paper of dimensions 200 × 60 mm glued along the long edge with a 4-mm overlap (shown folded). The adhesive did not produce any contamination in soil extracts.

The filters described are part of an NRDC Patent Application, No. 8024164.

Received *September 2nd*, 1980
Accepted *October 1st*, 1980

Book Reviews

EXPERIMENTS IN ENVIRONMENTAL CHEMISTRY. A LABORATORY MANUAL. By P. D. VOWLES and D. W. CONNELL. *Pergamon Series on Environmental Science. Volume* 4. Pp. viii + 102. Pergamon Press. 1980. Price $20, £9 (hardback); $9.95, £4.50 (softback). ISBN 0 08 024010 1 (hardback); 0 08 024009 7 (softback).

It would have been nice to have said that this book answers all the needs of those lecturers seeking a good laboratory guide for environmental chemistry. Unfortunately, despite the fact that many people will find it useful, it suffers from many shortcomings.

The authors have built the book around their own laboratory programme for environmental chemistry at Griffith University in Australia. It is divided into five parts covering aquatic systems, toxic substances, food additives, chemical ecology and a field survey. Within these five parts is a total of 14 laboratory or field experiments ranging from the measurement of biochemical oxygen demand and water pollutants through to LD_{50} values for shrimps and thin-layer chromatography on synthetic food colorants.

The book is useful in that it contains ideas that can serve as the basis for other modified laboratory procedures. I do, however, question the wisdom of including a laboratory procedure for the assay of aflatoxins in groundnuts for undergraduates. Despite the necessary precautions that the authors have clearly indicated, this is an area of study which even highly experienced chemists treat with considerable caution and should not be in a book at this level.

Course organisers in Britain and other parts of Europe will also have considerable difficulty in carrying out studies on the larvae of the monarch (milkweed) butterfly as it is uncommon, and in any case it is a protected species.

In publishing the book Pergamon Press have sacrificed any real attempt at quality with only a moderate saving in cost for the purchaser. To achieve this it has been reproduced from the authors' original typescript, with all the limitations in print size, headings and layout inherent in such a procedure. This in itself would not be too bad if greater care had been taken over editing. As it is, the number of spelling mistakes and inconsistencies is too large, and for the chemist there are several important errors.

The book contains several tables and diagrams borrowed from other sources, but these have usually been incorporated without adequate accompanying explanations or details. As a result the reader is left rather in the air. Similarly an aerial photograph of a sewage treatment plant is too small and unlabelled, so that individual sections cannot be identified and related to the text.

The authors have failed to differentiate between adsorption and absorption in chromatography; they have also labelled the ordinate on an infrared spectrum as absorption when it is clearly an absorbance scale.

Apart from typing errors like, poisioning twice on page 51, too instead of to, titrade, curvette and yeilds, the authors are inconsistent in their use of chemical names, their presentation of multiple charges on ions and their use of hyphens. These are just a few of the criticisms that can be made about the text.

I regret to say that the closer I examined this book the more disheartened I became. Whilst I do not question its undoubted value for some laboratory work I greatly regret that the workmanship in producing it is so poor.

R. C. DENNEY

ANALYTICAL ATOMIC ABSORPTION SPECTROSCOPY. SELECTED METHODS. By JON C. VAN LOON. Pp. xii + 337. Academic Press. 1980. Price £19.50. ISBN 0 12 714050 6.

Most people active in the research and development of procedures for atomic-absorption analysis, will find some reservations with respect to the selection of the individual methods included in this book. However, it must be welcomed as a valuable addition to the atomic-absorption literature. The author has had a wide experience of the development and practical application of atomic-absorption methods to many types of sample and this experience has been distilled and set out for the benefit of both the new and established user. The format includes an introductory chapter on general principles and a final chapter on possible new developments both of which are written in a very practical context, with little detailed theory and reference to other material. Whilst other texts can be consulted for more detailed theory, this volume gives many

useful practical tips not commonly found elsewhere, together with the personal views of the author on the advantages and limitations of atomic-absorption spectroscopy in its various forms.

In the 8 chapters that constitute the major part of the book, the author gives atomic-absorption procedures in full detail for determinations applicable to waters, geological materials, organic samples, metals and alloys, air samples, petroleum and petroleum products, industrial compounds and the determination of metal compounds. He has wisely chosen those for which the accuracy has been established by the use of standard reference materials, and in each instance gives his own comments on the method. All of the methods are well tested and established and should therefore be reliable. Many have been developed in the author's own laboratory and the others have been taken from the published literature. Methods covered include flame atomisation, electrothermal atomisation, hydride generation, Delves' cup and cold-vapour (Hg) procedures. The collection presents a balanced and useful coverage of the applications of atomic absorption. Some procedures do emphasise one problem that has resulted from the rapid development of commercial electrothermal atomisers in the past 2–3 years. Developments in temperature control, matrix modification, mode of atomisation, *e.g.*, from a platform, have rendered some early procedures obsolete in the sense that improved procedures can now be adopted on more modern instruments. Many of the problems that appeared in early electrothermal atomisers have now been resolved and whilst the author discusses this in his introduction, it is not explicitly explained in the relevant application sections. For the same reasons, the authors statement that he "cannot recommend the direct analysis of high salt content waters using electrothermal devices," would be challenged by many workers (including the reviewer) for many elements. The chapter on the determination of metal compounds is a notable innovation. Methods for the speciation of metals, many of them developed by the author and his co-workers, are given and indicate the likely future development of many procedures in environmental and biochemical analysis.

Without doubt this will be a very useful bench work book of particular benefit to new workers in routine analytical laboratories. There is a wealth of detailed practical information, which has not been accumulated in this form in any other text. J. M. OTTAWAY

ANALYSIS OF AIRBORNE POLLUTANTS IN WORKING ATMOSPHERES: THE WELDING AND SURFACE COATINGS INDUSTRIES. By JANET MORETON and N. A. R. FALLA. *Analytical Sciences Monographs*, No. 7. Pp. viii + 184. The Chemical Society. 1980. Price £12; $32 (RSC Members £9). ISBN 0 85186 860 6.

Following a brief but comprehensive introduction covering general legislative and occupational hygiene aspects within the UK and elsewhere, and something of the philosophy governing the measurement of airborne pollutants in industry, the contents of this book split into two parts, each written by a separate author.

The first part deals with the welding industry and opens with a chapter describing the potential of the various processes used for giving rise to a variety of atmospheric pollutants, mainly as fume and dust, but including several inorganic gases. The next chapter, on sampling, is wide-ranging, dealing both with breathing zone and with background situations. The equipment to be used in each situation is treated in some detail, particular note being made of the choice of filtering materials and pumps. The concept of fume boxes for sampling and determining the amount of airborne pollutants generated by each consumable welding unit is also described. To an analytical chemist the following chapter on the analysis of welding fumes and polluting gases undoubtedly appears lightweight and superficial. However, in the interests of maintaining a balance of content throughout the volume, sufficient information is given to indicate the applicability of a variety of physical techniques for the analysis of collected fume samples ranging from simple gravimetry to the most modern methods. Suitable methods are also reviewed for the analysis of the inorganic gases associated with welding.

The first of the three chapters in Part Two identifies the potential hazards associated with each of the several components used in the surface coating industry, namely, binders, solvents, pigments and specialist additives. Whereas the majority of pigments and pigment extenders are inorganic materials, most of the basic substances are organic and volatile, or give rise to volatile thermal degradation products and the next chapter reviews the analytical options for the determination of a variety of volatile substances. These include solvents, products of thermal decomposition, isocyanates, vinyl monomers, amines, bis(chloromethyl) ether, phenols and mercury. The choice of method in most instances is wide-ranging, from indicator tubes through to the use of gas - liquid

chromatography - mass spectrometry. It is, however, surprising to find the Health and Safety Executive method for mercury and compounds of mercury, generally recognised to be unacceptably cumbersome, still being recommended. Finally, methods are described for the collection and analysis of both organic and inorganic particulates. In general these serve to illustrate, especially in the handling of organic material, a continuing need for the thinking analyst who can decide on how best to treat a collected particulate sample in order that he may perform analyses that will yield a complete identification and quantification of its constituents.

The title of this book may give the impression that it is only for the restricted interest of those concerned with the measurement of atmospheric contaminants in the welding and surface coatings industries. This is unfortunate because this well produced volume, well referenced up to 1978, will be of interest and value and can be generally recommended to all industrial hygienists and analytical chemists involved in the monitoring of the atmospheres of industrial workplaces. R. WOOD

METHODS FOR ANALYSIS OF MUSTS AND WINES. By M. A. AMERINE and C. S. OUGH. Pp. x + 341. John Wiley. 1980. Price £19. ISBN 0 471 05077 6.

This latest volume from Amerine and Ough is essentially a re-vamp of "Wine and Must Analysis," published in 1974. There are numerous sections that are reproduced verbatim; however, there are some additions to the new volume.

The book presents a comprehensive survey of standard, and well documented, AOAC and OIV methods for analysis of wines and musts, together with a literature review up to 1979.

Most of the errors in the previous edition have been corrected, but have now been replaced by other, different, errors.

The additional material can be summarised as follows. The section on alcohol discusses losses during the fermentation process and also covers enzymatic methods for strength measurement. Surprisingly, however, there is no comprehensive comparison of different alcohol tables as expected from such a volume.

Chapter 5 is a new, albeit succinct, chapter on esters covering their formation during fermentation and typical values for ester levels. Methods for total and individual esters (including methyl anthranilate) are described.

The chapter on nitrogen compounds now includes methods for determination of arginine, and biogenic amines such as histamine and tyramine.

The subject of chemical additives has been expanded. The binding mechanism of sulphur dioxide is described and the Lieb-Zacheol apparatus is included as a method for free sulphur dioxide. Several methods for sorbic acid are included and the formation of 2-ethoxyhexadiene is discussed. Other "additives" cited are 5-nitrofurylacrylic acid, dialkyldicarbonates, sodium azide, betaine, β-Asarone, coumarin, vinyl chloride, styrene, PVPP and aflatoxins.

An extra section has been added to cover trace elements. This results from the advances in atomic-spectroscopic methods. Fluoride is mentioned in passing.

Chapter 12 is entitled "General Chemical and Equipment Information and Theory," and comprises 37 pp. It gives a somewhat basic summary of the principles of modern instrumental methods, which will be of limited use to a section of its intended audience. Incidentally the basic objective of the book is "to explore optimal procedures for the most important constituents" (of wines) and is "intended for students of enology and technical/research and development personnel."

The price of the volume is £19, which is 250% up on the previous edition. Although the content has not increased by such a figure, the book will undoubtedly find itself established as a "reference" edition in many wine and spirit companies. There is a useful selection of standard tables. D. W. CLUTTON

AQUAMETRY. PART III. A TREATISE ON METHODS FOR THE DETERMINATION OF WATER. Second Edition. By JOHN MITCHELL, JR., and DONALD MILTON SMITH. *Chemical Analysis, Volume* 5. Pp. xviii + 851. John Wiley. 1980. Price £41. ISBN 0471 02266 7.

The authors have abbreviated the sub-title of the equivalent volume of the First Edition: "Application of the Karl Fischer reagent to Quantitative Analyses Involving Water" to "The Karl Fischer Reagent." This is a particularly unfortunate contraction, as it implies that the book is concerned with Karl Fischer reagent rather than its uses. That such an impression is

incorrect may be gleaned from even a swift perusal of the comprehensive list of contents (9 pp.) and the extensive index (19 pp.).

The structure of this volume is similar to that of the First Edition, the first part dealing, in a great wealth of detail, with the determination of water in a wide range of substances and the second part with the application of Karl Fischer reagent to the determination of numerous organic functional groups.

Part A commences with a resumé of alternative methods for the determination of water and, in order to assist the analyst in selecting the most appropriate method for his particular requirement, the index includes a large number of references to Parts I and II of this treatise on Aquametry (Part I deals with general methods, and Part II with electrical methods for water determination). Following this introduction, the authors move on to the problems associated with water determination in the presence of a wide range of organic functional groups and for many analysts this will be the most useful chapter in the volume.

The nature of Karl Fischer reagent and possible alternative formulations are covered in an interesting chapter, which is followed by a wide ranging review of methods for the preparation and standardisation of the reagent, together with a comprehensive review of methods of performing the titration. This chapter covers methods of end-point detection, with an unfortunate emphasis on the usefulness of visual methods, and concludes with a section covering methods of drying solvents and analytical methods for liquids, solids and gases. Part A concludes with a section, covering the determination of water in organic compounds, which duplicates some of the earlier contents dealing with organic functional groups, and chapters covering the determination of water in commercial organic materials and inorganic compounds, an often neglected area of application for the Karl Fischer method. The final chapter of this section deals with the reactions of the reagent with inorganic compounds and is essential reading for the analyst who wishes to ensure that his water determinations are free of interference from a number of inorganic materials that react with the reagent or products derived from it.

Part B deals with the determination of a very wide range of organic functional groups via reactions that involve liberation or consumption of water, including hydroxyls, carboxylic acids anhydrides, carbonyls, amines, nitrites and peroxides, concluding with an extensive section on specific compounds, both organic and inorganic. The coverage of compounds in this section could give the impression that Karl Fischer reagent is an almost universal panacea, however, the authors have avoided giving any such impression by including a large number of examples and results that adequately illustrate the shortcomings, both in terms of specificity and precision, of such methods. However, this section remains an invaluable and unique compilation of uses of Karl Fischer reagent for the indirect determination of substances other than water.

As with other volumes in this series, the standard of production is high and the few typographical errors, such as a failure to label section "e" in Fig. IV-99 or the transposition of references 212 and 213 on p. 255 are unlikely to detract from its usefulness to any analyst concerned with the determination of water. A particularly valuable feature is the extensive bibliography that covers the literature on applications of Karl Fischer reagent up to 1978, with approximately 1150 entries. However, one area in which the current volume is open to criticism is a failure of the authors to dispense with some of the older material, which, while relevant at the time of the First Edition (1948), is now only of historical interest. In particular, the amount of space devoted to visual methods of end-point detection and apparatus for such titrations is excessive in view of the minimal use of such methods today. A more critical approach in this direction would have led to a somewhat slimmer volume at a more accessible price, without measureably detracting from the usefulness of the work.

C. A. WATSON

ELEMENTAL ANALYSIS OF BIOLOGICAL MATERIALS. CURRENT PROBLEMS AND TECHNIQUES WITH SPECIAL REFERENCE TO TRACE ELEMENTS. By INTERNATIONAL ATOMIC ENERGY AGENCY. *Technical Reports Series No.* 197. Pp. viii + 371. International Atomic Energy Agency. 1980. Price Sch.530. ISBN 92 0 115080 6.

This book consists of 15 chapters contributed by 15 authors from 8 countries. The subject matter is arranged in four sections: (*i*) "On the Need for Trace Element Analyses in the Life Sciences" (54 pp.); (*ii*) "Sampling and Sample Preparation for Trace Element Analysis" (48 pp.); (*iii*) "Analytical Techniques for Trace and Minor Elements in Biological Materials" (198 pp.); and (*iv*) "Analytical Quality Control" (46 pp.). Two appendices contain data on reference materials and on

the suitability of techniques for their analysis. The chapters on technique were first presented as review papers at an IAEA Symposium on "Nuclear Activation Techniques in the Life Sciences" held in Vienna in 1978 at which one of the themes was the comparison of neutron activation analysis with other trace analysis techniques. The most recent references quoted are dated 1978 with the majority relating to 1977 or earlier.

The declared aim of the book is to "be a valuable aid to analysts who wish to identify suitable analytical techniques for the determination of trace and minor elements in biological matrices." The book succeeds admirably in fulfilling that aim. A particularly attractive feature of the book is the availablity of information on all aspects of trace and minor elements in biology within one cover. Inevitably the treatment of any one topic cannot be regarded as exhaustive of that topic but the reader is given a sound introduction from which he can decide for himself whether to study the subject further or not.

The emphasis of the book is on analytical aspects; hence those chapters on the role of trace elements in biological systems are of limited scope, but nevertheless they provide the analyst with some indication of the breadth and importance of the subject. Chapters on sample collection, preparation and contamination demonstrate clearly that frequently it is not the sensitivity of the analytical method that limits the study of trace elements but rather the sample and its manipulation. The analytical techniques presented are neutron activation, atomic absorption, inductively coupled plasma atomic-emission spectroscopy, mass spectrometry, X-ray methods, chemical methods and electrochemical methods. Each chapter presents the theory of the technique, a description of the instrumentation and methodology employed and its cost, followed by discussion of the applicability of the technique to biological material. An important table that facilitates comparison of techniques is presented in an appendix where the precision of determination of elements in biological reference materials by a variety of methods is summarised. A chapter devoted to reference samples and associated analytical problems lists the sample types available and the certified values and notes that "reference materials for whole blood, serum and bone are also still urgently needed for comparative studies in clinical chemistry and biomedical research."

This book is not an analytical handbook, but rather a text-book from which those concerned with the analysis of biological material may broaden their knowledge on many aspects of the subject; it can be read profitably by both student and practising analyst alike. J. B. DAWSON

TREATISE ON ANALYTICAL CHEMISTRY. PART I. THEORY AND PRACTICE. Second Edition. Volume 2: Section D (continued): SOLUTION EQUILIBRIA AND CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING. Pp. xxviii + 815. John Wiley. 1979. Price £39.60. ISBN 0 471 05510 7.

This volume contains Chapters 15–23 of the Treatise's review of "Solution Equilibria and Chemistry," which began in Part I, Volume I with chapters on, "Transfer Activity Coefficients," "Electrode Potentials" and "Concept and Determination of pH." It should be noted that the Second Edition is not a volume-by-volume revision; although some chapters are updated from the First Edition, others are new in concept and content.

The first two chapters, by E. Högfeldt, are on related material, "Graphic Presentation of Equilibrium Data" (61 pp., 34 references) and "Graphic and Computational Methods in the Evaluation of Stability Constants" (65 pp., 51 references). In the latter the author shows, by example from a variety of experimental methods, how with the aid of modern programmable pocket calculators even complicated systems can be evaluated with little effort and he concludes that for good data the results from graphic and from computer methods are the same.

Acid - base equilibria in a variety of solvents are given extensive and systematic treatment. Firstly, "Concepts of Acids and Bases" (27 pp., 41 references) are outlined by the doyen of American analytical chemistry, I. M. Kolthoff. The chapter "Acid - Base Equilibria, Buffers and Titrations in Water" by D. Rosenthal and P. Zuman (79 pp., 226 references) includes a discussion of the effects of structure and solvent on dissociation constants and a summary of the principal methods of estimating equivalence points of titrations. The "General Introduction to Acid - Base Equilibria in Nonaqueous Solvents" (63 pp., 141 references) by I. M. Kolthoff and M. K. Chantooni, Jr., is an individual treatment of the subject and contains much from the authors' researches. The chapters devoted to solvent types are as follows: "Amphiprotic Solvents" (41 pp., 151 references), A. I.

Popov and J. A. Caruso; "Dipolar Aprotic Solvents" (35 pp., 128 references), I. M. Kolthoff and M. K. Chantooni, Jr.; "Inert Solvents" (39 pp., 129 references), J. Steighman; and lastly a chapter on "Inorganic Aprotic Solvents" (8 pp., 50 references) by A. I. Popov, which although from the point of view of a practical analytical chemist is fairly useless, is none-the-less important in the understanding of the chemistry it is possible to carry out in non-aqueous systems.

"Complexation Reactions," a major section (137 pp., 245 references), by (the late) A. Ringbom and E. Wänninen is a systematic and thorough treatment of the subject and their manifold analytical applications and includes a large number of useful constants and α coefficients presented in tabular format. The specific topic of "Masking and Demasking in Analytical Chemistry" (45 pp., 87 references) is dealt with with the clarity one has come to expect from its author, D. D. Perrin.

The chapter on "Mechanisms of Oxidation - Reduction Reactions," (54 pp., 122 references), by R. G. Linck, is a most useful introduction to a fundamental topic that has much developed in recent years and has implications in the application of redox reactions in systems as diverse as volumetric analysis to rapid scan electroanalytical methods. "Induced Reactions" are shown by L. J. Csanyi (94 pp., 201 references) to be important in chemical analysis using a variety of redox, complex and free radical reactions as illustrations; the author purposely excludes induced precipitation from the discussion.

The over-all standards of presentation and scholarship are high. The volume is not likely to be considered for purchase by individual chemists in industry, public service or academic analytical chemistry unless a major chapter is of particular research interest but should be a mandatory purchase by tertiary level academic libraries. D. THORBURN BURNS

TREATISE ON ANALYTICAL CHEMISTRY. PART II. ANALYTICAL CHEMISTRY OF INORGANIC AND ORGANIC COMPOUNDS. Volume 16. FUNCTIONAL GROUPS. By R. F. Muraca. Pp. xxii + 560. John Wiley. 1980. Price £26.95; $56.55. ISBN 0 471 05857 2.

The scope of this Treatise has now become so vast that the General Editors have engaged Specialist Editors to advise on specific areas. For the section on "Organic Analysis" they have had the advice of Dr. E. W. D. Huffman and of Mr. J. Mitchell, Jr. The General Editors are to be congratulated on their good fortune in securing the services of such highly respected authorities in this field.

Volume sixteen has been written entirely by Dr. R. F. Muraca and the various sections are classified as follows: azoxy groups, nitro and nitroso groups, nitrate and nitrite ester groups, and nitrile, isocyanide, cyanamide and carbodiimide groups. Every type of known method is discussed, and selected procedures are described. Presumably the recommended methods are those under the heading "Laboratory Procedures" at the end of each section.

I had formed the impression that organic functional group analysis was now the machinist's paradise and the beautiful and elegant chemical methods of former times were now out of fashion. However, most of the recommended methods are of the classical type, although details of instrumental methods are provided in the general text. I hope that this is a fair reflection of general practice.

This volume is of the same impeccable style we have learnt to expect from experience with earlier volumes and it is difficult to find fault with any of the sections. One very minor criticism is that one or two expected references are missing. Nevertheless this is an outstanding contribution to an area of chemistry which can present an array of major problems and this volume will rank as one of the best available accounts of this subject. R. BELCHER

ELECTROANALYSIS IN HYGIENE, ENVIRONMENTAL, CLINICAL AND PHARMACEUTICAL CHEMISTRY. PROCEEDINGS OF A CONFERENCE, ORGANISED BY THE ELECTROANALYTICAL GROUP OF THE CHEMICAL SOCIETY, LONDON, HELD AT CHELSEA COLLEGE, UNIVERSITY OF LONDON, APRIL 17th–20th, 1979. Edited by W. FRANKLIN SMYTH. *Analytical Chemistry Symposia Series, Volume* 2. Pp. xii + 473. Elsevier. 1980. Price $70.75; Dfl145. ISBN 0 444 41850 4 (Vol. 2); ISBN 0 444 41786 9 (Series).

This volume contains 39 papers that represent the proceedings of a symposium devoted to electroanalytical chemistry. The contributions are divided into a number of sections: applications in clinical chemistry; electroanalysis in industrial hygiene; pharmaceutical and pharmacological applications; trace analysis of drugs and metabolites; and on-line analysis and environ-

mental electroanalysis (inorganic and organic). The papers cover potentiometric and voltammetric methods and are concerned with how they can be used to solve a wide variety of analytical problems. Some papers are of a review nature while others present original work.

The standard of the papers and their presentation varies considerably; however, the plenary lecture by Simon on new ion-selective electrodes along with those by Palecek on the polarographic analysis of nucleic acids and Nurnberg's critical assessment of the voltammetric approach for the study of toxic trace metals in biological specimens are particularly recommended for reading.

Errors abound and the responsibility for these must rest upon not only the editor but also the authors as the volume has been produced directly from the original typescripts. Even the editor's introduction contains a typographical error.

High cost and the wide range of topics will deter individuals from buying personal copies; however, most electroanalysts will find a few papers of interest and would be advised to inspect a copy. For the casual or non-specialist reader the unfortunate absence of an index will considerably decrease the value of the book.

R. D. JEE

ARCHAEOLOGICAL CHEMISTRY. A SOURCEBOOK ON THE APPLICATIONS OF CHEMISTRY TO ARCHAEOLOGY. By ZVI GOFFER. *Chemical Analysis, Volume* 55. Pp. xviii + 376. John Wiley. 1980. Price £15.80. ISBN 0 471 05156 X.

At first sight this volume appears to be a comprehensive review of the applications of chemistry to archaeology, indeed a note on the dust-jacket makes the claim that it is the first such book. Also it is sub-titled a "source book." However, the applications of chemistry to archaeology are now very extensive and unless a source book compiler is in close contact with many of these, omissions can occur. It must also be said at the outset that although this book is volume 55 of "Chemical Analysis," the author has addressed it primarily to archaeologists and has therefore pitched the chemistry at an appropriate level.

There are 23 chapters divided into four main sections: Chemistry; Ancient Technology and Materials; Decay and Restoration; and Dating. The contents range from definitions of elements, compounds, atoms and molecules in the chemistry section to a chapter on palaeotemperatures and palaeoclimates in a fifth miscellaneous section titled "Anelecta."

Dr. Goffer has ranged widely in collecting his information and, in some instances, is well abreast of current developments. For example, a page is devoted to the comparatively recent use of particle accelerators for the radio-carbon dating of small samples. At the same time there are omissions in the discussion of well established fields of work, which creates a certain lack of balance For example, apart from the novel use of particle accelerators, the reader might get the impression that gas counting is the only existing standard method for radiocarbon assay as there is no mention of liquid scintillation counting, which has replaced it in a number of dating laboratories.

Also, of the analytical methods employed in archaeology, some get more detailed treatment than others. For example, Mössbauer spectroscopy, which is of limited applicability, has a page and a half devoted to it whereas the possibly more important use of X-ray diffraction gets a briefer mention. There is also the surprising statement that atomic-absorption spectroscopy has, up to the present, not been much used in the study of archaeological material. In the UK at least, it is a standard tool in archaeological laboratories.

Similarly, in the section on conservation, the use of benzotriazole is not listed with the other standard methods for the stabilisation of corroded bronze artifacts although it is probably now the most important, but strangely, it is mentioned in the glossary at the end of the chapter. Also, the stabilisation of corroded iron artifacts remains a considerable research problem and an understanding of the detailed nature of iron corrosion products is critical to the solution of this. It is possibly not very helpful, therefore, to be given (in the chapter on the corrosion of metals) the sort of simplistic equations for the rusting of iron that one finds in outdated elementary chemistry text-books.

These selected examples suffice to demonstrate the problems that can be encountered in surveying a comparatively large field to produce a source book, and finding the right level at which to pitch it, and regretfully one must draw the conclusion that what promised to be a fine conception has fallen between two stools. There is insufficient depth to satisfy a practising chemist, for whom it might have been much better to have written the book in the first place. At the same time

there is some detail that will be of only passing interest to the lay reader (including archaeologists) and insufficient detail (with significant omissions) for others, including practising conservators to whom this book is also addressed. However, in fairness to the author it must be pointed out that in the Preface he says that he found it a continual problem to decide what to put in and what to omit, and that he was not wholly satisfied with the result. Nevertheless, the book does contain some useful data for the discriminating reader who can recognise the author's problems in compiling it.

J. MUSTY

ANALYTIKER-TASCHENBUCH. Band 1. Edited by H. KIENITZ, R. BOCK, W. FRESENIUS, W. HUBER and G. TÖLG. Pp. viii + 439. Springer-Verlag. 1980. Price DM78; $43.70. ISBN 3 540 09594 2; 0 387 09594 2.

The concept of a book that contains all of the most important information about analytical chemistry, yet fits into the pocket, is exciting. In practice, the overwhelming amount of material that might claim a place in such a book might necessitate a rucksack to accommodate the inevitably numerous tomes. This text seems to be a compromise between these extremes. The first volume indeed fits into a reasonable-sized pocket, and the material is wide ranging but selective. No doubt the complete set of volumes will provide a comprehensive coverage.

Volume 1, written in German, covers sampling of solids, sample dissolution, on-line data processing and statistical treatment of errors; electrochemical techniques, flame atomic-absorption spectrometry, gas chromatography, detector tubes, polarimetric methods, ion-selective electrodes, energy-dispersive spectrometry, use of the scanning electron microscope, and methods of surface analysis; enzymatic analysis, mycotoxins, qualitative analysis of dyestuffs, detection of stimulants and dope in urine, mercury and organomercurials in water and the analysis of plutonium. The treatment is concise but a great deal of information and data is squeezed in. The chapter on electrochemical techniques, for example, has one of the most useful descriptions of the various methods that can be crammed into twenty pages of tables. Thus, for anyone who needs to have data on the topics covered readily at hand, this book will be a valuable companion.

A. TOWNSHEND

SEPARATION PROCEDURES IN INORGANIC ANALYSIS. A PRACTICAL HANDBOOK. By ROLAND S. YOUNG. Pp. viii + 475. Charles Griffin. 1980. Price £23. ISBN 0 85264 247 4.

The subject matter of this handbook is divided up into 55 chapters, the majority of which each deal with the separation of a single element from a great many others. Only the lighter alkali metals, lithium to potassium, and the Rare Earths (taken to be yttrium and the elements in the sequence lanthanum to lutecium) are considered collectively. Fluorine and chlorine are given separate chapters but bromine and iodine are omitted as is scandium, which many would have included as a Rare Earth. The heavy alkali metals, rubidium and caesium are also omitted.

Nevertheless, the task of organising information on the chosen elements or groups is obviously a formidable one. The approach adopted by the author is to first give a group separation from which individual components are later isolated. The general group scheme employed is based on precipitation reactions leading to the formation of insoluble acids (silicic, tungstic, etc.), sulphides from acidic and later alkaline solution, hydroxides, oxalates and phosphates. Frequent use is made of the fire assay method to isolate noble metals. Remaining elements or groups, such as boron, carbon, halogens, alkali metals, nitrogen, oxygen and sulphur, are considered independently. Within each group, elements are separated by precipitation, volatilisation, electrodeposition, solvent extraction, ion-exchange or other chromatographic methods. The choice here must depend to some extent on personal preference and experience, and the author generally adopts a somewhat conservative approach making use of well established methods that may not always be the most convenient or best available.

The mode of presentation of the subject matter leads to an increasing use of cross-references to earlier chapters as the book progresses with the result that the last chapter on zirconium consists of little else but lists of page references. Each chapter is terminated by a list of references with the entries referring almost entirely only to other monographs and there is a good deal of overlap from chapter to chapter with the result that, on the whole, the total body of supporting literature is somewhat limited. An example is provided by the frequent reference to the author's earlier

monograph, "Chemical Analysis in Extractive Metallurgy," published by Charles Griffin in 1971 and, incidentally, with which there is some overlap of subject matter. While it can be argued that this is as it should be in a practical handbook for use at the laboratory bench, a case can also be made for up-dating and broadening the scope by inclusion of references to recent reviews and some key original papers.

In a task as formidable as that tackled by the author, the aforementioned defects are relatively minor. For the analyst working in the metallurgical or general inorganic fields and confronted with the problem of performing an analysis on a sample containing components outside normal working experience, there is likely to be useful advice available within the covers of this handbook. It can be recommended to such analysts and to students and other research workers as a good starting point in setting up methods to meet special requirements in the analysis of complex inorganic materials.

S. J. Lyle

Advances in X-Ray Analysis. Volume 23. Edited by John R. Rhodes, Charles S. Barrett, Donald E. Leyden, John B. Newkirk, Paul K. Predecki and Clayton O. Ruud. Pp. xviii + 390. Plenum Press. 1980. Price $45. ISBN 0 306 40435 4.

This series reports the papers presented at the annual Denver X-ray conferences. Traditionally a particular aspect of X-ray analysis is given special attention and the theme chosen for this, the 28th Annual Conference in 1979, was the analysis of rocks, ores and coal by X-ray fluorescence. Of the 48 papers reprinted here, 11 were directly related to the chosen theme, a further 14 were concerned with X-ray diffraction, the remainder covered other aspects of X-ray fluorescence. In addition, the titles of 22 papers, which were presented at the conference but not published, are listed in the introductory pages.

Most of the reprints concerned with X-ray fluorescence describe the so-called "energy-dispersive" systems using cooled solid-state detectors. These cover the analysis of soils, stream sediments, mine products (including on-stream applications), brines, uranium-bearing rocks and ores, coal for ash, coal for metallic elements and sulphur, complex silicates, waters for sulphate using co-precipitation techniques, nuclear fuels, electronics products, inks on paper, air-borne particulates and bio-medical substrates. There is a fairly even split between the use of isotopes and X-ray tubes as excitation sources although some of the accounts were fairly sketchy in their description of instrumentation. Papers that appeared to be of general or unusual interest included a report by Ahlgren, Gronberg and Mattsson who determined lead in finger-bone, cadmium in kidneys and iodine in finger-tips. The analyses were carried out *in vivo* using isotope sources of 20–300 mCi and a Ge(Li) detector in conjunction with some apparently sophisticated scanning and focusing mechanisms that were not described in detail. Workers at the University of Southern California described an energy dispersive system using a HgI_2 detector that operates at room temperature without the need for a cryostat. Comparisons with Si(Li) and proportional counters suggest a resolution only slightly inferior to the former. A number of papers reported the application of microprocessors to the deconvolution of energy-dispersive "spectra" and to the correction of matrix effects. Of particular interest was the instrument developed at Outokumpu in Finland, which uses a deconvolution routine to enable discrimination between adjacent elements of the Periodic Table. The instrument employs a sealed isotope excitation source; detection is by a sealed proportional counter operating at low gas gain of which there are very few details; no filters were used. The multi-element analysis capability appeared to be limited only by the type of source used and the number of channels available.

Of the papers dealing with X-ray diffraction, several are devoted to computer matching of powder patterns with reference files. These are either reviews or synopses of recently introduced programs. A number of reports describe the use of XRD methods for determining residual strain in bearings and resins.

This volume compares favourably with others in the series and is, at least, part consolation for those readers who were unable to get to Denver.

R. Smith

Fluorimetric Assay of Polyanions in Complex Fluids: Carrageenan Stabilisers in Dairy Products and Heparin in Hog Mucosa Extracts

The spectrofluorimetric assay of several polyanions, particularly κ-carrageenan and heparin, is discussed. The presence of lipid and protein significantly affects the assay and techniques are described to overcome these interactions. These methods are then extended to complex real systems, in particular to carrageenan-stabilised milk products and heparin-containing hog mucosa extracts. The significance of the findings in terms of a general assay for biological fluids is discussed, as well as a consideration of the standards and conditions required for complete characterisation.

Keywords: *Spectrofluorimetry*; *carrageenan*; *heparin*; *biological fluids*; *polyanions*

D. MURRAY and R. B. CUNDALL
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Analyst, 1981, **106**, 335–343.

Use of Thiaminase I in the Determination of Thiamine in Biological Materials

The usefulness of thiaminase I enzyme in preparing "thiamine-free" blanks for the determination of non-thiochrome fluorescence in thiamine determinations has been evaluated. The level of thiaminase I has been optimised and an improved thiochrome procedure for the assay of thiamine developed. The modified procedure involves the direct measurement of thiamine in crude extracts and thus is much simpler and more rapid than other procedures that involve ion-exchange purification of the thiamine. Unlike the chemical procedures used for the preparation of thiamine-free blanks, thiaminase I is highly specific.

Keywords: *Thiamine determination*; *thiaminase I*; *thiochrome*; *animal tissues*; *cereal products*

CHRISTINE A. KENNEDY and BARRY V. McCLEARY
Biological and Chemical Research Institute, N.S.W. Department of Agriculture, P.M.B. 10, Rydalmere, 2116, N.S.W., Australia.

Analyst, 1981, **106**, 344–351.

Profiles of Organic Volatiles in Biological Fluids as an Aid to the Diagnosis of Disease

The transevaporator sampling technique is described for the solvent stripping of the organic volatile fraction from 5–500-μl samples of biological fluids prior to analysis by capillary column gas chromatography. The reproducible nature of the isolation and separation technique enables quantitative comparisons to be made between normal and pathological samples and marker substances of use for the diagnosis of the diseased state to be identified. Electron-capture detector profiles provide complementary information to the flame-ionisation detector that may prove to be very useful in developing biological correlations.

Keywords: *Organic volatiles*; *biological fluids*; *capillary column gas chromatography*; *electron-capture detection*; *clinical diagnosis*

ALBERT ZLATKIS, COLIN F. POOLE, ROSWITHA BRAZELL, KWAN Y. LEE, FRANCIS HSU and SUREERAT SINGHAWANGCHA
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Analyst, 1981, **106**, 352–360.

Characterisation of Candicidin and Levorin by Means of High-performance Size-exclusion Liquid Chromatography

A bimodal high-performance size-exclusion liquid chromatographic method for the comparison of aromatic heptaene macrolide antibiotics has been developed. Candicidin from different sources and levorin were found to contain components of high relative molecular mass but with different elution patterns. Ultraviolet - visible spectrophotometry of fractions collected during the size-exclusion chromatography demonstrated that both the components of high relative molecular mass and the constituents that were retained by adsorption to the packing material contain a heptaene moiety. The latter was found to be identical with the free aromatic heptaene macrolides previously characterised by reversed-phase high-performance liquid chromatography.

Keywords: *Candicidin*; *levorin*; *heptaene macrolides*; *high-performance size-exclusion liquid chromatography*; *high-performance liquid chromatography*

PER HELBOE and MOGENS THOMSEN

National Board of Health, Drug Standardisation Laboratory, 378 Frederikssundsvej DK-2700 Brønshøj, Denmark.

Analyst, 1981, **106**, 361–364.

Determination of Allopurinol in Tablets by Differential-pulse Polarography

Short Paper

Keywords: *Allopurinol assay*; *dosage forms*; *differential-pulse polarography*

L. G. CHATTEN, MARILYNN BOYCE, R. E. MOSKALYK, B. STANLEY PONS and D. K. MADAN

Faculty of Pharmacy and Pharmaceutical Sciences and the Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2N8, Canada.

Analyst, 1981, **106**, 365–368.

Anodic Voltammetry of 12-Molybdophosphate and Molybdenum Blue at a Stationary Glassy Carbon Electrode

Short Paper

Keywords: *Orthophosphate determination*; *12-molybdophosphate*; *molybdenum blue*; *anodic voltammetry*

A. G. FOGG and N. K. BSEBSU

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Analyst, 1981, **106**, 369–373.

Simple Method for the Differentiation of Fuel Oil and Weathered Crude Oil Pollutants

Short Paper

Keywords: *Weathered crude oil*; *fuel oil*; *differentiation*; *pollutants*

J. D. NEAL

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Analyst, 1981, **106**, 373–374.

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FLAP A

A Novel Filtration Technique for Soil Extracts

Short Paper

Keywords: *Filtration*; *soil extracts*

J. HUNT
National Vegetable Research Station, Wellesbourne, Warwick, CV35 9EF.

Analyst, 1981, **106**, 374–375.

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ANALAO 106 (1260) 257–384 (1981) March 1981
ISSN 0003–2654

THE ANALYST

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Printed by Heffers Printers Ltd Cambridge England
Entered as Second Class at New York, USA, Post Office