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Determination of Silver, Lead and Bismuth in Glasses by Atomic-absorption Spectrometry with Introduction of Solid Samples into Furnaces

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Atomic-absorption spectrometry with an induction or resistively heated furnace has been used for the determination of 0.007–22 μg g^{-1} of silver, 1.5–40 μg g^{-1} of lead and 0.02–15 μg g^{-1} of bismuth in 0.1–10-mg samples of standard, forensic, archaeological and ancient stained glass added directly to the furnace. Calibration graphs of peak area *versus* the mass of trace element have been constructed by using NBS standard glasses for silver and lead and standard solutions of bismuth nitrate. Information is presented on the accuracy and precision of the method for 9–29 glasses depending on the element under investigation. The limits of detection are silver 0.004, lead 0.014 and bismuth 0.02 μg g^{-1}. The significance of the results is described with particular reference to forensic work and stained glasses.

Keywords: *Silver, lead and bismuth determinations; glass analysis; atomic-absorption spectrometry; furnace atomisation; solid samples*

The determination of trace elements in glasses is important when these glasses are used for optical fibres and is helpful in establishing the origins or types of archaeological and forensic glass samples. When forensic scientists wish to establish that suspect and control samples of glass are from the same batch, they resort to the measurement of physical properties, such as density and refractive index, and to chemical analysis for major and minor elements. However, modern glasses are made by carefully controlling the composition of the major elements for batches of the same type of glass and, if significant differences in the composition of glasses of the same type are to be revealed, this is most likely to be among the minor elements and even more so among the trace elements. Catterick and Hickman[1] used inductively coupled plasma emission spectrometry to determine minor and trace concentrations of aluminium, barium, iron, magnesium and manganese in 0.2–0.5-mg samples of glass with limits of detection of 500, 5, 50, 500 and 10 μg g^{-1}, respectively, for 0.5 mg of glass. However, glasses contain many other elements at trace or ultra-trace levels and, if the list of elements that can be determined is to be extended significantly, then a convenient method is required for concentrations down to 0.1 μg g^{-1}. Lead, silver and bismuth are trace elements that occur in glasses at concentrations often considerably less than 5 μg g^{-1} and were selected in this study partly for that reason.

It is of considerable importance to establish where archaeological glasses and similar materials were made, and chemical analysis for elements in such materials is useful in tracing their origin. This can be illustrated with Scottish faience beads, where the results of chemical analysis for magnesium, aluminium, titanium, barium and silver enabled archaeologists to refute the theory that these Bronze Age artifacts originated from the Eastern Mediterranean. They were actually made in Scotland and showed that its people had a more advanced civilisation at that time than had previously been believed.[2] In matching glasses to kiln waste materials, the most convincing evidence for a perfect match is produced when minor and trace element concentrations are in good agreement in addition to a good match for concentrations of the major elements. Trace element concentrations in archaeological glasses are frequently higher than those in modern glasses but, for many elements, they are still below 10 μg g^{-1}. Useful elements in archaeological and forensic work are those whose concentrations vary widely throughout a selection of glasses.

The most widely used methods for the determination of trace elements in glass have been spark-source mass spectrometry (SSMS),[3,4] neutron activation analysis (NAA)[5,6] and furnace atomic-absorption spectrometry (FAAS).[7,8] All of these have their disadvantages. Expensive instrumentation is required for SSMS and, for very low concentrations of trace elements, long exposure times are needed. Access to a nuclear reactor is usually required for NAA. In conventional FAAS a dissolution step and often a pre-concentration step are required. This results in long analysis times and the possibility of introducing errors, either by contamination or loss of analyte, is increased.

AAS with the introduction of solid samples into furnaces has been used successfully for the determination of trace elements in metals.[9] It was decided to investigate this technique for the analysis of solid chips of glass. Most of the work undertaken refers to silver, lead and bismuth, but preliminary investigations have also been made for thallium, antimony, manganese, iron, zinc and cadmium. The only solid sampling work done previously with glass has involved mixing powdered glass with either graphite[10,11] or graphite and potassium chloride.[12]

Experimental

Materials and Solutions

Standard glasses. These were standard reference materials SRM 613, 614 and 616 from the National Bureau of Standards, Washington, DC, USA.

Glasses for analysis. These were obtained from the British Glass Industry Research Association, Sheffield, the Society of Glass Technology, Sheffield, the Metropolitan Police Forensic Science Laboratory, London, and the Department of Ceramics, Glasses and Polymers, University of Sheffield. The types and forms of the glasses are shown in Table 1. The ancient stained glasses from York Minster were small sheets, approximately 3 mm thick, with red staining extending about half-way through the otherwise colourless material. These sheets were sawn parallel to their large faces to produce both red and colourless samples for analysis.

All glass samples were cleaned by washing with ethanol and immersing in 1.5 M nitric acid for 30 min. They were then rinsed with distilled water and air-dried between filter-papers. The samples were then wrapped in thick polythene sheeting,

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Table 1. Types and forms of glasses analysed

Code	Type of glass	Form
EC1.1*	Na_2O - MgO - CaO - SiO_2	Float glass
EC1.2*	Na_2O - MgO - CaO - SiO_2	Sheet glass
SGT2†	Na_2O - B_2O_3 - Al_2O_3 - SiO_2	Broken rod
SGT3†	K_2O - PbO - SiO_2	Broken tumblers
SGT4†	Fluoride-opal	White opal sheet
SGT5†	Na_2O - MgO - CaO - SiO_2	Tableware
SGT6†	Na_2O - CaO - SiO_2	Broken bottles
SGT7†	Na_2O - CaO - SiO_2	Flat-sided bottles
SGT9†	K_2O - PbO - SiO_2	Flat disc
A - F‡	Na_2O - CaO - SiO_2	
MC 791‡	Na_2O - MgO - CaO - SiO_2	Sheet glass
YM1, YM3, YM4, YM6§	Ancient stained glass	Window fragments
Hutton, Rosedale, Bagots Park and Kimmeridge¶	Na_2O - K_2O - CaO - SiO_2	Ancient containers (*ca.* 1600 A.D.)

* From British Glass Industry Research Association.
† From Society of Glass Technology.
‡ From Metropolitan Police Forensic Science Laboratory.
§ YM1 and YM3 are 12th century glass; YM4 and YM6 are 14th century glass; all from York Minster.
¶ From Department of Ceramics, Glasses and Polymers, University of Sheffield.

Table 2. Instrumental settings for the induction furnace and the Perkin-Elmer 300S

Element under study	Core temperature/ °C	Resonance line/nm	Slit width/nm
Lead	2 100–2 400	283.3	0.7
Silver	2 300–2 400	328.1	0.7
Iron	2 350	248.3	0.2
Thallium	2 250	276.8	0.7
Antimony	2 250	217.6	0.2
Manganese	2 400	279.5	0.2
Zinc	1 870–2 300	213.9	0.7
Cadmium	2 350	228.8	0.7

which has been soaked overnight in 8 M nitric acid and dried, and crushed with a plastic mallet so as to produce chips that weighed up to 10 mg. Such chips were added directly to the furnaces. However, for wet-chemical analysis of the glasses, crushing was continued until no single piece weighed more than 0.5 mg.

Hydrofluoric acid. Aristar grade, 40% *m/m*, BDH Chemicals.

Nitric acid. Aristar grade, sp.gr. 1.42, BDH Chemicals.

Standard silver solutions. Dissolve 0.394 g of silver nitrate (Engelhard, photographic quality) in 25 ml of distilled water. Add 25 ml of 1.6 M nitric acid and make up to 250 ml so as to give a solution 0.16 M in nitric acid and 1 mg ml^{-1} in silver. Prepare more dilute solutions when required by dilution with 0.16 M nitric acid.

Standard bismuth solutions. Dissolve 0.250 g of bismuth powder (Koch-Light Laboratories, 99.999 5%) in a minimum volume of 4 M nitric acid. Dilute the solution to 250 ml with 2 M nitric acid to give a solution 1 mg ml^{-1} in bismuth and approximately 2 M in nitric acid. Prepare more dilute solutions when required by dilution with 2 M nitric acid.

Apparatus

Induction furnace

This was identical with that previously described,[13] except that the graphite core and side-arms were made from AGTS (British Acheson Electrodes) or UF4S (Ultra Carbon) grade graphite and the window mounts were modified to accommodate the flow of a window gas as described by Baker and Headridge.[14] The graphite core, side-arms and powder were baked under vacuum for 10 h at approximately 1 500 °C (35 h for lead). Absorbances were measured on a Perkin-Elmer 300 atomic-absorption spectrometer fitted with a JJ Instruments CR 552 recorder.

Resistively heated furnace

This was an Instrumentation Laboratory 555 furnace fitted with a square-section graphite cuvette to accommodate graphite microboats. It was fitted within a Varian AA-6 atomic-absorption spectrometer whose amplifier response had been speeded up according to the modification of Lundberg.[15] Absorbance - time peaks were recorded on a JJ Instruments CR 552 recorder. The flow-rate of argon within the furnace was always maintained at 20 standard cubic feet per hour (*ca.* 9.5 l min^{-1}).

Measurement Procedures for a Series of Solid Samples

Induction furnace

The furnace was operated in the manner already described.[13] The following flow-rates of argon were employed: purge gas, 40 ml min^{-1}, except when determining silver, antimony and thallium, when the flow-rates were 60, 50 and 30 ml min^{-1}, respectively; stir gas, 300 ml min^{-1}; and window gas 1 l min^{-1}. Other instrumental settings are shown in Table 2. With the appropriate hollow-cathode lamp in position, recordings of absorbance *versus* time were obtained for each sample. Peak areas (absorbance × time in seconds) were determined by multiplying the peak height by the width at half-height. Sample masses were within the range 0.1–10 mg and were determined with a five-place balance. Glass chips could be added to the furnace every 2 min. Each sample should consist of a single chip.

Resistively heated furnace

A chip of glass (0.1–2 mg) was placed on a microboat (uncoated graphite, Ringsdorff RWO 322) and inserted into the cuvette at room temperature. The cuvette was then heated to a high temperature in a programmed manner and allowed to cool to near room temperature before insertion of the next sample. Peak areas were determined as for the induction furnace. Samples could be processed every 2–3 min. These masses were determined with a five-place balance.

Calibration graphs with the induction furnace

For the determination of silver, lead and iron in glass samples, calibration graphs of peak area *versus* mass of element were obtained by adding increasing masses of SRM 614 or SRM 616 to the graphite core at a constant temperature (see Table 2) to

Table 3. Instrumental settings for the IL 555 furnace and Varian AA-6. The microboats were cleaned by holding at 2 800 °C for 5 s at the end of each programme

Element under study	Resonance line/nm	Slit width/nm	Temperature programme/°C
Silver (solid samples)	328.1	0.5	Ambient $\xrightarrow[5\text{ s}]{\text{ramp}}$ 1 000, hold 5 s $\xrightarrow{\text{step}}$ 2 700, hold 5 s
Silver (solutions)	328.1	0.5	Ambient $\xrightarrow[20\text{ s}]{\text{ramp}}$ 60 $\xrightarrow[20\text{ s}]{\text{ramp}}$ 120 $\xrightarrow[20\text{ s}]{\text{ramp}}$ 500, hold 5 s $\xrightarrow{\text{step}}$ 2 800, hold 5 s
Bismuth (solid samples)	306.8	0.4	Ambient $\xrightarrow[5\text{ s}]{\text{ramp}}$ 1 200, hold 5 s $\xrightarrow{\text{step}}$ 2 700, hold 5 s
Bismuth (solutions)	306.8	0.4	Ambient $\xrightarrow[20\text{ s}]{\text{ramp}}$ 60 $\xrightarrow[20\text{ s}]{\text{ramp}}$ 120 $\xrightarrow[20\text{ s}]{\text{ramp}}$ 400, hold 5 s $\xrightarrow{\text{step}}$ 2 700, hold 5 s

produce absorbances up to 1.0. Nine samples were used to obtain a calibration graph. Sample masses should not exceed 10 mg.

In a further limited study, calibration graphs were also obtained for thallium, antimony, manganese, zinc and cadmium using standard glass samples added directly to the furnace, to assess the potential of the method for determining these elements in glasses. The absorbances were obtained using the experimental conditions shown in Table 2.

Calibration graphs with the IL 555

For the determination of silver in glass samples, calibration graphs of peak area *versus* mass of element were obtained by adding increasing masses of SRM 614 (0.2–0.6 mg) to uncoated graphite microboats, which were heated under the conditions shown in Table 3 so as to produce absorbances up to 0.7. Nine samples were again used to obtain a calibration graph. For bismuth, calibration graphs were produced using 1–10-μl volumes of a 0.2 μg ml^{-1} solution of bismuth as nitrate dispensed on to microboats coated with pyrolytic graphite. (Solutions tend to sink into uncoated microboats. This results in curved calibration graphs.)

Procedure for the Determination of Silver, Lead, Bismuth and Iron in Glasses Using the Furnaces

Induction furnace

When a series of glasses is to be analysed for a particular element, suitable masses (six of each glass) are introduced into the induction furnace whose core has been constructed from the densest graphite available (see Discussion). During the same run, various masses of a standard glass are also added for the purpose of constructing a calibration graph. The maximum number of glass samples that can be added to the core before its replacement is about 500, corresponding to approximately 2.5 g of glass. When the run involving 33 samples has been completed, the calibration graph is drawn and the mass of element in each sample is obtained from the graph. The concentrations of element in the samples are then calculated.

Resistively heated furnace

The procedure is similar to that for the induction furnace except that samples are placed on microboats which are pushed horizontally into the IL 555 furnace. Glass chips weighing more than 2 mg are too large to pass through the entrance slit of the cuvette. Masses should be restricted within the range 0.1–2 mg. The total mass of samples that can be added to any microboat for repeated introduction to the furnace is 10 mg. Accumulating masses in excess of 10 mg produces a hole in the bottom of the microboat as a result of the reaction of silica with hot graphite.

Procedures for the Determination of Silver and Bismuth in Glasses After Dissolution

Because only one standard glass (SRM 614, 0.42 μg g^{-1} of silver) was available with a silver content near to those of the glasses being analysed, it was considered necessary to analyse a few glasses for silver after dissolution, to check on the accuracy of the FAAS method with solid samples. This was done by the well established method of FAAS using solutions and the method of standard additions. Likewise, a glass was analysed for bismuth after dissolution as no glasses of known bismuth content were available, and it cannot be assumed without additional evidence that aqueous bismuth nitrate solutions can be used to construct a calibration graph for the accurate analysis of solid samples of glasses added directly to a furnace.[16]

Method for silver

Dissolve an appropriate mass of glass within the range 0.1–0.4 g in a PTFE beaker with 10 ml of concentrated hydrofluoric acid. Heat until all the glass has dissolved and evaporate to dryness. Add 10 ml of water, swirl the solution until all the solids have dissolved, then add 5 ml of concentrated nitric acid and evaporate to dryness. Repeat this procedure. Dissolve the residue in 10 ml of water and dilute to 25, 50 or 100 ml in a calibrated flask with nitric acid and water such that the final concentration of nitric acid is about 2 M. Prepare a blank solution in an identical manner by adding all the reagents but no glass.

Pipette 5 ml of the blank solution and 5-ml aliquots of the glass solution into dry flasks labelled blank, 0, 1, 2, 3 and 4. Add 10-, 20-, 30- and 40-μl volumes of standard silver solution into flasks 1, 2, 3 and 4, respectively, and mix thoroughly (the volume change is negligible). The mass of glass taken and the concentration of the standard silver solution should be such that the amount of silver added in the first standard addition (10 μl) should be similar to that to be expected in 5 ml of the glass solution.

Pipette 10 μl of blank solution, using an all-glass micropipette, into a new pyrolytic graphite microboat and determine the peak absorbance for silver using the IL 555 furnace with the instrumental settings shown in Table 3. Repeat this operation with the same tray, processing the solutions in the following order to correct for any decrease in sensitivity with time: another blank, 0, 0, 1, 2, 3, 4, 4, 3, 2, 1, 0, 0, blank, blank. Average the results for each concentration, subtract the average blank level and determine the concentration of silver in flask 0 from the standard addition plot. Repeat this procedure for the series of flasks twice more and average the three results to obtain the silver content of the glass.

Method for bismuth

This is identical with that for silver except that the temperature programme for the IL 555 furnace is that shown for bismuth in Table 3.

Results

Results for the determination of lead in glasses using the induction furnace and SRM 616 (1.85 μg g^{-1} of lead) to construct the calibration graphs, and a core made from UF 4S

Table 4. Results for the determination of lead in glasses using the induction furnace

Glass	Lead content found/μg g^{-1}	R.s.d., %
SGT5	10	2
	11	10
SGT6	4.4	5
	5.0	9
SGT7	4.8	5
	4.8	7
EC1.1	5.0	3
EC1.2	2.3	8
A	1.8	5
B	2.3	14
C	1.5	13
D	1.7	18
E	2.0	11
F	1.8	6
MC 791	5.0	4
	5.2	11
Hutton	13	5
Bagots Park	23	12
SRM 614*	2.22	5
SRM 613†	36	6
	38	2
	35	12

* Certificate value 2.32 μg g^{-1} of lead.
† Certificate value 38.6 μg g^{-1} of lead.

Table 5. Approximate results for the determination of lead in some glasses using the induction furnace. The standard used was SRM 616 (1.85 μg g^{-1} of lead)

Glass	Lead content found/μg g^{-1}	Glass	Lead content found/μg g^{-1}
SGT4	100	YM3, stained	>500
Rosedale	60	YM4, clear	60
Kimmeridge	100	YM4, stained	150
YM1, clear	70	YM6, clear	50
YM1, stained	200	YM6, stained	250
YM3, clear	100		

graphite, are shown in Table 4. Some approximate results for lead in glasses containing more than 40 μg g^{-1} of lead are shown in Table 5. The characteristic mass of lead, *i.e.*, the mass of element for 1% absorption, varies with core temperature, being 90 pg at 2 100 °C and 30 pg at 2 400 °C.

Results for the determination of silver in the glasses using the induction furnace, and for some glasses the resistively heated furnace, employing SRM 614 (0.42 μg g^{-1} of silver) to construct the calibration graphs, are shown in Table 6, together with the silver contents of three glasses obtained by the standard additions method after dissolution. The characteristic mass for silver was 55 pg at 2 400 °C using the induction furnace and 3.3 pg using the IL 555.

Because no glasses standardised for bismuth were available, it was necessary to construct calibration graphs from standard solutions of bismuth. These are much more readily handled with the IL 555 furnace and hence all the results reported for bismuth in Table 7 were obtained with this furnace. Peak-height rather than peak-area measurements were employed for peaks with absorbances less than 0.05. The characteristic mass for bismuth was 16 pg. Glass SGT3 was also analysed for bismuth by AAS after dissolution and a result of 13 μg g^{-1} was obtained.

From calibration graphs obtained for thallium, antimony, manganese, iron, zinc and cadmium, using standard glass samples added directly to the induction furnace, the following characteristic masses were obtained after correcting for any background absorption: thallium 110 pg (2 250 °C), antimony 550 pg (2 250 °C), manganese 550 pg (2 400 °C), iron 1 100 pg (2 350 °C), zinc 13 pg (2 300 °C) and cadmium 14 pg (2 350 °C); the temperatures in parentheses are those of the induction furnace for the element under study. When SRM 614 was analysed for iron using a calibration graph prepared with the induction furnace from SRM 616 (11 μg g^{-1} of iron), an iron content of 13 μg g^{-1} was obtained, identical with the certificate value.

Discussion

The results in Table 4 for the determination of lead are of acceptable accuracy. It will be noticed that the average result for SRM 613 (36.3 μg g^{-1} of lead) is slightly low. This is because SRM 613 produces a wider peak of absorbance *versus* time than does SRM 616 and it is likely that a small amount of lead escapes through the walls of the graphite core before the bulk of the lead reaches the light path. This is borne out by the fact that in earlier determinations the core was constructed from the less dense graphite AGTS (sp.gr. 1.60) and the average lead content for SRM 613 was calculated as 30 μg g^{-1}. Diffusion of lead through the walls is greatly reduced by using the denser UF4S graphite (sp. gr. 1.72). The average relative standard deviation for the determination of lead in glass is 8%, which is similar to that reported for the determination of trace levels of lead in metals.[9] A limit of detection cannot be obtained from the results reported in Table 4 because all of these concentrations are very much greater than the limit of detection. However, it is possible to calculate an approximate limit of detection using the equation previously reported by Headridge and Nicholson[17]:

$$\text{Estimated limit of detection} = \frac{\text{mass of element for 1\% absorption} \times 0.2 \times \text{average r.s.d. of the method (\%)}}{\text{mass of sample}}$$

This was calculated as 0.014 μg g^{-1} of lead in glass at 2 100 °C using a 10-mg sample.

The results shown in Table 5 are only approximate because the method is too sensitive to produce accurate results for lead at concentrations above 40 μg g^{-1} owing to the very small masses of glass that have to be analysed. They are included mainly to show that the ancient glasses usually have lead contents much higher than modern glasses. It is interesting that the stained part of the York Minster glasses always contains more lead than the clear part. The staining is caused by copper dispersed throughout the glass and this copper probably contains lead, which produces the elevated lead levels in the stained portions.

The results in Table 6 for the determination of silver are of acceptable accuracy, agreement between the three methods being very satisfactory. The results for silver obtained using the induction furnace are probably slightly low for a few glasses, such as SRM 613 because these results were obtained early in the investigation when cores were constructed from AGTS graphite rather than the denser UF4S. However, glasses produced silver absorption peaks similar in width or not much wider than that for the standard (SRM 614) and results that were significantly low, because of increased loss of silver by diffusion through the core walls, were not expected, in contrast to the situation with lead. Even so, it is suggested that the denser grades of graphite should be used for core construction if results of the highest accuracy are to be obtained for silver.

The average relative standard deviation for the determination of silver in glass using the induction furnace is 11% if the results for MC 791 and the stained York Minster glasses are not included. This is similar to the relative standard deviations reported for the determination of silver in nickel-base alloys.[18] The limit of detection for silver in glass calculated from the data obtained for glass MC 791 was 0.004 μg g^{-1}. It will be

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Table 6. Results for the determination of silver in glasses, with relative standard deviations in parentheses

Glass	Silver content found/μg g^{-1}		
	Induction furnace	IL 555	Standard additions method after dissolution
SGT2	0.035 (14)		
SGT3	0.74 (4)	0.89 (5)	0.73
SGT4	0.32 (2)	0.19 (8), 0.23 (6)	
SGT5	0.088 (18)	0.11 (39)	
SGT6	0.024 (16)		
SGT7	0.030 (10), 0.026 (8)		
SGT9	3.1 (1)		3.0
EC1.1	0.028 (13), 0.025 (14)		
EC1.2	0.058 (10), 0.065 (13)		
A	0.34 (11)	0.33 (14)	
B	0.67 (10)	0.89 (10)	0.78
C	0.41 (7)	0.43 (4)	
D	0.37 (10)	0.41 (15)	
E	0.31 (9)	0.26 (19)	
F	0.40 (14)	0.46 (9)	
MC 791	0.007 (30)		
Hutton	0.093 (18)		
Bagots Park	0.16 (14)		
Rosedale	0.67 (6)	0.87 (8)	
Kimmeridge	0.36 (12)	0.40 (9)	
YM1, clear	0.37 (12)		
YM1, stained	4.9 (26)		
YM3, clear	0.39 (11)		
YM3, stained	7.3 (9)		
YM4, clear	0.54 (6)		
YM4, stained	3.8 (32)		
YM6, clear	0.23 (16)		
YM6, stained	1.7 (32)		
SRM 613*	19 (16), 21 (7), 19 (7), 19 (7)		

* Certificate value 22 μg g^{-1} of silver*

Table 7. Results for the determination of bismuth in glasses using the IL 555

Glass	Bismuth content found/μg g^{-1}	R.s.d., %
SGT2	0.02*	47
SGT3	15	11
SGT4	0.15	20
	0.20	14
SGT5	0.07	4
SGT6	0.05*	19
SGT7	0.03*	35
Kimmeridge	0.49	14
Rosedale	8.3	3
Bagots Park	0.12*	21

* Using peak height.

noticed from Table 6 that the silver concentrations of the stained parts of the York Minster glasses are very much higher than those in the clear glasses. As for lead, the elevated silver levels in the stained parts probably arise from the presence of silver in the copper used to produce the red stain.

Because the induction furnace is situated within a Perkin-Elmer 300, which is not fitted with a background corrector, it was necessary to check that background absorption was negligible under the conditions employed for the determination of lead and silver. This was done using a hydrogen lamp at the wavelength employed for lead and silver. At no time was the absorbance in excess of 0.01.

The results in Table 7 for the determination of bismuth are only approximate as aqueous bismuth solutions were used to construct the calibration graphs, but the results are probably accurate to within 15%, as results of this accuracy have been obtained for the determination of bismuth in steels, nickel-base alloys and coppers, when calibration graphs were also constructed from standard bismuth nitrate solutions.[16] This conclusion seems reasonable because the bismuth content of glass SGT3 obtained by analysing the solid and by AAS after dissolution is 15 and 13 μg g^{-1}, respectively. The dissolution method is not sufficiently sensitive to determine levels of bismuth below 0.5 μg g^{-1}.

For the determination of bismuth in solid samples of glasses added to the IL 555 furnace, the average relative standard deviation for results based on peak area was 11%, which is usual for this technique. The limit of detection as calculated from the peak-height results for SGT2, SGT6 and SGT7 was 0.02 μg g^{-1} of bismuth.

In conclusion, it can be stated that a very sensitive method of acceptable accuracy and precision has been developed for the determination of silver, lead and bismuth in glasses. With a background corrector fitted to the Perkin-Elmer 300 atomic-absorption spectrometer used with the induction furnace, it should also be possible to determine very low levels of thallium, zinc and cadmium in glasses and concentrations of antimony, manganese and iron in excess of approximately 0.2 μg g^{-1}. Undoubtedly this list of elements could be extended if trace element contents of other volatile elements in the standard reference materials SRM 613, 614 and 616 became available. This would be particularly helpful in connection with the determination of trace elements in optical fibres. The concentrations of light-absorbing elements in glasses used for optical fibres must be extremely low if long-distance laser communication systems using fibre optic waveguides are not to suffer unacceptable levels of attenuation (the power loss must be <20 dB km^{-1}).[19] The light-absorbing ions are V(III), Mn(III), Cr(III), Fe(II), Co(II), Ni(II) and Cu(II). The

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quality control of these glasses requires an analytical technique that is both extremely sensitive and reasonably rapid. It should be possible to extend the furnace methods with solid samples to the analysis of optical fibres. It has been shown that iron can be determined in glass using AAS and the induction furnace and it seems reasonable to assume that this method could be applied to other transition elements when suitable standards become available.

The concentrations of bismuth and silver in glasses have been found to be more variable than those of lead and, for forensic purposes, bismuth and silver would appear to be better trace elements than lead for distinguishing between samples.

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Quantitative Determination of Lead and Cadmium in Foods by Programmed Dry Ashing and Atomic-absorption Spectrophotometry with Electrothermal Atomisation

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This paper describes the simultaneous determination of lead and cadmium in foods by programmed dry ashing, in the presence of magnesium nitrate, complexing with sodium diethyldithiocarbamate, extraction with isobutyl methyl ketone and atomic-absorption spectrophotometry using the graphite furnace technique. Recoveries are 94–100% for lead and 94–109% for cadmium.

Keywords: *Lead and cadmium determination; programmed dry ashing; atomic-absorption spectrophotometry; electrothermal atomisation; foods*

The determination of lead and cadmium in food products for both man and animals has already received considerable attention and plays an important role in trace element analysis. The principal sources of environmental contamination of foods by lead are exhaust gases, industrial discharge and its presence in the soil; industrial food processing can also give rise to lead contamination. Cadmium contamination is largely accounted for by the volatilisation of the metal from metallurgical and other industrial plants, but the intensive application of fertilisers can lead to soil being a source as well.[1,2]

Because lead and cadmium are both highly toxic, sufficiently sensitive methods are required for their determination. However, the volatile character of the two metals, in an elemental as well as in an organic-bound form, not only contributes to their spreading over large areas, but complicates the analysis because their volatilisation must be prevented in an ashing procedure prior to determination in the food product. In the literature, numerous procedures for the ashing are described, including dry ashing with or without ashing aids and with or without temperature programming[3–7] and wet digestion with mixtures of sulphuric and nitric acids, sulphuric acid and hydrogen peroxide and nitric and perchloric acids.[8,9] Further expedients include high-pressure digestion in a decomposition vessel and low-pressure, low-temperature ashing.[10]

All of these techniques have advantages and limitations. In this study programmed temperature ashing with magnesium nitrate as an ashing aid, added in ethanolic solution, was chosen[11,12]—ashing can be carried out at night, which saves time, and a relatively large sample can be analysed (5 g).

After dissolving the ash in acid, sodium diethyldithiocarbamate (NaDDTC) was added as a complexing agent, followed by extraction of the complex in isobutyl methyl ketone (IBMK). In the extract, the levels of lead and cadmium were measured by atomic-absorption spectrophotometry with the graphite furnace technique. The method is highly sensitive and has good reproducibility.

Experimental

Apparatus

The following apparatus was used: 30- and 50-ml quartz dishes, warmed with 7 N nitric acid and rinsed thoroughly with de-mineralised or distilled water; an electrical hot-plate or drying oven; a muffle furnace with temperature programming; 50-ml polythene bottles, rinsed thoroughly before use with demineralised or distilled water; a pH-meter; and a Perkin-Elmer Model 430 atomic-absorption spectrophotometer with a Model HGA 500 graphite furnace, a Model AS-1 autosampler and PTFE sample cups.

Reagents

Magnesium nitrate solution, 10% m/V in 95% ethanol. Analytical-reagent grade.

Nitric acid, 65% (sp. gr. 1.40). Analytical-reagent grade.

Ammonia solution, 25% (sp. gr. 0.91). Analytical-reagent grade.

Isobutyl methyl ketone. Analytical-reagent grade.

Phenol red solution, 1% m/V. Dissolve 1 g of phenol red in 100 ml of de-mineralised water, heat on a water-bath, cool and filter the solution with a white ribbon filter.

Potassium cyanide solution, 10% m/V in de-mineralised water.

Sodium diethyldithiocarbamate (NaDDTC) solution, 1% m/V in de-mineralised water. Prepare fresh daily and purify by extracting twice with IBMK.

Ammonium citrate buffer solution. Dissolve 400 g of citric acid (analytical-reagent grade) in 100 ml of de-mineralised water. Add 400 ml of ammonia solution and 40 ml of a 1% phenol red solution. Adjust the pH to 8.8 by adding ammonia solution or citric acid solution, using a pH-meter. Bring the volume to 1 l. Transfer the buffer into a 2-l separating funnel, add 25 ml of NaDDTC solution, mix, add approximately 80 ml of IBMK and shake for 2 min. Allow to separate and discard the organic layer. Repeat the procedure and store the buffer solution in a polythene bottle.

Lead stock standard solution (1 ml = 1 mg *of lead*). Weigh 1.598 5 g of $Pb(NO_3)_2$ and rinse into a 1-l calibrated flask with de-mineralised water. Add 70 ml of 65% nitric acid and make up to volume. Store the solution in a polythene bottle.

Cadmium stock standard solution (1 ml = 1 mg *of cadmium*). Weigh 2.282 0 g of $3CdSO_4.8H_2O$ and proceed as described for the lead stock standard solution.

Combined lead - cadmium standard solution, containing 1 p.p.m. *of lead and* 0.1 p.p.m. *of cadmium.* Pipette 500 µl of the lead stock standard solution and 50 µl of the cadmium stock standard solution into a 500-ml calibrated flask and make up to volume with 3% *V/V* nitric acid. Mix and transfer into a polythene bottle. Prepare fresh daily.

Procedure

Weigh into a quartz dish 1.0–5.0 g of sample and add 5 ml of the magnesium nitrate solution. Dry on a hot-plate by gently raising the temperature until the sample is completely dry. Place in the muffle furnace and apply the following temperature programme: 2 h at 150 °C, 2 h at 250 °C, 3 h at 350 °C and 6 h at 450 °C. If the ashed residue contains residual carbon, wet it with 1 ml of de-mineralised water, add 1 ml of 65% nitric acid, dry on the hot-plate and place the samples in the muffle furnace at 350 °C for 30 min.

Take up the fully ashed sample in 1 ml of water and 5 ml of 65% nitric acid, warm on a hot-plate for a few minutes and rinse with 25 ml of de-mineralised water into a 50-ml polythene bottle. Add 4 ml of ammonium citrate buffer solution and enough ammonia solution to obtain a deep red colour. Check the pH with the pH-meter and adjust to 8.8 ± 0.2. Allow to cool, add 1 ml of potassium cyanide solution and mix. After 5 min add 5 ml of the NaDDTC solution, mix and allow to stand for 10 min. Add 5.0 ml of IBMK and shake for 1 min. After separation of the organic phase transfer the IBMK layer, the sample extract, into a PTFE cup of the autosampler for atomic-absorption measurement.

Prepare reference extracts as follows: pipette 25 ml of de-mineralised water and 5 ml of nitric acid into each of five polythene bottles and add 0, 125, 250, 375 and 500 µl of the combined lead-cadmium standard solution. Proceed as above, *i.e.*, starting from "Add 4 ml of ammonium citrate buffer." The lead concentrations in the reference extracts will thus be 0, 25, 50, 75 and 100 parts per billion (p.p.b.) and the cadmium concentrations 0, 2.5, 5, 7.5 and 10 p.p.b. All extracts are measured in succession on the day of their preparation. The concentrations in the sample extracts are found by comparing their peak heights with those of the reference extracts.

Measurement Conditions

Lead

Using an electrodeless discharge lamp and a molybdenum-coated graphite tube,[13] measurements for lead were carried out under the following conditions: background correction, on; wavelength, 283.3 nm; slit width, 0.7 nm; integration time, 8 s; and recorder, 1 V.

The peak height was measured using the following programme:

Step 1	100 °C	25 s ramp	20 s hold
Step 2	110 °C	10 s ramp	10 s hold
Step 3	120 °C	10 s ramp	10 s hold
Step 4	600 °C	25 s ramp	25 s hold
Step 5	2300 °C	3 s ramp	7 s hold

An internal gas flow-rate of 10 ml min^{-1} of argon, a 2-s read time and a 2-s record time were also used.

Cadmium

Similarly, using an electrodeless discharge lamp and a normal graphite tube, measurements for cadmium were carried out under the following conditions: background correction, on; wavelength, 228.8 nm; slit width, 0.7 nm; integration time, 8 s; and recorder, 1 V.

The peak height was measured using the following programme:

Step 1	110 °C	20 s ramp	20 s hold
Step 2	110 °C	10 s ramp	10 s hold
Step 3	120 °C	10 s ramp	10 s hold
Step 4	450 °C	25 s ramp	25 s hold
Step 5	2000 °C	3 s ramp	7 s hold

An internal gas flow-rate of 50 ml min^{-1} of argon, a 2-s read time and a 2-s record time were also used.

Results and Discussion

For the determination of lead and cadmium in food products, good results have already been attained with the application of NaDDTC as a complexing agent.[14] In order to obviate interference from large amounts of copper, zinc or iron, which are possibly present in the food product, a masking process with potassium cyanide has been simultaneously applied.[14] The addition of phenol red to the ammonium citrate buffer solution speeds up the crude neutralisation of the acidic sample solution by visual observation. The pH value with which an optimum result is obtained (9.0 ± 0.5) is beyond the neutral point of the indicator and has to be checked with a pH-meter.

As a check on the ashing procedure, we conducted a series of tests in which identical amounts of lead (in the 0–500 ng range) and cadmium (in the 0–50 ng range) were subjected to analysis without and with previous ashing. The resulting absorbance values are given in Table 1; intercomparison shows that the ashing procedure with the specified temperature programme does not measurably affect the results. Statistically, using a paired *t*-test, the difference was found to be insignificant at the 95% confidence level.

The extraction of the two elements was then examined. To that end, identical amounts of complexed lead and cadmium

Table 1. Standard amounts of lead and cadmium subjected to analysis without and with ashing. All results are the means of two duplicate analyses; the differences between the blanks without and with ashing are caused by magnesium nitrate and results are given as peak heights to to give a comparison between the sensitivity to both elements

	Lead absorbance, arbitrary units					Cadmium absorbance, arbitrary units			
	Without ashing		With ashing			Without ashing		With ashing	
Sample/ng	Uncorrected	Corrected for blank	Uncorrected	Corrected for blank	Sample/ng	Uncorrected	Corrected for blank	Uncorrected	Corrected for blank
Blank	48	—	105	—	Blank	41	—	65	—
125	173	125	225	120	12.5	162	121	185	120
250	291	243	335	230	25	270	229	299	234
375	407	359	447	342	37.5	380	339	410	345
500	535	487	555	450	50	500	459	504	439

Table 2. Extraction of identical amounts of lead (100 ng) and cadmium (10 ng) in different volumes of IBMK. The values of the absorbance were corrected for the total blank, consisting of the constant contribution from the IBMK (lead = 0, cadmium = 4 units) plus the proportional contribution from the blank, which for 5 ml was 33 units for lead and 24 units for cadmium

	Lead		Cadmium	
IBMK/ml	Concentration after complete extraction, p.p.b.	Absorbance	Concentration after complete extraction, p.p.b.	Absorbance
5	20	84	2	129
4	25	130	2.5	165
3	33	167	3.3	225
2	50	241	5	335
1	100	500	10	675

Table 3. Effect of internal gas flow-rate on peak height for cadmium. Test solution: IBMK extract containing 2.5 p.p.b. of cadmium

Internal gas flow-rate/ml min^{-1}	Peak height
0	493
10	421
30	302
50	193
80	117
100	101

Table 4. Recovery of lead and cadmium in samples of chicken meat and eggs, spiked with 50 p.p.b. of lead and 5 p.p.b. of cadmium. All results are the means of duplicate analyses.

Sample	Lead, p.p.b.	Recovery, %	Cadmium, p.p.b.	Recovery, %
Chicken meat A	13		6	
Chicken meat A, spiked	60	95	10.7	97
Chicken meat B	19		3	
Chicken meat B, spiked	66	96	7.5	94
Eggs A	9		<0.1	
Eggs A, spiked	59	100	5	100
Eggs B	3		0.3	
Eggs B, spiked	50	94	5.8	109

Table 5. Determination of lead in milk powder. Results are of the duplicate analyses

Sample	Lead concentration/mg kg^{-1}: Values found with this method	Values of inter-laboratory tests
A	0.70, 0.65	0.63, 0.68
B	1.25, 1.28	1.30, 1.35
C	1.87, 1.87	1.94, 1.99
1	1.08, 1.04	1.03, 1.07
2	2.05, 2.05	2.05, 2.09

were extracted in 5 ml of IBMK, shaking for different lengths of time. The same absorbance was obtained with extraction times of 30, 60 and 120 s. In the final extraction a shaking time of 1 min was used.

The volume of IBMK was also varied. As shown in Table 2, the absorbance was inversely proportional (as expected) to the volume of IBMK over the range 1–5 ml. Therefore, a 1-ml, rather than a 5-ml, volume of IBMK is to be preferred if the highest sensitivity is required or when the sample size is limited, as may be so with blood, serum or other biological materials.

It proved to be essential to purify the NaDDTC solution by extracting twice with IBMK and the ammonium citrate buffer solution by treating with NaDDTC and extracting twice with IBMK, in order to obtain low blanks for lead and cadmium. In the furnace of the HGA 500 an adjustable flow of gas can pass through the graphite tube during the atomisation step (step 5 of the temperature programme). By interrupting or accelerating the gas flow-rate, one can adjust the retention time of the vaporised atoms in the light beam and thereby vary the peak height. Table 3 gives the results of a typical test series.

The sensitivity of the lead measurement can be increased considerably by coating the graphite tube with molybdenum.[13] In this investigation a coated tube was used for more than 200 measurements without any change in sensitivity being observed.

Performance Tests

The method described has been applied to a number of samples of chicken meat and eggs. Recoveries were checked by adding 250 mg of lead and 25 ng of cadmium. The results, showing a recovery of 97 ± 3% for lead and 100 ± 10% for cadmium, are summarised in Table 4.

Five samples of milk powder that had been used in inter-laboratory tests for the presence of lead were analysed as described under Procedure. The results in Table 5 obtained using this procedure are in good agreement with those obtained by the participants of the inter-laboratory test. Six participants used atomic-absorption spectrophotometry and two differential-pulse anodic-stripping voltammetry. Statistically there is no significant difference at the 95% confidence level.

From participating in inter-laboratory tests it has been shown during the last few years that this method is also applicable to fish products, bread, vegetables and animal feedstuffs. If the dry-ashing procedure is replaced by wet digestion with 5 ml of concentrated sulphuric acid and nitric acid followed by extraction of the nitrous fumes with de-mineralised water, the method can be followed as described under Procedure starting at ". . . and rinse with 25 ml of de-mineralised water . . ." Statistically there is no significant difference between the methods as results for samples of grass meal, milk powder and eggs have shown.

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Extraction - Atomic-absorption Spectrophotometric Determination of Antimony by Generation of Its Hydride in Non-aqueous Media

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A method for covalent hydride generation in a non-aqueous extraction phase is proposed. The hydride generation is carried out in an aliquot of metal - complex extraction solution by sodium tetrahydroborate(III) in *N,N*-dimethylformamide solution and anhydrous acetic acid. The proposed method gives improved sensitivity, eliminates interferences and enables hydride generation of difficult elements to be carried out. Antimony is extracted by ammonium pyrrolidine-1-carbodithioate into chloroform and after hydride generation by the proposed method, it is determined by flame AAS. The method is applied to the determination of antimony in BCS standard steels with good accuracy and precision.

Keywords: *Antimony determination; atomic-absorption spectrophotometry; hydride generation; non-aqueous media*

The determination of As, Bi, Ge, Pb, Sn, Sb and Te by atomic-absorption spectrophotometry (AAS) with covalent hydride generation using sodium tetrahydroborate(III) solution is a well established analytical procedure. In the future development of determinations with hydride generation, there are two possible areas, improved automation and methods of lowering detection limits,[1] which may improve as manufacturers introduce improved designs for their instruments and reduction vessels or using combinations of hydride generation with techniques such as atomic-fluorescence spectrophotometry,[2] d.c. arc discharges[3] or inductively coupled plasmas.[4] A further area of research in which much useful work may yet be done is in reduction or elimination of interference effects.

In this paper, a procedure for covalent hydride generation from a non-aqueous extraction phase is proposed in which the organic phase after extraction is treated with sodium tetrahydroborate(III) solution in *N,N*-dimethylformamide (DMF) and an acid. This covalent hydride generation in a non-aqueous medium with a previous extraction stage gives improved sensitivity and eliminates interferences. Also, elements that form hydrides with difficulty in aqueous solution, such as lead, are reduced more easily in non-aqueous media, owing to the greater range of electroactivity of some organic solvents than water.

In the determination of antimony by hydride generation followed by AAS, an electrically heated silica tube has been used as an alternative to a flame. Detection limits reported with this type of atomisation are 0.5 μg l^{-1} of Sb[5] and 0.2 μg g^{-1} in geological samples.[6] The Analytical Methods Committee[7] reported sufficient sensitivity and quantitative recovery from solutions containing 50 μg l^{-1} of Sb in organic matter or foodstuffs. Chapman and Dale[8] obtained a sensitivity of 2.6 ng of Sb using an argon - hydrogen flame for atomisation. This lower sensitivity was ascribed to the lower temperature of the flame.

In this work, antimony was extracted with ammonium pyrrolidine-1-carbodithioate into chloroform at pH 6.9 with phosphate buffer solution. The hydride was generated in an aliquot of the chloroform extract with sodium tetrahydroborate(III) in DMF and anhydrous acetic acid. The generation of hydride was carried out in an inexpensive generator[9,10] with direct introduction into an air - acetylene flame through the nebulisation device of the spectrophotometer. The proposed method has been applied to the determination of antimony in BCS standard steels with excellent agreement.

Experimental

Apparatus

A Pye Unicam SP-9 atomic-absorption spectrophotometer with a three-slot burner, equipped with a Pye Unicam antimony hollow-cathode lamp, was used. An Orion Research Microprocessor Ionanalyzer 901 was used for pH measurements.

A Haake mechanical shaker was employed.

Reagents and Solutions

All chemicals were of analytical-reagent grade, obtained from Merck.

Standard antimony solution, 1.000 μg ml^{-1} *of Sb*. Dissolve 0.598 6 g of antimony trioxide in 10 ml of concentrated hydrochloric acid (sp. gr. 1.16) and dilute to 500 ml in a calibrated flask with 3 M hydrochloric acid. The working solutions were prepared by diluting this solution immediately before use.

Sodium tetrahydroborate(III) solution. Dissolve 1 g of sodium tetrahydroborate(III) in 100 ml of DMF. This solution can be used for 2 weeks.

Sodium sulphite solution, 50 g l^{-1}. Dissolve 5 g of anhydrous sodium sulphite in 100 ml of water. Prepare this solution as required.

Ammonium pyrrolidine-1-carbodithioate solution, 0.25% *m/V*. Prepare just before use.

Sodium diethyldithiocarbamate solution, 0.25% *m/V*. Prepare just before use.

Clark and Lubs buffer solution (*pH* 6.9). 0.1 M phosphate - sodium hydroxide.[11]

N,N-*Dimethylformamide*. Purify by distillation, collecting the fraction boiling between 148 and 150 °C.

Chloroform.

Toluene.

Isobutyl methyl ketone (IBMK).

Procedure

Weigh exactly 0.2–0.4 g of steel (containing up to 250 μg of Sb) and dissolve it in 10 ml of concentrated hydrochloric acid (sp. gr. 1.16). Add 2 ml of concentrated nitric acid (sp. gr. 1.47), heat until nitrogen dioxide has been eliminated and boil for 5 min. Dilute to 50 ml in a calibrated flask with water.

Place 10 ml of the steel solution into a separating funnel and add 1 ml of sodium sulphite solution and 15 ml of buffer

solution (pH 6.9), then 2 ml of pyrrolidine-1-carbodithioate solution. Extract with 10 ml of chloroform by mechanical shaking for 5 min. Leave the phases to separate then dry the chloroform phase by filtering it through cotton-wool.

Place 1 ml of the chloroform extract into the hydride generator and add 3 ml of anhydrous acetic acid (sp. gr. 1.05). Inject 4 ml of $NaBH_4$ solution through the septum membrane. Measure the peak height of the atomic-absorption signal of antimony at 217.59 nm.

Prepare a calibration graph for known amounts of antimony using the standard antimony solution following the same procedure.

Results and Discussion

Solubility and Stability of $NaBH_4$ Solution in DMF

The solubility of $NaBH_4$ in DMF at 25 °C was studied. Using a gravimetric method, a solubility of 60 g l^{-1} was found. After optimising the proposed method, solutions of $NaBH_4$ of concentration 1% *m/V* in DMF were used. This solution, as distinct from aqueous $NaBH_4$ solution,[12,13] was useful for 2 weeks, as shown by the signals obtained for hydride generation. Its stability was comparable to that of an aqueous solution containing a pellet of sodium hydroxide and filtered through a 0.45-μm membrane filter.

Acids for Hydride Generation

An acid is needed for covalent hydride generation and the simultaneous evolution of hydrogen by $NaBH_4$ in DMF. The acid used must be miscible with DMF and the extraction solvent. The possibilities studied were hydrogen chloride gas dried and dissolved in DMF or dioxane, anhydrous acetic acid and a mixture of glacial acetic acid and concentrated sulphuric acid (sp. gr. 1.84) (3 + 1).

The HCl contents in DMF or dioxane solutions saturated at 1 atm were determined by neutralisation titrimetry. The HCl concentration in DMF solution was found to be 3.9 M and the solution remained stable for 2 weeks, whereas a dioxane solution (1.4 M HCl) rapidly lost the HCl.

The best results for the antimony peak height were obtained by using either anhydrous acetic acid or a mixture of acetic and concentrated sulphuric acids. When HCl in DMF solution or a mixture of acetic and sulphuric acids was used, a white crystalline precipitate was formed (owing to the formation of NaCl or Na_2SO_4, respectively) that was hardly soluble in the organic solvents. With anhydrous acetic acid, the solution remained clear.

The hydride generation and the simultaneous evolution of hydrogen were immediate and the reaction was complete in less than 30 s. Therefore, there was no need for collection devices and the inexpensive generator described elsewhere[9,10] can be used.

Solvents

A necessary condition for the accurate and rapid evolution of the volatile hydride is miscibility of the extraction solvent, the $NaBH_4$ solution in DMF and the acid used. Chloroform, toluene and IBMK were studied as suitable solvents for metal-complex extraction. It was found that there was good miscibility of these solvents with DMF and anhydrous acetic acid or the mixture of acetic and sulphuric acids (3 + 1) in the volume proportions used in the proposed method, so that hydride evolution was carried out in the homogeneous phase.

Optimisation of Hydride Generation and Atomic-absorption Signal

The optimisation of the atomic-absorption signal with this hydride generation device has been discussed in previous papers.[9,10] Recommended instrumental parameters were acetylene flow-rate, 1.0 l min^{-1}; air flow-rate, 5.0 l min^{-1}; burner height, 8 mm; spectral slit width, 0.5 nm; wavelength, 217.59 nm; and lamp current, 12 mA.

The conditions for hydride generation were studied and the best conditions were as follows: 1 ml of antimony extract, 3 ml of anhydrous acetic acid or mixture of acetic and sulphuric acids (3 + 1) and 4 ml of 1% $NaBH_4$ solution 1% in DMF injected through the septum membrane of the generator.

Antimony Extraction

Antimony has been extracted into toluene from hydrochloric acid solutions, but the extraction yield has always been less than 85%. Therefore, the antimony extraction was carried out with sodium diethyldithiocarbamate[12] or ammonium pyrrolidine-1-carbodithioate[13] into chloroform, which also separates Sb(III) from Sb(V).[14] In this work total antimony was determined, as the Sb(V) formed was reduced to Sb(III) by the addition of sodium sulphite solution.[7]

Calibration Graph

The peak height of the atomic-absorption signal for antimony was linear between 0.05 and 5 μg of Sb (in 1 ml of extraction solution), with a regression coefficient of 0.999 1. The noise level of the blanks was indistinguishable from the noise of the determination up to a setting of 4 on the expansion scale of the spectrophotometer.

The sensitivity (1% absorption) and the detection limit were 0.03 and 0.01 μg ml^{-1} of Sb in the extraction solution, respectively. Ten replicate determinations of 2 μg of Sb gave a relative standard deviation of 2.0%.

Interference Study

Interferences from different elements in the determination of antimony were studied. Up to 300 mg of Al, Ca, Mg, Ni, Cr(III), Fe(III), Fe(II), Sn(IV), Sn(II), As(V), As(III), silicate, fluoride, sulphate, phosphate or tartrate did not interfere in the determination of 50 μg of Sb. Up to 1 mg of Se(IV), Te(IV), Bi(III), Pb(II), Cu(II) or Ge(IV) did not interfere but larger amounts produced a decrease in the atomic-absorption signal for 50 μg of antimony. As a result of the extraction process and the non-aqueous hydride generation, the interference limits were higher than for generation in an aqueous medium.[10]

Applications

The proposed method has been applied to the determination of antimony in BCS standard steels, as shown in Table 1.

The determination of 25 μg of antimony in spiked samples gave a 98.1% recovery with a relative standard deviation of 1.8% (ten determinations).

The method has also been applied to the determination of antimony in air particulates, with good precision.

Table 1. Determination of antimony in BCS steels

Sample	Sb certified, %	Sb found, %*	Relative standard deviation,* %
BCS 329 mild steel	0.018	0.019	2.0
BCS 457 mild steel	0.029	0.030	1.7
BCS 458 mild steel	0.070	0.068	1.6

* Means and relative standard deviations for ten replicate determinations.

Conclusion

The extraction and hydride generation in non-aqueous media with $NaBH_4$ in DMF eliminates interferences and improves sensitivity. Elements that have difficulty in forming hydrides, such as Pb, Te and Sn, have also been studied. They are reduced more easily than in water and will be the topic of a later paper.

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Analytical Application of Inorganic Salt Standards and Mixed-solvent Systems to Trace-metal Determination in Petroleum Crudes by Atomic-absorption Spectrophotometry

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The determination of Ni, Cu, Zn, Na, Pb, Cd and Fe in petroleum crudes by means of a toluene - acetic acid mixed-solvent system, inorganic salt standards and atomic-absorption spectrophotometry is presented. The low systematic errors and good burning characteristics of this mixed-solvent system led to its choice. Good recoveries of metals added to petroleum crudes are demonstrated. Coefficients of variation of 8.4 for Ni, 7.0 for Cu, 7.8 for Zn, 11.2 for Na, 9.8 for Pb, 10.2 for Cd and 13.9% for Fe are obtained. The validity of the method is shown by comparison of results obtained with those of an established method.

Keywords: *Metal determination; petroleum crudes; mixed solvents; inorganic salt standards; atomic-absorption spectrophotometry*

The occurrence of trace metals in petroleum crudes or fractions is useful analytically. For instance, the nickel to vanadium ratio is used in the characterisation of petroleum crudes.[1] Additionally, the determination of elements such as lead, copper and zinc in both fuel oils and gasolines is often required because these metals accelerate the oxidative deterioration of the products or otherwise reduce the storage ability.[2] The analysis of the metal contents of petroleum crudes and products could give an idea of the amount of heavy metal fall-out into the environment as a result of the burning of oil fuels.

Atomic-absorption spectrophotometry has been widely applied to the determination of trace metals in petroleum products. As most of the metals in petroleum are in the form of organometallic compounds, organometallic standards are often used in their determination.[3] Omang[4] and Chuang and Winefordner[5] have described the determination of metals in mineral oils and jet-engine oils, respectively, using electrothermal atomisation with a graphite tube. The analytical application of emulsions in the determination of trace metals in gasoline by atomic-absorption spectrophotometry has also been described by Polo-Díez *et al.*[6]

Most methods for determining trace metals in petroleum and their products by atomic-absorption spectrophotometry involve dilution of the sample with a suitable organic solvent, usually xylene, followed by the use of organometallic standards. The organometallic standards are very difficult to obtain and often expensive. Further, different results are obtained when a metal is present in different forms, *e.g.*, the amount of lead obtained with the same organometallic standard would be different when it is present as tetramethyllead or tetraethyllead. As a result of these problems, the need to develop a new and relatively inexpensive method of sample preparation prior to analysis by atomic-absorption spectrophotometry has become necessary.

Some workers have developed mixed-solvent systems for the determination of metals in lubricating oils by atomic-absorption spectrophotometry using inorganic salt standards. Holding and co-workers[7,8] used the cyclohexanone - butan-1-ol - industrial methylated spirit - hydrochloric acid and 2-methylpropan-2-ol - toluene mixed-solvent systems and inorganic salt standards in the determination of trace amounts of Ca, Zn and Ba in unused lubricating oils and obtained results that were in good agreement with those obtained by X-ray fluorescence and established Institute of Petroleum chemical procedures. Wittmann[9] reported the use of a toluene - acetic acid (1 + 4) solvent and inorganic salt standards for the determination of Ca, Mg and Zn in lubricating oils by atomic-absorption spectrophotometry with good precision. The analytical application of inorganic salt standards and mixed-solvent systems for the determination of metals in petroleum crudes has been investigated in this work.

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Experimental

Preliminary Investigation of the Choice of Mixed Solvent

The choice of solvents as components of a mixed-solvent system depends on the solubility of the various petroleum crudes and that of the inorganic salt standards. The solubilities of various Nigerian petroleum crudes were tested in several solvents, *viz.*, benzene, toluene, dioxane, 4-methylpentan-2-one, xylene, propanone, 2-methylpropan-1-ol and acetic acid. The petroleum crudes were soluble in all except acetic acid but, because of its good flame characteristics,[10] its use as a component of the mixed solvent was considered.

The dioxane - acetic acid and 4-methylpentan-2-one - acetic acid systems were found to be unsuitable as there was emulsion formation whenever acetic acid was added to a solution of the oil in either dioxane or 4-methylpentan-2-one. The inorganic salt standards were also insoluble in the mixed solvent systems. Both benzene - acetic acid (1 + 4) and toluene - acetic acid (1 + 5) solvent systems were found to be suitable for analysis. The choice of the latter system for this study is due to the less smokey characteristics of the flame produced.

Apparatus

A Varian AA 475 atomic-absorption spectrophotometer, with various metal hollow-cathode lamps, was used with an air - acetylene flame and other conditions stated in the operations manual.

Reagents

All chemicals were of analytical-reagent grade unless otherwise stated. Solvents that were not of analytical-reagent grade were purified before use as described in the literature.[11]

Benzene.
Toluene.
Xylene.
Propanone.
(2)-*Methylpropan*-1-*ol.*
4-*Methylpentan*-2-*one.*

Copper.
Zinc. AnalaR grade.
Sodium chloride. AnalaR grade.
Potassium chloride. AnalaR grade.
Nickel nitrate. AnalaR grade.
Lead nitrate. AnalaR grade.
Cadmium chloride. AnalaR grade.
Iron(III) nitrate. AnalaR grade.
Hydrochloric acid. AnalaR grade.
Nitric acid. AnalaR grade.
Ethanol. AnalaR grade.
Nickel cyclohexylbutyrate.
Zinc cyclohexylbutrate.

Preparation of Stock Solutions

A modification of the method of Wittman[9] is used for stock solution preparation. Appropriate amounts of copper and zinc metals are dissolved in nitric and hydrochloric acids, respectively, diluted with a small volume of distilled, de-ionised water and finally made up to the required volume with ethanol to give a 1 000 p.p.m. solution. Stock solutions of Na, Ni, Pb, Cd and Fe are prepared from their chloride or nitrate salts by dissolving in a small volume of distilled, de-ionised water and diluting with ethanol to the required volume. Potassium chloride, of about 2 000 p.p.m. with respect to potassium, is added to the sodium solution as an ionisation suppressant.

Standard Working Solutions

These are freshly prepared daily by serial dilution of appropriate volumes of the stock solution of each metal with toluene - acetic acid (1 + 5).

Sample Preparation

Weigh into a weighing bottle about 0.2 g of crude oil. Add 3.0 ml of toluene and stir well before transferring into a 25-ml calibrated flask. Wash the bottle with more toluene followed by acetic acid and transfer quantitatively into the flask. Finally, dilute to the mark with glacial acetic acid. When benzene - acetic acid is used the oil should be dissolved first in benzene before adding acetic acid, as the reverse order results in precipitation of the oil. The standard additions method was used in the analysis of the crude samples to minimise errors due to matrix effects.

Results and Discussion

The use of a toluene - acetic acid mixed-solvent system and inorganic salt standards was applied to the analysis of trace metals in Nigerian petroleum crudes from seven terminals. The choice of this system resulted from the relatively low systematic errors (between 2 and 25%) compared with those obtained with the benzene - acetic acid system, which ranged from 15 to 48% for most of the metals determined. This also allowed the use of the inorganic salt standards as there was no precipitation as observed in the preparation of cadmium working solutions with the benzene - acetic acid system. The efficacy of the standard additions method employed was ascertained by recovery studies on some of the petroleum crudes quoted in Table 1. This was carried out by spiking about 0.2 g of sample with known amounts of mixed-metal standards. The recoveries obtained for most of the metals were good, as there was no metal for which the recovery was less than 90%. The coefficients of variation, calculated for seven replicate determinations, were 8.4% for nickel at the 18.6 p.p.m.level, 7.0% for copper at the 2.94 p.p.m. level,

Table 1. Average percentage recovery of trace metals in some petroleum crudes by the proposed method

	Recovery of trace metals from spiked crude petroleum samples, %						
Sample terminal	Ni	Cu	Zn	Na	Pb	Cd	Fe
Shell Forcardos	105.41	99.33	93.74	130.49	123.81	—	104.57
Brass Blended	103.83	92.48	107.93	120.36	97.28	104.76	92.56
Bonny Medium	97.53	102.49	99.73	103.36	108.38	108.38	94.45

Table 2. Trace metal content as determined by the mixed-solvent system and inorganic salt standards

	Metal content, p.p.m.						
Element	Shell Forcardos	Texaco M.V. Oloibiri	Gulf Escravos	Brass Blended	Bonny Light	Qua Iboe T. Blended	Bonny Medium
Ni	12.75 ± 1.45	21.20 ± 2.06	19.14 ± 4.69	15.96 ± 1.99	18.59 ± 2.42	25.28 ± 1.23	16.72 ± 1.29
Cu	4.48 ± 0.11	2.68 ± 0.15	2.00 ± 0.24	1.69 ± 0.24	2.94 ± 0.04	3.64 ± 0.37	3.58 ± 0.44
Zn	5.23 + 0.63	2.88 + 0.38	3.74 + 0.14	3.38 + 0.35	3.62 + 0.08	4.32 + 0.09	3.40 + 0.36
Na	9.21 ± 1.10	17.01 ± 1.90	6.23 ± 0.24	3.75 ± 0.41	20.22 ± 3.61	20.35 ± 2.18	22.22 ± 2.92
Pb	6.10 ± 0.97	1.47 ± 0.11	3.38 ± 0.17	3.94 ± 0.45	1.16 ± 0.08	2.10 ± 0.36	2.69 ± 0.12
Cd	N.D.*	N.D.*	0.11 ± 0.02	1.39 ± 0.27	0.44 ± 0.06	3.02 ± 0.41	2.10 ± 0.14
Fe	9.05 ± 0.73	12.09 ± 1.65	5.38 ± 0.84	4.50 ± 0.84	7.37 ± 1.73	6.73 ± 0.64	7.81 ± 0.64

* Not detectable by this method.

Table 3. Comparison of results for nickel and zinc in Nigerian petroleum crudes

	AAS using organometallic standards and xylene as solvent[3]		AAS using inorganic salt standards and toluene - acetic acid mixed-solvent system	
Sample/terminal	Ni, p.p.m.	Zn, p.p.m.	Ni, p .p.m.	Zn, p.p.m.
Shell Forcardos	9.86 ± 1.02	5.73 ± 0.72	12.75 ± 1.45	5.23 ± 0.63
Texaco M.V. Oloibiri	20.38 ± 1.90	2.81 ± 0.32	21.20 ± 2.06	2.88 ± 0.38
Gulf Escravos	21.05 ± 2.98	4.48 ± 0.51	19.14 ± 4.69	3.74 ± 0.14
Brass Blended	14.65 ± 2.89	3.53 ± 0.19	15.96 ± 1.99	3.39 ± 0.35
Bonny Light	15.61 ± 3.14	4.28 ± 0.17	18.59 ± 2.42	3.62 ± 0.08
Bonny Medium	20.26 ± 2.71	2.94 ± 0.41	16.72 ± 1.29	3.40 ± 0.36

7.8% for zinc at the 3.62 p.p.m. level, 11.2% for sodium at the 20.22 p.p.m. level, 9.8% for lead at the 1.16 p.p.m. level, 10.2% for cadmium at the 0.44 p.p.m. level and 13.9% for iron at the 7.37 p.p.m. level. The levels of these metals found in the petroleum crudes are shown in Table 2.

Good agreement is obtained when the results obtained with six of the crudes by this method are compared with those of the established method of Means and Raclifte.[3] Nickel and zinc were compared by the two methods as shown in Table 3. For both metals, the Student *t*-test showed that there was no significant difference between results obtained by the two methods.

Conclusion

The use of inorganic salt standards and a toluene - acetic acid mixed-solvent system in the determination of trace metals in petroleum crudes by atomic-absorption spectrophotometry possesses considerable advantages of time and cost over the use of organometallic standards. A precision of less than 10% was obtained for most metals determined by the method. The validity of the method was shown by comparison of results obtained with those of an established method.

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Modifications of Standard Methods for Coal Analysis for Sulphur and Nitrogen Determinations in Oil Shales

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Investigations were made to determine if methods that are satisfactory for determining total sulphur and total nitrogen in coals are also suitable for determinations when using compounds that contain some organic structures to be expected in oil shale kerogens. Sulphur recoveries in an excess of 98%, and typically better than 99%, were obtained using modifications of ASTM method D3177 (75). It was found that the semi-micro Kjeldahl method in the BS 1016 : Part 6 : 1977 was not suitable for the determination of nitrogen in aromatic nitrogen compounds. For example, the recovery of nitrogen from 8-hydroxyquinoline was increased from approximately 30 to 96% by increasing the digestion time to a minimum of 2 h. Variation in the initial amount of sulphuric acid in the digestion mixture between 3 and 5 ml did not alter the recovery. The use of the modified semi-micro Kjeldahl method for an oil shale sample gave a higher nitrogen recovery and better precision of results than the unmodified standard method.

Keywords: *Sulphur determination; nitrogen determination; oil shale analysis; coal*

Oil shale reserves are a significant component of Australia's over-all energy resources. Analytical methods of demonstrated suitability for oil shales are therefore of great importance. This paper describes a preliminary investigation of the methods used for determining the sulphur and nitrogen contents of compounds of types expected in oil shales.

Sulphur Determination

Zinc oxide - sodium carbonate Eschka fluxes are known to be suitable for the determination of sulphur in sulphide minerals, and they are particularly suitable if free silica is present.[1] The zinc oxide reacts with free silica to form zinc silicates, which have lower water solubilities than, for example, the magnesium silicates formed when magnesium oxide - sodium carbonate fluxes are used. Many oil shales contain high concentrations of free silica, *e.g.*, typically 39% in both Julia Creek shale (Queensland, Australia)[2] and Anvil Points shale (Colorado, USA).[3] The zinc oxide - sodium carbonate fluxes are unsuitable for sulphur determinations in materials that contain either fluorine or tungsten,[1] but as these elements usually occur only at very low concentrations in oil shales,[2–4] a zinc oxide - sodium carbonate flux may be as suitable for sulphur determination in oil shales as the magnesium oxide - sodium carbonate flux recommended for coal analysis.[5]

It was for this reason that a preliminary investigation was made in order to determine if the zinc-based Eschka flux, which is suitable for determining sulphur in sulphide minerals, is also suitable for determining the sulphur in the organic forms that can be expected in oil shales.[6]

Nitrogen Determination

Aliphatic and aromatic nitrogen compounds, including substituted pyridines and quinolines, have been identified in shale oil,[7,8] and it is reasonable to assume that both types of compound exist in the parent oil shales. Therefore, any method for the determination of nitrogen in oil shale must be suitable for the determination of nitrogen in both aliphatic and aromatic compounds.

Nadkarni[9] has recently shown that the Kjeldahl method is not generally suitable for determining the nitrogen contents of oil shales. His opinion is that this probably indicates the presence of different specific nitrogen compounds in oil shales that result from their different genesis.

Bradstreet[10] noted that compounds having pyridine structures are "refractory," *i.e.*, very stable, and are difficult to determine by the Kjeldahl method. This conclusion was borne out by the results obtained from the initial experiments when only 30% of the nitrogen in 8-hydroxyquinoline was recovered using a method recommended for determining the nitrogen content of coals.[5] However, as will be discussed later, the same standard method was much more satisfactory for the determination of nitrogen in diphenylamine.

Superficially, the Dumas method should be a suitable alternative to the Kjeldahl method. However, it requires scrupulous control of reagent purity and combustion assembly parameters to obtain reliable results,[11,12] and is then less attractive than the Kjeldahl method as a routine method. It was therefore decided to determine whether the semi-micro Kjeldahl method[5] could be modified so as to permit the satisfactory determination of nitrogen in both refractory and non-refractory nitrogen compounds.

In the initial stage of his work, the semi-micro Kjeldahl method was modified to give satisfactory recoveries of nitrogen from selected organic compounds. The modified and unmodified methods were then used for determining the nitrogen content of an oil shale and the results were compared.

Experimental

Methods

Sulphur

An ASTM method for sulphur determination in coals[13] was chosen as the basis for this work. The modifications to the method and the reasons for making them are as follows:

(i) A mixture (4 + 1) of zinc oxide and sodium carbonate was used as the flux for the reasons discussed earlier.
(ii) The flux topping was increased from 1 g to a minimum of 2 g. The effect of varying the amount of topping is discussed below.
(iii) A 100-mg mass of sodium peroxide was added to the fusion frit - hot water slurry to precipitate any manganese as manganese dioxide before filtration.
(iv) The bromine water oxidation step was omitted because the sodium peroxide treatment served the same purpose.
(v) The precipitated barium sulphate was ignited to constant mass at 800 °C instead of 925 °C, as the lower temperature was found to be satisfactory.

This method is used in the authors' laboratories for routine determinations of sulphur in ores and concentrates.

Nitrogen

The modifications made to the semi-micro Kjeldahl method[5] that were used in all tests are as follows:

(i) A 10-ml volume of 750 g l^{-1} sodium hydroxide solution was used instead of the 20 ml of 400 g l^{-1} sodium hydroxide solution specified in the standard, as splash-over tended to occur when the larger volume was used. The problem did not occur when the smaller volume of higher concentration solution was used.
(ii) A distillation time of 25 min.[14] Waanders *et al.*[14] have shown that the evolution of ammonia from diluted digest, after the addition of alkali, may not be complete if a distillation time of 6 min is used,[5] but is complete after 20 min.
(iii) A sample mass of 20–40 mg for the organonitrogen compounds.

A selenium - vanadium(V) oxide catalyst was chosen for this work because of local difficulties in disposing of spent mercury-based catalyst.

The variables investigated were the total digestion time and the volume of concentrated sulphuric acid in the digestion mixture.

Materials and Determinations

Sulphur

The reagents used in the experiments were elemental sulphur, benzothiazole-2-thiol and calcium sulphate dihydrate. Sulphur was chosen as a source of volatile sulphur, as it melts at approximately 119 °C and boils at 445 °C. Benzothiazole-2-thiol was selected as a relatively stable source of organic sulphur as it contains both a thiol group and sulphur in a thiazole ring. It melts at approximately 180 °C. Many oil shales contain calcium carbonate in the form of calcite, *e.g.*, 40% in Julia Creek shale.[2] To check for interference, a calcium carbonate - benzothiazole-2-thiol mixture was also used in the experiments. Calcium sulphate may be formed in oil shale processes if the retorted shales are calcitic, contain sulphur and are combusted to provide process heat, and it was therefore included in the list of compounds.

Nitrogen

The three organonitrogen compounds used in these experiments were (i) diphenylamine (≥99.0% purity), which may be considered as a substituted ammonium compound and hence easily determined by Kjeldahl analysis, (ii) benzothiazole-2-thiol (nominal purity 99%), which contains nitrogen in a thiazole ring and was expected to be easily determined owing to the relative instability of the five-membered ring, and (iii) 8-hydroxyquinoline (≥99.5% purity), which contains nitrogen in a very stable aromatic ring.

A spot sample of oil shale, denoted PC in reference 15, was also used.

Results and Discussion

Sulphur

The results for the recovery of sulphur (Table 1) show that, for the levels of sulphur recorded, significant improvements in recovery were obtained by increasing the covering layer of zinc oxide-based Eschka flux for sulphur and benzothiazole-2-thiol. Provided the topping layer of Eschka flux was approximately 6 g, the method proved capable of recovering approximately 99% of the theoretical sulphur content of benzothiazole-2-thiol and sulphur samples. The addition of calcium carbonate to the organic compound was found to decrease the recovery of sulphur slightly. Calcium sulphate gave the highest recovery of any of the reagents used (99.6%), even with a 2-g flux covering, as this layer was not required to trap volatile (and hence highly mobile) sulphur prior to oxidation. The results also show that an ignition temperature of 800 °C was satisfactory.

The sulphide-sulphur method was thus considered satisfactory when applied to elemental sulphur, organic sulphur compounds and calcium sulphate, and hence should be suitable for oil shales. For optimum recovery it may be necessary to adjust the thickness of the covering layer of flux mixture according to the proportion of organic sulphur in the sample. As indicated in Table 1, the amount of flux topping may need to be large if the sample contains a high concentration of calcium carbonate.

Nitrogen

The results for the recovery of nitrogen from the organonitrogen compounds used are reported in Table 2. The effects of variation of digestion time for 8-hydroxyquinoline and diphenylamine are illustrated in Figs. 1 and 2. The results for determinations of nitrogen in the oil shale are shown in Table 3.

It is clear from the results in Fig. 1 and Table 2 that the BS 1016 : Part 6 : 1977 method with a 25-min distillation time is not adequate for determining the nitrogen content of

Table 1. Recovery of sulphur

Compound	Approx. sample mass/mg	Flux cover/g	Recovery of sulphur,* %
$CaSO_4.2H_2O$	270	2	99.6
Sulphur	100	3	97.7
Sulphur	100	6	99.3
Benzothiazole-2-thiol	230	3	96.6
Benzothiazole-2-thiol	220	6	99.0
Benzothiazole-2-thiol†	120	3	95.5
Benzothiazole-2-thiol†	110	6	98.2

* Results reported are averages of duplicate determinations. In the calculations the assumption was made that the reagents were pure.
† 0.9 g of $CaCO_3$ was mixed with the benzothiazole-2-thiol.

Table 2. Recovery of nitrogen in organonitrogen compounds

Compound	Total digestion time/h	No. of assays	Recovery of nitrogen,* %
Diphenylamine	0.5	7	97.9 ± 3.0
Diphenylamine	2–4	12	97.8 ± 1.3
Benzothiazole-2-thiol	0.5	7	~85
Benzothiazole-2-thiol	2–4	9	97.9 ± 1.8
8-Hydroxyquinoline	0.5	3	~30
8-Hydroxyquinoline	2–4	10	96.2 ± 1.7

* Results are reported assuming the reagents were pure; where limits are given these represent the 95% confidence level.

Table 3. Determination of nitrogen in oil shale

		Results, % *m/m*	
Digestion time	No. of tests	Mean	Standard deviation
25–30 min	7	0.60	0.14
3 h	7	0.81	0.06

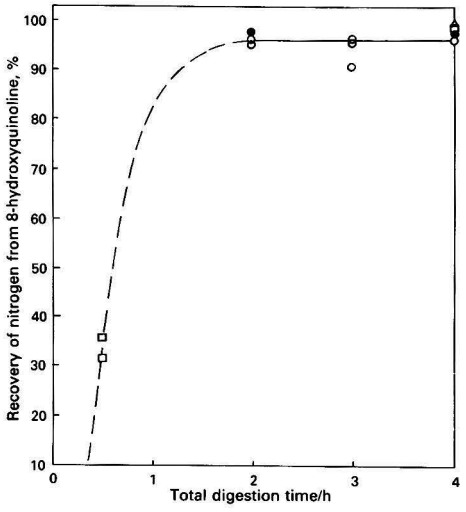

Fig. 1. Effect of digestion conditions on recovery of nitrogen from 8-hydroxyquinoline. Volume of acid in digest: ●, 3.0 ml; □, 4.0 ml; △, 4.5 ml; and ○, 5.0 ml

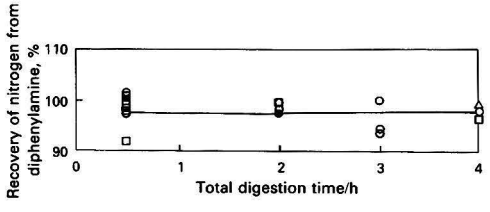

Fig. 2. Effect of digestion conditions on recovery of nitrogen from diphenylamine. Volume of acid in digest: ●, 3.0 ml; □, 4.0 ml; △, 4.5 ml; and ○, 5.0 ml

8-hydroxyquinoline or of benzothiazole-2-thiol. Much more acceptable results can be obtained by simply prolonging the digestion period from 30 min to 2 h. The data in Figs. 1 and 2 for 8-hydroxyquinoline and diphenylamine, respectively, show that the recoveries of nitrogen were not significantly different after digestion times of 2, 3 or 4 h. Therefore, in Table 2 the results for 2-, 3- and 4-h digestion periods for the organonitrogen compounds were grouped together. The effect of variation in the volume of concentrated sulphuric acid in the digestion vessel from 3 to 5 ml was insignificant (see Figs. 1 and 2).

The best results for the recovery of nitrogen were found to be low for all materials used. The error varied from a nominal value of 2% for benzothiazole-2-thiol to nearly 4% for 8-hydroxyquinoline. The assumption of 100% purity of the reagents, as used in the calculations, no doubt contributed to a small error, particularly with benzothiazole-2-thiol; however, this could not explain errors as large as those observed here. When using long digestion times evaporation can cause the temperature to rise to levels where nitrogen dissolved in the digest as ammonium salt can be lost by oxidation. For example, Baker[16] has stated that the critical temperature for the loss of nitrogen when using a selenium - vanadium(V) oxide catalyst is approximately 387 °C. However, in these experiments, even when 3 ml of acid were used, digestion temperatures did not rise above 345–350 °C, so that loss of nitrogen from the digestion mixture by oxidation was not the cause of the slightly low results listed here. In further support of this contention, as already stated, the results from 4-h digestions were not significantly different from those from the 2-h digestions. The results obtained for the oil shale show that the nitrogen recovery is improved by using long digestion times and that the precision of the results is probably improved also. However, the repeatability specified in the standard method was not achieved even with the long digestion time.

Conclusions

A method for determining sulphur based on the use of a zinc-based Eschka flux, which is known to be satisfactory for sulphide ores and concentrates, is also suitable for determining the sulphur contents of compounds that contain sulphur in forms that can be expected in oil shales.

The semi-micro Kjeldahl method for determining nitrogen was modified so that it was satisfactory for either aromatic or non-aromatic nitrogen compounds.

A comparison of the results from the modified and unmodified semi-micro Kjeldahl method when applied to an oil shale showed that the recovery of nitrogen was increased by using the modified method. The precision of the results was probably improved by using the modified method.

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Differential-pulse Polarographic Determination of Trace Heavy Elements in Coal Samples

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The conditions for the differential-pulse polarographic determination of trace heavy elements in a coal have been investigated. By means of polarograms taken at two different pHs of a solution of 0.5 M EDTA + 0.5 M sodium acetate, it has been shown that Fe, Cu, Pb, Cd, Ti, Sb, Mo, As and Zn could be determined in coal, a complex matrix, within a reasonably short time.

Keywords: *Trace heavy element determination; differential-pulse polarography; coal analysis*

Pulse polarography has been used in such diverse research fields as biological, metallurgical, sea water, minerals and pharmaceutical analysis. Although it is suitable for the analysis of single elements, it can also be used for the simultaneous determination of a number of elements in a sample.[1–6] The range of its applications has increased since 1960 with the introduction of single-sweep, square-wave and a.c. polarography.

In this study, polarography has been used for the determination of elements in coal samples. Although for such complex matrices atomic-absorption spectrometry, neutron-activation analysis and X-ray fluorescence are usually considered as standard methods for the determination of trace elements,[7,8] it has been found that polarography can also be used with success. However, if the E_{peak} values of certain elements are very near to each other or identical, interferences occur. Nevertheless, the possibility of determining a set of elements simultaneously certainly shortens the analysis time required, which is an advantage.

Overlapping peaks in one electrolyte can be separated by changing either the electrolyte or the pH. By choosing an electrolyte that is convenient to use at different pH values, it is possible to determine many elements by measuring only two polarograms.

In this work the determination of elements in coal was investigated. The most suitable electrolyte and pH values at which the elements gave distinct E_{peak} values were established.

Experimental

Apparatus

A pulse polarographic system of standard design and a normal polarograph, both built in the Physics Department at Hacettepe University, together with a Tektronix 510 N,D-15 oscilloscope, were used. The normal polarograph was similar to a Heath Model EUW-198 with IC operational amplifiers in place of the older vacuum tube types. It had an additional ramp output section that was connected to the pulse polarograph.

The system of the pulse polarograph was as follows. The potential applied to the electrodes in the system was obtained from a circuit consisting of an external ramp of a normal polarograph from a unit that produced an initial d.c. potential, from a reed relay and a potential adder circuit. The reference potential was divided by a Zener from a regulated power supply of ±15 V. It could be accurately adjusted by means of a ten-circuit (±3 V maximum) helipot. Potential pulses were obtained by opening or closing the reed relay at convenient times.

The adder, consisting of a 741 OpA (operational amplifier), served to apply to the electrodes the pulses that were superimposed on the external ramp. Two current samples were taken, one just before the pulse had been applied (τ') and the other just before the drop had fallen (τ). A difference amplifier and a sample and hold circuit were used to plot $i(\tau)-i(\tau')$ *versus* base potential. Observation of the oscilloscopic trace ensured that the pulses were being applied just before the mercury drops fell. The ramp and pulse were observed on the screen of this memory oscilloscope. A three-electrode system with a saturated calomel electrode as reference was used. The dropping-mercury electrode was part of a Heath polarography system (Model EUW-198) and the drop time was about 2–3 s. To prevent the capillary from plugging, it was stored in distilled water when not in use. The polarograms were recorded with a Heath - Schlumberger SR-255B recorder.

Reagents

The electrolytes and acids used were obtained from Merck (pro analysi grade). The standard solutions of elements were prepared with analytical-reagent grade salts. The water used was distilled approximately eight times in a specially designed packed distillation column. Its conductivity was less than 1 μmho.

The mercury used in the dropping-mercury electrode was obtained from Merck (pro analysi). The contaminated mercury was cleaned by passing it successively through dilute nitric acid and water columns in the form of fine droplets. The collected mercury was dried between filter-papers. A polarogram of this mercury was taken before use to ensure the absence of impurities.

Procedure

A coal sample (25 mg) was digested in a Parr acid digestion bomb with 1–2 ml of acid mixture ($HClO_4$ - HNO_3 - H_2SO_4) (24 + 24 + 1) by heating for 2 h at 150 °C. After digestion, most of the acids were fumed, 10–15 ml of electrolyte were added, the pH was adjusted to the desired value and nitrogen was passed through the cell for 30 min.

The ramp and reference voltages were adjusted by observing them on the oscilloscope screen, so that the pulse would be applied just before the release of the mercury drops. Another advantage of following the ramp and pulse on the oscilloscope was that a very small peak on the recorder could be

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distinguished from the noise. The applied pulse had an amplitude of 50 mV and the pulse duration could be chosen as 40 or 80 ms. The potential sweep was adjusted between the limits of 0 and −0.8 V or 0 and −1.2 V, depending on the pH of the solution. The recorder rate was adjusted to 1 in min^{-1} and the potential sweep rate was 0.2 V min^{-1}.

A 50–500 µl volume of the standard solution (10^{-3} M) of one of the elements found in coal was added to the electrolyte under test and its peak was recorded. By repeating the same procedure for the appropriate elements and comparing the clarity of polarograms obtained, the most suitable electrolyte for identification of these elements was chosen. This electrolyte was added to the solution of the coal sample and its polarogram was taken. By using the standard additions method the elements were determined both qualitatively and quantitatively.

Results and Discussion

Although combustion is used most frequently for the digestion of coal samples, partial loss of elements during combustion makes the method unfavourable. The approximate losses at 550 °C are 54% for Pb and Sb, 61% for Zn and 47% for Cd.[9] Therefore, acid digestion was used in this study. It was found that an acid mixture consisting of $HClO_4$ - HNO_3 - H_2SO_4 (24 + 24 + 1) was the most favourable mixture for digestion. The coal sample could be digested in a Parr acid digestion bomb at 150 °C in 1–2 h. In an attempt to determine the most suitable electrolyte for a coal sample that was later analysed to contain the elements listed in Table 2, KCl, HCl, $HClO_4$, $NaNO_3$ and EDTA + sodium acetate solutions were individually tested. Of these, the most suitable electrolyte was 0.05 M EDTA + 0.5 M sodium acetate. One advantage of this electrolyte was that it allowed polarograms to be obtained at different pH values. They differed in the degree of separation of overlapping peaks, thus allowing the best pH to be chosen. It should also be noted that the peaks were shifted by the variation in pH. This provided valuable information, as polarograms of known elements could be obtained at the same pH values and from the extent of the shift the presence of certain elements in the unknown sample could be ascertained. In quantitative determinations, after taking the polarogram of the sample at a certain pH, the pH of the sample was changed and a new polarogram taken. The concentrations of trace elements obtained at each pH level had to be checked closely before the results were considered to be accurate.

Table 1. Approximate peak potentials for a coal sample using 0.05 M EDTA + 0.5 M sodium acetate electrolyte

Element	*E*/V *vs.* S.C.E. pH 5.5	pH 2.5
Fe^{3+}	−0.10	−0.05
Cu^{2+}	−0.25	−0.10
Ti^{4+}	−0.40	−0.22
Pb^{2+}	No peak	−0.38
Bi^{3+}	−0.55	−0.60
Sb^{3+}	−0.67	−0.65
Mo^{6+}	−0.52	−0.53
As^{3+}	−0.85	No peak
Cr^{6+}	No peak	No peak
Co^{2+}	No peak	No peak
Ni^{2+}	No peak	No peak
Cd^{2+}	No peak	−0.85
Zn	−0.93	−0.95

Table 2. Differential-pulse polarographic (DPP) analysis of two lignite coal samples

Element	Amount ± standard deviation, p.p.m. Seyitomer lignite (DPP)	Aşkale lignite DPP	Aşkale lignite AAS
Cu^{2+}	110 ± 20	185 ± 25	160 ± 30
Ti^{4+}	980 ± 90	705 ± 55	—
Mo^{6+}	320 ± 45	87 ± 22	—
Sb^{3+}	125 ± 30	220 ± 35	—
Cd^{2+}	40 ± 11	95 ± 18	100 ± 15
As^{3+}	105 ± 25	55 ± 13	—
Fe^{3+}	3300 ± 120	1210 ± 70	—

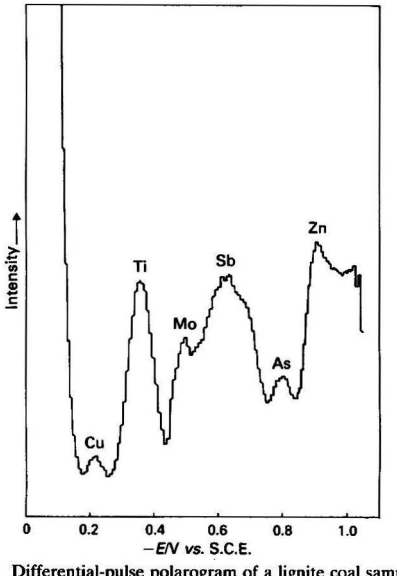

Fig. 1. Differential-pulse polarogram of a lignite coal sample from Seyitömer (pH 5.5)

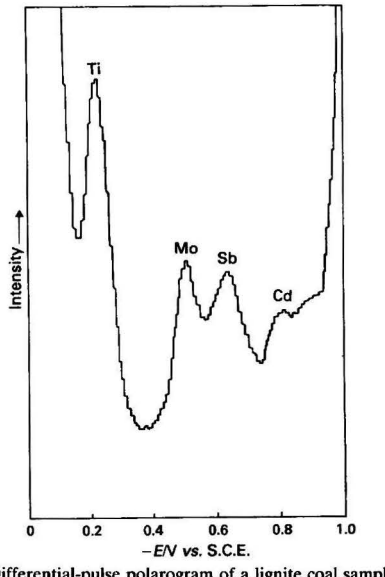

Fig. 2. Differential-pulse polarogram of a lignite coal sample from Seyitömer (pH 2.5)

The peak potentials in coal samples using EDTA and sodium acetate as the electrolyte at pH 2 and 5.5 are given in Table 1. After the peak potentials of individual elements had been determined in coal samples, the polarograms could be analysed for the elements.

Figs. 1 and 2 show polarograms of a coal solution at two different pH values. To assign a peak to a certain element, a standard solution of this element had to be added; an appropriate increase in the peak height confirmed the assignment. If the presence of one element was doubtful, the pH was changed and the newly formed peak investigated. If the peak appeared at the expected potential, and if the amount calculated was the same as that found at the previous pH, the identification was confirmed.

Fig. 1 (pH 5.5) shows a peak that is off-scale, followed by peaks at −0.23, −0.36, −0.50, −0.63, −0.80 and −0.92 V. When the sensitivity was decreased 100-fold the off-scale peak became measurable and, by addition of an iron(III) salt, it was identified as an iron peak. The peak at −0.23 V, according to Table 1, had to be copper, as there was no other peak at that potential in this medium. At pH 2, however, the copper peak moved to more positive potentials (Fig. 2) and thus overlapped with the off-scale iron peak. At this pH the peaks were at −0.22, −0.52, −0.65 and −0.86 V.

The element that gave peaks at −0.36 V (pH 5.5) and −0.22 V (pH 2.5) was titanium, as on the addition of a standard solution of titanium the peak heights increased proportionally and the amounts of titanium calculated at the two pH values were the same. The presence of molybdenum and antimony in coal was confirmed by the same procedure.

The polarogram at pH 2.5 had a small peak at about −0.85 V that moved to more negative potentials with increasing pH. Also, as the addition of cadmium salt increased the peak height, this peak was assigned to cadmium. The peak at −0.90 V (pH 5.5) was similarly shown to be that of arsenic.

In order to determine the amount of impurities that may have been introduced by the reagents, water and glassware, a blank test was carried out. No peak was observed when a polarogram was taken at the same sensitivity as for the coal samples.

Two different lignite samples, one from the Seyitömer and the other from the Aşkale mines in Turkey, were analysed using the above procedure and the results are given in Table 2. Standard deviations were calculated in 5–8 samples. In the analysis of lignite coal samples, at least two standard additions were made for each element.

To check the validity of the method, one coal sample was analysed by both AAS and the proposed method. As only Cd and Cu lamps were available, these ions could be determined. Results are given in Table 2, and the agreement is considered to be satisfactory.

In conclusion, it may be stated that it is possible to analyse a coal sample for heavy metals, both qualitatively and quantitatively, by taking polarograms at two different pH values.

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Anodic Voltammetry of Butyrophenone Neuroleptics

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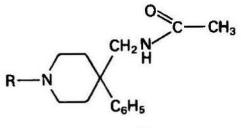
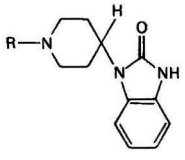
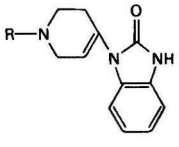
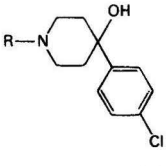
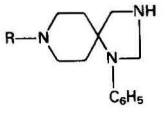
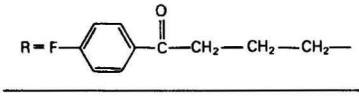
Example drugs have been examined at platinum and gold rotating disc electrodes in 0.1 mol l^{-1} sulphuric acid, and for spiperone over the pH range 0–7. Aceperone and haloperidol show neither anodic nor cathodic activity. Benperidol and droperidol are anodically active, but the voltammograms do not obey the Levich relationship, and adsorption destroys electrode activity. Spiperone gives a two-electron wave for oxidation of the diazo ring; electrode kinetic parameters have been evaluated. Although relative standard deviations of less than 1% are obtainable in the determination of spiperone, adsorption is severe and caution is required.

Keywords: *Butyrophenone neuroleptics; rotating disc electrode voltammetry; oxidation mechanism; electrode kinetic parameters*

Butyrophenones are widely used major tranquillisers of recent development. These long acting neuroleptics are particularly useful in the prevention of psychotic relapse in schizophrenic patients after discharge on remission of symptoms. Peak plasma levels occur in 2–6 h and may attain a plateau for 72 h and persist detectably for several weeks. Reduction at a dropping-mercury electrode has been reported.[1,2] Coulometric determination of the number of electrons in alkaline media gave the value as two and the half-wave potential is pH-dependent. Determinations have been reported by GC,[3–6] TLC,[7–9] fluorimetry[10] and radioimmunoassay.[11] The anodic behaviour of the compounds listed in Table 1 at rotating disc platinum and gold electrodes in 0.1 mol l^{-1} sulphuric acid and for spiperone in buffer media is described in this paper.

Table 1. Butyrophenones examined. All compounds were supplied by Janssen Pharmaceuticals Ltd.

C.A. number	Generic name	Structure*	Batch No.	Proprietary name
807-31-8	Aceperone		3248	Aceperone
2062-84-2	Benperidol		A19/1	Anquil
548-73-2	Droperidol		A7801	Droleptan
52-86-8	Haloperidol		D0301	Haldol
749-02-0	Spiperone		A3301	Spiperol



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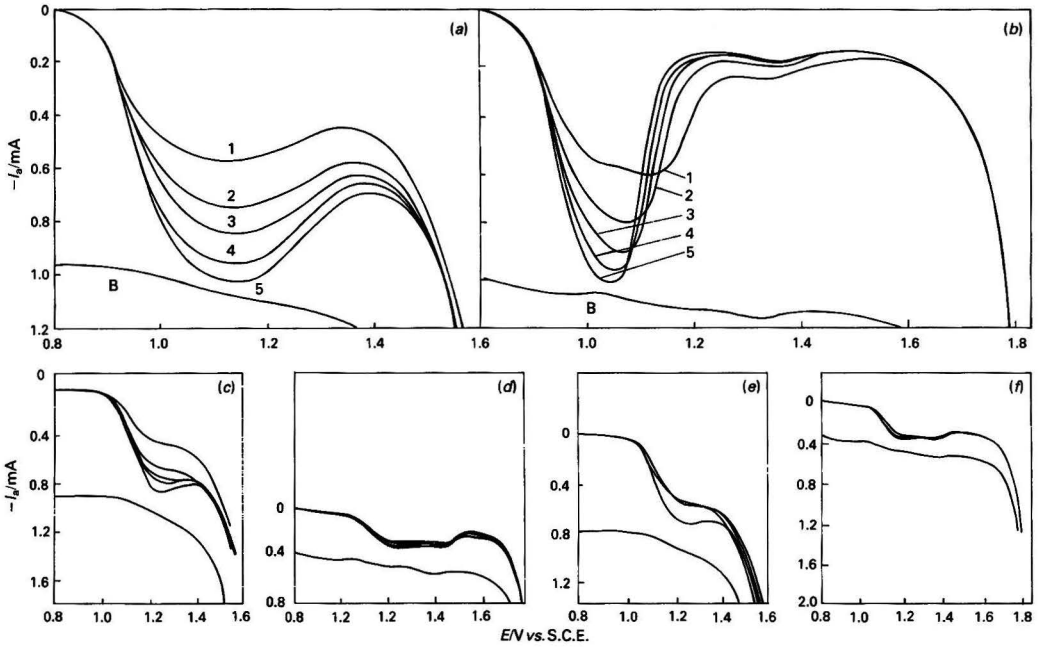


Fig. 1. Rotating disc electrode voltammograms of 0.002 mol l^{-1} butyrophenones in 0.1 mol l^{-1} sulphuric acid: (*a*) and (*b*) spiperone, (*c*) and (*d*) benperidol and (*e*) and (*f*) droperidol, at (*a*, *c* and *e*) platinum and (*b*, *d* and *f*) gold electrodes. Electrode area, 0.503 cm^3; temperature, 25 °C. Curves 1–5, nominal rotation speeds 10, 20, 30, 40 and 50 Hz, respectively; offset curves (B) represent a re-scan at 50 Hz without intervening activation of the electrode

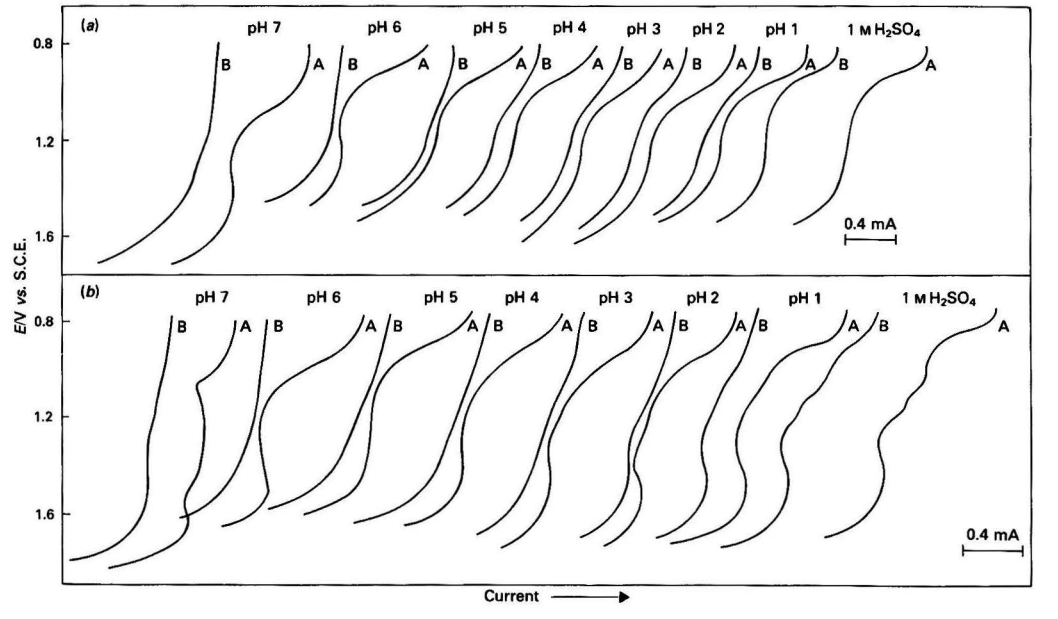


Fig. 2. Influence of pH on the anodic voltammograms of spiperone at (*a*) platinum and (*b*) gold electrodes. Curves A, activated electrode; B, re-scan without intervening activation. Concentration, 10^{-3} mol l^{-1}; rotation speed, 50 Hz; temperature, 25 °C; media, sulphuric acid (pH = 0, 1) or citrate - phosphate buffer adjusted to exact unit pH value

Table 2. Electrode kinetic parameters for spiperone. Rotation speed, 50 Hz; electrode area, 0.503 cm²; medium 0.1 mol l⁻¹ sulphuric acid; reference potential, E_i at 25 °C

	Platinum electrode				Gold electrode			
C_R[b]/10⁻³ mol l⁻¹	E_i/V vs. S.C.E.	β	k_m/ 10⁻⁶ l cm⁻² s⁻¹	k/ 10⁻⁶ l cm⁻² s⁻¹	E_i/V vs. S.C.E.	β	k_m/ 10⁻⁶ l cm⁻² s⁻¹	k/ 10⁻⁶ l cm⁻² s⁻¹
1.999 87	0.955	0.35	5.37	5.60	0.940	0.41	5.77	6.43
4.999 69	0.960	0.32	5.05	5.55	0.945	0.39	5.45	6.12
7.999 51	0.965	0.34	4.71	4.78	0.950	0.37	4.97	5.89
9.999 39	0.965	0.34	4.32	4.25	0.955	0.40	4.78	5.65

Experimental

Apparatus, procedures, electrode activation and solution manipulation have been described.[12] The samples, which were of Drug Standard grade, were supplied by Jenssen Pharmaceuticals Ltd.

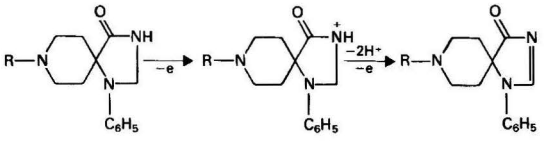
Results and Discussion

Spiperone at platinum electrodes in 0.1 mol l⁻¹ sulphuric acid gave well formed waves as broad peaks followed by troughs [Fig. 1(*a*)], which virtually disappeared when an unactivated electrode was used. The peak potential moved to more positive values as the rotation speed increased. The behaviour was similar but more marked at gold [Fig. 1(*b*)], and the peak potential became less positive as rotation speed increased; there was also a diminishing after-wave. The Levich plots of peak current against square root of frequency are poor (the RSD of the slope is 4% at platinum electrodes and 8% at gold electrodes), suffering a change of slope rather than curvature and having non-zero intercepts. The peak current *versus* concentration plots, however, are exceptionally good, especially at gold, with excellent zero intercepts and RSDs <0.4%. The voltammograms and frequency graphs are characteristic of adsorption, and suppression of the waves at unactivated electrodes identifies the adsorbate as the oxidation product.

The influence of pH on the voltammetry of more dilute spiperone is illustrated in Fig. 2. At platinum, a single good wave is formed at all pH values, with a maximum height in 0.1 mol l⁻¹ sulphuric acid, but the wave dwindles and vanishes at an unactivated electrode as the pH increases. At an activated gold electrode three waves, the second ill defined, in sulphuric acid become two at pH 2–4, one at pH 5–6 and two peaks at pH 7. The first wave attains maximum height in 0.1 mol l⁻¹ sulphuric acid. Again, increasing adsorption gradually destroys the wave as the pH increases if an unactivated electrode is used.

Benperidol gave good waves at platinum at low rotation speeds, but peaking, overlapping and deviation from Levich dependence occurred at higher rotation speeds, as can be seen in Fig. 1(*c*). Oxidation did occur at gold, Fig. 1(*d*), but the waves showed little dependence on rotation speed. The behaviour of droperidol, Fig. 1(*e*) and (*f*), was similar. For both compounds, a repeat scan at 50 Hz without intervening activation of the electrode showed virtual suppression of the waves, as the offset curves in Fig. 1 demonstrate. This indicates that adsorption of oxidation product generates deviation and malfunction and that this is aggravated by high concentrations and rotation speeds.

Determination of the number of electrons involved in the oxidation of spiperone by amperostatic coulometry[12] gave a value of 2.0. Aceperone and haloperidol are anodically inactive, which eliminates the butyrophenone and piperidine fragments, leaving the benzamidazol fragment in benperidol and droperidol and the diazo ring in spiperone as the sites for oxidation. In spiperone, the carbonyl group further fixes the reaction site as shown.



Here R is the 4-fluorophenyl-4-oxobutyl group, which, by elimination, can be suggested as the adsorptive site. Electrode kinetic parameters have been derived for spiperone by pattern theory,[13] and are listed in Table 2.

The dominance of adsorption of reaction product on the electrode surface prevents voltammetry from being a useful analytical method for the butyrophenones examined other than perhaps spiperone. The square root of frequency graphs raise no great expectations, but the spiperone calibration graphs are unusually good, and sets of four measurements on each of five Drug Standard solutions in 0.1 mol l⁻¹ sulphuric acid at activated platinum gave relative standard deviations of 0.53, 0.69, 0.89, 0.20 and 0.34%, while at activated gold the results were 0.57, 0.37, 0.32, 0.20 and 0.54%, in the concentration range 10⁻³–10⁻² mol l⁻¹. Nevertheless, the potential interference presented by adsorption counsels caution in the application.

We thank Janssen Pharmaceuticals Ltd., for the gift of materials listed in Table 1, and the Royal Society for the SEL transfer standard DVM. W. H. thanks the Government of Pakistan for the award of a Scholarship and the University of Karachi for the grant of leave of absence.

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Anodic Voltammetry of Codeine and Dihydrocodeine at Rotating Disc Electrodes of Platinum and Gold

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Of the analgesics examined, phenylbutazone and oxyphenylbutazone showed neither anodic nor cathodic activity, while codeine is oxidised by a single four-electron step and dihydrocodeine by a six-electron step to the same products. Electrode kinetic parameters have been evaluated, and the reaction mechanisms elucidated. Determination of codeine and dihydrocodeine by anodic voltammetry is simple and rapid, and gives relative standard deviations of 2–3%, and is free from interference from adsorption on the electrodes in 0.1 mol l^{-1} sulphuric acid. The influence of pH in the range 0–11.5 has been examined.

Keywords: *Codeine and dihydrocodeine determination; rotating disc electrode voltammetry; coulometry; electrode kinetic parameters; reaction mechanisms*

Codeine is well known for its antitussive, antidiarrhoeal and mild analgesic action. Dihydrocodeine, produced by reduction of the 7,8-double bond, is 2–4 times more potent as an analgesic.[1] It is curious, in view of the structure, that no electroanalytical examination has been reported. Indeed, codeine is stated to be devoid of electroactivity,[2,3] whereas morphine is electroactive.[4] Many methods of determination have been described, including spectrophotometry,[5] titrimetry,[6] conductimetry,[7] chromatography[8–10] and GC - MS.[11] A full investigation was therefore undertaken of the anodic voltammetry at rotating disc electrodes (RDEs) of platinum and gold, including the electrode kinetics and the reaction mechanisms.

Experimental

The dihydrocodeine tartrate Drug Standard was supplied by Glaxo Laboratories: codeine phosphate was purchased from Macfarlane Smith Ltd. Apparatus, instrumentation, electrode activation, solution manipulation and procedures have been decribed.[12] The normal scan speed was 5 mV s^{-1}.

Results and Discussion

Voltammetry

Oxidation in 0.1 mol l^{-1} *sulphuric acid*

Typical sets of voltammograms for codeine are shown in Fig. 1. The better plateaux at gold are not reflected in the charge-transfer coefficients. The Levich dependences are statistically reasonable; the limiting current *versus* square root of frequency plots tend to bend, rather than curve, towards the time axis at higher mass transport rates, and the intercepts are non-zero. The limiting current *versus* concentration plots similarly show a tendency to shift (axehead) towards the current axis at higher mass transport rates, but up to at least 5 $\times 10^{-3}$ mol l^{-1} at up to 50 Hz the plots show good linearity and zero intercepts. Dihydrocodeine behaves in the same way, except that the wave height is about 50% greater. Repeat scans at 50 Hz with either electrode for either compound, without intervening reactivation of the electrodes, superimpose, so the system is free from adsorptive effects in this medium.

Influence of pH

As may be seen from Fig. 2, a single wave is displayed at either electrode throughout the pH range of 0–11.5 and the combination of best definition and greatest wave height occurs in 0.1 mol l^{-1} sulphuric acid for platinum and in citrate buffer at pH 3 for gold. Good waves are available in the neutral region, particularly at gold, and at pH 9 at platinum. The negative movement of the background wave with mounting hydroxyl concentration brings mergence of sample and solvent waves at more extreme pH values. Adsorption on an unactivated platinum electrode is slight, but is marked at an unactivated gold electrode and suppresses the wave at pH >3. All waves are shifted gradually to less positive potentials as the pH rises.

Analytical appraisal

An analysis of an example calibration for each electrode and compound is given in Table 1, along with the precision of the results of rapid determination of the two compounds at the alternative electrode. The latter involved the measurement of the limiting current for a Drug Standard solution at 1.45 V *versus* S.C.E. at each of 5 rotation speeds and calculation of the concentration from the slope and intercept of the calibration graph, so that propagation of errors from five calibrations affects the result. Each set of measurements required less than 10 min, including activation of the electrode. A relative standard deviation of 2–3% is readily attainable.

Electrode kinetics

Half-wave potentials, mass- and charge-transfer rate constants and charge-transfer coefficients have been definitively determined in 0.1 mol l^{-1} sulphuric acid and a tabulation is

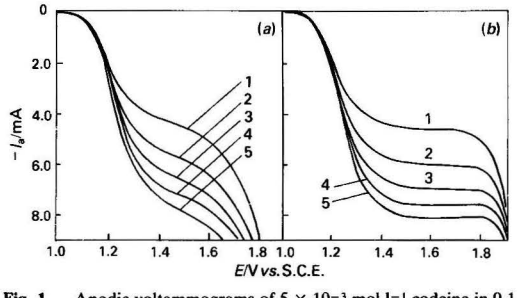

Fig. 1. Anodic voltammograms of 5 $\times 10^{-3}$ mol l^{-1} codeine in 0.1 mol l^{-1} sulphuric acid at (*a*) platinum and (*b*) gold RDE. Electrode area, 0.503 cm^2; scan speed, 5 mV s^{-1}; temperature, 25 °C; nominal rotation speeds for curves 1–5 are 10, 20, 30, 40 and 50 Hz, respectively

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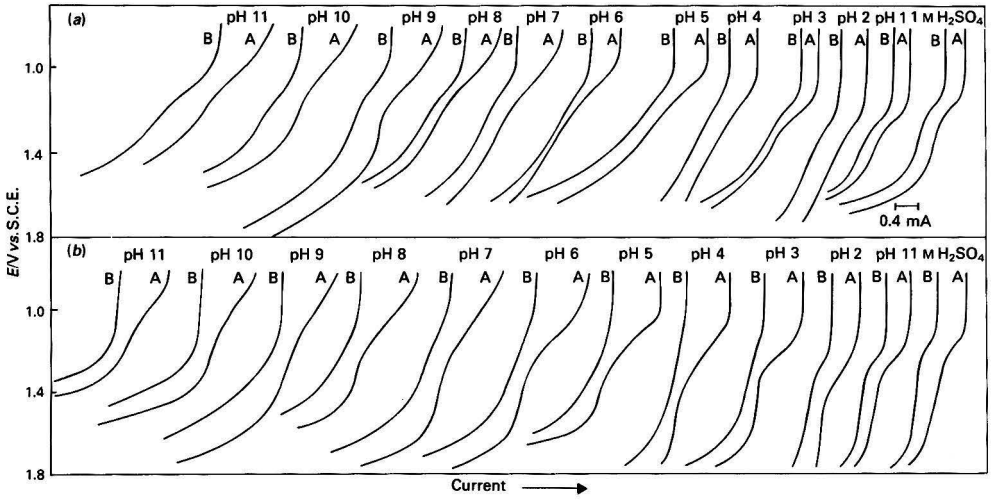

Fig. 2. Influence of pH on the anodic voltammograms of 10^{-3} mol l^{-1} codeine in sulphuric acid (pH = 0, 1) citrate - phosphate buffer (pH = 2–9) or sodium carbonate (pH = 10, 11) adjusted to exact unit value at (*a*) platinum and (*b*) gold RDE. Curves: A, activated electrode; B, re-scan without intervening electrode activation. Rotation speed, 50 Hz

Table 1. Analytical performance of RDE voltammetry

Sample	Nominal rotation speed/Hz	Slope/mA l mmol^{-1}	Intercept/mA	Correlation coefficient	SD of residuals/mA	SD of slope/mA 1 mmol^{-1}	RSD of slope, %
		Calibration analysis					
Codeine (0–5 × 10^{-3} mol l^{-1}, platinum electrode)	10	0.382 11	0.028 42	0.998 16	0.041 29	0.011 60	3.04
	20	0.478 95	0.015 79	0.999 64	0.022 94	0.006 45	1.35
	30	0.548 68	0.019 74	0.999 57	0.028 68	0.008 06	1.47
	40	0.619 74	0.003 95	0.999 99	0.005 74	0.001 61	0.26
	50	0.659 21	0.011 84	0.999 89	0.017 21	0.004 83	0.73
Dihydrocodeine (0–10 × 10^{-3} mol l^{-1}, gold electrode)	10	0.431 73	0.938 46	0.999 99	0.006 93	0.001 18	0.27
	20	0.685 71	0.002 38	0.999 99	0.010 91	0.001 46	0.21
	30	0.655 38	1.250 77	1.000 00	0.005 55	0.000 94	0.14
	40	0.778 95	0.794 74	0.999 86	0.022 94	0.006 45	0.83
	50	0.811 04	1.159 21	0.999 61	0.040 15	0.011 28	1.39

Precision of determination, n = 5

Gold electrode		Platinum electrode	
Codeine/10^{-3} mol l^{-1}	RSD %	Dihydrocodeine/10^{-3} mol l^{-1}	RSD %
2.000 14	3.61	2.000 12	2.78
5.000 35	1.73	5.000 30	0.17
8.000 56	2.79	8.000 48	2.89

available from the authors. Half-wave potentials are about 30 mV higher at gold, around 1.2 V *versus* S.C.E., charge-transfer rate constants range from 1.5 to 5 × 10^{-6} l cm^{-2} s^{-1}, and the charge-transfer coefficients, β, show very little variation around 0.17. Values are referenced to the half-wave potentials, because conditional potentials are not accessible, and calculated from pattern theory.[13]

Other analgesics

Oxyphenylbutazone (*C.A.* 129–20–4, batch 79R344, Geigy Pharmaceuticals, Tenderil) and phenylbutazone (*C.A.* 50–33–9, batch 78R505, Geigy Pharmaceuticals, Butacote) displayed no electroactivity at either platinum or gold RDE when scanned anodically or cathodically within the potential range of the aqueous medium (0.1 mol l^{-1} sulphuric acid).

Coulometric Determination of *n*-values

Amperostatic coulometry[12] gives a decrease in limiting current of 25% per electron equivalent of charge passed for codeine, identifying a four-electron reaction. With dihydrocodeine, the decrease is 16.7% per electron equivalent and the reaction is therefore six-electron. Both reactions occur in a single wave and the oxidation product is stable, giving no

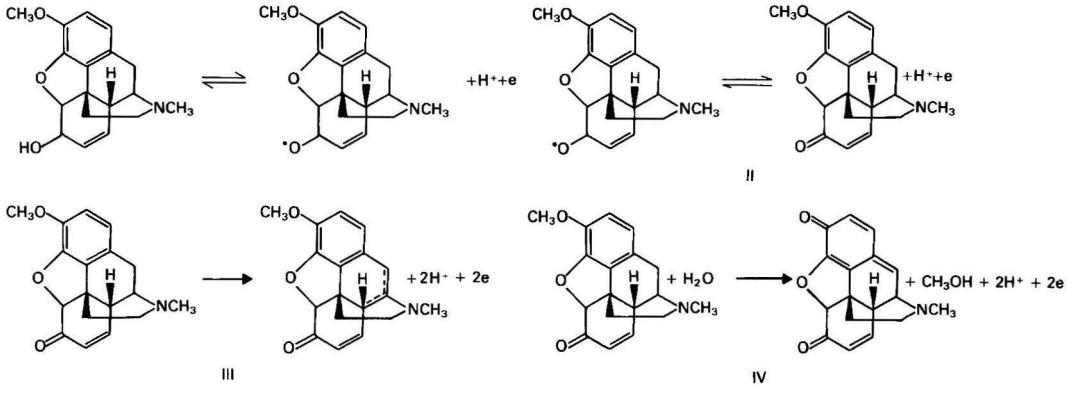

Fig. 3. Reaction mechanism

further reaction or regeneration. Potentiostatic coulometry gives a more precise determination of both compounds than does voltammetry, but is much more time consuming.

Reaction Mechanism

Although no intermediate steps are revealed by voltammetry, it is likely that fast steps lead up to the final slow step, which is rate determining. As shown in Fig. 3, in step I the 6-hydroxy group is deprotonated to the radical, from which the ring proton is lost in step II to form the carbonyl group. The next step is slow and may be either III or IV in which the 9,10- or the 9,14-hydrogens are removed from ring 3 as in III, or the 3-methoxy group is attacked together with the 10-hydrogen by demethoxylation and formation of the quinonoid structure in IV. For dihydrocodeine, which is saturated in the 7,8-positions, a fast first step is the two-electron dehydrogenation to give codeine and the reaction then proceeds as for codeine.

We thank Glaxo Laboratories for the gift of dihydrocodeine, Geigy Pharmaceuticals for the gift of oxyphenylbutazone and phenylbutazone and the Royal Society for the SEL transfer standard DVM. W. H. thanks the Government of Pakistan for the award of a Scholarship and the University of Karachi for the grant of leave of absence.

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Bioelectrochemical Detection Systems for Substrates of Dehydrogenases

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In the context of the development of enzyme electrodes for the determination of the substrates of dehydrogenases, different systems for the oxidation of NADH were tested. In a homogeneous system with the dehydrogenase, NAD^+ and phenazine methosulphate, the oxygen consumption caused by oxidation of the substrate was determined with a Clark electrode. Satisfactory sensitivity and reproducibility were obtained in the range 0.04–1 mM for several substrates, but the enzyme was rapidly denatured by the mediator. The physical entrapment of dehydrogenases together with a water-forming NADH oxidase on a Clark electrode yielded enzyme electrodes for different substrates with linear responses in the range 0.1–10 mM and a response time of 2 min. Owing to the instability of the NADH oxidase, the half-life of the electrodes was less than 5 h. The most promising systems were obtained by the coupling of the dehydrogenases to 3-β-naphthoyl-Nile Blue-modified graphite electrodes leading to oxygen-independent catalysed electrochemical NADH oxidation. The systems were optimised with regard to the NAD^+ concentration in the solution and the pore size of the membranes covering the electrodes. By immobilisation of the enzymes, mainly by chemical binding to Eupergit, a considerable increase in their stability could be achieved, and electrodes were obtained with a half-life of 8.5 d, a response time, $\tau_{\frac{1}{2}}$, of 3.5 min and linear responses over four orders of magnitude.

Keywords: *Enzyme electrodes; NADH oxidation; immobilised enzymes; dehydrogenase substrate determination*

Many of the amperometric enzyme electrodes reported so far are based on platinum electrodes, mainly on the Clark oxygen electrode.[1–3] By combination of these electrodes with oxidases, devices for the determination of the corresponding substrates are obtained.[4,5] Less common are systems for monitoring substrates of dehydrogenases[6,7] and, in most instances, their function is, as with oxidase electrodes, dependent on the oxygen saturation of the sample.

Most of the oxidoreductases are coenzyme dependent, so the use of these enzymes for the construction of electrodes suitable for monitoring their substrates requires the oxidation of a reduced coenzyme, preferably NADH. This can be performed by means of a mediator or an enzyme in the presence of oxygen or by a catalysed electrochemical process. In this investigation we have compared these three possibilities and optimised the most promising system, catalytic NADH oxidation.

Experimental

Chemicals

Most of the enzymes and coenzymes used were purchased from Boehringer, Mannheim, FRG, and most of the other chemicals and some of the supports for the enzyme binding from E. Merck, Darmstadt, FRG. Leucine dehydrogenase (E.C. 1.4.1.9) from *Bacillus sphaericus* and polyethylene glycol 10 000-bound NAD^+ (PEG-NAD^+ 10 000)[8] were a kind gift from Dr. M. R. Kula, Gesellschaft für Biotechnologie, Stöckheim, FRG. and the pre-polymerised oligomer ETN-2000[9] was a gift from Prof. S. Fukui, Kyoto, Japan.

NADH oxidase was isolated from *Streptococcus faecalis* as reported elsewhere.[10] Phenoxyacetylcellulose (PAC) was obtained from Sigma, St. Louis, MO, USA; phenyl-Sepharose and CNBr-activated Sepharose from Pharmacia, Uppsala, Sweden; Eupergit (epoxyacrylic resin granules) from Röhm Pharma, Weiterstadt, FRG; Blue Gel A and UM 500 ultrafiltration membranes from Amicon, Lexington, MA, USA; cellulose acetate membranes for electrophoresis from Sartorius, Göttingen, FRG; RO-100 membranes from Spectrum Medical Industries, Los Angeles, CA, USA; Nucleopore membranes from Nucleopore, Pleasanton, CA, USA; Porlisan from Percola, Munich, FRG; and dialysis membranes from Serva, Heidelberg, FRG (Visking) and from Union Carbide, Chicago, IL, USA.

Measuring Devices and Solutions

Mediated oxygen consumption

The mediated oxygen consumption was determined in a Clark electrode measuring device from Bachofer, Reutlingen, FRG. The medium (total volume 2.5 ml) consisted of 0.2 M Tris buffer (pH 8.0) - 1 mM NAD^+ - 2 mM phenazine methosulphate (PMS^+) - 1.5 mM KCN. The reagent was stored in the dark in order to prevent decomposition of PMS^+ by light. After addition of the substrate (2–40 μl of a 100 mM solution) the background oxygen consumption was observed for 5 min, then the reaction was started by addition of 10 U ml^{-1} of the corresponding enzyme. The initial velocity of the oxygen consumption as a function of the substrate concentration in the medium on a double-logarithmic scale yielded the calibration graphs.

Enzyme-catalysed NADH oxidation

For the enzyme-catalysed NADH oxidation the Clark electrode (Type E 5046, Radiometer, Copenhagen, Denmark) was covered with a small piece of cigarette paper that was impregnated with 5 μl of an NADH oxidase solution (*ca.* 4 U, enzyme from *Streptococcus faecalis*[10]) and 5 μl of the dehydrogenase solution (4–8 U). It was fixed to the electrodes by means of a dialysis membrane (Union Carbide, relative molecular mass cut-off 10 000–20 000) and an O-ring. The medium (10 ml total) was 0.5 M phosphate buffer with a pH between the optimum of the dehydrogenase and that of the oxidase (pH 6.8) with 1 mM NAD^+. After addition of the substrate the initial velocity or the maximum oxygen consumption was determined (Radiometer PHM 71 MK 2) and plotted as a function of the substrate concentration on a double-logarithmic scale.

Catalytic NADH oxidation

The details of the measuring device for the catalytic NADH oxidation are shown in Fig. 2. The measuring electrode was prepared by application of 5 μl of a 1 mM ethanolic solution of 3-β-naphthoyl-Nile Blue[11] to the top of a graphite electrode (diameter 6.5 mm) and evaporation of the solvent. The dehydrogenase (a few microlitres of solution, suspension or immobilised enzyme) was placed on the top of the electrode and fixed by means of a dialysis membrane (Visking 36/32, relative molecular mass cut-off 10 000–20 000). The measuring solution (25 °C) was 0.2 M Tris buffer (pH 8.0), usually with 1

mM NAD^+. After addition of the substrate the initial velocity of the current change and the maximum current were used for the calibration graphs.

Immobilisation of Enzymes

For the physical adsorption of enzymes, 1 g of polymer support was suspended in a solution of 10 mg of lyophilised enzyme protein in 2.5 ml of water. After 1 h the gel was isolated by suction and applied to the electrode without further treatment. The covalent binding of enzymes to CNBr-Sepharose or to Eupergit was performed according to the recommendations of the manufacturer and the immobilisation of enzymes by pre-polymerised oligomers after Tanaka *et al.*[9] The preparation of dextran-NAD^+ has been reported earlier.[12]

Results

Oxygen-dependent Systems for NADH Oxidation

Phenazine methosulphate (PMS^+) can transfer hydrogen from NADH to O_2,[13] and this oxidation has already been used for the determination of NADH by means of a Clark electrode.[14] The combination of this reaction with an NADH-producing dehydrogenation yielded a system suitable for the determination of the corresponding substrate through the oxygen consumption. Calibration graphs for ethanol, lactate and glutamate in the 0.1–1.0 mM range were obtained from the initial velocity of the oxygen consumption after addition of the enzyme to solutions of the substrate, PMS^+ and NAD^+. The reproducibilities and accuracies of substrate determinations were within 2%. With this system, dehydrogenase substrates can be determined in analysers for substrates of oxidases. However, as the enzymes are readily denatured by PMS^+, only a limited application is possible.

Microbial and enzymatic coenzyme recycling has been applied several times for the development of dehydrogenase electrodes, mostly with non-specific NADH oxidation.[6,7] For specific NADH oxidation in coupled systems, Gwak *et al.* [15] recently purified an NADH-oxidising multi-enzyme complex, and we succeeded in isolating a single-chain water-forming "NADH oxidase" from *Streptococcus faecalis*.[10] In coupled systems of this enzyme with dehydrogenases the oxidation of the corresponding substrates could be measured from the oxygen consumption.

The coupled enzymes were physically entrapped by a dialysis membrane on top of a Clark electrode, and the substrates were determined in 1 mM NAD^+ solution. The calibration graphs (Fig. 1), obtained from the initial velocity of the oxygen consumption, are straight lines for substrate concentrations in the range 0.1–10 mM. The half response time (time needed to attain half of the maximum response), $\tau_{\frac{1}{2}}$, of the systems was about 2 min and the accuracy of substrate determination was ±3%. Mainly owing to the instability of the NADH oxidase, the sensitivity of the electrodes decreased by 50% within a few hours ($t_{\frac{1}{2}} < 5$ h; the stability is defined as the time required until half of the initial maximum response is observed). By co-immobilisation of the dehydrogenase and NADH oxidase with ETN-2000[9] stabilisation could be attained, but at the expense of response time and sensitivity.

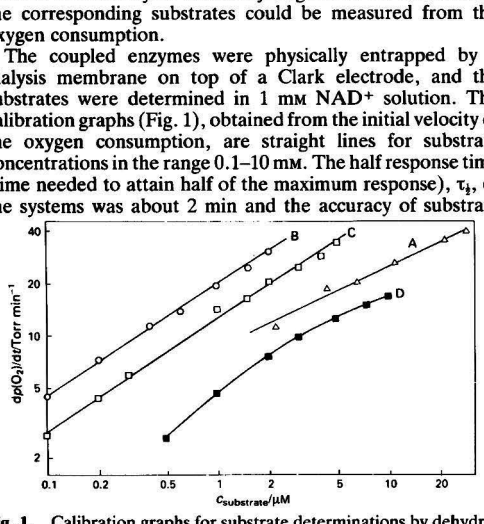

Fig. 1. Calibration graphs for substrate determinations by dehydrogenases coupled with NADH oxidase. Enzymes (4–8 U) bound to a Radiometer Type E 5046 Clark electrode by means of a dialysis membrane. 0.5 M phosphate buffer (pH 7–8), 1 mM NAD^+. A, Ethanol with alcohol dehydrogenase from yeast; B, lactate with lactate dehydrogenase from hog muscle; C, glutamate with glutamate dehydrogenase from beef liver; and D, as C, enzyme immobilised with ETN-2000[13]

Systems with Catalytic NADH Oxidation

The function of the electrodes described so far is dependent on oxygen saturation of the sample, which reduces considerably their field of application. In order to eliminate this problem, Enfors[16,17] used the response current of an oxidase electrode to control electrolytic oxygen production near the electrode. Oxygen-independent systems with mediated electron transfer are restricted to a very limited number of enzymes, *e.g.*, L(+)-lactate : cytochrome c oxidoreductase (E.C. 1.1.2.3).[18] The possibility of direct electrochemical oxidation of NADH by platinum electrodes has been demonstrated several times[19–22]; however, this oxidation implies high overvoltages and is therefore accompanied by non-specific side-reactions, including some NAD^+ decomposition.

By systematic investigations with cyclic voltammetry we succeeded in finding new substances that catalyse NADH oxidation on graphite electrodes at low voltages.[11,23] Graphite electrodes impregnated with 3-β-naphthoyl-Nile Blue[11] (cat.) in combination with dehydrogenases (DH) are suitable for the determination of the corresponding substrates (SH_2) through an electric current.

$$SH_2 + NAD^+ \overset{DH}{\rightleftharpoons} S + NADH + H^+$$

$$NADH \xrightarrow{cat.} NAD^+ + H^+ + 2e^-$$

$$SH_2 \xrightarrow[NAD^+]{DH,\ cat.} S + 2H^+ + 2e^-$$

In the potentiostatic measuring device (Fig. 2), the electron current is determined from a voltage drop on the resistance R_a. The initial velocity of this current change or the maximum current can be used to establish calibration graphs, but only the first value is attained within a reasonable time after addition of the substrate. In order to optimise this system the influence of different parameters on the sensitivity, response time and stability (for definitions see Tables 1 and 2) of the electrodes has been determined.

Influence of the NAD^+ concentration

The total reaction of the substrate oxidation indicates that NAD^+ has only a mediator function; however, maximum sensitivity of an electrode should be attained only with coenzyme saturation of the enzyme. According to Fig. 3, high sensitivity and independence of NAD^+ concentration are attained with a glutamate electrode only at a coenzyme concentration above 1 mM in the sample solution. With polymer-bound NAD^+ (dextran or polyethylene glycol as substituent) entrapped within the protecting membrane of the electrode, substrate-proportional responses were also observed, but the system strongly decreased in sensitivity and stability.

Influence of the pore size of the membrane

With the aim of enclosing unsubstituted NAD^+ together with the enzymes, the relative molecular mass cut-off limit of the membrane was decreased. This modification led to a dramatic change in sensitivity and response time (Table 1). The results also indicate that not only the pore size but also the membrane thickness influences the diffusion of substrates and coenzyme. In order to retain high sensitivity and the shortest half response time possible, dialysis membranes were used throughout in further experiments.

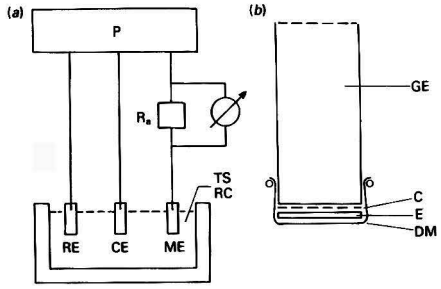

Fig. 2. Measuring device for the determination of dehydrogenase substrates by means of modified graphite electrodes. (*a*) Three-electrode arrangement consisting of potentiostat, P (Minipotentiostat MP 75, Bank Elektronik, Göttingen, FRG; Kip & Zonen recorder), reference electrode, RE (calomel); counter electrode, CE (Pt); and measuring electrode, ME. (*b*) Details of ME: a graphite electrode, GE, impregnated with the catalyst, C, and coated with the enzyme, E (dehydrogenase, suspension or support-bound), and protected by a dialysis membrane, DM, fixed by an O-ring

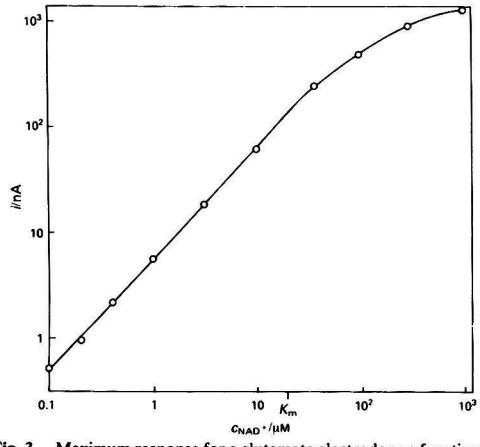

Fig. 3. Maximum response for a glutamate electrode as a function of the NAD^+ concentration in the measuring cell. Constant glutamate concentration, 1 mM

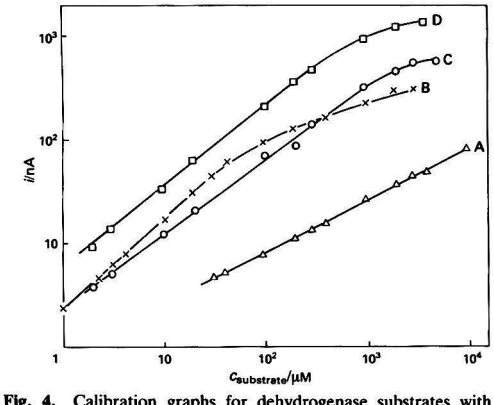

Fig. 4. Calibration graphs for dehydrogenase substrates with a 3-β-naphthoyl-Nile Blue-modified graphite electrode, using enzyme suspensions behind the dialysis membrane. 0.2 M Tris buffer (pH 7–8), 1 mM NAD^+, 0.02% sodium azide. A, Ethanol with 18 U of alcohol dehydrogenase from yeast ($t_{\frac{1}{2}}$ = 20 h; $i_{max.}$ = 440 nA); B, alanine with 7 U of alanine dehydrogenase from *Bacillus subtilis* ($t_{\frac{1}{2}}$ = 33 h; $i_{max.}$ = 330 nA); C, lactate with 5.5 U of lactate dehydrogenase from hog muscle ($t_{\frac{1}{2}}$ = 50 h; $i_{max.}$ = 600 nA); and D, glutamate with 4.8 U of glutamate dehydrogenase from beef liver ($t_{\frac{1}{2}}$ = 65 h; $i_{max.}$ = 1050 nA). Response time, $\tau_{\frac{1}{2}}$ = 0.6 min

Table 1. Influence of membrane pore size on the characteristics of an ethanol electrode based on a 3-β-naphthoyl-Nile Blue-modified graphite electrode. Eupergit-immobilised alcohol dehydrogenase, NAD^+ in solution. Sensitivity = $i_{max.}$ observed in a 3 mM substrate solution; half response time $\tau_{\frac{1}{2}}$ = time needed to attain half maximum response; HCLR = highest concentration of linear response

Membrane	Relative molecular mass cut-off	Sensitivity, *i*/nA	$\tau_{\frac{1}{2}}$/ min	HCLR/ mM
Visking dialysis 36/32 ..	10000	740	3.5	0.30
Reversed osmosis RO 100	100	270	8.0	0.10
Nucleopore (0.015 nm) ..	—	180	5.0	0.50
Ultrafiltration UM 500 ..	500	2	20.0	—

Table 2. Influence of enzyme immobilisation on the characteristics of an ethanol electrode based on a 3-β-naphthoyl-Nile Blue-modified graphite electrode. Response time τ, see Table 1; stability $t_{\frac{1}{2}}$ = time for decline of the sensitivity to half its initial value; sensitivity = $i_{max.}$ at substrate saturation. A Visking dialysis membrane was used throughout

Support	Binding method*	Sensitivity, *i*/nA	$\tau_{\frac{1}{2}}$/ min	$t_{\frac{1}{2}}$/h
None, 30% solution ..	—	440	0.6	20
Cellulose acetate (Sartorius)	S	32	2.5	190
Phenoxyacetyl cellulose PAC (Sigma)	A	460	4.0	145
Silica gel (Merck)	A	700	5.0	120
Porlisan (Percola)	A	1050	4.0	21
Cellit (Merck)	A	900	4.0	18
Phenyl Sepharose (Pharmacia)	A	880	2.0	69
Blue Gel A (Amicon) ..	A	380	3.0	40
Sepharose, CNBr-activated (Pharmacia)	CB	250	2.5	145
Sepharose, CNBr-activated (Pharmacia) + 0.5 M NaCl	CB	700	2.5	60
Eupergit (Röhm Pharma)	CB	740	3.5	206

* A = adsorption; S = solution; CB = covalent binding. A and S: 10 mg of enzyme + 1 g of carrier per 2.5 ml of water; CB according to manufacturer of carrier.

As already observed with the NADH oxidase system (Fig. 1), in calibration graphs for electrodes with physically entrapped enzymes no correlation between the total enzyme activity applied and the sensitivity of the electrode or the slope of the calibration graph was found (Fig. 4). This is mainly because the steady-state NADH concentration within the enzyme layer is not only determined by different diffusion processes but also by the velocities and by the equilibrium constants of the dehydrogenase reactions. In addition, for practical reasons, enzyme suspensions were used (the activities given in Fig. 4 correspond to the total amount of enzyme applied), but the actual enzyme activities within the electrodes could not be determined.

Theoretically, the influence of the parameters discussed could be eliminated by applying conditions that would lead to a purely diffusion-controlled process. The enlargement of the diffusion layer was achieved by the simple application of a double dialysis membrane. The glutamate electrode thus obtained showed the expected 45° slope of the double-logarithmic calibration graph, which would be the same for all enzymes. Naturally, the electrode was less sensitive and had nearly twice the response time in comparison with the normal glutamate electrode.

Importance of enzyme immobilisation

The limited stability of the electrodes under working conditions (25 °C) always required repeated calibration. Therefore,

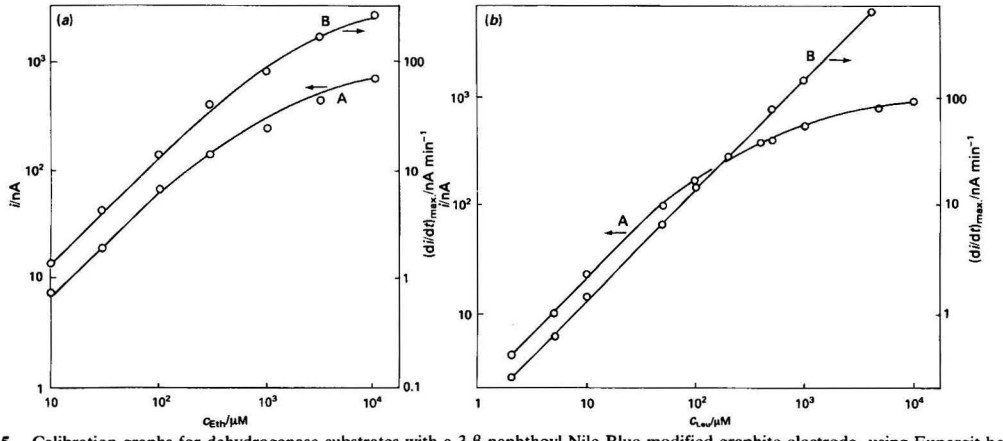

Fig. 5. Calibration graphs for dehydrogenase substrates with a 3-β-naphthoyl-Nile Blue-modified graphite electrode, using Eupergit-bound enzymes behind the dialysis membrane. For buffer solution, see Fig. 4. A, Initial response velocity; B, maximum response. (*a*) Ethanol with alcohol dehydrogenase from yeast ($t_{\frac{1}{2}}$ = 8.6 d; $i_{max.}$ = 750 nA); (*b*) leucine with leucine dehydrogenase from *Bacillus sphaericus* ($t_{\frac{1}{2}}$ = 14 d; $i_{max.}$ = 850 nA)

systematic investigations with the aim of prolonging the lifetime of the enzymes by physical or chemical immobilisation were performed. Alcohol dehydrogenase was chosen for the experiments, because it is one of the most sensitive dehydrogenases. As the volume needed for an enzyme bound to an insoluble support is always larger than that for an enzyme solution of the same activity, a longer diffusion path is implied with immobilisation, and hence the response time was increased for all electrodes with immobilised enzymes (Table 2). However, in most instances their sensitivity was enhanced, and in some also their stability. For some electrodes a rapid decline of sensitivity in the first 2 d was followed by a slow decline over a longer period. This is probably due to the denaturation or bleeding of a small amount of non-immobilised enzyme. When only the slow decline is taken into account, the stability of some electrodes is even better than indicated in Table 2.

The most advantageous combination was obtained with the enzyme bound to Eupergit granules. The corresponding electrode with a half-life of 8.5 d needed a calibration (measurement of a standard) only once a day. A further advantage of this type of electrode is that in the steady-state linear calibration graphs with 45° slopes (double-logarithmic plot) were obtained (Fig. 5). This indicates optimum adaptation of diffusion and reaction velocities. The calibration graph for the leucine electrode, using the initial velocity of the current change as a parameter, is linear for more than four orders of magnitude.

Discussion

Coenzyme recycling is a prerequisite for the development of enzyme electrodes for dehydrogenase substrates. As shown in this investigation, systems with oxygen-dependent mediated or enzymatic NADH oxidations are unstable and not versatile, but can be applied in some instances to the determination of dehydrogenase substrates in analysers for oxidase substrates.

The catalysed electrochemical NADH oxidation is more versatile and suited for general practical application. Our investigations demonstrate that with this system high sensitivities and large ranges of linear response can be obtained, and that by immobilisation of the enzymes satisfactory stability of the electrodes is attained. Further progress concerning response time and stability is expected by the use of enzymes with higher specific activity and by the development of new immobilisation methods. Maybe even more selective membranes could become available that would in addition permit the coenzyme to be entrapped within the electrode chamber. A time-saving improvement is also expected from the use of an automated electronic evaluation of the initial slope of the response curves. The final step will be the adaptation of the systems for continuous control of substrate concentration in biological liquids.

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Studies on the Selective Separation of Rubidium on a Titanium(IV) Tungstoarsenate Column

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The heteropolyacid salt titanium tungstoarsenate has been prepared by adding titanium(IV) chloride to a mixture of sodium arsenate and tungstate. The product, prepared under optimum conditions, has an exchange capacity of 0.86 mequiv. g^{-1} and is stable in acidic and salt solutions. It is possible to separate selectively Rb^+ from Ag^+, Pb^{2+}, Ba^{2+}, Sr^{2+}, Mg^{2+}, Cd^{2+}, Fe^{3+}, Cr^{3+}, etc., ions on a column of this exchanger material. It can also be used for the sorption and removal of some stable radioactive pollutants.

Keywords: *Titanium(IV) tungstoarsenate; inorganic ion exchanger; heteropolyacid salt; rubidium ion exchange*

Heteropolyacid salts of quadrivalent metal ions provide suitable materials for the column separation of alkali metals.[1,2] In a recent paper[3] zirconium molybdophosphate columns were utilised for the specific separation of Tl(I) ions from a mixture of other cations. This paper describes the investigations on the exchange properties of titanium(IV) tungstoarsenate gel. The compound exhibits a specific selectivity of Rb^+ ions and it has been possible to adsorb and elute quantitatively even very small amounts of this metal ion in the presence of other cations.

Reagents

All reagents used were of analytical-reagent grade unless otherwise specified.

Titanium(IV) chloride (BDH Chemicals Ltd.), sodium arsenate heptahydrate (E. Merck) and sodium tungstate (AnalaR, BDH Chemicals Ltd.) were used.

The radioisotopes ^{86}Rb, ^{134}Cs, ^{204}Tl, $^{110}Ag^m$, $^{85+87}Sr$, $^{115}Cd^m$, ^{203}Hg and ^{51}Cr were obtained from Bhabha Atomic Research Centre, Bombay.

Instrumentation

The following apparatus was used: an expanded-scale pH meter, pH-823, for pH measurements; a Spectronic-20, for recording the absorbance; a Sico temperature-controlled shaker; a scintillation counter for gamma-ray counting; and a Geiger - Müller counter for beta-ray counting.

X-ray spectra were obtained by a Philips X-ray diffractometer employing Mo Kα (λ = 0.0711 nm) radiations; IR spectra of the sample (dried at 50 °C) were recorded in potassium bromide pellets, on a Beckman IR-120 spectrophotometer; and thermogravimetric analysis of the product was carried out on a Stanton Redcroft thermogravimetric balance with a heating rate of 10 °C min^{-1}.

Synthesis of Titanium(IV) Tungstoarsenate

Titanium(IV) tungstoarsenate was prepared by adding 0.25 M titanium(IV) chloride solution to a mixture of 0.25 M sodium arsenate and 0.25 M sodium tungstate in the volume proportions 2 + 1 + 1. The pH of the mixture was adjusted to 1.0 and the precipitate obtained was allowed to stand for 24 h at room temperature. It was then washed by decantation and afterwards with 2 M nitric acid. The gel, dried at 50 °C, can be ground to the desired particle size. The conditions for the preparation of the product (which were elucidated after much preliminary investigation) are those at which a stable gel showing good sorption properties are obtained. The reproducibility of the samples prepared by this method is evident by the fact that the exchange capacity of different samples of the same batch had a standard deviation of 0.01 mequiv. per gram and the standard deviation in exchange capacity of samples of different batches was 0.03 mequiv. per gram. The exchanger was converted into the hydrogen form by treatment with 1.0 M nitric acid.

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Chemical Composition

A 200-mg amount of the exchanger material was dissolved in sodium hydroxide solution. Titanium was determined by the hydrogen peroxide method,[4] arsenic by titrimetry[5] and tungsten by gravimetry as barium tungstate.[6] The Ti : W : As ratio was found to be 1 : 4 : 2. The results of three simultaneous runs are given in Table 1.

Dissolution of Titanium(IV) Tungstoarsenate Gel

A 400-mg portion of the exchanger material was refluxed in a flask with 50 ml of various solvents in a temperature-controlled shaker for 10 h. The undissolved portion of the exchanger was removed by filtration, and titanium, arsenic and tungsten were determined spectrophotometrically.

Ion-exchange Capacity

The exchange capacity of the material (hydrogen form) was determined by a standard method.[7] A change in the exchange capacity of the product was also observed by heating the sample at 150, 200 and 300 °C in an oven for 20 h.

Distribution Coefficients

The distribution coefficients (K_d) of various cations were determined at pH 2 for a metal ion concentration of 0.002 M. For this purpose, 0.1 g of the exchanger was shaken with 10 ml of the metal ion solution for 10 h. The amount of cation in the solution was determined radiometrically, spectrophotometrically and in some instances by EDTA titration. K_d values were calculated from the equation

$$K_d \text{ (ml g}^{-1}) = \frac{I - F}{F} \cdot \frac{10}{0.1}$$

where I is the volume of titrant (or counts) for the original solution and F is the volume of titrant (or counts) for the solution after equilibrium.

Column Operation

For separation studies, a glass column (30 × 0.50 cm) having 1.0 g of exchanger (100–200 mesh particle size obtained after sieving the product) on a glass-wool support was used. The exchanger column was loaded with mixture of pairs of ions until these were completely adsorbed on the column material. Elution was started after 15 min at a flow-rate of 0.2 ml min^{-1}. Cations eluted were determined in a 2-ml fraction of the eluent collected.

Results and Discussion

Titanium(IV) tungstoarsenate was obtained in the form of hard granules that could be easily sieved to a desired particle size. It was stable in acids and salt solutions but dissolved to a considerable extent in bases (especially sodium hydroxide solution).

Chemical analysis of the material (samples were run in triplicate) gave the ratio of Ti : W : As as 1 : 4 : 2 (Table 1) and thermogravimetric analysis of the product exhibited complete loss of water at 700 °C for a total water content of 19% (heating was continued to 1 000 °C but no further loss in mass was observed). The absence of lines in the X-ray diffraction pattern reflects the amorphous nature of the product and the infrared spectrum of the compound shows bands at 3 420, 1 620, 960, 895, 850, 780 and 500 cm^{-1}. The strong band at 3 420 cm^{-1} indicates interstitial water molecules and the 1 620-cm^{-1} band is due to the HOH bending mode. Bands at 960, 895, 850 and 780 cm^{-1} are characteristic frequencies of the tungstoarsenate anion[8] and that at 500 cm^{-1} can be assigned to a Ti–O stretching vibration.[9]

Table 1. Analysis of titanium(IV) tungstoarsenate

Sample	Amount of exchanger taken/mg	Composition observed/mg		
		Ti	W	As
1	200	19.96	80.38	39.98
2	200	18.02	79.82	39.94
3	200	19.78	79.98	39.96

The ion-exchange capacity of the product was determined by equilibrating the original compound (hydrogen form) in 1 M potassium chloride for 10 h. The concentration of electrolyte and the equilibration time was fixed by trial; the equilibration time of this material was much less than that reported for other synthetic inorganic ion exchangers (24–48 h). The exchange capacity of the product was found to be 0.86 mequiv. g^{-1} of the exchanger material and the value decreased for a product dried at higher temperatures (Table 2).

The distribution coefficients (K_d) for various cations (Table 3) show the specific affinity of this material for Rb^+. Besides Rb^+ the material also exhibits a significant uptake of Cs^+, Tl^+, Cd^{2+}, Hg^{2+}, Sr^{2+}, Ba^{2+}, Zn^{2+} and Fe^{3+} ions; the

Table 2. Properties of titanium(IV) tungstoarsenate (in hydrogen form) heated at different temperatures

Temperature/°C	Colour	Ion-exchange capacity/mequiv. g^{-1}
50	White	0.86
150	White	0.63
200	White	0.33
300	Dirty white	0.052

Table 3. K_d values (ml g^{-1}) of metal ions on titanium(IV) tungstoarsenate dried at 50 °C at pH 2

Metal ion	K_d/ml g^{-1}	Metal ion	K_d/ml g^{-1}
Na^+	85.00	Sr^{2+}	107.40
K^+	75.00	Ba^{2+}	123.20
Rb^+	390.00	Zn^{2+}	101.95
Cs^+	202.00	Fe^{2+}	0
Tl^+	192.00	UO_2^{2+}	8.80
Ag^+	112.50	ZrO_2^{2+}	37.80
Cd^{2+}	102.88	Co^{2+}	0
Pb^{2+}	25.64	Mn^{2+}	12.42
Mg^{2+}	0	Cr^{3+}	0
Ca^{2+}	17.94	Fe^{3+}	103.70
Ni^{2+}	5.90	Bi^{3+}	70.80
Hg^{2+}	117.96	Th^{4+}	22.58

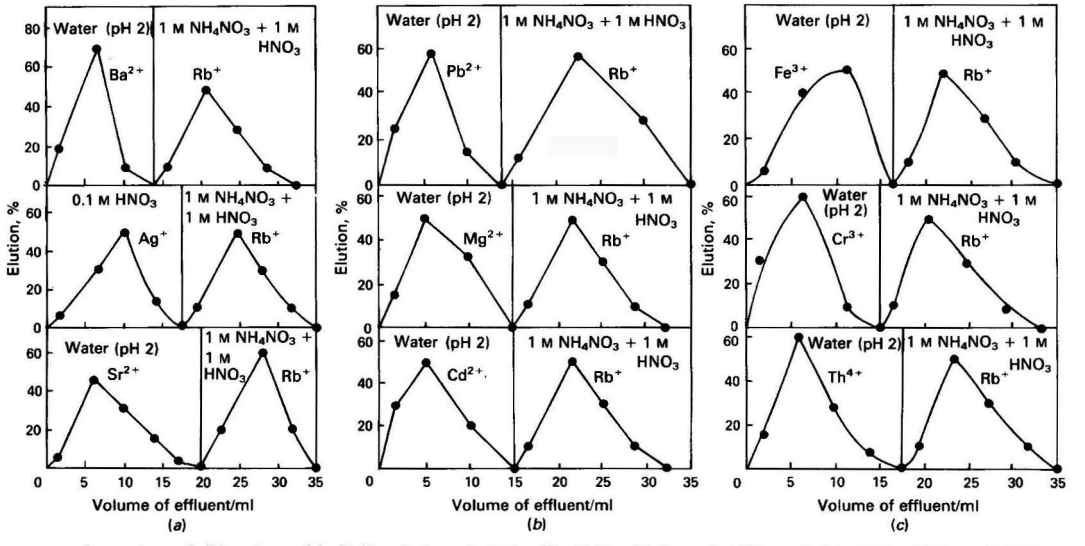

Fig. 1. Separation of Rb^+ from (*a*) Ba^{2+}, Ag^+ and Sr^{2+}, (*b*) Pb^{2+}, Mg^{2+} and Cd^{2+} and (*c*) Fe^{3+}, Cr^{3+} and Th^{4+} on a titanium(IV) tungstoarsenate column

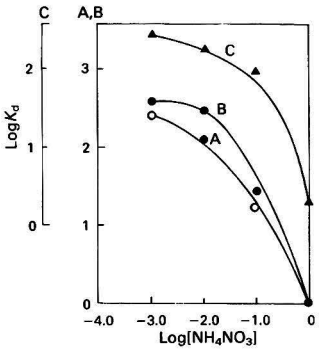

Fig. 2. Variation in K_d of (A) Rb^+, (B) Cs^+ and (C) Tl^+ (0.002 M) on a titanium(IV) tungstoarsenate column with varying NH_4NO_3 concentration

sorption of other ions is almost negligible. On the basis of this selectivity pattern the separation potential of the product was explored, and it was found to be a good scavenger of Rb^+ ions from a mixture of other cations. Consequently, a large variety of separations involving Rb^+ and other cations could be performed on the columns of this exchanger material and the results are shown in Fig. 1 (*a*, *b* and *c*). All of the separations are clean without any contamination and give a 100% recovery. It has been possible to adsorb and separate 100 μg ml^{-1} of Rb in the original solution along with other bivalent and trivalent metal ions. The adsorption of other ions (Table 3) on this exchanger material suggests the utility of the product in the removal of some stable radioactive pollutants such as Cs, Tl, Cd, Hg and Sr. It is possible to use the material for eight to ten cycles without any loss in its adsorption capacity or separation efficiency.

The variation of distribution coefficient with HNO_3 and NH_4NO_3 concentration for Rb^+, Cs^+ and Tl^+ ions is shown in Figs. 2 and 3. The results indicate that in competition with NH_4^+ or H^+ ions the uptake of Tl^+ and Cs^+ is slightly more than that of Rb^+ but in general the sorption capacity of the product is high for the three cations and it can easily trap these ions from moderately concentrated acidic or salt solutions. Further, it was observed that the sorption decreased with increasing NH_4^+ or acid concentration. A maximum decrease was observed for Rb^+ ions and consequently even smaller amounts of this cation were completely recovered with the addition of 1 M NH_4NO_3 and HNO_3, while only 70% recovery was possible for Cs^+ or Tl^+ ions, even with 3 M NH_4NO_3 and HNO_3. Efforts to separate Rb^+, Cs^+ and Tl^+ from each other were not successful owing to the low separation factor between these ions. Poor linearity of the graphs (Figs. 2 and 3) and the value of the slope obtained from the linear portions of the graphs reveal that the uptake of these cations does not strictly proceed via an ion-exchange mechanism.

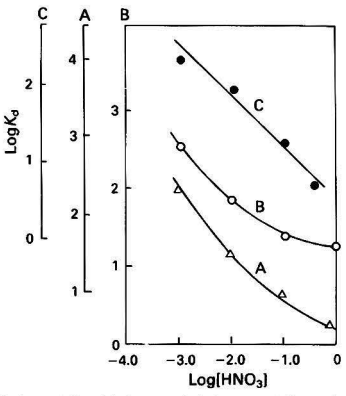

Fig. 3. Variation of K_d of (A) Rb^+, (B) Cs^+ and (C) Tl^+ (0.002 M) on a titanium(IV) tungstoarsenate column with varying HNO_3 concentration

A simulated low-level radioactive waste liquid, with a composition similar to that of an AGNS fuel reprocessing plant in Barnwell[10] (a complete simulation was not possible), was prepared from a mixture of the following solutions: Rb^+, 0.007 M; Sr^{2+}, 0.016 M; Ag^+, 0.001 M; Cd^{2+}, 0.002 M; and Cs^+, 0.014 M. The mixture was loaded on to the exchanger column and Rb^+ and Ag^+ were completely recovered without any contamination although Sr^{2+} was contaminated with Cd^{2+}.

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High-performance Liquid Chromatographic Separation and Determination of Fluorescent Whitening Agents in Detergents

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A simple, rapid, precise and accurate method for determining the fluorescent whitening agents commonly used in detergents is described. The method is based on simple extraction from the detergent by methanol and on direct injection of the extract, without further purification. The extracted whitening agents were separated using a reversed-phase column (RP-18) and by ion-pair formation. The mobile phase was methanol - water (43 + 57) containing 0.005 M triethylammonium chloride, 0.002 5 M sodium acetate and 0.002 5 M acetic acid. Depending on the type of whitening agent present, the determination can be simplified by using methanol - water (1 + 1) as the mobile phase. Fluorimetric detection makes the method highly sensitive and specific.

Keywords: *Fluorescent whitening agents; detergents; optical brightening agents; high-performance liquid chromatography; fluorimetric detection*

Fluorescent whitening agents are present in detergents at concentrations ranging from 0.02 to 0.5% *m/m*.[1] They play an important role because they create an impression of superior brightness in fabrics after laundering, and the consumer relates the superior brightness to cleaner fabrics and to a more efficient detergent. Several fluorescent substances belonging to different chemical classes have been suggested as whitening agents in detergents, such as diaminostilbenedisulphonic acid derivatives, coumarin, biphenylpyrazoline, naphthotriazole, benzoxazole and distyrylbiphenyl derivatives,[2–5] but only a few of these are actually used in the formulation of detergents. This limits our field of investigation, thus making easier the problems connected with analytical controls.

Nowadays the technique most used for the separation, identification and determination of fluorescent whitening agents in detergents is thin-layer chromatography (TLC) followed by a visual or instrumental evaluation of the intensity of the fluorescence emitted by the spots excited by exposure of the plates to the ultraviolet radiation.[6,7] With this technique good qualitative and quantitative results can be obtained by using different eluent systems in order to carry out the separation of the mostly used fluorescent whitening agents.

In order to simplify the separation of these compounds and to make it faster, high-performance liquid chromatography (HPLC) with different columns and different analytical conditions has been used, but the mobile phases used were not compatible with a long life of the stationary phase.[8,9] McPherson and Omelczenko[10] carried out the separation and determination of some fluorescent whitening agents in detergents using several types of reversed-phase columns and several mobile phases.

In this paper we describe the determination by HPLC of fluorescent whitening agents commonly used in detergents, which were separated using a single reversed-phase column (RP-18) and ion-pair formation according to a method described previously.[11]

Experimental

Apparatus

A Perkin-Elmer Model 3B liquid chromatograph equipped with 6 000 lb in^{-2} pumps was used. Injection was achieved by using a Rheodyne Model 7120 syringe-loading sample injector fitted with a 10-μl loop.

The spectrofluorimetric detector was a Perkin-Elmer Model MPF-3 with a 20-μl microcell connected to a Perkin-Elmer Model 56 recorder. For the spectrofluorimetric detection an excitation wavelength of 340 nm and an emission wavelength of 440 nm were used.

The chromatographic separations were carried out with a stainless-steel HIBAR (250 × 4 mm i.d.) column, pre-packed with LiChrosorb RP-18 (7 μm) and thermostated at 60 °C. The flow-rate was 1.5 ml min^{-1} and the volumes injected were between 1 and 10 μl. The eluents were filtered before use with Millipore filters.

The peak areas were measured using a Perkin-Elmer Minigrator M-2 calculating integrator.

Reagents

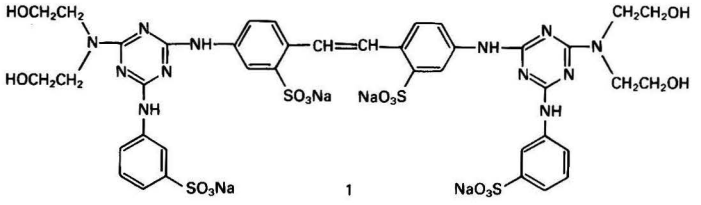
Methanol (absolute) was glass distilled before use. The other chemicals used, sodium acetate, acetic acid and triethylammonium chloride, were of analytical-reagent grade.

Fluorescent Whitening Agents

The following standard whitening agents, the structures of which are shown in Fig. 1, were used: (**1**) bis(anilinodihydroxyethylaminotriazinylamino)stilbene tetrasulphonate; (**2**) bis(anilinodihydroxyethylaminotriazinylamino)stilbene disulphonate (Ciba-Geigy); (**3**) bis(anilinomethylaminotriazinyl amino)stilbene disulphonate (Mobay Chemical Corp.); (**4**) bis(anilinohydroxyethylmethylaminotriazinylamino)-stilbene disulphonate (Ciba-Geigy); (**5**) dimethylaminomethylcoumarin (American Cyanamid); (**6**) bis(phenyltriazolyl)stilbene disulphonate (Mobay Chemical Corp.); (**7**) bis(styryl sulphonate)biphenyl (Ciba-Geigy); (**8**) bis(anilinomorpholinotriazinylamino)stilbene disulphonate (Ciba-Geigy); (**9**) (chlorophenylpyrazolinyl)benzenesulphonamide (Bayer); (**10**) diethylaminomethylcoumarin (American Cyanamid); and (**11**) naphthotriazolylstilbene sulphonate (Ciba-Geigy).

Detergent Sample Preparation

About 200 mg of detergent, accurately weighed, were placed in a dark 100-ml bottle and extracted twice with 40-ml portions of methanol in a mechanical agitator for 10 min at room temperature. The extracts, after centrifugation, were combined in a 100-ml calibrated flask and made up to volume with methanol. After filtering with Millipore FHLP 01300 filters, the sample, kept in the dark in order to avoid *cis–trans* isomerisation, was ready for analysis.



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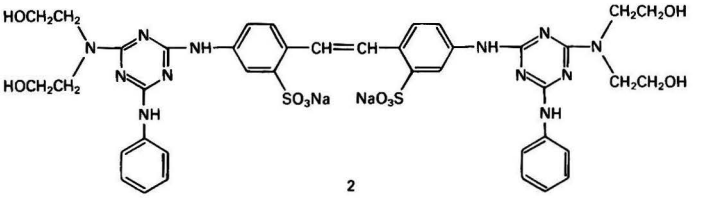

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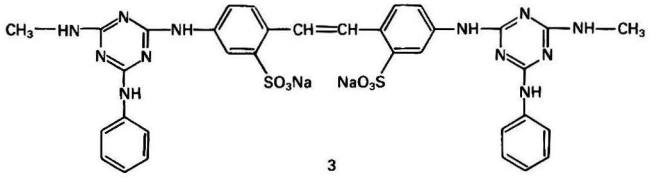

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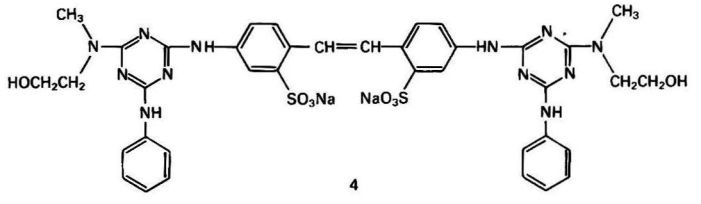

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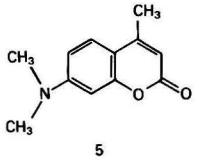

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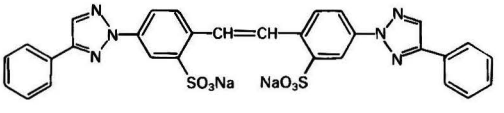

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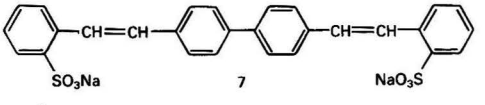

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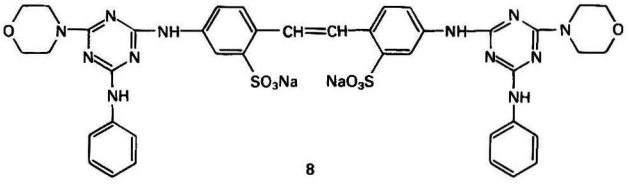

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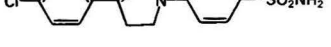

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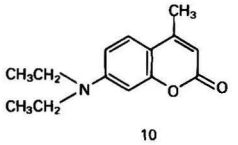

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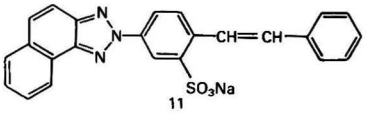

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Fig. 1. Formulae of fluorescent whitening agents

Chromatographic Analysis

Analyses of standard fluorescent whitening agents and detergent samples were carried out by reversed-phase ion-pair chromatography with an RP-18 column, using triethylammonium chloride as ion-pairing agent. The column was thermostated at 60 °C, the flow-rate was 1.5 ml min^{-1} and the mobile phase was methanol - water (43 + 57) containing 0.005 M triethylammonium chloride, 0.002 5 M sodium acetate and 0.002 5 M acetic acid. The pH of the mobile phase was 4.5. Depending on the type of fluorescent whitening agent present, the determination can be simplified by using methanol - water (1 + 1) as the mobile phase, excluding the ion-pair formation.

Table 1. Fluorescent whitening agents in commercial samples of detergents

Application of detergents	Sample No.	Compound present
Washing machines	1–5	**8**
Hand washing	6	**6** and **8**
	7–9	**8**
	10–12	**7** and **8**
Delicate fibres	13	**7** and **8**
	14	**8** and **9**
	15	**8** and **10**

Table 2. Quantitative analysis carried out on commercial samples of detergents

		Fluorescent whitening agent, % *m/m*		
Application of detergent	Sample	**8**	**9**	**10**
Washing machines	A	0.153	—	—
	B	0.050	—	—
	C	0.058	—	—
	D	0.070	—	—
	E	0.053	—	—
Delicate fibres	F	0.109	—	0.019
	G	0.026	0.028	—
Hand washing	H	0.130	—	—
	I	0.041	—	—
	J	0.069	—	—

Table 3. Concentration of compound **8** found in five separate analyses of different amounts of the same detergent (sample H, Table 2)

Sample No.	Fluorescent whitening agent, % *m/m*
1	0.130
2	0.129
3	0.131
4	0.132
5	0.129
Mean	0.130
Standard deviation	0.001

Peaks in the chromatograms of commercial samples were identified by comparing the retention times with those of the standards, determining the excitation and emission spectra directly on the eluted fraction by using the stopped-flow method. In order to have a further confirmation of the identification of the fluorescent whitening agents in commercial products, the analysis was also carried out by TLC following the methods of Schulze *et al.*[6] and the Norme Sostanze Tensioattive (N.S.T.).[7] The results agreed with those obtained by HPLC according to the proposed method, and no peaks other than those of the fluorescent whitening agents were found in the chromatograms.

Calibration graphs were obtained by analysing solutions containing 3×10^{-6} mg μl^{-1} of each fluorescent whitening agent prepared by dilution of standard solutions that contained 30 mg (accurately weighed) of pure fluorescent whitening agent in 100 ml of methanol. The volumes injected, via the 10-µl loop, were between 1 and 10 µl. An individual fluorescent whitening agent was determined by interpolation on a calibration graph obtained by plotting peak area against amount injected (1–10 µl) of one calibration standard. The response of the detector was linear over the interval measured up to 0.015 mg.

In order to evaluate the reproducibility and accuracy of the proposed method, several analyses, on different amounts of the same detergent, were carried out and various amounts of fluorescent whitening agent added to samples of detergents, previously analysed in order to establish their qualitative and quantitative composition, were recovered.

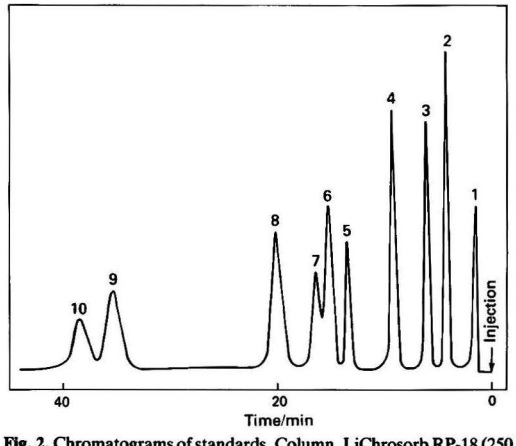

Fig. 2. Chromatograms of standards. Column, LiChrosorb RP-18 (250 × 4 mm i.d.) (7 µm); mobile phase, methanol - water (43 + 57) containing 0.005 M triethylammonium chloride, 0.002 5 M sodium acetate and 0.002 5 M acetic acid; flow-rate 1.5 ml min^{-1}; pressure, 12 MPa; temperature 60 °C; fluorimetric detection, $\lambda_{exc.}$ 340 nm and $\lambda_{em.}$ 440 nm

Table 4. Recovery of some fluorescent whitening agents

Fluorescent whitening agent	Sample (Table 2)	Amount of fluorescent whitening agent/mg				
		Present	Added	Total	Found	Recovery, %
8	F	0.222	0.150	0.372	0.373	100.2
		0.223	0.075	0.298	0.295	99.0
	G	0.053	0.075	0.128	0.127	99.3
		0.058	0.150	0.208	0.208	100.0
	H	0.285	0.250	0.535	0.540	100.9
		0.267	0.500	0.767	0.750	97.7
9	G	0.057	0.075	0.132	0.131	99.2
		0.058	0.150	0.208	0.209	100.4
10	F	0.039	0.063	0.102	0.101	99.0
		0.039	0.125	0.164	0.164	100.0

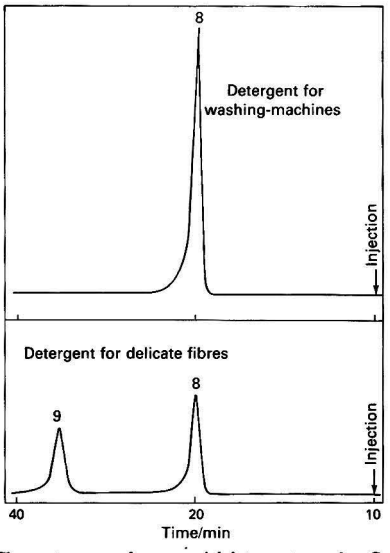

Fig. 3. Chromatograms of commercial detergent samples. Conditions as in Fig. 1

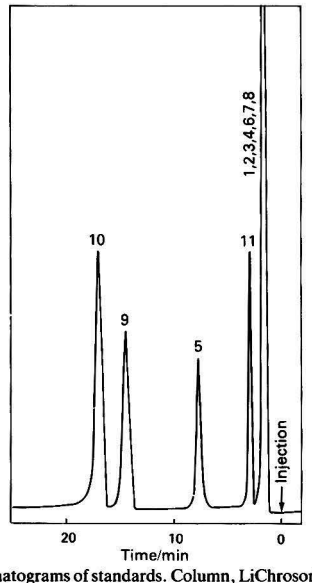

Fig. 4. Chromatograms of standards. Column, LiChrosorb RP-18 (250 × 4 mm i.d.) (7 μm); mobile phase, methanol - water (1 + 1); flow-rate, 1.5 ml min^{-1}; pressure, 8 MPa; temperature, 60 °C; fluorimetric detection, $\lambda_{exc.}$ 340 nm, and $\lambda_{em.}$ 440 nm

Results and Discussion

Fig. 2 shows a chromatogram of the standards and Fig. 3 shows examples of chromatograms of commercial detergents.

The HPLC method yields a sharp separation of compounds **1–10** (Fig. 2); compound **11** does not appear on the chromatogram because its retention time is about 90 min. This compound was absent from samples of the commercial detergents.

Using the method described, we analysed several samples of detergents designed for washing machines, hand washing and for delicate fibres, chosen from the most representative national brands. Table 1 shows the results of the qualitative analysis. In the samples analysed not more than two fluorescent whitening agents are present; compound **8** is present in all samples. The extensive use of this compound is due to its bleaching action, which is not affected by the kind of tensioactive substances present in the formulation, and it can be used without harm with other fluorescent whitening agents that are substantive to synthetic fibres.

Table 2 shows examples of quantitative analyses carried out on commercial samples of detergents using the experimental conditions in Fig. 2. Tables 3 and 4 show the results of the reproducibility and accuracy of the proposed method.

Depending on the type of fluorescent whitening agent present, the determination can be simplified by using methanol - water (1 + 1) as the mobile phase, excluding ion-pair formation. If only some or all of components **5, 9, 10** and **11** are present in a sample, these can be separated and determined by using the conditions reported in Fig. 4. Under these conditions the sulphonate compounds **1, 2, 3, 4, 6, 7** and **8,** owing to their polarity, are not distributed in the non-polar stationary phase and are not separated. In contrast, the non-sulphonate fluorescent whitening agents **5, 9** and **10** and the sulphonate compound **11,** in which the lipophilicity of the molecule seems to balance the polarity of the functional group, are completely separated. When this simplification is possible, the method is even faster.

Conclusion

The proposed method, based on simple extraction from the detergent and direct injection of the extract without further purification, is rapid, precise and accurate.

The results of analyses of commercial detergent samples show that in the formulations of those examined the number of fluorescent whitening agents present simultaneously is limited.

The method described is simple because it allows the separation and determination of all the common fluorescent whitening agents with a single reversed-phase column (RP-18); fluorimetric detection makes the method highly sensitive and specific.

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Extraction - Spectrophotometric Determination of Iron with 2-[2-(3,5-Dibromopyridyl)azo]-5-dimethylaminobenzoic Acid

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An extraction - spectrophotometric method for the determination of trace amounts of iron based on its extraction into chloroform with 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid from a weakly acidic medium has been developed. The maximum absorbance of the extracted species occurs at 615 nm. The reagent was very sensitive and reacted with iron(II) to form a stable, blue 1 : 2 (Fe : reagent) complex, which was extracted into chloroform. The apparent molar absorptivitiy of the iron(II) complex was 9.36×10^4 l mol^{-1} cm^{-1} at 615 nm in chloroform. The method has been applied to the determination of iron in various samples, such as aluminium alloys, silicon carbide powders and well waters.

Keywords: *Iron determination; spectrophotometry; 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid*

Many spectrophotometric methods have been proposed for the determination of iron, and methods based on colour reactions with various heterocyclic azo reagents have been studied extensively.[1–4] Generally, these reagents have the following advantages: (1) high sensitivity and selectivity; (2) a simple and less time-consuming procedure; (3) good reproducibility; and (4) very low absorbance of the reagent blank. In this work, an extraction - spectrophotometric method has been developed for the determination of iron using a sensitive reagent 2-[2-(3,5-dibromopyridyl)azo]-5-dimethylaminobenzoic acid (3,5-diBr-PAMB), with which micro-amounts of iron in aluminium alloy, silicon carbide and well water were determined satisfactorily.

Experimental

Apparatus

Spectrophotometric measurements were carried out using Hitachi Model 101 and 356 spectrophotometers with 10-mm glass cells. Extractions were carried out by shaking on an Iwaki Model KM shaker. The pH measurements were carried out with a Denki-Kagaku Model HG-2 pH meter.

Reagents

2-[2-(3,5-*Dibromopyridyl)azo*]-5-*dimethylaminobenzoic acid*. The reagent was obtained by coupling diazotised 2-amino-3,5-dibromopyridine with *N,N*-dimethylaminobenzoic acid in an ethanolic solution at 30–40 °C as described previously.[5,6] This reagent can be purchased commercially from Dojindo Laboratories, Kumamoto, Japan.

Dimethylformamide solution, 0.05% *m/V*. Prepared from pure materials. This solution was stable for several months when stored in an amber-coloured bottle.

Iron(II) standard solution, 1.0 g l^{-1} *of iron(II)*. A 7.021-g mass of ammonium iron(II) sulphate hexahydrate was dissolved in 10 ml of hydrochloric acid (1 + 1) and diluted to 1 000 ml with re-distilled water in a calibrated flask.

Buffer solutions. Solutions of 1 M hydrochloric acid - 1 M sodium acetate (pH 1.0–6.0), 0.2 M boric acid, potassium chloride - 0.2 M sodium hydroxide (pH 7.0–10) were used for pH adjustment.

Buffer solution, pH 5.5. This solution was prepared by adding acetic acid, with mixing, to 1 M sodium acetate solution until a pH of 5.5 was obtained, as measured by a pH meter.

Ascorbic acid, 1% *m/V*. This solution was freshly prepared daily.

Dimethylglyoxime, 1% *m/V*.

Procedure

Transfer 20 ml of slightly acidic sample solution containing up to 5.5 μg of iron into a 100-ml separating funnel. Add 0.5 ml of 1% *m/V* ascorbic acid solution and 5 ml of acetic acid buffer solution, then dilute to about 30 ml with re-distilled water. Add 0.4 ml of 0.05% *m/V* 3,5-diBr-PAMB solution and mix the solution by stirring. Add 10 ml of chloroform and shake vigorously for 2 min. Transfer the organic layer into a 10-mm glass cell after drying with cotton fibre and measure the absorbance at 615 nm against a reagent blank.

Results and Discussion

Absorption Spectra, Organic Solvent and Molar Absorptivity

Iron(II) and 3,5-diBr-PAMB form a blue complex that can be extracted into various organic solvents. On the other hand, the reactivity of iron(III) with 3,5-diBr-PAMB was almost negligible. The iron(II) complex was extracted into chloroform, dichloromethane and nitrobenzene, of which chloroform was preferred because it gave the largest absorbance. The absorption spectrum of the iron(II) complex of 3,5-diBr-PAMB in chloroform was recorded and is shown in Fig. 1. The iron(II) complex in chloroform exhibited an absorbance maximum at 615 nm, where the apparent molar absorptivity was 9.36×10^4 l mol^{-1} cm^{-1}.

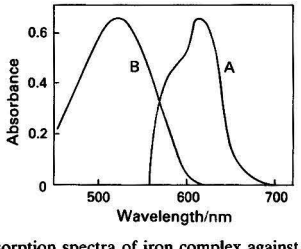

Fig. 1. Absorption spectra of iron complex against reagent blank; concentration of iron(II), 0.4 μg ml^{-1}. A, Iron(II) complex extracted into chloroform at pH 5.5; B, reagent blank measured against chloroform

Effect of pH on Extraction

The absorbance was at a maximum at pH 2.0–7.0, and was constant throughout this range (Fig. 2). Subsequent determinations were carried out at pH 5.5.

Effect of Reagent Concentration

The absorbances of a series of solutions containing 4.0 μg of iron and various amounts of 1% *m/V* ascorbic acid solution were measured. It was found that 0.2 ml of reducing agent sufficed to reduce 4.0 μg of iron. Next, the effect of an excess of the chromogenic reagent was examined. It was found that 0.2 ml of 0.05% *m/V* 3,5-diBr-PAMB solution sufficed to complex 4.0 μg of iron; with higher concentrations the absorbance remained essentially constant.

Shaking Time and Stability

The minimum shaking time for complete extraction of the complex with chloroform was found to be 1 min at room temperature. The absorbance at 615 nm was then stable for at least 24 h.

Nature of the Complex

The empirical formula of the iron(II) complex was studied by the continuous variation and molar ratio methods. A typical graph obtained by the former method showed unequivocally that a 1 : 2 (Fe : reagent) complex is formed (Fig. 3). Similar results were obtained from a molar ratio plot.

Calibration Graph

The calibration graph obtained by the procedure showed good linearity over the range 0–5.5 μg of iron per 10 ml of chloroform. Reproducibility tests for 20 results at the 3.0 μg of iron level showed a relative standard deviation of 0.6%. The Sandell sensitivity was 6.0×10^{-4} μg cm^{-2} of iron.

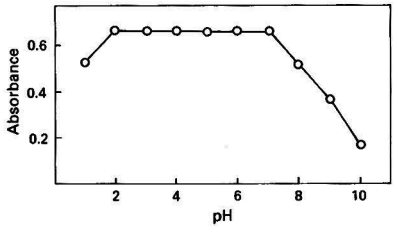

Fig. 2. Effect of pH on the formation and extraction of iron(II) complex. Absorbance measured at 615 nm against reagent blank

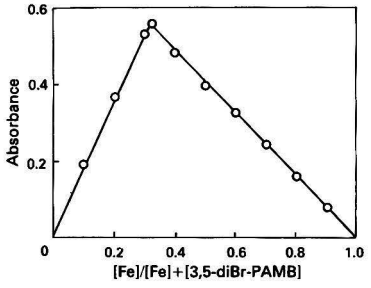

Fig. 3. Composition of iron(II) complex by the continuous variation method. Iron(II) complex extracted into chloroform under the following conditions; pH, 5.5; wavelength, 615 nm; and concentration, 4×10^{-5} M

Effect of Diverse Ions

The selectivity of 3,5-diBr-PAMB is good. Numerous cations and anions were examined by applying the method to a fixed amount of iron in the presence of increasing amounts of the ion being studied. The tolerance limit was taken as the amount giving an error of ±2% in the absorbance.

For the determination of 3.0 μg of iron by this method, foreign ions can be tolerated at the levels given in Table 1. Chloride, nitrate, perchlorate and sulphate can be tolerated at levels of 500 mg, and sodium citrate, sodium tartrate, sodium thiosulphate, sodium fluoride and thiourea at levels of 100 mg. Cations such as silver(I), aluminium(III), arsenic(III), cadmium(II), manganese(II), lead(II), silicon(IV), titanium(IV) and zinc(II) can be tolerated at levels of 1 mg. However, cobalt(II), copper(II), nickel(II), palladium(II) and vanadium(V) form intensely coloured complexes with 3,5-diBr-PAMB. These ions therefore interfere in the general procedure for the determination of iron, but if necessary they can be removed by the addition of various masking agents, as shown in Table 2. They can also be removed by the addition of 0.01 M EDTA solution after the formation of iron(II) complex of 3,5-diBr-PAMB, as shown in Table 3. As the removal of interferences by addition of 0.01 M EDTA is simple, this method was applied to the determination of iron. However,

Table 1. Tolerance limits for the determination of iron. The solution contained 3.0 μg of iron(II)

Ion added	Amount tolerated/mg
Cl^-, NO_3^-, ClO_4^-, SO_4^{2-}	500
Sodium citrate, sodium tartrate, sodium thiosulphate, sodium fluoride, thiourea	100
Ag(I), Al(III), As(III), Ba(II), Bi(III), Ca(II), Cd(II), Hg(II), Li(I), Mg(II), Mn(II), Mo(VI), Pb(II), Rb(I), Sb(III), Se(IV), Si(IV), Sn(IV), Sr(II), Ti(IV), Tl(I), Zn(II)	1
Cr(VI), W(VI)	0.2

Table 2. Elimination of interferences by addition of 100 mg of masking agent. The solution contained 3.0 μg of iron(II)

Ion	Amount tolerated/μg: Without masking agent	With masking agent	Masking agent
Co(II)	0.2	10	Sodium citrate
Cu(II)	0.5	1 000	Sodium thiosulphate
		1 000	Thiourea
Ni(II)	0.1	0.5	Sodium citrate
Pd(II)	1	50	Sodium thiosulphate
V(V)	5	1 000	Sodium tartrate

Table 3. Elimination of interferences by addition of 0.01 M EDTA. The solution contained 3.0 μg of iron(II)

Ion	Amount tolerated/μg	Volume of 0.01 M EDTA/ml
Co(II)	200	3
Cu(II)	20	3
Ni(II)	0.1	3
Pd(II)	5.0	3
V(V)	1 000	3

Table 4. Determination of iron in aluminium alloy standard reference materials

Sample*	Composition (elements other than iron), %	Iron content, % Certified value	Found Mean†	Found Range
1019	Cr, 0.10; Cu, 3.43; Mg, 0.41; Ni, 0.20; Si, 8.42, V, 0.05; Zn, 0.51; remainder Al	0.83	0.830	0.826–0.836
920	Co, 0.10; Cr, 0.27; Cu, 0.71; Mg, 0.46; Ni, 0.29; Si, 0.78; V, 0.15; Zn, 0.80; remainder Al	0.72	0.724	0.722–0.728
916	Co, 0.03; Cr, 0.05; Cu, 0.27; Mg, 0.10; Ni, 0.06; Si, 0.41; V, 0.02; Zn, 0.30; remainder Al	0.54	0.546	0.544–0.552

* Sample from the Nippon Litemetal Research Laboratory.
† Means of three determinations.

Table 5. Determination of hydrochloric acid solubilised iron in silicon carbide powders

Sample	Iron content/μg g^{-1} 3,5-DiBr-PAMB method Mean*	3,5-DiBr-PAMB method Range	Atomic-absorption spectrophotometric method Mean*	Atomic-absorption spectrophotometric method Range
Cerac 1058	344	341–349	342	331–352
Stark A-10	24.3	18.3–30.0	21.9	18.8–25.8
Abrasive 2000 ..	807	790–822	789	772–815
Abrasive 120 ..	261	251–272	249	233–255
Abrasive 80	235	220–246	233	223–245

* Means of three determinations.

Table 6. Determination of iron in well waters

Sample No.	Iron content/μg ml^{-1} 3,5-DiBr-PAMB method Mean*	3,5-DiBr-PAMB method Range	1,10-Phenanthroline method Mean*	1,10-Phenanthroline method Range
1	0.048	0.042–0.053	0.045	0.037–0.054
2	0.042	0.037–0.048	0.045	0.040–0.052
3	0.039	0.034–0.043	0.040	0.029–0.047
4	0.010	0.006–0.013	0.011	0.006–0.014
5	0.026	0.023–0.028	0.023	0.017–0.028

* Means of three determinations.

for the determination of iron in a synthetic sample that contained 3.0 μg of iron, 10 μg of cobalt, 20 μg of copper, 10 μg of nickel, 10 μg of palladium and 50 μg of vanadium, the tolerance limit for the nickel was still low. Therefore, to remove large amounts of nickel, an extraction with dimethylglyoxime was used.[7] The analysis of synthetic sample solutions of iron using dimethylglyoxime and EDTA gave good results.

From the above studies, the following procedure was formulated.

Procedure for the Analysis of Synthetic Sample Solutions

Transfer an aliquot of synthetic sample solution into a 100-ml separating funnel. Adjust the pH of the solution to 5.5 with ammonia solution. Add 1 ml of 1% *m/V* dimethylglyoxime and 5 ml of pH 5.5 buffer solution, then dilute to about 30 ml with re-distilled water. Add 10 ml of chloroform and shake vigorously for 5 min. Discard the organic layer. Add 0.5 ml of 1% *m/V* ascorbic acid solution and 0.4 ml of 0.05% *m/V* 3,5-diBr-PAMB solution to the aqueous phase and mix the solution by stirring. Add 3 ml of 0.01 M EDTA solution and 10 ml of chloroform, shake vigorously for 2 min, allow the phases to separate and transfer the organic layer into a 10-mm glass cell after drying with cotton fibre. Measure the absorbance at 615 nm against a reagent blank.

Applications

The 3,5-diBr-PAMB method has been applied satisfactorily to the determination of iron in various materials, as follows.

Determination of iron in aluminium alloy standard reference materials

A 0.1-g sample of aluminium alloy in a 200-ml beaker was treated with 50 ml of a mixture of hydrochloric acid, nitric acid and re-distilled water (3 + 2 + 8) and the mixture was heated on a hot-plate, covering with a watch-glass. The solution was then cooled to room temperature and transferred into a 200-ml calibrated flask. Suitable aliquots of this solution were taken and the determination was carried out according to the above extraction procedure.

Several aluminium alloy standard reference materials were analysed and the results are presented in Table 4. The results obtained agreed with the certified values.

Determination of hydrochloric acid solubilised iron in silicon carbide powders

A 0.1-g sample of silicon carbide powder in a 100-ml beaker was treated with 50 ml of hydrochloric acid (1 + 1) and the mixture was heated on a hot-plate for 10 min. The solution was cooled to room temperature and filtered through a 5B filter-paper and the residue was washed with hydrochloric acid (2 + 100). The filtrate was transferred into a 100-ml calibrated flask. Suitable aliquots of this solution were taken and the determination was carried out according to the above extraction procedure.

Several silicon carbide powders were analysed and the results are compared with those obtained by atomic-absorption spectrophotometry in Table 5.

Determination of iron in well waters

A 100-ml volume of well water was measured into a 200-ml beaker and 3 ml of hydrochloric acid were added. The solution was boiled on a hot-plate until the volume was reduced to 30–40 ml, then cooled to room temperature and transferred into a 50-ml calibrated flask. Suitable aliquots of this solution were taken and the determination was carried out according to the above extraction procedure.

Several well waters were analysed and the results are compared with those obtained by the 1,10-phenanthroline method in Table 6.

Conclusion

2-[2-(3,5-Dibromopyridyl)azo]-5-dimethylaminobenzoic acid can react with iron(II) to form an intensely coloured complex that can be extracted into chloroform. The iron(II) complex formed is very stable in the organic phase and the stoicheiometric ratio is 1 : 2 (Fe : reagent). The calibration graph is linear over the range 0–5.5 μg of iron in 10 ml of chloroform and the apparent molar absorptivity is 9.36×10^4 l mol^{-1} cm^{-1} at 615 nm. 3,5-DiBr-PAMB reacts with only a few metal ions. The method is relatively free from interferences because most other metallic complexes of 3,5-diBr-PAMB can be decomposed by the addition of 0.01 M EDTA solution after formation of iron(II) complex. The results of the analysis of aluminium alloy standard reference materials, silicon carbide powders and well waters were satisfactory.

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Spectrophotometric Determination of Cobalt after Separation by Adsorption of Its Ternary Complex with 4′-(*p*-Methoxyphenyl)-2,2′:6′,2″-terpyridine and Tetraphenylborate on to Microcrystalline Naphthalene

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A spectrophotometric method for the determination of cobalt after adsorption of its ternary complex with 4′-(*p*-methoxyphenyl)-2,2′:6′,2″-terpyridine (MPTP) and tetraphenylborate (TPB) on to microcrystalline naphthalene has been developed. The insoluble cobalt ternary complex formed at pH 4.0–11.1 has an absorption maximum at 518 nm and a molar absorptivity of 3.3×10^3 l mol^{-1} cm^{-1}. The complex is quantitatively adsorbed on to microcrystalline naphthalene by vigorous shaking for a few seconds and is stable in acetonitrile for more than 6 d. Simple and direct spectrophotometric methods are described for the determination of cobalt in reference materials and practical samples.

Keywords: *Cobalt determination; spectrophotometry; naphthalene adsorption; 4′-(p-methoxyphenyl)-2,2′:6′,2″-terpyridine*

Many sensitive complexing reagents have been developed for the solvent extraction and spectrophotometric determination of trace metal ions. Solvent extraction is an excellent technique for the concentration and separation of metal ions, but it cannot be applied directly to the extraction of metal ions which form complexes with complexing reagents at high temperature or if the solubility of their complexes is low at room temperature. However, by extraction with molten naphthalene, such complexes have been successfully applied to the separation and concentration of metal ions.[1–5] The main advantages of this method are that the equilibrium distribution in the two phases is achieved within a few seconds and metal complexes are dissolved merely by contact with the molten naphthalene. Because a very small amount (2 g) of the organic phase is required for the complete extraction, the sensitivity is enhanced. In contrast, the method becomes more complicated than the conventional one using extraction directly into immiscible organic solvents because of operating the extraction at high temperature. In order to overcome the drawback, we have developed a spectrophotometric method involving solid - liquid separation after adsorption of metal complexes on to microcrystalline naphthalene.

With this method, many metal complexes can be easily collected on microcrystalline naphthalene by vigorous shaking for a few seconds at room temperature and the trace metals are determined spectrophotometrically.[6–10] It is a very convenient, useful and interesting technique in which an acetone solution of naphthalene, on mixing with an aqueous phase, yields microcrystalline naphthalene that shows excellent adsorption characteristics towards metal complexes. Further, the method can be applied to metal complexes that are insoluble in non-aqueous organic solvents such as benzene, chloroform, nitrobenzene and isoamyl alcohol.

Since 1965, 4′-(*p*-methoxyphenyl)-2,2′:6′,2″-terpyridine (MPTP) has been used as an extremely sensitive reagent for the colorimetric determination of iron(II).[11–13] This reagent also reacts with cobalt ions to give an intense red, water-soluble complex cation, which is neither very soluble in non-aqueous organic solvents nor adsorbed on to microcrystalline naphthalene. In the presence of the tetraphenylborate (TPB) anion, the cobalt complex cation forms a thermally very stable, bulky, water-insoluble, ion-associated complex. The complex is hardly extracted into any non-aqueous organic solvents because of the low solubility of the complex and also because of the formation of an emulsion in two phases by shaking. This complex can be easily adsorbed on to microcrystalline naphthalene by vigorous shaking for a few seconds at room temperature. This paper describes conditions for the adsorption of cobalt with MPTP and TPB on naphthalene and its subsequent determination. The interference of the various ions has been studied and conditions have been developed for the determination of cobalt in practical samples.

Experimental

Reagents

All reagents, unless otherwise specified, were of analytical-reagent grade.

Standard cobalt solution, 10 p.p.m. Prepared by diluting 10 ml of 1 000 p.p.m. standard cobalt solution to 1 000 ml with doubly distilled water.

MPTP solution, 0.04%. Prepared by dissolving 0.04 g of MPTP in 2–3 drops of hydrochloric acid and diluting to 100 ml with water.

Sodium tetraphenylborate (TPB) solution, 1% *in water.*

Naphthalene solution, 20% *in acetone.*

Buffer solutions. Prepared by mixing 1 M acetic acid and 1 M ammonium acetate solution for pH 3–6 and 1 M ammonia solution and 1 M ammonium acetate solution for pH 8–11.

Naphthalene.

Acetone.

Acetonitrile.

Apparatus

A Hitachi Model 200-20 spectrophotometer, with 10-mm glass cells, was used for absorbance measurements and a Toa-Dempa Model HM-6A pH meter was employed for all pH measurements.

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Procedure

Transfer 35 ml of sample solution containing 10–180 μg of cobalt into an 80-ml Erlenmeyer flask and add 4.0 ml of 0.04% MPTP solution, 2.0 ml of acetate buffer solution and adjust to pH 5.5 with dilute ammonia solution. Add 2.0 ml of 1% TPB solution, mix the solution well and allow to stand for 15 min at room temperature for complete formation of the complex. Add 2.0 ml of 20% solution of naphthalene in acetone and shake vigorously for 30 s. Filter through a filter-paper placed flat on a PTFE filter-plate in a funnel or a sintered-glass filter (No. 3) by filtration. Wash with water and dry in an oven at 55–60 °C. Dissolve the naphthalene in acetonitrile and dilute to 10 ml. Measure the absorbance of the solution against a reagent blank.

Results and Discussion

Absorption Spectra

The absorption spectra of the MPTP - TPB reagent and the Co - MPTP - TPB complex in naphthalene - acetonitrile solution, measured against water, are shown in Fig. 1. The cobalt complex absorbed strongly at 518 nm, at which wavelength the reagent showed negligible absorption. A wavelength of 518 nm was therefore chosen as the optimum.

Effect of pH

Adsorptions of the cobalt complex were carried out at various pH values, with other factors kept constant. The optimum pH range is 4.0–11.1.

Effect of MPTP and TPB Concentration

Adsorptions were carried out at a fixed pH but with various MPTP concentrations and it was observed that the adsorptions were quantitative for 2.2–7.0 ml of 0.04% MPTP solution. Similar studies with TPB showed that adsorptions were almost constant with the addition of 0.2–4.0 ml of 1% TPB solution. A relatively larger amount of TPB was effective for the formation of bulky precipitates of the complex. Thus 4.0 ml of 0.04% MPTP solution and 2.0 ml of 1% TPB solution were added for the adsorption.

Effect of the Addition of Buffer Solution and of Naphthalene

Various volumes of acetate buffer were added to the samples containing 80 μg of cobalt and the same procedure was followed. No change was observed in the absorbance with addition of 1.0–5.0 ml of the buffer solution. The amount of naphthalene (20% in acetone) was varied from 0.5 to 3.5 ml and adsorption was carried out by the same procedure. The adsorption was constant irrespective of the amount of naphthalene. Hence in all of the experiments, 2.0 ml of the buffer and 2.0 ml of 20% naphthalene solution were added.

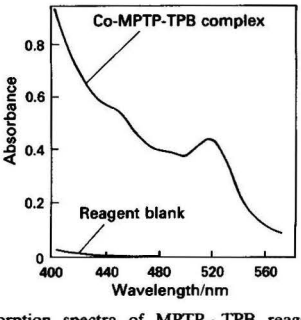

Fig. 1. Absorption spectra of MPTP - TPB reagent and Co - MPTP - TPB complex in naphthalene - acetonitrile solution. Co, 80 μg; pH, 5.5; 0.04% MPTP , 4.0 ml; 1% TPB, 2.0 ml; 20% naphthalene, 2.0 ml. Digestion time, 15 min; shaking time, 30 s; standing time, 15 min. Reference, water

Effect of Shaking and Standing Time

The Co - MPTP - TPB complex in the solution containing 80 μg of cobalt was allowed to stand at room temperature over the range 5–30 min for complete formation of the complex and then adsorbed on to microcrystalline naphthalene. The rate of formation of the complex is fast at room temperature and the absorbance remained constant in this range. The adsorption of the complex on to microcrystalline naphthalene was very fast and was completed by vigorous shaking for a few seconds. A mixture of the complex and naphthalene was dissolved in acetonitrile, and the effect of standing time on the absorbance was studied. The colour of the complex in the acetonitrile solution was stable for more than 6 d.

Choice of Solvent

Various organic solvents were tested to dissolve the adsorbed complex along with naphthalene. The complex mixture was not soluble in benzene, toluene, xylene, chlorobenzene, *o*-dichlorobenzene, nitrobenzene, chloroform, carbon tetrachloride, 1,2-dichloroethane, isoamyl acetate, diethyl ether, methanol, ethanol, dioxane, pentan-1-ol and pentan-2-ol, but was soluble in dimethyl sulphoxide, dimethylformamide, propylene carbonate and acetonitrile, which are miscible with water. Dimethyl sulphoxide and propylene carbonate are better solvents, but expensive and viscous. The complex is unstable in dimethylformamide.

Effect of the Volume of Aqueous Phase

As the volume of the organic phase (0.4 g) is small compared with that of the aqueous phase, it was essential to study the effect of the volume of the aqueous phase on the adsorption. When the volume of aqueous phase was varied between 30 and 280 ml, the adsorption remained constant up to a volume of 150 ml. Above this volume the adsorption was not quantitative.

Calibration Graph

The absorbances with various concentrations of cobalt were measured at 518 nm against a reagent blank under the optimum conditions described above. It was found that Beer's law was obeyed over the concentration range 10–180 μg of cobalt in 10 ml of acetonitrile. The molar absorptivity and

Table 1. Effect of diverse ions. Co, 80 μg; pH, 5.5; 0.04% MPTP, 4.0 ml; and 20% naphthalene, 2.0 ml

Salt	Tolerance limit	Metal ion	Tolerance limit
NaI	15 g	Mg^{2+}	1.4 g
KNO_3	7 g	Ca^{2+}	300 mg
$CH_3COONa.3H_2O$	5 g	Mn^{2+}	20 mg
Na_2SO_4	2 g	Pb^{2+}	4 000 μg
Sodium tartrate	2 g	Pt^{4+}	500 μg
KH_2PO_4	0.5 g	Cd^{2+}	300 μg
$NaClO_4$	9 g	Hg^{2+}	700 μg
NaCl	5 g	Cr^{6+}	1 000 μg
KSCN	1 g	Ni^{2+}	300 μg
Sodium citrate	500 mg	Sn^{2+}	100 μg
Sodium oxalate	500 mg	Al^{3+}	100 μg
KCN	500 μg	Cu^{2+}	100 μg
Disodium EDTA	40 μg	Zn^{2+}	70 μg
		Bi^{3+}	10 μg
		Fe^{3+}	1 μg

Table 2. Analysis of samples for cobalt

Sample	Composition, %	Cobalt certified value, %	Cobalt content,* %	
			Present method	2-Nitroso-1-naphthol method
JSS 611-7, high-speed steel	C (0.88), Si (0.30), Ni (0.064), S (0.002 2), Cu (0.036), W (6.18), Mn (0.33), P (0.017), Cr (4.07), Mo (5.01), V (1.92), N (0.031 2)	0.21	0.20 ± 0.02	0.21 ± 0.01
JSS 654-7, stainless steel	C (0.060), Mn (1.69), Cu (0.028), Cr (24.84), Si (1.29), P (0.031), Ni (20.33), Mo (0.016), N (0.025 8)	0.45	0.44 ± 0.02	0.45 ± 0.01
JSS 175-3, carbon steel	C (0.038), V (0.093), Al (0.054), B (0.009 1), Zr (0.031), Sb (0.019 6), Nb (0.011)	0.011	0.010 ± 0.002	0.010 ± 0.001
Stainless steel	—	—	0.24 ± 0.01	0.24 ± 0.02
Carbon steel	—	—	0.037 ± 0.001	0.038 ± 0.001
$NiCl_2.6H_2O$	—	—	0.001 2 ± 0.000 3	0.001 0 ± 0.000 2

* Average value of five individual determinations.

sensitivity were calculated to be 3.3×10^3 l mol^{-1} cm^{-1} and 0.017 8 μg cm^{-2}, respectively. Ten replicate analyses of a sample solution containing 80 μg of cobalt gave a mean absorbance of 0.449 with a relative standard deviation of 0.9%.

Effect of Diverse Ions

Sample solutions containing 80 μg of cobalt and diverse metal ions or alkali metal salts were prepared and the determination of cobalt was studied. The pH of the solution was adjusted to 5.5 and the procedure was applied. The tolerance limits of diverse ions on the system are summarised in Table 1 (error 2%). Large amounts of alkali metal salts, NaI, KNO_3, $NaClO_4$, NaCl and $CH_3COONa.3H_2O$, did not interfere; only EDTA interfered considerably. Most metal ions did not interfere when present in the same amount as cobalt. However, small amounts of iron(III) and bismuth(III) interfered in the determination. Hence in practical samples these must be completely eliminated by preliminary extraction with organic solvents.

Determination of Cobalt in Steels

The proposed method has been successfully applied to the determination of cobalt in reference materials (steels) and practical samples. The results (Table 2) are in reasonable agreement with those obtained by the conventional 2-nitroso-1-naphthol - benzene extraction procedure.[14] For the analysis of steel samples, a 1–2 g mass of steel was completely dissolved in 20–40 ml of hydrochloric acid (1 + 1) on a water-bath and then 3–5 ml of 30% hydrogen peroxide were added. The excess of hydrogen peroxide was decomposed by heating the solution on a water-bath. This mixture was cooled and filtered through a filter-paper. The filtrate was diluted to 100 ml with water. A few millilitres of this sample were taken in a separating funnel and about 20 ml of 2 N hydrochloric acid were added. The iron(III) in this sample was completely removed by extracting with three 25-ml portions of a 5% xylene solution of trioctylamine.[15] The aqueous solution was evaporated to 1–2 ml on a hot-plate at 60 °C. This was diluted with 20 ml of 8 N hydrochloric acid and the cobalt was extracted by two 20-ml portions of a 5% xylene solution of trioctylamine. Cobalt was then back-extracted from the organic phase with two 20-ml portions of 0.5 N nitric acid. The aqueous phase was evaporated to about 10 ml and the proposed method was applied.

Generally, non-aqueous organic solvents are soluble in water to a certain degree and the interfacial separation between water and organic solvent becomes incomplete owing to the formation of an emulsion on shaking. This causes an error in the determination of metals owing to the change in the volume of organic to aqueous phase, whereas naphthalene is solid, completely immiscible with water and hence completely separated from the aqueous phase. Also, the system described causes an emulsion to form between water and organic solvent and the ternary complex cannot be extracted into a non-aqueous solvent.

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Application of the Copper - Cadion 2B - Triton X-100 System to the Spectrophotometric Determination of Micro-amounts of Cyanide in Waste Water

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A sensitive colour reaction of copper with Cadion 2B in the presence of Triton X-100 and its application to the spectrophotometric determination of cyanide in waste water are described.

Keywords: *Cyanide determination; Cadion 2B; spectrophotometry; waste water*

Cadion 2B (4-nitronaphthyldiazoaminoazobenzene) forms coloured complexes with a number of metal ions in weakly acidic and alkaline solution[1] and has been used as a reagent for spot tests of cadmium, magnesium[2] and mercury.[1] In the last few years, it has also been employed as a chromogenic agent in the spectrophotometric determination of cadmium,[3] mercury,[4,5] palladium[6] and silver.[7] During studies on the spectrophotometric determination of silver, we noticed that at pH 9.2 and in the presence of the non-ionic surfactant Triton X-100, Cadion 2B forms a red - violet complex with copper. The complex has an absorption maximum at 535 nm and the molar absorptivity at this wavelength is 1.31×10^5 l mol^{-1} cm^{-1}. We also observed that the development of the colour of copper was apparently suppressed by cyanide and the decrease in absorbance of the copper complex was directly related to the cyanide concentration.

This work was undertaken to establish optimum conditions for the colour reaction of copper with Cadion 2B in the presence of Triton X-100 and to develop a method of determining cyanide in industrial waste water. The advantages of this method in comparison with current spectrophotometric methods for determining cyanide with pyridine - pyrazolone and isonicotinic acid - pyrazolone are the broad pH range of colour development, the high stability of the coloured solution, the lack of toxicity of the reagents and the good precision and accuracy.

Experimental

Apparatus

A Zeiss Specord double-beam automatically recording UV - visible spectrophotometer was used for measuring absorbances in glass cells of 1-cm path length. A Leici Model 25 pH meter equipped with a combined electrode was used for measuring pH.

Reagents

All reagents were of analytical-reagent grade and distilled water was used.

Standard cyanide solution, 1 mg ml^{-1}. Dissolve 0.250 3 g of potassium cyanide in distilled water and dilute with distilled water to 100 ml in a calibrated flask. Store the solution in a polyethylene bottle. This solution is unstable and should be standardised titrimetrically with silver nitrate. Prepare a 10.0 μg ml^{-1} solution by dilution with distilled water.

Standard copper solution, 1 mg ml^{-1}. Dissolve 0.1000 g of pure copper wire (99.99%) in a small volume of dilute nitric acid and dilute to 100 ml with distilled water in a calibrated flask. Prepare a 10.0 μg ml^{-1} solution by dilution with distilled water.

Cadion 2B solution, 0.05% *m/V in ethanol.* Obtained from Beijing Chemical Works, China.

Triton X-100 solution, 5% *V/V.*

Sodium tetraborate(III) solution, 5% *m/V.*

Recommended Procedures

Procedure for investigating the colour reaction of copper

Pipette an aliquot of standard copper solution containing not more than 10.0 μg of copper into a 25-ml calibrated flask and, with swirling, add 3 ml of sodium tetraborate(III) solution (pH 9.2), 1 ml of 5% Triton X-100 and 1.2 ml of 0.05% Cadion 2B solution. Dilute to volume with distilled water and mix well. Measure the absorbance at 535 nm in a 1-cm cell against a reagent blank after 20 min.

Procedure for determination of cyanide

Transfer 0, 10.0 and 10.0 μg of copper and 0, 0 and 4.0 μg of cyanide into three 25-ml calibrated flasks and to each add 3 ml of sodium tetraborate(III) solution (pH 9.2), 1 ml of 5% Triton X-100 and 1.2 ml of 0.05% Cadion 2B solution. Measure the absorbances of two solutions, one of which contains copper and the other copper and cyanide, in a 1-cm cell at 535 nm against a reagent blank without copper and cyanide, and calculate the difference in absorbance (ΔA) of the coloured solutions.

Procedure for determination of cyanide in industrial waste water

Adjust the pH of the waste water sample to above 11, and add powdered lead carbonate a little at a time, with stirring, until the black lead sulphide precipitate no longer appears. Centrifuge the mixture, transfer the clear supernatant liquid into a 1 000-ml distillation flask and add distilled water to give a volume of about 500 ml. Add 3 drops of 0.1% methyl orange and 2 g of tartaric acid, the solution now changing colour from yellow to red. Then distil the solution immediately at a distillation rate of 4–5 ml min^{-1}, the distillate containing hydrogen cyanide being absorbed in 5 ml of 0.25 N sodium hydroxide solution. Continue to distil until 45 ml of distillate have been collected and finally add distilled water to give a volume of 50 ml. Pipette an aliquot of the sample solution and carry out the cyanide determination as outlined above.

Results and Discussion

Studies on the Copper - Cadion 2B - Triton X-100 System

Absorption spectra

At pH 9.2 in the presence of the non-ionic surfactant Triton X-100, the absorption spectra of Cadion 2B and its copper

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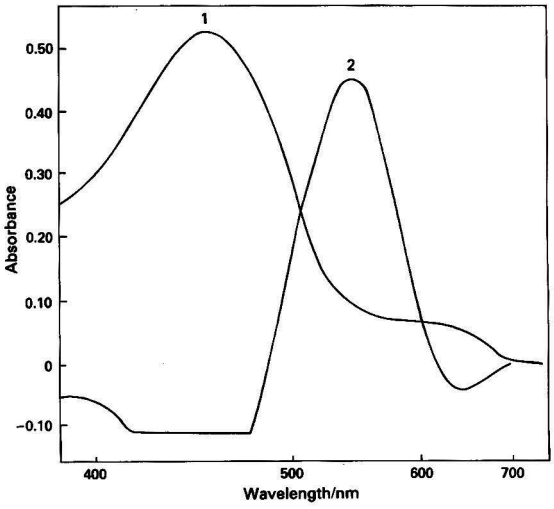

Fig. 1. Absorption spectra of Cadion 2B and its copper complex at pH 9.2, with a 1-cm cell. 1, 1.6×10^{-5} M Cadion 2B against solvent; 2, 4.0×10^{-6} M copper and 7.5×10^{-5} M Cadion 2B against reagent blank

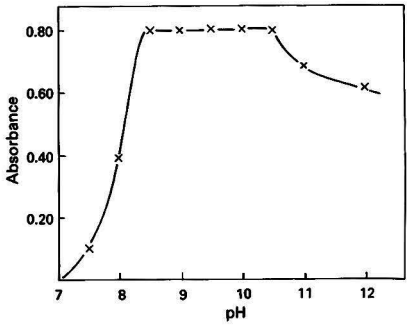

Fig. 2. Effect of pH on absorbance. Copper taken, 10 μg

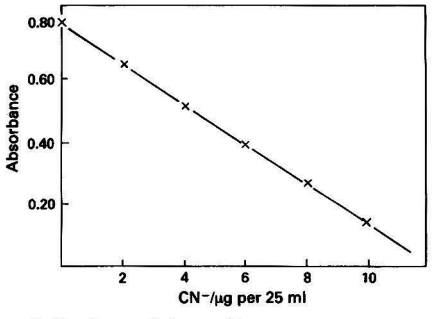

Fig. 3. Calibration graph for cyanide

Table 1. Results of recovery of added standard cyanide

Standard cyanide added/μg	Cyanide found/ μg*	Recovery, %
10.0	10.1	101
20.0	20.0	100
50.0	49.2	98.4
100.0	97.0	97.0

* Each value is the average of three determinations.

complex are as shown in Fig. 1. The maximum absorbance of the reagent was at 445 nm and that of the red - violet complex of copper at 535 nm. Therefore, all subsequent measurements were made at this wavelength.

Effect of pH

Buffer solutions of sodium tetraborate(III) - hydrochloric acid (or sodium hydroxide) were used and their pH values were measured with a pH meter. The optimum pH range for use with the copper complex (Fig. 2) was 8.5–10.5. Therefore, all studies were carried out at pH 9.2.

Effect of Cadion 2B concentration

At least 0.75 ml of 0.05% ethanolic Cadion 2B solution was found to be necessary for the full development of colour with 10.0 μg of copper per 25 ml of the aqueous solution. The colour remained constant with the addition of up to 3 ml of the reagent. Hence, 1.2 ml of the reagent were used.

Effect of Triton X-100 concentration

Cadion 2B is hardly soluble in pH 9.2 buffer solution and copper therefore does not form a coloured complex with this reagent. However, in 25 ml of test solution containing more than 0.5 ml of Triton X-100, a red - violet complex was formed quickly, and maximum colour formation with constant absorbance was attained when 5% Triton X-100 was added in the range 0.5–5 ml. Therefore, 1 ml of 5% Triton X-100 was the preferred additive.

Effect of times and stability of the complex

The formation of the coloured complex of copper with Cadion 2B in the presence of Triton X-100 occurs within 1 min. Maximum colour development is attained within 5 min and the coloured solution is stable for at least 24 h.

Adherence to Beer's law

A series of standard copper solutions was prepared and the absorbance of each was measured at 535 nm in a 1-cm cell. Beer's law was obeyed over the range 0–12 μg of copper per 25 ml. The average molar absorptivity of the complex was 1.31×10^5 l mol^{-1} cm^{-1} and the Sandell sensitivity (based on an absorbance of 0.001 unit) of the colour reaction was 4.85×10^{-4} μg cm^{-2}. Thus, Cadion 2B is considered to be one of the most sensitive chromogenic reagents for copper.

Table 2. Analytical results for some waste water samples

Sample No.	Source	Cyanide content, p.p.m.	Average value, p.p.m.	Relative standard deviation, %
1	Contaminated river water	0.016, 0.016, 0.014, 0.016	0.016	7.21
2	Contaminated river water	0.006 1, 0.006 0, 0.006 2, 0.006 0	0.006 1	1.64
3	Carbonisation factory	4.00, 3.90, 4.16, 4.00	4.02	2.48
4	Carbonisation factory	76.8, 80.0, 76.8, 80.0, 84.0, 76.8, 84.0, 76.8	79.2	4.28
5	Machinery electroplating workshop	51.2, 51.2, 50.0, 51.2, 51.2, 51.2, 51.2, 51.2	51.2	0.88
6	Meter electroplating factory	24.6, 24.6, 25.2, 25.2, 25.5, 24.0, 24.6, 24.6	24.8	1.81

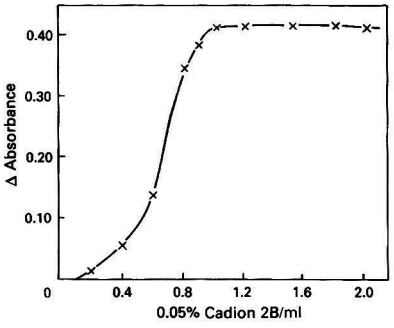

Fig. 4. Effect of Cadion 2B concentration. Cyanide taken, 6.0 μg

Table 3. Comparison of the proposed method with the current method

Sample No.	Cyanide content, p.p.m. This work	Cyanide content, p.p.m. Isonicotinic acid - pyrazolone method
1	0.016	0.018
3	4.02	3.93
5	51.2	54.1
6	24.8	25.5

Optimum Conditions for the Spectrophotometric Determination of Cyanide

Correlation between the decrease in absorbance and the concentration of cyanide

Cyanide forms a very stable complex ion with copper and suppresses the formation of the red - violet complex of copper with Cadion 2B in the presence of Triton X-100. Fig. 3 shows that, using the procedure for the determination of cyanide, the decrease in absorbance of the coloured complex is proportional to the concentration of cyanide in the range 0–10.0 μg per 25 ml.

Effect of Cadion 2B concentration

The reactions of copper with Cadion 2B and cyanide with copper are competitive, so that the amount of Cadion 2B used may affect these two competing reactions. Fig. 4 shows that, using the procedure for determining cyanide and varying the amount of Cadion 2B, the addition of 1.0–2.0 ml of 0.05% Cadion 2B solution in determining 6.0 μg of cyanide, the ΔA value obtained is a maximum and constant. If the amount of Cadion 2B solution added is less than 1.0 ml, then the ΔA value obtained is significantly lower.

Effect of order of addition of reagents

It was verified experimentally that the order of addition of the reagents is not important. The following two sequences of addition of the reagents gave identical results: CN^- + Cu^{2+} + Triton X-100 + Cadion 2B and Cu^{2+} + Cadion 2B + Triton X-100 + CN^-. In the former instance, the copper remaining after complexation with cyanide reacts with Cadion 2B to give the coloured complex, whereas in the latter instance, cyanide displaces copper from the copper complex with decreasing absorbance of the coloured complex. Hence, the order of addition of the reagents indicated that the complexing action of copper with cyanide is much stronger than that of copper with Cadion 2B.

Stability of the Coloured Systems

It was shown that the two coloured systems of Cu^{2+} - CN^- - Cadion 2B - Triton X-100 and Cu^{2+} - Cadion 2B - Triton X-100 are stable for 24 h.

Precision and Accuracy

Using the procedure for the determination of cyanide in waste water, the recovery of standard cyanide added to four alkaline solutions was in the range 97.0–101%, as shown in Table 1. Some results for six samples of waste water are shown in Table 2, and indicate that the method is reproducible. The recoveries of standard cyanide added to water samples were in the range 95.0–100%.

The results of this method are in good agreement with those of the isonicotinic acid - pyrazolone method (see Table 3).

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Collaborative Study of a Method for the Determination of Residues of Halofuginone in Chicken Tissue

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A procedure is described for the determination of halofuginone residues in chicken tissues. The halofuginone is extracted as the free base with ethyl acetate after digestion of the tissues with trypsin and then partitioned into an aqueous ammonium acetate buffer. After further clean-up and concentration using a Sep-Pak C_{18} cartridge, the extract is examined by high-performance liquid chromatography using a reversed-phase column and a UV detector. The procedure was tested by carrying out procedural recoveries from spiked samples and also by a collaborative exercise using samples of tissues from birds fed on a diet containing halofuginone. The efficiency of the extraction procedure was assessed by using samples of a chicken that had been fed with ^{14}C-labelled halofuginone.

Keywords: *Halofuginone determination; chicken tissues; high-performance liquid chromatography; collaborative studies*

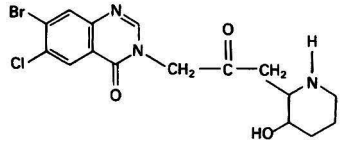
The Analytical Methods Committee has received and approved for publication the following report from its Veterinary Residues in Fresh Meat Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was Dr. J. F. C. Tyler (Chairman), Mr. J. B. Aldred, Mr. A. Anderson, Mr. P. M. Brown, Dr. N. T. Crosby, Mr. J. Ganley, Mr. H. L. Hatfield (from October 1982), Mr. A. Hobson-Frohock, Mr. A. F. Lott, Mr. A. F. Machin, Mr. R. Ryden, Dr. G. Shearer, Mr. G. M. Telling and Mr. J. J. Wilson as Secretary.

Introduction

Halofuginone, (±)-*trans*-7-bromo-6-chloro-3-[3-(3-hydroxy-2-piperidyl)acetonyl]quinazolin-4(3*H*)-one, has the structure



It is used as an aid in the prevention of coccidiosis in broiler chickens and is added to feeds at a final concentration of 3 mg kg^{-1}. It is the principal ingredient of proprietary mixtures marketed under the name of Stenorol (Hoechst). Medicated feed is administered continuously to broilers until 5 d before slaughter. Halofuginone is added to feeds as hydrobromide and thoughout this report halofuginone means the hydrobromide unless stated otherwise. The Chemical Abstract Registry Numbers are: halofuginone hydrobromide 64924-67-0 and halofuginone (free base) 55837-20-2. The factor for converting the hydrobromide into the free base is 0.8361.

The Sub-Committee was charged with the task of developing and evaluating a method for the determination of halofuginone in chicken tissues (liver, kidney, skin, fat and breast muscle) that would determine levels down to at least 0.1 mg kg^{-1}. A method developed by a member of the Sub-Committee from the Huntingdon Research Centre (HRC) was made available to the Sub-Committee. In this procedure,[1] halofuginone is extracted from the macerated tissue as the free base with ethyl acetate and subsequently partitioned into aqueous acetate buffer solution. Further clean-up of the extract is achieved by using a Sep-Pak C_{18} cartridge prior to final determination by high-performance liquid chromatography (HPLC) on a reversed-phase column with a UV detector.

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Experimental

During the development of the method the Huntingdon Research Centre determined the distribution of halofuginone in the tissues and the efficacy of the extraction from a radio-labelling experiment. A single dose of 5 mg of [^{14}C]-halofuginone per kg of body mass with a specific activity of 3.5×10^8 Bq g^{-1} was administered orally to a chicken, which was slaughtered 48 h later. The data they obtained from kidney and liver tissues was as follows. Unchanged halofuginone (mg kg^{-1}) in tissues from radioactivity measurements: 2.0 and 2.2 for kidney and 4.6 and 4.9 for liver; and halofuginone in tissues (mg kg^{-1}) from HPLC measurements: 1.9 and 2.0 for kidney and 3.8 and 4.2 for liver. This shows that 93% of all radioactivity is extracted from kidney by using ethyl acetate and 84% is similarly extracted from liver.

In developing the analytical method, HRC[1] obtained the results shown in Table 1.

The method was then examined by members of the Sub-Committee. As a result of this work a number of minor modifications were suggested. In particular, it was found to be essential to ensure that the pH did not rise too high for too long and to remove all residual ethyl acetate from the ammonium acetate buffer solution. The suggested amendments have been incorporated into the procedure described in the Appendix.

Table 1. Determination of halofuginone in chicken tissue

Tissue	Fortification range/ mg kg^{-1}	No. of determinations	Mean procedural recovery, %	Recovery range, %	Coefficient of variation, %
Liver	0.015–1.03	18	81.0	68–93	15.5
Kidney	0.015–1.03	17	84.3	69–100	10.2
Breast muscle	0.03	7	98.7	74–117	17.3
Skin and fat	0.03	10	87.3	72–104	10.8

* One recovery was obtained at 124% (0.03 mg kg^{-1}).

Table 2. Determination of halofuginone in chicken tissue—preliminary experiments

Laboratory	Tissue	Fortification level/ mg kg^{-1}	Number of determinations	Mean recovery, %	Relative standard deviation, %	Coefficient of variation, %
A	Muscle	1.0	2	95	—	—
	Muscle	0.1	5	87	3	3
B	Muscle	0.1	4	80	4	5
C	Muscle	0.1	10	66	10	15
	Liver	0.1	7	67	11	17
D	Muscle	0.01	6	82	5	6
E	Muscle	0.1	2	70	—	—
	Liver	0.1	2	55	—	—
Mean (muscle)				80		
Range				66–95		

Table 3. Recovery of halofuginone in chicken tissue—collaborative trial

Laboratory	Halofuginone recovered from standard solution,* %	Halofuginone recovered from fortified tissue†, %			
		Liver	Kidney	Muscle	Fat
1	—	75	75	85	—
2	68, 75	67, 79	92, 88	75, 93	75, 76
3	99, 100	64, 73	66, 66	90, 91	68, 53
4	—	82, 86	—	72, 78	83, 71
5	52, 68	36, 56, 44	45	82, 68, 62	48, 68
6	84, 70	73, 89	—	88, 88	76, 77
7	54, 54, 44, 39, 54, 64, 62, 74	54, 72	55, 39	39, 56	54, 37
8	83, 84	85	75	81	67
Mean	68	69	67	77	66
Range	39–100	36–89	39–92	39–93	37–83

* Fortification level, 2 μg. † Fortification level, 0.1 mg kg^{-1} for all tissue.

Table 4. Halofuginone in chicken tissue trial—blank values

Laboratory	Halofuginone found in untreated tissue from locally purchased birds/mg kg^{-1}			
	Liver	Kidney	Muscle	Fat
1	—	—	—	—
2	—	0.01	0.01	0.01
3	N.d.*	N.d.	N.d.	N.d.
4	—	—	—	—
5†	0.25	0.12	N.d.	0.17
	0.38		0.20	0.13
	0.40		N.d.	
6	N.d.	N.d.	N.d.	N.d.
7	0.08, 0.08	N.d.	N.d.	N.d.
	N.d.			
8†	N.d.	N.d.	N.d.	N.d.

* N.d., not detected.
† Locally reared birds.

Following these preliminary studies in which members had acquired some experience with the method, a collaborative study was initiated. The Sub-Committee readily accepted the offer made by HRC to provide samples of chicken tissue from a fast growing strain of birds (Ross I), fed for 8 weeks on a feed medicated with 3 mg kg^{-1} of halofuginone and slaughtered on June 10th 1982 with no withdrawal period. Samples of muscle, liver, kidney and fat were homogenised and divided into 12 portions. These were packaged and, with sufficient dry ice to keep them frozen for 24 h, were distributed by hand to members of the Sub-Committee. On receipt in individual laboratories, the test samples were stored at −18 °C until analysis. Unfortunately, no chickens reared on a halofuginone-free diet were available. Members therefore used locally purchased birds for "blank" values and procedural recovery tests. The halofuginone content of the trial samples was unknown, although from the experience of earlier feeding trials it was expected to be in the region of 0.04 mg kg^{-1} for muscle tissue and as high as 1.0 mg kg^{-1} in liver. In the event, the levels found were considerably lower. Analysis of the samples was carried out over the period of June 1982 to January 1983. A further set of samples was kept at −18 °C and analysed approximately 1 year later to check on possible loss of halofuginone during storage.

Collaborative Study

The agreed programme of work is described. All samples were analysed in duplicate on separate days. Duplicate injections were made for each analysis. Sample masses of 20 g were taken, except for kidney where there was only sufficient material for 10 g per analysis. Samples examined were as follows: 1, 2.00 μg of halofuginone hydrobromide standard in solution; 2, samples of muscle, liver, kidney and fat from locally purchased chickens as a check on blank values; 3, samples of 2 above fortified with halofuginone to a level of 0.1 mg kg^{-1}; and 4, samples of chicken tissue supplied by the Huntingdon Research Centre.

Results and Discussion

The initial evaluation of the method by members of the Sub-Committee is shown in Table 2. Recoveries were generally thought to be satisfactory, although a number of difficulties were encountered and several members decided to carry out further experimental work before starting on the collaborative exercise.

Some laboratories reported that the efficiency of their HPLC columns was below the minimum specified in the method (*i.e.*, 2 000 theoretical plates, expressed with respect to halofuginone). Frequently, deterioration in column performance occurred during the exercise leading in one instance to a column with only 500 plates. Nevertheless, the separation

Table 5. Halofuginone in chicken tissue—feeding trial results

Laboratory	Date of analysis	Halofuginone found/mg kg^{-1} Liver	Kidney	Muscle	Fat
1	June 82	0.24, 0.22	0.16, 0.13	0.02, 0.02	0.02, 0.02
		0.23, 0.21	0.16, 0.12	0.02, 0.02	0.01, 0.01
		0.21, 0.22	0.14, 0.14	0.02, 0.02	0.02, 0.02
2	July 82	0.17, 0.16	0.12, 0.11	0.01, 0.02	0.01, 0.01
3	Aug/Sept 82	0.13, 0.18	0.11, 0.10	0.01, N.d.	
	June 1983	0.14, 0.19	0.11, 0.11	0.02, N.d.	0.02, N.d.
4	Sept 82	0.19, 0.19	0.11, 0.11	0.03, 0.01	0.02, N.d.
		0.19	0.15, 0.15	0.01	N.d.
5	July/Aug 82	0.14, 0.18	0.16, 0.19	0.02, 0.02	0.01, 0.01
6	Oct 82	0.18, 0.17	0.12, 0.11	0.02, 0.03	0.02, 0.02
			0.13		
7	Oct 82	0.14, 0.15	0.08, 0.14	0.01	N.d.
8	Dec/Jan 82/3	0.08, 0.11	0.11, 0.09	N.d., 0.01	N.d.
		0.10	0.11	0.01	
Mean		0.17	0.13	0.01	0.01
Range		0.08–0.24	0.08–0.19	N.d.–0.03	N.d.–0.02
Ratio (to level found in liver)		1.00	0.74	0.08	0.06

* N.d., not detected.

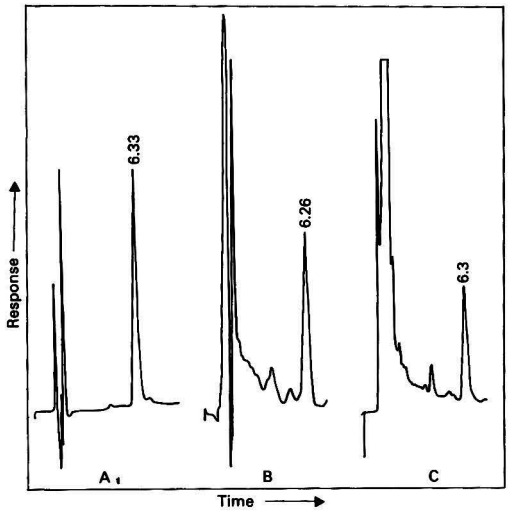

Fig. 1. Typical chromatograms for halofuginone in chicken tissue. A, standard (5 μg ml^{-1}); B, kidney (0.11 mg kg^{-1}, 0.02 a.u.f.s.); and C, liver (0.17 mg kg^{-1}, 0.04 a.u.f.s.)

achieved with these columns was generally adequate and no interference from co-extractives was observed. A typical chromatogram is shown in Fig. 1. Columns other than a Waters Associates μBondapak C_{18} were operated successfully and one laboratory used a paired-ion separation mode. Some variation in response factor was observed both from day to day and even within the same day.

The recovery of halofuginone from samples of locally purchased chickens fortified to a level of 0.1 mg kg^{-1} is summarised in Table 3. Although these are slightly lower than expected on the basis of the preliminary studies (Table 2), they are thought to be satisfactory at a level of 0.1 mg kg^{-1}. Of greater concern, is the wide range of mean values encountered. However, the over-all aim of the work of the Sub-Committee is to develop methods of analysis that will allow the determination of residues present in different tissues. Halofuginone residues, especially at levels below 0.1 mg kg^{-1}, will vary widely from one animal to another and will be dependent on feeding history and other factors. Hence, mean recovery values in the range 64–74% (Table 3) are considered to be satisfactory for this purpose. Perez[2] has stated that for FDA purposes an assay is considered satisfactory if measurements fall within the range 60–110% of the true value and a minimum recovery of 60% is required for residues at the μg kg^{-1} level.

As can be seen from Table 4, high and variable blank values were reported by laboratory 5. No rational explanation has been found for these results, although the birds were experimental animals and not purchased from local shops. All possible sources of contamination were examined without success.

The results of determinations on the feeding trial samples of chicken tissues supplied by HRC are shown in Table 5. Clearly, all values reported for liver, kidney and muscle are very much lower than the levels expected from the incorporation of 3 mg kg^{-1} in the feed. There is no explanation for this discrepancy, but the levels found in the different tissues are in the expected ratios. In view of the high variability of the method at these low levels of determination, no statistical analysis of the results has been carried out except as shown in Table 5. However, the coefficient of variation found (3–17%, Table 2) compares favourably with the values reported by Horwitz *et al.*[3] for collaborative studies at the 0.1 mg kg^{-1} level. The Sub-Committee believes the lower limit of the determination is best judged from a comparison of the results on the untreated tissues in Table 4 with those of the treated tissues in Table 3 and that it is about 0.01 mg kg^{-1}.

The values for halofuginone residues in the set of samples stored at −18 °C for approximately 1 year were not significantly different, as shown in Table 5 under laboratory 3.

Although it was not possible to carry out a final collaborative trial with circulated samples containing a known level of halofuginone, the procedure adopted by the Sub-Committee in testing tissues obtained from chickens, fed a diet containing levels of the coccidiostat used in practice, is considered superior, as it provides a more realistic test of the method. Very high dosage levels would have been required to secure more easily measurable levels in the fat and muscle tissues in particular. Further, the preparation and distribution of such tissues and subsequent fortification with known amounts of halofuginone could also present difficulties unconnected with the method. Hence, the approach adopted was thought to be a more suitable test of the problems likely to be encountered in practice in that material added *in vivo* is more difficult to recover than material added *in vitro*.

Conclusion

The method given in the Appendix is satisfactory for the determination of halofuginone in chicken tissues and is recommended by the Sub-Committee.

APPENDIX

Determination of Halofuginone Residues in Chicken Tissue

Principle

Halofuginone is extracted as the free base with ethyl acetate and then partitioned into aqueous buffer. After further clean-up and concentration using a Sep-Pak C_{18} cartridge, the extract is examined by high-performance liquid chromatography using a reversed-phase column and a UV detector.

Reagents

Acetic acid, glacial.

Acetonitrile. HPLC grade.

Ammonium acetate.

Ammonium acetate buffer solution, 0.25 M, *pH* 4.3. Dissolve 19.27 g of ammonium acetate (analytical-reagent grade) and 30 ml of glacial acetic acid in water and dilute to 1 l.

Ammonium acetate buffer solution, 0.125 M, *pH* 4.3. Dilute 500 ml of 0.25 M ammonium acetate buffer solution to 1 l with water.

Ethyl acetate. HPLC grade.

Halofuginone hydrobromide. Available from Roussel Uclaf, Paris.

Halofuginone standard solutions. Dissolve 50.0 mg of halofuginone hydrobromide in ammonium acetate buffer solution (0.25 M, pH 4.3) and dilute to volume in a 250-ml calibrated flask. This stock solution is stable for 3 weeks at 5 °C. Dilute the stock standard solution with HPLC mobile phase to provide calibration solutions in the range 0–5 μg halofuginone hydrobromide per millilitre as required.

Methanol. HPLC grade.

Mobile phase, acetonitrile - acetate buffer (0.25 M) *- water,* 5 + 3 + 12. Adjust to pH 4.3 with acetic acid after mixing.

Salt-saturated sodium carbonate solution, 5% *m/V.*

Sep-Pak C_{18} cartridge. Waters Associates Ltd., Northwich, Cheshire or one of equivalent performance. Wash the cartridge sequentially with 2 ml of methanol and 5 ml of water before use.

Sodium chloride.

Sodium carbonate, anhydrous. Analytical-reagent grade.

Sodium carbonate solution, 10% *m/V.*

Trypsin. Laboratory-reagent grade, Fisons Ltd., or similar.

Apparatus

Centrifuge. Operating at 2000 rev min^{-1} and capable of accepting 200-ml bottles.

Liquid chromatograph. Waters Associates Ltd. 6000A pump in conjunction with a UV detector, or any equivalent system.

Liquid chromatographic column. Waters Associates Ltd. μBondapak C_{18}, 30 cm × 3.9 mm i.d. or equivalent. A column of about 2000 theoretical plates is required.

Liquid chromatograph operating conditions. These are as follows: flow-rate, 2 ml min^{-1}; injection volume, 40–100 μl; detector wavelength, 243 nm; and temperature, ambient.

Under these conditions, the retention volume of halofuginone is approximately 14 ml, corresponding to a retention time of 7 min.

Macerator.

Recorder/integrator.

Rotary film evaporator.

Water-bath.

Procedure

Extraction

Add 0.5 g of trypsin and 10 ml of water to 20 g of homogenised tissue in a centrifuge bottle. Mix and adjust the pH to between 7 and 8 by the dropwise addition of sodium carbonate solution. Incubate for 3 h in a water-bath at 40 °C. Cool. Add 10 ml of sodium carbonate solution and 100 ml of ethyl acetate. Macerate for 3 min without allowing the temperature to rise; cool the container in ice - water if necessary. Centrifuge for 2 min and decant the ethyl acetate phase into a 500-ml separating funnel. A dropping pipette may be required to remove the last trace amounts of ethyl acetate. Add a further 100 ml of ethyl acetate to the residue and macerate for 3 min as before. Centrifuge and add the ethyl acetate phase by decantation to the first extract. Wash the combined extracts for 1 min with salt-saturated sodium carbonate solution and discard the aqueous layer.

Caution—During the above operations, halofuginone (as the free base) should not remain in the ethyl acetate phase for more than 30 min.

Extract the ethyl acetate layer for 1 min with 50 ml of ammonium acetate buffer solution (0.125 M) and run the lower layer into a 250-ml separating funnel. Re-extract the ethyl acetate with a second 50-ml portion of ammonium acetate buffer solution (0.125 M) and combine the aqueous extracts. Wash the combined buffer extracts with 10 ml of ethyl acetate by gentle swirling. Avoid vigorous shaking in order to prevent emulsion formation. Transfer the aqueous layer quantitatively into a round-bottomed flask and discard the organic layer. Evaporate the ethyl acetate from the buffer solution under reduced pressure until the solution clears. The smell of ethyl acetate may persist in the buffer solution after removal from the rotary evaporator. Excessive evaporation should be avoided. A pressure below 20 Torr and a temperature below 40 °C are recommended. Quantitatively transfer the remaining aqueous solution into a 100-ml calibrated flask, dilute to volume with ammonium acetate buffer solution (0.125 M) and mix. Filter through a Whatman glass-fibre filter-paper, discarding the first few millilitres of filtrate. Fit a prepared Sep-Pak cartridge to the end of a 10-ml glass syringe. Pass 10 ml of ammonium acetate buffer extract through the cartridge. Repeat using 2 further 10-ml portions of extract. Wash the cartridge with 3 ml of water and discard the washings. Blow air through the cartridge to remove the last trace amount of water. Elute halofuginone from the cartridge with 10 ml of methanol and collect in a 50-ml pear-shaped flask. Evaporate to dryness on a rotary evaporator, dissolve the residue in 0.5 ml of mobile phase, mix and take suitable aliquots for liquid chromatography.

Calculation of Results

Construct a calibration graph of chromatographic peak height or area *versus* halofuginone concentration. For each injection of extract, calculate the peak height or area of the peak at the characteristic retention time for halofuginone and by interpolation from the calibration graph, the concentration of halofuginone in the extract. Hence, calculate the concentration of halofuginone in the original sample.

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SHORT PAPERS

Measurement of Phenol in Urine Using a High-performance Liquid Chromatographic Method

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Keywords: *Phenol determination; urine analysis; high-performance liquid chromatography; exposure monitoring*

Following exposure to either benzene or phenol, phenol is excreted in human urine as the sulphate and glucuronic acid conjugates. Occupational exposure to these compounds can be monitored by measurement of the concentrations of free and conjugated urinary phenol. The Van Haaften and Sie method[1] involves injection of a 1 + 1 mixture of urine sample and phosphoric acid into a gas - liquid chromatograph. Phenol conjugates are hydrolysed on a coarse glass powder pre-column and the phenol is then separated from other urinary components (*e.g.*, cresols) on an analytical column with subsequent flame-ionisation detection. The limit of detection of the method is 1–2 p.p.m. of phenol in urine (equivalent to a daily excretion of 2–3 mg of phenol). Baldwin *et al.*[2] have suggested the introduction of safeguards into the above procedure to ensure that it is working correctly. These modifications, however, do not overcome the difficulty of the easily contaminated glass powder pre-columns, which need regular checking and renewing.

The uncertainty of the method of Van Haaften and Sie led to the investigation of a method involving enzymatic hydrolysis followed by headspace gas chromatography.[3] This approach, however, although very reliable, was limited by the long elapsed time required for complete hydrolysis. This work describes an investigation into an alternative high-performance liquid chromatographic (HPLC) method for the measurement of urinary phenol.

Experimental

Chemicals

Phenyl β-D-glucuronide monohydrate. Obtained from Koch-Light Laboratories.

Phenol. Minimum purity 99%, obtained from Hopkins and Williams Ltd.

Phenyl sulphate, potassium salt. Prepared according to Feigenbaum and Neuberg.[4]

Dibutyl ether. Laboratory grade, obtained from Fisons Scientific Apparatus.

Concentrated hydrochloric acid. AnalaR grade, obtained from BDH Chemicals.

Chloroform. AnalaR grade, obtained from BDH Chemicals.

Apparatus

Normal-phase HPLC was performed at ambient temperature using a Pye Unicam Model PU4010 pulse-free pump and a Model PU4020 variable-wavelength ultraviolet spectrophotometric detector operated at 265 nm. The analytical column, 12.5 cm × 5 mm i.d. stainless steel, was packed with Spherisorb NH_2, 5 μm (Phase Separations). Injection of samples and standard solutions on to the column was via a Rheodyne Model 7125 injection valve with an external 20-μl loop. The mobile phase was 2% *V/V* propan-2-ol in hexane maintained at a flow-rate of 2 ml min^{-1}. Under these conditions the phenol had a retention time of 10 min. Quantitation was effected using peak-height measurement against external standards.

Human Urine Samples

Plant worker urines were provided by Stanlow Medical Centre. Human urines for control and recovery analyses were provided by staff in the Environmental Analysis and Industrial Hygiene groups in the Environmental and Biochemical Toxicology Division, Sittingbourne Research Centre.

Procedure

The basis of the method is hydrolysis of phenyl sulphate and phenyl glucuronide in urine to phenol and subsequent solvent extraction of the total free phenol (originally in free and conjugated forms) from hydrolysed urine. Separation of the phenol from other co-extracted compounds, *e.g.*, cresols, is achieved using normal-phase HPLC with UV detection.

The analytical procedure was applied to control human urine samples (to establish both a range of background urinary phenol levels and a limit of detection) and to control samples to which known amounts of phenol, phenyl sulphate and phenyl glucuronide had been added (to establish the recovery of the method for each compound and its stability in urine). The procedure was also applied to urine samples from plant workers exposed to benzene at a petrochemical plant, previously analysed by the current Van Haaften and Sie GLC method (to compare the two methods).

Results and Discussion

Hydrolysis of Phenol Conjugates to Phenol

Known amounts of the conjugates (phenyl sulphate or phenyl glucuronide, 20 μg ml^{-1}) were added to control urine (5 ml) and hydrolysed with concentrated hydrochloric acid (1 ml) in a water-bath at 100 °C. The conversion of each conjugate to phenol at intervals of 30 min was determined after extraction of the free phenol with dibutyl ether (5 ml) (Table 1).

The optimum period chosen for efficient hydrolysis of benzene metabolites is 1.5 h.

Extraction of Phenol from Hydrolysed Urine

Urines containing phenol, phenyl sulphate or phenyl glucuronide were hydrolysed (as above) for 1.5 h and the recovery of each compound, as phenol, was determined using different volumes of dibutyl ether. An increase in the volume of dibutyl ether used for the extraction from 5 to 10 ml (*i.e.*, solvent to urine ratio from 1 + 1 to 2 + 1) did not improve the extraction efficiency of phenol. When centrifugation was employed at a

Table 1. Effect of variation of the hydrolysis time on the recovery of benzene metabolites in urine

Metabolite	Urinary concentration/ μg ml^{-1}	Reaction time/h	Proportion of metabolite in urine extracted as phenol after hydrolysis, %
Phenyl sulphate . .	20	0.5	79
		1.0	84
		1.5	85
		2.0	83
Phenyl glucuronide . .	20	0.5	46
		1.0	61
		1.5	75
		2.0	76

Table 2. Stability of urinary phenol in control urine and in control urine fortified with individual metabolites at 4 °C

Concentration of phenol in control urine and fortified urine/μg ml^{-1}

Period of storage/d	With chloroform				Without chloroform			
	Control urine	Control urine fortified with			Control urine	Control urine fortified with		
		Phenol*	Phenyl sulphate†	Phenyl glucuronide‡		Phenol*	Phenyl sulphate†	Phenyl glucuronide‡
0	5	41	18	12	5	41	16	12
14	6	43	19	12	10	41	18	12
26	5	43	18	12	11	40	19	12
72	5	42	19	12	9	39	18	13

* Equivalent to 50 μg ml^{-1} of phenol, final concentration corrected for control figure.
† Equivalent to 23 μg ml^{-1} of phenol, final concentration corrected for control figure.
‡ Equivalent to 18 μg ml^{-1} of phenol, final concentration corrected for control figure.

Table 3. Comparison of urinary phenol analyses from plant workers by the HPLC and Van Haaften and Sie methods

Phenol content found/μg ml^{-1}	
HPLC method	Van Haaften and Sie "on-column" hydrolysis
19	17
1	<2
4	5
21	26
7	5
10	16
39	40
29	27
10	9
38	38

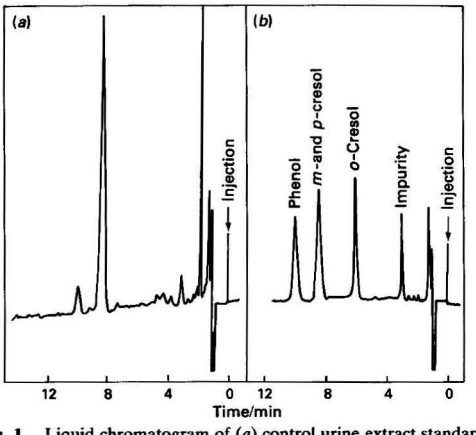

Fig. 1. Liquid chromatogram of (*a*) control urine extract standards and (*b*) phenol and *o*-, *m*- and *p*-cresol. Column: 12.5 cm × 5 mm i.d. Spherisorb NH_2 (5 μm). Mobile phase: 2% *V/V* propan-2-ol in hexane. Flow-rate: 2 ml min^{-1}. Detection: UV absorbance at 265 nm. Sample: (*a*) control urine extract (5 ml of urine ≡ 5 ml of dibutyl ether); (*b*) phenol, *o*-, *m*- and *p*-cresol in dibutyl ether (10 μg ml^{-1} each). Injection volume: 20 μl

solvent to urine ratio of 1 + 1 there was a slight improvement in the extraction of phenol. This extraction efficiency was noticeably enhanced (10% overall) when centrifugation was coupled with an increase in solvent volume to 10 ml.

Analysis of Control Human Urine and Recovery Experiments

Control human urine contained on average 3 μg ml^{-1} of phenol (range 1–6 μg ml^{-1}; No. of subjects = 6) after hydrolysis. The limit of detection for phenol was <0.5 μg ml^{-1} in urine (equivalent to a daily excretion of <1 mg). Control urine to which known amounts of phenol, phenyl sulphate or phenyl glucuronide had been added (ranging from 20 to 100 μg ml^{-1}, as phenol) were similarly analysed. From each of twelve samples the average recovery of phenol, phenyl sulphate and phenyl glucuronide, as phenol, was 91, 85 and 70%, respectively, and the relative standard deviations were 3.5, 2.9 and 4.2%, respectively. Typical HPLC traces of a control urine extract and phenol and cresol standards are shown in Fig. 1.

Stability of Free and Conjugated Phenol in Urine

Urine samples, without fortification and also fortified individually with phenol, phenyl sulphate or phenyl glucuronide (50 μg ml^{-1} each), were analysed on several occasions during storage at 4 °C. The results of the storage stability study over a period of 72 d are given in Table 2. The results for the fortified urines are corrected for the phenol content of control urine, which showed some variation when stored without chloroform preservative. There was no significant decrease in phenol, phenyl sulphate or phenyl glucuronide concentrations in urine with or without 1% *V/V* of chloroform preservative over the period of study, confirming that these metabolites are stable in urine. In view of the increase in the phenol content of control urine when stored without chloroform preservative, it is recommended that urine samples for phenol analysis should contain chloroform as a preservative and be stored at 4 °C.

Comparison of Urine Analyses from Plant Workers Using the HPLC and Modified Van Haaften and Sie Methods

Urine samples, previously analysed by the modified Van Haaften and Sie method at Stanlow Medical Centre, were re-analysed using the HPLC method at Sittingbourne Research Centre. A comparison of the two sets of results shown in Table 3 indicates that there is good agreement between the two methods.

Conclusions

The use of HPLC offers a reliable and sensitive analytical technique for urinary phenol analysis. This method provides results that compare favourably with those obtained by the Van Haaften and Sie method and avoids the disadvantages of pre-column acid hydrolysis inherent in the latter method.

The authors thank H. Bloomberg of Stanlow Medical Centre, Shell Chemicals UK Ltd., who carried out all the phenol analyses using the Van Haaften and Sie method reported in this paper.

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High-performance Liquid Chromatographic Determination of Di(2-ethylhexyl) Phthalate in Blood Stored in PVC Blood Bags

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Keywords: *Di(2-ethylhexyl) phthalate determination; blood; PVC blood bags; high-performance liquid chromatography*

Poly(vinyl chloride) (PVC) materials in medical applications have produced remarkable therapeutic benefits to patients.[1,2] However, it was recognised by Trimble *et al.*[3] and Guess *et al.*[4] that the plasticiser di(2-ethylhexyl) phthalate (DEHP) is leached from PVC by blood and various injection solutions. The leachability of phthalate plasticisers from PVC containers and their subsequent presence in intravenous fluids, blood and blood products have been extensively documented[1,5-8] and the toxic hazard associated with phthalate plasticisers has been a matter of serious concern.[2,6,9,10] Transfusion of blood stored in PVC containers might lead to risks due to the plasticiser[5] used.

As part of a development programme with disposable PVC* blood bags at this Institute, it was necessary to monitor the extent of plasticiser leachability from our Chitra bags into blood and to compare it with the data obtained for similar blood bags currently in use (Tuta, Fenwal and Russian). Squirrell[11] has reported that high-performance liquid chromatography (HPLC) has now become a valuable tool for plastics analysis, particularly in the additive field. We report here the results of an HPLC procedure for determining plasticiser leachability into blood stored in disposable blood bags.

Experimental

Apparatus

The HPLC system was a Waters Model 6000 A solvent delivery pump equipped with a U6K injector, a μStyragel column (30 × 0.78 cm i.d., pore size 100 Å) and a Model 440 absorbance detector. Chromatograms were obtained on a Houston Instruments Omni-Scribe recorder and peak absorbance values were read directly from the digital display of the Model 440 system. Linearity of the absorbance scale was checked using chloroform solutions with known concentrations of DEHP.

HPLC Conditions

A flow-rate of 0.2 ml min^{-1} of the mobile phase was used and the absorbance was monitored at 254 nm.

Reagents

DEHP (Indo-Nippon) was used for preparing plasma standards and Hatcol 200 (Hatco Chemical Company, Fords, NJ) (an ester of a branched oxo alcohol and 1,3-dihydro-1,3-dioxo-5-isobenzofurancarboxylic acid) was used as the internal standard. Spectroscopic-grade methanol (S.D. Chemicals, Bombay) and freshly distilled analytical-reagent grade chloroform (Glaxo Laboratories, Bombay) were used, the latter being the mobile phase.

Plasma Samples

Plasma samples were obtained from calf blood stored at 4 °C in indigenous (Chitra) and imported PVC blood bags for 1, 7 and 21 d. The blood from the same animal was simultaneously stored in a glass bottle to serve as a control in each individual set of experiments.

Sample Preparation

The concentration of DEHP in the standard plasma samples ranged from 10 to 100 μg ml^{-1}. These standards were prepared by evaporating to dryness aliquots from a 200 μg ml^{-1} chloroform stock solution of DEHP and dissolving the residue in 5 ml of control plasma.

DEHP was extracted from the plasma standards and unknown samples by the following procedure. A 10-ml volume of methanol was added to 5 ml of plasma to precipitate the plasma proteins and the supernatant was collected after centrifuging at 1 000 *g* for 15 min. Methanol was evaporated by heating the supernatant at 60 °C and the residue was extracted with three batches of 5 ml of chloroform in a separating funnel. To 5 ml of concentrated chloroform extracts a 1.5-μl aliquot of a 5.47 mg ml^{-1} chloroform solution of Hatcol 200 (internal standard) was added. A 100-μl volume of the chloroform extract was then injected on to the column.

Independent checks confirmed that the evaporation steps did not lead to contamination from the solvents and there was no detectable loss of DEHP.

Results and Discussion

Under the experimental conditions used, DEHP and Hatcol 200 had retention times of 23.2 ± 0.1 and 20.9 ±0.1 min, respectively. Fig. 1 is a chromatogram of DEHP (77.5 μg ml^{-1}) and Hatcol 200 (15.6 μg ml^{-1}) in the mobile phase. Fig. 2(*a*) is a representative chromatogram of a control plasma with the internal standard added. All controls revealed the presence of DEHP. This contamination comes from tapping of the blood into a plastic (PVC) set before transferring into glass bottles to serve as controls. Identical blood tapping

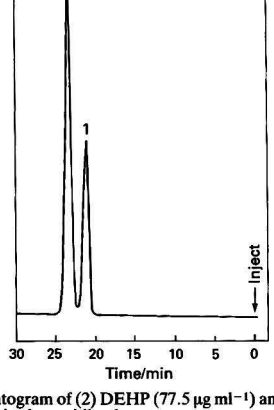

Fig. 1. Chromatogram of (2) DEHP (77.5 μg ml^{-1}) and (1) Hatcol 200 (15.6 μg ml^{-1}) in the mobile phase

* Compound No. 42, Polymer Technology Division.

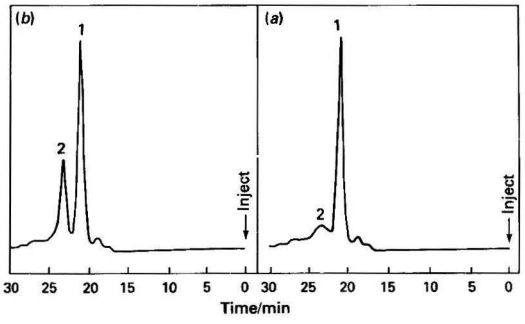

Fig. 2. Chromatograms obtained from calf plasma samples. (*a*) Control plasma sample stored in a glass bottle with (1) the internal standard Hatcol 200 added. Peak 2 corresponds to DEHP contamination of the control, giving an upper limit of 6.4 μg ml^{-1}. (*b*) Plasma sample from blood stored in a PVC bag. The DEHP peak (2) corresponds to a plasma concentration of 36.6 μg ml^{-1}

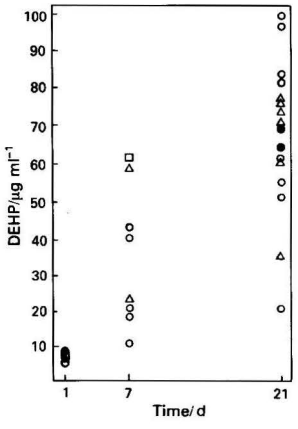

Fig. 3. Extent of leaching of DEHP by blood stored at 4 °C for 1, 7 and 21 d in different containers, showing individual variations. Blood bags: ○, Chitra; △, Tuta; □, Fenwal; and ●, Russian

procedures ensured that the extent of contamination due to this source, prior to storage, was the same for the sample and its control. The DEHP concentrations in the controls generally varied from 2 to 12 μg ml^{-1} (in two controls, however, values as high as 17 and 24 μg ml^{-1} were detected). These values are significant, as prior to storage contamination due to this source also occurs in the usage of blood bags. Vessman and Rietz,[12] for instance, in a study with Fenwal bags, found high levels of DEHP in blank samples corresponding to zero storage time. The limit of detection was about 2 μg ml^{-1} and was essentially imposed by the blood tapping procedure employed. This was satisfactory for the present purpose as the expected values for the unknown samples were much higher. Moreover, the determinations of DEHP in the unknown samples were made against their respective controls as reference blanks.

Fig. 2(*b*) shows a representative chromatogram of an unknown sample with the internal standard added. The extraction and detection procedures used show a relatively clean plasma background (the most intense absorbance peak in the control still being due to DEHP contamination occurring prior to storage) in the chromatogram obtained.

Fig. 3 shows the extent of leaching of DEHP by blood stored at 4 °C for 1, 7 and 21 d in different containers. The values obtained in this study are of the same order of magnitude as those found by Marcel and Noel,[13] Piechoki and Purdy[14] and Vessman and Rietz.[12] It should be noted that there is a significant bag-to-bag variation for both indigenous and imported bags for the same storage duration. The values ranged from 21 to 95.5 μg ml^{-1} of plasma in blood samples stored in eight indigenous bags for 21 d. This, to a certain extent, may be the result of possible inhomogeneities in the viscous sample. However, differences between individual bags (the DEHP content in ten different bags varied from 16 to 120 μg ml^{-1}) were also observed by Vessman and Rietz in their study of Fenwal blood bags.[12]

Hatacol 200 has been reported to show much less leachability than DEHP[15] and might replace the latter in PVC formulations used in medical applications (the present procedure can be easily adapted for the determination of Hatcol 200 in plasma with DEHP as the internal standard).

While much concern has been shown for the contamination of blood with plasticiser during storage and the better plasticisers are being sought, it should be noted that significant contamination of blood might also occur during tapping (body temperature) and prior to storage at 4 °C. Some attention should also be paid to this aspect.

Plasma samples were supplied by Mr. S. N. Pal, to whom thanks are due for providing the necessary details. A gift of Hatcol 200 from Mr. K. Rathinam is gratefully acknowledged.

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Gas-chromatographic Method for the Determination of Volatile Fatty Acids (C_1–C_7) and Lactic Acid

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Keywords: *Volatile fatty acids determination; lactic acid determination; benzylation; gas chromatography; flame-ionisation detection*

In a project concerning the feeding value for poultry of flocculation - flotation (f - f) sludge from slaughterhouses and dairy factories, a simple and reliable method for the determination of volatile fatty acids and lactic acid was needed. As the formation of these acids, as an indication of the microbiological decomposition of de-contaminated and preserved f - f sludge, should be determined, and also the amounts of some of these acids added as preservatives, detection at both the p.p.m. and the percentage levels was necessary. Therefore, gas chromatography with flame-ionisation detection was chosen, which implied that the acids would have to be derivatised, as formic acid, and one of the preservatives used had to be detected also.

Methyl, ethyl esters, etc., of volatile fatty acids are too volatile, resulting in too large losses and/or separation problems with regard to the solvent peak. Therefore, the method, initially described by Bethge and Lindström[1] and used by Jones and Kay[2] and the Agricultural Development and Advisory Service of the UK Ministry of Agriculture, Fisheries and Food,[3] seemed to be appropriate for our sludge samples because of the ion-exchange pre-separation and the benzylation. However, our results with this method were not satisfactory.[4,5] Firstly, titration with tetrabutylammonium hydroxide (TBAH) to pH 8 was a problem, as a stable pH could not be obtained. Secondly, the results were variable, especially for the longer chain acids.

In this paper the results obtained with some other tetra-alkylammonium hydroxides (TAAHs) as possible substitutes for TBAH and the influence of acetone - water mixtures on the elution of the acids from the ion-exchange column are presented.

Experimental

Apparatus

A gas chromatograph equipped with a flame-ionisation detector (Packard Model 427) was used, with a glass column (1.8 m × 4 mm i.d.) containing 10% *m/m* Carbowax 20M on Chromosorb W AW DMCS (100–120 mesh). The carrier gas was helium at a flow-rate of 60 ml min^{-1}. The initial column temperature was 85 °C (6 min), programmed at 6 °C min^{-1} to a final temperature of 195 °C (10 min). The injection port was maintained at 195 °C and the detector at 250 °C. The electrometer was linked via an A/D converter to a Hewlett-Packard 3354 Laboratory Automation System for data processing.

A digital pH meter with a combined electrode (Philips PW-9409) and a glass column (*ca.* 100 × 10 mm i.d.) containing Duolite C225 SRC 14 ion-exchange resin (52–100 mesh) in the H^+ form were also used.

Reagents

Acetone.

Benzyl bromide.

Tetraalkylammonium hydroxides. TBAH, 40%; tetraethylammonium hydroxide (TEAH), 40%; tetramethylammonium hydroxide (TMAH), 25%; tetrapropylammonium hydroxide (TPAH), 20%. Prepare aqueous solutions of the TAAHs at a concentration of *ca.* 0.5 M and standardise them with standard hydrochloric acid, using a pH meter to detect the end-point.

Standard acid solutions. Transfer 0.5 ml of formic acid into a weighed 50-ml calibrated flask containing *ca.* 10 ml of water, stopper, re-weigh and dilute to 50 ml. Similarly, prepare solutions containing, in 50 ml of water, *ca.* 2 ml of glacial acetic acid, *ca.* 2 ml of propionic acid, *ca.* 2 ml of isobutyric acid, *ca.* 2 ml of butyric acid and *ca.* 5 g of lactic acid.

Similarly, prepare solutions containing, in 25 ml of acetone and 25 ml of water, *ca.* 2 ml of isovaleric acid, *ca.* 2 ml of valeric acid and *ca.* 2 ml of caproic acid, and in 50 ml of acetone and 50 ml of water, *ca.* 0.3 g of heptanoic acid.

Standard acid mixture. Pipette 1 ml of each standard acid solution (except heptanoic acid) into a 100-ml calibrated flask and dilute to 100 ml.

Procedure

Sample treatment

Pipette 5 ml of the standard acid mixture and 1 ml of the heptanoic acid solution on to the ion-exchange column, elute slowly (*e.g.*, 1 drop s^{-1}) into a 100-ml beaker until the column is almost dry and wash with one of the mixtures as shown in Table 1. Titrate with a standardised TAAH solution to pH 9–9.5, using a pH meter, and record the volume of TAAH consumed (V ml). Transfer the beaker into an oven with forced air circulation (fan or a stream of, *e.g.*, nitrogen) and heat at 90 °C until evaporation is complete and a syrupy residue remains. Immediately remove the beaker from the oven and allow it to cool. If the residue is too dry (crystals instead of a syrup) add a few drops of water. Add 5 ml of acetone to the cold residue, swirl to dissolve and transfer into a glass-stoppered graduated test-tube. Repeat with 2 × 5 ml of acetone, collect the acetone fractions and evaporate the solvent until the 10-ml mark. Add V^* µl of benzyl bromide from a microsyringe, mix well and allow to stand for at least 2 h. Inject 2 µl of the acetone solution of benzyl esters into the gas-chromatographic column.

Calculations

Calculation of volume V^* has been performed with the BASIC program BENZBR:TS.† In the literature,[1,2] two equations are given for calculating the amount of benzyl bromide needed:

$$V_1 = 10 + \left[V \times M \times \frac{M_r\,(\text{benzyl bromide})}{d\,(\text{benzyl bromide})}\right]$$

or

$$V_1\ (\mu l) = 10 + V \times M \times 119$$

and

$$V_2\ (\mu l) = 120 \times V \times M \times (1 + k)$$

where V is the volume (ml) of TAAH solution used, M is the molarity of the solution, M_r is the relative molecular mass, d is the specific gravity and k is an arbitrary factor, to be chosen in

† Details of the program are available from the author on request.

the range 0.1–1 (we used 0.5). In this work we chose average values of V_1 and V_2, so

$$V^* (\mu l) = \frac{V_1 + V_2}{2}$$

For the preparation of an external standard calculation method[6] (with absolute response factors), a calibration run was made with a sample of 5 ml of the standard acid mixture and 1 ml of the heptanoic acid solution. After dilution to *ca.* 25 ml with distilled water, analysis was carried out exactly as under *Sample treatment* except for the ion-exchange step. The external standard method thus obtained was used for quantifications.

Table 1. Composition of tested eluents

Mixture	Acetone	Water
A. . . .	None	4 × 5 ml
B. . . .	1 × 5 ml of 50% acetone*	3 × 5 ml
C. . . .	2 × 5 ml of 50% acetone	2 × 5 ml
D. . . .	1 × 5 ml of 75% acetone	3 × 5 ml
E. . . .	2 × 5 ml of 75% acetone	2 × 5 ml
F. . . .	1 × 5 ml of 100% acetone	3 × 5 ml

* 50% acetone = 1 + 1 water - acetone, etc.

Table 2. Influence of the composition of the eluent on the recoveries of the acids. Duplicates were run on two similar ion-exchange columns

Ion-exchange column	Acid	Recovery after elution with mix, %					
		A	B	C	D	E	F
1	Formic	103	100	98	97	100	98
	Acetic	99	97	99	96	99	101
	Propionic - isobutyric	98	97	100	97	100	101
	Butyric	97	96	97	96	98	99
	Isovaleric	95	97	100	97	100	101
	Valeric	90	95	99	96	100	99
	Caproic	75	92	96	96	98	98
	Heptanoic	39	87	94	94	98	95
	Lactic	97	97	100	88	101	98
2	Formic	102	98	98	100	98	95
	Acetic	98	97	98	98	99	102
	Propionic - isobutyric	98	97	99	98	100	101
	Butyric	96	94	97	96	97	100
	Isovaleric	94	95	99	98	100	101
	Valeric	89	94	97	97	99	101
	Caproic	76	89	95	96	98	99
	Heptanoic	42	83	93	93	97	97
	Lactic	96	98	96	99	100	99

Results and Discussion

As the solubility of the acids in water decreases with increasing chain length, it might be necessary to use a water - acetone mixture to elute all of the acids from the ion-exchange column. Table 1 shows the acetone - water mixtures tested.

In Table 2 the results obtained with a series of samples of the standard acid mixture eluted with the different mixtures are shown.

Table 2 shows clearly that elution of the acids with water alone leads to tremendous losses for the acids with five, six and seven carbon atoms. The best over-all results were obtained after elution with 1 × 5 ml of acetone and 3 × 5 ml of water and with 2 × 5 ml of 75% acetone and 2 × 5 ml of water, although the differences with the other acetone - water mixtures are small and probably not significant. The use of pure acetone (1 × 5 ml) leads to air bubbles in the ion-exchange column, which should be avoided.

Preliminary studies with TMAH, TEAH, TPAH and TBAH showed that the best over-all results were obtained with TPAH. TPAH was not only the best reagent for titration (pH stable between 9 and 9.5) but also gave the highest recoveries.[7] Therefore, subsequent treatment of the acids involved titration with TPAH and, from the high recoveries in Table 2, it can be concluded that this reagent gives satisfactory results.

Summarising, the best over-all results are obtained by eluting the acids with 2 × 5 ml of 75% acetone and 2 × 5 ml of water, followed by titration with TPAH to pH 9–9.5 and dissolution of the syrupy residue in acetone prior to benzylation.

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Significant Reactions of Aluminium, Magnesium and Fluoride During the Graphite Furnace Atomic-absorption Spectrophotometric Determination of Arsenic in Coal

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Keywords: *Arsenic determination; atomic-absorption spectrophotometry; aluminium, magnesium and fluoride reactions; coal*

In the determination of arsenic by graphite furnace atomic-absorption spectrophotometry (AAS) at 193.7 nm, spectral interference occurs if aluminium is present in the analyte solution.[1] The emission lines[2] of Al^{2+} at 193.58 nm and Al^{+} at 193.47 and 193.45 nm are sufficiently near to overlap the resonance line of arsenic. Interference by aluminium is to be expected in the analysis of the acid digests of materials such as rocks, soils, sediments, coal and fly ash. However, a method suitable for the direct determination of arsenic in these materials has been described by Haynes,[3] who observed no interference by aluminium at 193.7 nm. This method is used in the author's laboratory for the determination of arsenic in coal, although to eliminate any possibility of spectral interference by aluminium, measurement at the alternative 197.2-nm resonance line is preferred. The reasons why spectral interference is not observed in Haynes' method are discussed in this paper.

Haynes' procedure requires that coal be ashed at 500 °C, with nickel and magnesium nitrates present as a volatilisation suppressant and ashing aid, respectively. The ash is digested with nitric and hydrofluoric acids and arsenic is determined by graphite furnace AAS. The nickel salt prevents loss of arsenic during both the ashing and preliminary stages of the subsequent AAS measurement.[4] Although magnesium nitrate is often used as an ashing aid,[5] it performs another important function in this procedure. In the dissolution of rocks with hydrofluoric acid, the insoluble complex $MgAlF_5.xH_2O$ can form[6] and it is probable that the formation of this complex during the Haynes' decomposition procedure effectively removes aluminium from solution. To confirm this, the insoluble residue from the digestion of the standard reference coal, NBS 1632a (which contains 3.1% of aluminium) was analysed and the amount of aluminium in solution was determined. The effects on the absorbance by aluminium at 193.7 nm, when solutions containing magnesium, nickel and fluoride ions were added as matrix modifiers directly to the graphite furnace, were also studied.

Experimental

Solutions were prepared from analytical-reagent grade chemicals and glass-distilled water. The dissolution procedure is that used in the author's laboratory for determining arsenic and antimony in coal, and is based on the procedure of Haynes.[3] A 1-g amount of NBS 1632a was weighed into a 30-ml platinum crucible, to which were added 1 ml of ethanol and 5 ml of a solution containing 15% *m/m* of $Mg(NO_3)_2.6H_2O$ and 12.5% *m/m* of $Ni(NO_3)_2.6H_2O$. The sample was dried and transferred into a muffle furnace at 100 °C. The temperature was increased to 500 °C over a 2-h period, and the sample was allowed to ash overnight (14 h). The ash was cooled and 5 ml of water and 5 ml of concentrated nitric acid were added. The crucible was warmed until any visible reaction ceased and 5 ml of hydrofluoric acid (40%) were added. The solution was evaporated to dryness and soluble salts were dissolved by heating in 5 ml of concentrated nitric acid plus 20 ml of water. The solution and insoluble residue were transferred into a tared 100-ml polyethylene bottle and the volume was adjusted by mass to 50 g with distilled water.

The solution was decanted from the residue, which was dried over a water-bath. Conventional flame AAS was used to determine the aluminium concentration of the solution (Table 1). A portion of the residue was digested with perchloric and nitric acids and the aluminium and magnesium contents were also determined by flame AAS. A fusion - ion-selective electrode method[7] was used to determine the fluoride content of the precipitate (Table 1). Other cations were determined by emission spectrography.

Changes in the absorbance by aluminium at 193.7 nm were studied, when solutions containing magnesium, nickel and fluoride ions were added as matrix modifiers directly to the graphite furnace. Aluminium solutions were prepared from $Al(NO_3)_3.9H_2O$ in 1% *V/V* nitric acid. Nickel and magnesium solutions were prepared from their nitrates dissolved in 5% *V/V* nitric acid and 5% *V/V* nitric acid - 1% *V/V* hydrofluoric acid. Absorption measurements were performed at 193.7 nm using a Perkin-Elmer Model 4000 atomic-absorption spectrophotometer equipped with an HGA-500 graphite furnace, an AS-40 autosampler and an arsenic electrodeless discharge lamp source. The spectral band width was set at 0.7 nm. Continuum source background correction was used for all measurements. The furnace parameters (Table 2) are those used for the determination of arsenic. However, a lower char temperature (900 °C) was also used to study its effect. The matrix modifiers were added at the furnace using the autosampler facility.

Table 1. Analysis of solution and residue

	Solution
Al determined	23 μg ml^{-1}
Al theoretically	620 μg ml^{-1}

Residue	Concentration, %	Atomic ratio
Al	12.2	1.0
Mg	11.6	1.1
F	41.7	5.0
Ca, Fe, Ni, Si, Ti	Minor amounts	

Table 2. Furnace parameters

Step	Temperature/ °C	Ramp/s	Hold/s	Ar flow-rate/ ml min^{-1}
1	120	20	20	300
2	1 200 (or 900)	30	5	300
3*	2 700	0	5	10
4	2 700	2	1	300

* Read: peak height.

Results and Discussion

Analysis of the solution and residue (Table 1) obtained by the dissolution of NBS 1632a using Haynes' procedure indicated that less than 5% of the total aluminium is in solution; most is precipitated[6] as $MgAlF_5.xH_2O$. This precipitation minimises the possibility of any spectral interference in the measurement of arsenic at 193.7 nm. A combination of the magnesium and fluoride ions, when added directly to the graphite furnace, also suppresses the absorption by aluminium at this wavelength (Table 3). Thus the presence of these ions in solution contributes to the suppression of spectral interference in Haynes' method. However, the addition of nickel with or without fluoride, or magnesium alone, actually increases the observed absorbance at 193.7 nm (Table 3). This increase reflects an inability of the continuum background corrector to cope with all the species present.

Although it is apparent that in the presence of magnesium and fluoride ions aluminium does not form the ionic species responsible for the spectral interference, the reactions involved are not clear. When sufficient fluoride is present in the graphite furnace, aluminium ions are not produced. Generally, fluoride is lost at the charring stage[8] but in the presence of magnesium it is likely that a stable magnesium aluminium fluoride complex forms in the furnace and survives charring partially at 1 200 °C and more readily at 900 °C (Table 3). The retained fluoride thus prevents ionisation of the aluminium.

Table 3. Absorbance of aluminium solutions (10 µl) at 193.7 nm with the addition of matrix modifiers (10 µl)

	Modifier					
Al/ µg g^{-1}	None	0.1% Ni	0.1% Ni, 1% HF	0.1% Mg	0.1% Mg, 1% HF	0.1% Mg, 1% HF*
100	0.118	0.337	0.339	0.057	0.001	0.000
200	0.403	0.823	0.900	0.517	0.030	0.001
400	0.920	>1	>1	>1	0.653	0.005
600	>1	>1	>1	>1	0.930	0.015
800	>1	>1	>1	>1	>1	0.022
1000	>1	>1	>1	>1	>1	0.041

* 900 °C char (step 2 in furnace parameters).

Conclusion

When Haynes' procedure is used for the determination of arsenic in coal (and similar materials) by graphite furnace AAS, chemical reactions involving magnesium, aluminium and fluoride provide the explanation for the absence of spectral interference at 193.7 nm. The spectral interference is caused by aluminium ions. In this procedure, most of the aluminium precipitates as $MgAlF_5.xH_2O$ and any residual aluminium reacts in the graphite furnace with the magnesium and fluoride also in solution. Thus, as only a small amount of aluminium is actually present in the graphite furnace, and this reacts to form fluorides rather than ions, no spectral interference is observed.

N. Morgan is thanked for the optical emission spectrographic results.

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Spectrophotometric Determination of Boron with Curcumin after Extraction with 2-Methylpentane-2,4-diol - Chloroform

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Keywords: *Boron determination; 2-methylpentane-2,4-diol extraction; curcumin; spectrophotometry*

The determination of microgram amounts of boron in natural products (water, soils, ores and plants), steel and alloys has been an analytical problem not totally resolved by the different methods proposed owing to the lack of reproducibility and the pre-separation necessary in order to eliminate interferences.[1]

Interferences caused by oxidising agents and Fe(III) produced in the dissolution of steel make it necessary or recommendable to separate boron prior to the various spectrophotometic methods for its determination with curcumin,[2,3] quinalizarin, purpurine, carminic acid, azomethine H[4] and 1,1-dianthrimide.[5] This separation can be effected by distillation as methyl borate, by ion exchange or by extraction. Also, the use of concentrated sulphuric acid to form the boric acid - reagent complex is needed. Another method, based on spectrophotometric measurement of the methylene blue - fluoroborate complex after its extraction into dichloroethane,[6,7] has been little used owing to the poor reproducibility and the critical conditions of acidity and fluoride concentration required for the formation of the complex.

Aznárez and co-workers[8–10] have used the simplicity and selectivity of extraction with 1,3-diols in order to extract boric acid into 4-methylpentan-2-one (isobutyl methyl ketone) containing 2-methylpentane-2,4-diol (MPD). After this extraction boron was determined with curcumin in a portion of the extract by flame photometry, atomic-absorption spectrophotometry and spectophotometry using concentrated phosphoric acid as a dehydrating agent.

This paper describes the spectrophotometric determination of boron with curcumin in an acetic acid - concentrated sulphuric acid medium after its extraction into chloroform containing MPD. The solution was diluted with *N,N*-dimethylformamide (DMF). The basic character of this solvent leads to the destruction of the protonated curcumin. Hence a better separation between the absorption peaks of the reagent and the complex is achieved.

The proposed method is sensitive and specific, and large amounts of iron and moderate amounts of fluoride do not interfere. It has been applied to the determination of boron in plants and steels with good precision and accuracy.

Experimental

Apparatus

Absorbance measurements were made with a Pye Unicam SP8-100 spectrophotometer.

To avoid losses of boric acid by volatilisation, polyethylene bottles with air condensers (3 mm i.d. and 50 mm long) were used for the decomposition of solid samples. The use of borosilicate glassware was avoided throughout. Also, 100-ml polyethylene separating funnels were used. A Beckman Research pH meter with glass and calomel electrodes was used for the measurement of pH.

Reagents

All reagents were of analytical-reagent grade.

Standard boron solution, 1 000 μg ml^{-1}. Dissolve 5.715 g of pure boric acid in water in a 1-l calibrated flask, dilute to volume, mix thoroughly and transfer into a plastic bottle. Dilute solutions are prepared just before use.

Curcumin, 0.1% *m/V solution in glacial acetic acid.* This solution is stable for 1 month.

2-Methylpentane-2,4-diol (MPD), 20% *V/V solution in chloroform.*

Sulphuric acid - acetic acid solution, 50% *V/V.* Prepared fresh daily.

Zirconium sulphate, 10% *m/V solution in* 2 M *sulphuric acid.*

Procedure

Steel sample preparation

Weigh a sample of steel (less than 0.5 g) into the polyethylene bottle for its decomposition. Add 10 ml of 6 M hydrochloric acid, 1 ml of concentrated nitric acid and 1 ml of hydrofluoric acid. Close with an air condenser and heat in a water-bath at 70–80 °C for 2 h. Allow the mixture to cool to room temperature, transfer it into a 50-ml calibrated flask and dilute to volume with 6 M hydrochloric acid.

Plant sample preparation

Weigh 0.2–0.5 g of sample, depending on the boron content, after pulverising and drying at 60 °C in a platinum crucible. Place the crucible in a muffle furnace at 500 °C for 4 h, then allow it to cool inside the furnace. Add 10 ml of 6 M hydrochloric acid and heat the mixture carefully to 80 °C on a hot-plate. Allow it to cool to room temperature and dilute to 50 ml with 6 M hydrochloric acid.

Losses of boron during dry ashing of plant material in the muffle furnace were not observed, although they have been reported by other workers.[11]

Boron extraction and complex formation

To an aliquot of the sample solution containing up to 3 μg of boron add 1 ml of zirconium sulphate solution and 10 ml of MPD solution. Shake the mixture for 5 min with a mechanical shaker and allow the phases to separate. Filter the chloroform extract through cotton-wool in order to remove any remaining aqueous phase.

To 3 ml of the organic phase add 3 ml of curcumin - acetic acid solution and 0.5 ml of the sulphuric acid - acetic acid mixture. Heat in a water-bath at 80 °C for 15 min, then allow it to cool to room temperature and dilute to 10 ml with DMF. Measure the absorbance at 555 nm with DMF as a reference.

Calibration graph

Dilute aliquots of the standard boron solution covering the range 0–3 μg of boron to the volume required with 6 M hydrochloric acid. After extraction and complex formation have been carried out as indicated above, prepare a calibration graph by plotting concentration *versus* the net absorbance of the standard solutions.

Results

Spectrophotometric Determination of Boron with Curcumin

The use of 3 ml of the MPD - chloroform extract for complex formation avoids the difficulties mentioned by Donaldson[6]

Table 1. Results of the determination of boron in plant material (n= 10)

Sample	Mean value/ μg g^{-1}	Relative standard deviation, %	Recovery, %
Eucalyptus globulus	35.1	1.8	99.0
Zea mays	20.8	2.0	97.8
Olea europea	19.1	2.2	98.5
Gossypium herbaceum	23.0	1.8	97.0
Vitis vinifera	46.1	1.5	98.7

Table 2. Results of the determination of boron in BCS mild steel samples (n= 10)

Sample	Certified B value and range, %	Mean value obtained in this work, %	Relative standard deviation, %
BCS 457 ..	0.002 5† (0.002 5–0.003)	0.003 0	3.2
BCS 458 ..	0.005 (0.000 4 5–0.005)	0.004 5	2.1
BCS 329 ..	0.008 (0.007–0.008)	0.007 4	1.7

* Methods used: direct with carminic acid; 1,1′-dianthrimide; by distillation of 4-methyl ester and determination with curcumin; pyrohydrolysis with argon plasma spectrophotometry.

† Certified value based on the two results shown in parentheses.

due to the excess of MPD that is not totally removed in the heating process at 80 °C. This greater volume of extract permits a higher sensitivity of the method. Beer's law is obeyed for amounts of boron up to 2.5 μg. The molar absorptivity is 1.82 × 10^{-5} l mol^{-1} cm^{-1} at 555 nm, the wavelength of maximum absorption. This is slightly higher than the values reported by other investigators,[6,12] *viz.*, 1.65 × 10^{5} and 1.46 × 10^{5} l mol^{-1} cm^{-1}, but is similar to other calculated values,[13] *viz.*, 1.85 × 10^{5} l mol^{-1} cm^{-1}. The absorbance of the reagent blank against DMF as reference is 0.010 ± 0.002 at 555 nm for ten determinations. This low absorbance value and its standard deviation permitted the use of DMF as a reference. No change in absorbance at 555 nm was observed during 3 h at room temperature in the dark.

Separation of Boron by Extraction of the Borate - MPD Complex into Chloroform

We found that up to 5 μg of boron can be quantitatively extracted (yield >97%) into chloroform containing 20% of MPD in a single extraction from 2 to 6.5 M hydrochloric acid when the volume ratio of the aqueous to the organic phase is 2.5 : 1. The recoveries of boron from hydrochloric acid at different concentrations were as follows: 1 M, 95.0%; 2 M, 97.5%; 3 M, 97.5%; 4 M, 98.8%; 5 M, 99.0%; 6 M, 99.0%; 6.5 M, 98.8%; and 7.0 M, 98.4%.

Effect of Diverse Ions

The separation of boron by chloroform extraction of boric acid with diols is highly selective. Agazzi[14] found that Sr, Ca, Ba, Mg, Cr(III), Zn, Fe(III), Co, Cu(II), Ni, Pd, Al, Bi, V(V) and phosphate do not interfere in the extraction of boron and only Mn, Ti, Mo and W are significantly co-extracted.

Interference from fluoride, which is used in the sample decomposition step, is eliminated by complexing it with zirconium before the extraction. If the extraction is from 6 M hydrochloric acid solution, small amounts of fluoride do not interfere. In this work, the following species were found not to interfere in the determination of 5 μg of boron up to at least the amounts indicated: Fe, Mg and Ca, 500; Co, Cu, Sr, Ba and PO_4^{3-}, 100; Zn, Al and SO_4^{2-}, 200; Pb, Bi and Cr(III), 50; Ti, Mo(VI) and Mn(II), 10; and F^-, 0.05 mg.

Applications

The proposed method was applied to the determination of boron in plants from the Comité Inter-Instituts of Nimes, provided by M. Pinta, and BCS steel samples.

The results obtained for plant samples are shown in Table 1. As no standard plant samples containing boron were available, the standard additions method was applied and mean recoveries of 98.2 ± 1.5% (ten determinations) were obtained. Good precision was also observed.

Table 2 shows the results obtained with the BCS steel samples. Acid-insoluble boron was not considered.

Conclusion

Extraction into chloroform containing MPD achieves both the separation and concentration of boron. This extraction step with formation of the curcumin using DMF as the solvent constitutes an excellent method for the determination of microgram amounts of boron in steels and plant ashes, with a high molar absorptivity at 555 nm and a low absorbance of reagent blank due to the use of the mentioned solvent.

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BOOK REVIEWS

Polymer Characterization: Spectroscopic, Chromatographic, and Physical Instrumental Methods
Edited by Clara D. Craver. *Advances in Chemistry Series, 203.* Pp. xvi + 791. American Chemical Society. 1983. Price $69.95 (USA and Canada); $83.85 (Export). ISBN 0 8412 0700 3.

This volume is based on a symposium sponsored by the Macromolecular Secretariat of the American Chemical Society at the 181st meeting of the American Chemical Society held at Atlanta, Georgia, from 29 March to 3 April 1981—a total of 42 papers.

The book is arranged in eight sections that encompass physical properties (10 papers), thermal methods (3), chromatographic methods (5), electron microscopy (4), nuclear magnetic resonance (6), infrared spectroscopy (6), analytical pyrolysis/gas chromatography/mass spectrometry (6), and "spectral topics" (covering the scattering of light, X-rays and neutrons and a technique known as "excimer fluorescence"—used as a molecular probe of blend miscibility and compared with differential-scan calorimetry) (2).

As with many collections of symposia papers, rather a hotchpotch has resulted—which is of course no reflection on the Editor, who appears to have been presented with a task of rather more than usual difficulty.

The text is very much in the American idiom, and unfortunately it is not the American of H. D. Thoreau and E. B. White. In some places indeed (as was written of another US author) the clarity of the prose might have been improved merely by deleting each alternate sentence.

Among the variety of topics covered by the individual papers, those in the sections devoted to electron microscopy, chromatography and infrared spectroscopy may perhaps be of particular interest. Taking the text as a whole, some of the papers presented lend themselves fairly easily to general practical applications, but others do not; the balance of the book, unfortunately (in the opinion of the reviewer), is rather more towards the latter than the former group.

However, this book is likely to be purchased by research libraries, both industrial and academic, and the content is such as to find favour with higher research students who have access to such libraries.

D. Simpson

Chromatography in Biochemistry, Medicine and Environmental Research, 1. Proceedings of the 1st International Symposium on Chromatography in Biochemistry, Medicine and Environmental Research, Venice, June 16–17, 1981
Edited by Alberto Frigerio. *Analytical Chemistry Symposium Series, Volume 13.* Pp. x + 278. Elsevier. 1983. Price $72.25 (USA and Canada); Dfl 170 (Rest of World). ISBN 0 444 42016 9 (Volume 13); 0 444 41786 9 (Series).

This book contains 27 papers and 1 plenary lecture presented at the chromatography symposium held in Venice in 1981 and organised by the Italian Group for Mass Spectrometry in Biochemistry and Medicine in collaboration with the International Scientific Center. It covers a broad range of topics, as reflected by the sections devoted to drug, environmental and biomedical studies. Drug studies are represented by papers on the analysis of silymarin and the quantification of quinidine, ergot alkaloids, theophylline, vinpocetine, DL 111-IT (a new antifertility drug), and 19-*nor*-testosterone, and a paper in which GC - MS is compared with conventional techniques for screening in cases of acute drug intoxication. Under the heading of environmental studies are papers on priority pollutants, the herbicide atrazine, alkyl disulphides, polychlorinated naphthalenes, organochlorine insecticides and plutonium. Biomedical studies are represented by papers on the analysis of the actinide elements, of technetium-99*m* and of gallium-67, the determination of aliphatic aldehydes, biogenic amines and phylloquinone, and the application of liquid, gas and adsorption chromatography to a variety of problems. Automated headspace gas chromatography as a diagnostic aid in clinical microbiology is the subject of the plenary lecture by L. Larsson.

The book is well produced by reproduction of the typed manuscripts and concludes with an author index. Unfortunately, there is no subject index. Although symposia of this type undoubtedly serve a valuable purpose in bringing together scientists with a common interest, in this instance the biomedical applications of chromatography, the published proceedings often tend to be of less value as they generally consist of a limited number of specialist research papers scattered over a broad field. This book is no exception. Although the quality of work presented is generally high, it would have been of more use if the book had contained more in the way of overview papers and general introductions to new techniques. As far as the general analytical chemist is concerned, it would be difficult to justify spending over $70 on what is essentially a set of research papers.

D. J. Harvey

Chromatography and Mass Spectrometry in Biomedical Sciences, 2. Proceedings of the International Conference on Chromatography and Mass Spectrometry in Biomedical Sciences, Bordighera, Italy, June 20–23, 1982
Edited by Alberto Frigerio. *Analytical Chemistry Symposium Series, Volume 14.* Pp. xii + 506. Elsevier. 1983. Price $106.50 (USA and Canada); Dfl 250 (Rest of World). ISBN 0 444 42154 8 (Volume 14); 0 444 41786 9 (Series).

The annual Symposia organised by the Italian Group for Mass Spectrometry in Biochemistry and Medicine have become a well established part of the mass spectroscopists calendar. The 11th meeting in this series was held in Bordighera, Italy, in June 1982 and the 47 papers and plenary lectures presented at the symposium are published in this book. The scope of the meeting was broader than in previous years in that it covered biomedical applications of chromatography in general as well as applications of mass spectrometry. In fact, mass spectrometry featured in only 26 of the presentations. To some extent this lessens the usefulness of the book as it merely becomes the record of a fairly general meeting and as such has little appeal to the specialist. There appears to be little difference between the contents of this book and a volume of, say, the *Journal of Chromatography*. However, it does provide a general coverage of a large number of chromatographic techniques, several of them new, and illustrates the increasing usefulness of these methods in the biomedical sciences.

The papers themselves have, in many instances, been written by experts and are of a generally high standard. Topics covered include drug studies (12 papers), developments in methodology (10), endogenous compounds (15), clinical studies (2), toxins (1) and environmental studies (2). The reviews on combined gas chromatography - Fourier transform

infrared spectroscopy (T. L. Isenhour and J. C. Marshall), quantitative ion-exchange thin-layer chromatography (A. Varadi, S. Pongor and T. Devenyi), chromatographic techniques for the determination of some calcium antagonist drugs in biological fluids (G. B. Gervasi) and tandem mass spectrometry (J. De Graeve and J. L. Delaval) are particularly interesting and there is a useful set of mass spectra of the *tert*-butyldimethylsilyl derivatives of steroids included in the paper by B. P. Lisboa and I. Ganschow. Newer techniques include metastable mapping in mass spectrometry (R. S. Stradling, G. A. Warburton and D. Hazelby), FAB mass spectrometry (L. C. E. Taylor and D. Hazelby) and the use of fused-silica capillary columns for gas chromatography. A new device for GC - MS coupling based on a supersonic seeded beam separator is also described (M. R. Cimberle *et al.*).

The book, as with other volumes in the series, has been produced by photoreproduction of the typed manuscripts and the format is generally clear and well organised. There is a short author index but the book could have been improved by inclusion of a subject index. Unfortunately, as with so many other books of this type, the price is much higher than most people would be willing to pay for a set of conference proceedings.

D. J. Harvey

Ion Exchange Membranes
Edited by D. S. Flett. Pp. 210. Ellis Horwood for the Society of Chemical Industry. 1983. Price £19.50. ISBN 0 85312 640 2 (Ellis Horwood); 0 470 27452 2 (Halstead Press).

This volume presents the texts of lectures and papers presented at a Symposium on the theme of its title. These are directed to the use of ion-exchange membranes in the process industries and especially the adoption of ion-exchange membrane technology in the chloralkali industry. Membranes for hydrometallurgy, high-temperature work and process control are covered and within this block the only contribution of potential analytical interest is the contribution on ion exchange and ion-selective electrodes by A. K. Covington. Even this is of limited interest to practising analytical chemists for its discussion is essentially restricted to the role of ion-exchange processes in the functioning of ion-selective electrodes with very little attention to the other intended objective of discussing their use as sensors in process control.

The above comments on the restricted analytical chemical content are not intended to imply that there is no worthwhile reading for analytical chemists. On the contrary, there is much within this volume that can broaden the background knowledge that they often need to have in order to appreciate the variety of problems encountered in their work.

J. D. R. Thomas

A Bibliography of Applications of Near Infrared Reflectance Spectroscopy to Food Analysis
Compiled by B. G. Osborne. Pp. 56. Flour Milling and Baking Research Association. 1983. Price £2 (Members); £12 (Non-members). ISBN 0 907503 01 2.

This comprehensive bibliography contains 483 references on quantitative analysis by near-infrared and mid-infrared transmittance and reflectance spectroscopy. Methods of quality assessment of intact biological samples using research composition analysers operating in the visible region are also covered. Most of the references relate to food and agricultural commodities; relevant papers from a large number of different disciplines are included.

McGraw-Hill Encyclopedia of Chemistry
Edited by Sybil P. Parker. Pp. x + 1195. McGraw-Hill. 1983. Price £39.95. ISBN 0 07 045484 1.

Because of the very high cost of producing (and therefore purchasing) multi-volume reference books, scientific establishments are increasingly relying on single-volume dictionaries and encyclopaedias for that purpose. New publications of this type are therefore of considerable interest. The "McGraw-Hill Encyclopedia of Chemistry" is a large volume, even by reference standards, the monographs within which have been selected by its Consulting Editors and editorial staff from the "McGraw-Hill Encyclopedia of Science and Technology." The dust-jacket claims that the encyclopaedia covers all aspects of inorganic, organic, analytical and physical chemistry, as well as selected topics from nuclear physics that are essential to atomic theory; it contains 790 entries (with 800 illustrations) and a 5 000-entry index.

The monographs in this encyclopaedia are somewhat shorter than in, for example, the "Encyclopedia of Physics" and the bulk of them are aimed at the non-specialist chemist (although a reasonable amount of scientific knowledge on the part of the reader is obviously assumed). However, some are far more technical in nature. In all, the book is pitched at such a level as to be of maximum appeal and is reasonably priced at £39.95 for nearly 1 200 pages.

R. A. Young

Analytical Techniques in Environmental Chemistry 2. Proceedings of the Second International Congress, Barcelona, Spain, November 1981
Edited by J. Albaiges. *Pergamon Series of Environmental Sciences, Volume 7.* Pp. xii + 473. Pergamon Press. 1982. Price $75. ISBN 0 08 028740 9.

There are some 51 chapters covering a wide variety of analytical methodologies in this conference proceedings book. Several of the chapters give accounts of current developments in techniques that have newly emerged into the environmental field, such as HPLC, ICP and XRF, or have become more readily available, such as GC - MS. While these reports are unlikely to give the specialist reader new information, taken together they do offer the worker who has to maintain a current awareness of the available analytical techniques for environmental chemistry a useful source book. The other half of the chapters tend to give more detailed accounts of particular environmental investigations in areas of the world as diverse as Kuwait's marine environment, the St. Lawrence Estuary, Venezuelan air and the Rhône. Some of these chapters expound useful case histories but others merely re-cover old ground with few fresh insights. As often in the environmental field, many of the contributions are from workers in government or academic laboratories, with very few accounts from active industrialists.

As frequently seen in multi-author volumes, the quality of the presentation, English and even the camera-ready copy is very variable. More regrettably, the quality of the analytical chemistry is variable. Some of the work presented is of a very high standard, and it is to be hoped that this will be noted by those who have presented material with inadequate validation of analytical methods or who have built over-definitive conclusions upon inadequate data. At least in producing a book with variations in quality the Editor can justifiably claim to be reflecting the real state of environmental analysis.

There is a useful 9-page index that helps to unify a book which cannot pretend to be other than a collection of conference papers. As such, it will be of interest to those

active in the field, provided they do not expect a comprehensive, or in-depth, treatment of environmental analytical chemistry.

L. Ebdon

Short-term Bioassays in the Analysis of Complex Environmental Mixtures. III
Edited by Michael D. Waters, Shahbeg S. Sandhu, Hoellen Lewtas, Larry Claxton, Neil Chernoff and Stephen Newsnow. *Environmental Science Research, Volume 27.* Pp. xvi + 589. Plenum. Price $69.50. ISBN 0 306 41191 1.

This text is a valuable addition to the *Environmental Science Research* series and provides those of us interested in genetic toxicology and environmental protection with an up-to-the-minute handbook of short-term assay procedures provided by notable investigators.

The volume is conveniently divided into six chapters and includes such essential topics as collection and preparation of samples, development of biological tests for mutagens, carcinogens and teratogens and the application of the methods to complex mixtures and concentrates derived from air and water.

Much of the volume is concerned with the analysis of diesel engine emissions and other combustion products, an inevitable consequence of the high level of interest shown in the subject by both industry and government regulators, and indeed the work has resulted in the production of overwhelming evidence against nitro-polynuclear aromatics as the bad-actors of exhaust emissions. There are also contributions addressing the question of mutagens in drinking water, a subject that continues to cause hot debate amongst environmentalists. The geographic location of such mutagens could of course be related eventually to the distribution of human cancer and found to have significant cause - effect relationship. However, what is still missing from the whole procedure is the extrapolation of these sort of data to human health hazard and risk assessment. Nevertheless, the text provides an insight into the current efforts to identify and assess health hazards in our environment and I recommend it as a valuable update to all genetic toxicologists.

E. Longstaff

Methods of Enzymatic Analysis. Third Edition. Volume 1. Fundamentals
Edited by Hans Ulrich Bergmeyer. Pp. xxiv + 574. Verlag Chemie. 1983. Price £50, DM170 (subscription); £68, DM230 (individual). ISBN 3 527 26041 2 (Verlag Chemie); 0 89573 231 9 (Verlag Chemie International).

Methods of Enzymatic Analysis first appeared in 1962 as a single volume written in German, with the First English Edition being published in 1963. The Second English Edition appeared in 1974, having been expanded to four volumes, and for many years it became the standard text for those involved in enzymatic analysis. Volume 1, "Fundamentals," marks the start of publication of a much improved and expanded (to ten volumes) Third Edition, which is proposed to be finally completed by 1986.

Volume 1, as its title implies, deals with the fundamental aspects of enzymology and enzymatic analysis with the subject matter being divided into three major parts. Each part consists of a number of chapters written by internationally renowned scientists who have either developed a method or are most familiar with its application. This policy has ensured that the information given is reliable and contains sufficient detail to allow the application to be achieved, even in difficult circumstances.

The short introductory part proceeds from a chapter on definitions, nomenclature and units, through chapters on reliability and practicability of methods, standardisation of procedures and economic considerations to end with a chapter devoted to the present status and future aspects of enzymatic analysis. The second part, which occupies about 35% of the volume, deals with theoretical considerations such as reaction kinetics, determination of Michaelis and inhibitor constants, catalytic activity and metabolite concentrations, design of indicator reactions and the principles of enzyme immunoassays.

The remainder of the volume (which occupies about half the volume) is taken up by the third section, which deals with the techniques of measurement and instrumentation used. The nine chapters in this section deal not only with the more common measurements of volume and spectrophotometric techniques, but also nephelometry, turbidimetry, reflectance photometry, fluorimetry and luminometry with some discussion on techniques such as potentiometry, polarography, conductimetry, enthalpimetry, radiometry and applications of immobilised enzymes. Development of the modern techniques of automation, micro-determination and the use of radiobiochemicals are also covered.

Each contribution is written clearly with many diagrams and tables to make the reading enjoyable as well as informative. Each chapter contains a list of references that represent the key publications of the past 10 years or so, and is complete in its coverage up to 1982. The Editor and his staff have done an admirable job of ensuring a uniformity of style and spelling without eliminating the personal approach of the authors, a number of whom do not use English as their major language.

This book represents a series that is obligatory reading to all involved in enzymatic analysis, irrespective of their field of study. The subject matter is equally applicable to the industrial analyst, clinician, research worker, academic teacher and student and the series should find a place in the libraries of many institutions. The total price of the series perhaps excludes personal subscription, but the individual volume price may well allow the purchase of a single volume to be justified for personal use, particularly the later, more specialised volumes.

John F. Kennedy
Charles A. White

Analytical Techniques for Heavy Metals in Biological Fluids. Lectures of a Course Held at the Joint Research Centre, Ispra (Italy), 22–26 June 1981
Edited by S. Facchetti. *Ispra Courses on Chemical and Environmental Science.* Pp. xii + 228. Elsevier. 1983. ISBN 0 444 42212 9.

This book is based on 13 lectures given by experts from five European countries during the title course. The content of the book is broader than implied by its title and approximately one third of the text is devoted to non-analytical subjects such as the assessment of human exposure to pollutants, biological monitoring and the kinetics and biological effects of heavy metals. Reference is also made to hard and soft tissues and to body fluids. The elements to which greatest attention is given are cadmium and lead followed by arsenic, chromium, mercury and nickel.

The analytical papers also cover a wide range of subject matter. There are reviews of spectrochemical techniques, atomic emission and absorption analysis, voltammetry and

isotope dilution mass spectrometry, with critical comparisons of the methods available for the determination of cadmium, lead and nickel in biological material. More detailed accounts of procedures for individual elements and a well documented and comprehensive review (433 references) of ashing in trace element analysis are also presented. Serveral contributions incorporate a substantial body of information on sample handling, quality control procedures, reference materials and inter-laboratory comparisons. In view of the breadth of coverage of the book it is surprising that there is no contribution devoted to the all important, but difficult, subject of sampling.

The book, which is printed from camera ready copy, is well presented and generally free from typographical errors. Most papers include references to the literature up to 1981. This provides an adequate introduction to the literature on heavy-metal monitoring but in this continuously developing field, supplementation of this database with a wider literature search and more recent publications would generally be necessary when in-depth knowledge is required.

As this book is a multi-authored compilation of independent contributions, it inevitably lacks the smooth flow that facilitates continuous reading of a book from cover to cover and there is some overlap between one contribution and another. It is a book to be sampled; each contribution is complete in itself and may be enjoyed in the manner of a collection of short stories.

The literature on heavy metal pollution, its measurement and consequences is volumious. The course organisers have rendered a valuable service to the new worker in the field, to the general scientist, who among other duties has the responsibilities for heavy-metal pollution monitoring, and to the expert requiring a compact reference book, by bringing together within a single volume a wide range of basic information.

J. B. Dawson

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