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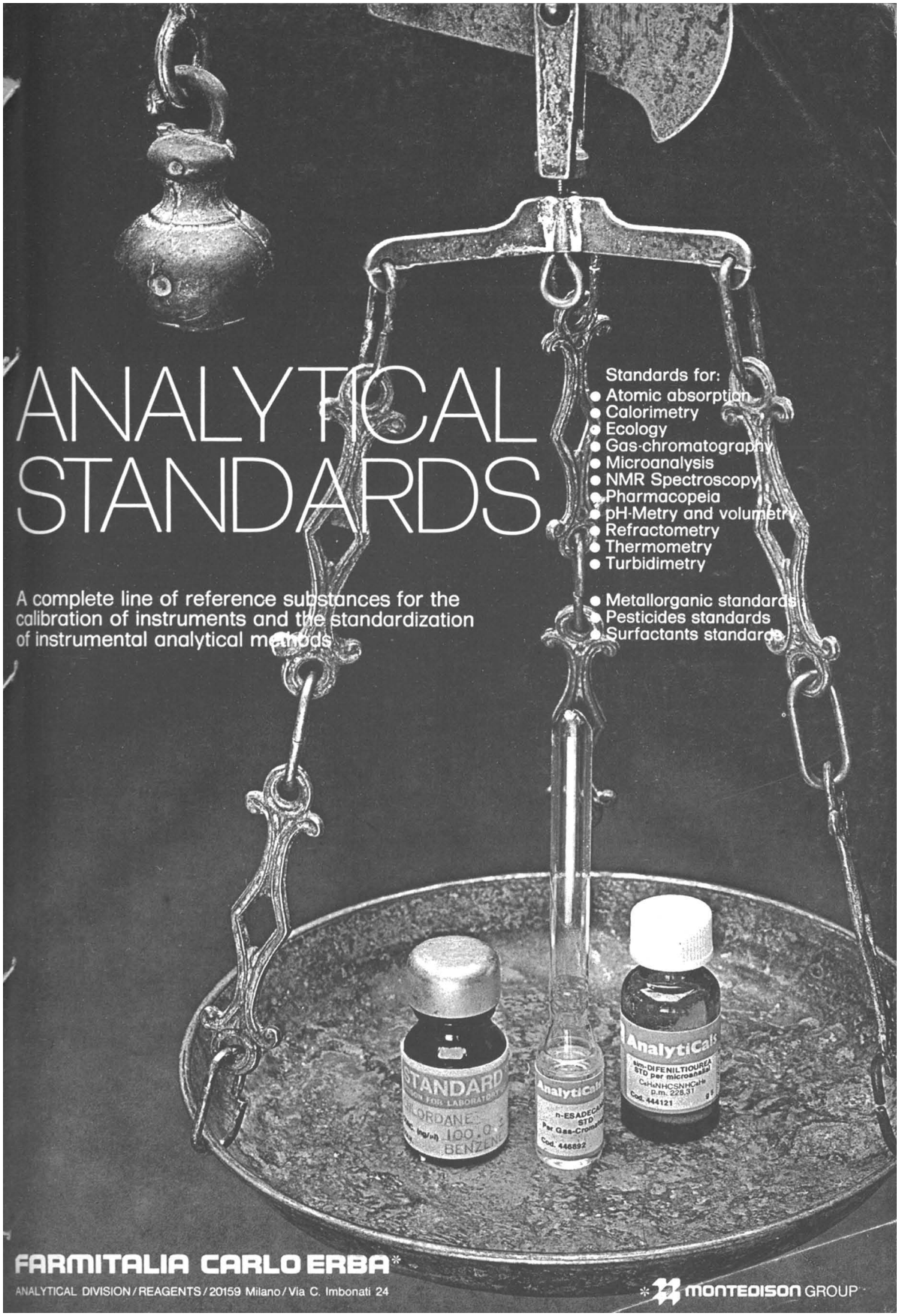
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Summaries of Papers in this Issue

Analytical Applications of Emulsions in Atomic-absorption Spectrophotometry: Determination of Zinc in Undecenoate Ointments Using Aqueous Inorganic Standards

An atomic-absorption spectrophotometric method for the determination of zinc in zinc undecenoate ointments has been developed, based on solubilisation of the sample in benzene, the formation of a stable oil-in-water emulsion using a suitable emulsifier and aspiration into the flame. Aqueous inorganic standards are used for calibration, which for emulsions might often be possible by suitable choices of both the nature and the percentage of the emulsifier. The accuracy and precision of the method are similar to those of the complexometric standard method involving mineralisation of the ointment. The proposed method has the advantages of simplicity and speed.

Keywords: *Zinc determination*; *emulsions*; *atomic-absorption spectrophotometry*; *zinc undecenoate ointments*

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Analyst, 1981, **106**, 737–742.

Method for Improving the Determination of Silicon by Atomic Absorption Spectrometry Using a Tantalum-coated Carbon Furnace

A process is described in which carbon furnace tubes are treated internally with a coating of sputtered tantalum. The method provides a three-fold increase in sensitivity over reported electrothermal methods for the determination of silicon. A practical limit of detection of 3 $\mu g\ kg^{-1}$ of silicon has been determined and the method has been shown to be suitable for the determination of total silicon in water at low levels. Calculations suggest that the signal enhancement may be a kinetic rather than a thermodynamic effect.

Keywords: *Silicon determination*; *atomic-absorption spectrometry*; *electrothermal atomisation*; *tantalum-coated furnace*

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Analyst, 1981, **106**, 743–750.

Flotation - Spectrophotometric Determination of Palladium with Thiocyanate and Methylene Blue

A sensitive and precise method for the determination of microgram amounts of palladium, based on the ion associate of the anionic palladium(II) - thiocyanate complex with Methylene Blue (MB), has been developed. The ion associate is floated with benzene and dissolved in acetone, and its molar absorptivity is found to be 1.7×10^5 l mol^{-1} cm^{-1} at 660 nm. The formation of the ion associate takes place over a wide pH range (1–6). The method is selective; interfering metals include silver, platinum, ruthenium, molybdenum and tungsten. The composition of the ion associate has been established as $[MB^+]_2[Pd(SCN)_4^{2-}]$. The proposed method has been applied to the determination of trace amounts of palladium in high-purity silver after its preliminary separation from the matrix as palladium dimethylglyoximate.

Keywords: *Palladium determination*; *spectrophotometry*; *thiocyanate*; *Methylene Blue*; *high-purity silver analysis*

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Analyst, 1981, **106**, 751–756.

Rapid Determination of Trace Amounts of Selenium in Biological Samples by Gas Chromatography with Electron-capture Detection

A rapid, simple and sensitive method of determining total selenium in biological samples is described. Selenium(IV) reacts with 1,2-diamino-3,5-dibromobenzene to form 4,6-dibromopiazselenol, which is detected by means of a gas chromatograph equipped with an electron-capture detector. In order to complete the mineralisation of selenium in seven organic compounds, a wet digestion method was employed. Thirty determinations can be made in 8 h by a modified method. Trace amounts of total selenium in NBS standard reference materials, human blood and serum were determined and compared with the certified or reported values.

Keywords: *Selenium determination*; *biological samples*; *gas chromatography*

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Analyst, 1981, **106**, 757–762.

Measurement of Phenol in Urine by the Method of Van Haaften and Sie: A Critical Appraisal

The method of Van Haaften and Sie for the measurement of urinary phenol gives better than 80% of the expected response for conjugates of phenol. However, the response is strongly dependent on instrumental conditions and safeguards must be built into the method to ensure that hydrolysis of conjugates is satisfactory.

Keywords: *Phenol*; *urine analysis*; *exposure monitoring*

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Analyst, 1981, **106**, 763–767.

Polarographic Determination of Temazepam in Soft Gelatin Capsule Formulations

Precise and accurate procedures have been developed for the polarographic determination of temazepam in soft gelatin capsules. The capsule contents can be determined either by direct-current or by differential-pulse polarography. In order to cover the possibility of temazepam having diffused into the capsule shell a whole capsule assay has been developed based on direct-current polarography: the differential pulse signal is considerably suppressed by the gelatin present in the shell. For capsules that have undergone storage tests at high temperatures leading to denaturisation, an enzyme dissolution step is included. The procedures are not subject to interference from known degradation products, and can therefore be used for stability determinations.

Keywords: *Temazepam determination*; *direct-current polarography*; *differential-pulse polarography*; *enzymatic dissolution*; *soft gelatin capsules*

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Analyst, 1981, **106**, 768–775.

Solvent Extraction - Spectrophotometric Determination of Boron in Steel with 2,4-Dinitronaphthalene-1,8-diol and Brilliant Green

On the basis of previous work, a solvent extraction procedure has been applied to the determination of boron in steel. The boron (as boric acid) is converted to the monovalent complex anion, BR_2^- (where H_2R is 2,4-dinitronaphthalene-1,8-diol), in acetic acid medium, extracted as a coloured ion associate with Brilliant Green into toluene and determined spectrophotometrically. Steel samples were dissolved in a mixture of dilute hydrochloric acid and hydrogen peroxide and large amounts of iron and other metal ions in the solution were removed either by solvent extraction with 4-methylpentan-2-one or by precipitation as hydroxides or oxinates. Micro amounts of boron in steel samples (0.0013–0.012%) were determined.

Keywords: *Boron determination*; *2,4-dinitronaphthalene-1,8-diol and Brilliant Green reagent*; *solvent extraction*; *spectrophotometry*; *steel analysis*

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Analyst, 1981, **106**, 776–781.

Determination of Residues of Dithiocarbamate Pesticides in Foodstuffs by a Headspace Method

Report by the Panel on Determination of Dithiocarbamate Residues

Keywords: *Dithiocarbamate residues analysis*; *headspace analysis*; *carbon disulphide*

COMMITTEE FOR ANALYTICAL METHODS FOR RESIDUES OF PESTICIDES AND VETERINARY PRODUCTS IN FOODSTUFFS OF THE MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (N. A. SMART, SECRETARY)
Ministry of Agriculture, Fisheries and Food, Harpenden Laboratory, Hatching Green, Harpenden, Hertfordshire, AL5 2BD.

Analyst, 1981, **106**, 782–787.

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Analytical Applications of Emulsions in Atomic-absorption Spectrophotometry: Determination of Zinc in Undecenoate Ointments Using Aqueous Inorganic Standards*

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An atomic-absorption spectrophotometric method for the determination of zinc in zinc undecenoate ointments has been developed, based on solubilisation of the sample in benzene, the formation of a stable oil-in-water emulsion using a suitable emulsifier and aspiration into the flame. Aqueous inorganic standards are used for calibration, which for emulsions might often be possible by suitable choices of both the nature and the percentage of the emulsifier. The accuracy and precision of the method are similar to those of the complexometric standard method involving mineralisation of the ointment. The proposed method has the advantages of simplicity and speed.

Keywords: *Zinc determination*; *emulsions*; *atomic-absorption spectrophotometry*; *zinc undecenoate ointments*

Zinc is often present in pharmaceutical creams, pastes and ointments for external application as stearate, naphthenate and/or undecenoate salts, the therapeutic properties of which are well known. All of these compounds and their bases are generally insoluble in water; therefore, most methods for the determination of zinc in these samples involve a mineralisation step. Subsequently, zinc is titrated complexometrically with EDTA[1] or determined by atomic-absorption spectrophotometry (AAS).[2] Alternatively, methods based on isotopic dilution[3] or neutron activation[4] may be used.

Recently, we have developed a method for the determination of metallic elements in liquid organic samples based on the formation of an oil-in-water emulsion from a small amount of the sample and a larger amount of water in the presence of a suitable emulsifier; this emulsion is aspirated into the flame of an atomic-absorption spectrophotometer. We have applied this method successfully to the determination of lead in used lubricant oils[5] and gasoline.[6] In addition to its simplicity, the proposed method overcomes or minimises most of the known problems derived from the introduction of organic solvents into the flame.[7] Regarding calibration graphs, emulsions were used, which for oils were prepared from inorganic standard salts.

In this paper, we examine the results obtained when this method was applied to the determination of zinc in a zinc undecenoate ointment. Particular attention is devoted to the determination of experimental conditions for using aqueous inorganic standards, without forming emulsions, to prepare calibration graphs.

Experimental

Apparatus

A Pye Unicam atomic-absorption spectrophotometer was used, measuring the zinc line at 213.9 nm. Experimental parameters of the instrument were adjusted to obtain maximum sensitivity. A Kerry Pulsatron 250 ultrasonic bath with an oscillating frequency of 50 kHz was used to prepare the emulsions.

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

Reagents

In order to apply the complexometric standard method, reagents specified in the British Pharmacopoeia were used.[1]

The emulsion method requires the use of benzene as a solvent and a stock solution of zinc nitrate containing 100.0 p.p.m. of zinc (both reagents of analytical-reagent grade), together with Tween 80 non-ionic lipophilic emulsifier [polyoxyethylene-20-sorbitan monooleate, hydrophilic lipophilic balance (HLB) 15.0].

Caution—Benzene is highly toxic and appropriate precautions should be taken.

Tween 20 non-ionic lipophilic emulsifier (polyoxyethylene-20-sorbitan monolaureate, HLB 16.7) and Brij 30 non-ionic lipophilic emulsifier (polyoxyethylene-4-lauryl ether, HLB 9.7) were also used in some experiments.

Sample

Undecenil ointment from UCB-Pevya S.A. (Spain) was used, with the following approximate composition per 100 g of sample: 20 g of zinc undecenoate, 5 g of undecenic acid and 2 g of geraniol and ointment base.

Benzene sample solution

A 0.4479-g amount of the sample was transferred into a vessel and heated gently in a water-bath. A few millilitres of benzene were added and heating was continued until total solubilisation took place. The solution was transferred into a 250-ml calibrated flask and diluted to volume with benzene.

Procedures

Standard method

The method specified in the British Pharmacopoeia was applied.[1]

Emulsion method

To a 50-ml flask were added about 25 ml of distilled water, 1.50 g of Tween 80 emulsifier (with gentle shaking) and 1 ml of the benzene sample solution, the volume being made up with distilled water. After shaking the flask for a few seconds, it was placed in an ultrasonic bath for 5 min and the resulting emulsion was aspirated into the flame. The calibration graph was obtained by using aqueous zinc nitrate standards in the range 0.1–1 p.p.m. of zinc.

Results and Discussion

Solubilisation of the sample

Although the emulsion could be prepared directly from a small amount of sample, the high content of zinc makes this unsatisfactory because emulsion volumes of about 10–25 ml are sufficient in atomic-absorption spectrophotometry and errors might be introduced with amounts of sample less than 0.1 g. However, as might be predicted from the nature of the sample, qualitative solubility tests showed that it is soluble in non-polar organic solvents. Among those most commonly used in analytical laboratories, benzene, which is easily emulsified, seems the most suitable (solubility about 0.2 g per 100 ml). Therefore, in spite of its carcinogenicity, this solvent was used to dissolve the sample.

In order to obtain oil-in-water emulsions, preferably with concentrations of organic solvent below 10%, small volumes of the benzene solution must be emulsified. This factor, in addition to the percentage of zinc in the sample, the solubility of the sample in benzene and the sensitivity of zinc in the emulsion, was taken into account in selecting the mass of the sample, the volume of benzene in which it was dissolved to prepare the stock solution and the dilution factor of this solution for obtaining the emulsions. In this work, 1 ml of benzene solution produced suitable absorbance values of about 0.2–0.3.

Choice of emulsification method

Benzene solutions of the sample are easily emulsified. With regard to the stability of emulsions and the sensitivity and reproducibility of the zinc absorbance, the most suitable method for the preparation of emulsions was that based on dissolving the emulsifier in the aqueous phase and adding the organic solution to it.

Effects of HLB and nature of the emulsifier

Obviously, in order to be of use analytically, an emulsion must be stable long enough to be handled. For our purposes, an emulsion was assumed to be stable when no significant differences in the absorption signal were observed on aspirating samples of emulsion taken from different points in the bulk sample.

It is known[6] that stable emulsions may be obtained in the presence of a small amount of a suitable emulsifier. The ability of an emulsifier to stabilise an emulsion depends on its nature, which is mainly indicated by its HLB, especially for non-ionic emulsifiers, although other properties must also be taken into account. Foaming emulsifiers should be avoided because they may introduce heterogeneities in the concentration of the element in the emulsion. On the other hand, water-soluble emulsifiers are to be preferred as they avoid the need for emulsification of additional organic phases in which the emulsifier might be solubilised.

The effect of the emulsifier HLB on sensitivity to zinc is shown in Fig. 1. Emulsifiers with the HLB values shown were prepared by suitable mixing of Brij 30 and Tween 20 emulsifiers, the HLB value of the mixture being the average of those of the components. Although the differences were not high, maximum sensitivity was obtained for HLB values close to 15, which was considered to be the optimum value. Stabilities were similar for all emulsions for at least 5 h.

The nature of the emulsifier affects the sensitivity to zinc, as can be deduced by comparing the above results with those in Table I. Again, maximum sensitivity was obtained for an HLB value of 15, supplied by the Tween 80 emulsifier. It must be emphasised that this sensitivity is about 10% higher than that obtained with the mixed Brij 30 - Tween 20 emulsifier, and this was also true for the other HLB studied.

TABLE I

EFFECT OF HLB ON SENSITIVITY TO ZINC

Volume of benzene solution, 1 ml; emulsification time, 5 min.

Percentage of emulsifier in the emulsion				
Brij 30	Tween 80	Tween 20	HLB	Absorbance
1.15	1.86	—	13	0.143
0.57	2.43	—	14	0.146
—	3.00	—	15	0.150
—	1.19	1.81	16	0.141
—	—	3.00	16.7	0.134

Effect of the percentage of emulsifier

The percentage of the emulsifier affects the nature of the emulsion. Qualitatively, milky emulsions obtained with low percentages of emulsifier become translucid in the presence of high percentages, which may correspond to the formation of micro-emulsions involving a decrease in the mean droplet diameter of the dispersed benzene phase. By taking this qualitative criterion as a basis, the percentage of the emulsifier necessary to produce this change is roughly proportional to the volume of the benzene phase, as may be deduced from Fig. 2.

The quantitative effect of the percentage of emulsifier on sensitivity to zinc for two emulsifiers is shown in Fig. 3. Sensitivity increases with percentage of emulsifier up to a limit of 3%, which corresponds approximately to the change from an emulsion to a micro-emulsion.

Effect of emulsification time

As has been mentioned earlier,[6] in order to obtain an emulsion it is necessary to supply a certain amount of energy. Mechanical techniques may be used; however, with respect to reproducibility for analytical purposes ultrasonic techniques seem more suitable, particularly when the organic phase is lighter than water, apart from being easier to handle.

The effect of emulsification time is shown in Fig. 4. Sensitivity increases with emulsification time and becomes independent after about 5 min. Emulsions are stable for at least

5 h for emulsification times greater than 5 min; however, with shorter emulsification times, both sensitivity and stability decrease significantly.

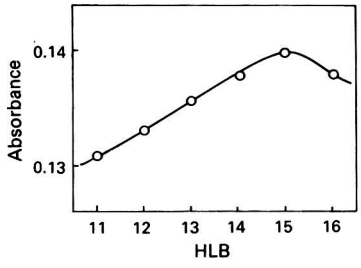

Fig. 1. Effect of the HLB on sensitivity to zinc. Volume of the benzene solution, 1 ml; HLB values obtained from Tween 20 and Brij 30 emulsifiers; emulsifier concentration, 3%; emulsification time, 5 min.

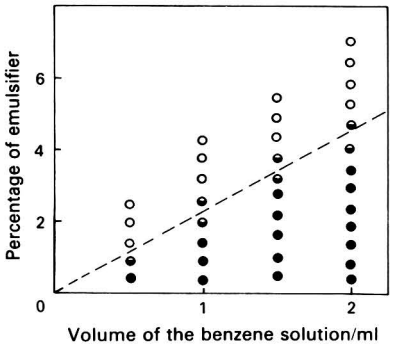

Fig. 2. Effect of percentage of emulsifier on the nature of emulsions. Emulsifier, Tween 80; emulsification time, 5 min. Appearance of emulsions: ●, milky; and ○, translucent.

Choice of emulsifier and its percentage when using aqueous standards for calibration

It can be deduced from the above results that the sensitivity to zinc in emulsions depends on both the nature and the percentage of the emulsifier. Hence it may be possible to adjust the experimental conditions, by a suitable choice of these two variables, in order to equalise the sensitivity to zinc in emulsions and aqueous solutions.

The effect of the percentage of emulsifier on sensitivity to zinc is shown in Fig. 5. Emulsions were prepared from several aliquots of benzene solutions containing different concentrations of zinc and using Tween 80 emulsifier (the concentration of zinc in the sample was determined by the standard method[1]). This procedure was applied in order to avoid any effect on sensitivity of the percentage of benzene in emulsions. However, no significant differences were observed between these calibrations and those obtained with different volumes of benzene solution, at least for volumes below 3 ml. As shown in Fig. 5, the slope of calibrations increases with increasing percentage of emulsifier and attains a limit, which is in agreement with the results shown above. This limiting slope is higher than that obtained

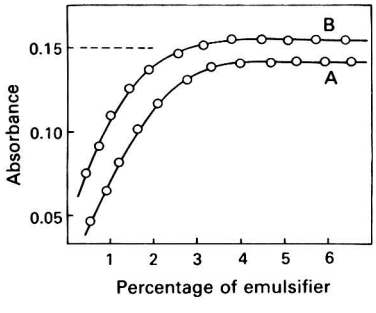

Fig. 3. Effect of percentage of emulsifier on sensitivity to zinc. Volume of the benzene solution, 1 ml; emulsification time, 5 min. Emulsifiers: A, Tween 20 - Brij 30 (3 + 1); and B, Tween 80. Dotted line: aqueous nitrate solution containing 1 p.p.m. of zinc.

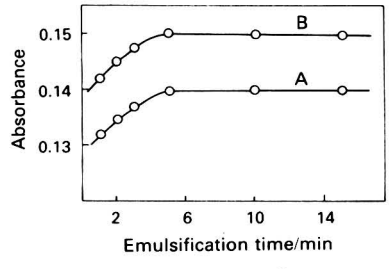

Fig. 4. Effect of emulsification time on sensitivity to zinc. Volume of the benzene solution, 1 ml; emulsifier concentration, 3%. Emulsifiers: A, Tween 20 - Brij 30 (3 + 1); and B, Tween 80.

from aqueous nitrate solutions, which shows the possibility of equalising the slopes of both emulsion and aqueous zinc nitrate calibrations by choosing a suitable percentage of Tween 80 emulsifier. It must be emphasised that, according to Fig. 3, this is not possible when using the mixed Tween 20 - Brij 30 emulsifier because the limiting sensitivity is lower than that for aqueous nitrate solutions.

A simple linear interpolation between two percentages giving sensitivities higher and lower than that of the aqueous nitrate solution in Fig. 3 should allow one to determine the percentage of emulsifier for which the sensitivity in aqueous solutions is equal to that in emulsions. However, as the points for these two percentages are affected by random errors, it was preferred to carry out this interpolation between the slopes of calibrations obtained from the two percentages in Fig. 5. The percentage of emulsifier calculated in this way is 3.00, which is easy to adjust by adding the emulsifier drop by drop from a pipette, each drop containing a few centigrams of material. In order to confirm this calculation, a calibration for this percentage was obtained (Fig. 5, C). Apparently, it is linear and coincident with that obtained from aqueous nitrate solutions for zinc concentrations below 1 p.p.m., although differences become apparent at higher concentrations.

Taking as a basis the points on the emulsion and aqueous calibrations between which linearity occurs, equations for the corresponding straight lines were determined by the least-squares method. In all instances, the independent terms in these equations were negligible, being lower than the precision of both the concentration and absorption measurements. This permitted an easy statistical comparison of slopes by applying Student's *t*-test. The results showed that small differences observed in the slopes may be attributed to random errors.

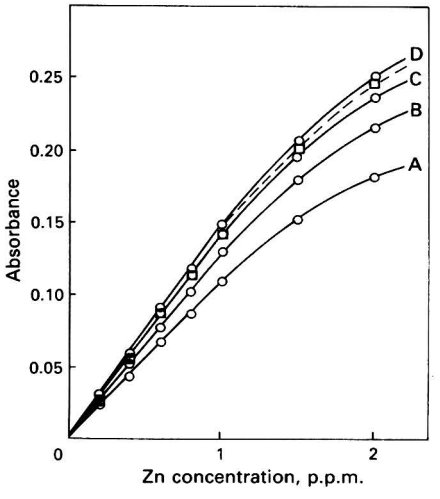

Fig. 5. Effect of the percentage of emulsifier on sensitivity to zinc in emulsion calibrations. Volume of benzene solution, 1 ml; emulsifier, Tween 80; emulsification time, 5 min. Emulsifier concentrations: A, 1%; B, 2%; C, 3%; and D, 4%. Dotted line: aqueous zinc nitrate calibration.

Determination of zinc in samples

In order to determine the zinc content in the sample, emulsions were prepared by the procedure specified under Experimental, using 3.00% of Tween 80 emulsifier. The reproducibility was determined by applying this procedure to ten replicates of the sample and the results are shown in Table II.

TABLE II

COMPARISON OF STANDARD COMPLEXOMETRIC AND EMULSION METHODS

	Method	
Parameter	Complexometric	Emulsion
$\bar{x}$*	3.05†	3.03†
s	0.013	0.023
s_r	0.43	0.76

* Average of ten determinations.
† Percentage of zinc in the sample.

The accuracy was determined by comparing the results obtained by the emulsions and standard methods when applied to ten replicates of the sample. The latter method is based on mineralisation of the sample by refluxing with hydrochloric acid and further complexometric titration of zinc. The F-factor showed that there were no significant differences between the standard deviations of the two methods at the 95% probability level. On the other hand, when the two methods were compared, the calculated Student's t-value was less than the tabulated value, which indicates that the small differences observed in the percentages of zinc determined by these methods may be attributed to random errors (see Table II).

It must be emphasised that the proposed method is simpler than the standard method; as well as avoiding the mineralisation step, only a few, common reagents are used. In addition, problems derived from the direct introduction of organic solvents into the flame are minimised or even obviated.

Conclusions

In this paper, the utility of oil-in-water emulsions in combination with AAS is established. A method for the determination of zinc in a pharmaceutical ointment containing zinc undecenoate has been developed. The sample is dissolved in benzene, an aliquot is emulsified in the presence of Tween 80 emulsifier and the zinc content in the emulsion is determined using a standard aqueous solution of zinc nitrate. This was possible because the sensitivity to zinc in emulsions depends on both the nature and the percentage of the emulsifier; thus, by a suitable choice of these parameters, the sensitivities of aqueous and emulsion calibrations can be equalised. The precision of the emulsion method is similar to that of the standard method. A statistical comparison with the standard method indicated that the small differences in the results of the proposed method may be attributed to random errors. The method may be applied to other similar samples that are soluble in organic solvents. The emulsion method avoids mineralisation steps and also minimises or even obviates problems due to the aspiration of organic solvents into the flame; consequently, it is simpler than the standard complexometric method. The results are promising with regard to the possibility of using aqueous inorganic standards for calibration for the determination of metallic elements in organic samples after solubilisation in organic solvents and the formation of oil-in-water emulsions.

References

1. "British Pharmacopoeia 1973," H.M. Stationery Office, London, **1973**, p. 505.
2. Moody, R. R., and Taylor, R. B., *J. Pharm. Pharmacol.*, 1972, **24**, 848.
3. Tolgyessy, J., Sarsunova, M., and Majer, J., *Cesk. Farm.*, 1959, **8**, 565.
4. Margoris, M., and Lambert, J. P. F., *J. Pharm. Sci.*, 1971, **60**, 592.
5. Hernández Méndez, J., Polo Díez, L., and Bernal Melchor, A., *Anal. Chim. Acta*, 1979, **108**, 39.
6. Polo Díez, L., Hernández Méndez, J., and Pedraz Penalva, F., *Analyst*, 1980, **105**, 37.
7. Cresser, M. S., "Solvent Extraction in Flame Spectroscopic Analysis," Butterworths, London, 1978.

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Method for Improving the Determination of Silicon by Atomic-absorption Spectrometry Using a Tantalum-coated Carbon Furnace

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A process is described in which carbon furnace tubes are treated internally with a coating of sputtered tantalum. The method provides a three-fold increase in sensitivity over reported electrothermal methods for the determination of silicon. A practical limit of detection of 3 μg kg^{-1} of silicon has been determined and the method has been shown to be suitable for the determination of total silicon in water at low levels. Calculations suggest that the signal enhancement may be a kinetic rather than a thermodynamic effect.

Keywords: Silicon determination; atomic-absorption spectrometry; electrothermal atomisation; tantalum-coated furnace

Silicon occurs in water in several forms, not all of which can be determined directly by the spectrophotometric method, based on the formation of reduced β-molybdosilicic acid, developed by Morrison and Wilson.[1] The several forms of silicon may be conveniently classified as either "reactive" silicon or "non-reactive" silicon, terms which are defined, respectively, as those forms of silicon which react or do not react with acidified ammonium molybdate in 10 min under the conditions specified by Webber and Wilson.[2] These two forms of silicon make up the total silicon content of water, the determination of which has previously involved conversion of the "non-reactive" form of silicon to the "reactive" form by methods that either are time consuming or lack the precision required by the electricity generating industry.[3–5] The elimination of such a conversion stage, particularly by replacement with a rapid method of analysis capable of detecting silicon concentrations below 20 μg kg^{-1} and having a standard deviation of about 2 μg kg^{-1}, was evidently desirable.

Atomic-absorption spectrometry provides the required speed of analysis, and recent work on electrothermal atomisation techniques[6–8] showed that the desired precision might also be attainable. This paper describes a method of carbon furnace-tube pre-treatment that is shown to enhance the atomisation of silicon, and compares it with other procedures that have been used to obtain improved sensitivities for other elements.[9,10]

Experimental

Apparatus

A Varian Techtron CRA-90 carbon rod atomiser was used in conjunction with a Varian Techtron, Model 1150, atomic-absorption spectrometer. Peak signals were recorded using an Oxford Instruments, Series 3000, fast-response, flat-bed recorder. A Varian Techtron single-element hollow-cathode lamp was used to determine silicon at 251.6 nm. A lamp current of 10 mA and spectral band width setting of 0.2 nm were used for all measurements except where stated otherwise. The carbon furnace tubes supplied by Varian Techtron incorporate a pyrolytic carbon surface and consist of a graphite tube of length 9 mm, outside diameter 5 mm and wall thickness 1 mm. The purge gas used in the CRA-90 work-head was nitrogen.

The equipment used for furnace-tube pre-treatment was a vacuum chamber manufactured by Edwards High Vacuum Ltd., which incorporated appropriate vacuum-sealed electrical connections, and valves for the admission of a suitable gas, in this instance argon.

Reagents

"Reactive" silicon standard solution. A stock solution was prepared by fusing 1.000 g of pure, dry silica with 5.000 g of anhydrous sodium carbonate in a platinum crucible at red

heat and dissolving the cooled melt in distilled water. The resulting solution was diluted to 1 dm^3. A working solution was prepared by dilution of the stock solution.

"Non-reactive" silicon standard solution. A stock solution was prepared by adding bentonite clay to silicon-free water and stirring the resulting mixture until a uniform suspension was obtained. This was centrifuged until very little clay was removed during 1 h of centrifuging. The total silicon content of the final suspension was determined using the method of Morrison and Wilson.[11] A working "solution" was prepared by dilution of the stock suspension.

The "reactive" silicon content of the suspension was determined by the method of Webber and Wilson[2] and was found to be less than 1% of the total silicon concentration.

Tantalum hydroxide suspension, 2%. Tantalum powder (2 g) was dissolved in a mixture of 8 cm^3 of hydrofluoric acid (40%) and 10 cm^3 of nitric acid (70%). The solution was diluted to 100 cm^3 and an excess of sodium hydroxide solution was added. The precipitate of tantalum hydroxide was washed and centrifuged three times and was finally shaken with and suspended in 100 cm^3 of silicon-free water. It was necessary to shake the suspension vigorously on each occasion before use.

Tantalum foil, thickness 0.25 mm. Koch-Light Laboratories Ltd.

Tantalum wire, diameter 0.25 mm. Koch-Light Laboratories Ltd.

Ammonium nitrate solution, 1000 mg kg^{-1}. Analytical-reagent grade.

Ascorbic acid solution, 1000 mg kg^{-1}. Analytical-reagent grade.

Carbon Furnace Pre-treatment

Each furnace-tube to be used subsequently in the CRA-90 carbon rod atomiser was pre-treated using one of the following procedures. The effectiveness of the pre-treatment was assessed by measuring the instrument response when the treated furnace tube was used to determine a standard amount of silicon. An untreated furnace tube was used as a reference.

Tantalum foil lining

The furnace tube was drilled out to a larger inner diameter so that insertion of a tantalum foil tube would restore the original dimensions. The tantalum foil tube was a close fit inside the furnace and incorporated a small hole aligned to allow addition of the silicon solution to the furnace.

Pyrolysis of tantalum hydroxide

Procedure (a). The furnace tube was placed in position in the CRA-90 work-head and three 10-μl portions of a tantalum hydroxide suspension (2%) were added to it. After each single addition, the furnace tube was subjected to the following atomisation cycle: drying at 100 °C for 75 s, ashing for 1000 °C for 30 s and atomising at 2600 °C for 5 s, the heating ramp rate being 400 °C s^{-1}.

Procedure (b). A tantalum hydroxide suspension (50 μl) was added to the furnace tube, which had been dried at 105 °C for 5 min in a hot air oven. The furnace tube was then placed in the CRA-90 work-head and subjected to a cycle that involved ashing at 500 °C for 5 s and atomising at 2500 °C for 2 s, the ramp rate being 400 °C s^{-1}. This procedure was used to examine the effect of varying the amount of tantalum hydroxide up to a maximum of 150 μl of a 2% suspension.

High-temperature evaporation of tantalum.

The furnace tube was placed in a vacuum chamber with a tantalum wire stretched along its axis between two electrodes. The chamber was evacuated to a pressure of 5×10^{-5} mmHg and the temperature of the wire was raised by resistance heating to a value just below its melting-point. These conditions could be maintained for periods of up to about 5 min. In order to increase the total treatment time, evaporation of tantalum from several lengths of wire was used in some instances to treat a single furnace-tube.

Tantalum coating by sputtering.

This method involved passing an electrical discharge between two electrodes at low gas pressure, under which conditions the cathode surface is slowly disintegrated by the bombard-

ment of the ionised gas molecules, either to be condensed on the surfaces surrounding the cathode or to be returned to the cathode by collision with gas molecules. Cathodic sputtering is influenced by many factors, among which are the applied voltage, the gas pressure, the glow discharge current and the electrode geometry. It is also necessary to eliminate or at least to suppress undesirable glow discharges resulting, for example, from lead-in electrodes. This was done by adequate shielding of all cathode material apart from the actual material to be sputtered.

The most suitable arrangement was found to be that depicted in Fig. 1, in which the carbon furnace tube is located above the cathode terminal with its axis vertical. The tantalum wire cathode (diameter 0.25 mm, length 20 mm) was a loose fit inside a hole drilled in the cathode terminal and was arranged vertically along the axis of the furnace tube. The cathode terminal was fitted with a turned and threaded PTFE collar to prevent undesirable discharges. Sputtering from the end of the cathode terminal was minimised by fitting an earthed ring above the Teflon collar. The whole of the cathode - substrate assembly was enclosed in the vacuum chamber.

In order to carry out a coating procedure, the chamber was evacuated to a pressure of about 0.01 mmHg and a potential was applied between the tantalum wire cathode and the furnace-tube anode. Argon gas was slowly admitted into the chamber and the applied potential was changed to obtain an optimum position for the discharge boundary within the furnace tube. Applied potentials of 0.5–1.0 kV and argon gas pressures of about 1.0 mmHg gave acceptable results. Several furnace tubes were treated in this way for various periods and at different potentials and discharge current values.

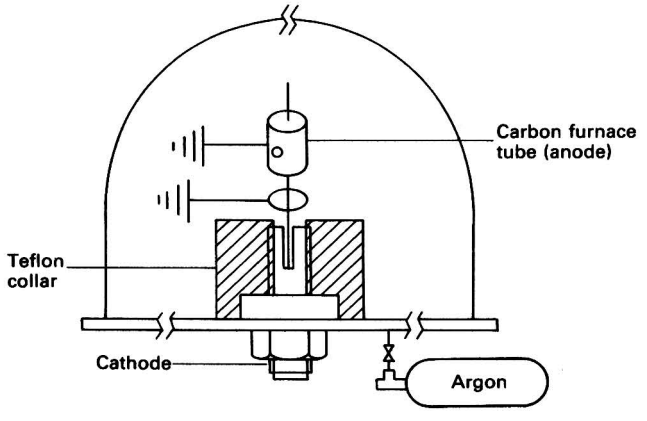

Fig. 1. Sputtering assembly.

The use of additives to the test solution

Both oxidising and reducing agents have been reported to have a beneficial effect on the determination of various elements using electrothermal atomisation techniques.[9,10] A series of solutions was prepared that contained ammonium nitrate at concentrations between 20 and 500 mg kg^{-1}. A second series of solutions was prepared containing ascorbic acid at the same concentrations. Each solution contained 5 mg kg^{-1} of silica. The instrument response to the silica content of each solution was determined using an untreated furnace-tube and a furnace-tube that had been treated by sputtering.

Preliminary Work

The effects of ashing time and temperature, atomisation temperature and rate of heating were examined for untreated furnace tubes (and subsequently for sputtered furnace tubes); the final choice of operating conditions is listed in Table I together with details of other basic parameters.

TABLE I

OPERATING CONDITIONS

Drying temperature and time	100 °C for 75 s
Ashing temperature and time	1000 °C for 5 s
Atomisation temperature and time	2600 °C for 1 s
Heating rate	400 °C s^{-1}
Lamp current	10 mA
Spectral band width	0.2 nm
Wavelength	251.6 nm
Operating mode	Absorbance
Test solution volume	10 μl
Test solution concentration*	5 mg kg^{-1} of silica

* The quoted concentration is equivalent to 2.34 mg kg^{-1} of silicon as Si and therefore corresponds to an absolute mass of 23.4 ng of silicon.

A calibration graph was prepared using an untreated furnace tube and was shown to be linear over the range 0–1 mg kg^{-1} of silica. The conditions specified in Table I were used with the exception that the instrument was used in the concentration mode to give about a three-fold scale expansion. The linearity of the calibration graph over this range was subsequently confirmed for a sputtered furnace tube, when the instrument was used in the absorbance mode. Measurements for more concentrated solutions (up to 5 mg kg^{-1} of silica) indicated that a significant deviation from linearity occurred above about 4 mg kg^{-1}.

Preliminary work using untreated furnace tubes showed that the instrument response to silicon in the form of a bentonite clay suspension ("non-reactive" silicon) was equal to that from a solution of sodium silicate ("reactive" silicon). The "reactive" silicon solution was used to test for inherent differences between furnace tubes supplied by the manufacturer. For this purpose, five successive determinations of silicon in the standard solution were made using each of four untreated furnace tubes. Examination of the measured absorbance figures by the analysis of variance method did not reveal any significant differences between these furnace tubes. The remaining work was carried out using furnace tubes from a single batch delivery.

Results

Initially, the instrument response obtained from the atomisation of 10 μl of a solution containing 5 mg kg^{-1} of reactive silica was measured. The results from the various treatments are summarised in Table II.

For sputter-coated furnace tubes, it was later shown that identical instrument responses were obtained from the "reactive" silicon solution and from the bentonite clay, *i.e.*, "non-reactive" silicon suspension. These values are included in Table V.

Although improved sensitivities were obtained by pyrolysis of tantalum hydroxide, this was not considered to be a satisfactory procedure because reproducible improvements could not be obtained. However, pyrolysis by procedure (*b*) extended the useful life of the furnace tubes in their improved condition and reduced the between-tube response differences. Furnace tubes so treated were used to examine the effect of the amount of tantalum hydroxide used. The results are given in Table III.

Visual examination of furnace tubes treated by high temperature evaporation showed a roughening of the interior surface and an unevenly distributed deposit of metallic appearance. The effect of treatment time is given in Table IV.

Furnace tubes that were subjected to the sputtering process were observed to be coated internally with an apparently uniform film of a strongly adherent material of metallic appearance. The effect of using different values of time, current and potential is given in Table V.

Sensitivity and Detection Limits

Tests were carried out on water from an industrial de-ionisation process which contained 0.005 mg kg^{-1} of silicon according to an analysis by the method of Webber and Wilson.[3] Because of the low silicon concentration in this water, the instrument was used in the concentration mode with scale expansion and the results were converted to silicon concentration

TABLE II

EFFECTS OF VARIOUS FURNACE-TUBE TREATMENTS

Treatment	Effect on sensitivity compared with an untreated tube	Comments
Tantalum foil lining	No response	No improvement when atomisation temperature increased
Pyrolysis of tantalum hydroxide, procedure (*a*)	3-fold increase	Rapid deterioration. Variable improvement between tubes
Pyrolysis of tantalum hydroxide, procedure (*b*)	3-fold increase	Less scatter between tubes than for procedure (*a*). Improved sensitivity maintained for up to 50 cycles. Amount of tantalum hydroxide has an effect—see Table III
High-temperature evaporation	3-fold increase	Treatment conditions difficult to reproduce. Treatment time has an effect—see Table IV
Sputtering	3-fold increase	Treatment reproducible. Improved sensitivity maintained for over 300 cycles
Additives	No improvement	Some evidence of tube degradation

TABLE III

EFFECT OF THE AMOUNT OF TANTALUM HYDROXIDE USED

Volume of 2% tantalum hydroxide/μl	0	10	20	50	100	500
Absorbance (means of 5 determinations)	0.192	0.531	0.500	0.584	0.558	0.593

by comparison with a standard 0.2 mg kg^{-1} silicon solution. Ten replicate determinations were made for both an untreated furnace-tube and a sputter-coated furnace-tube. The results are shown in Table VI.

Discussion

This paper has shown that carbon furnace tubes can be treated to improve the sensitivity of the determination of silicon, suggesting that the surface properties of the furnace-tube are important in the production of the silicon atomic vapour. The part played by the surfaces of untreated furnace tubes has been considered by Campbell and Ottaway,[12] who established a correlation between the "appearance temperature*" of an element M, and the temperature at which the standard free energy change becomes negative for the reaction

$$MO_{(s)} + C_{(s)} \rightarrow CO_{(g)} + M_{(g)}$$

This indicates that although there is an almost complete lack of reducing environment inside a carbon furnace tube, it is probable that many metal oxides are converted into metal atoms by reaction with the carbon surface. Fuller[13] adopted a kinetic approach to explain the

TABLE IV

EFFECT OF HIGH-TEMPERATURE EVAPORATION TREATMENT TIME

Treatment time/min	0	0.1	2.5	10.5	17.0
Absorbance (means of 5 determinations)	0.201	0.228	0.289	0.581	0.466

* The appearance temperature was defined as the lowest temperature at which a substantial proportion of atoms of the element appeared in the furnace-tube under specified conditions.

TABLE V

EFFECT OF TREATMENT WITH SPUTTERED TANTALUM

Furnace tube No.	Sputtering parameters: Time/ min	Current/ mA	Potential/ kV	Estimate of sputtered material*	Absorbance† (*a*)	Absorbance† (*b*)
Untreated	0	0	0	0	0.196	0.204
1	5	2	0.4	4	0.422	0.415
2	10	5	0.5	25	0.538	0.533
3	10	5	0.6	30	0.589	0.602
4	2	27	0.7	37.8	0.545	0.541
5	15	7	0.5	52.5	0.521	0.539
6	60	1	1.0	60	0.617	0.595
7	20	3	1.0	60	0.627	0.620
8	15	7	0.7	73.5	0.600	0.612
9	15	14	0.5	105	0.566	0.592
10	4	40	0.7	112	0.577	0.569
11	30	10	0.5	150	0.532	0.551
12	60	10	0.6	360	0.560	0.575

* Based on time × current × potential.
† Values are means of 5 determinations; (*a*) "reactive" silicon and (*b*) "non-reactive" silicon.

mechanism of the atomisation process and demonstrated the importance of reaction rates in producing an absorption signal.

However, the efficiency of atomisation for treated furnace tubes may also depend on the ease with which competing reactions can occur. Therefore, standard free energy ($\Delta G^{\ominus}$) calculations have been carried out for the reactions listed in Table VII in order to determine a possible explanation for the improved sensitivity obtained in the presence of tantalum.

Reactions (1)–(4) represent possible reactions that produce free silicon atoms, whereas reactions (5) and (6) are suggestions for mechanisms that reduce the free atom population by involving the furnace-tube surface. Reactions (7) and (8) have been considered as possible important reactions that affect the surface of the furnace tube. Calculations were carried out at 2600 °C, this being the lowest temperature at which maximum sensitivity was achieved in the experimental work.

The standard free energy changes at 2600 °C suggest that: free silicon atom production is thermodynamically easier from a carbon surface than from a tantalum carbide surface; removal of silicon from the atom population is thermodynamically easier at a tantalum carbide surface than at a carbon surface; reaction (7) is unlikely to contribute; and tantalum carbide is thermodynamically stable as a surface coating at 2600 °C.

A possible mechanism for the production of silicon atoms at a carbon surface may therefore be represented as

$$2C_{(s)} + SiO_2 \rightarrow 2CO_{(g)} + Si_{(g)}$$

and

$$Si_{(g)} + C_{(s)} \rightarrow SiC_{(s)}$$

where the latter reaction represents a process that reduces the silicon atoms available for determination; these reactions are in accord with the process for the commercial manufacture of carborundum, which involves heating silica and an excess of carbon electrically. Although a similar mechanism may be postulated for a tantalum carbide surface, thermodynamic calculations suggest that this would not lead to the increased sensitivity observed. It is likely, therefore, that the effect is kinetic, either the release of silicon is faster at a tantalum carbide surface than at a carbon surface or the rate of reaction of free silicon atoms at a tantalum carbide surface is slower than at a carbon surface.

The significance of kinetic effects was investigated for copper by Fuller,[13] who was able to demonstrate a difference between the time - absorbance profiles for the atomisation of copper

TABLE VI

PRECISION MEASUREMENTS

Furnace tube	Mean concentration of silicon/mg kg^{-1}	Standard deviation/ mg kg^{-1}	Sensitivity/ mg kg^{-1}	Detection limit/ mg kg^{-1}
Untreated	0.005	0.005	0.029	0.010
Sputtered	0.005	0.0015	0.008	0.003

without and with a tantalum liner fitted to a graphite furnace. An attempt to confirm the above conclusions in this work by carrying out analogous time - absorbance measurements using silica, and treated and untreated furnace tubes, was not successful.

The main object of the investigation was to find a rapid, sensitive method for the determination of total silicon in power-station feed waters. This aspect of the work has proved to be very successful and consideration of Table II indicates that there is a three-fold improvement in sensitivity for tantalum-treated furnace tubes over untreated furnace tubes. This is supported by the values in Table V, which relate absorbance to an estimate of the sputtered material. As the time, current and potential all have an effect on the sputtering rate, the estimate has been left dimensionless. It is apparent, however, that enhancement of the sensitivity has an upper limit.

The use of two techniques, sputtering and tantalum hydroxide pyrolysis, to reinforce each other did not give any further improvement in sensitivity. This finding is consistent with work carried out by Thompson *et al.*,[14] who found that the use of pyrolytic coatings and the addition of a large excess of calcium nitrate, although separately enhancing the silicon absorption signal, failed to have an additive effect. Similar results were obtained by Hocquellot and Labeyrie,[9] who worked with tantalum carbide coated furnaces and used ammonium nitrate as a "releasing agent."

TABLE VII

STANDARD FREE-ENERGY VALUES FOR COMPETING ATOMISATION REACTIONS

	Reaction	$\Delta G^{\ominus}_{\text{reaction}}$ at 2600 °C/kJ mol^{-1}	Temperature of sign change/°C
(1)	$2C_{(s)} + SiO_{2(s)} \rightarrow 2CO_{(g)} + Si_{(g)}$	−237	2470
(2)	$C_{(s)} + SiO_{2(s)} \rightarrow CO_{2(g)} + Si_{(g)}$	+678	2100
(3)	$2TaC_{(s)} + SiO_{2(s)} \rightarrow 2CO_{(g)} + Si_{(g)} + 2Ta_{(s)}$	−33.2	2620
(4)	$TaC_{(s)} + SiO_{2(s)} \rightarrow CO_{2(g)} + Si_{(g)} + Ta_{(s)}$	+542	2200
(5)	$Si_{(g)} + C_{(s)} \rightarrow SiC_{(s)}$	−153	2700
(6)	$Si_{(g)} + TaC_{(s)} \rightarrow SiC_{(s)} + Ta_{(s)}$	−206	2870
(7)	$SiO_{2(g)} + C_{(s)} \rightarrow SiC_{(s)} + O_{2(g)}$	+347	4000
(8)	$Ta_{(s)} + C_{(s)} \rightarrow TaC_{(s)}$	−135	4000

Determination of silicon in solutions containing either ascorbic acid or ammonium nitrate at concentrations up to 500 mg kg^{-1} did not show any improvement in sensitivity whether or not the furnace tube had been treated with sputtered tantalum. Moreover, for ascorbic acid and untreated furnace tubes, successive atomisation cycles led to reduced sensitivities. A similar effect, although less pronounced, was observed for sputtered furnace tubes.

In addition to increasing the sensitivity, treatment with sputtered tantalum greatly improved the lifetime of the furnace tubes and 300 atomisation cycles at 2600 °C have been achieved.

The limit of detection of silicon for a sputtered furnace tube has been calculated to be 0.003 mg kg^{-1} using the SAC[15] definition of "twice the standard deviation of at least ten determinations at or near the blank level." Using the procedure described above, this concentration corresponds to a detection limit of 30 pg of silicon.

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References

1. Morrison, I. R., and Wilson, A. L., *Analyst*, 1963, **88**, 100.
2. Webber, H. M., and Wilson, A. L., *Analyst*, 1964, **89**, 632.
3. Webber, H. M., and Wilson, A. L., *Analyst*, 1969, **94**, 110.
4. Kosfeld, E. G., *Tech. Ueberwach.*, 1967, **8**, 133.
5. Baker, P. M., and Farrant, B. R., *Analyst*, 1968, **93**, 732.
6. Massman, H., *Proc. Anal. Div. Chem. Soc.*, 1976, **13**, 258.
7. Johnson, D. J., West, T. S., and Dagnall, R. M., *Anal. Chim. Acta*, 1973, **67**, 79.
8. Norval, E., Human, H. G. C., and Butler, L. R. P., *Anal. Chem.*, 1979, **51**, 2045.
9. Hocquellot, P., and Labeyrie, N., *Analusis*, 1975, **3**, 505.
10. Regan, J. G. T., and Warren, J., *Analyst*, 1976, **101**, 220.
11. Morrison, I. R., and Wilson, A. L., *Analyst*, 1963, **88**, 446.
12. Campbell, W. C., and Ottaway, J. M., *Talanta*, 1974, **21**, 837.
13. Fuller, C. W., *Analyst*, 1974, **99**, 739.
14. Thompson, K. C., Godden, R. G., and Thomerson, D. R., *Anal. Chim. Acta*, 1975, **74**, 289.
15. Platt, P., *Sel. A. Rev. Anal. Sci.*, 1971, **1**, 177.

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Flotation - Spectrophotometric Determination of Palladium with Thiocyanate and Methylene Blue

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A sensitive and precise method for the determination of microgram amounts of palladium, based on the ion associate of the anionic palladium(II) - thiocyanate complex with Methylene Blue (MB), has been developed. The ion associate is floated with benzene and dissolved in acetone, and its molar absorptivity is found to be 1.7×10^5 l mol^{-1} cm^{-1} at 660 nm. The formation of the ion associate takes place over a wide pH range (1–6). The method is selective; interfering metals include silver, platinum, ruthenium, molybdenum and tungsten. The composition of the ion associate has been established as $[MB^+]_2[Pd(SCN)_4^{2-}]$. The proposed method has been applied to the determination of trace amounts of palladium in high-purity silver after its preliminary separation from the matrix as palladium dimethylglyoximate.

Keywords: Palladium determination; spectrophotometry; thiocyanate; Methylene Blue; high-purity silver analysis

The increasing importance of trace metal analysis has led to great interest in the development of sensitive spectrophotometric methods such as flotation - spectrophotometry, which is among the most sensitive.[1] It is based on the formation of ion associates between anionic complexes of elements and basic dyes.[2] Shaking an acidic aqueous solution of the metal with a non-polar solvent causes the sparingly soluble ion associate to accumulate at the phase boundary or on the walls of the separating funnel. After separation of the phases and washing the separated compound, the latter is dissolved in a suitable polar solvent. The absorbance of the solution obtained is proportional to the amount of the element present.

An examination of the systems that could be used in a flotation - spectrophotometric method for the determination of palladium revealed the palladium - thiocyanate - Methylene Blue system.

Flotation - spectrophotometric methods for the determination of platinum,[3] rhodium[4] and osmium[5] have already been published.

Experimental

Apparatus

The absorbance was measured using a VSU2-P spectrophotometer and the absorption spectra were recorded using a Specord ultraviolet - visible spectrophotometer. In all measurements 1-cm cells were used. The pH measurements were carried out using an ELPO N-512 pH meter.

Reagents

Methylene Blue (MB) solution, 10^{-3} M (*about* 0.04%). The compound supplied by Merck was used without further purification. The molar absorptivity of its aqueous solution was 8.4×10^4 l mol^{-1} cm^{-1} at a wavelength of 670 nm. The literature value[6] for the molar absorptivity of an aqueous solution of Methylene Blue (6.35×10^{-6} M) is 8.0×10^4 l mol^{-1} cm^{-1}.

Potassium thiocyanate solution, 0.25 M (*about* 2.5%).

Palladium(II) standard solution, 1 mg ml^{-1}. An appropriate amount of palladium chloride was dissolved in water containing hydrochloric acid. The solution was diluted in a calibrated flask and the concentration of palladium was determined gravimetrically using dimethylglyoxime. The solution (of palladium concentration higher than 1 mg ml^{-1}) was then diluted with water to obtain a solution containing exactly 1 mg ml^{-1} (in about 0.2 M hydrochloric acid).

Procedure

Place a solution containing not more than 12 μg of palladium in a 100-ml separating funnel. Add 2 ml of the potassium thiocyanate solution and dilute with water to about 20 ml. Adjust the pH of the solution to about 2.5 using dilute hydrochloric acid and ammonia solution then add 2 ml of the Methylene Blue solution and float the resulting ion associate by shaking with 10 ml of benzene for 45 s. Discard the water phase. Wash the precipitate and the organic phase with three 10-ml portions of water, then discard the benzene phase and dissolve the separated compound in acetone. Transfer the solution thus obtained into a 25-ml calibrated flask, then dilute to volume with acetone and measure the absorbance of the solution at 660 nm against a reagent blank.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

Results and Discussion

Preliminary Experiments

In order to find an ion associate of palladium that could be either extracted or floated quantitatively, a number of coloured ternary systems of palladium were examined. Ligands used for the formation of the anionic complex were chloride, bromide, iodide, azide, thiocyanate, 4-(2-pyridylazo)resorcinol (PAR) and 4-(2-thiazolylazo)resorcinol (TAR); the basic dyes used were Rhodamine B and Rhodamine 6G (xanthene dyes), Crystal Violet (a triphenylmethane dye), Methylene Blue, Meldola Blue and Capri Blue (azine dyes) and Bindschedler's Green (an indamine dye).

The experiments were carried out in the acidity range from 1 M hydrochloric acid to pH 4.5 (acetate buffer solution). The concentrations of the basic dyes were 10^{-4} M, the final volumes of the solutions (before shaking with the solvent) were about 25 ml and the amount of palladium in all of the samples was 10 μg. The aqueous phase was shaken with 10 ml of benzene or chloroform for 1 min. The ion associates formed in the palladium - azide - Methylene Blue and palladium - thiocyanate - Crystal Violet systems can be extracted with chloroform and both of these systems have been used already in the extraction - spectrophotometric methods for the determination of palladium.[7,8]

The ion associates of palladium formed with iodide - Methylene Blue, thiocyanate - Methylene Blue, iodide - Capri Blue and thiocyanate - Capri Blue can be floated with chloroform, whereas those formed with thiocyanate - Crystal Violet, thiocyanate - Capri Blue, thiocyanate - Methylene Blue, bromide - Rhodamine 6G, PAR - Crystal Violet and PAR - Rhodamine B can be floated with benzene.

However, only for the palladium - bromide - Rhodamine 6G - benzene and the palladium - thiocyanate - Methylene Blue - benzene systems are the blank values low and consequently only these two systems can be used for the flotation - spectrophotometric determination of palladium. This paper deals with the palladium - thiocyanate - Methylene Blue (Pd - SCN^- - MB) system.

Formation of the Pd - SCN^- - MB Ion Associate

Formation of the Pd - SCN^- - MB ion associate requires suitable molar excesses of thiocyanate ions and of Methylene Blue. In order to determine the necessary excesses of these reagents, experiments were carried out using 10 μg of palladium.

It has been found that a 30-fold excess of Methylene Blue and a relatively high thiocyanate concentration are necessary to convert the palladium into the ion associate quantitatively. The solution before flotation should be not less than 10^{-5} M in Methylene Blue and 2×10^{-2} M in thiocyanate ions.

As the ion associate is formed over a wide pH range (1–6) no buffer solutions are necessary.

It has been found that the formation of the thiocyanate - palladium complex proceeds quickly at room temperature. Heating an aqueous solution containing palladium and thiocyanate (without the dye) at a temperature of 90 °C for 20 min has no effect on the flotation. The time taken between mixing the reagents (palladium, thiocyanate and pH adjustment), and adding the dye and flotation has no effect on the final result. The measured absorbances are constant for up to 60 min.

Composition of the Ion Associate

The absorption spectrum of the solution of the ion associate in acetone is identical with the spectrum of a solution of Methylene Blue (chloride salt) in acetone (the same $\lambda_{max.}$ and shape of curve). It has been found that, under the optimum conditions, palladium is converted completely into the sparingly soluble ion associate. Comparison of the molar absorptivity of the ion associate solution (containing a known amount of palladium) with the molar absorptivity of a solution of Methylene Blue in acetone ($\epsilon = 7.7 \times 10^4$ l mol^{-1} cm^{-1}) indicates that the molar ratio of palladium to Methylene Blue in the ion associate is 1:2. This was confirmed using the logarithmic method.[9] The dependence of $\log A_x/(A_0 - A_x)$ (where A_x is the absorbance at a given concentration of Methylene Blue in the aqueous phase and A_0 is the maximum absorbance) on the concentration of the dye in the aqueous phase is shown in Fig. 1.

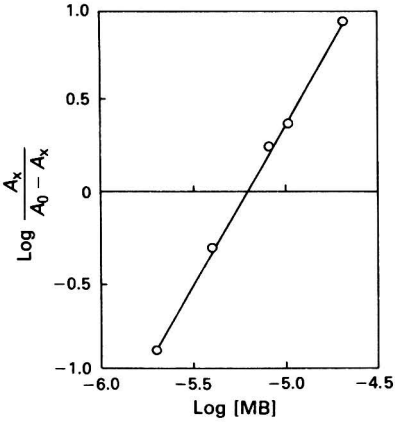

Fig. 1. Estimation of the molar ratio Pd:MB in the ion associate by the logarithmic method.

According to Shlenskaia *et al.*,[10] under the flotation conditions used (high concentration of thiocyanate ions and low concentration of chloride ions), palladium occurs in the aqueous phase exclusively as the anionic complex $Pd(SCN)_4^{2-}$. Taking this into account, the formula $[MB^+]_2[Pd(SCN)_4^{2-}]$ can be proposed for the ion associate (the dye reacts with the anionic complex of palladium as a monopositive cation).[2]

The equation used for the calculation of the apparent equilibrium constant of the flotation reaction was

$$\log \frac{A_x}{A_0 - A_x} = \mathrm{f}(\log C_{MB})$$

where C_{MB} is the concentration of the Methylene Blue solution.

According to the reaction

$$2\ MB^+ + Pd(SCN)_4^{2-} \rightleftharpoons [MB^+]_2[Pd(SCN)_4^{2-}]$$

the equilibrium constant K is given by

$$K = \frac{[MB^+]_2[Pd(SCN)_4^{2-}]}{[MB^+]^2[Pd(SCN)_4^{2-}]}$$

and

$$\log K = \log D - 2\log[MB^+]$$

where

$$\log D = \log \frac{A_x}{A_0 - A_x} = \log \frac{[MB^+]_2[Pd(SCN)_4^{2-}]}{[Pd(SCN)_4^{2-}]}$$

The equilibrium constant calculated for five different concentrations of Methylene Blue solution was $2.6 \pm 0.4 \times 10^{10}$ with a 90% probability level.

Flotation and Dissolution of the Ion Associate

It has been shown experimentally that the ion associate of palladium is floated quantitatively by benzene but not by toluene and diisopropyl ether. In addition, toluene gives a high blank value. Chloroform and carbon tetrachloride cannot be used as flotation agents owing to the occurrence of high blank values and difficulties connected with quantitative isolation of the floated compound.

The effect on the flotation of the time taken for shaking the phases has been examined. It has been shown that the results are constant and reproducible in the time range 45–60 s. Longer shaking results in higher blank values. As an ion associate between the dye and thiocyanate ions is also formed and partly undergoes flotation, it is necessary to wash the precipitate with water. Washing the organic phase and the separated compound with three 10-ml portions of water results in a decrease of the blank value to about 0.05.

The floated and washed ion associate is soluble in polar organic solvents. It has been found that complete dissolution of the precipitate is obtained most readily in acetone, the absorbance of the solutions obtained being constant for 1 h. A solution in methanol shows similar properties. Dissolution of the ion associate in ethanol or dimethylformamide is incomplete.

Absorption spectra of the ion associate in various solvents are shown in Fig. 2. The absorption maxima ($\lambda_{max.}$) are shifted with respect to each other and with respect to an aqueous solution of Methylene Blue (655 nm in methanol, 660 nm in ethanol and acetone, 670 nm in dimethylformamide, 672 nm in aqueous solution). The variation of the $\lambda_{max.}$ value of the dye in the different solvents is due to interactions of the dye cation with solvent molecules.

Method for the Determination of Palladium

Sensitivity and precision

The proposed method is more sensitive than other spectrophotometric methods for palladium.[11] The molar absorptivity (ϵ) is 1.7×10^5 l mol^{-1} cm^{-1} (specific absorptivity a = ϵ/relative atomic mass $\times$ 1000 = 1.6) at a $\lambda_{max.}$ value of 660 nm. The calibration graph obtained under the optimum conditions (see Procedure) obeys Beer's law in the palladium concentration range 0.03–0.5 μg ml^{-1}. The precision data for the method are given in Table I for four different amounts of palladium.

TABLE I

PRECISION DATA FOR THE DETERMINATION OF PALLADIUM

Palladium		Standard	Relative standard	
Added/μg	Found/μg	deviation*/μg	deviation, %	Confidence limits†
2.00	1.92	0.11	5.7	1.92 ± 0.10
4.00	3.96	0.18	4.5	3.96 ± 0.17
8.00	7.92	0.25	3.2	7.92 ± 0.23
12.00	11.88	0.41	3.4	11.88 ± 0.37

* For seven determinations.
† Probability level = 0.95.

Interferences

The effect of foreign metal ions on the determination of palladium has been examined. The results are presented in Table II. In addition, it has been found that gold at a 100-fold excess with respect to palladium does not interfere. Also, vanadium(V), rhenium(VII),

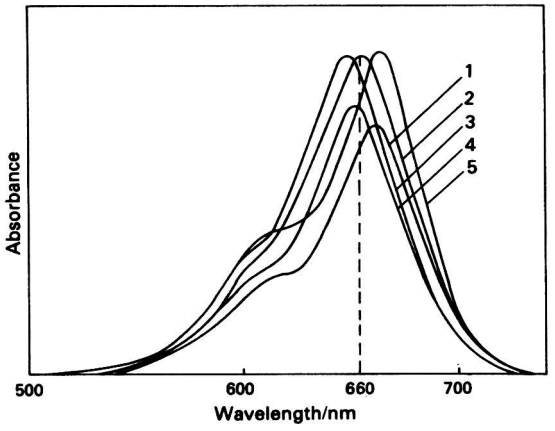

Fig. 2. Absorption spectra of solutions of the ion associate in: dimethylformamide (1); acetone (2); methanol (3); ethanol (4); and an aqueous solution of Methylene Blue (5). The concentration of Methylene Blue is the same in all solutions.

titanium, cadmium, gallium, indium, thallium, aluminium and scandium at 1000-fold excesses do not interfere. Chloride, sulphate and nitrate up to concentrations of 2 M (in the solution before flotation) do not interfere. Perchlorate interferes at a concentration higher than 2×10^{-2} M (positive errors).

The determination of palladium in the presence of a large excess (*e.g.*, 1000-fold) of some metals requires a higher excess of thiocyanate (for metals that form thiocyanates) than that given under Procedure.

Determination of Palladium in High-purity Silver

The method has been used to determine trace amounts of palladium in high-purity silver samples. As silver interferes seriously in the determination, a preliminary separation of palladium from the matrix is necessary. Extraction of palladium dimethylglyoximate with chloroform, according to the method proposed by Young[12] (except that the silver is dissolved in nitric acid), was used for this purpose.

Dissolve the silver sample (4 g) in nitric acid (about 2 ml of nitric acid per 1 g of sample). Transfer the solution into a 100-ml separating funnel and dilute with water to approximately 50 ml. Add 2 ml of 1% sodium dimethylglyoximate solution and mix the solution thoroughly. After 10 min, extract the palladium dimethylglyoximate with two 5-ml portions of chloroform (shaking time 30 s). Evaporate the combined extracts to dryness on a water-

TABLE II

EFFECT OF METAL IONS ON THE DETERMINATION OF PALLADIUM (8 μg)

Metal	Concentration of metal relative to Pd	Positive error, %	Metal	Concentration of metal relative to Pd	Positive error, %
Silver	1	20	Osmium	100	30
Platinum	5	20	Iridium	500	10
Molybdenum	5	20	Bismuth	1000	25
Ruthenium	10	15	Zinc	1000	15
Tungsten	10	10	Nickel	1000	10
Copper	50	25	Cobalt	1000	10
Rhodium	50	20	Manganese	1000	10

TABLE III

DETERMINATION OF PALLADIUM IN HIGH-PURITY SILVER

Sample mass/g	Palladium		Standard deviation*/μg	Confidence limits†	
	Added/μg	Found/μg		μg	% × 10^{-5}
4.0	—	1.0	0.3	1.0 ± 0.4	2.5 ± 0.8
2.0	1.0	1.4	0.2	1.4 ± 0.3	2.0 ± 0.9

* For five determinations.
† Probability level = 0.95.

bath. Mineralise the residue by evaporation with a mixture of 3 ml of concentrated hydrochloric acid and 2 ml of concentrated nitric acid. Dissolve the precipitate in 1 M hydrochloric acid, transfer the solution to a separating funnel, adjust the pH to approximately 2.5 and determine palladium according to the method given under Procedure (using 10-ml calibrated flasks).

Palladium was determined for 4-g samples and for 2-g samples to which palladium had been added. The results, shown in Table III, can be considered satisfactory, if one takes into account the low levels of palladium present in the silver analysed.

References

1. Marczenko, Z., *Crit. Rev. Anal. Chem.*, 1981, **10**, in the press.
2. Marczenko, Z., *Mikrochim. Acta*, 1977, **11**, 651.
3. Marczenko, Z., and Maruszak, J., *Chem. Anal. (Warsaw)*, 1979, **24**, 341.
4. Marczenko, Z., and Kowalczyk, E., *Anal. Chim. Acta*, 1979, **108**, 261.
5. Marczenko, Z., and Uścińska, J., *Anal. Chim. Acta*, 1981, **123**, 271.
6. Michaelis, L., and Granick, S., *J. Am. Chem. Soc.*, 1945, **67**, 1212.
7. Kuroda, R., Yoshikuni, N., and Kamimura, Y., *Anal. Chim. Acta*, 1972, **60**, 71.
8. Khvatkova, Z. M., and Golovina, V. V., *Zh. Anal. Khim.*, 1979, **34**, 2035.
9. Bent, M., and French, C., *J. Am. Chem. Soc.*, 1941, **63**, 568.
10. Shlenskaia, V. I., Khvostova, V. P., and Peshkova, V. M., *Zh. Anal. Khim.*, 1962, **17**, 598.
11. Marczenko, Z., "Spectrophotometric Determination of Elements," Ellis Horwood, Chichester, 1976.
12. Young, R. S., *Analyst*, 1951, **76**, 49.

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Rapid Determination of Trace Amounts of Selenium in Biological Samples by Gas Chromatography with Electron-capture Detection

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A rapid, simple and sensitive method of determining total selenium in biological samples is described. Selenium(IV) reacts with 1,2-diamino-3,5-dibromobenzene to form 4,6-dibromopiazselenol, which is detected by means of a gas chromatograph equipped with an electron-capture detector. In order to complete the mineralisation of selenium in seven organic compounds, a wet digestion method was employed. Thirty determinations can be made in 8 h by a modified method. Trace amounts of total selenium in NBS standard reference materials, human blood and serum were determined and compared with the certified or reported values.

Keywords: *Selenium determination*; *biological samples*; *gas chromatography*

Trace amounts of selenium in biological samples have been determined by fluorimetry using naphthalene-2,3-diamine,[1,2] by gas chromatography using 1,2-diamino-3,5-dibromobenzene,[3] and by neutron-activation analysis.[4] Most methods except neutron-activation analysis require the complete conversion of selenium in biological samples to inorganic selenium(IV) because, in these methods, the reaction of *o*-diamines with inorganic selenium(IV) is used. Many types of method for the decomposition of selenium compounds have been proposed,[5] but wet digestion methods are preferable for routine analysis. As digestion reagents, nitric acid, nitric acid - perchloric acid, nitric acid - perchloric acid - sulphuric acid, and nitric acid - molybdate have been used. Most wet digestion methods have been studied using biological samples spiked with inorganic selenium or using NBS standard reference materials and studies using organic selenium compounds have rarely been reported.[6] This paper describes complete digestion for the determination of total selenium in seven organic compounds that contain selenium in different oxidation states. All of the compounds examined were completely decomposed to yield inorganic selenium(IV) using a mixture of nitric and perchloric acids as the digesting medium. Selenium was coprecipitated with lanthanum hydroxide and was then heated with 1,2-diamino-3,5-dibromobenzene monohydrochloride (DDB) solution to form the piazselenol, which was extracted into 1 ml of toluene and injected into a gas chromatograph equipped with an electron-capture detector. By this method, 30 determinations were possible in 8 h.

Experimental

Reagents

All of the reagents used were of analytical-reagent grade.

1,2-Diamino-3,5-dibromobenzene monohydrochloride, 0.05%. The synthesis of this reagent has been reported elsewhere.[7] DDB (0.1 g) was dissolved in 200 ml of perchloric acid (1 + 1 *V*/*V*) and washed with toluene (10 ml) to remove toluene-soluble matter. This solution can be used for at least 1 month.

Selenium(IV) stock solution. Selenium dioxide (1.4 g) was dissolved in 1 l of distilled water and standardised gravimetrically. The solution was stable for 6 months. Working solutions were prepared by appropriate dilution.

Selenium(VI) stock solution. About 220 mg of selenic acid monohydrate were dissolved in 100 ml of distilled water. The exact concentration of the solution was determined by heating the solution with concentrated hydrochloric acid, to reduce selenium(VI) to selenium(IV), forming the piazselenol with DDB and then determining this by gas chromatography.

Lanthanum nitrate solution. Lanthanum nitrate (25 g) was dissoved in 500 ml of distilled water.

Apparatus

A Shimadzu, Model GC-5A, gas chromatograph equipped with an electron-capture detector (Shimadzu Seisakusho Ltd., Kyoto, Japan) was used. A glass column (1 m × 3 mm i.d.) was packed with 15% of SE-30 on 60–80-mesh Chromosorb W. The column and detector temperatures were maintained at 200 and 280 °C, respectively, and the nitrogen flow-rate was 28 ml min^{-1}. A Shimadzu, Model R 101, recorder was used at a chart speed of 5 mm min^{-1}.

Procedure

The sample (20–500 mg) is placed in a 100-ml conical flask. After adding 10 ml of concentrated nitric acid and 1 ml of perchloric acid, the mixture is heated for 1 h on a sand-bath (160–170 °C) in a fume-hood. The temperature of the sand-bath is then raised to 220–230 °C. White fumes of perchloric acid appear after about 30 min and the digestion is continued for a further 30 min to decompose selenium compounds completely. During this procedure no loss of selenium is observed. After cooling, 0.5 ml of concentrated hydrochloric acid is added and the mixture is heated on a sand-bath for 10 min at about 100 °C to reduce selenium(VI) to selenium(IV). A 2-ml volume of 1 M urea solution is then added and the mixture again heated on a sand-bath for 10 min to decompose the oxides of nitrogen. After cooling, the contents are transferred into a 10-ml calibrated centrifuge tube with 5 ml of distilled water. To each tube are then added first 0.5 ml of lanthanum nitrate solution (5%) and then 2 ml of ammonia solution (25%), the solution is then shaken before being centrifuged at 3000 rev min^{-1} for 5 min. The supernatant liquid is discarded and the precipitate washed with 3 ml of 1 M ammonia solution. The precipitate is then dissolved by the addition of 3 ml of DDB solution (0.05%) and the reaction completed by heating in a water-bath at 60 °C for 2 min. The 4,6-dibromopiazselenol formed is then extracted into 1 ml of toluene by shaking for 2 min. The toluene extract is washed with 3 ml of perchloric acid (1 + 1 *V*/*V*) by shaking for 2 min. A portion of the toluene extract (2 μl) is then injected into the gas chromatograph and the peak height measured.

Results and Discussion

Synthesis of Organic Selenium Compounds

Organic selenium compounds are less stable than the corresponding sulphur compounds. It has been reported that selenium compounds seldom exist in plant and animal tissues, any that were present would exist as selenide, selenite or selenate. In order to study the digestion method for determination of total selenium in biological materials, organic selenium compounds were used. The organic selenium compounds used are shown in Fig. 1. The selenide compounds were piazselenol (**I**), trimethylselenonium iodide (**II**) selenohypoxanthine (**III**),

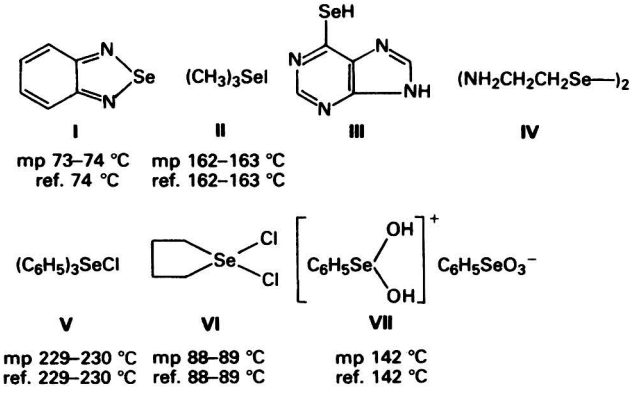

Fig. 1. Organic selenium compounds studied.

selenocystamine (**IV**) and triphenylselenonium chloride (**V**). The selenite compounds were cycloselenobutane 1,1-dichloride (**VI**) and the benzeneselenonic salt of benzeneseleninic acid (**VII**). Compound **VII** was also used as selenate. Compounds **I**, **II**, **V** and **VII** were synthesised by published methods[8–11] and identified by their melting-points. Compounds **III** and **IV** were of commercial origin.

Digestion of Selenium Compounds

The rapid digestion method for total selenium in biological materials was investigated using the organic selenium compounds listed above. The compound (50–100 mg) was accurately weighed and dissolved in distilled water or acetone in a 100-ml conical flask. After appropriate dilution, the solution (0.2–1 ml) containing 15–30 ng of selenium was transferred by pipette into a 100-ml conical flask. Filter-paper (0.2 g) was added as organic matter and the digestion performed by one of the two methods described below.

(*i*) Concentrated nitric acid (10 ml) and 1 ml of perchloric acid are added to the flask containing the sample and heated for 1 h on a sand-bath (160–170 °C). The sand-bath temperature is then raised to 220–230 °C and maintained until the first appearance of dense white fumes of perchloric acid.

(*ii*) The mixed acid and the sand-bath temperatures are the same as in method (*i*), but the digestion is continued for a further 30 min after the first appearance of dense white fumes.

After digestion by method (*i*) or (*ii*), 0.5 ml of concentrated hydrochloric acid is added and the mixture heated to reduce selenium(VI) to selenium(IV). Milk products were easily decomposed with concentrated nitric acid,[12] and some of the organic selenium compounds were also decomposed completely. However, trimethylselenonium iodide is not digested by concentrated nitric acid and in this instance the digestion temperature should be raised, by the addition of perchloric acid to the nitric acid, and the digestion time prolonged to obtain complete digestion. The other organic selenium compounds are completely digested by method (*i*). The recovery of trimethylselenonium iodide by method (*ii*) was in agreement with that obtained by Olson *et al.*[6] The results are shown in Table I. Therefore, for the digestion of biological materials to determine total selenium, digestion method (*ii*) was employed. Using this procedure less than 0.5 g of the sample is completely decomposed.

TABLE I

DETERMINATION OF SELENIUM IN ORGANIC SELENIUM COMPOUNDS

Compound*	Sample mass/ng	Calculated mass of selenium in sample/ng	Selenium found/ng	Standard deviation §/ng
Piazselenol	45.6	19.7	19.8†	0.9
Trimethylselenonium iodide	45.4	22.5	0.0†	
	45.4	22.5	22.1‡	0.5
Selenohypoxanthine	49.4	19.6	19.8†	0.7
Selenocystamine	64.2	15.9	15.6†	0.7
Triphenylselenium chloride	125.1	28.6	28.3†‡	0.5
Cycloselenobutane 1,1-dichloride	70.4	27.0	26.5†	0.7
Benzeneselenonic salt of benzeneseleninic acid	110.3	22.0	21.8†	0.5

* See Fig. 1 for structures.
† Digestion was stopped at the appearance of perchloric acid fumes.
‡ Digestion was continued for 30 min after perchloric acid fumes appeared.
§ The results are the means of five determinations.

Reduction of Selenium(VI)

In digestion method (*ii*) selenium is oxidised to some extent to selenium(VI). This oxidised selenium must be reduced to selenium(IV) because the reagent reacts only with selenium(IV) to form the piazselenol. Perchloric acid remains in the conical flask after digestion. The effect of the volume of concentrated hydrochloric acid used on the reduction of selenium(VI) in the presence of perchloric acid was therefore studied. Selenium(VI) (23.6 ng) and filter-paper (0.2 g) were placed in a conical flask and digested by method (*ii*). To the resulting solution was added concentrated hydrochloric acid (0.2–2.0 ml) and the mixture was then

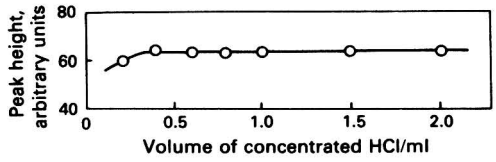

Fig. 2. Effect of the volume of concentrated hydrochloric acid on the reduction of selenium(VI).

heated at 100 °C for 10 min. As shown in Fig. 2, more than 0.5 ml of concentrated hydrochloric acid reduced selenium(VI) completely to selenium(IV). In the same way, the effect of the reduction time was studied using 0.5 ml of concentrated hydrochloric acid. Selenium(VI) was reduced quantitatively by heating at about 100 °C for at least 4 min. The reduction was therefore carried out by heating for 10 min at about 100 °C, with the addition of 0.5 ml of concentrated hydrochloric acid.

Coprecipitation by Lanthanum Hydroxide

The electron-capture detector is very sensitive to compounds containing electrophilic groups such as nitro or halogen. It is therefore necessary to remove these compounds, which otherwise produce unknown peaks in the chromatogram, before the extraction of piazselenol. In a previous method, the digest was washed with toluene but separation of the two phases took a long time. A simple and rapid procedure for removing interferences was therefore studied. Lanthanum hydroxide coprecipitation gave the best results. The effect of the volumes of lanthanum nitrate and ammonia solutions on the quantitative selenium recovery was studied. Azad *et al.*[13] reported that lower recoveries were obtained as the time between precipitation and filtration was increased. As shown in Fig. 3, however, a constant peak height was obtained for up to 30 min after precipitation and the average recovery was 98%.

Effect of Heating on Complex Formation

It is well known that the reaction between *o*-diamine and selenium(IV) takes place rapidly at higher temperatures. The determination, however, suffered from interference by unknown products in the digest when the reaction mixture was heated. On separation of selenium from these unknown products by lanthanum hydroxide coprecipitation, the determination was no longer obstructed by overlapping unknown peaks when the selenium was reacted with the reagent at high temperature. The effect of temperature on the reaction of the reagent with selenium(IV) is shown in Fig. 4. The piazselenol formation reaction was quantitative after heating for 2 min at 60 °C in a water-bath.

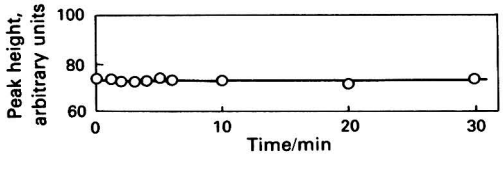

Fig. 3. Effect of time elapsed before centrifugation.

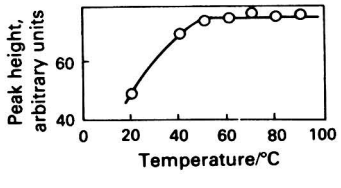

Fig. 4. Effect of reaction temperature.

Accuracy and Precision

The accuracy of the procedure was evaluated by recovery experiments, the recovery being established by digesting NBS SRM 1571 orchard leaves to which a known amount of selenium(IV) or trimethylselenonium iodide had been added, using the standard procedure. As shown in Table II, complete recovery was obtained within experimental error. The precision of the method was evaluated by analysing ten 200-mg samples of the orchard leaves. The mean result obtained was 0.076 $\mu g\ g^{-1}$ of selenium, with a standard deviation of 0.003 $\mu g\ g^{-1}$ and a relative standard deviation of 3.9%.

TABLE II

RECOVERY TEST USING ORCHARD LEAVES

Mass of orchard leaves taken/mg	Selenium(VI) added as H_2SeO_4/ng	Selenium(IV) added as $(CH_3)_3SeI$/ng	Selenium found/ng	Selenium recovered/ng	Recovery, %
100.0	None	None	7.7	—	—
104.0	11.8	—	20.3	12.3	104
112.2	11.8	—	19.2	10.6	90
105.4	11.8	—	19.8	11.7	99
108.5	11.8	—	19.6	11.2	95
110.1	11.8	—	21.3	12.8	108
119.8	—	10.7	19.8	10.6	99
122.7	—	10.7	20.7	11.3	106
130.2	—	10.7	19.3	9.3	87
110.4	—	10.7	19.1	10.6	99
124.2	—	10.7	20.6	11.0	103

Calibration Graphs

Calibration graphs (Fig. 5) were prepared for selenium(IV) standard solution, with and without lanthanum hydroxide coprecipitation, by the standard procedure without digestion or reduction. The calibration graph after coprecipitation gave values of about 98% of those obtained using the standard selenium solution. The calibration graph should therefore be prepared by the coprecipitation procedure. The selenium content was calculated by subtracting the blank value of 0.2 g of filter-paper from the peak height of piazselenol.

Determination of Selenium in Biological Samples

Total selenium in NBS standard reference materials (20–400 mg), whole blood (0.1 ml) and serum (0.2 ml) were determined by the procedure described above. The results are shown in Table III. The selenium concentrations agree with the certified or reported values within experimental error. The determination of selenium was carried out using the two digestion procedures previously described. The results for the NBS standard reference materials and for the whole blood obtained by the two digestion procedures were the same, but results for serum by procedure (*ii*) were about 10% higher than those obtained by procedure (*i*). Therefore, serum may contain trimethylselenonium ion or similar selenium compounds. As the selenium concentration in the erythrocytes in whole blood is 2–3 times higher than that in the serum, the difference between the two procedures would not appear so clearly in whole blood

TABLE III

DETERMINATION OF SELENIUM IN BIOLOGICAL SAMPLES

Sample		Selenium concentration*/μg g^{-1}			
Reference number	Type	(*i*)‡	(*ii*)§	Certified value	Reference
NBS SRM 1577 ..	Bovine liver	1.10 ± 0.05	1.10 ± 0.06	1.1 ± 0.1	14
NBS SRM 1571 ..	Orchard leaves	0.077 ± 0.002	0.076 ± 0.003	0.08 ± 0.01	14
NBS SRM 1575 ..	Pine needle	0.043 ± 0.001	0.043 ± 0.001	0.04 ± 0.01 0.05 ± 0.01	15
NBS SRM 1570 ..	Spinach	0.033 ± 0.003	0.033 ± 0.003	0.04 ± 0.01	15
NBS SRM 1573 ..	Tomato leaves	0.061 ± 0.002	0.057 ± 0.003	0.05 ± 0.01	15

	Selenium concentration†/μg ml^{-1}	
	(*i*)‡	(*ii*)§
Whole blood 1	0.250 ± 0.005	0.257 ± 0.008
Whole blood 2	0.203 ± 0.006	0.199 ± 0.001
Serum 1	0.118 ± 0.003	0.130 ± 0.003
Serum 2	0.114 ± 0.001	0.124 ± 0.002

* Values are averages for five samples.
† Values are averages for three samples.
‡ Digestion was completed at the appearance of perchloric acid fumes.
§ Digestion was continued for 30 min after perchloric acid fumes appeared.

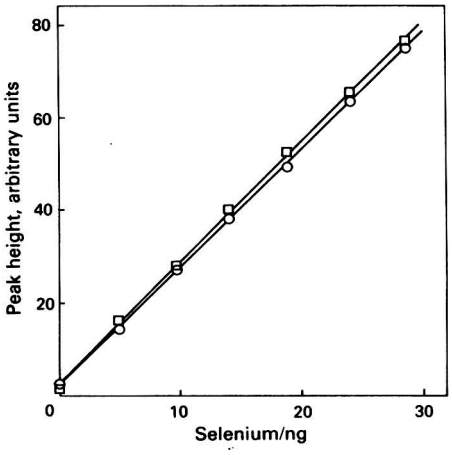

Fig. 5. Calibration graphs for selenium: (□) without and (○) with coprecipitation procedure, respectively.

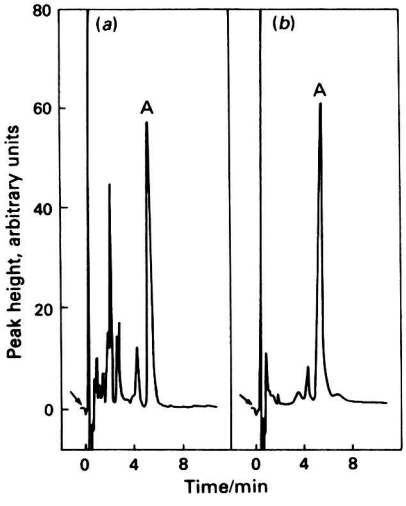

Fig. 6. Determination of selenium in 0.1 ml of whole blood. (*a*) Digestion was completed at the first appearance of perchloric acid fumes. (*b*) Digestion was continued for 30 min after the appearance of perchloric acid fumes. Peak A is due to 4,6-dibromopiazselenol.

analyses. The gas chromatograms of whole blood (0.1 ml) treated by the two procedures are shown in Fig. 6. The sample used is more completely decomposed by increasing the digestion period to 30 min after perchloric acid fumes appear, and most of the unknown peaks in the chromatogram disappear if this procedure is used. This method is very simple, rapid and sensitive and no special skill is necessary for its use.

References

1. Parker, C. A., and Harvey, L. G., *Analyst*, 1962, **87**, 558.
2. Hall, R. J., and Gupta, P. L., *Analyst*, 1969, **94**, 292.
3. Kurahashi, K., Inoue, S., Yonekura, S., Shimoishi, Y., and Tôei, K., *Analyst*, 1980, **105**, 690.
4. Bowen, H. J. M., and Cawse, P. A., *Analyst*, 1963, **88**, 721.
5. Masson, M. R., *Mikrochim. Acta*, 1976, I, 419.
6. Olson, O. E., Palmer, I. S., and Cary, E. E., *J. Assoc. Off. Anal. Chem.*, 1975, **58**, 117.
7. Shimoishi, Y., and Tôei, K., *Anal. Chim. Acta*, 1978, **100**, 65.
8. Hashimoto, T., Sugita, M., Kitano, H., and Fukui, K., *Nippon Kagaku Zasshi*, 1967, **88**, 991.
9. Morgan, H. T., and Burstall, F. H., *J. Chem. Soc.*, 1929, 1096.
10. Doughty, H. W., *Am. Chem. J.*, 1909, **41**, 326.
11. Klayman, D. L., and Günther, W. H. H., *Editors*, "Organic Selenium Compounds: Their Chemistry and Biology," Wiley-Interscience, New York, 1973, p. 142.
12. Shimoishi, Y., *Analyst*, 1976, **101**, 298.
13. Azad, J., Kirkbright, G. F., and Snook, R. D., *Analyst*, 1979, **104**, 232.
14. "Catalog of NBS Standard Reference Materials," Office of Standard Reference Materials, NBS Special Publication, No. 260, National Bureau of Standards, Washington, D.C., 1975–76.
15. Knab, D., and Gladney, E. S., *Anal. Chem.*, 1980, **52**, 825.

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Measurement of Phenol in Urine by the Method of Van Haaften and Sie: A Critical Appraisal

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The method of Van Haaften and Sie for the measurement of urinary phenol gives better than 80% of the expected response for conjugates of phenol. However, the response is strongly dependent on instrumental conditions and safeguards must be built into the method to ensure that hydrolysis of conjugates is satisfactory.

Keywords: *Phenol*; *urine analysis*; *exposure monitoring*

Phenol is excreted in urine as the sulphate or glucuronic acid conjugate (Fig. 1) (or both) following exposure to either phenol or benzene. The concentration of phenol (free and combined) in the urine is an indicator of the degree of exposure to those compounds. Van Haaften and Sie[1] measured the concentration of phenol in urine by gas - liquid chromatography. The urine sample is mixed with an equal volume of phosphoric acid and injected into a gas - liquid chromatograph. Conjugates are hydrolysed on a pre-column containing coarse glass powder and the phenol is separated from other urinary components on an analytical column. Detection is by flame ionisation. A few parts per million of phenol in urine can be detected.

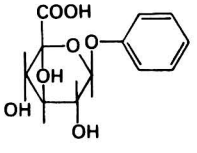

Fig. 1. Structure of phenyl glucuronide. Relative molecular mass of monohydrate = 288.3.

The original report of this method did not give data showing the efficiency of the hydrolysis of phenol conjugates. In a preliminary study of the method it was found that hydrolysis of phenol glucuronide was not complete at the temperatures recommended by Van Haaften and Sie. Increasing the temperature of the pre-column to give quantitative hydrolysis resulted in unusually large residues of phenol being found in some samples. This work describes an investigation into the method and makes recommendations for its improvement.

Experimental

Chemicals

Phenol β-D-glucuronide monohydrate. Koch-Light Laboratories.

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

Phenyl sulphate potassium salt. Prepared according to Feigenbaum and Leuberg.[2]

Phosphoric acid. Analytical-reagent grade material, 88% H_3PO_4, was obtained from BDH Chemicals.

Coarse glass powder. Prepared by crushing Fenske helices of 4 mm diameter (Jobling Laboratory Division, Cat. No. FC 8/45) and sieving to the required particle size.

Silanised glass-wool. Obtained from Field Instruments Co. Ltd.

Apparatus

Gas - liquid chromatography was performed using an F & M, Model 1402, high-efficiency gas chromatograph. The U-shaped column, total length 1.1 m × 6 mm o.d. and 3 mm i..d, was made of borosilicate glass. The first portion of the column was empty to allow for injection using a 2-in needle. Various pre-columns, each 10 cm long, were investigated, these are described under Results. Each pre-column was fitted with a heater so that it could be heated independently of the main oven, the temperature of the pre-column being measured with a thermocouple. Some pre-columns were detachable from the main column whereas others were part of the main column tube. The main oven was kept between 150 and 165 °C, which was the temperature necessary to give good chromatograms. The nitrogen carrier gas flow-rate was approximately 45 ml min^{-1}. Injection volumes were 5 or 6 μl.

Procedure

The method was evaluated using several pre-column packings under different conditions, by analysing standard solutions of phenol, phenyl sulphate or phenyl glucuronide in water or human urine. Urine and water were used to check for ghost peaks. These are phenol peaks obtained when solutions containing no phenol are injected on to the column after it has been used for samples containing large amounts of phenol. Their formation may result from hydrolysis of conjugates of phenol contained on the column, which can only take place in the presence of water. The necessary conditions are achieved at the moment of injection of aqueous samples.

Pre-column Packing

The following pre-column packings were used:

(*a*) 30–50-mesh sieved glass powder.
(*b*) Silanised glass-wool.
(*c*) Silanised glass-wool soaked in 10% *V*/*V* of phosphoric acid in water, drip-dried and heated to 110 °C.
(*d*) Broken Fenske helices sieved to remove material of less than 50 mesh, with a 1-cm section of 30–50-mesh glass powder next to the analytical column. This pre-column was more open in texture than (*a*), the particles being predominantly thin crescents.
(*e*) 30–50-Mesh sieved glass powder packed in a disposable glass pre-column connected to the main column by a stainless-steel Swagelok coupling. The coupling had been drilled out to allow the pre-column and the analytical column to be butted end to end.

Results

Pre-column (a)

This pre-column is similar to that recommended by Van Haaften and Sie, which contained 30–50-mesh glass powder but had an inner diameter of 6 mm. Samples were analysed after mixing with an equal volume of phosphoric acid.

When the pre-column was operated at 150 °C the peak heights obtained from solutions of the sulphate and the glucuronide were similar (80% or more) to those obtained from equivalent amounts of phenol. The chromatography was acceptable and no ghost peaks were observed. The temperature of the pre-column was increased in 50 °C steps to 250 °C but this led to no significant increase in recovery. After less than 1 week of operation the base line became noisy and the pre-column was found to have become heavily contaminated. It was unpacked with difficulty, having set to a solid mass.

Pre-column (b)

This pre-column was prepared in the hope that it would be easier to clean than one containing 30–50-mesh glass powder. The performance of the system at a pre-column temperature of about 150 °C was close to that described under (*a*), but slightly inferior. It was recognised that the main cause of contamination of the pre-column was phosphoric acid and its polymers which, having a low volatility, accumulated in the pre-column. Experiments were carried out to determine whether the amount of phosphoric acid mixed with a sample could be reduced without serious effect on the recovery of phenol from its conjugates. It was found that some

phenol was liberated when only 5% of the volume of the injected sample was phosphoric acid but that recoveries were always reduced when the amount of phosphoric acid in the injection mixture was below that recommended, *i.e.*, equal volumes of sample and phosphoric acid.

Pre-column (c)

This pre-column was prepared to determine whether conjugates could be hydrolysed on an acidic pre-column without mixing samples with phosphoric acid before analysis. The initial results were encouraging. Good chromatograms and recoveries were obtained when solutions of phenol conjugates in water were injected at a pre-column temperature of about 160 °C. However, the performance with conjugates deteriorated after a few injections of urine although the performance with aqueous solutions of free phenol was not affected.

Pre-column (d)

This type of pre-column was used both with and without phosphoric acid pre-treatment. In both instances the pre-column temperature was about 160 °C. A small amount of phosphoric acid was first injected into the pre-column and samples were injected without pre-mixing with phosphoric acid. Recovery of phenol from conjugates was poor. An identical pre-column was then used with samples pre-mixed with phosphoric acid. In this instance, the recovery of conjugated phenol was good and no ghost peaks were observed after continuous use for 1 d. In general, the chromatograms obtained using this pre-column were not as good as those obtained with some other pre-columns. However, it is unwise to assume that one column of the type is typical, and coarse glass pre-columns may be of value in reducing contamination problems.

Pre-column (e)

All samples used with this pre-column were mixed with an equal volume of phosphoric acid before analysis. Results of analyses of solutions of phenol and its conjugates in water or urine are shown in Table I. The apparent recovery of phenol from its conjugates at a pre-column temperature of 160 °C was usually above 80% and no ghost peaks were observed. After continuous use for about 2 d the contaminated section of the pre-column had reached to within 1 cm of the main column. The pre-column was replaced simply by unscrewing the coupling and inserting a fresh column. This system was the most satisfactory of those investigated. Systems (*a*) and (*e*) were equivalent except for the convenience of the disposable pre-column (*e*).

TABLE I

APPARENT RECOVERY OF PHENOL FROM SOLUTIONS OF PHENOL CONJUGATES IN WATER OR URINE

Conjugate	Medium	Number of experiments	Equivalent concentration of phenol in solution/ $\mu g\ ml^{-1}$	Average recovery of phenol, %	Relative standard deviation, %
Sulphate	Water	2	50	87	—
		1	100	98	—
	Urine	4	50	91	5.8
		5	100	92	4.1
Glucuronide	Water	2	50	87	—
		1	100	89	—
	Urine	4	50	82	5.0
		4	100	82	11.2

Over-all average recovery = 88.5%

Discussion

The experiments described show that the method of Van Haaften and Sie does determine phenol in the free state and as the glucuronic acid and sulphate conjugates. The average recovery from conjugates was about 89% and was therefore good but not quantitative.

There are, however, problems of contamination with the method and it is necessary to introduce safeguards into the procedure to ensure that it is working correctly,

If a 10-cm pre-column containing 30–50-mesh glass is used, and samples are mixed with an equal volume of phosphoric acid before analysis, the method works well at a pre-column temperature of 150 °C. This procedure is close to that recommended by Van Haaften and Sie. Their pre-column had an inner diameter of 6 mm whereas that used in this work had an outer diameter of 6 mm and an inner diameter of about 3 mm. The larger diameter pre-column could tolerate more phosphoric acid and would therefore have an extended life. However, both would eventually become contaminated with phosphoric acid. Silanised glass-wool has a pre-column performance almost as good as 30–50-mesh glass powder.

Good recoveries of phenol from conjugates were not obtained when samples were injected, without pre-mixing with phosphoric acid, on to an acidic pre-column at 150 °C. This effect would not be detected if the performance of the method was monitored only with aqueous solutions of free phenol.

Our preliminary experiments used pre-columns at 250 °C to obtain complete hydrolysis of phenol-β-D-glucuronide. (It was assumed that sulphate conjugates would react faster than the glucuronide.) At that temperature equivalent amounts of phenol and the glucuronide gave the same peak heights. However, this procedure is not recommended. Phenol is predominantly or entirely present as conjugates in urine. Conjugates of other compounds are also present. Raising the temperature of the pre-column may improve the apparent degree of hydrolysis of phenol conjugates, but it also increases the risk of formation of artefacts by pyrolysis of other urinary components. It seems unnecessary and inappropriate to operate in this way, considering the acceptable recoveries obtained at lower pre-column temperatures. Peak-height equivalence for free phenol and conjugates is probably unnecessary, aqueous solutions of free phenol having little merit as standards when analysing for conjugated phenol. It is better to use solutions of conjugates as standards, in which event a lower efficiency of hydrolysis will have less effect on the result. As a general procedure the method described below is recommended.

Using aqueous solutions of phenol and of conjugated phenol, check that the peak heights obtained from the sulphate and glucuronic acid conjugates are greater than 80% of those obtained from an equivalent amount of free phenol.

As a working standard use solutions of either phenyl glucuronide or phenyl sulphate. To ensure comparability between different laboratories it would be best to select one conjugate as a standard. The sulphate has the advantage of being available as a stable potassium salt.

The pre-column should not be heated much above the temperature of the main column but a pre-column temperature about 10 °C above the main column temperature may be necessary to give good chromatograms.

It was observed that urine from subjects taking aspirin (acetylsalicylic acid) gives a response for phenol at a pre-column temperature of 250 °C. Phenol has not been reported as a metabolite of aspirin.[3] However, salicylic acid is a metabolite of aspirin and can lose carbon dioxide on heating to give phenol.

A solution of salicylic acid was analysed on pre-column (*e*) at pre-column temperatures of 160 and 250 °C. At 160 °C no phenol was detected. At 250 °C a phenol peak corresponding to about 3% of the salicylic acid injected was observed. This may be one example of a general phenomenon. The hotter the pre-column the more probable it is that components in a sample will pyrolyse to give phenol.

The history of the application of the Van Haaften and Sie method appears to have been one of continuous modification. To a large extent this has been necessary because repeated injection of aqueous solutions of phosphoric acid has a detrimental effect on the pre-column and will eventually cause it to become partially blocked. Phosphoric acid has a low volatility and remains near the point of injection, even at 250 °C, where it dehydrates to give a mixture of pyrophosphoric acid and metaphosphoric acid. It is likely that the phenomenon of ghost peaks results from the use of contaminated pre-columns. The effect was not observed during this work but no pre-column was used for more than a few days. The best solution to the problem of pre-column contamination seems to be the use of disposable pre-columns which can be changed when necessary. The pre-column should be replaced if the peak height from either the glucuronide or sulphate conjugates of phenol is less than 80% of that obtained from the equivalent amount of phenol, or if ghost peaks are observed.

Conclusions

On the basis of this work the following recommendations can be made:

1. The performance of the method of Van Haaften and Sie should be checked at least once per day to ensure that phenyl sulphate and phenyl glucuronide give not less than 80% of the response given by the equivalent quantity of phenol.
2. If the response from conjugated phenol is less than 80% of the response from free phenol, the pre-column should be changed.
3. If ghost peaks are observed the pre-column should be changed.
4. The use of aqueous phenol solutions as working standards should be discontinued and solutions of conjugated phenol should be used instead. The conjugate to be used should be standardised between different laboratories.
5. Pre-columns should not be heated to more than about 10 °C above the temperature of the main oven, *i.e.*, to about 165 °C.
6. Users of the method should evaluate pre-columns that may be detached from the main analytical column, as a method of minimising contamination problems.

References

1. Van Haaften, A. B., and Sie, S. T., *Am. Ind. Hyg. Assoc. J.*, 1965, **26**, 52.
2. Feigenbaum, J., and Leuberg, C. A., *J. Am. Chem. Soc.*, 1941, **63**, 3529.
3. Sano, M., Votsui, V., Abe, H., and Sasaki, S., *Biomed. Mass Spectrom.*, 1976, **3**, 1.

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Polarographic Determination of Temazepam in Soft Gelatin Capsule Formulations

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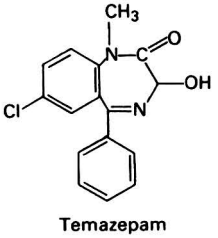
Precise and accurate procedures have been developed for the polarographic determination of temazepam in soft gelatin capsules. The capsule contents can be determined either by direct-current or by differential-pulse polarography. In order to cover the possibility of temazepam having diffused into the capsule shell a whole capsule assay has been developed based on direct-current polarography: the differential pulse signal is considerably suppressed by the gelatin present in the shell. For capsules that have undergone storage tests at high temperatures leading to denaturisation, an enzyme dissolution step is included. The procedures are not subject to interference from known degradation products, and can therefore be used for stability determinations.

Keywords: *Temazepam determination*; *direct-current polarography*; *differential-pulse polarography*; *enzymatic dissolution*; *soft gelatin capsules*

Temazepam (7-chloro-2,3-dihydro-3-hydroxy-1-methyl-5-phenyl-1*H*-1,4-benzodiazepin-2-one) is a rapidly acting hypnotic drug, and unpublished ultraviolet spectrophotometric procedures had been developed previously for the determination of the drug in soft gelatin capsules. In these, the active ingredient is determined either by extracting the capsule contents with a suitable organic solvent such as ethanol or by dissolving the whole capsule in a mixture of water and ethanol and then measuring the absorbance at 230 nm. These methods cannot differentiate between the active constituent and the degradation products present in the formulation and therefore cannot be used for stability determinations. Therefore, alternative methods, *e.g.*, polarography and high-performance liquid chromatography (HPLC), are required for the determination of specific temazepam contents after storage. This paper describes the development of a polarographic procedure for the determination of temazepam in soft gelatin capsules that can differentiate between the temazepam and its degradation products and can therefore be used for stability determinations.



Temazepam

Oelschläger and Oehr have studied the mechanism of the polarographic reduction of temazepam[1] and have produced a method of assay in Britton - Robinson buffer (pH 5–7) containing 10% of dimethylformamide (DMF). The use of DMF is necessary in order to obtain complete dissolution of the active constituent before polarographic determination.

The polarographic method, which involves the reduction of the azomethine bond, is very sensitive and is less time consuming than the spectrophotometric methods recommended for the determination of temazepam. As temazepam is extracted from the dosage form and determined in a well controlled solvent system at its characteristic half-wave potential, the method is adequately specific.

The object of the present work was to investigate the application of polarography to the rapid determination of temazepam in soft gelatin capsules. The suppressive effect of certain electrochemically inactive organic substances used in the gelatin capsules, *e.g.*, polyethylene glycol (PEG 400) and gelatin, was also studied.

Initially, the method was used for the determination of the capsule contents only. However, in order to cover the possibility of temazepam having diffused into the capsule shell, a procedure for the dissolution of the complete capsule was developed. Capsules stored at moderate temperatures dissolve readily in aqueous medium but the gelatin shell of those stored at high temperatures becomes resistant to dissolution. As prolonged heating could cause degradation of the active ingredient, enzymatic hydrolysis was used to dissolve the complete capsule.

Experimental and Results

Apparatus

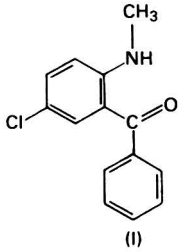
All experiments were performed using a Princeton Applied Research (PAR), Model 174, polarographic analyser equipped with a PAR, Model 172A, drop timer and electrode assembly and an Advance *X* - *Y* recorder (Model LR100). A three-electrode system containing a dropping-mercury working electrode (D.M.E.), a saturated calomel reference electrode (S.C.E.) and a platinum counter electrode was used.

Polarograms, obtained in both the direct-current (d.c.) and differential-pulse (d.p.) modes, were scanned from −0.60 to −1.30 V (*versus* S.C.E.) at a scan rate of 5 mV s^{-1}. A 50 mV pulse and 1.0 s drop were applied in the d.p. polarographic (d.p.p.) procedure. The current sensitivity required ranged from 5 to 50 μA full scale. A low-pass filter 3 and a 1-s forced drop time were used in the d.c. procedure.

A water-bath maintained at 50 ± 0.5 °C was used.

Reagents

Impurities and temazepam degradation products. These compounds were prepared in the Wyeth Laboratories, their purities being confirmed by thin-layer chromatography: 2-methylamino-5-chlorobenzophenone (**I**), 7-chloro-5-phenyl-4,5-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2,3-(1*H*)-dione (**II**), oxazepam, 7-chloro-2,3-dihydro-3-hydroxy-5-phenyl-1*H*-1,4-benzodiazepin-2-one (**III**), 6-chloro-4-hydroxy-1-methyl-4-phenyl-1,4-dihydroquinazoline (**IV**) and 6-chloro-4-phenyl-1-methyl-3,4-dihydroquinazoline-2-one (**V**).



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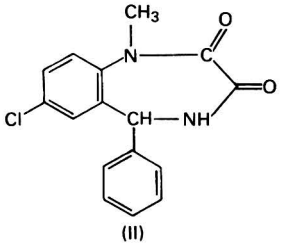

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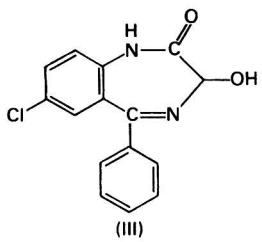

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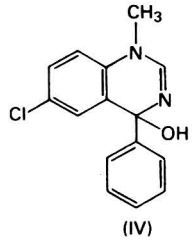

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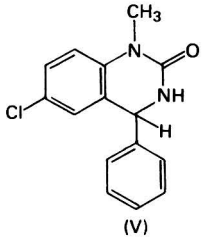

(V)

Temazepam and placebo capsules. All of the temazepam and placebo capsules examined contained PEG 400 USP and had dry shells consisting of Gelatin BP, Glycerine BP, sodium ethyl *p*-hydroxybenzoate and sodium propyl *p*-hydroxybenzoate; the temazepam capsules also contained temazepam.

Dimethylformamide. Laboratory-grade dimethylformamide (DMF) was purified by allowing it to stand over molecular sieve 5A (calcium aluminosilicate) for at least 24 h.

Acetate buffer solution, pH 4.7. Prepared by dissolving 8.2 g of anhydrous sodium acetate in 1 l of distilled water containing 5.8 ml of glacial acetic acid.

Phosphate buffer solution, pH 7.4. Prepared by dissolving 1.18 g of potassium dihydrogen orthosphosphate (KH_2PO_4) and 10.9 g of disodium hydrogen orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$) in 2 l of distilled water.

Enzyme solution, 4 mg ml^{-1}. Prepared by dissolving 1 g of pancreatin grade VI (Sigma London Chemical Company Ltd.) in 250 ml of the phosphate buffer solution (pH 7.4).

Preparation of Calibration Graphs

Solutions of temazepam (10–100 μg ml^{-1}) were prepared by suitable dilution of a working standard (1.0 mg ml^{-1}) with the acetate buffer solution containing 10% of DMF.

Preliminary Studies on Temazepam Raw Material

Some of the work of Oelschläger and Oehr[1] was repeated and polarographic studies were carried out on temazepam in Britton - Robinson buffer solutions at varying pH values using both the d.c. and d.p. modes of the instrument. As Oelschläger and Oehr[1] had observed, there was only one polarographic wave for the whole pH range (2–11) but the limiting current showed significant changes depending on the pH value of the buffer solution and the wave height was less at pH 3 than at pH values between 3 and 5. In the weakly acidic to strongly alkaline range, a deformation of the plateau resulted. The half-wave potential was given by $E_{\frac{1}{2}} = E^\circ - 0.059\text{pH}$.

It has been shown that temazepam is relatively unstable in strongly acidic or alkaline buffer solutions and consequently the optimum pH range for polarographic determination is 4.5–7.5. It is recommended that samples should be diluted with the buffer solution shortly before the determination.

DMF is used to dissolve the drug before dilution with the buffer solution. Alternative solvents including methanol, 95% ethanol and isopropanol had been used but none of them were as rapid as DMF at the concentrations used. In this work, an aqueous acetate buffer solution (pH 4.7) was used as the supporting electrolyte to dilute the sample solution. The polarogram was very similar to that obtained using Britton - Robinson buffer solution (pH 5.0), but had slightly better separation of sample wave and cut-off potential. In subsequent work, DMF was used as the solvent and acetate buffer solution (pH 4.7) as the supporting electrolyte.

Typical d.c. and d.p. polarograms of temazepam at the 40 μg ml^{-1} level are shown in Fig. 1. The peak occurs at -0.92 V (*versus* S.C.E.), corresponding to the half-wave potential potential ($E_{\frac{1}{2}}$) of the d.c. wave.

For the d.c. polarographic procedure, the calibration graph obtained for the drug in the range 10–100 μg ml^{-1} of the final solution was rectilinear. For the d.p.p. procedure, at higher concentrations, there is a levelling effect and the calibration graph for the drug is no longer rectilinear over the range studied. The results are shown in Fig. 2. The coefficients of variation for the determination of the drug obtained by carrying out 10 replicate analyses on the raw material at the 40 μg ml^{-1} level by both the d.c. and d.p.p. procedures were 1.4 and 0.6%, respectively.

Application to Soft Gelatin Capsule

Effect of solvent and capsule constituents on polarographic reduction of temazepam

Polyethylene glycols and gelatin are known to be adsorbed at the D.M.E. and to suppress some polarographic waves.[2] The suppressive effect of these electrochemically inactive organic substances was studied. Calibration graphs obtained for temazepam and the effect of four 10-mg placebo capsule contents and four 10-mg complete placebo capsules on the suppression of d.c. wave height/d.p. peak height for temazepam in the range 10–100 μg ml^{-1}

are shown in Fig. 2. The amounts of PEG 400 and gelatin had little effect on the d.c. wave height but suppressed the d.p. peak height considerably over the whole concentration range. Further, results obtained by the d.p. method were low and less reproducible than those for the d.c. method when the whole capsule was used. Therefore, only the d.c. method is suitable for the determination of temazepam in whole gelatin capsules. In the d.p. technique the peak current is governed by the rate of the electron transfer reaction and therefore is sensitive to the exact matrix used. A slight change in matrix composition can alter the peak current considerably.[3]

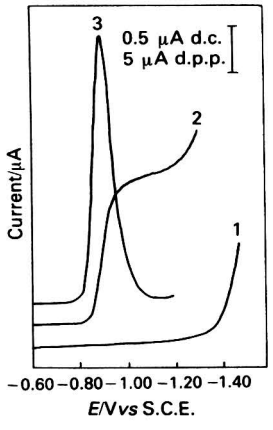

Fig. 1. Typical polarograms obtained with standards using the recommended polarographic procedures: (1) blank; (2) d.c. polarogram of temazepam at a level of 40 μg ml^{-1}; and (3) d.p. polarogram of temazepam at a level of 40 μg ml^{-1}.

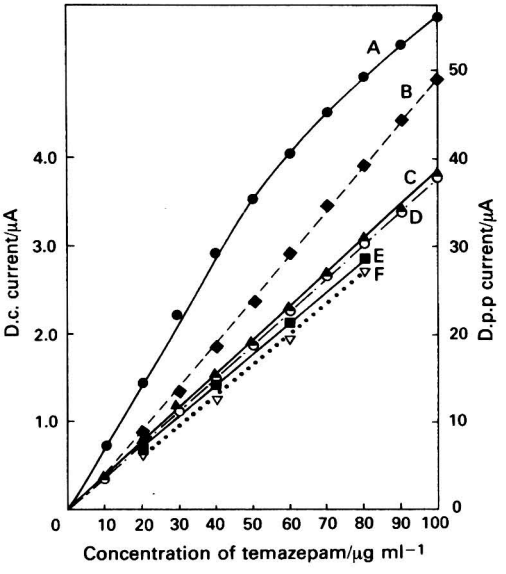

Fig. 2. Calibration graphs for temazepam and the effect of placebo capsules on the suppression of d.c. wave/d.p. peak height for temazepam in the region 10–100 μg ml^{-1}. A, Temazepam standards only, d.p.p.; B, temazepam standards containing placebo extracts, d.p.p.; C, temazepam standards only, d.c.; D, temazepam standards containing placebo extracts, d.c.; E, temazepam standards containing whole placebos, d.c.; F, temazepam standards containing whole placebos, d.p.p.

The suppression that is obtained in both the d.c. and d.p. methods on adding an increasing number of placebo capsule contents and complete placebo capsules to aliquots of a temazepam standard solution containing 60 μg ml^{-1} of temazepam is shown in Fig. 3. It can be seen that the minimum number of placebo capsules that can be used for the analysis of the capsule content and the whole capsule are four and six, respectively. This produces constant suppression but involves a considerable loss in sensitivity.

Enzymatic pre-treatment of gelatin capsules

Samples stored at high temperatures have proved difficult to analyse as the capsule shells are more difficult to dissolve. Therefore, proteolytic enzymes were used to aid complete dissolution of the capsule shell and prevent coagulation during the extraction stage. Enzymes covering a wide range of activities were examined, as shown in Table I, and it was observed that pancreatin gave the best results for the lowest cost.

The presence of the enzyme did not interfere with the polarographic determination. The drug was shown to be sufficiently stable in the enzyme solution as, after storage for more than 3 h, polarograms identical with the originals were obtained.

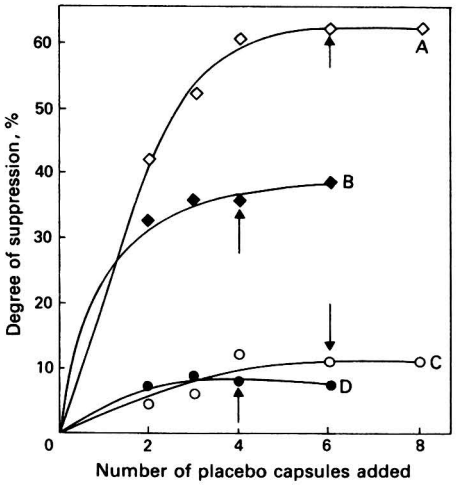

Fig. 3. Effect of placebo capsules on limiting currents for temazepam (60 μg ml^{-1}) using both d.c. and d.p. polarography. Arrows indicate the maximum suppression reached for a particular method. A, Whole capsule, d.p.p.; B, capsule content only, d.p.p.; C, whole capsule, d.c.; and D, capsule content only, d.c.

Determination of temazepam content of soft gelatin capsules by d.c. and d.p.p. procedures

The recommended procedure for the determination of the temazepam contents of soft gelatin capsules is as follows:

Sample. Place four capsules in a 100-ml stoppered conical flask and add 20 ml of DMF. Open the capsules by cutting them in half with scissors under the surface of the DMF. Wash the scissors into the flask with a further 5 ml of DMF using a Pasteur pipette. Pour off the extract through a small funnel into a 100-ml calibrated flask. Wash the conical flask and capsule shells with two 25-ml portions of DMF, transferring each portion into the 100-ml calibrated flask. Use a further 20 ml of DMF to wash the stopper, flask rim and funnel into the calibrated flask, then dilute to volume with DMF and mix well. Dilute 5 ml of the resulting solution to 50 ml using the acetate buffer solution. De-oxygenate a portion of this solution in a polarographic cell and record the polarogram between −0.60 and −1.30 V. (The above procedure applies to 10-mg capsules. For 20-mg capsules, collect the extract in a 200-ml calibrated flask and dilute to volume).

TABLE I

ENZYMES FOR THE DISSOLUTION OF TEMAZEPAM SOFT GELATIN CAPSULES

Enzyme*	Temperature/ °C	pH	Buffer solution	Time†/min (*a*)	Time†/min (*b*)	Cost of enzyme per gram/£	Cost per assay/£
Trypsin (Type III) ..	50	8.4	0.1 N $NaHCO_3$	10	20	5.0	0.5
Pepsin (crystallised) ..	50	3.0	0.001 N HCl	20	60	1.9	0.19
Protease (Type VIII) ..	50	7.5	Phosphate	5	15	30.0	3
Pancreatin (Grade VI) ..	50	7.5	Phosphate	10	25	0.05	0.005

* Enzyme concentration was 100 mg per 25 ml.

† Time required to dissolve completely (*a*) six fresh capsules and (*b*) six capsules stored at 37 °C for 6 months in 25 ml of enzyme solution at 50 °C with occasional shaking.

Standard. Weigh accurately 40 mg of temazepam into a 100-ml calibrated flask. Extract four placebo capsules according to the procedure adopted for the sample, collecting the extract in the calibrated flask containing the standard. Dilute to volume with DMF and

mix well. Dilute 5 ml of the resulting solution to 50 ml using the acetate buffer. De-oxygenate a portion of this solution in a polarographic cell and record the polarogram between −0.60 and −1.30 V.

Compare the wave height of the d.c. polarogram and the peak height of the d.p. polarogram with those obtained for the standards.

The method involves extraction of the capsule contents with DMF, dilution with aqueous acetate buffer solution (pH 4.7) and comparison with standards containing placebo extracts.

Calibration graphs for both the d.c. and d.p.p. procedures were rectilinear in the range 10–100 μg ml^{-1} (Fig. 2). Coefficients of variation and mean assay figures (8 determinations) for the 10-mg capsules were 1.42 (9.9) and 0.76% (10.0 mg per capsule), respectively, for both the d.c. and d.p.p. determinations.

Determination of temazepam in whole soft gelatin capsules by d.c. polarography following aqueous dissolution

The recommended procedure for the determination of temazepam in whole soft gelatin capsules following aqueous dissolution is as follows.

Sample. Place six 10-mg or three 20-mg capsules in a 100-ml calibrated flask and add 25 ml of distilled water. Warm in a water-bath at 50 °C, shaking periodically by hand, until the capsules have dissolved. Add 50 ml of DMF and shake vigorously for 30 min. Dilute to volume with DMF, allow the flask to cool to room temperature, re-adjust to volume with DMF and mix well. Pipette 5 ml of this solution into a 50-ml calibrated flask and dilute to volume with acetate buffer solution shortly before the polarographic determination. De-oxygenate a portion of this solution in a polarographic cell and record the polarogram between −0.60 and −1.30 V.

Standard. Accurately weigh approximately 60 mg of temazepam into a 100-ml calibrated flask. Add six 10-mg or three 20-mg placebo capsules and proceed as above. Compare the wave height of the d.c. polarogram with those obtained for the standards.

The method involves dissolution of the capsule shell in water, extraction with DMF and dilution with aqueous acetate buffer (pH 4.7). Results are compared with a standard solution containing an equivalent number of placebo capsules in the preparation. The coefficient of variation and mean temazepam content obtained using the procedure for the 10-mg capsule were 0.82% and 9.90 mg per capsule, respectively, based on 10 determinations.

Determination of temazepam in whole soft gelatin capsules by d.c. polarography following enzymic dissolution

The recommended procedure for the determination of temazepam in whole soft gelatin capsules following enzymic dissolution is as follows.

Sample. Place six 10-mg or three 20-mg capsules in a 100-ml calibrated flask and add 25 ml of enzyme solution. Warm in a water-bath at 50 °C, shaking periodically by hand, until the capsules have dissolved. (Complete dissolution of capsules takes about 10 min, but the solution should be maintained at 50 °C for 30 min before the addition of DMF.) Add 50 ml of DMF and shake vigorously for 30 min. Dilute to volume with DMF, allow the flask to cool to room temperature, re-adjust to volume with DMF and mix well. Pipette 5 ml of this solution into a 50-ml calibrated flask and dilute to volume with acetate buffer solution shortly before the polarographic determination. De-oxygenate a portion of this solution in a polarographic cell and record the polarogram between −0.60 and −1.30 V.

Standard. Accurately weigh approximately 60 mg of temazepam into a 100-ml calibrated flask. Add six 10-mg or three 20-mg placebo capsules and proceed as above. Compare the wave height of the d.c. polarogram with those obtained for the standards.

This method involves enzymic dissolution of the capsule shell, extraction with DMF and dilution with acetate buffer solution (pH 4.7). Results are compared with a standard solution containing an equivalent number of placebo capsules in the preparation. The coefficient of variation and mean result for the procedure for the 10-mg capsule were 1.0% and 9.85 mg per capsule, respectively, based on six determinations.

Stability determinations using the polarographic method

A solution of temazepam was degraded in PEG 400 (10 per 228 mg) at 100 °C and the solution was analysed polarographically and by quantitative thin-layer chromatography

(QTLC) after 17, 23, 39 and 62 h. The results are shown in Table II. The QTLC method involved separation of the main spot from impurities, collection and extraction of the main spot with a solvent, followed by determination by ultraviolet spectrophotometry. Some of the degradation spots from QTLC were also isolated and examined using both ultraviolet spectrophotometry and polarography. None of the spots showed any polarographic activity but one non-fluorescent spot [R_F 0.54 using chloroform - methanol (92.5 + 7.5) as the eluent and silica gel $60F_{254}$ pre-coated commercial plates as the stationary phase] exhibited a strong ultraviolet peak at 250 nm, a wavelength at which temazepam itself absorbs strongly. Therefore, the polarographic method is better than the ultraviolet spectrophotometric method for the determination of temazepam in the presence of its degradation products and so can be used for stability studies.

TABLE II

COMPARISON OF QTLC AND POLAROGRAPHIC DETERMINATIONS OF TEMAZEPAM SOLUTIONS DEGRADED AT 100 °C IN PEG 400

	Temazepam remaining, %	
Time/h	QTLC	D.c. polarography
17	90.2	88.0
23	90.3	85.8
39	87.6	84.8
62	83.4	81.9

Polarographic studies of some possible impurities and degradation products in temazepam

Possible impurities in temazepam raw material and formulated products fall into two categories, *viz.*, those arising from the method of manufacture, which include 2-methylamino-5-chlorobenzophenone (**I**), 7-chloro-5-phenyl-4,5-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2,3-(1*H*)-dione (**II**) and oxazepam (**III**), and possible degradation products, which include 6-chloro-4-hydroxy-1-methyl-4-phenyl-1,4-dihydroquinazoline (**IV**) and 6-chloro-4-phenyl-1-methyl-3,4-dihydroquinazoline-2-one (**V**).

The d.c. polarographic behaviour of 10^{-4} M solutions of each was tested in acetate buffer solution containing 10% of DMF with the results shown in Table III, whereas Table IV shows the effect on temazepam itself.

Discussion

This paper describes a simple and reliable polarographic method for the determination of temazepam in soft gelatin capsule formulations without the need for prior separation of the drug compound from capsule matrices by solvent extraction or chromatography, and which is suitable for use in stability studies. Enzymatic dissolution of the gelatin capsule shell was found to be particularly useful in obtaining solutions of the capsules prior to polarographic determination.

Although HPLC has been used widely for drug analysis, it has been shown that the presence of gelatin in the solution forms a coating on the column packing materials and renders the

TABLE III

POLAROGRAPHIC DATA ON TEMAZEPAM AND COMPOUNDS I–V

Compound	Concentration $\times 10^{-4}$/M	$E_{\frac{1}{2}}$/V *vs.* S.C.E.	Current/μA
Temazepam ..	2	−0.94	2.23
I	2.5	−1.0	0.18
II	2.0	*	—
III	2.0	−0.91	2.39
IV	2.2	−0.75	0.33
V	2.2	*	—

* These compounds did not exhibit polarographic activity.

TABLE IV

EFFECT OF COMPOUNDS I–V ON THE POLAROGRAPHIC DETERMINATION OF TEMAZEPAM (2×10^{-4} M)

Compound added	Concentration $\times 10^{-4}$/M	Current/μA	Recovery, %
None	—	2.23*	—
I	2.50	2.73	122
I	0.25	2.30	103
II	2.00	2.18	98
III	0.20	2.21	99
III	0.40	2.41	108
IV	0.22	2.24	100
V	2.20	2.06	92.5
V	0.22	2.25	101

* This value is for 2×10^{-4} M temazepam solution.

separation difficult. A preliminary clean-up step that involves solvent extraction is therefore required before the sample is applied to the column.

This method has been adopted for routine use in studies of the stability of temazepam in soft gelatin capsules. Therefore, automation is desirable when large numbers of determinations are necessary, for example, in quality control and stability work. An AutoAnalyzer system for automated polarographic analyses has been described in detail by Cullen *et al.*[4] for the determination of another important benzodiazepine, lorazepam, in tablet and capsule formulations. A microprocessor controlled polarograph with data handling facilities coupled to an autoanalyser unit would allow more samples per hour to be assayed with a high precision.

References

1. Oelschläger, V. H., and Oehr, H. P., *Pharm. Acta Helv.*, **1974**, **49**, 179.
2. Lukaszewski, Z., *Talanta*, **1977**, **24**, 603.
3. Osteryoung, J., and Haseba, K., *Rev. Polarogr.*, **1976**, **22**, 1.
4. Cullen, L. F., Brindle, M. P., and Papariello, G. J., *J. Pharm. Sci.*, **1973**, **62**, 1708.

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Solvent Extraction - Spectrophotometric Determination of Boron in Steel with 2,4-Dinitronaphthalene-1,8-diol and Brilliant Green

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On the basis of previous work, a solvent extraction procedure has been applied to the determination of boron in steel. The boron (as boric acid) is converted to the monovalent complex anion, BR_2^- (where H_2R is 2,4-dinitronaphthalene-1,8-diol), in acetic acid medium, extracted as a coloured ion associate with Brilliant Green into toluene and determined spectrophotometrically. Steel samples were dissolved in a mixture of dilute hydrochloric acid and hydrogen peroxide and large amounts of iron and other metal ions in the solution were removed either by solvent extraction with 4-methylpentan-2-one or by precipitation as hydroxides or oxinates. Micro amounts of boron in steel samples (0.0013–0.012%) were determined.

Keywords: *Boron determination*; *2,4-dinitronaphthalene-1,8-diol and Brilliant Green reagent*; *solvent extraction*; *spectrophotometry*; *steel analysis*

Many spectrophotometric methods for boron have been reported. Of these, the method using curcumin is probably the most sensitive: the molar absorptivity has been reported to be 17.4×10^4 (ref. 1) and 16.2×10^4 l mol^{-1} cm^{-1} (ref. 2). However, methods using curcumin are troublesome and the absorbance of the reagent blank is very large, which is a problem in the sensitive and reproducible determination of micro amounts of boron.

The solvent extraction - spectrophotometric determination of boron with 2,4-dinitronaphthalene-1,8-diol (DNNDO) and Brilliant Green that was developed in our laboratory is more suitable for routine boron determinations than the other spectrophotometric methods so far reported. The method has the following advantages: (*i*) high sensitivity (the molar absorptivity in toluene at 639 nm is 10.3×10^4 l mol^{-1} cm^{-1}) and high selectivity for boron; (*ii*) a simple and less time-consuming procedure; (*iii*) common laboratory apparatus is used; (*iv*) good reproducibility; and (*v*) very low absorbance of the reagent blank (below 0.03).[3] In this work, micro amounts of boron in steel samples were determined using DNNDO and Brilliant Green.

Experimental

Apparatus

Absorption measurements were made on a Hitachi Perkin-Elmer, Model 139, spectrophotometer in a glass cell of 10-mm path length. An Iwaki, Model V-S, Type KM, shaker was used to shake the separating funnel.

Reagents

2,4-Dinitronaphthalene-1,8-diol (DNNDO). The parent compound (naphthalene-1,8-diol) was synthesised according to the literature[4]; this method is different from that used in previous work.[3] A mixture of 22.4 g of 1-naphthylamino-8-sulphonic acid (Tokyo Kasei Co.), 20 g of sodium hydroxide and 180 ml of water was transferred into an autoclave and heated with stirring at 290–300 °C and 200–300 atm for 6 h. After cooling, the solution was shaken with diethyl ether to remove by-products. The aqueous solution was then saturated with carbon dioxide and filtered. The filtrate was acidified to about pH 1 with hydrochloric acid and the precipitate (naphthalene-1,8-diol) formed was collected, dried and recrystallised from benzene (melting-point 143–145 °C; reported value[5] 140 °C). This synthesis is less expensive and gives a higher yield than the method used in previous work. The synthesis of DNNDO from naphthalene-1,8-diol was carried out as previously described.[3] The product was recrystallised from toluene (melting-point 180–182 °C; reported value[5] 180–182 °C). The elemental analysis of DNNDO gave the following results: H 2.13, C 47.92 and N 11.24%; calculated values for $C_{10}H_6N_2O_6$, H 2.42, C 48.01 and N 11.20%.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

DNNDO - acetic acid solution. Recrystallised DNNDO (0.064 g) was dissolved in glacial acetic acid to give 100 ml of solution (2.5×10^{-3} M).

Brilliant Green solution. Commercially available Brilliant Green was dissolved in de-ionised water to give a 1×10^{-3} M solution.

Sodium acetate solution. Sodium acetate ($CH_3COONa.3H_2O$) was dissolved in de-ionised water to give a 1×10^{-2} M solution. This solution was stored in a polyethylene bottle.

Mannitol solution. Mannitol was dissolved in de-ionised water to give 1×10^{-3} M solution. This solution was stored in a polyethylene bottle.

Standard boron solution. Boric acid (H_3BO_3) (0.618 g) was dissolved in de-ionised water to give 100 ml of solution (1.000×10^{-2} M). This solution was stored in a polyethylene bottle and used after accurate dilution.

8-Hydroxyquinoline solution. Commercially available 8-hydroxyquinoline (16 g) was dissolved in acetone (100 ml).

Preparation of Sample Solution

Weigh the required amount of the steel sample into a 100-ml silica beaker. Add 5 to 15 ml of dilute hydrochloric acid (4 M) and then gradually add 5 ml of 30% *m/V* hydrogen peroxide solution. When about 0.1 and 1 g of steel sample were dissolved, 5 and 15 ml of dilute hydrochloric acid solution, respectively, were used. After dissolving the sample, heat the solution on a hot-plate (40–50 °C) for a few minutes, until effervescence ceases. Subsequently boil the solution on the hot-plate for about 5 min in order to complete the removal of hydrogen peroxide. The solution is then treated by one of the three methods for removing co-existing metal ions described below.

For extracting with 4-methylpentan-2-one, make the steel solution 6 M in hydrochloric acid and shake with 5 ml of 4-methylpentan-2-one. The extraction procedure is repeated two or three times until the organic phase is no longer coloured. The pH of the aqueous phase is then adjusted to 3–4 by adding sodium hydroxide solution (6–10 M). The solution is then accurately diluted to the required volume with de-ionised water (solution A).

To remove co-existing metal ions as precipitated hydroxides, add 0.1 g of cellulose powder (100–200 mesh) and 10 ml of sodium hydroxide solution (2 M) to the steel solution. Heat this solution for 5–10 min on a hot-plate (about 100 °C). After cooling the solution to room temperature, adjust to pH 10 with dilute hydrochloric acid. Filter the solution through filter-paper (Toyo Roshi No. 5C), which has been previously washed with sodium hydroxide solution (by filtering 20–30 ml of a 2 M solution). Wash the precipitate with 10^{-4} M sodium hydroxide solution. Transfer the filtrate and washings (about 70, 150 and 200 ml, respectively) into a calibrated polyethylene flask (100, 200 or 250 ml, as appropriate) and dilute to volume with de-ionised water (solution B).

To remove co-existing metal ions as precipitated 8-hydroxyquinoline complexes, add 2 ml of sodium acetate solution (10^{-2} M) and 8-hydroxyquinoline solution (about 8 ml per 0.1 g of steel sample) or, if necessary, transfer the steel solution into a 100-ml calibrated flask and dilute to volume with de-ionised water. An accurately measured volume of this diluted solution is transferred into a silica beaker before the addition of the sodium acetate and 8-hydroxyquinoline solutions. Adjust the pH of the solution to 7–7.5 with dilute sodium hydroxide solution and heat at 60–70 °C on a water-bath or hot-plate for 30 min. Filter the precipitate through a filter-paper (No. 5C), which has been previously washed with sodium hydroxide solution. Wash the precipitate with 30–50 ml of de-ionised water. Transfer the filtrate and washings into a calibrated 100-ml polyethylene flask and dilute to volume with de-ionised water (solution C).

Procedure for the Determination of Boron

Transfer the sample solution, containing up to 0.5 μg of boron, into a porcelain evaporating dish and add 1 ml each of mannitol and sodium acetate solutions. Evaporate the solution to dryness on a water-bath. Add 3 ml of glacial acetic acid and 1.5 ml of DNNDO - acetic acid solution and allow to stand for at least 1 h. Transfer this acetic acid solution into a separating funnel. Add 1 ml of 1 M sodium hydroxide solution and then dilute the solution to about 30 ml with water. Add 0.5 ml of Brilliant Green solution and 5 ml of toluene. Shake the solution for 10 min and then discard the aqueous phase. Add 10 ml of 2 M hydrochloric acid and shake for a further 10 min. Measure the absorbance of the toluene layer at 637 nm in a glass cell of 10-mm path length.

Results and Discussion

Removal of Excess of Hydrogen Peroxide

Large amounts of hydrogen peroxide interfere in the determination of boron by oxidising the cationic dye. Hydrogen peroxide can be easily decomposed by heating. However, it appeared that boron in the acidic solution is easily volatilised by heating. In previous work[3] it had been found that volatilisation of boron did not occur until the volume reached about 5 ml. To confirm this conclusion, the volatility of boron in acidic solution was examined. Table I shows recoveries of boron obtained after boiling the boron solution for up to 20 min on a hot-plate (about 200 °C). From the results shown, it is clear that volatilisation of boron from large volumes of solution is insignificant. In this work, therefore, the removal of the excess of hydrogen peroxide was carried out by heating the solution at 40–50 °C until effervescence ceased.

The solution was then boiled for about a further 5 min in order to decompose the hydrogen peroxide completely.

TABLE I

EFFECT OF BOILING TIME ON THE VOLATILISATION OF BORON

About 8 ml of boron solution containing 11.2 μg of boron in 4 M hydrochloric acid solution was boiled in a 100-ml silica beaker, which was capped with a silica watch-glass. After boiling, the solution was neutralised with sodium hydroxide solution and diluted to 100 ml with water. The boron concentration in the dilute solution was then measured.

Boiling time/ min	Recovery of boron, %
0	100
1	98.2
2	98.5
3	98.5
5	99.7
10	99.7
20	97.9

Effect of Co-existing Ions

As described previously,[3] high concentrations of metal ions will result in negative errors in the determination of boron. For example, copper, cobalt, nickel and manganese at concentrations above 3 μg ml^{-1}, iron above 8 μg ml^{-1} and aluminium above 1 μg ml^{-1} will interfere.

In the determination of boron in steel samples containing down to 10^{-3}% of boron, the concentration of iron in the sample solution will be about 1 mg ml^{-1}. Therefore, large amounts of iron must be removed prior to the determination procedure.

In general, iron(III) is removed from about 6 M hydrochloric acid solution by shaking with 4-methylpentan-2-one. This removal procedure, although very simple and not time consuming, requires the very acidic solution to be neutralised before use; further, other metal ions such as copper, manganese, nickel and chromium, which exist in the steel sample solutions in relatively high concentrations, are not removed to any significant extent.

Removal of metal ions by precipitation as their hydroxides has the advantage that the procedure does not require any special reagents. However, as described later, there is the serious disadvantage that micro amounts of boron are removed by adsorption on to the hydroxide precipitates.

Removal of metal ions by precipitation as chelates was examined using cupferron and 8-hydroxyquinoline. Cupferron was not suitable because it was easily decomposed and the reagent blank increased. Oxinate and 8-hydroxyquinoline are very stable and, further, a large number of oxinates are slightly soluble in water at pH 7–7.5.

The suitability of each of these three removal procedures was examined using the standard steel samples. In addition, the extraction of the boron complex into 4-methylpentan-2-one with 2-ethylhexane-1,3-diol (10–0.1% *m*/*V*), according to the procedure proposed by Spielholtz *et al.*,[6] was also examined. The extract was evaporated to dryness in the presence of

sodium acetate and mannitol solutions in a porcelain dish and the boron was determined according to the procedure described here. The maximum recovery of boron was about 20% using a 0.5% *m*/*V* solution of 2-ethylhexane-1,3-diol in 4-methylpentan-2-one; further, it required longer to evaporate the mixture of the organic extract and the aqueous solution to dryness than to evaporate only an aqueous solution. It was therefore concluded that this extraction procedure for boron was not suitable for use with the DNNDO and Brilliant Green method.

Determination of Boron in Standard Steel Samples

Boron sample solutions were prepared and their boron contents determined by the procedures previously described here.

In Table II, the results obtained for the boron content of JSS (Japanese Standards of Iron and Steel) 161-3 (certified boron content = 0.012%) using solutions A, B and C are shown. In all instances, the mean values were in good agreement with the certified value. However, with solution B the results varied more widely than those with solution C. In Table II, results of recovery tests for boron are also shown; the results are satisfactory.

TABLE II

DETERMINATION OF BORON IN JSS 161-3

Certified boron content = 0.012%.*

Sample solution	Concentration of steel	Boron added†	Volume of solution taken/ ml	Boron added/ μg‡	Boron found/ μg	Boron content,§ %	Recovery %
A	0.1032 g per 100 ml	0	1	0	0.118	0.0114	
		0	1	0.116	0.233		99.1
	0.1101 g per 100 ml	0	1	0	0.149	0.0135	
		0	1	0.116	0.262		97.4
	0.1022 g per 100 ml	11.6 μg per 100 ml	1	0	0.255		100.9¶
B	0.0636 g per 250 ml	0	5	0	0.155	0.0122	
	0.0623 g per 250 ml	11.6 μg per 250 ml	5	0	0.388		101.7‖
	0.1088 g per 250 ml	0	5	0	0.227	0.0104	
	0.0330 g per 100 ml	0	2	0	0.0750	0.0114	
C	0.1142 g per 1000 ml	0	5	0	0.0718	0.0126	
	0.0147 g per 100 ml	0	5	0	0.0896	0.0122	
	0.0141 g per 100 ml	0	5	0	0.0925	0.0131	

* Other components: Mo, 0.31; V, 0.01; Co, 0.02; Ti, 0.30; As, 0.01; Sn, 0.01; Nb, 0.01%.
† The boron solution was added to the steel sample in a beaker. The sample was then dissolved.
‡ The boron solution was added to the sample solution in a porcelain dish.
§ Calculated from the mean of three spectrophotometric measurements.
¶ Boron content was taken to be 0.0135%.
‖ Boron content was taken to be 0.0122%.

In Table III results for the boron content of NBS 364 (certified boron content = 0.0106%) using solutions B and C are shown. In both instances the results obtained are in good agreement with the certified value.

In Table IV results for the boron content of JSS 160-3 (certified boron content = 0.0063%) using solutions A, B and C are shown. In all instances the mean values are in good agreement with the certified value, but in solution B the values vary more widely than those in solution C.

TABLE III

DETERMINATION OF BORON IN NBS 364

Certified boron content = 0.0106%.*

Sample solution	Concentration of steel	Volume of solution taken/ ml	Boron content,† %
B	0.0653 g per 250 ml	5	0.0105
	0.1088 g per 250 ml	5	0.0102
C	0.1274 g per 400 ml	5	0.0105
	0.1552 g per 400 ml	5	0.0102

* Other components: C, 0.87; Mn, 0.26; P, 0.01; S, 0.03; Si, 0.07; Cu, 0.25; Ni, 0.14; Cr, 0.06; V, 0.11; Mo, 0.49; W, 0.10; Co, 0.15; Ti, 0.24; As, 0.05; Sn, 0.01; Nb, 0.16; Ta, 0.11; Pb, 0.02; Zr, 0.07; Sb, 0.03%.
† Calculated from the mean of three spectrophotometric measurements.

TABLE IV

DETERMINATION OF BORON IN JSS 160-3

Certified boron content = 0.0063%.*

Sample solution	Concentration of steel	Volume of solution taken/ ml	Boron content,† %
A	0.5377 g per 100 ml	1	0.00617
B	0.1052 g per 250 ml	5	0.00618
	0.1599 g per 250 ml	5	0.00604
	0.0736 g per 200 ml	5	0.00642
C	0.1126 g per 100 ml	5	0.00639
	0.1189 g per 100 ml	5	0.00616
	0.1268 g per 200 ml	5	0.00619

* Other components: Mo, 0.16; V, 0.10; Co, 0.06; Ti, 0.09; As, 0.05; Sn, 0.06; Nb, 0.11%.

† Calculated from the mean of three spectrophotometric measurements.

In Table V results for the boron content of NBS 362 (certified boron content = 0.0025%) using solution C are shown. The results obtained were in good agreement with each other; however, they were about 15% higher than the certified boron contents. The cause of the error with this particular sample is not apparent.

TABLE V

DETERMINATION OF BORON IN NBS 362

Certified boron content = 0.0025%.*

Sample solution	Concentration of steel	Volume of solution taken/ ml	Boron content,† %
C	0.1068 g per 100 ml	5	0.00283
	0.1096 g per 100 ml	5	0.00289
	0.1093 g per 100 ml	5	0.00288

* Other components: C, 0.16; Mn, 1.04; P, 0.04; S, 0.04; Si, 0.39; Cu, 0.50; Ni, 0.59; Cr, 0.30; V, 0.04; Mo, 0.07; W, 0.20; Co, 0.30; Ti, 0.08; As, 0.09; In, 0.02; Al, 0.10; Nb, 0.29; Ta, 0.20; Zr, 0.19; Sb, 0.01%.

† Calculated from the mean of three spectrophotometric measurements.

In Table VI results for the boron content of JSS 159-3 (certified boron content = 0.0013%) using solutions A, B and C are shown. With solutions A and B, the values obtained are all less than the certified value (with solution B, the mean value is about 20% below the certified value), because of adsorption of boron on to the precipitated metal hydroxide. With solution C, the values obtained varied widely when about 0.1 g of steel sample was used; however, the mean value is in good agreement with the certified value. About 1 g of steel sample was dissolved, the solution diluted to 100 ml with de-ionised water and a 10-ml aliquot was used for removal of metal ions. After filtering, the filtrate was diluted to 100 ml and the boron content determined, each value obtained was in good agreement with the certified value. From these results, it must be concluded that micro amounts of boron may be adsorbed on to the precipitated hydroxide and that in the steel sample micro amounts of boron are heterogeneously distributed.

Conclusion

The solvent extraction - spectrophotometric method for the determination of micro amounts of boron was applied to the determination of boron in steel samples. The samples were dissolved in hydrochloric acid and hydrogen peroxide.

Three kinds of preparation method for the sample solution were examined. Removal of metals with 4-methylpentan-2-one may be used for a sample containing relatively large amounts of boron. Removal of metals as precipitated hydroxides is not recommended for

TABLE VI

DETERMINATION OF BORON IN JSS 159-3

Certified boron content = 0.0013%.*

Sample solution	Concentration of steel	Volume of solution taken/ ml	Boron content,† %
A	0.1617 g per 100 ml	5	0.00092
B	0.3208 g per 250 ml	5	0.00094
	0.2080 g per 250 ml	5	0.00099
	0.2012 g per 250 ml	5	0.00097
	0.3102 g per 250 ml	5	0.00104
	0.2047 g per 250 ml	5	0.00127
	0.4808 g per 250 ml	5	0.00103
	0.1467 g per 100 ml	5	0.00091
		Mean value:	0.00102
C	0.1145 g per 100 ml	5	0.00166
	0.1147 g per 100 ml	5	0.00153
	0.1094 g per 100 ml	5	0.00113
	0.1032 g per 100 ml	10	0.00120
	0.1138 g per 100 ml	10	0.00127
		Mean value:	0.00135
	1.0345 g per 1000 ml	5	0.00127
	1.0618 g per 1000 ml	5	0.00130
	1.0144 g per 1000 ml	5	0.00135
		Mean value:	0.00131

* Other components: Mo, 0.01; V, 0.31; Co, 0.11; Ti, 0.02; As, 0.10; Sn, 0.11; Nb, 0.21%.

† Calculated from the mean of three spectrophotometric measurements.

the determination of micro amounts of boron because of the adsorption of boron on to the precipitate, although it does not require any special reagents. Removal of metals as precipitated oxinates was the recommended method for the determination of micro amounts of boron. The advantages of this method are (*i*) micro amounts of boron are scarcely adsorbed on the precipitate, (*ii*) almost all of the other metal ions are removed as precipitated oxinates and (*iii*) the precipitates are easily formed and can be filtered with filter-paper.

The proposed method for the preparation of the sample solution and for the determination of micro amounts of boron can be easily applied to many other samples.

References

1. Goto, H., Kakita, Y., and Takada, K., *Bunseki Kagaku*, **1969**, **18**, 52.
2. Hayes, M. R., and Metcalfe, J., *Analyst*, **1962**, **87**, 956.
3. Kuwada, K., Motomizu, S., and Tôei, K., *Anal. Chem.*, **1978**, **50**, 1788.
4. Fierz, H. E., *Helv. Chim. Acta*, **1920**, **3**, 318.
5. Erdmann, H., *Justus Liebigs Ann. Chem.*, **1888**, **247**, 356.
6. Spielholtz, G. I., Toralballa, G. C., and Willsen, J. J., *Mikrochim. Acta*, **1974**, 649.

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Determination of Residues of Dithiocarbamate Pesticides in Foodstuffs by a Headspace Method

Report by the Panel on Determination of Dithiocarbamate Residues

OF THE COMMITTEE FOR ANALYTICAL METHODS FOR RESIDUES OF PESTICIDES AND VETERINARY PRODUCTS IN FOODSTUFFS OF THE MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Keywords: *Dithiocarbamate residues analysis*; *headspace analysis*; *carbon disulphide*

In 1977 the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs of the Ministry of Agriculture, Fisheries and Food (MAFF) set up a Panel on determination of dithiocarbamate residues with the following terms of reference: 'To investigate and, as appropriate, validate by collaborative study methods of analysis for dithiocarbamate fungicide residues and toxic degradation products in vegetables and fruit." This work was undertaken, primarily, as a consequence of a requirement of the Working Party on Pesticide Residues of MAFF to have information on the occurrence of certain fungicide residues and, particularly, of dithiocarbamate residues in lettuce. Representation on the Panel came from both central and local government, from the food industry and from pesticide manufacturers (including workers from France and Switzerland). The participating laboratories with their representatives are listed in Appendix II.

Although dithiocarbamates are used primarily on field crops and cereals in the UK, appreciable areas of lettuces and other vegetables and fruits are also treated. Maximum residue limits for dithiocarbamates (expressed as carbon disulphide) under consideration at the FAO/WHO Codex Committee on Pesticide Residues range from 0.1 mg kg^{-1} in potatoes to 5 mg kg^{-1} in celery; in lettuce they are 1 mg kg^{-1}. In the European Economic Community tolerances for dithiocarbamates are drafted as 2–7 mg kg^{-1} (as carbon disulphide) and in the USA they are about 10 mg kg^{-1} calculated as pesticide (the various pesticides contain about half their masses of available carbon disulphide). Terminal residues from the use of ethylenebisdithiocarbamate fungicides on crops can include the degradation product ethylenethiourea (the determination of which is not dealt with here).

The most widely used procedures for determining dithiocarbamate residues in fruits and vegetables are those involving a spectrophotometric determination of carbon disulphide liberated following a hot acid digestion of the foodstuff.[1,2] The sample is covered with hot acid (frequently sulphuric acid) and heated gently to boiling. The carbon disulphide evolved is passed through the apparatus by means of a stream of nitrogen, firstly into an absorption solution (such as lead acetate) to remove hydrogen sulphide and other interfering gases, and finally into a second absorption flask where the carbon disulphide is absorbed (usually by a solution of an amine with transition metal salt). The type of substrate, and also the particular dithiocarbamate, influence the yield of carbon disulphide.[3,4] The procedure used until recently by Public Analysts in the UK has been published[5] and the food industry has thoroughly investigated another variant.[6] A different type of procedure being increasingly used is headspace analysis, first published by McLeod and McCully,[7] in which the sample is digested in a hot acidic reducing medium. The digestion flask is closed at the neck with a silicone-rubber septum. After a fixed and sufficiently long period, some of the headspace gas is withdrawn through the septum, using a gas-tight syringe, and injected directly into a gas chromatograph. The carbon disulphide content of the solution is determined by comparison with standards. Polarographic[8] and gel permeation[9] techniques have also been used. The use of paper or thin-layer chromatography has been mainly confined to pure substances or formulations, although the solubility characteristics of dithiocarbamates, particularly in the presence of co-extracted materials, are a difficult problem. A high-performance liquid chromatographic procedure for the determination of thiram in lettuces[10] has been developed by some of the participants in the current work alongside the recommended method (Appendix I).

Comparison of Methods

At about the time the Panel started work, a comparison between one of the spectrophotometric procedures and a headspace method was published by van Haver and Gordts.[11] About 100 samples of lettuces treated with zineb were analysed by both methods with good agreement, a correlation factor $r = 0.9903$ being obtained. When the Panel considered a comparison of these types of method, two laboratories organised their own comparison of a spectrophotometric and a headspace procedure, using individual half-heads of ten lettuces treated experimentally, one of the methods being used in each laboratory. However, results obtained by the headspace procedure were higher than those obtained by the spectrophotometric method and these differences were ascribed, at least partly, to variability in sampling, transit and treatment of samples on arrival.

Solvent-layer Procedure

In the headspace procedures the liberated carbon disulphide is directly sampled with a syringe, through a silicone-rubber septum. Both syringe and septum must be completely gas-tight. The Laboratory of the Government Chemist has introduced a method[12] in which the sample being digested with warm acid is layered with an inert solvent, such as hexane, so that liberated carbon disulphide is absorbed by the organic solvent. The organic solvent is separated from the aqueous acid digest and sampled with a normal gas - liquid chromatography syringe for liquids. This technique would, it was hoped, help to reduce certain types of error inherent in the headspace procedure.

Initially, in order to obtain experience with the methods, members examined both the solvent-layer procedure (above) and the headspace procedure (basically that in Appendix I) in their own laboratories without using common samples. After discussion of points that arose, a first collaborative exercise was carried out using a revision of the solvent-layer method on 25 g of lettuce (common samples) spiked in collaborators' own laboratories with a suspension of nominally 5 or 50% zineb wettable powder (from a common source). Blank lettuce samples were also run. Recoveries at the 2 mg kg^{-1} level of pesticide were generally satisfactory, 65–96% in four laboratories (mean with a coefficient of variation of $83 \pm 12\%$), but at 0.2 mg kg^{-1} chromatograms were rarely distinguishable from those of the blanks. Some difficulties were encountered in satisfactorily stirring the two phases so as to keep the fragments of lettuce leaves in the aqueous layer or at the interface.

Individual laboratories then further accustomed themselves independently to a revision of this method and also to the headspace method as applied by J. W. Edmunds at the Agricultural Science Service, Harpenden Laboratory, for dithiocarbamates in lettuces (basically that in Appendix I).

Collaborative Studies of the Solvent-layer and Headspace Methods

When all collaborators felt that they were familiar with both methods a second collaborative exercise was carried out using them on lettuces (common samples) spiked in members' own laboratories with suspensions of zineb wettable powders (from a common source) at levels of 0.2 or 2.0 mg kg^{-1} of zineb, equivalent to about 0.1 and 1.0 mg kg^{-1} of carbon disulphide, respectively. Untreated controls were also run. For the headspace method 50 g of lettuce were used whereas for the solvent-layer method 25 g were taken, both having been scaled up from their original versions. Six laboratories participated, although not all six contributed results for both methods. Recoveries averaged 74 and 79% at the 0.2 and 2.0 mg kg^{-1} levels, respectively, by the headspace method and 75% at the 2.0 mg kg^{-1} level by the revised solvent-layer method. Standard deviations were about 10%. The detection limit of the solvent-layer method was too near the 0.2 mg kg^{-1} level for accurate measurement. Blank results were occasionally high because of apparent contamination of the lettuce or impurities in the hexane. In one laboratory heptane rather than hexane was used for extraction with the solvent-layer method and in another 2,2,4-trimethylpentane was sometimes used; hexane from certain sources could cause more interference than that from others. On the basis of this collaborative exercise the Panel considered that the headspace procedure was probably the more satisfactory method to study further as there were fewer difficulties at the 0.2 mg kg^{-1} level than with the solvent-layer method, although the latter was probably satisfactory for determining residues in lettuces at about the maximum residue limits (about 2 mg kg^{-1} of dithiocarbamate).

Collaborative Studies of the Headspace Method for Several Dithiocarbamates

A third collaborative exercise was then carried out using only the headspace procedure, now slightly modified and using larger amounts of sample, on lettuces spiked with four different dithiocarbamates (zineb, maneb, mancozeb and thiram) as their 5 or 50% wettable powders (each laboratory using materials from a common source). A common sample of lettuces was again despatched and seven laboratories participated in the exercise, although only six followed the exact instructions as the seventh was using a new highly sensitive gas - liquid chromatography detector that easily became saturated. Recovery data and blanks for the six laboratories are given in Table I and are corrected to two significant figures, although the statistical summary was obtained from the uncorrected data. The seventh laboratory obtained 92, 95, 93, 86, 97 and 89% recovery of zineb at the 2.0 mg kg^{-1} level. Results were satisfactory for zineb, maneb and thiram, but those for mancozeb were low. Statistical analysis of the individual determinations showed that four out of the eight sets of results had a skewed distribution but logarithmic transformation converted them into a normal Gaussian form. The coefficients of variation averaged about 20% at the 2 mg kg^{-1} level but at the 0.2 mg kg^{-1} level they were about 25–35%.

Vigorous shaking of the reaction flask is necessary at the appropriate stage and the septum must fit well and not leak.

These results were considered too variable to be acceptable without further investigation to define the procedure more closely. Therefore, a fourth collaborative exercise was undertaken with a further modification of the procedure (Appendix I) on lettuces spiked with suspensions of (about) 50% wettable powders of the same pesticides at, nominally, 4 or 10 mg kg^{-1} levels of dithiocarbamate in the crop. Both crop and formulations were distributed to the seven laboratories from common sources. The nominally 50% wettable powders were analysed in two laboratories by the CIPAC 61/1/M/1 method[13] to determine their precise pesticide content and hence obtain accurate (mean) values for the amount added in each residue recovery analysis. Mean residue recovery figures for the seven laboratories and values for the blanks are all satisfactory (Table II, where results are corrected to two significant figures, although the statistical summary was obtained from the uncorrected data); the coefficients of variation were notably high only with maneb. In comparison with the third collaborative exercise, mean recovery figures were higher with less spreading (six of the eight were 80–100%, two 70–80% and none was below 70%) and the mean of the coefficients of variation was 20% compared with about 25% in the third study. Statistical analysis of the individual results showed that two of the eight sets of results had a skewed distribution

TABLE I

RECOVERY OF DITHIOCARBAMATES FROM LETTUCE, THIRD COLLABORATIVE STUDY

Results expressed as pesticide.

Laboratory	Blank/ mg kg^{-1}	Zineb added*/ mg kg^{-1} net		Maneb added*/ mg kg^{-1} net		Mancozeb added*/ mg kg^{-1} net		Thiram added*/ mg kg^{-1} net	
		0.18	1.9	0.17	1.7	0.20	2.0	0.20	2.0
1	<0.01	0.16	1.3	0.10	1.1	0.09	1.4	0.16	1.3
		0.13	1.5	0.08	1.1	0.09	1.3	0.13	1.3
2	<0.02	0.18	1.8	0.18	1.3	0.18	1.6	0.19	1.8
		0.19	1.7	0.18	1.5	0.18	1.4	0.19	1.7
3	<0.01	0.15	1.3	0.12	1.1	0.11	1.2	0.15	1.6
		0.14	1.3	0.11	1.0	0.11		0.13	1.4
4	0	0.16	1.4	0.07	1.5	0.14	1.6	0.24	1.6
		0.14	1.8	0.10	1.6	0.20	1.6	0.25	1.6
5	<0.02	0.15	1.3	0.11	1.2	0.07	1.1	0.13	1.2
		0.14	1.2	0.11	1.2	0.07	1.0	0.12	1.4
6	0.04	0.25	2.0	0.18	1.8	0.13	1.8	0.16	1.1
	0.06	0.25	2.2	0.18	2.2	0.12	1.7	0.17	1.8
Mean result†/mg kg^{-1}		0.17	1.5(5)	0.13	1.3(9)	0.12	1.4(2)	0.17	1.4(8)
Recovery, %		94	83	77	83	60	71	85	74
n		12	12	12	12	12	11	12	12
Standard deviation†, / mg kg^{-1}		0.04(1)	0.3(3)	0.04(2)	0.3(4)	0.04(4)	0.2(5)	0.04(3)	0.2(3)
Coefficient of variation, %		24	21	32	24	37	18	25	16

* Based on analyses of wettable powders used for spiking.
† Digits in parentheses are not precise.

TABLE II

RECOVERY OF DITHIOCARBAMATES FROM LETTUCE, FOURTH COLLABORATIVE STUDY

Results expressed as pesticide.

Laboratory	Blank/ mg kg^{-1}	Zineb added*/ mg kg^{-1} net		Maneb added*/ mg kg^{-1} net		Mancozeb added*/ mg kg^{-1} net		Thiram added*/ mg kg^{-1} net	
		3.7	9.3	3.0	7.5	3.9	9.6	3.6	9.0
1	<0.01	3.8	9.0	3.7	8.0	3.5	8.6	3.8	10.7
	<0.01	3.8	9.7	3.8	7.5	3.4	8.7	3.8	10.2
	<0.01	3.8	10.8	3.8	7.3	3.6	8.6	3.8	10.2
2	<0.04	2.0	8.0	1.5	5.4	2.2	6.7	3.1	8.9
		2.7	8.0	1.6	3.8	2.3	6.2	3.1	7.6
		2.4	8.7	1.8	3.5	2.3	5.1	3.4	7.5
3	<0.02	3.6	9.4	2.3	6.7	3.4	7.3	3.2	9.3
		3.6	9.6	2.7	6.5	3.7	7.8	3.3	9.2
		3.7	9.4	2.6	4.4	3.5	8.1	3.4	9.6
4	0.10	3.1	8.4	2.7	7.0	2.8	8.4	2.8	7.6
	0.25	3.3	8.2	2.3	7.4	2.9	8.9	2.7	7.5
	0	3.1	8.1	2.3	6.7	3.1	8.6	3.2	7.5
5	<0.02	3.1	8.7	1.8	5.2	2.7	7.0	3.4	9.4
		3.2	8.7	1.8	5.6	3.0	7.4	3.5	10.0
		3.1	8.1	1.9	5.4	2.7	7.3	3.5	9.6
6	<0.01	3.1	7.3	2.4	4.8	2.9	7.3	3.0	7.0
		2.9	7.4	2.1	6.1	4.8	6.9	3.6	7.4
7	<0.01	2.6	4.9	2.4	4.3	2.6	4.1	2.9	4.7
		2.7	4.7			2.5	5.0	2.8	5.1
			4.9			2.7	6.5	2.8	
Mean result†/mg kg^{-1}		3.1(4)	8.1(0)	2.4(2)	5.8(7)	3.0(3)	7.2(2)	3.2(6)	8.3(7)
Recovery, %		85	87	81	78	78	75	91	93
n		19	20	18	18	20	20	20	19
Standard deviation†, / mg kg^{-1}		0.5(1)	1.6(3)	0.7(2)	1.3(6)	0.6(2)	1.3(5)	0.3(5)	1.6(9)
Coefficient of variation, %		16	20	30	23	20	19	11	20

* Based on analyses of wettable powders used for spiking.
† Digits in parentheses are not precise.

but logarithmic transformation converted these into a normal Gaussian form. The method as carried out in this exercise is therefore an improvement upon that used in the previous study, although some uncertainty in describing the shaking procedure adequately still persists.

Use of the Headspace Method on Other Foodstuffs

One laboratory, acting independently, also obtained satisfactory recovery data for the above four dithiocarbamates added at the 0.2 or 2.0 mg kg^{-1} levels to tomatoes, blackcurrants, potatoes, strawberries (frozen) and raspberries. Another laboratory obtained satisfactory recovery of zineb added to cassava and total diet groups at the 1 mg kg^{-1} level of pesticide. Canned fruit and vegetables were also analysed for dithiocarbamate residues using the method but blank values for vegetables are sometimes found that are as high as the tolerance levels.

The recommended method (Appendix I) was therefore agreed to be satisfactory for determining zineb, maneb, mancozeb and thiram in lettuces and appeared to be suitable for determining dithiocarbamates in a wider range of foodstuffs. Laboratories using the method should satisfy themselves thoroughly as to its applicability to these other commodities before obtaining residue data.

Experience has shown the method to be equally reliable at higher concentrations of pesticide (10–1000 mg kg^{-1}) in lettuces.

There are indications that, in a few instances, the presence of appreciable residues of copper compounds together with those of dithiocarbamates will lower the amount of carbon disulphide liberated from a foodstuff and so give a low residue figure.

Sampling

In several laboratories the decomposition of incurred dithiocarbamate residues on lettuce between actual cutting of the lettuce and insertion in the bottle for headspace analysis was investigated. Generally, as has been found by other workers for kale,[14] the residues of dithiocarbamates as measured by evolution of carbon disulphide decrease as the interval between commencement of cutting and insertion into the bottle increases. Consequently,

the more time spent in cutting and the finer the division (using a Hobart chopper or knife) before transferring to the bottle before analysis, the lower the residue level appears to be. It was noted that these effects are more marked with those dithiocarbamates which are more easily decomposed chemically as pure substances or in formulations, *e.g.*, zineb, and less with the more stable ones, *e.g.*, maneb. It is clear that vegetables and fruits must be analysed for residues as soon as possible after cutting or picking and any further cutting, picking or dicing of the whole commodity should be carried out immediately before placing in the reaction flask and should be kept to a minimum. Foodstuffs should be frozen whole, when this becomes necessary, and chopped and mixed in the frozen state immediately before taking the analytical samples. Deep-frozen samples that have been diced or chopped into small pieces before storage will always give unacceptably low results. Sampling procedures for pesticide residues in foodstuffs agreed by international organisations may have to be modified to accommodate dithiocarbamates.

APPENDIX I

Recommended Method for Determination of Dithiocarbamate Residues in Lettuces Using a Headspace Procedure

Reagents

Hydrochloric acid, concentrated.

Tin(II) chloride solution. Dissolve 15 g of analytical-reagent grade tin(II) chloride in an appropriate volume of 5 M hydrochloric acid (prepared by diluting 430 ml of concentrated hydrochloric acid to 1000 ml with water) and dilute to 1000 ml with 5 M hydrochloric acid.

Acetone. AnalaR or Distol grade. Check for freedom from interfering compounds by gas chromatography before use.

Carbon disulphide standard solution. Weigh accurately 0.4–0.6 g of carbon disulphide (infrared spectroscopy grade) into a 50-ml calibrated flask containing a small amount of acetone and dilute to 50 ml with acetone. Prepare other standard solutions containing 1 and 0.1 mg ml^{-1} of carbon disulphide by dilution of this solution with acetone. All standard solutions should be kept in a refrigerator when not in use and prepared freshly each week.

Apparatus

Bottle. Glass, 250 ml, with a screw-cap drilled with a 3-mm hole and fitted with a silicone-rubber septum (*e.g.*, V. A. Howe & Co. Ltd., London, Ref. Nos. 388–48 and 4.708.72, or Fisons, Cat. No. 21–801–365; QQ flasks with Subaseal caps have also been used successfully).

Water-bath, 80 °C.

Syringes, 100–250 μl, *gas-tight.* Check the performance of the syringes from time to time as they wear and cease to be gas-tight.

Gas chromatograph, with a flame-photometric detector in its sulphur mode. An electron-capture detector can be used as an alternative. The column is not critical: 2 m 2% OV-1 on 80–100-mesh Gas-Chrom Q has been used successfully with an oven temperature of 60 °C and an injection port temperature of 100 °C.

Procedure

Samples of vegetables and fruits for analysis should be subdivided and mixed for sub-sampling as soon as possible after cutting or picking. Keep further dicing and cutting to a minimum. Carry out the following analytical method as soon as possible.

Place a representative 50-g sample in a 250-ml glass bottle having a screw-cap and a silicone-rubber septum. Add 50 ml of 1.5% tin(II) chloride - hydrochloric acid solution and immediately screw on the cap containing a gas-tight septum. Place in the water-bath at 80 °C. After 15 min, remove the closed bottle from the water-bath and shake vigorously by hand for 2 min. Return the bottle to the water-bath and repeat the shaking every 15 min until 1 h has elapsed from the time the bottle was placed in the water-bath (also shake the bottle at the end of the 1-h period). Remove a suitable aliquot (10–100 μl) from the headspace (at 80 °C) using a gas-tight syringe and immediately inject it into the gas chromatograph. Compare the peak height with a calibration graph and record the level of carbon disulphide in the sample.

Calibration graph

Place 50 ml of water into each of four sample bottles and add 50 ml of 1.5% tin(II) chloride - hydrochloric acid solution. Fit the screw-caps containing the silicone-rubber septa. Inject through the different septa microlitre aliquots of carbon disulphide standard solution in acetone to provide a suitable calibration range (*e.g.*, 20, 40, 50 and 60 μl of the 1.0 mg ml^{-1} solution). Place the bottles in the water-bath and follow the procedure described above for treated samples. Construct a calibration graph from the chart recorder responses against amounts of carbon disulphide added. Note that as the response from the flame-photometric detector follows approximately a square function, results should be plotted as logarithms to give linearity.

Precautions

Determine the saturation level of the detector periodically by injecting a range of standards containing up to 500 μg of carbon disulphide.

Ensure that each septum is thoroughly gas-tight in its screw-cap and, when using it several times, pierce it at different points on the circumference of the hole with the needle.

Rinse the gas-tight syringes with distilled water at about 80 °C immediately prior to injections (three rinses should be sufficient).

Shaking of the sample with acid in the digestion flask from time to time should be thorough; a horizontal-action mechanical shaker is a suitable type to use if hand-shaking is too time consuming.

APPENDIX II

Membership of the Panel

The following laboratories, represented by the workers named, contributed to the work of the Panel: Agricultural Science Service, Ministry of Agriculture, Fisheries and Food, Slough Laboratory and Headquarters Staff [R. H. Thompson (Chairman)], Harpenden Laboratory, [J. W. Edmunds, N. A. Smart (Secretary)]; Campden Food Preservation Research Association (H. A. Blundstone); Staffordshire County Council, County Analyst's Department (R. S. Hatfull, B. S. Nichols, V. M. Stanway); Dr. Bernard Dyer and Partners (P. S. Hall); Du Pont de Nemours International SA (E. Raleigh); Agricultural Scientific Services, Department of Agriculture and Fisheries for Scotland (K. Hunter); Laboratory of the Government Chemist, Department of Industry (R. A. Hoodless); Murphy Chemical Ltd. (R. Rowe); Pan Britannica Industries Ltd. (D. Russell); Robinson Brothers Ltd. (A. Stevenson); Rohm and Haas France SA (B. J. de la Gravière); and Unilever Research (G. M. Telling, C. J. Ebden).

References

1. Cullen, T. E., *Anal. Chem.*, 1951, **23**, 1842.
2. Keppel, G. E., *J. Assoc. Off. Anal. Chem.*, 1971, **54**, 528.
3. Keppel, G. E., *J. Assoc. Off. Anal. Chem.*, 1969, **52**, 162.
4. Benson, W. R., *J. Assoc. Off. Anal. Chem.*, 1969, **52**, 266.
5. "Joint Survey of Pesticide Residues in Foodstuffs sold in England and Wales. (Third Year). 1 January 1972–31 December 1972," Association of Public Analysts, London, 1973.
6. Unilever Research, personal communication.
7. McLeod, H. A., and McCulley, K. A., *J. Assoc. Off. Anal. Chem.*, 1969, **52**, 1226.
8. Engst, R., and Schnaak, W., *Z. Lebensm.-Unters.-Forsch.*, 1967, **134**, 216, and 1969, **139**, 149.
9. Ebing, W., and Pflugmacher, J., personal communication.
10. Stevenson, A., personal communication.
11. van Haver, W., and Gordts, L., *Z. Lebensm.-Unters. -Forsch.*, 1977, **165**, 28.
12. Hoodless, R. A., personal communication.
13. Ashworth, R. de B., Henriet, J., and Lovett, J. F., *Compilers, in* Raw, G. R., *Editor*, "CIPAC Handbook. Volume I. Analysis of Technical and Formulated Pesticides," Collaborative International Pesticides Analytical Council, Harpenden, 1970.
14. Howard, S. F., and Yip, G., *J. Assoc. Off. Anal. Chem.*, 1971, **54**, 1371.

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Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs (Dr. N. A. Smart, Secretary), Ministry of Agriculture, Fisheries and Food, Agricultural Science Service, Hatching Green, Harpenden, Hertfordshire, AL5 2BD

Analyst, July, 1981, Vol. 106, pp. 788–789

Analytical Methods Committee

REPORT PREPARED BY THE ANTIBIOTICS IN ANIMAL FEEDINGSTUFFS SUB-COMMITTEE

Microbiological Assay of Low Levels of Monensin in Animal Feeds

The method for the determination of monensin in animal feedingstuffs published previously was developed at a time when this material was being incorporated in feeds at the 100 mg kg^{-1} level. As a need has arisen for a method that would determine monensin when present in amounts of 20–40 mg kg^{-1}, the original method has been re-examined and a concentration step introduced that enabled satisfactory results to be obtaincd on a sample of feed containing 18 mg kg^{-1} of monensin.

Keywords: *Monensin assay*; *animal feeds*; *microbiological assay*

The Analytical Methods Committee has received and approved for publication the following report from its Antibiotics in Animal Feedingstuffs Sub-Committee.

Report

The constitution of the Sub-Committee responsible for the preparation of this Report was: Mr. R. S. Hatfull (Chairman), Mr. M. Bentley, Mr. R. Goodey, Mr. H. L. Hatfield, Mr. A. F. Lott, Mr. G. S. Meadows, Mr. R. Ryden (to October 1979), Mr. G. F. Snook (from October 1979), Mr. R. Smither and Mr. L. D. Ward, with (the late) Dr. N. W. Hanson (to July 1979) and Mr. J. J. Wilson (from September 1979) as Secretaries.

Introduction

A previous Report recommended a method for the assay of monensin at levels of 50 mg kg^{-1} or greater; it was designed specifically for the examination of animal feeds containing monensin at the then recommended level of 100 mg kg^{-1}. Subsequently, however, the use of monensin was extended to feeds for ruminants, in which the recommended level of incorporation was 20–40 mg kg^{-1}. Therefore, the published method[1] was re-examined and a modification developed to make it suitable for the assay of monensin at such levels.

Experimental

Early experiments with much larger amounts of sample were unsuccessful because of practical difficulties but it was found that the necessary concentration could be achieved by rotary evaporation of the extract.

The final test solution was found to be turbid owing to the presence of "oily" matter, which caused interference and apparently low recoveries, but this problem was overcome by centrifuging the final solution. (An attempt to remove this interference by a preliminary extraction of the feed with light petroleum gave very low recoveries owing to loss of monensin.)

The column chromatography stage in the published method was slightly modified to take account of the smaller volume of concentrated extract.

Details of the modification to the method are given in the Appendix.

Results and Discussion

The recommended method was subjected to a collaborative trial in which Experts from a number of other EEC countries participated, using a beef ration containing 18 mg kg^{-1} of monensin. The results obtained in eleven laboratories are given in Table I, which includes, by agreement with the EEC Committee of Experts on the Determination of Antibiotics in Animal Feedingstuffs, results from the other EEC countries.

The mean recovery from 28 results was 18.0 mg kg^{-1} with a standard deviation of 1.76 mg kg^{-1} and a coefficient of variation of 9.80%.

TABLE I

DETERMINATION OF MONENSIN IN BEEF RATION AT A LEVEL OF 18.0 mg kg^{-1} BY THE RECOMMENDED METHOD

Laboratory	Monensin found/mg kg^{-1}	Assay levels used
A	18.5	4
B	16.6, 16.0	4
C	17.7, 16.9	4
D	19.8, 19.2, 19.6, 19.9	4
E	15.9, 16.7	4
F	17.8, 18.3, 18.2, 18.8	4
G	17.5, 15.1, 16.4	4
H	23.8, 16.8, 16.3	4
I	19.3, 17.6, 18.4, 18.6	4
J	16.4	4
K	19.9, 19.0	2

APPENDIX

Recommended Method for the Determination of Monensin

Scope and Field of Application

The method details a modification to the original method,[1] which makes it suitable for the determination of monensin at concentrations of less than 50 mg kg^{-1}, to a lower limit of approximately 10 mg kg^{-1}.

Procedure

The procedure is modified as follows.

Extraction

Add a new section:

(*c*) *Feedingstuffs with a presumed monensin level of less than 50 mg kg*$^{-1}$. Take 10.0–20.0 g of the sample, weighed to the nearest 0.01 g, add 100 ml of 90% methanol and homogenise for 15 min. Centrifuge until a clear supernatant liquid is obtained. Take an aliquot of the supernatant liquid (*e.g.*, 80 ml of extract from 20 g of a feed containing 10 mg kg^{-1} of monensin) and evaporate to dryness in a rotary evaporator at a temperature below 40 °C. Dissolve the residue in 10 ml of 90% methanol.

Prepare a column of aluminium oxide as follows. Take a glass tube of dimensions 300 × 11 mm i.d., tapered at one end to 2 mm i.d. Insert a plug of cotton-wool and push it down to the tapered end, then while gently tapping the tube, fill it with aluminium oxide to a height of 80 mm. Pass the 10 ml of dissolved extract through the prepared column and wash the column with sufficient 90% methanol to produce a total eluate volume of 20 ml. Evaporate the eluate to dryness in a rotary evaporator at a temperature below 40 °C. Dissolve the residue in 10 ml of anhydrous methanol and dilute to 20 ml with distilled water. Centrifuge at greater than 4000 rev min^{-1} for at least 10 min. If necessary, make subsequent dilutions of the supernatant liquid with 50% methanol in order to obtain a solution having an expected monensin content of 8 μg ml^{-1} (U_8). Prepare solutions U_4, U_2 and U_1 by successive dilution (1 + 1) with 50% methanol.

The assay is then completed as in the original method.[1]

Reference

1. Analytical Methods Committee, *Analyst*, 1977, **102**, 206.

SHORT PAPERS

Simultaneous Determination of Copper, Lead, Cadmium and Zinc in Non-ferrous Smelter Products by Differential-pulse Polarography

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Keywords: Non-ferrous smelter; copper, lead, cadmium and zinc determination; differential-pulse polarography; orthophosphoric acid

Although polarographic techniques are well regarded for their sensitivity and selectivity, there have been relatively few reports of applications to samples as varied and complex as those of environmental or geological origin. An examination of the literature reveals that applications of polarography to metallurgical samples have focused largely on finished products such as steels and base metal alloys. These materials contain a limited number of components and are usually amenable to dissolution by classical acid digestion procedures. In contrast, a typical non-ferrous smelter product may contain 20–30 different elements ranging in concentration from major to trace. Often, the main difficulty associated with the analysis of such samples is the preparation of the sample solution[1] and it is necessary to find suitable decomposition procedures and base electrolytes before multi-element voltammetric analysis of such complex matrices can be carried out.

The early literature on polarography contains several descriptions of the analysis of non-ferrous ores and smelter products, including some for the determination of copper, lead, cadmium and zinc in zinc ores.[2–4] These methods were remarkable for their accuracy, despite the lengthy digestion and separation procedures required for the elimination of interfering "pre-waves" associated with the d.c. technique. In the mid-1950s Milner and co-workers[5–7] described the application of square-wave polarography to the determination of copper, lead, cadmium and zinc in light alloys. Polarograms for these elements were taken in orthophosphoric acid electrolyte (1 M) after an initial digestion of the alloy in nitric or hydrochloric acid.

It has been established that orthophosphoric acid is a particularly useful agent for the decomposition of oxides,[8] having found its widest use in the treatment of refactory chromites.[9,10] Recently, Mizoguchi and Ishii have described the use of condensed orthophosphoric acid (*i.e.*, 85% H_3PO_4 heated to 300 °C) in a procedure for the titrimetric determination of iron(II) and total iron in iron ores[11] and for the spectrophotometric determination of aluminium, iron and titanium in bauxite.[12] A mixture of equal volumes of perchloric and orthophosphoric acids has also been found to be an excellent solvent for sulphide ores prior to the titrimetric determination of their copper[13] and iron[14] contents.

This paper deals with the use of orthophosphoric acid with perchloric and hydrofluoric acids for the decomposition of a variety of non-ferrous smelter products. After the removal of the latter two acids by evaporation, simultaneous polarographic determinations were made of the copper, lead, cadmium and zinc contents of the orthophosphoric acid solution. The same smelter dusts were then subjected to the acid digestion procedures used in the determination of the elements by atomic-absorption spectrophotometry.

Experimental

Apparatus

Electrochemical analysis

A PAR 174A Polarographic Analyser was used in the differential-pulse mode of operation with a conventional three-electrode system. A saturated calomel electrode (S.C.E.) was used as a reference and the counter electrode was of platinum wire. Voltage scans were

made from +0.2 to −1.3 V at a rate of 2 mV s^{-1}, a modulation amplitude of 50 mV and a drop time of 2 s. Microlitre additions of standard multi-element solutions, containing copper, lead, cadmium and zinc, were made to the cell with the aid of an Oxford precision pipette.

Spectrophotometric analysis

A Perkin-Elmer 503 spectrophotometer employing a hollow-cathode lamp and an air - acetylene atomiser was used for atomic-absorption determinations. The absorbance values for lead, copper, zinc and cadmium were measured at 283.3, 424.8, 213.9 and 228.8, respectively.

Reagents

Analytical-reagent grade chemicals were used throughout. Multi-element polarographic standards were prepared by digesting high-purity metals with 10 ml each of concentrated hydrochloric and nitric acids, heating to near dryness and treating the salts with 15 ml of concentrated orthophosphoric acid until dissolution was complete. A typical standard would contain 0.20 g of copper, 0.25 g of lead, 0.05 g of cadmium and 0.80 g of zinc per 500 ml of acid solution. For the spectrophotometric work, commercially available atomic-absorption standards were employed. Distilled water with a specific resistance greater than 10 MΩ cm was taken from a Millipore Super-Q water system.

Sample Pre-treatment

Eight non-ferrous dust samples were selected from various process sites at five Canadian smelters. Approximately 10-g portions were ground with a mortar and pestle and passed through an 80-mesh stainless-steel sieve. Each dust was dried at 105 °C for 1 h before weighing.

Polarographic Analysis

To 0.2 g of sample weighed into a 400-ml PTFE beaker were added 15 ml each of orthophosphoric and perchloric acids. The beaker was placed on a hot-plate, which had been pre-heated to 260–290 °C, and 10 ml of hydrofluoric acid (49%) were added upon the first appearance of fumes (water vapour). The digestion was continued to a final solution volume of 10–15 ml. The resulting solution was poured into a measuring cylinder, diluted to 50 ml with concentrated orthophosphoric acid and transferred into a 250-ml calibrated flask with careful rinsing of the cylinder and beaker. The final concentration of orthophosphoric acid was 3 M.

A 50-ml aliquot of the sample solution was pipetted into the polarographic cell and the solution was de-aerated for 10 min with a stream of pre-purified nitrogen. A preliminary polarogram was run from +0.2 to −1.1 V (*versus* S.C.E.) in order to establish the current range for each of the four metals. This was followed by duplicate polarograms over the voltage range appropriate to each element.

The current peak heights were compared with those of the calibration graphs measured at 25 °C (the correlation coefficient was 0.995 or greater for each metal over a concentration range of four orders of magnitude) and the volume of standard solution required to yield a peak height approximately twice that of each element in the sample was calculated. The concentrations of the four elements were then determined, starting with the standard addition for the element requiring the least volume of standard solution and progressing to that requiring the greatest. For each metal, duplicate polarograms were obtained and the average value of the peak height was recorded.

Spectrophotometric Analysis

The procedures adopted for the determination of the four metals by atomic-absorption spectrophotometry were based on those given by Donaldson[15,16] in a useful compendium of methods for the analysis of ores, rocks and mill products. One procedure was used for the determination of copper, cadmium and zinc in a common filtrate[15] but a separate technique[16] was used for the determination of lead, because the oxidative digestion of non-ferrous smelter products may yield insoluble lead sulphate.

Results and Discussion

A typical differential-pulse polarogram for a non-ferrous dust is that of the NBS standard reference concentrate 113-a shown in Fig. 1. The current peaks are well resolved and limited in number to the four metals of interest by the choice of the base electrolyte and the oxidative character of the digestion. For the samples studied here, only iron(III) and nickel(II) ions were found to interfere in the determination of copper(II) and zinc(II) ions, respectively. By spiking sample solutions with standards it was found that the determination of copper(II) ion in the presence of iron(III) ion was limited to those samples in which the molar ratio of iron(III) to copper(II) ion was no greater than 100:1, whereas the determination of zinc(II) ion in the presence of nickel(II) ion was limited to samples in which the metals were present in approximately equimolar amounts.

The average results of duplicate analyses for eight non-ferrous smelter dusts are given in Table I. Agreement between the polarographic and spectrophotometric methods is satisfactory; a paired Student's *t*-test indicated that there is no significant difference in the methods at the 95% confidence level.

TABLE I

POLAROGRAPHIC AND ATOMIC-ABSORPTION SPECTROPHOTOMETRIC ANALYSES OF SMELTER DUSTS

Sample	Concentration of Cu, %		Concentration of Pb, %		Concentration of Cd, %		Concentration of Zn, %	
	P*	AAS*	P	AAS	P	AAS	P	AAS
Reactor slag	4.90	4.85	2.14	2.01	0.02	0.02	5.88	5.94
Reactor dust	6.09	6.77	30.4	29.5	9.05	8.82	9.26	9.33
Stack dust	2.57	2.50	5.59	5.75	0.68	0.65	42.9	44.2
Flue dust	0.66	0.62	0.23	0.24	0.01	0.02	0.07	0.07
Stack dust	3.21	3.39	15.4	15.2	1.43	1.41	31.9	31.2
Stack dust	3.49	3.78	17.1	17.7	1.19	1.31	28.3	29.0
Roaster dust	21.1	20.7	0.57	0.59	0.02	0.02	4.30	4.39
Flue dust	8.82	8.58	20.6	20.7	0.37	0.35	7.20	7.11

* P, polarography and AAS, atomic-absorption spectrophotometry.

A comparison of the accuracy and precision of the two techniques is provided by the data given in Table II. Each technique yielded data in excellent agreement with the certified values of the standard reference materials. Although the polarographic data may appear to be more scattered, a comparison of the variances using the *F*-test indicated that no difference existed in the precision of the two methods (95% confidence).

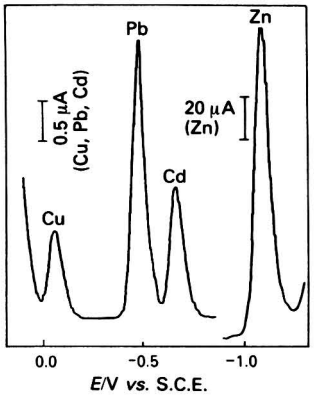

Fig. 1. Differential pulse polarogram of NBS concentrate 113-a.

TABLE II

ACCURACY AND PRECISION FOR THE POLAROGRAPHIC AND ATOMIC-ABSORPTION SPECTROPHOTOMETRIC TECHNIQUES

Eight replicate determinations were made in each instance.

Sample	Cu		Pb		Cd		Zn	
	Concentration, %	Standard deviation, %	Concentration, %	Standard deviation, %	Concentration, %	Standard deviation, %	Concentration, %	Standard deviation %
CANMET MP–1 (Zn, Sn, Cu, Pb ore)—								
Polarography	2.16	0.102	1.87	0.053	0.05(6)	0.004	15.6	0.504
Atomic-absorption spectrophotometry	1.93	0.090	1.82	0.021	0.05(3)	0.003	15.3	0.215
Certified value	2.09		1.88		—		15.9	
NBS 113–a (Zn concentrate)—								
Polarography	0.31	0.011	2.78	0.131	0.79	0.036	57.1	0.438
Atomic-absorption spectrophotometry	0.28	0.009	2.74	0.083	0.78	0.024	56.6	0.964
Certified value	0.31		2.80		0.78		57.3	

The efficiency of the orthophosphoric, perchloric and hydrofluoric acid mixture (3 + 3 + 2) as a reagent for the decomposition of non-ferrous smelter products is confirmed by these results. Hoyle and Diehl[13] observed that the dissolution of a sulphide ore under reflux in a mixture of equal volumes of perchloric and orthophosphoric acids could be completed, except for the silica present, in 3–5 min. Similar results had been reported previously by Goetz and Wadsworth[14] for iron ores. When the technique was applied to the dusts studied in this work, it was found that sample decomposition was not as complete as that obtained in open digestion wherein the full oxidative power of perchloric acid comes into effect (*i.e.*, after the evaporation of water) and that hydrofluoric acid was still required for maximum dissolution.

Conclusion

A simple, rapid method has been developed for the simultaneous polarographic determination of copper, lead, cadmium and zinc in non-ferrous smelter dusts. The low volatility of orthophosphoric acid during sample digestion and its properties as a base electrolyte permit determinations of the four elements to be made with no additional sample manipulation. The close agreement between polarographic and spectrophotometric data confirms the efficiency of the digestion procedure employing orthophosphoric acid and supports the contention that electro-analytical techniques may be applied to complex inorganic matrices with minimum sample preparation.

References

1. Slavin, W., "Atomic Absorption Spectroscopy," Interscience, New York, 1968, pp. 74 and 243.
2. Kraus, R., and Novak, J. V. A., *Collect. Czech. Chem. Commun.*, 1938, **10**, 534.
3. Strubl, R., *Collect. Czech. Chem. Commun.*, 1938, **10**, 466.
4. Semerano, G., and Gagliardo, E., *Anal. Chim. Acta*, 1950, **4**, 422.
5. Ferrett, D. J., and Milner, G. W. C., *Analyst*, 1955, **80**, 132.
6. Ferrett, D. J., and Milner, G. W. C., *Analyst*, 1956, **81**, 193.
7. Milner, G. W. C., and Slee, L. J., *Analyst*, 1957, **82**, 139.
8. Sulcek, A., Pavondra, P., and Dolezal, J., *Crit. Rev. Anal. Chem.*, 1977, **6**, 255.
9. Lucas, R. P., and Ruprecht, B. C., *Anal. Chem.*, 1971, **43**, 1013.
10. Hofton, M. E., and Baines, S., PB Report, No. 204596, NTIS, U.S. Department of Commerce, 1975.
11. Mizoguchi, T., and Ishii, H., *Talanta*, 1978, **25**, 311.
12. Mizoguchi, T., and Ishii, H., *Talanta*, 1979, **26**, 33.
13. Hoyle, W. C., and Diehl, H., *Talanta*, 1971, **18**, 1072.
14. Goetz, C. A., and Wadsworth, E. P., *Anal. Chem.*, 1956, **28**, 375.
15. Donaldson, E. M., "Methods for the Analysis of Ores, Rocks and Related Materials," Mines Branch Monograph 881, Department of Energy, Mines and Resources, Ottawa, 1974, p. 15.
16. Donaldson, E. M., "Methods for the Analysis of Ores, Rocks and Related Materials," Mines Branch Monograph 881, Department of Energy, Mines and Resources, Ottawa, 1974, p. 19.

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Versatile Fusion Method for the Dissolution of Refractory Materials

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Keywords: Fusion technique; refractory materials; atomic-absorption spectrophotometry

The increasing use of instrumental methods in the analysis of ceramics has underlined a problem that has long existed in this field of chemistry. The different types of refractory materials that may be encountered in routine work are numerous, and for each type several different decomposition techniques are feasible. Methods in current use include acid dissolution in pressure vessels and fusion techniques using a variety of flux mixtures.[1,2] Recent refinements in atomic spectroscopy [*e.g.*, atomic-absorption spectrophotometry (AAS)[3,4] and inductively coupled plasma spectrometry[5]] now permit the accurate determination of the main constituents, silica and alumina. However, the silica must remain in solution in a stable form throughout the analysis. For optical spectrophotometry this is also desirable as the analysis of minor constituents may then be carried out without the necessity for a silica separation.

For routine work fusion methods are preferred because they are usually more rapid than acid dissolution, and a number of samples may be analysed simultaneously. Hitherto, it has been generally accepted that each type of sample material of a particular chemical composition requires its own individual flux.[2,6] However, this imposes several practical complications, *e.g.*, the necessity for keeping numerous calibration graphs and standard solutions and the frequent need to check reagent blanks. Moreover, it impedes the efficient flow of routine samples through the laboratory.

The ideal situation in which only one flux is needed for all types of sample so far has not been realised. However, we have developed a method that goes a long way towards this objective, and we use it as standard practice in the preparation of solutions of a wide range of refactory and ceramic materials for routine analysis.

Development of a General Method

The primary requirement that prompted this work was to produce sample solutions that could be used for the determination of silica and alumina by atomic-absorption spectrophotometry. An early paper by Katz[7] suggested the use of sodium hydroxide as a flux for this purpose, but we found this to be unsatisfactory for the range of materials that we analyse; high alumina materials, for example, are only partially attacked. Further, the ratio of flux to the mass of sample required for easy fusion was considered excessive, and the sample solutions were inevitably contaminated with nickel from the crucible.

A flux that we have found to be particularly useful for fusing refractory alumina consists of a mixture of sodium carbonate and boric acid, 2.4 + 0.4 g, respectively, for 1 g of sample. The fusion can be carried out at 1200 °C in a platinum crucible without any appreciable attack on the platinum. This technique was also found to be satisfactory for the fusion of other refractory materials. However, two important disadvantages are that the fusion melt is slow to dissolve in dilute hydrochloric acid and that when used with samples of high silica content, such as aluminosilicates, much of the silica is precipitated as a gel during dissolution. Both of these problems were overcome by the addition of potassium carbonate* after the initial fusion; the crucible was then reheated for a short period to obtain a glassy melt. Complete dissolution of the melt in dilute hydrochloric acid was achieved in about 20–30 min. For materials of high silica content it was found necessary to agitate the solution at intervals in order to ensure that no silica was precipitated. Dissolution of other materials could be hastened by gentle heating. In all instances exceptionally clear solutions were obtained.

* Potassium carbonate was chosen in preference to sodium carbonate for the second stage as potassium salts are commonly added to solutions for atomic-absorption spectrophotometry to prevent ionisation interference.

Procedure

Weigh 0.500 g of the sample, ground to 150 mesh, and transfer it into a 35-ml platinum crucible. Add 1.4 g of flux (1.2 g of sodium carbonate + 0.2 g of boric acid), mix well and cover with a platinum lid. Heat at 1200 °C for 25 min. Remove from the furnace, add 1 g of potassium carbonate to the melt surface and heat at 1200 °C for 5 min. Remove and quench the base of the crucible in distilled water. Place the crucible and lid in a 250-ml squat-form beaker containing 60 ml of 1 + 1 hydrochloric acid. Leave in a warm place, agitating the solution at intervals, until dissolution is complete. Dilute to the appropriate volume.

Results and Discussion

Using the procedure detailed above, completely clear solutions were obtained indicating that silica was retained in solution. As evidence of this, the results for silica obtained by atomic-absorption spectrophotometry are compared with accepted values in Table I.

TABLE I

COMPARISON OF SILICA CONTENTS DETERMINED BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY WITH ACCEPTED VALUES

	Silica, %	
Sample	Determined by AAS	Accepted value
BCS 269 Firebrick	56.6	56.7
BCS 309 Sillimanite	34.2	34.1
BCS 315 Firebrick	50.9	51.2

Confirmation of complete dissolution of other components is furnished by the results given in Table II for the full analysis of a variety of materials dissolved by the proposed method. In all instances silica, alumina, calcium oxide and magnesium oxide were determined by atomic-absorption spectrophotometry using a Pye Unicam SP 2900 spectrophotometer. Iron(III) oxide and titanium(IV) oxide were determined by spectrophotometry. Sodium oxide and potassium oxide were determined by flame photometry on a separate solution prepared by acid dissolution.

Solutions obtained by the proposed method have also been analysed for silica and alumina by wet-chemical methods; good agreement with results from atomic-absorption spectrophotometry was obtained. Silica was determined gravimetrically after precipitation with hydrochloric acid and subsequent dehydration; alumina was determined complexometrically (without the necessity for a silica separation) after removal of iron, titanium, etc., by extraction with cupferron - chloroform.

TABLE II

RESULTS FOR ANALYSIS OF REFRACTORY MATERIALS DISSOLVED BY THE PROPOSED METHOD

	Composition, %			
Component	Talc	Ball clay	Kyanite	Graphite ash
Loss on ignition ..	7.2	8.9	0.07	—
SiO_2	60.2	52.5	39.8	55.7
Al_2O_3	0.38	31.5	57.9	30.4
Fe_2O_3	0.30	1.5	0.35	10.9
TiO_2	0.16	1.5	1.5	0.64
CaO	0.22	0.28	0.01	0.88
MgO	31.7	0.59	0.04	0.85
Na_2O	0.04	0.37	0.02	0.09
K_2O	0.01	2.8	0.01	0.71
Total	100.2	99.9	99.7	100.2

During the first stage of the fusion the type of reaction will depend on the composition of the sample. With materials of high silica content, for example, complete fusion is obtained, but materials of low silica content such as fused alumina will only sinter with the

flux. For this reason, it is important that the flux constituents are ground together to a fine, free-flowing powder and that the flux and sample are intimately mixed together. For the best results it is recommended that some samples of alumina are ground to pass a 200-mesh sieve.

Of the materials that we have examined so far, we have obtained successful dissolution for the following: aluminous materials (up to 95% alumina); aluminosilicates, *e.g.*, clays, graphite and coal ashes, sillimanite and kyanite; materials of high silica content (up to 95% silica); and miscellaneous materials such as blast furnace slag, zircon, titanium dioxide, barium titanate and soapstone. However, our experiments with fused zirconia invariably yielded a few milligrams of undecomposed material after dissolution. With chrome-bearing materials, *e.g.*, chrome ore and magnesite - chrome, best results were obtained by reducing the sample mass to 0.25 g.

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References

1. Langmyhr, F. J., and Paus, P. E., *Anal. Chim. Acta*, 1968, **43**, 508.
2. Oliver, G. J., *Proc. Anal. Div. Chem. Soc.*, 1979, **16**, 151.
3. Price, W. J., and Whiteside, P. J., *Analyst*, 1977, **102**, 664.
4. Boar, P. L., and Ingram, L. K., *Analyst*, 1970, **95**, 124.
5. Walsh, J. N., *Spectrochim. Acta, Part B*, 1980, **35**, 107.
6. BS 1902 (1964), Parts 2A, 2B and 2C, British Standards Institution, London.
7. Katz, A., *Am. Mineral.*, 1968, **53**, 283.

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Coulometric Determination of Herbicides Containing Nitro Groups by Reduction with Electro-generated Chromium(II)

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Keywords: *Nitro-containing herbicide determination*; *coulometry*; *chromium(II) generation*; *nitro group reduction*

Compounds used as herbicides often contain nitro groups. For example, 2,4-dinitro-6-*sec*-butylphenol (DNBP, dinoseb) is used as a pre- and post-emergence herbicide for the control of weeds in a variety of agricultural crops; it has also been recommended as a pre-harvest top killer for potato vines and a growth enhancer for corn. Related compounds employed as selective herbicides or insecticides include 4,6-dinitro-*o*-cresol (DNOC, Ditrosol), 2,6-dinitro-3-amino-4-trifluoromethyl-*NN*-diethylaniline (dinitramine, Cobex) and 2,6-dinitro-4-trifluoromethyl-*NN*-dipropylaniline (trifluoralin, Treflan).

The widespread use of these chemicals has created a need for reliable analytical procedures, and several methods have been proposed for their measurement in commercial formulations, including ultraviolet spectrophotometry,[1] gas chromatography[2] and liquid chromatography.[3,4] This paper reports an electrochemical procedure that can be employed at the micromolar level for the determination of the purity of DNOC. It has also been applied to the determination of pure DNBP and dinitramine in a commercial formulation.

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Experimental

Chemicals and Apparatus

4,6-Dinitro-*o*-cresol (DNOC) (Eastman) was recrystallised once from ethanol (reported melting-point, 85–87.5 °C; found, 85 °C). DNBP was provided by Dow Chemical of Canada as a commercial formulation marketed under their trademark as Premerge 360 and consisting of an aqueous solution of a mixture of the mono-, di- and triethanolammonium salts of DNBP. Crystalline trifluoralin was provided by Elanco Products, Canada. The remaining commercial formulations—dinitramine (as Cobex E-C, trademark of US Borax), trifluoralin (as Treflan, trademark of Rohm and Haas) and nitrofen (as TOK, trademark of Dow Chemical)—were provided by A. Vandenborn of the University of Alberta.

The remaining chemicals and the coulometer, titration cell arrangement and catholyte composition were as previously described.[5] Other constant-current coulometric devices could be used instead of the one described; for example, the Princeton Applied Research, Model 179, coulometer or equivalent would serve equally well.

Procedure for Titration

Into the anode compartment of the coulometer cell place 50 ml of a solution 0.01 M in hydrochloric acid and 1.0 M in potassium chloride. Into the cathode compartment place 70 ml of a solution 0.1 M in $CrBr(H_2O)_5Br_2$ [added as chromium(III) bromide] and 1.5 M in hydrochloric acid. De-aerate the catholyte with oxygen-free nitrogen, then introduce the mercury-pool cathode and adjust the nitrogen inlet so that the stream of nitrogen passes over the surface of the solution. Inject into the cathode compartment through a rubber serum cap a portion of sample solution containing about 1 mg of the compound to be determined (see below for the procedure for preparation and measurement of sample solutions), and generate chromium(II) at the mercury cathode at a current level of about 20 mA until the potential between the indicating electrodes begins to decrease. At this point generate current in small increments, waiting for the potential to stabilise after each increment and then recording the total time of current generation and the potential reading. Determine the time required from a plot of the potential against time as shown in Fig. 1, and then calculate the milliequivalents of compound present from a knowledge of the generating current and the time of current generation.

Add a second weighed portion of herbicide sample solution and repeat the titration. Two to four samples can be titrated in the same portion of catholyte before it must be replaced. A blank should be run on each fresh portion of catholyte solution by adding a portion of sample solution and titrating as outlined previously; this serves to establish an initial end-point. Alternatively, the first sample can be run and treated as a blank, that is, not used in the calculations of sample composition. The total time required to titrate each sample after injection into the cell is 15–20 min.

Sample solutions were prepared for injection as follows. Liquid commercial formulations were shaken well and a 70–250-mg portion was weighed, to the nearest 0.1 mg, into a flask. Approximately 30 ml of acetonitrile (water was satisfactory for Premerge 360) was added and the flask re-weighed. After mixing, approximately 0.5-g portions of the mixture were withdrawn in a hypodermic syringe and the syringe plus contents weighed to the nearest 1 mg on a top-loading balance. The contents were then injected into the coulometer cell, the syringe was re-weighed and the mass of sample obtained by difference. For pure DNOC and trifluoralin, solutions containing about 1 mg ml^{-1} were prepared in acetonitrile as solvent (acetonitrile was employed because most of the compounds concerned are only sparingly soluble in water).

Results

Results for those compounds which were titrated successfully are given in Table I. For purposes of calculation, the active component of Premerge 360 was assumed to be the diethanolammonium salt of DNBP, with a relative molecular mass of 345. The end-points for DNOC and DNBP are sharper (Fig. 1) and so give better precision than the end-point for nitramine in the formulation studied. End-points for nitrofen (as TOK) and trifluoralin (as Treflan and as the pure crystalline material) were too sluggish and drawn out to be satisfactory for analytical use.

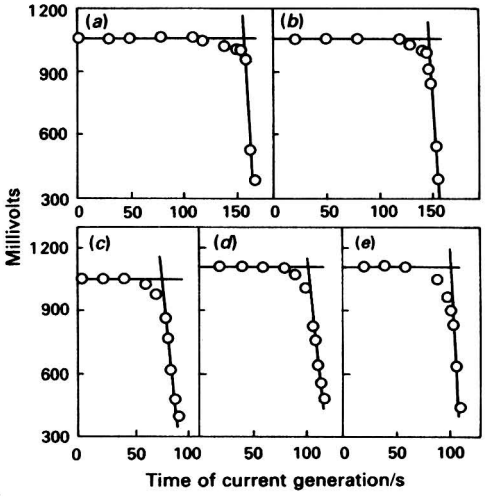

Fig. 1. Plots of potential between polarised platinum indicating electrodes in millivolts and time of current generation for sets of titrations of dinitro-*o*-cresol (DNOC) [(*a*) blank; and (*b*) sample 1] and dinitro-*sec*-butylphenol (DNBP) [(*c*) pre-titration; (*d*) blank; and (*e*) sample 1]. End-points are obtained by extrapolation of lines drawn through points early in the titration and through the steepest portion of the potential change region.

No attempt was made to establish the lower limit of the method, but smaller amounts should be determinable through use of smaller generating currents or shorter times. Interferences in the method include substances that are reduced by chromium(II) or that form stable complexes with it. Nitro compounds present as starting materials for, or decomposition products of, the active herbicide or insecticide under study would, therefore, probably react.

TABLE I

RESULTS OF TITRATIONS OF DNBP IN PREMERGE 360 AND 4,6-DINITRO-*o*-CRESOL WITH COULOMETRICALLY GENERATED CHROMIUM(II)

Compound	Set*	Composition, %
DNBP in commercial formulation (Premerge 360)	1	50.9, 50.7, 51.1
	2	50.8, 50.5, 49.1, 51.0
	3	49.8, 51.1
	4	50.4, 51.0
	Average	50.6 (RSD† 0.62%); reported by Dow, ~50%
4,5-Dinitro-*o*-cresol (DNOC)	1	100.5, 100.9, 100.9
	2	99.7, 99.8, 101.9
	Average	100.6 (RSD† 0.83%)
Dinitramine in commercial formulation (Cobex E-C) ..	1	27.5, 28.4, 29.8
	2	29.8, 28.8, 27.4
	3	28.4, 27.4, 26.7
	Average	28.2 (RSD† 1.1%)

* Each set consisted of a series of measurements on successive samples in a single solution of catholyte. Sets were run on at least two different days and on different sample solutions.

† Relative standard deviation.

Among other compounds containing nitro groups that are similar to DNOC and DNBP and would probably be determinable by the procedure described are dinocap, dinobuton and binapacryl, none of which were available at the time of this study.

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References

1. Hebbourn, R. G., personal communication.
2. Hrivnak, J., and Stota, Z., *J. Gas Chromatogr.*, 1968, **6**, 9.
3. Lawrence, J. F., and Turton, D., *J. Chromatogr.*, 1978, **159**, 207.
4. Raw, G. R., *Editor*, "CIPAC Handbook. Volume I. Analysis of Technical and Formulated Pesticides," Collaborative International Pesticides Analytical Council, Harpenden 1970, p. 337.
5. Al-Daher, I. M., and Kratochvil, B., *Anal. Chem.*, 1979, **51**, 1480.

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Spectrophotometric Determination of Copper in Blood Serum with 4-(2-Quinolylazo)phenol

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Keywords: *4-(2-Quinolylazo)phenol*; *copper(I) determination*; *blood serum*; *spectrophotometry*

Cuproine (2,2′-biquinolyl) and its analogues have been used in the spectrophotometric determination of copper(I). The methods, though selective, involve time-consuming extraction procedures. This paper reports the use of 4-(2-quinolylazo)phenol[1] (*p*-QAP), a heterocyclic azo dye, as a selective and sensitive reagent for copper(I). The reagent forms a greenish yellow complex ($\lambda_{max.}$ 440 nm) over the pH range 2.9–4.0 in a medium containing 40% of ethanol. The complex is stable for more than 24 h. Under these conditions there is no interference from other metal ions and copper(I) can be determined selectively. The method has also been used for the selective determination of copper in blood sera and the results compare favourably with those obtained by use of other published methods.[2–4]

Experimental

Apparatus

A Unicam SP600 spectrophotometer with matched 10-mm glass cells was used for recording the spectra. A Beckman Expandomatic SS-2 pH meter was used for the pH adjustments.

Reagents

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

p-*QAP solution*. *p*-QAP was prepared by the method of Barua *et al.*[1] A 1×10^{-3} M solution of the reagent was prepared by dissolving 0.249 g in 1 l of ethanol. The solution was stable for several days.

Copper(II) solution. A stock solution of copper(II) (2×10^{-2} M) was prepared by dissolving an appropriate amount of analytical-reagent grade copper(II) sulphate pentahydrate in water

and was standardised complexometrically with EDTA using 1-(2-pyridylazo)-2-naphthol (PAN) as indicator.[5] Subsequent dilutions were made whenever necessary.

Ascorbic acid. A fresh 2% solution of ascorbic acid was prepared and stored in an amber-coloured bottle.

Trichloroacetic acid. A 20% (*m*/*V*) solution of trichloroacetic acid was used for precipitating proteins from blood sera.

Determination of Copper(I)

To a suitable volume of sample containing 1.1–11.4 μg of copper(II), add 2.0 ml of 2% ascorbic acid solution. Allow the mixture to stand for about 5 min and add 1.0 ml of 1×10^{-3} M *p*-QAP solution. Adjust the pH to 3.3 with dilute sodium hydroxide solution or hydrochloric acid and dilute the volume to 10.0 ml with doubly distilled water, maintaining the ethanol concentration at 40% (*V*/*V*). Record the absorbance of the solution at 440 nm against a reagent blank prepared under identical conditions. The amount of copper(I) in the unknown sample can be determined from a calibration graph prepared from known concentrations of copper as described above.

Selective Determination of Copper in Blood Sera

Pipette 0.50 ml of serum sample into a 13 × 100-mm test-tube and add 2.0 ml of 2% ascorbic acid. Allow the mixture to stand for about 10 min. Add 0.5 ml of 20% trichloroacetic acid solution, mix, stopper the tube and centrifuge it until the supernatant solution is clear. Then pipette out 1.0 ml of the supernatant liquid into a 10.0-ml calibrated flask, add 1.0 ml of 1×10^{-3} M *p*-QAP solution, adjust the pH to 3.3 with dilute sodium hydroxide solution and dilute the mixture to 10.0 ml, keeping the ethanol concentration at 40%. Measure the absorbance of the solution at 440 nm against a reagent blank. Calculate the amount of unknown copper in the sample from a calibration graph obtained under identical conditions.

Results and Discussion

Spectral Behaviour and Characteristics of Copper(I) - *p*-QAP Complex

An ethanolic solution of *p*-QAP forms a greenish yellow complex with copper(I) that has a maximum absorbance at 440 nm in the pH range 2.9–4.0. The complex remains stable for 24 h but it precipitates out if the concentration of ethanol is less than 30%; all studies were therefore carried out in 40% ethanolic solutions. Three moles of the reagent are required for full colour development. The composition of the complex, as determined by Job's method of continuous variation and the molar-ratio method, was found to be 1:2 (metal to ligand). Beer's law was valid for up to 1.25 p.p.m. of copper. With the particular conditions adopted here 0.11–1.14 p.p.m. of copper can be determined accurately. The Sandell's sensitivity[6] [the amount of copper(I) corresponding to an absorbance of 0.001] of the colour system is 0.0012 μg cm^{-2} with a molar absorptivity of 5.15×10^4 l mol^{-1} cm^{-1} at 440 nm. The relative standard deviation in the determination of 0.64 p.p.m. of copper(I) (six replicates) was found to be less than 1%.

The sensitivities of various reagents for the determination of copper(I) are compared in Table I.

Studies in the Presence of Diverse Ions

p-QAP also forms coloured complexes ($\lambda_{max.}$ above 520 nm) with copper(II), silver(I), gold(III), zinc(II), cadmium(II), mercury(II), cobalt(II), nickel(II), palladium(II) and osmium(VIII) at higher pH ranges (above 8.0) and different ethanol concentrations.

The tolerance limits of various ions (in parts per million) that caused a deviation of less than ±2% in absorbance in the determination of 0.64 p.p.m. of copper(I) are as follows: nitrate, sulphate and sulphite, 5000; chloride and bromide, 400; citrate and tartrate, 100; borate and oxalate, 500; fluoride and iodide, 20; magnesium(II), calcium(II), barium(II), strontium(II), aluminium and lanthanum(III), 500; uranium(VI), zinc(II), cadmium(II), 80; silver(I), nickel(II), cobalt(II), mercury(II), lead(II), platinum(IV), iridium(III), rhodium(III), iron(II) and manganese(II), 50; gold(III), vanadium(V) and molybdenum(VI), 20; osmium(VIII), 10; and palladium(II), 2.

TABLE I

SENSITIVITIES OF VARIOUS REAGENTS FOR THE SPECTROPHOTOMETRIC DETERMINATION OF COPPER(I)

Reagent	Sensitivity to Cu(I) at wavelength indicated/ g cm^{-2}	Reference
Cuproine (2,2′-biquinolyl) - isoamyl alcohol	0.0098 (546 nm)	9
	0.0012 (358 nm)	9
Neocuproine (2,9-dimethyl-1,10-phenanthroline) - isoamyl alcohol	0.0080 (454 nm)	7
Neocuproine and Rose Bengal - chloroform	0.0010 (570 nm)	8
Bathocuproine (2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline) - isoamyl alcohol	0.0044 (479 nm)	9
2,3,8,9-Dibenzo-4,7-dimethyl-5,6-dihydro-1,10-phenanthroline - chloroform	0.00799 (554 nm)	10
4,4′-Dihydroxy-2,2′-biquinoline - isoamyl alcohol	0.0092 (525 nm)	11
3,3′-Dimethylene-4,4′-diphenylbiquinolyl - isoamyl alcohol	0.00648 (555 nm)	12
6,7-Dimethyl-2,3-di(2-pyridyl)quinoxaline and methyl orange - dichloroethane	0.0021 (418 nm)	13
2,9-Dimethyl-4,7-dihydroxy-1,10-phenanthroline	0.0055 (400 nm)	14
4-(2-Quinolylazo)phenol	0.0012 (440 nm)	This work

Sulphide, thiosulphate, thiocyanate and nitrite ions interfere seriously; 40 p.p.m. of EDTA can be tolerated after the formation of the copper(I) - *p*-QAP complex but otherwise it interferes.

Determination of Copper in Blood Sera

Results obtained for the determination of copper in blood sera of humans, monkeys and fish are summarised in Table II. A known amount of copper (0.32 p.p.m.) was added to the serum sample and the recovery of the copper obtained using the recommended procedure was satisfactory (Table II).

TABLE II

RESULTS FOR DETERMINATION OF COPPER IN BLOOD SERA

Sample	No. of analyses	Copper present, p.p.m.			Copper added, p.p.m.	Recovery of copper, %	
		Min.	Max.	Average		Min.	Max.
Fish—							
Singi	5	0.240	0.310	0.290	0.320	98	105
Magur	5	0.270	0.300	0.295	0.320	102	103
Sol	3	0.270	0.370	0.330	0.320	101	95
Human—							
Male	14	0.980	1.220	1.100	0.320	95	99
Female	11	1.080	1.360	1.221	0.320	97	104
Rhesus monkey (pooled)	5	0.830	0.970	0.932	0.320	97	101

Conclusion

The proposed method, using *p*-QAP as the reagent, compared favourably with other sensitive methods known for the determination of copper(I). The method is simple and sensitive and the determination of copper(I) can be carried out selectively in the presence of large amounts of other ions. The other advantages of the method are that no extraction step is necessary, the colour development is instantaneous and the complex is highly stable.

References

1. Barua, S., Garg, B. S., Singh, R. P., and Singh, I., *Analyst*, 1980, **105**, 996.
2. Dezso, I., and Fulop, T., *Mikrochim. Acta*, 1959, 592.
3. Traister, G. L., and Schilt, A. A., *Anal. Chem.*, 1976, **48**, 1216.
4. Versieck, J., and Cornelis, R., *Anal. Chim. Acta*, 1980, **116**, 217.
5. Schwarzenbach, G., Flaschka, H., and Irving, H. M. N. H., "Complexometric Titrations," Methuen, London, 1969, p. 257.

6. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Interscience, New York, 1959.
7. Smith, G. F., and McCurdy, W. H., Jr., *Anal. Chem.*, 1952, **24**, 371.
8. Bailey, B. W., Dagnell, R. M., and West, T. S., *Talanta*, 1966, **13**, 753.
9. Marczenko, Z., "Spectrophotometric Determination of Elements," John Wiley, New York, 1976.
10. Ackerman, G., and Angermann, W., *Talanta*, 1968, **15**, 79.
11. Schilt, A. A., and Hoyle, W. C., *Anal. Chem.*, 1969, **41**, 344.
12. Uhlemann, E., and Waiblinger, K., *Anal. Chim. Acta*, 1968, **41**, 161.
13. Foster, D., and Trussell, F. C., *Anal. Chim. Acta*, 1969, **47**, 154.
14. Dunbar, W. E., and Schilt, A. A., *Talanta*, 1972, **19**, 1025.

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Differential-pulse Polarographic Determination of Norpace in Plasma

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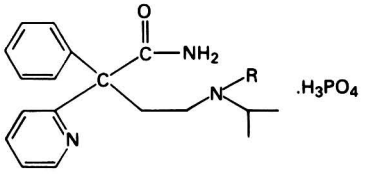
Keywords: *Norpace determination*; *differential-pulse polarography*

Norpace (**I**) (diisopyramide phosphate) is an antiarrhythmic agent[1,2] that has been shown to be of use in clinical medicine.[3,4] The metabolism of this compound has been investigated in several animal species[5,6] and it has been shown that the major metabolic routes involve aromatic hydroxylation and *N*-dealkylation reactions. In man, however, the compound is excreted predominately unchanged in the urine and only the *N*-dealkylated or secondary amino compound (**II**) has been identified as a minor metabolite.



(I) R=Isopropyl
(II) R=H

Norpace has been determined using a fluorescence spectrophotometric technique[7] but this method was unable to differentiate the parent compound from its secondary amino metabolite. Gas-chromatographic methods show greater selectivity with limits of detection of about 0.5 μg ml^{-1}.[8,9] A differential-pulse polarographic (d.p.p.) method has been evaluated for the determination of Norpace in plasma following a nitration procedure,[10] but this method suffers from the presence of substances co-extracted from the plasma, which are nitrated under the same conditions and interfere with the reduction peak of the nitrated derivative of Norpace. Therefore, having considered the existence in the Norpace nucleus of three oxidative centres, *i.e.*, pyridyl, amide and diisopropylamino groups, it was decided to investigate the oxidative

polarographic behaviour of Norpace and its secondary amino metabolite to see if a polarographic technique could provide a sensitive and selective method for their determination in body fluids.

Experimental

Apparatus

Polarographic measurements were carried out using a PAR, Model 174A, polarographic analyser in conjunction with a three-electrode cell system having either a dropping-mercury or glassy carbon indicator electrode, a saturated calomel reference electrode (S.C.E.) and a platinum counter electrode.

Reagents

Samples of Norpace and its secondary amino metabolite were provided by G. D. Searle & Co. Ltd. Stock solutions of these compounds (1 mg ml^{-1}) were made up in distilled water and kept in the dark under refrigeration. A stock Britton - Robinson (BR) buffer solution, 0.04 M in glacial acetic, orthophosphoric and boric acids, was prepared from analytical reagent grade reagents; buffer solutions of varying pH were prepared by the dropwise addition of 0.2 M sodium hydroxide solution whilst measuring the pH using a glass electrode. The mineral acids and solvents used were of analytical-reagent grade.

Procedures

Nitration method

Preliminary studies showed that Norpace could be nitrated, then the optimum conditions for nitration were assessed and the products were isolated and subjected to both polarographic analysis (in buffered media) and also to mass spectrometry, using a Finnigan 4000 mass spectrometer in the chemical ionisation mode. The nitration procedure was then applied to the determination of the drug in plasma. The details of the extraction method are as follows. Plasma (1 ml) was made alkaline with 0.5 ml of potassium hydroxide solution (1 M) extracted with three 5-ml volumes of diethyl ether and the combined extract was evaporated to dryness under nitrogen at 45 °C. The residue was then nitrated, using a mixture of 50 μl of concentrated sulphuric acid and 50 μl of concentrated nitric acid, and after 5 min the reaction was quenched with 9.9 ml of potassium hydroxide solution (1 M), which was sufficient to take the pH to greater than 12. The resulting solution was re-extracted with 20 ml of chloroform and after 10 min the layers were separated by centrifugation (for 5 min at 3000 rev min^{-1}) and an 18-ml aliquot of the chloroform layer was taken. This was evaporated to dryness under nitrogen and the residue was dissolved in 0.5 ml of methanol and 4.5 ml of BR buffer solution (pH 1.8). The resulting solution was polarographed at a scan rate of 2 mV s^{-1} with a 2 s drop time at a modulation amplitude of 100 mV, from an initial potential of 0.15 V.

Oxidation method

Investigations into the oxidative polarographic behaviour of 10^{-4} M concentrations of Norpace and its secondary amino metabolite at the glassy carbon electrode were carried out by recording current - potential curves in the sampled d.c. and the d.p. modes. A starting potential of 0.4 V, a scan rate of 1–2 mV s^{-1} and a controlled drop time of 1 s were typically employed in these investigations. A modulation amplitude of 50 mV was used in the d.p.p. investigation. For the determination of Norpace in plasma, 2 ml of plasma were taken and made alkaline by the addition of 1 ml of potassium hydroxide solution. The plasma was then extracted three times for 5 min each with 5-ml portions of diethyl ether. The combined extract was then evaporated to dryness under nitrogen, the residue taken up in 0.4 ml of methanol and diluted to 4 ml with BR buffer solution (pH 8.0). The d.p. polarogram was then recorded using the conditions stated above.

Results and Discussion

Nitration of Norpace and its Application to Plasma Analysis

A d.p.p. analysis of nitrated Norpace in acidic solution (BR buffer solution, pH 2) reveals a main wave at −0.04 V and a second small peak at −0.24 V. This latter peak is broad and

probably corresponds to the reduction of the hydroxylamine group to the amine. Sampled d.c. polarography gives two waves at −0.05 and −0.21 V with relative heights 2.26:1, respectively. The introduction of one nitro group into Norpace is supported by chemical ionisation mass spectral data, which shows a single peak (*m/e* 384). Only a very minute trace of the parent compound (*m/e* 340) can be observed. Norpace is most probably nitrated at the 4-position of either the phenyl or pyridyl moieties.

Extractions from reconstituted plasma were carried out and a linear calibration graph of peak height *versus* concentration was constructed. Norpace was determined in the concentration range 0.8–3.2 μg ml^{-1}. Variable backgrounds did pose a slight problem in the accuracy of determination of the drug, especially as the sample peak occurred on a sloping background.

The over-all extraction efficiency for this method (*i.e.*, for two extractions) was found to be 67.5% (83.3% efficiency for the first diethyl ether extraction and 81% for the chloroform extraction). When this method was applied to real human plasma samples, measurement of the drug concentrations was rendered highly inaccurate owing to co-extractable, co-nitratable interferences present in the samples. Also, the nitration method could not provide any differentiation between Norpace and its *N*-dealkylated metabolite.

Voltammetric Behaviour of Norpace

No voltammetric response at the glassy carbon electrode was observed for Norpace (**I**) or its secondary amino metabolite (**II**) for pH values of 6 or less in both sampled d.c. and d.p. modes. In the pH range 7–13, Norpace gave a main wave that was constant in height over this range. In the pH range 9–13, the plateau of the d.c. wave was complicated by a distortion that manifested itself in the d.p. mode as an "apparent" second peak. This is illustrated in Fig. 1. The $E_{\frac{1}{2}}$ value corresponding to the d.c. wave became constant for pH values greater than ten. The secondary amino metabolite of Norpace exhibited no voltammetric response in either the d.c. or d.p. modes in the pH range 7–13.

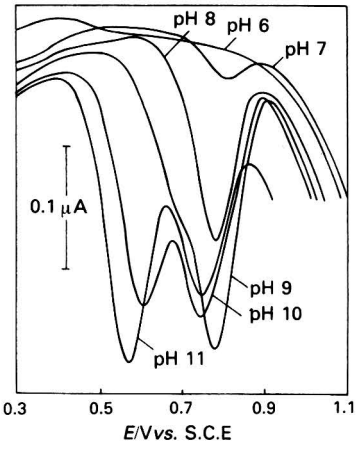


Fig. 1. Effect of pH on the differential-pulse voltammetric behaviour of a 1 × 10^{-4} M solution of Norpace. Conditions: scan rate, 2 mV s^{-1}; drop time, 1 s; and modulation amplitude, 50 mV.

The pK_b value of Norpace of 8.36[11] indicates that only the neutral form of the molecule is oxidised at the electrode surface. A cyclic polarographic examination of Norpace in BR buffer solution (pH 8.0) showed that the process giving rise to the oxidation process was irreversible in nature. As the secondary amino metabolite does not exhibit any voltammetric behaviour at the glassy carbon electrode it can reasonably be assumed that the oxidation process in

Norpace is due to an oxidation occurring at the tertiary amino moiety in the molecule, probably *N*-oxidation. The distortion on the plateau of the sampled d.c. wave is presumably due to reactant adsorption effects.

Choice of Wave for Analytical Purposes

From Fig. 1 it can be seen that the best peak for analytical purposes is obtained in BR buffer solution (pH 8.0). The effect of increasing modulation amplitude (ΔE) on the height of this peak was studied and it was found that the peak current was proportional to the modulation amplitude and that the maximum peak current was obtained for a modulation amplitude of 100 mV. However, measurement of the peak current at a modulation amplitude of 100 mV was made difficult by the increasing slope of the background electrolyte decay and, for analytical purposes, a modulation amplitude value of 50 mV was found to be more suitable. The effect of scan rate on the height of this peak was also investigated and, although larger peak currents were obtained for faster scan rates (5–20 mV s^{-1}), problems were again encountered with sloping base lines and a scan rate of 2 mV s^{-1} was found to give the optimum results. Using this set of conditions, a calibration graph was constructed for Norpace and was found to be linear in the concentration range 1–5 μg ml^{-1}.

Application of Differential-pulse Voltammetry to the Determination of Norpace in Human Plasma

Using the method described under Experimental, it was found that the recovery of Norpace from plasma was 81.2 $\pm$ 3.0%. The limit of detection of the method was found to be 1.5 μg ml^{-1} when a 2-ml sample of plasma was extracted and analysed in a final volume of 4 ml.

TABLE I

COMPARISON OF DIFFERENTIAL-PULSE VOLTAMMETRIC AND GAS-CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF NORPACE IN HUMAN PLASMA

Time following intravenous administration/min	Concentration of Norpace in plasma/μg ml^{-1}	
	Voltammetry	Gas chromatography
10	5.95	6.10
60	2.83	3.40
90	2.80	3.50
240	2.50	2.20

When the method was applied to plasma samples taken from a patient who had been given an intravenous dose of Norpace, the results shown in Table I were obtained. The plasma levels obtained using the d.p.p. and gas-chromatographic methods[9] show a broad agreement both in quantitative terms and in their drug distribution profiles. An oral dosing was also monitored after 20 and 65 min and the levels obtained by polarographic and gas-chromatographic determination were also in agreement, *i.e.*, 20 min (1.9 and 2.1 μg ml^{-1}, respectively) and 65 min (1.75 and 1.8 μg ml^{-1}).

References

1. Mokler, C. M., and Van Arman, C. G., *J. Pharmacol. Exp. Ther.*, 1962, **113**, 114.
2. Dean, R. R., and Ferguson, D. M., *Arch. Int. Pharmacodyn. Ther.*, 1971, **190**, 183.
3. Katz, M. J., Meyer, C. E., El-Etr, A., and Sladki, S. J., *Curr. Ther. Res. Clin. Exp.*, 1963, **5**, 343.
4. "Norpace Seminar," *Angiology*, 1975, **26**, Suppl., Part 2.
5. McMahon, R. E., *J. Pharm. Sci.*, 1966, **55**, 457.
6. Karim, A., Ranney, R. E., and Kraychy, S., *J. Pharm. Sci.*, 1972, **61**, 888.
7. Ranney, R. E., Dean, R. R., Karim, A., and Radzialowski, F. M., *Arch. Int. Pharmacodyn. Ther.*, 1971, **191**, 162.
8. Duchateau, A. M. J. A., Merkus, F. W. H. M., and Schobben, F., *J. Chromatogr.*, 1975, **109**, 432.
9. Hutsell, T. C., and Staakelski, S. J., *J. Chromatogr.*, 1975, **106**, 151.
10. Burmicz, J. S., *PhD Thesis*, University of London, 1979.
11. Chien, Y. W., Akers, M. J., and Yonan, P. K., *J. Pharm. Sci.*, 1975, **64**, 1632.

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Separation of Scandium(III) from Succinate Solution by Extraction with Liquid Ion Exchangers

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Keywords: Scandium separation; solvent extraction; liquid ion exchangers; succinate

Few solvent extraction methods are known for scandium. Diethyl ether,[1] acetylacetone,[2] 1,1,1-trifluoro-3-(2-thienyl)propan-2-one (TTA)[3] and tributyl phosphate (TBP)[4] have been used for the extraction of scandium from thiocyanate solutions. Extraction from perchlorate media using acetylacetone[5] and di(2-ethylhexyl)phosphoric acid[6] has also been reported. Similarly, TBP[7] and dibutylphosphoric acid[8] have been used for the extraction of scandium from hydrochloric acid and nitric acid solutions, respectively, but a systematic study of the separation of scandium from other elements is lacking. This paper describes a simple, rapid procedure for the extraction of scandium from succinate solution using high relative molecular mass amines such as trioctylamine (TOA), triisooctylamine (TIOA) and Aliquat 336 S as extractants. Scandium(III) is extracted quantitatively from 0.06 M sodium succinate solution using a 5% solution of one of these amines in benzene and, after re-extraction from the organic phase, is determined spectrophotometrically using Arsenazo I at 570 nm.[9]

Experimental

Apparatus

A Zeiss spectrophotometer with 1-cm quartz cells and a Philips pH meter were used for measuring absorbance and pH values, respectively.

Reagents

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

Scandium(III) solution was prepared by dissolving 0.15 g of scandium oxide (Koch-Light) in 3 ml of concentrated hydrochloric acid. The solution was evaporated to dryness and the residue was taken up in 100 ml of 0.1 M hydrochloric acid. The stock solution was standardised using EDTA[10] and further diluted as required.

Solutions of TOA, TIOA (Koch-Light) and Aliquat 336 (Fluka), 5% in benzene, were equilibrated for 10 min with equal volumes of 2 M sodium succinate solution before use.

Arsenazo I (Loba Chemie, Austria) was used as a 0.1% aqueous solution for the determination of scandium.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

General Extraction Procedure

Sodium succinate (0.41 g) was added to an aliquot of a solution containing 10–80 μg of scandium, giving a sodium succinate concentration of 0.06 M in a total volume of 25 ml. The pH of the solution, measured using a pH meter, was adjusted to 4.5 by the addition of sodium hydroxide solution and hydrochloric acid and then the mixture was equilibrated for 5 min in a separating funnel with two 5-ml portions of 5% TOA solution. The organic phase was collected and the scandium re-extracted using three 5-ml portions of 1 M hydrochloric acid. The combined aqueous phases were equilibrated with benzene in order to remove trace amounts of dissolved TOA and then evaporated to dryness. The residue was taken up in water containing 0.1 M hydrochloric acid, and 2 ml of 0.1% Arsenazo I solution, 2.5 ml of 1 M acetate buffer solution (pH 6.1) and 1 ml of 0.1 M ammonia solution were added. The coloured solution was transferred into a 25-ml calibrated flask, diluted to volume with distilled water and its absorbance was measured at 570 nm, using a reagent blank as the reference. The amount of scandium was determined from the calibration graph.

Results and Discussion

The extraction of scandium was studied at various pH values (3–9) and sodium succinate concentrations (0.02–0.07 M). It was found that 5% TOA solution extracts scandium (10–80 μg) quantitatively from 0.06 M sodium succinate solution at pH 4.5, whereas 5% TIOA and 5% Aliquat extract scandium from 0.06 and 0.07 M sodium succinate solutions, respectively, most effectively at pH 7 (Fig. 1).

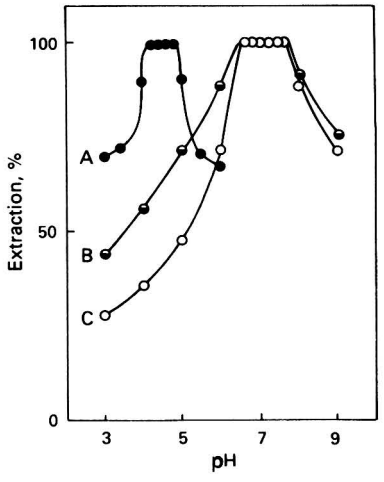

Fig. 1. Extraction behaviour of scandium(III) as a function of pH. A, Extraction with 5% TOA, from 0.06 M sodium succinate solution. B, Extraction with 5% TIOA from 0.06 M sodium succinate solution. C, Extraction with 5% Aliquat from 0.07 M sodium succinate solution.

Variation of the amine concentration (using benzene as the diluent) showed that a 5% solution of the amine is required for quantitative extraction of scandium. The logarithmic plot of the distribution ratio *versus* the amine concentration at fixed sodium succinate concentrations (0.02 and 0.04 M) gave a slope of unity for all amines, indicating that the metal to amine ratio in the extracted species is 1:1. Similarly, the logarithmic plot of distribution ratio *versus* the sodium succinate concentration gave a slope of nearly two, indicating that the succinate to metal ratio in the extracted species is 1:2. The probable extracted species is $[R_3NH^+Sc(C_4H_4O_4)_2^-]$ or $[R_4N^+Sc(C_4H_4O_4)_2^-]$ where R_3NH^+ represents TOA or TIOA and R_4N^+ represents Aliquat.

An interference study showed that the following ions do not affect the extraction and determination of scandium (25 μg): silver(I), zinc(II), cadmium(II), nickel(II), barium(II), calcium(II), bismuth(III), molybdenum(VI), ascorbate, phosphate, nitrate, sulphate, thiocyanate and thiourea, 2500 μg; cobalt(II), manganese(II) and uranium(VI), 2000 μg; lead(II) and chromium(VI), 1500 μg; and mercury(II), tin(II), arsenic(III), iron(III) and vanadium(V), 1000 μg. However, zirconium(IV), cerium(IV), copper(II), lanthanum(III), thorium(IV), EDTA, oxalate and citrate interfere seriously. Copper (up to 200 μg) is masked by thiourea, and interference due to lanthanum and thorium is eliminated by their prior extraction with 0.05 M hydrochloric acid.

Separation of Scandium from Iron, Molybdenum and Uranium

Iron, molybdenum and uranium remain in the aqueous phase when scandium is extracted from 0.06 M sodium succinate solution at pH 4.5 using 5% TOA. This facilitates the separation of scandium from iron, molybdenum or uranium in binary mixtures. Iron and molyb-

TABLE I

ANALYSIS OF SYNTHETIC MIXTURES

Sample No.	Composition of synthetic mixture: Sc/μg	Other elements/μg	Scandium: Recovery, %	Scandium: Relative error, %	Added ions: Recovery, %	Added ions: Relative error, %
1	25	Mo, 1000	99.8	0.2	99.0	1.0
2	25	Mo, 2500	99.8	0.2	99.2	0.8
3	10	Mo, 1000	99.8	0.2	99.6	0.4
4	25	U, 1000	99.2	0.8	99.0	1.0
5	25	U, 2500	99.2	0.8	99.0	1.0
6	10	U, 1000	99.6	0.4	99.4	0.6
7	25	Fe, 500	99.2	0.8	99.0	1.0
8	25	Fe, 1000	99.2	0.8	99.0	1.0
9	10	Fe, 500	99.4	0.6	99.6	0.4
10	25	La, 40	99.0	1.0	99.0	1.0
11	10	La, 40	99.4	0.6	99.4	0.6
12	25	Th, 1000	99.0	1.0	99.0	1.0
13	25	Th, 2000	99.0	1.0	99.0	1.0
14	10	Th, 1000	99.6	0.4	99.2	0.8
15	25	Cr, 1000	99.2	0.8	99.0	1.0
16	25	Cr, 500	99.2	0.8	99.0	1.0
17	10	Cr, 500	99.6	0.4	99.5	0.5
18	25	V, 500	99.6	0.4	—	—
19	25	V, 1000	99.6	0.4	—	—
20	25	Y, 50	99.2	0.8	99.0	1.0
21	10	Y, 50	99.5	0.5	99.2	0.8
22	25	La, 40; Y, 50	99.2	0.8	—	—
23	10	La, 40; Y, 50	99.8	0.2	—	—
24	25	Fe, 500; Mo, 1000; Cr, 1000; U, 1000	99.4	0.6	—	—
25	50	Fe, 500; Mo, 1000; Cr, 1000; U, 1000	99.4	0.6	—	—
26	25	Fe, 500; Mo, 1000; V, 1000	99.2	0.8	—	—
27	50	Fe, 500; Mo, 1000; V, 1000	99.2	0.8	—	—

denum in the aqueous phase were determined photometrically using the thiocyanate method[11] and uranium was determined by the 4-pyridylazoresorcinol method.[12] Scandium is subsequently re-extracted from the organic phase and determined by the method described under General Extraction Procedure. The results are reported in Table I.

Separation of Scandium from Vanadium, Chromium, Lanthanum, Thorium and Yttrium

Vanadium(V), chromium(VI), lanthanum(III), thorium(IV) and yttrium(III) are co-extracted with scandium from 0.06 M sodium succinate solution at pH 4.5 by 5% TOA solution. Lanthanum and yttrium can be removed from the organic phase using water (three 5-ml portions) containing hydrochloric acid (0.05 M) and determined spectrophotometrically.[13,14] Thorium can be removed similarly using water (three 5-ml portions) containing 0.05 M nitric acid and determined titrimetrically.[15] Scandium is finally re-extracted with 1 M hydrochloric acid and determined as described under General Extraction Procedure. Scandium and chromium can be separated by first extracting the scandium using 1 M hydrochloric acid and then back-extracting the chromium with 2 M ammonia solution (two 5-ml portions). The chromium can then be determined spectrophotometrically using diphenylcarbazide.[16] Scandium and vanadium are similarly separated by extracting the scandium with 1 M hydrochloric acid, leaving the vanadium in the organic phase. Vanadium(V) at milligram levels was not removed from the organic phase completely by any of the extractants tried. However, at low concentrations (10–40 μg) it could be extracted using 5 ml of acetate buffer solution (prepared by dissolving 27.2 g of sodium acetate in 200 ml of distilled water, adding 17 ml of glacial acetic acid and diluting to 1 l with water) and then determined spectrophotometrically.[17]

TABLE II

PRECISION DATA

Metal concentration/μg	Absorbance*	Standard deviation	Coefficient of variation, %
10	0.097	0.002	2.0
20	0.188	0.005	2.6
25	0.24	0.005	2.1
30	0.27	0.006	2.3
40	0.35	0.006	1.7
50	0.45	0.009	2.0
80	0.72	0.016	2.2

* Mean of six determinations.

The results for these separations are reported in Table I. The recovery of scandium and that of added ions is greater than 99%, with a relative error of 0.2–1%. The precision data are given in Table II.

The total period required for extraction and determination of scandium by the general extraction procedure is 40 min. The wide applicability of the method is shown by the satisfactory analysis of a variety of synthetic mixtures.

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References

1. Bock, R., *Fresenius Z. Anal. Chem.*, 1951, **133**, 110.
2. Shigematsu, T., Tabushi, M., Matsui, M., Nishikawa, Y., and Goda, S., *Nippon Kagaku Zasshi*, 1963, **84**, 263.
3. McIntyre, R. T., Berg, E. W., and Campbell, D. N., *Anal. Chem.*, 1956, **28**, 1316.
4. Yoshida, H., *Bunseki Kagaku*, 1963, **12**, 169.
5. Sekine, T., Koizumi, H., and Sakairi, M., *Bull. Chem. Soc. Jpn.*, 1966, **39**, 2681.
6. Peppard, D. F., Mason, G. W., Maier, J. L., and Driscoll, W. J., *J. Inorg. Nucl. Chem.*, 1957, **4**, 334.
7. Peppard, D. F., Mason, G. W., and Maier, J. L., *J. Inorg. Nucl. Chem.*, 1956, **3**, 215.
8. Samodelov, A. P., *Radiokhimiya*, 1964, **6**, 286.
9. Shimizo, T., *Anal. Chim. Acta*, 1967, **37**, 75.
10. Welcher, F. J., "The Analytical Use of EDTA," Van Nostrand, New York, 1958, p. 175.
11. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience, New York, 1965, pp. 534 and 649.
12. Busev, A. I., and Ivanov, V. M., *Vestn. Moskov. Univ., Ser. Khim.*, 1960, **3**, 52; *Anal. Abstr.*, 1961, **8**, 4612.
13. Shibata, S., Takevchi, F., and Matsumae, T., *Anal. Chim. Acta*, 1959, **21**, 177.
14. Munshi, K. N., and Dey, A. K., *Anal. Chem.*, 1964, **36**, 2003.
15. Fritz, J. S., and Ford, J. J., *Anal. Chem.*, 1953, **25**, 1640.
16. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience, New York, 1965, p. 397.
17. Gagliardi, E., and Ilmaier, B., *Mikrochim. Acta*, 1967, 180.

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Qualitative and Semi-quantitative Determination of Chromium(VI) in Aqueous Solution Using 1,5-Diphenylcarbazide-loaded Foam

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Keywords: *Chromium detection*; 1,5-*diphenylcarbazide foam test*

The detection and determination of low concentrations of chromate ion in aqueous solution are important for many purposes, particularly in environmental pollution analyses. Many spot-test reagents have been suggested[1] for this purpose. 1,5-Diphenylcarbazide appears to

be one of the most sensitive and satisfactory of the reagents available. Chromium(VI) gives a violet colour with this reagent.[2–4]

The sensitivity and selectivity of spot reactions have been greatly improved by impregnating the reagents on filter-paper,[1] by the ring oven technique,[5] and by the resin spot-test method.[6] The introduction of polyurethane foam[7,8] as a separatory medium in gas and liquid chromatography seemed to be very attractive. Polyurethane foam loaded with Amberlite LA-1 has been employed for the qualitative and semi-quantitative determination of cobalt(II) in aqueous thiocyanate solution.[9]

In this work the application of polyurethane foam, previously treated with 1,5-diphenylcarbazide reagent, for the detection and semi-quantitative determination of chromium(VI) in batch and flow experiments has been investigated.

Experimental

Reagents and Materials

Unless otherwise specified all reagents were of analytical-reagent grade. Tricaprylylamine (Alamine-336), pure grade, was used without further purification. Polyurethane foam, an open-cell, polyether type (bulk density 30 kg m^{-3}), was supplied by Greiner KG, Schaumstoff-Werk-Kremsmunster, Austria. The foam material (cubes of 5-mm edge) was washed with 1 M hydrochloric acid followed by distilled water until the washings were free from chloride ion. The foam was then washed with acetone and dried at 80 °C.

A stock solution containing 1 mg ml^{-1} of chromium(VI) was prepared by dissolving potassium dichromate in 0.01 M perchloric acid. 1,5-Diphenylcarbazide solution was prepared by dissolving 0.1 g of the solid material in 10 ml of benzene followed by the addition of 10 ml of the amine.

Caution—Benzene is highly toxic and appropriate precautions should be taken.

Reagent foam preparation

One gram of the dried foam cubes (white) was equilibrated with 3 ml of the 1,5-diphenylcarbazide solution with efficient stirring and was then allowed to remain in contact with the solution for about 1 h to ensure complete equilibration. The loaded foam material (pale yellow) was then dried between two sheets of filter-paper to remove the excess of reagent solution.

Apparatus

Glass columns of 5-mm diameter and 10-cm length were used in the dynamic experiments.

TABLE I

EFFECT OF DIFFERENT CATIONS ON THE DETECTION OF 1 μg OF CHROMIUM(VI)

Foreign ion	Compound added	Maximum tolerable amount of the foreign ion/mg*	Maximum tolerable concentration of interfering ion relative to Cr(VI)
La(III)	$La(NO_3)_3$	10	10^4
Ce(IV)	$Ce(SO_4)_2$	10	10^4
Co(II)	$Co(NO_3)_2.5H_2O$	1	10^3
Fe(III)	$FeCl_3$†	10	10^4
Ni(II)	$NiSO_4.7H_2O$	10	10^4
Tl(I)	$TlNO_3$	1	10^3
In(III)	$InCl_3.7H_2O$	1	10^3
Ba(II)	$BaCl_2$	10	10^4
Ca(II)	$CaCl_2$‡	10	10^4
Sr(II)	$SrCl_2$	10	10^4
Rb(I)	$RbNO_3$	10	10^4
Mg(II)	$MgCl_2$	10	10^4
NH_4^+	NH_4Cl	10	10^4

* The amount of foreign ion below which the determination of 1 μg of chromium(VI) in 2 ml of aqueous solution can be easily achieved.

† The foam colour in the blank test [*i.e.*, in the absence of chromium(VI)] was yellow.

‡ A few drops of 1 N perchloric acid were added to the $CaCl_2$ solution.

The foam columns were prepared using the vacuum method of foam column packing described previously.[10,11] About 0.3 g of the loaded foam was employed.

Results and Discussion

The application of polyurethane foam to the detection and semi-quantitative determination of lead, copper and cobalt in aqueous solution has been reported previously.[9] Fundamental data and the application to the detection of 1 μg of cobalt in the presence of relatively high concentrations of various other elements showed the usefulness of the method in trace element analysis. The use of this method for the determination of chromium(VI) was the subject of the present investigation.

1,5-Diphenylcarbazide was dissolved in benzene, an equal volume of tricaprylylamine was then added. The added amine has two functions: it acts as a plasticiser to the foam material and as an anion exchanger, which allows the collection of chromate ion on the foam matrix.

The colour reaction of chromium(VI) and 1,5-diphenylcarbazide could be carried out on the foam material simply by mixing one cube of the plasticised 1,5-diphenylcarbazide foam

TABLE II

EFFECT OF VARIOUS ANIONS ON THE DETECTION OF 1 μg OF CHROMIUM(VI)

Foreign ion*	Compound added	Maximum tolerable amount of the foreign ion/mg†	Maximum tolerable concentration of interfering ion relative to Cr(VI)	Notes
Acetate ..	CH_3COONa	10	10^4	Drops of 0.1 N perchloric acid were added
Oxalate ..	$Na_2C_2O_4$	10	10^4	Oxidation by bromine water was carried out followed by addition of drops of 0.1 N perchloric acid
Tartrate ..	$KNaC_4H_4O_6$	10	10^4	Conditions as mentioned for oxalate
$S_2O_3^{2-}$ ..	$Na_2S_2O_3$	0.1	10^2	Oxidation by bromine water was carried out and the excess of bromine was eliminated by heating
Cl^-	NaCl	10	10^4	
Br^-	NaBr	10	10^4	
CNS^- ..	KCNS	10	10^4	Drops of 0.1 N perchloric acid were added
PO_4^{3-} ..	Na_3PO_4	10	10^4	pH adjusted to about 2
HPO_4^{2-} ..	Na_2HPO_4	10	10^4	pH adjusted to about 2
$H_2PO_4^-$ ..	NaH_2PO_4	10	10^4	
HCO_3^- ..	$KHCO_3$	10	10^4	pH adjusted to about 2
$B_4O_7^{2-}$ ..	$Na_2B_4O_7.10H_2O$	10	10^4	pH adjusted to about 2
NO_2^-	$NaNO_2$	10	10^4	Oxidation by Br_2 was carried out followed by adjusting pH to about 2
I^-	NaI	10	10^4	
Formate ..	HCOONa	10	10^4	Oxidation by Br_2 was carried out followed by adjusting pH to about 2
SO_3^{2-}	Na_2SO_3	0.1	10^2	
SO_4^{2-}	Na_2SO_4	0.1	10^2	
VO_3^-	NH_4VO_3‡	0.1	10^2	
MoO_4^{2-} ..	$(NH_4)_2MoO_4$‡	10	10^4	Drops of a saturated solution of oxalic acid were added
MnO_4^- ..	$KMnO_4$	0.5	2×10^2	
L-Ascorbate ..	L-Ascorbic acid	0.1	10^2	Oxidation by bromine water was carried out followed by removal of excess of bromine

* With metal ions that interfere due to their own colour in solution, it was found better to take out the foam cube (after shaking with the test solution) and to shake it with a few millilitres of water. The red - violet colour that appears in the presence of chromium(VI) was then better observed.

† The amount of the foreign ion below which the detection of 1 μg of chromium(VI) in 2 ml of aqueous solution can be easily achieved.

‡ The foam colour in the blank test [*i.e.*, in the absence of chromium(VI)] was yellow.

with 1–2ml of an aqueous, acidic solution of chromium (pH 2–4). Using this method, as little as 0.1 μg of chromium(VI) could be detected, a red - violet colour appearing on the foam cube after shaking for 1–2 min.

The detection limits for chromium using the 1,5-diphenylcarbazide reagent in a normal spot test,[1] on impregnated filter-paper[1] and in the proposed foam test were found to be 5.0, 0.5 and 0.1 p.p.m., respectively. These results indicate clearly that the proposed foam test is by far the most sensitive.

Initial results for the semi-quantitative determination of chromium(VI) using the proposed method were satisfactory. The colour density on the reagent foam cube was found to be proportional to the concentration of chromium(VI) in the aqueous solution. A standard colour scale for 0.1, 0.5, 1 and 5 p.p.m. of chromium(VI) was found to be suitable.

The 1,5-diphenylcarbazide foam was examined for the detection of 1 μg of chromium(VI) in the presence of relatively high concentrations of a wide range of different cations and anions in aqueous solutions. It was shown that the foam test for chromium is selective. The ratios of the concentrations of chromium(VI) detected to the concentrations of the accompanying ions are shown in Tables I and II.

The 1,5-diphenylcarbazide-loaded foam was next applied in column operations. The fact that polyurethane foam is easily packed in a column having good hydrodynamic properties suggests the possibility that even lower concentrations of chromium(VI) could be detected using 1,5-diphenylcarbazide foam columns. Small amounts of chromium could be collected from 100 ml of aqueous solution (pH 2–4) by passing it through the foam column at reasonable flow-rates (3–5 ml min^{-1}). The change in colour of the foam bed due to the coloured products of the collected chromate ion on the reagent foam is a test for chromium(VI). It was further observed that the length of the coloured zone is approximately proportional to the concentration of chromium in the aqueous solution. Semi-quantitative determination of chromium(VI) at the parts per 10^9 (p.p.b.) level could, therefore, be achieved. The following colour scale proved to be satisfactory: 10, 20, 30, 40 and 50 p.p.b. The lengths of the coloured zones formed were found to be 2, 4.5, 10, 12 and 15 mm, respectively, at the specified flow-rate. These results offered further evidence of the advantages of the proposed foam test.

Conclusion

The semi-quantitative determination of as little as 0.1 p.p.m. and the detection of 10 p.p.b. of chromium(VI) in aqueous solution was achieved in batch and column experiments, respectively. The semi-quantitative determination of chromium(VI) was also carried out using a suitable colour scale. The detection of chromium was successful in the presence of relatively high concentrations of various other elements.

References

1. Feigl, F., and Anger, V., "Spot Tests in Inorganic Analysis," Sixth Edition, Elsevier, Amsterdam, 1972.
2. Cazeneuve, P., *C.R. Acad. Sci.*, 1900, **131**, 346.
3. Cazeneuve, P., *Bull. Soc. Chim. Fr.*, 1900, **23**, 701.
4. Cazeneuve, P., *Bull. Soc. Chim. Fr.*, 1901, **25**, 761.
5. Weisz, H., *Mikrochim. Acta*, 1954, 140.
6. Fujimoto, M., *Chemist Analyst*, 1965, **54**, 58 and 92.
7. Braun, T., and Farag, A. B., *Anal. Chim. Acta*, 1978, **99**, 1.
8. Moody, G. J., and Thomas, J. D. R., *Analyst*, 1979, **104**, 1.
9. Braun, T., and Farag, A. B., *Anal. Chim. Acta*, 1974, **73**, 301.
10. Braun, T., and Farag, A. B., *Anal. Chim. Acta*, 1972, **61**, 265.
11. Braun, T., and Farag, A. B., *Anal. Chim. Acta*, 1972, **62**, 476.

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Book Reviews

WILSON AND WILSON'S COMPREHENSIVE ANALYTICAL CHEMISTRY. Volume XI. Edited by G. SVEHLA. Pp. xiv + 407. Elsevier. 1981. Price $119.50; Dfl245. ISBN 0 444 41886 5 (Vol. XI); 0 444 41735 4 (Series).

Most of the individual earlier volumes of this series have contained contributions on a common theme. By comparison, mathematical statistics, mass spectrometry and ion-selective electrodes as the chapter contributions of the present volume are strange companions. However, all three topics are of paramount importance in analytical chemistry; they have a vast published literature to the extent that in each case there are rather better treatments to be found elsewhere at a more moderate price.

Mathematical statistics in relation to analytical chemistry was well covered by E. C. Wood in Volume IA; it forms the basis of the reviewer's treatment of the subject in his lecture courses. The present chapter by P. Moritz builds on the earlier work under the main headings of basic concepts of mathematical statistics, the law of propagation of errors and its application to various analytical methods, and the methodology of setting up mathematical models. The text is appropriately interwoven with tailor-made computer programs, which makes it a very practical contribution. There are, however, one or two blemishes, such as the inconsistencies in the symbolism of flow-chart modes. Despite this minor criticism, the chapter is the best and major part of the book; its 170 pages take up over two fifths of the complete work.

Mass spectrometry by J. Grimshaw is introduced as a new field to the series, with the promise of a return to more specialised aspects at a later stage. Its 104 pages are a very useful, but rather dated introduction to the subject. The instrumentation section covers historical development, sample flow, sample inlet systems, ion sources, mass analysers, resolving power, detectors and recorders. Applications take up the major part under the headings of pure organic compounds (fragmentation theories and patterns, and molecular formulae determination), mixtures of gaseous and volatile compounds (with GLC - MS interfaces) and elemental analysis of involatile inorganic samples (spark ionisation).

Ion-selective electrodes follow an earlier consideration (in Volume VIII) of enzyme electrodes and the chapter is justified by the fact that the subject cannot be omitted from the series. The author (W. E. van der Linden) has probably been restricted in the amount of space available; nevertheless, he has not fully succeeded in the choice and treatment of subject content within the 118 pages used. The treatment of liquid membrane electrodes is contrary to practice, and the use of "phosphate" for "phosphonate" when dealing with phosphonate solvent mediators on the bottom of page 346 will be confusing to readers. Although the discussion progresses systematically through membrane potentials, electrode types and properties, instrumentation, methods of analysis and applications, it is very much regretted that the work is cursory. Readers will be far better served by turning to the texts in the author's reference list, especially as some of these have been revised. Also, other good texts have recently been published.

In conclusion, this volume takes the series forward by a short step. It will be needed by those who wish to complete the series, but unfortunately there are many loose points other than those mentioned above, and they are not difficult to find. For example, on page 239 "loose" appears for "lose" and "principle" for "principal," and on page 325 "Karh" appears for "Kahr."

J. D. R. THOMAS

ION-SELECTIVE ELECTRODES IN ANALYTICAL CHEMISTRY. Volume 2. Edited by HENRY FREISER. Pp. xii + 291. Plenum. 1980. Price $35. ISBN 0 306 40500 8.

This book rounds off the venture on ion-selective electrodes in analytical chemistry of the *Modern Analytical Chemistry Series* of Plenum, which previously was devoted essentially to spectroscopic themes. Its Volume 1 partner, reviewed in *The Analyst* (1979, **104**, 896), covered "Theory and Principles of Membrane Electrodes" (R. P. Buck), "Precipitate-based Ion-selective Electrodes" (E. Pungor and K. Tóth), "Ion-selective Electrodes Based on Neutral Carriers" (W. E. Morf and W. Simon), "Poly(vinyl chloride) Matrix Membrane Ion-selective Electrodes" (G. J. Moody and J. D. R. Thomas), "Sources of Error in Ion-selective Electrode Potentiometry" (R. A. Durst) and "Applications of Ion-selective Electrodes" (G. J. Moody and J. D. R. Thomas). Volume 2 makes

up for the very obvious omissions by having chapter contributions on "Potentiometric Enzyme Methods" (R. K. Kobos), "Coated Wire Ion-selective Electrodes" (H. Frieser) and "Chemically Sensitive Field Effect Transistors" (J. Janata and R. J. Huber). The work is completed by a 114-page tabular compilation of membrane electrode papers "based on previous, more comprehensive reviews" (R. P. Buck, J. C. Thompson and O. R. Melroy).

Following a brief introduction and sections devoted to soluble and insoluble enzyme systems, the chapter on enzymes deals systematically with the various enzyme electrodes, followed by short collective considerations of trends, applications, theoretical aspects and response. The related bacterial electrodes are also discussed systematically along with a tissue-based electrode. The chapter is a very concise and pleasant to read coverage of its subject, while the crystal-gazing on the future of these devices is a clear indication that there is still plenty of "activity" left for the future.

The Editor, in the coverage of his special field, leads the reader very nicely into the intricacies of coated-wire electrodes and the constructional details on page 89 will be greatly valued. However, this reviewer believes that our modest Editor does himself less than justice, for coated-wire devices have been more widely studied and used than is indicated by his literature coverage.

CHEMFETs are extremely topical and the chapter on this theme will be the impetus for many to want to buy this book. They will not be disappointed, for there is a most inviting introduction preceding a stimulating discussion with easy to follow diagrams. The authors have well succeeded in their declared objective of presenting a common theoretical base for these sensors as well as summarising the results so far obtained. There is an invitation to readers to bring their appreciation even more up to date through the supplementary references for 1979 and 1980.

The classified literature compilation will be helpful to readers to find references to set subject areas, while the introduction to these highlights many of the significant developments and events. Deficiencies existing in our knowledge are also mentioned, especially that of the role of solvent mediators in liquid ion-exchange sensors.

This book is highly recommended and its modest price will ensure that it will be on as many shelves and as widely read as its elder companion, Volume 1. J. D. R. THOMAS

ENVIRONMENTAL CHEMISTRY OF THE ELEMENTS. By H. J. M. BOWEN. Pp. xvi + 333. Academic Press. 1979. Price £18.80. ISBN 0 12 120450 2.

At the present time, when the tasks or duties of the analytical chemist are constantly being reassessed so as to widen them to include interpretation of the gathered data to arrive at a meaningful solution to the problem, this book is most welcome. It is an extensive re-write, revision and renaming of the 1966 book "Trace Elements in Biochemistry." The need for the present book has arisen from the vast amount of data now forthcoming about the distribution of chemical elements in the environment and living organisms. No doubt this explosion of data is partly due to the use of atomic-absorption spectroscopy and the increasing interest in environmental matters over the last decade. Topics included in the book are the atmosphere, the hydrosphere, the elemental geochemistry of rocks, soils, the biosphere and elemental cycles, elemental composition of living matter, uptake and excretion of elements, deficiencies and toxicities of elements, chemical forms and functions of elements, biological fractionation of isotopes, radioactive nuclides as well as the environmental effects of human activities.

The text is packed with tables both large and small, all full of data which represent an encyclopaedic coverage. Some tables are so large as to render the presentation of the material difficult to read and interpret. However, the author is at pains to apologise for those facts which he has missed. It is these tables that will probably be most useful, as they act as a reference guide for what range of values for a particular element in a specified matrix is to be expected.

Little mention is made of the analytical techniques employed to gather the data, but the comments on the collecting and cleaning of biological material, analytical errors and the expression of the results are most appropriate. What is most stimulating is the suggestion that although much has been done, much data remain to be gathered. Thus little work is available on the lanthanide and platinum metals and almost nothing is known about the chemical species present in soils and water. In fact, the second paragraph of the Preface offers ideas for countless PhD students. The task of obtaining these data, particularly in speciation studies, will require the utmost professional skill the analytical scientist has to offer. All we require is the sympathetic Research Council to support such work, but no doubt few honours will be forthcoming for such essential work, as it will not be novel or timely enough!

All in all a stimulating book, one to be consulted but not one which will act as a workshop guide or recipe text for analysts. Frankly the book is much deeper than that; it offers some solutions, but raises many fascinating questions. A book for the reference shelf, which over a period of time will doubtless become well thumbed. G. NICKLESS

HYDROCARBONS AND HALOGENATED HYDROCARBONS IN THE AQUATIC ENVIRONMENT. Edited by B. K. AFGHAN and D. MACKAY. *Environmental Science Research, Volume* 16. Pp. xiv + 588. Plenum Press. 1980. Price $59.50. ISBN 0 306 40329 3.

The book is the proceedings of the International Symposium on the Analysis of Hydrocarbons and Halogenated Hydrocarbons in the Aquatic Environment, held in Ontario, Canada, on May 23rd–25th, 1978. The Symposium was organised by the National Research Institute, Canada Centre for Inland Waters and The Institute of Environmental Studies of the University of Toronto. The environmental contaminants given the widest coverage are those with which we have all become most familiar, namely organochlorine insecticides, polychlorinated biphenyls and polynuclear aromatic hydrocarbons. Several papers are devoted to or deal partially with trihalogenated methanes. These compounds are of particular concern in the aquatic environment, as they are formed during the disinfection of natural waters using chlorine. Chlorinated phenols are similarly formed from phenolic pollutants and receive some attention. Mineral oil-derived hydrocarbons are dealt with in several papers, as are volatile compounds that can be purged from water and sediment samples using a stream of inert gas.

Despite the title of the Symposium being specific to analysis, the papers themselves cover a field better described by the book title, and of the total of 44 papers presented only 19 deal with analytical methodology. The remainder give a good coverage of the field of environmental contamination, namely occurrence, speciation, routes of entry into the environment, degradation, toxicity and metabolism.

Gas chromatography, in many instances combined with mass spectrometry, is the major analytical technique used in the papers presented. Capillary column applications have advanced rapidly in recent years and such columns are now considered essential in the analysis of complex mixtures. The fact that some of the papers of this type in the book describe the use of packed columns is an indication of the rapidity of the advances.

The book is a wide-ranging collection of papers on its title subject, some of which are not published elsewhere. It should prove useful to the specialist in the field. B.T. CROLL

QUALITATIVE ANALYSIS OF FLAVOR AND FRAGRANCE VOLATILES BY GLASS CAPILLARY GAS CHROMATOGRAPHY. By WALTER JENNINGS and TAKAYUKI SHIBAMOTO. Pp. viii + 472. Academic Press. 1980. Price $39. ISBN 0 12 384250 6.

This is an excellent and most valuable book, for which the authors and publishers are to be congratulated wholeheartedly. At first sight it might seem an odd publication in that it comprises only 26 pages of descriptive text and some 435 pages of appendices! However, it is the latter that provides the main value of the book, although the title is somewhat misleading and does not really convey its true content.

The first part of the book (the 26 pages) deals with relevant analytical considerations covering GC system requirements, retention indices, selective GC detectors, ancillary reactions including reaction GC, and gas chromatography - mass spectrometry. The treatment is, of course, brief, but adequate under the circumstances, and even so there are many useful tips for the practising researcher struggling with glass capillary columns for the 1st—or *n*th—time. The appendices provide four compilations. The first three list the Kováts retention indices of approximately 1200 compounds determined on high-resolution glass capillary GC columns of OV-101 and Carbowax 20M. The compounds are first listed alphabetically, and then in order of elution in each of the two phases. It is noticeable that the authors have selected the two phases on which the majority of astute flavour researchers now conduct their analyses.

The fourth appendix gives the mass spectra, as full line diagrams, of about 70 % of the previously mentioned compounds, again listed in alphabetical order. It is difficult to see how better these spectra could have been sequenced in only one listing, but certainly this compilation in its present form is of little value if confronted by a totally unknown spectrum. Doubtless it was not intended

directly for that specific purpose, but it took us but a short time to transfer reference to these spectra to listings based on relative molecular mass or base peak. Perhaps it is a shame that such listings were not also included in the book.

Throughout the appendices the data are very well presented—particularly the mass spectra—and a quick survey did not bring to light any errors. Indeed, there are very few misprints to be found in the book, although one did manage to sneak into the Preface! It is obvious that there are many omissions from the compilations—and the authors would be the first to admit this—but this is understandable, and these data provide an excellent first step in any search for the identity of unknown components in flavour mixtures, either natural or artificial. Their main use, however, will be more in confirmation of already suspected identities. I would recommend purchase of this book to all those working in relevant fields, and particularly at the very reasonable price. I am sure that it will prove a successful publication and I trust that such success will encourage the authors to update the compilation regularly.

A. J. MacLeod

Pesticide Analytical Methodology. Edited by John Harvey, Jr., and Gunter Zweig. *ACS Symposium Series No.* 136. Pp. x + 406. American Chemical Society. 1980. Price $38. ISBN 0 8412 0581 7; ISSN 0097 6156.

A more appropriate title for this book would have indicated that it takes a broad view of recent developments and likely future trends of pesticide analysis. This volume is not a compilation of analytical procedures, although there are chapters on specialised topics such as the determination of 2,3,7,8-tetrachlorodibenzo-*p*-diozin (a significant slip in the contents list?) in human milk and the analysis of organotin residues by gas chromatography - mass spectrometry. Most methods considered have a wide range of potential uses and many aspects of the analysis of trace amounts of pesticides are discussed, including extraction, clean-up, detection and formation of derivatives. Various kinds of spectrophotometry are included, but chromatography remains a field of interest where techniques are still developing. Relatively little space is devoted to gas chromatography but about a third of the book is devoted to aspects of high-performance liquid chromatography and about a sixth to thin-layer chromatography. This reflects the methods where improvements in materials and equipment are most promising for the sensitive assay of chemicals irrespective of volatility and thermal stability. Generally, developments in electronics have produced improved instrumentation and computing facilities so that an increasing number of techniques are becoming available for analytical purposes. Among those considered, either individually or combined with other techniques, are Fourier transform infrared spectroscopy, spectrofluorimetry, mass spectrometry and electrochemical and radiometric determinations. Perhaps not in the same category, but dependent upon a range of techniques, the potential of immunochemistry for the identification and measurement of pesticides is considered.

Treatment of the topics varies, as does the style and content of individual chapters. Likewise, the references given at the ends of the chapters vary from a single one to over eighty, but they are generally pertinent and sufficient to provide a useful background, and incidentally could provide an industrious reader with an introduction to pesticide analysis. The compilations of information on optical properties and chemical reactions will doubtless prove useful, although past experience suggests that they must be used with caution. Doubtless there are more extensive reviews elsewhere but the juxtaposition of individual topics provides a stimulating review of developments in pesticide identification and assay which should promote further developments and applications.

K. A. Lord

Microbiological Assay of Low Levels of Monensin in Animal Feeds

Report prepared by the Antibiotics in Animal Feedingstuffs Sub-Committee

The method for the determination of monensin in animal feedingstuffs published previously was developed at a time when this material was being incorporated in feeds at the 100 mg kg^{-1} level. As a need has arisen for a method that would determine monensin when present in amounts of 20–40 mg kg^{-1}, the original method has been re-examined and a concentration step introduced that enabled satisfactory results to be obtained on a sample of feed containing 18 mg kg^{-1} of monensin.

Keywords: *Monensin assay*; *animal feeds*; *microbiological assay*

ANALYTICAL METHODS COMMITTEE

Royal Society of Chemistry, Burlington House, London, W1V 0BN.

Analyst, 1981, **106**, 788–789.

Simultaneous Determination of Copper, Lead, Cadmium and Zinc in Non-ferrous Smelter Products by Differential-pulse Polarography

Short Paper

Keywords: *Non-ferrous smelter*; *copper, lead, cadmium and zinc determination*; *differential-pulse polarography*; *orthophosphoric acid*

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Analyst, 1981, **106**, 790–793.

Versatile Fusion Method for the Dissolution of Refractory Materials

Short Paper

Keywords: *Fusion technique*; *refractory materials*; *atomic-absorption spectrophotometry*

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Analyst, 1981, **106**, 794–796.

Coulometric Determination of Herbicides Containing Nitro Groups by Reduction with Electro-generated Chromium(II)

Short Paper

Keywords: *Nitro-containing herbicide determination*; *coulometry*; *chromium(II) generation*; *nitro group reduction*

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Analyst, 1981, **106**, 796–799.

Spectrophotometric Determination of Copper in Blood Serum with 4-(2-Quinolylazo)phenol

Short Paper

Keywords: 4-(2-*Quinolylazo*)*phenol*; *copper*(*II*) *determination*; *blood serum*; *spectrophotometry*

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Analyst, 1981, **106**, 799–802.

Differential-pulse Polarographic Determination of Norpace in Plasma

Short Paper

Keywords: *Norpace determination*; *differential-pulse polarography*

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Analyst, 1981, **106**, 802–805.

Separation of Scandium(III) from Succinate Solution by Extraction with Liquid Ion Exchangers

Short Paper

Keywords: *Scandium separation*; *solvent extraction*; *liquid ion exchangers*; *succinate*

D. D. DESAI and V. M. SHINDE
Analytical Laboratory, Department of Chemistry, Shivaji University, Kolhapur-416 004, India.

Analyst, 1981, **106**, 806–809.

Qualitative and Semi-quantitative Determination of Chromium(VI) in Aqueous Solution Using 1,5-Diphenylcarbazide-loaded Foam

Short Paper

Keywords: *Chromium detection*; 1,5-*diphenylcarbazide foam test*

A. B. FARAG, A. M. EL-WAKIL and M. S. EL-SHAHAWI
Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

Analyst, 1981, **106**, 809–812.

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