The Analyst

The Analytical Journal
of The Royal Society
of Chemistry

A monthly international publication dealing
with all branches of analytical chemistry

Volume 105 No 1255 Pages 969–1008 October 1980

THE ANALYST

THE ANALYTICAL JOURNAL OF THE ROYAL SOCIETY OF CHEMISTRY

Published by The Royal Society of Chemistry

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

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

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

Volume 105 No 1255 October 1980

A220 for further information. See page xiv ⟶

Summaries of Papers in this Issue

Polarographic Behaviour and Analysis of Some Azo Dyes of Biological Significance

The polarographic behaviour of the azo dyes CI Direct Orange 34, Acid Red 73, Direct Blue 84 and Direct Red 80 over the pH range 1–13 was studied. Mechanisms of reduction are postulated, the optimum pH ranges for the determination of the dyes by differential-pulse polarography are selected and the appropriate linear ranges deduced from calibration graphs.

Keywords: *Azo dyes*; *polarography*

J. P. HART
Orthopaedic Department, Charing Cross Hospital, London, W.6.

and W. FRANKLIN SMYTH
Chemistry Department, University College, Cork, Republic of Ireland.

Analyst, 1980, **105**, 929–938.

A New Approach to the Quantitative Analysis of Overlapping Anodic-stripping Voltammograms

A method, analogous to that used for studying overlapping bands in absorption spectroscopy, has been developed for the quantitative analysis of overlapping anodic-stripping voltammograms. Six simultaneous equations were formulated to resolve general overlapping voltammograms. When the second species is not oxidised at the peak potential of the first species these six equations are reduced to four. Provided that the oxidation currents are additive, this approach is not limited by the degree of overlap. The method has been shown to be satisfactory by studying the cadmium - lead pair as an example.

Keywords: *Anodic-stripping voltammetry*; *overlapping voltammograms*; *quantitative analysis*

JUEI H. LIU
Department of Criminal Justice, University of Illinois at Chicago Circle, Chicago, Ill. 60680, USA.

Analyst, 1980, **105**, 939–943.

A New Type of Biological Reference Material for Multi-element Analysis—the Fungus *Penicillium ochro-chloron* ATCC 36741

Several series of standard disk samples were prepared using dried mycelia of *Penicillium ochro-chloron*, a fungus extremely tolerant to heavy metals. Because of the ready availability of the homogeneous dry material with arbitrary metal concentrations, the fungus is very suitable for calibration in metal analysis by X-ray fluorescence spectrometry, especially for biological samples.

Based on the calibration graph for the reference material, the metal contents of edible wild plants were determined. The reliability of the data was checked by using biological reference materials and by atomic-absorption spectrometry.

Keywords: *Biological reference material*; Penicillium ochro-chloron *fungus*; *heavy metals*; *biological materials*; *X-ray fluorescence spectrometry*

MIWAKO SUZUKI, YUKIKO DOKIYA, SUNAO YAMAZAKI and SHOZO TODA
Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, Japan 113.

Analyst, 1980, **105**, 944–949.

Spectrophotometric Determination of Hydrogen Peroxide Using Potassium Titanium(IV) Oxalate

A simple and rapid method for the spectrophotometric determination of hydrogen peroxide using potassium titanium(IV) oxalate is described. The method can be used to measure peroxide concentrations down to about 10 μM (0.3 mg kg^{-1}) under the most favourable conditions. A variety of complexing and reducing agents, and catalysts of peroxide decomposition, known to interfere with the alternative iodide method for peroxide determination, had no effect. Fluoride was found to interfere.

Keywords: *Hydrogen peroxide determination*; *spectrophotometry*; *titanium(IV) oxalate*

ROBIN M. SELLERS

Central Electricity Generating Board, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire, GL13 9PB.

Analyst, 1980, **105**, 950–954.

Improved Extraction Method for the Spectrophotometric Determination of Trace Amounts of Boron in River Water with 1,8-Dihydroxynaphthalene-4-sulphonic Acid and Removal of the Excess of Reagent

A simple method for removing the excess of co-extracted reagent in the ion-association extraction of metal complex anions with quaternary ammonium salts has been applied successfully to the spectrophotometric determination of boron at the parts per 10^9 level in river water with 1,8-dihydroxynaphthalene 4-sulphonic acid (DHNS) and tetradecyldimethylbenzylammonium chloride (zephiramine). The procedure using DHNS described here greatly improves the previous method using chromotropic acid. Acetate buffer (pH 3.8), EDTA and DHNS are added to the sample solution (less than 50 ml) and the pH of the resulting solution is adjusted to 10.2. Then sodium chloride is added and the mixture is shaken with 5 ml of a 2×10^{-3} M solution of zephiramine in 1,2-dichloroethane (DCE). The organic phase is washed once with 10 ml of a back-washing solution (1.0 M in sodium chloride, pH 10.2) and the absorbance of the organic phase is measured in a quartz cell. The boron complex with DHNS is extracted quantitatively into DCE and its apparent molar absorptivity in DCE is 2.4×10^4 l mol^{-1} cm^{-1} at 341 nm. The detection limit and precision achieved with the method are 1 μg l^{-1} and 5%, respectively. EDTA allows most interferences caused from metals to be suppressed, and other sources of bias due to the effect of co-extractable anions are almost eliminated by adding relatively large amounts of sodium chloride to the extraction system. Parts per 10^9 amounts of boron present as boric acid in river water are determined spectrophotometrically, and the results obtained are successfully compared with those obtained by the methylene blue method.

Keywords: *Boron trace determination*; *river water analysis*; *spectrophotometry*; *ion-association extraction*

TAKASHI KORENAGA, SHOJI MOTOMIZU and KYOJI TÔEI

Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Okayama-shi 700, Japan.

Analyst, 1980, **105**, 955–964.

Pyridine-2-acetaldehyde Salicyloylhydrazone as an Analytical Reagent and its Application to the Determination of Vanadium

The synthesis, characteristics and analytical applications of pyridine-2-acetaldehyde salicyloylhydrazone (PASH) are described. The reagent reacts with vanadium(V) to produce a yellow 1 : 1 complex ($\lambda_{max.} = 415$ nm, $\varepsilon = 1.87 \times 10^4$ l mol^{-1} cm^{-1} in chloroform). The yellow complex, extracted into chloroform, has been used for the spectrophotometric determination of vanadium in a steel, a lead - vanadium concentrate and a phosphoric acid sample. A procedure based on the standard additions method has been applied satisfactorily to the determination of trace amounts of vanadium at the parts per billion level (parts per 10^9).

Keywords: *Pyridine-2-acetaldehyde salicyloylhydrazone reagent*; *vanadium determination*; *standard additions method*; *spectrophotometry*

M. GARCIA-VARGAS, M. GALLEGO and M. DE LA GUARDIA

Department of Analytical Chemistry, Faculty of Sciences, University of Seville, Seville, Spain.

Analyst, 1980, **105**, 965–973.

Determination of Cyanide in Animal Feeding Stuffs

A method for the determination of cyanide in feeding stuffs has been developed. Naturally occurring cyano-substituted glycosides are subjected to enzymatic hydrolysis, the liberated cyanide is isolated by aeration and determined either by a spectrophotometric method or by gas chromatography. Recoveries of cyanide added to feeding stuffs at concentrations of 10 and 20 mg kg^{-1} were approximately 98%. The method is sensitive to as little as 1 mg kg^{-1} of cyanide.

Keywords: *Cyanide determination*; *animal feeding stuffs*; *enzymatic hydrolysis*; *spectrophotometry*; *gas chromatography*

J. R. HARRIS, G. H. J. MERSON, M. J. HARDY and D. J. CURTIS

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ.

Analyst, 1980, **105**, 974–980.

A Simple Non-dispersive Atomic-fluorescence Spectrometer for Mercury Determination, Using Cold-vapour Generation

Short Paper

Keywords: *Mercury determination*; *vapour generation*; *non-dispersive atomic-fluoresence spectrometry*; *estuarine samples*

R. C. HUTTON and B. PRESTON

Tioxide International Limited, Central Laboratories, Stockton-on-Tees, Cleveland, TS18 2NQ.

Analyst, 1980, **105**, 981–984.

OCTOBER 1980 Vol. 105 No. 1255

The Analyst

Polarographic Behaviour and Analysis of Some Azo Dyes of Biological Significance

J. P. Hart

Orthopaedic Department, Charing Cross Hospital, London, W.6

and W. Franklin Smyth

Chemistry Department, University College, Cork, Republic of Ireland

The polarographic behaviour of the azo dyes CI Direct Orange 34, Acid Red 73, Direct Blue 84 and Direct Red 80 over the pH range 1–13 was studied. Mechanisms of reduction are postulated, the optimum pH ranges for the determination of the dyes by differential-pulse polarography are selected and the appropriate linear ranges deduced from calibration graphs.

Keywords: *Azo dyes*; *polarography*

Azo compounds are widely used in industry as textile dyes, colouring agents in foods and pharmaceuticals, etc. As a result they can become environmental pollutants through discharge of the contents of plating baths from textile works into rivers, especially if not treated by the activated sludge process, as has been observed in certain instances.[1,2] They can also enter the body through the intake of certain foods and drugs that contain these azo compounds.

Concern has been voiced about the potential carcinogenicity of compounds containing azo group(s).[3–5] Therefore, a study of the mechanism of the polarographic reduction of azo compounds, which often parallels the metabolism of these compounds *in vivo* (*e.g.*, reductive fission of the azo linkage to the parent aromatic amines), is worthy of investigation. Differential-pulse polarography can also be used to determine the parent compounds and their electroactive metabolites at trace levels.

The electrochemical behaviour of a variety of azo compounds has been investigated over the years, and a brief survey of the literature on monoazo compounds alone illustrates the complexity of the electrode reactions involved.

Issa *et al.*[6] studied the polarographic reduction of some 4-hydroxy monoazo compounds containing different substituents, and found that reduction proceeded in acidic and alkaline solutions with the consumption of four and two electrons, respectively. In the same study the authors reported that 4-hydroxyazobenzene and azo compounds with weak donor or acceptor groups were capable of catalysing the reduction of H^+, this process producing a wave that gave the appearance of a maximum. Similar results were obtained by other workers[7–9] on compounds closely related to those mentioned above. In contrast, Solochrome mordant dyes were found by Malik and Gupta[10] to undergo reduction with the transfer of two electrons in both acidic and alkaline solutions. These dyes produced one wave in acidic solution, but in some instances two waves developed in alkaline media. It has been shown, in an investigation on some *para*-substituted azobenzenes, that electron-accepting substituents promote reduction to hydrazo derivatives, whereas electron donors drive the reaction partially or totally to the corresponding amines.[11] Florence and co-workers[12–18] have contributed a number of publications on the study of azo compounds. They suggested that certain species formed unstable hydrazo intermediates, and over-all polarographic n values of 4 were obtained.

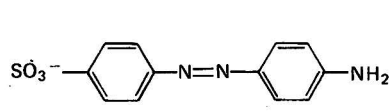
This paper is concerned with a polarographic study of the four azo dyes CI Direct Orange 34 (I), CI Acid Red 73 (II), CI Direct Blue 84 (III) and CI Direct Red 80 (IV) over the pH range 1–13.

Mechanisms of reduction are postulated on the basis of controlled-potential electrolysis at a large mercury pool and cyclic voltammetry as additional electroanalytical techniques. Optimum pH values for the determination of the azo dyes by differential-pulse polarography (DPP) are selected and the appropriate linear ranges deduced from calibration graphs.

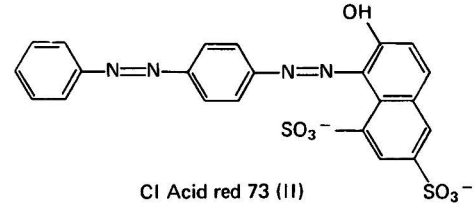
Experimental

Reagents and Chemicals

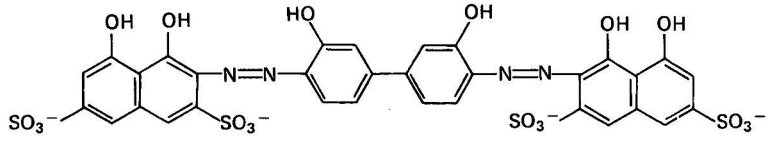
Britton - Robinson buffers of pH 2–12 were used and the pH range was extended with 0.1 N sulphuric acid and 0.1 N sodium hydroxide prepared in distilled water.



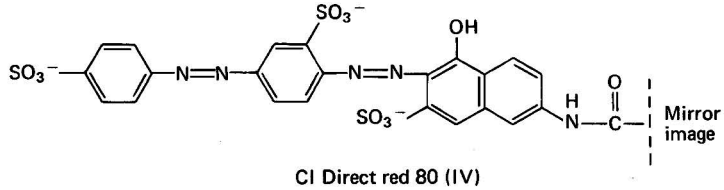
CI Direct orange 34 (I)



CI Acid red 73 (II)



CI Direct blue 84 (III)



CI Direct red 80 (IV)

All four dyes were recrystallised twice from ethanol - water (1 + 1) after which stock solutions were prepared by dissolving the appropriate amounts in distilled water to give concentrations of approximately 5×10^{-4} M.

Instrumentation

All pH measurements were made using an EIL, Model 23A, pH meter, incorporating a glass indicator electrode, saturated calomel reference electrode and a temperature compensator.

Polarography was carried out with a PAR, Model 174A, polarographic analyser operated in the differential pulse mode, and polarograms were recorded on an Advance HR2000 *X* - *Y* recorder. A three-electrode system was used for polarography and consisted of a saturated calomel reference electrode, a platinum counter electrode and dropping-mercury electrode (D.M.E.).

The dropping-mercury electrode used had the following characteristics: outflow velocity, $m = 1.57$ mg s^{-1}; drop time, $t = 4.3$ s at the potential of the saturated calomel electrode and at a mercury pressure of $h = 68$ cm in 1 M potassium chloride solution. For the analyser the controlled drop time was 0.5 s (sampled d.c. polarography), 1.0 s (DPP) with a modulation amplitude of 50–100 mV, scan speed 5 mV s^{-1} and low-pass filter 0.3 s. Cyclic voltammetry was performed with the aid of a PAR, Model 9323, hanging mercury-drop electrode. Controlled-potential electrolysis was carried out with the PAR 174A polarographic analyser and a cell containing a large mercury pool.

Experimental Techniques

Sampled d.c. polarography was performed on approximately 5×10^{-5} M solutions of the dyes in the pH range 1–13. Blanks were obtained by recording polarograms of the appropriate buffer solutions only, under the same conditions as used for sample solutions. All solutions were de-aerated for 5 min with oxygen-free nitrogen prior to polarography. From the polarographic data obtained by this technique graphs of $i_{lim.}$ *versus* pH and $E_{\frac{1}{2}}$ *versus* pH were constructed.

The nature of the electrode process was determined by recording the d.c. polarogram at varying heights of the mercury column in the appropriate buffer, and graphs of $i_{lim.}$ *versus* $h^{\frac{1}{2}}_{corr.}$ were constructed.

Controlled-potential electrolysis was performed on 5×10^{-4} M solutions of the dyes **I**–**IV** in 0.1 N sulphuric acid at potentials on the plateau of the most negative d.c. polarographic wave. Electrolysis was continued until the current decayed to zero and the electrolysed solution was diluted to give a final concentration of 5×10^{-5} M. This final solution was then submitted to spectral and d.c. polarographic analysis using the conditions given previously.

Differential-pulse polarography was performed on the four azo dyes **I**–**IV** at the same concentration and using the same pH range as for sampled d.c. polarography. Differential-pulse polarograms of buffer solutions only were recorded using the same conditions. From these polarographic data the optimum pH for determination of the dyes was chosen, and this was used in the construction of calibration graphs.

Cyclic voltammetry was performed on some of the dyes at a concentration of 5×10^{-5} M using a scan rate of 50 mV s^{-1}.

Results and Discussion

CI Direct Orange 34 (I)

The variations of $i_{lim.}$ *versus* pH and $E_{\frac{1}{2}}$ *versus* pH show that the dye was reduced in one main wave, i_1, which decreased in height at pH > 5. The height of this wave continued to decrease with increasing pH, then became constant in the pH range 11–13. A small pre-wave, i_2, also appeared on the i *versus* E curves in the pH range 4–13, the height of which remained independent of pH throughout the whole pH range. The $E_{\frac{1}{2}}$ *versus* pH relationship of i_1 in the pH range 1–6 was $E_{\frac{1}{2}} = 0.06 - 0.09$pH. A break occurred on the $E_{\frac{1}{2}}$ *versus* pH plot at pH 6, which is probably a pK_a value associated with the protonation of a nitrogen atom in **I**. Logarithmic analysis [*i.e.*, $E_{D.M.E.}$ *versus* log $i/(i_d-i)$] of the wave i_1 in 0.1 N sulphuric acid yielded an αn_a value of 1.55 and a p value of 2.3 for the rate-determining step evaluated from the equation

$$\frac{dE_{1/2}}{dpH} = \frac{-0.059p}{\alpha n}$$

The variation of limiting current with mercury pressure was determined in 0.1 N sulphuric acid and wave i_1 showed a linear relationship of $i_{lim.}$ *versus* $h^{\frac{1}{2}}_{corr.}$ indicating a diffusion-controlled current. When the drop time decreased from 18.2 to 7.0 s the half-wave potential shifted to more negative potentials by 10 mV, suggesting that the electrode reaction was not totally irreversible.

Electrolysis at a mercury pool in stirred solution was carried out as described earlier. At the end of electrolysis the colour and polarographic wave of the compound had both disappeared, and ultraviolet - visible spectral analysis of the electrolysed solution showed that the band at 420 nm had disappeared and a new band had appeared at 300 nm. This suggests that the dye had been reduced in strong acid (pH 1.0) to give a mixture of amines.

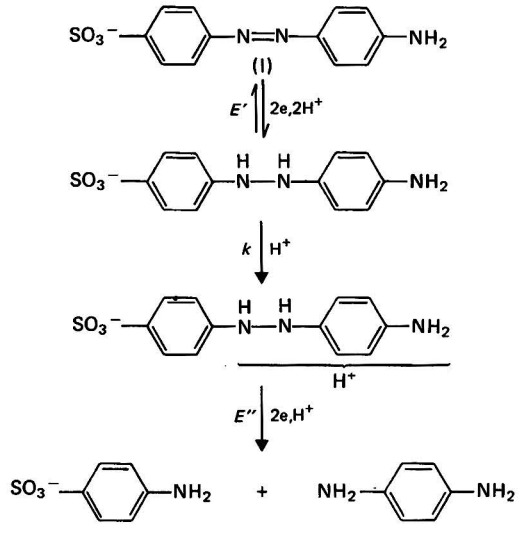
The mechanism of reduction of this dye (**I**) in acidic media (pH 1–6) would appear to involve formation of the hydrazo compound, which can then undergo protonation. This two-electron step (E') is then followed by another one of similar magnitude (E'') to produce a mixture of 4-aminobenzenesulphonic acid and 1,4-diaminobenzene. In alkaline media the reduction reaction is stopped at the hydrazo stage, which causes the wave height to decrease by half. The pre-wave i_2 is believed to be due to product adsorption and it disappeared with the addition of 50% methanol to the buffers at pH > 7.0. The mechanism of reduction can be illustrated as shown on p. 932.

CI Acid Red 73 (II)

The polarographic waves recorded for CI Acid Red 73 (**II**) over the pH range 2–12 show that **II** is reduced in one main wave (i_1) in the pH range 1–4. The $E_{\frac{1}{2}}$ *versus* pH relationship was $E_{\frac{1}{2}} = 0.1 - 0.077$pH. Logarithmic analysis at pH 2 yielded an αn_a value of 2.0 and a p value of 2.73 for the rate-determining step.

The variation of $i_{lim.}$ *versus* $h^{\frac{1}{2}}_{corr.}$ was determined in 0.1 N sulphuric acid and was found to be linear, indicating that the electrode process was diffusion controlled. When the drop time decreased from 16.8 to 6.8 s the $E_{\frac{1}{2}}$ value did not shift significantly, which indicates that the rate-determining step at pH 1.0 is reversible.

Electrolysis was performed at an applied potential of − 0.2 V for 40 min in a supporting electrolyte of 0.1 N sulphuric acid. At the end of the electrolysis period the red colour and polarographic wave (i_1) had disappeared, and no new waves were observed in the available potential region. Ultraviolet - visible spectral analysis of a portion of this electrolysed solution showed that the band at 513 nm had disappeared completely and the other bands at 346, 330(s) and 245 nm had moved towards the blue end of the spectrum.



It is expected that the azo group ($-\overset{\gamma}{N} = \overset{\delta}{N}-$) would be first to reduce in a step E', which can be deduced from the work of Florence and co-workers, who found that 1-phenylazo-2-naphthol was reduced at considerably more negative potentials than azobenzene. This is likely to be a reversible step involving 2e and $2H^+$, followed by protonation of the newly formed hydrazo group and subsequent 4e reduction (E'') of the other azo group ($-\overset{\alpha}{N} = \overset{\beta}{N}-$) to a mixture of amines. The species 4-aminohydrazobenzene can reduce in acidic media to the corresponding amines (E''').

As has been suggested by Florence and co-workers, the rate-determining step is protonation of the hydrazo group, which occurs very rapidly in the pH range 1–4 and thus processes E', E'' and E''' occur in one 8e step (i_1).

In the pH range 6.0–12.0 the main wave i_1 is replaced by two waves i_2 and i_3 (the latter at considerably more negative potentials) in the ratio 1:2 with respect to their limiting currents. In this pH range, protonation of the hydrazo compound is difficult and thus the over-all electrochemical process occurs in two steps, involving 2e and 4e, respectively, as shown in the scheme. Cyclic voltammetry of CI Acid Red 73 at 5×10^{-4} M (pH 7.0) showed that the steps corresponding to i_2 and i_3 are irreversible.

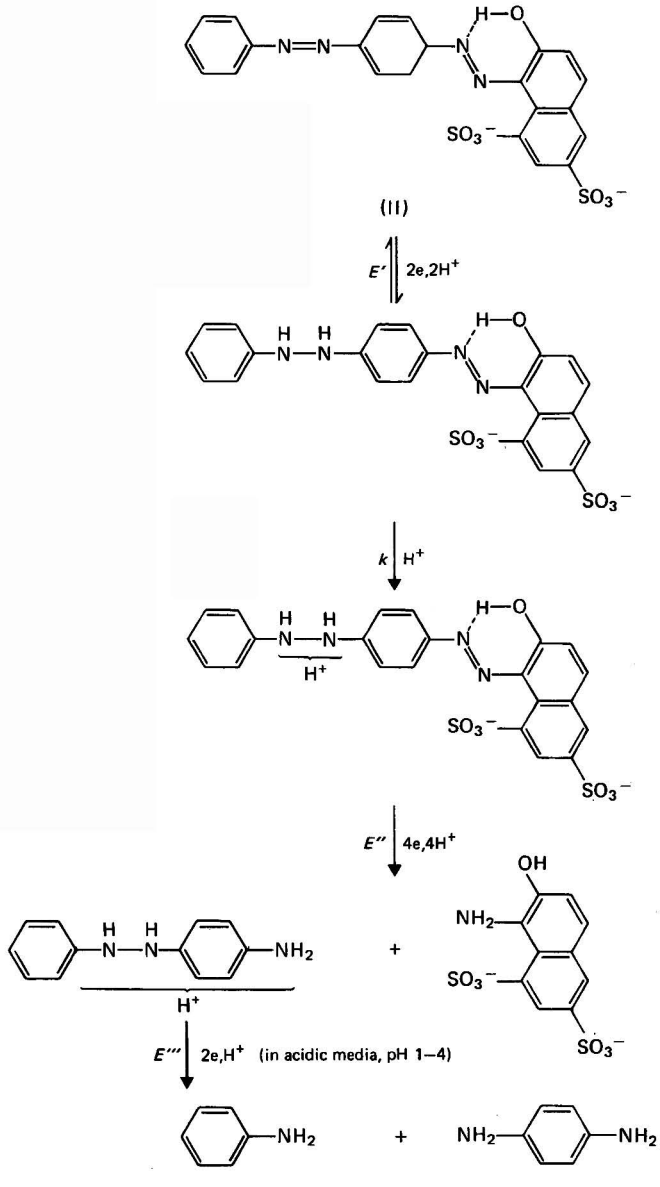
In more alkaline media and coinciding with ionisation of the hydroxy moiety ($pK_a = 11.2$),[19] the polarographic behaviour of II changes again in that three waves occur. This could possibly be explained by disproportionation reactions to products such as quinone hydrazones that are reduced in a different manner from azo-containing compounds.

CI Direct Blue 84 (III)

In buffer solutions of pH < 6 one wave only was observed, which showed a change in $E_{\frac{1}{2}}$ *versus* pH relationship at pH < 2. This probably arises as a consequence of ionisation of a

sulphonic acid group and also affected absorption bands in the ultraviolet spectrum around the same pH value. The $E_{\frac{1}{2}}$ *versus* pH relationship in the pH range 1–2 was $E_{\frac{1}{2}} = -0.43 - 0.04\text{pH}$.

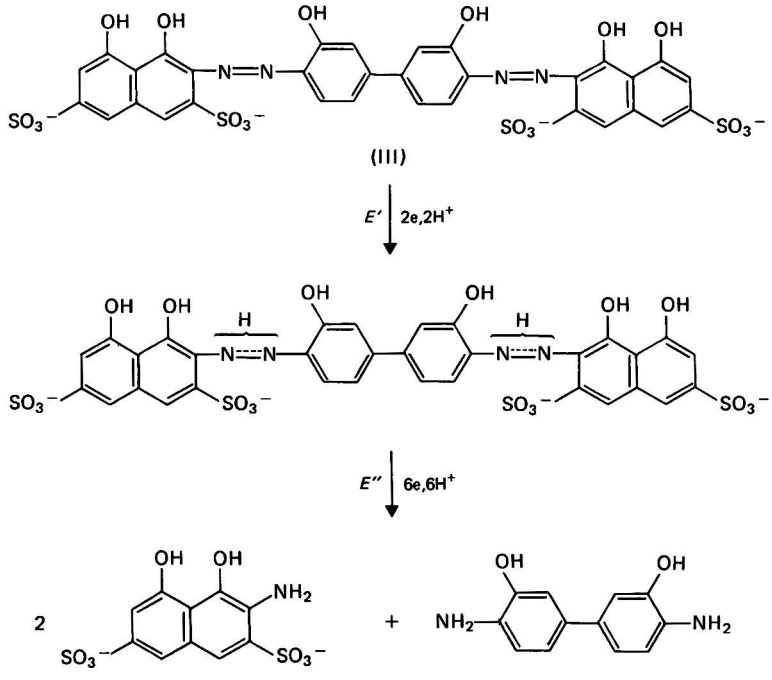
The variation of $i_{\text{lim.}}$ *versus* $h^{\frac{1}{2}}_{\text{corr.}}$ for **III** in 0.1 N sulphuric acid was linear and showed the current to be diffusion controlled. A change in drop time from 16.2 to 7.1 s caused the $E_{\frac{1}{2}}$ to shift to more negative potentials by 45 mV, which indicates that the electrode process is irreversible. Electrolysis was performed at a potential of −0.6 V for 40 min. At the end of the electrolysis time the visible band and ultraviolet bands at 290 and 350 nm had disappeared, and a low-intensity band appeared at 270 nm upon which two shoulders were



observed at 240 and 220 nm. The polarogram obtained after electrolysis at −0.6 V did not show any waves in the potential range 0 to −1.0 V, which again suggests the formation of amine derivatives upon electrolysis in strong acid (pH 1.0).

Logarithmic analysis of the polarographic waves obtained in Britton - Robinson buffers of pH 3.0 and 4.0 yielded αn_a values of 1.3 and 1.6 and p values of 1.5 and 1.9, respectively. This suggests that in acidic media the rate-determining step involves 2e and $2H^+$ and, unlike species I and II, that protonation of the hydrazo intermediate is not rate determining. The $E_{\frac{1}{2}}$ value of III in acidic solution is far more negative than that of either I or II, which illustrates both the effect of hydrogen bonding between the azo group and two *ortho*-hydroxy groups and inductive effects.

The mechanism of reduction of CI Direct Blue 84 in the pH range 1–6 can be represented by the scheme shown below, in which hydrogen bonding has been omitted.



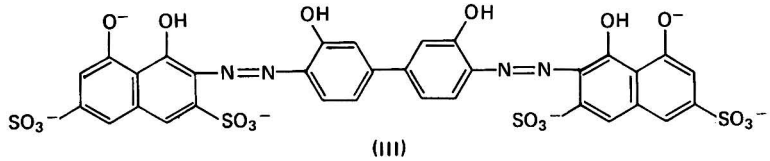
The changes in $E_{\frac{1}{2}}$ *versus* pH and $i_{lim.}$ *versus* pH graphs at pH 6 suggest an acid - base equilibrium with $pK_a = 6.0$. This is in reasonable agreement with the value obtained spectrophotometrically, *i.e.*, $pK_a = 5.2$, which was considered to reflect ionisation of the hydroxy moieties at the naphthalene 8-positions.[19] Between pH 6 and 12 a new wave, i_2, appeared on the current - potential curves, which increased in height with increasing pH while the height of the main wave i_1 decreased. At pH 12 the wave i_2 reached a height equivalent to a four-electron reduction.

One explanation for the appearance of wave i_2 is the reduction of a quinone hydrazone species (V), which is formed after the 8-hydroxynaphthalene group has ionised (*i.e.*, at pH 6.0). As the pH increased the formation of species V is facilitated, until at pH 12 maximum electro-reduction is attained. The equilibrium existing in solution at pH $\approx pK_a$ can be represented as shown on p. 935.

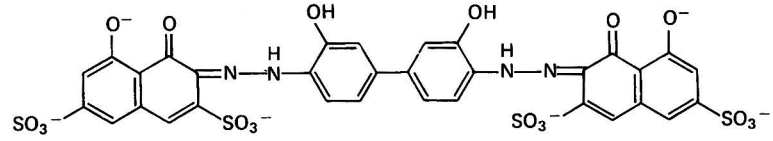
Tautomerism similar to that shown has been reported to exist for other dyes of closely related structure in aqueous solutions.

CI Direct Red 80 (IV)

The variations of $i_{lim.}$ *versus* pH and $E_{\frac{1}{4}}$ *versus* pH for CI Direct Red 80 show two waves, i_1 and i_2, with relative heights of 1:4, observed on the current - potential curves between pH 1 and 4 with $E_{\frac{1}{4}} = +0.1 - 0.066$pH and $E_{\frac{1}{4}} = 0 - 0.066$pH, respectively. The variations of limiting current and half-wave potential with mercury pressure were examined in 0.1 N sulphuric acid. Both i_1 and i_2 showed linear relationships in the graphs of $i_{lim.}$ *versus* $h^{\frac{1}{2}}_{corr.}$, indicating diffusion-controlled electrode reactions. When the drop time decreased from 18.7 to 6.8 s the $E_{\frac{1}{4}}$ value of i_1 and i_2 shifted by 10 mV, which suggests that the electrode processes giving rise to these waves are not totally irreversible.



(III)



(V)

Electrolysis was carried out at a stirred mercury pool held at a potential of -0.2 V for 40 min. At the end of the electrolysis period the red colour of the solution had disappeared and ultraviolet - visible spectral analysis showed that the visible bands at 420, 520 and 545 nm had completely disappeared and a new band of low intensity appeared at 340 nm. All the bands in the ultraviolet region showed blue shifts after electrolysis. Polarographic analysis was carried out on the same electrolysed solution and no waves appeared on the current - potential curve in the potential range $+0.160$ to -1.080 V. This indicates that reduction of **IV** in strong acid (pH 1.0) proceeds with the consumption of 16 electrons, giving the corresponding amines.

Logarithmic analysis of the wave i_1 at pH 2 gave an αn_a value of 2.3 and a p value of 2.7. The large αn_a value was considered to reflect the simultaneous addition of two electrons to each of the azo end-groups in the rate-determining step. However, although the p value apparently indicates the addition of three protons in the reaction, it was believed to involve six protons. This may be explained by considering that the linear portion of the $E_{\frac{1}{4}}$ *versus* pH graph of i_1 represents two identical graphs superimposed on each other. This is not unreasonable when it is noticed that the molecule is symmetrical and that the azo end-groups are in identical environments. Thus the $E_{\frac{1}{4}}$ values associated with the respective reductions of these two moieties might be expected to be so close that they would be indistinguishable. The αn_a and p values obtained for wave i_2 at the same pH were 2.7 and 2.8, respectively, which again suggests that four electrons and six protons are involved in the rate-determining step.

The mechanism of the reduction of CI Direct Red 80 in the pH range 1–4 may be represented as shown in Fig. 1. The wave i_1 arises from process E′ and i_2 from E'', E''' and E'''', which occur simultaneously. In the pH range 6–12 process E'''' no longer occurs because protonation of the hydrazo end-groups is difficult. This is reflected by the decrease in the height of i_2 to a value equivalent to eight electrons while i_1 remains independent of pH through the pH range 1–12. At pH greater than 12 both i_1 and i_2 decrease in height and two new waves, i_3 and i_4, appear on the current - potential curves. This indicates an acid - base equilibrium with pK_a = 12–13 and is associated with ionisation of hydroxy groups.[19]

Cyclic voltammetry was performed on a 5×10^{-5} M solution of the dye in 0.1 N sodium hydroxide solution and on scanning in a negative direction a peak was observed at −0.700 V and another broader peak at −0.860 V. On reverse scanning only one peak was observed, at −0.680 V, which was believed to arise from re-oxidation of the hydrazo derivative (**VI**) to the parent dye.

Variation of i_p with Concentration and Differentiation of I-IV by Differential-pulse Polarography

The optimum pH for analysis of the azo dyes **I–IV** was chosen from a consideration of both the magnitude and shape of the differential-pulse polarographic peaks, recorded at concentrations of approximately 5×10^{-5} M in the pH range 1–13.

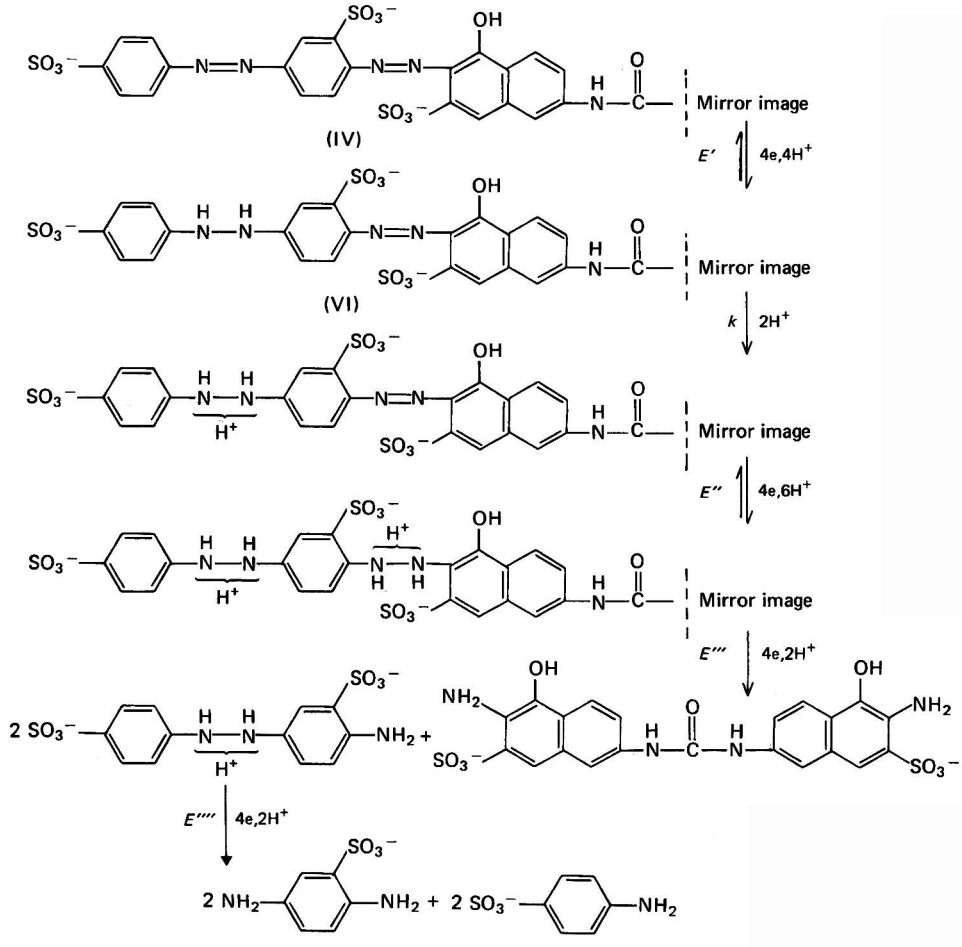


Fig. 1. Reduction scheme for CI Direct Red 80.

All four dyes showed the best defined peaks for quantitative analysis in acidic media, *e.g.*, the variation of log[peak current (μA)] *versus* log[concentration (μmol l^{-1})] for CI Direct Red 80 at pH 1.0 shows linearity of response over the concentration range 5×10^{-7}–4×10^{-5} M. Above 4×10^{-5} M the graph begins to level off, probably as the result of adsorption processes.

Table I summarises the differential-pulse polarographic data for the four dyes obtained at the selected pH values. It can be seen that the dyes showed linearity of response to varying

TABLE I

DIFFERENTIAL-PULSE POLAROGRAPHIC DATA FOR AZO DYES

Compound	Supporting electrolyte	$E_p^{\ominus}$ / V	Response factor/μA μmol^{-1} l	Range of concentration for linear response/M
CI Direct Orange 34 ..	Britton - Robinson buffer, pH 4.0	−0.280 ± 0.005	3.6×10^{-3}	5×10^{-7}–3×10^{-5}
CI Acid Red 73	Britton - Robinson buffer, pH 4.0	−0.220 ± 0.005	1.38×10^{-2}	5×10^{-7}–5×10^{-6}
CI Direct Blue 84 ..	Britton - Robinson buffer, pH 3.0	−0.605 ± 0.005	2.05×10^{-2}	1×10^{-6}–5×10^{-5}
CI Direct Red 80	0.1 N H_2SO_4	−0.010 ± 0.005	1.15×10^{-2}	5×10^{-7}–4×10^{-5}

extents, but all gave a linear response in the lower concentration range. The response factor was determined from the slope of the i_p (μA) *versus* concentration (μmol l^{-1}) graphs, where concentrations in the range 5×10^{-7}–5×10^{-6} M were investigated. Table I shows that all four dyes **I**–**IV** can be determined at the trace level by using differential-pulse polarography.

A further aspect of the differential-pulse polarographic technique is that it offers the possibility of differentiating and identifying the azo dyes in alkaline media. This is illustrated in Fig. 2, which shows the unique profiles obtained at concentrations of approximately 5×10^{-5} M in 0.1 N sodium hydroxide solution.

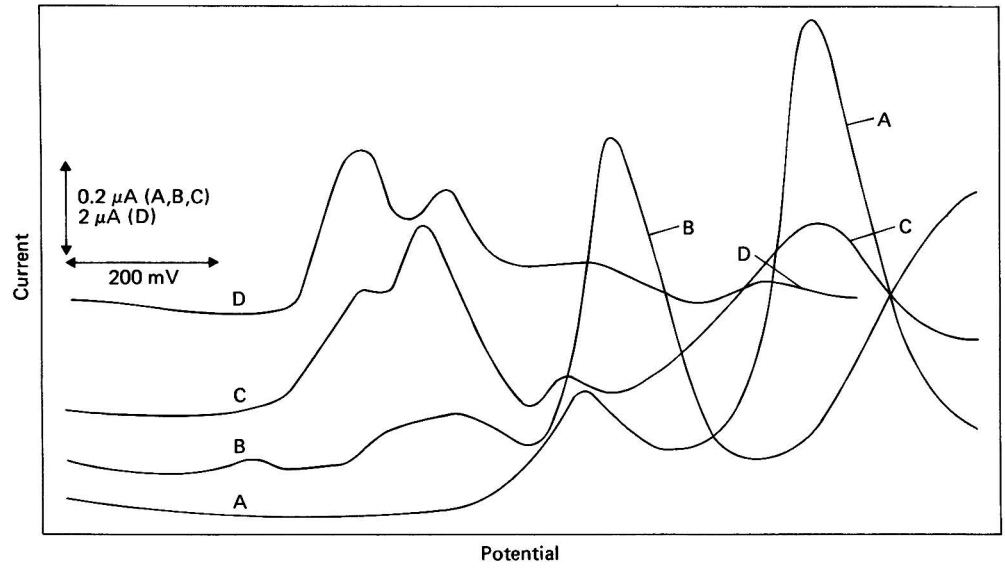


Fig. 2. Differential-pulse polarograms of (A) CI Direct Blue 84, (B) CI Direct Red 80, (C) CI Direct Orange 34 and (D) CI Acid Red 73. Initial potential, −0.2 V. Supporting electrolyte, 0.1 N sodium hydroxide solution.

Conclusion

The polarographic behaviour in acidic solution suggested that all four azo dyes were reduced to the corresponding amines. However, at pH $\geqslant$ 6 end-groups containing substituted azobenzenes in CI Direct Orange 34, CI Acid Red 73 and CI Direct Red 80 were reduced only to the hydrazo derivatives, whereas the remaining azo groups in the red dyes reduced to the amines. The polarographic behaviour of CI Direct Blue 84 in the same pH region was different to that of the other three dyes, which was considered to reflect the presence of tautomerism.

The optimum conditions for quantitative analysis of the four azo dyes were in acidic media, where as alkaline solutions proved more suitable for qualitative purposes.

References

1. Games, L. M., and Hites, R. A., *Anal. Chem.*, 1977, **49**, 1433.
2. Donaldson, E., DIFS, Belfast, personal communication.
3. Howe, J. R., *Lab. Pract.*, 1975, **24**, 457.
4. Miller, J. A., and Miller, E. C., *Adv. Cancer Res.*, 1953, **1**, 339.
5. Cilento, G., Miller, E. C., and Miller, J. A., *J. Am. Chem. Soc.*, 1956, **78**, 1718.
6. Issa, I. M., Issa, R. M., Temerk, Y. M., and Mahmoud, M. R., *Electrochim. Acta*, 1973, **18**, 139.
7. Holleck, L., and Holleck, G., *Naturwissenschaften*, 1964, **51**, 433.
8. Castor, C. R., and Saylor, J. H., *J. Am. Chem. Soc.*, 1953, **75**, 1427.
9. Shams El-din, A. M., *J. Electroanal. Chem. Interfacial Electrochem.*, 1969, **21**, 377.
10. Malik, W. V., and Gupta, P. N., *J. Electroanal. Chem. Interfacial Electrochem.*, 1974, **54**, 417.
11. Jannakoudakis, D., Kokkinidis, G., and Mauridis, P. G., *J. Chim. Phys. Chim. Biol.*, 1976, **73**, 872.
12. Florence, T. M., and Farrar, Y. J., *Aust. J. Chem.*, 1964, **17**, 1085.
13. Florence, T. M., and Aylward, G. H., *Aust. J. Chem.*, 1962, **15**, 65.
14. Florence, T. M., and Aylward, G. H., *Aust. J. Chem.*, 1962, **15**, 416.
15. Florence, T. M., *Aust. J. Chem.*, 1965, **18**, 609.
16. Florence, T. M., and Belew, W. L., *J. Electroanal. Chem.*, 1969, **21**, 157.
17. Florence, T. M., Johnson, D. A., and Batley, G. E., *J. Electroanal. Chem.*, 1974, **50**, 113.
18. Florence, T. M., *J. Electroanal. Chem. Interfacial Electrochem.*, 1974, **52**, 115.
19. Hart, J. P., and Franklin Smyth, W., *Spectrochim. Acta*, 1980, **36A**, 279.

Received *May 2nd*, 1979
Accepted *June 2nd*, 1980

Analyst, October, 1980, Vol. 105, pp. 939–943

A New Approach to the Quantitative Analysis of Overlapping Anodic-stripping Voltammograms

Juei H. Liu

Department of Criminal Justice, University of Illinois at Chicago Circle, Chicago, Ill. 60680, USA

A method, analogous to that used for studying overlapping bands in absorption spectroscopy, has been developed for the quantitative analysis of overlapping anodic-stripping voltammograms. Six simultaneous equations were formulated to resolve general overlapping voltammograms. When the second species is not oxidised at the peak potential of the first species these six equations are reduced to four. Provided that the oxidation currents are additive, this approach is not limited by the degree of overlap. The method has been shown to be satisfactory by studying the cadmium - lead pair as an example.

Keywords: *Anodic-stripping voltammetry*; *overlapping voltammograms*; *quantitative analysis*

Anodic-stripping analysis of mixtures often involves dealing with overlapping voltammograms. For quantitative determination of individual species the contribution of each species to the overlapped signal has to be resolved. The standard procedure[1–3] involves extrapolation of the preceding peak and determination of the peak height from this extrapolated base line. Sophisticated measurements[4] have also been made using an on-line computer. An empirical equation was first developed that described the general voltammograms for a wide variety of electroactive species, then this function was fitted to a number of standard voltammograms and the constants in the function, specifically determined for each species, were stored in a library. When analysing an unknown mixture these constants were used to regenerate the standard graph, a composite of which was then fitted to the unknown signal.

In this work an alternative approach, analogous to those used in handling overlapping bands in absorption spectroscopy, was developed for solving the problem of overlapping voltammograms. Resolution of overlapping voltammograms is achieved by solving four or six simultaneous equations, depending upon the severity of the overlap. This approach eliminates the uncertainty of the traditional extrapolation procedure[1–3] which cannot be used for severely overlapped voltammograms. In contrast to the numerical deconvolution method,[4] it does not require any additional instrumentation.

Essentially this approach involves the measurement of two current values at the peak potentials of the two species involved and the solution of equations derived from the following assumptions: (*i*) the current values measured are the sum of the oxidation currents of each species; (*ii*) the ratio of the currents contributed by the species in a peak potential is determined by their concentration ratio and their specific current height ratio; and (*iii*) peak current is proportional to concentration with a proportionality constant that can be obtained by plotting the current height *versus* concentration results obtained for each individual species in isolation. Information required for the interpretation of overlapping voltammograms can therefore readily be derived from the voltammograms of individual species in isolation. Two individual voltammograms are therefore obtained using known concentrations of the species under study. The information obtained is then used to solve the simultaneous equations derived, to obtain the concentration of each species in the mixture.

Experimental

Apparatus

All experiments were performed on a polarograph constructed in our laboratory (Fig. 1). A saturated calomel electrode (S.C.E.), a platinum electrode and a hanging mercury drop electrode were used as the reference, counter and working electrodes, respectively. A two-channel Hewlett Packard, Model 7100-15, recorder was used for recording the voltammograms and the anodic-voltage scans.

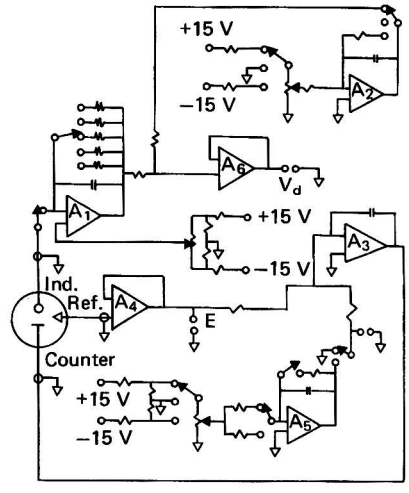


Fig. 1. Simplified schematic diagram of the polarograph.

Solution Preparation

A 1 M buffer solution of pH 5.9 ± 0.1 was prepared by dissolving 2.4 ml of 99.7% acetic acid and 129.2 g of sodium acetate trihydrate in de-ionised distilled water and diluting to 1 l. A 0.1 M acetate buffer, prepared from the 1 M stock solution, was used as the supporting electrolyte. The buffer solution was passed through a Chelex 100 (Bio-Rad Laboratories, Richmond, Calif.) column to remove trace amounts of copper.

Stock solutions (10^{-3} M) of cadmium and lead were prepared by dissolving appropriate amounts of analytical-reagent grade cadmium nitrate and lead nitrate in the 0.1 M acetate buffer.

Procedure

In a typical experiment the following conditions were used: (*i*) a mercury drop with radius and surface area of 0.0490 cm and 0.0302 cm^2, respectively; (*ii*) a 5-min de-aereation of 50 ml of 0.1 M acetate buffer solution in the electrolysis cell, before the addition of the solution containing the species to be determined; (*iii*) pre-electrolysis at −0.85 V (*versus* S.C.E.) for 3 min with stirring and then 1 min without stirring; and (*iv*) an anodic scan rate of 1.22 V min^{-1}. Concentrations of working solutions were changed as necessary by the addition of 2.5×10^{-4} M standard solutions.

Results and Discussions

Two types of overlapped anodic-stripping voltammograms are commonly observed. In most instances the second species is not oxidised at the peak potential of the first species (Type I). The overlapping therefore involves only the addition of the tailing oxidation current of the first species to the oxidation current of the second species. Severely overlapped voltammograms (Type II) also include the oxidation current of the second species at the peak potential of the first species.

The cadmium - lead voltammogram (Fig. 2 B) obtained under the experimental conditions described represents Type I overlapping. Type II overlapping would be shown on the same voltammogram by imagining that the peak potential of the cadmium voltammogram was −0.42 V (*versus* S.C.E.). Voltammograms preceding this potential are ignored. Current measured at this potential is then considered to be the peak current of the hypothetical cadmium species.

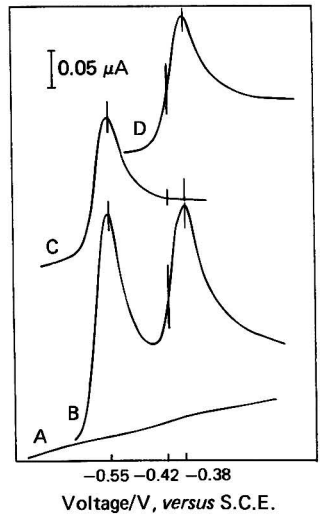


Fig. 2. Voltammograms of A, 0.1 M acetate buffer (pH = 5.9); B, 12.38 × 10^{-7} M Cd and Pb mixture; C, 7.48 × 10^{-7} M Cd; and D, 9.96 × 10^{-7} M Pb.

In the simultaneous determinations of several species by electrochemical methods, it is commonly assumed that the electrochemical behaviour of a species is not affected by the presence of the other species in the mixture by, for example, the formation of an inter-metallic compound. With this assumption six equations can be presented for the examples used in this work:

$$H_{\text{sum,Cd}} = H_{\text{Cd,Cd}} + H_{\text{Pb,Cd}} \quad \text{(1a)}$$

$$H_{\text{sum,Pb}} = H_{\text{Cd,Pb}} + H_{\text{Pb,Pb}} \quad \text{(1b)}$$

$$H_{\text{Cd,Cd}} = B_{\text{Cd}}C_{\text{Cd}} \quad \text{(2a)}$$

$$H_{\text{Pb,Pb}} = B_{\text{Pb}}C_{\text{Pb}} \quad \text{(2b)}$$

$$\frac{H_{\text{Cd,Cd}}}{H_{\text{Pb,Cd}}} = \frac{h_{\text{Cd,Cd}}}{h_{\text{Pb,Cd}}} \times \frac{C_{\text{Cd}}}{C_{\text{Pb}}} \quad \text{(3a)}$$

$$\frac{H_{\text{Cd,Pb}}}{H_{\text{Pb,Pb}}} = \frac{h_{\text{Cd,Pb}}}{h_{\text{Pb,Pb}}} \times \frac{C_{\text{Cd}}}{C_{\text{Pb}}} \quad \text{(3b)}$$

where C_w is the concentration of species w in the mixture, B_x is the slope of the peak current *versus* concentration graph for species x, $H_{y,z}$ is the current of species y measured at the peak potential of species z and $h_{y,z}$ is the same as $H_{y,z}$ but measured with the species in isolation. The values of $h_{y,z}$ in Equations (3a) and (3b) are obtained at the same concentrations and represent the currents produced by unit concentration of species y.

For the best statistical results[5] the $h_{y,z}$ ratios are replaced by the corresponding slope ratios from the current *versus* concentration graphs.

Equations (3a) and (3b) can therefore be rewritten as

$$\frac{H_{\text{Cd,Cd}}}{H_{\text{Pb,Cd}}} = \frac{b_{\text{Cd,Cd}}}{b_{\text{Pb,Cd}}} \times \frac{C_{\text{Cd}}}{C_{\text{Pb}}} \quad \text{(4a)}$$

$$\frac{H_{\text{Cd,Pb}}}{H_{\text{Pb,Pb}}} = \frac{b_{\text{Cd,Pb}}}{b_{\text{Pb,Pb}}} \quad \text{(4b)}$$

where $b_{y,z}$ is the slope of the current *versus* concentration graph of species y measured at the peak potential of species z measured with species y in isolation.

It should be noted that all currents are measured from the base line rather than from the extrapolated voltammogram of any species.

The cadmium - lead pair is an example of Type I overlapping. As lead is not oxidised at the cadmium peak potential, $H_{Pb,Cd} = b_{Pb,Cd} = 0$ (entries II B and II C in Table I) and equations (1a) and (4a) may be eliminated, leaving four simultaneous equations. Before the quantitative analysis of the overlapped unknown mixture data (entries III A and III F in Table I), B_{Cd} (= $b_{Cd,Cd}$), B_{Pb} (= $b_{Pb,Pb}$) and $b_{Cd,Pb}$ are derived from the current *versus* concentration graph for the species in isolation. Under the experimental conditions used, these values are 0.248, 0.174 and 0.0607, respectively (entries I C, II G and I G in Table I). Equations (1b), (2a), (2b) and (4b) are solved. The calculated values of C_{Cd} and C_{Pb} are 12.18×10^{-7} and 12.10×10^{-7} M, respectively, which are in excellent agreement with the expected value of 12.38×10^{-7} M.

TABLE I

ANODIC-STRIPPING VOLTAMMETRY DATA FOR CADMIUM, LEAD AND A CADMIUM - LEAD MIXTURE IN 0.1 M ACETATE BUFFER (pH = 5.9)*

		Column							
		A	B	C	D	E	F	G	
			At Cd peak potential†		At hypothetical Cd peak potential‡		At Pb peak potential§		
Row	Species	Concentration $\times 10^{-7}$ M	I¶	Slope of current *versus* concentration graph	I¶	Slope of current *versus* concentration graph	I¶	Slope of current *versus* concentration graph	
I	Cd	0.00	0.00	$B_{Cd} = b_{Cd,Cd}$	0.00	$B_{Cd} = b_{Cd,Cd}$	0.00	$b_{Cd,Pb} = 0.0607$	
		2.50	0.595	$= 0.248$	0.165	$= 0.0815$	0.165		
		4.99	1.18		0.335		0.290		
		7.48	1.80		0.603		0.395		
		9.96	2.44		0.802		0.630		
		12.4	3.08		0.980		0.755		
II	Pb	0.00	0.00	$B_{Pb,Cd} = b_{Pb,Cd}$	0.00	$B_{Pb,Cd} = b_{Pb,Cd}$	0.00	$B_{Pb} = b_{Pb,Pb} = 0.174$	
		2.50	0.00	$= 0.00$	0.150	$= 0.0532$	0.344		
		4.99	0.00		0.262		0.770		
		7.48	0.00		0.374		1.24		
		9.96	0.00		0.531		1.67		
		12.4	0.00		0.673		2.13		
III	Cd - Pb mixture each	12.38	$H_{sum,Cd}$		$H_{sum,Cd}$		$H_{sum,Pb}$		
			3.01		1.62		2.87		
			2.99		1.64		2.88		

* Symbols used in the table are defined in the test.
† −0.55 V (*versus* S.C.E.).
‡ −0.42 V (*versus* S.C.E.).
§ −0.38 V (*versus* S.C.E.).
¶ In units of 0.1 μA.

Assuming that currents measured at −0.42 V (*versus* S.C.E.) (Fig. 2) are peak currents of cadmium and, ignoring voltammograms preceding this potential, a Type II overlap is produced. The values of B_{Cd} (= $b_{Cd,Cd}$) and $B_{Pb,Cd}$ (= $b_{Pb,Cd}$) are now 0.0815 and 0.0532, respectively (entries I E and II E, respectively, in Table I). C_{Cd} and C_{Pb} are then calculated from equations (1a), (1b), (2a), (2b), (4a) and (4b); the values obtained are 12.27×10^{-7} and 12.11×10^{-7} M, respectively. These results are in excellent agreement with those expected. Compared with the peak lead current the hypothetical cadmium peak currents measured at −0.42 V (*versus* S.C.E.) (entry I D in Table I) are considerably lower. This indicates that this approach can be used even when the preceding species is in much lower concentration.

The consistently low concentrations calculated for both cadmium and lead with both types of overlap are probably caused by the reduction in the mercury surface available to each species in the mixture. This error should be reduced by working at lower concentration levels.

If the solution in the electrolysis cell is changed reproducible results may not be obtained. The approach described here requires three separate solutions, one for the unknown mixture, one for cadmium and one for lead. A constant concentration of bismuth was added to each solution before the solution was changed. The peak current of the constant concentration of bismuth in each solution was used to check the reproducibility and to make corrections if necessary.

A definite advantage of this approach compared with the traditional extrapolation method, is that all currents are measured from the supporting electrolyte solution base line. These measurements should eliminate the uncertainty caused by extrapolation. This procedure allows severely overlapped voltammograms to be resolved.

References

1. Delahay, P., "New Instrumental Methods in Electrochemistry," Interscience, New York, 1954, p. 129.
2. Ben-Bassar, A. M. I., Blinderman, J.-M., Salomon, A., and Wakshal, E., *Anal. Chem.*, 1975, **50**, 534.
3. Vydra, F., Stulik, K., and Julakora, E., "Electrochemical Stripping Analysis," Halsted Press, New York, 1976.
4. Gutknecht, W. F., and Perone, S. P., *Anal. Chem.*, 1970, **42**, 906.
5. Bauer, E. L., "A Statistical Manual for Chemists," Second Edition, Academic Press, New York, 1971, p. 140.

Received *March* 13*th*, 1980
Accepted *May* 15*th*, 1980

Analyst, October, 1980, Vol. 105, pp. 944–949

A New Type of Biological Reference Material for Multi-element Analysis—the Fungus *Penicillium ochro-chloron* ATCC 36741

Miwako Suzuki, Yukiko Dokiya,* Sunao Yamazaki and Shozo Toda

Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Bunkyo-ku, Tokyo, Japan 113

Several series of standard disk samples were prepared using dried mycelia of *Penicillium ochro-chloron*, a fungus extremely tolerant to heavy metals. Because of the ready availability of the homogeneous dry material with arbitrary metal concentrations, the fungus is very suitable for calibration in metal analysis by X-ray fluorescence spectrometry, especially for biological samples.

Based on the calibration graph for the reference material, the metal contents of edible wild plants were determined. The reliability of the data was checked by using biological reference materials and by atomic-absorption spectrometry.

Keywords: *Biological reference material*; Penicillium ochro-chloron *fungus*; *heavy metals*; *biological materials*; *X-ray fluorescence spectrometry*

Many standard reference materials or certified reference materials have been reported for use in calibration and checking the reliability of biological samples.[1–5] Kale powder[1], NBS orchard leaves and bovine liver[5] are the best known and widely utilised examples.

As the requirements for reference materials are still increasing, the authors have participated in studies to prepare new biological reference materials in Japan, such as tea leaves,[6] shark paste[7] and shark powder.[8]

In this paper, the preparation of a fungal-pellet reference material is described and proposed for use as a calibration material in the X-ray fluorescence determination of metals in biological samples. As a non-destructive multi-element method of determination, X-ray fluorescence spectrometry is widely applied to biological samples, but interference by matrix elements may limit the accuracy obtainable. Hence there is a need for reliable calibration utilising the reference material.

For the analysis of alloy and ore samples by X-ray fluorescence spectrometry, NBS SRM 625–629 zinc-base alloys, SRM 461–464 low-alloy steels, SRM 1636–1638 lead in fuel, SRM 330–333 copper in ores and so on are well established. However, no trials have been carried out with biological samples, partly because of the difficulty in obtaining appropriate concentration ranges using biological reference materials.

Penicillium ochro-chloron ATCC 36741 is a fungus tolerant to heavy metals that has been studied for several years in the authors' laboratory. This fungus can grow well even in media containing 10^5 μg ml^{-1} (saturated solution) of copper, zinc and manganese.[9] It also has a high tolerance against lead, cadmium and iron (2×10^5 μg ml^{-1}).[10] The fungus has the important specific characteristic that the cellular contents of metals can be controlled arbitrarily over a wide range for the metals added to the media. The ranges of cellular metal contents are much wider than for other biological samples.

Investigations were carried out with this fungus in order to prepare a suitable calibration material for X-ray fluorescence spectrometry. Incubation conditions of the fungus were studied, and determination of metals was performed by X-ray fluorescence spectrometry and atomic-absorption spectrometry.

Experimental

Preparation of *Penicillium ochro-chloron* Reference Material

After pre-incubation for 7 d on Czapek's agar slant, the spores of *P. ochro-chloron* were inoculated into 150 ml of sterilised culture media containing various concentrations of metals. The compositions of the basal medium and the metals and concentrations

* Present address: Geochemical Laboratory, Meteorological Research Institute, Nagamine, Yatabe, Tsukuba-gun, Ibaragi, Japan 305.

added are shown in Tables I and II. After incubation for 96 h on a rotary shaker at 30 °C, the fungal mycelia were harvested, lyophilised and dried at 105 °C to constant mass. The dried mycelia were pulverised and homogenised in an agate mortar.

TABLE I

COMPOSITION OF BASAL MEDIUM

Component	Content/g l^{-1}	Component	Content (as metal)/mg l^{-1}
Glucose	40	$FeSO_4$	5
$(NH_4)_2SO_4$	3.3	$ZnSO_4$	5
KH_2PO_4	2.5	$MnSO_4$	1
$MgSO_4.7H_2O$	1	Na_2MoO_4	0.5
$Ca(NO_3)_2.4H_2O$	0.5	$CuSO_4$	0.1
		$CoSO_4$	0.1
		$NaVO_3$	0.01

Determination of Metals

X-ray fluorescence spectrometry

To make standard disk samples for X-ray fluorescence calibration, 100 mg of the dried fungal powder were weighed, mixed with 400 mg of "binder" [made with poly(vinyl alcohol); Somar Co.] and pressed into disks (1500 kg cm^{-2} for 10 min) using an oil hydraulic press (Lumis Products Co.).

Copper, zinc and manganese were determined by X-ray fluorescence spectrometry using an Ortec TEFA 6110 energy-dispersive X-ray fluorescence spectrometer. The intensity of the K_α line of each metal was determined using a molybdenum target and molybdenum filter (40 kV, 50 A). A silicon (lithium) counter was used as the detector, requiring 200–1000 s per sample.

TABLE II

COMBINATIONS AND CONCENTRATIONS OF METALS ADDED TO THE BASAL MEDIA

First metal	Concentration/μg ml^{-1}	Second metal	Concentration/μg ml^{-1}
Cu	0.1 (control)	Ni	100
	10		200
	100	Co	100
	500		500
	1000		1000
	10000		2000
	50000	Cd	1000
	100000		5000
			10000
		Zn	1000
			10000
		Mn	1000
			10000
Zn	5 (control)	Ni	100
	100		200
	1000	Co	100
	10000		1000
	50000		2000
	100000	Cd	1000
			5000
			10000

Atomic-absorption spectrometry

A 500-mg amount of dried powder sample was transferred into Kjeldahl flasks and digested with a mixture of concentrated nitric and sulphuric acids. The digest was diluted to 50 ml with de-ionised water and used for determinations.

Copper, zinc and manganese were determined by atomic-absorption spectrometry using a Seiko SAS 721 atomic-absorption spectrophotometer. The analytical lines for copper, zinc and manganese were at 324.8, 213.8 and 279.5 nm, respectively, with a spectral band width of 1.0 nm. An air - acetylene flame was used under regular burning conditions.

Results and Discussion

Metal Characteristics in *Penicillium ochro-chloron*

The copper, zinc, manganese, cobalt, nickel, iron, potassium, calcium and magnesium contents in *P. ochro-chloron* are compared with those in NBS Biological Standard Reference Materials in Table III. The metal contents of the fungus were determined by atomic-absorption spectrometry. The most typical characteristic of this fungus is the high and wide concentrations of metals that can be controlled by appropriate addition of metals to the basal media. The ranges of the metal contents of the fungus are generally consistent for plants species.

TABLE III

ELEMENTAL COMPOSITIONS OF NBS BIOLOGICAL STANDARD REFERENCE MATERIALS AND *PENICILLIUM OCHRO-CHLORON*

	Metal content/μg g^{-1} (dry mass)								
Material	Cu	Zn	Mn	Co	Ni	Fe	K	Ca	Mg
Spinach	12 ± 2	50 ± 2	165 ± 6	(1.5)	(6)	550 ± 20	35 600 ± 300	13 500 ± 300	—
Orchard leaves ..	12 ± 1	25 ± 3	91 ± 4	(0.2)	1.3 ± 0.2	300 ± 20	14 700 ± 300	20 900 ± 300	6200 ± 200
Tomato leaves ..	11 ± 1	62 ± 6	238 ± 7	(0.6)	(0.6)	690 ± 25	44 600 ± 300	30 000 ± 300	(7000)
Pine needles ..	3.0 ± 0.3	61–74	675 ± 15	(0.1)	(3.5)	200 ± 10	3 700 ± 200	4 100 ± 200	—
Oyster tissue ..	63.0 ± 3.5	852 ± 14	17.5 ± 1.2	(0.4)	1.03 ± 0.19	195 ± 34	9 690 ± 50	1 500 ± 200	1280 ± 90
Bovine liver ..	193 ± 10	130 ± 10	10.3 ± 1.0	(0.18)	—	270 ± 20	9 700 ± 60	(123)	(605)
Ranges	3.0–193	25–852	10–675	0.1–1.5	0.2–6	195–690	3 700–44 600	123–30 000	605–7 000
P. ochro-chloron..	5.0–4 700	5.0–5 500	0–2 000	0–940	10–490	0–1300	140–40 000	0–900	10–5 000

The metal contents of ten samples of *P. ochro-chloron* (each 500 mg of powder of dried mycelia) selected at random are given in Table IV. The homogeneities with respect to copper, zinc and manganese were found to be within 6.5% (relative standard deviation).

Dried mycelia were preserved in a silica gel desiccator for 14 months. No apparent change in the colour or the state was observed. The change in mass was also negligible.

TABLE IV

ELEMENTAL COMPOSITION OF *PENICILLIUM OCHRO-CHLORON*

	Metal content/μg g^{-1} (dry mass)		
Bottle	Cu	Zn	Mn
a	1800	115	1500
b	1850	112	1500
c	1800	120	1500
d	1800	100	1500
e	1800	105	1700
f	1800	115	1500
g	1800	110	1500
h	1800	112	1500
i	1800	112	1700
j	1900	108	1400
Average	1820	110	1530
Relative standard deviation, %	1.8	4.7	6.2

Preparation of Calibration Samples for X-ray Fluorescence Spectrometry

In order to establish the appropriate mixing ratio of dried mycelia and binder [poly(vinyl alcohol) powder], X-ray fluorescence intensities were measured for amounts of mycelia from 100 to 400 mg *versus* 400 mg of binder. The results are summarised in Fig. 1. As linearity between the mass of mycelia and the X-ray fluorescence intensities was obtained, 100 mg of dried mycelia in 400 mg binder were adopted in further work.

The preservation of such "disk" samples was also tested. No visual changes and no changes in composition and metal contents occurred after 14 months in a desiccator. The mass of the disks increased slightly, but this might be improved by changing the binder material. In addition, samples in a disk shape are suitable for storage and repeated utilisation.

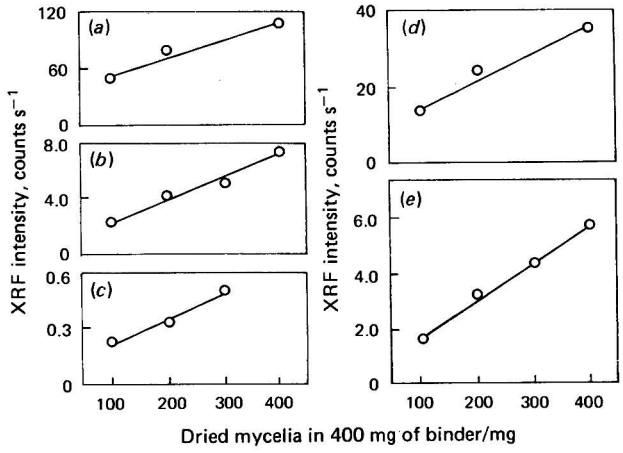


Fig. 1. Relationship between sample mass in disk binder and X-ray fluorescence intensities. Concentration of copper in culture media: (*a*) 4500 μg ml^{-1}; (*b*) 225 μg ml^{-1}; and (*c*) 5 μg ml^{-1}. Concentration of zinc in culture media: (*d*) 900 μg ml^{-1}; and (e) 115 μg ml^{-1}.

The relationships between the X-ray fluorescence intensities and the metal concentrations determined by atomic-absorption spectrometry were linear, as shown in Fig. 2. The correlation coefficients were 0.973, 0.987 and 0.989, respectively. Hence the calibration for copper, zinc and manganese in biological samples is reliable.

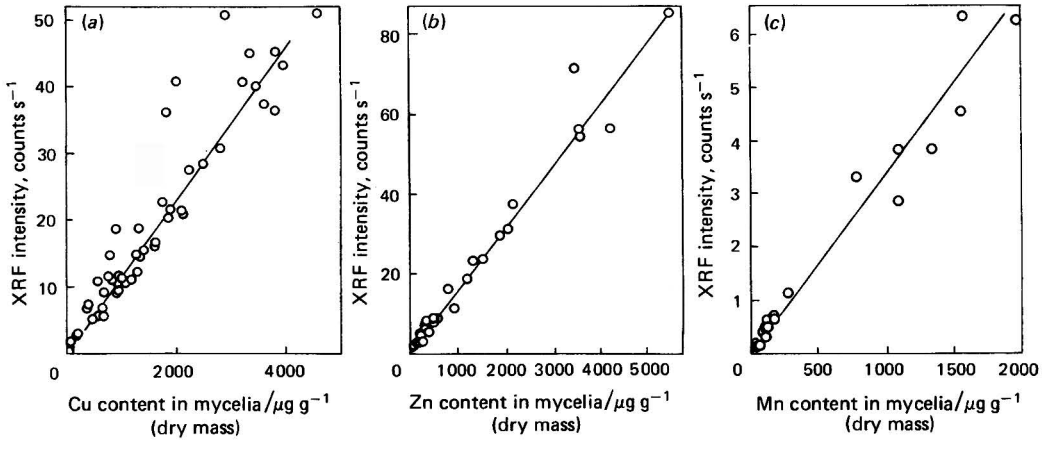


Fig. 2. Relationship between X-ray fluorescence intensities and metal contents determined by atomic-absorption spectrometry: (*a*) copper; (*b*) zinc; and (*c*) manganese.

Application of *Penicillium ochro-chloron* Calibration Graph to Other Biological Samples

Some NBS Standard Reference Materials and other biological materials whose metal contents are known were used to check the method. Table V shows a comparison between values obtained for most plant and animal samples, except for copper and zinc in orchard leaves, copper in pine needles and manganese in oyster tissue. It can be concluded that calibration for other biological materials is also reliable, with few exceptions.

TABLE V

RESULTS OF APPLICATION OF THE PROPOSED METHOD

		Metal content/μg g⁻¹ (dry mass)								
		Cu			Zn			Mn		
		Values determined by XRF*	Certified or reference values		Values determined by XRF*	Certified or reference values		Values determined by XRF*	Certified or reference values	
Type	Material		Source†	Value		Source†	Value		Source†	Value
Plants ..	Orchard leaves ..	30	A	12 ± 1	45	A	25 ± 3	98	A	165 ± 6
	Pine needles ..	53	A	3.0 ± 0.3	67	B	61–74	727	A	675 ± 15
	Tea leaves B ..	15	C	12–15	56	C	56–66	532	C	530–620
	Tea leaves D ..	18	C	27–31	56	C	65–95	760	C	890–1010
	Alfalfa	6	D, E	9	23	D, E	27	69	D, E	48
	Timothy	20	D, E	13	39	D, E	50	21	D, E	25
Animals ..	Bovine liver ..	167	A	193 ± 10	112	A	130 ± 10	19	A	10.3 ± 1.0
	Oyster tissue ..	61	A	63.0 ± 3.5	750	A	852 ± 14	3	A	17.5 ± 1.2
	Shark powder 1 ..	11	F	1.4	12	F	10–22	19	F	< 1
	Shark powder 2 ..	10	F	1.1	14	F	14	—	F	—

* X-ray fluorescence spectrometry.
† A, Certified values for NBS Biological Standard Reference Materials[5]; B, H. L. Rook personal communication; C, certified values for Japanese tea leaves[6]; D, samples provided by Dr. Jones, Jr., of the Ohio Agricultural Research and Development Centre, Wooster, Ohio; E, values determined by atomic-absorption spectrometry; F, T. Uchida, personal communication.

Next, the metal contents of wild plants were determined by using the proposed method. Some edible wild plants that have traditionally been used in daily dishes in Japan have recently aroused interest from the point of view of sources of trace metals for human nutrition. Fifty kinds of edible wild plants were collected in Fukushima Prefecture, dried at 105 °C to constant mass and homogenised in an agate mortar. A 100-mg amount of each was mixed with 400 mg of binder and pressed in the same way as for *P. ochro-chloron*. Their metal contents were then measured by X-ray fluorescence and atomic-absorption spectrometry. Some results obtained by X-ray fluorescence analysis utilising the calibration graphs for *P. ochro-chloron* reference materials are shown in Table VI, and the values are compared with those obtained by atomic-absorption spectrometry.

TABLE VI

DETERMINATION OF METALS IN EDIBLE WILD PLANTS

		Metal content/μg g⁻¹ (dry mass)					
		Cu		Zn		Mn	
Botanical name	Japanese name	XRF*	AAS†	XRF*	AAS†	XRF*	AAS†
Sambucus sieboldiana (bud)	Niwatoko-no-me	43	38	59	67	184	190
Houttuynia cordata	Dokudami	14	11	81	67	151	140
Petasites japonicus (bud)	Fuki-no-me	22	11	22	25	112	110
Laportea macrostachya (leaf)	Miyama-irakusa-no-ha	20	14	88	85	103	70
Vicia unijuga	Nanten-hagi	25	13	49	53	83	85
Plantago asiatica	Ohbako	19	11	31	33	112	110
Acarthopanax spinosum (bud) ..	Tara-no-me	20	14	74	75	268	240
Matteuccia struthiopteris	Kogomi	35	35	63	90	41	40
Elatostema umbellatum	Uwabami-so	11	10	24	29	89	82
Cacalia farfaraefolia	Tamabuki	15	19	65	65	78	100

* X-ray fluorescence spectrometry.
† Atomic-absorption spectrometry.

From these results, it is concluded that the dried mycelia of *P. ochro-chloron* serve as a good calibration material for the determination of metals in biological samples by X-ray fluorescence spectrometry.

The next step in this study might be to find better binder ingredients and to determine the optimum diameter and thickness of the disks. Further studies on other metals such as cobalt and nickel are under investigation.

The authors thank Dr. Yoko Saito of Koriyama Women's College for her help in collecting the Japanese wild plants, and Mr. Toshihiro Aota and Mr. Nobuo Matsumori of Daini Seiko-sha for their assistance in measuring the elements in fungal mycelia and wild plants by X-ray fluorescence spectrometry.

References

1. Bowen, H. J. M., *Analyst*, 1967, **92**, 124.
2. Jones, J. B., Jr., "Abstracts of the Pittsburg Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburg, Pa., 1967," paper no. 200.
3. Yamagata, N., *Bunseki Kagaku*, 1971, **20**, 515.
4. Fukai, R., "Intercalibration of Analytical Methods on Marine Environmental Samples," Progress Report No. 13, International Laboratory of Marine Radioactivity, Monaco, 1976.
5. "Catalog of NBS Standard Reference Materials," NBS Special Publication 260, 1978–79 Edition, US Department of Commerce, Washington, D.C., 1978.
6. Fuwa, K., Notsu, K., Tsunoda, K., Kato, H., Yamamoto, Y., Okamoto, K., Dokiya, Y., and Toda, S., *Bull. Chem. Soc. Jpn.*, 1978, **51**, 1078.
7. Dokiya, Y., Taguchi, M., Toda, S., and Fuaw, K., *Anal. Chem.*, 1978, **50**, 533.
8. Dokiya, Y., Kurosawa, S., Toda, S., and Fuwa, K., *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3649.
9. Okamoto, K., and Fuwa, K., *Agric. Biol. Chem.*, 1974, **38**, 1405.
10. Okamoto, K., Suzuki, M., Fukami, M., Toda, S., and Fuwa, K., *Agric. Biol. Chem.*, 1977, **41**, 17.

Received *March 20th*, 1980
Accepted *April 24th*, 1980

Spectrophotometric Determination of Hydrogen Peroxide Using Potassium Titanium(IV) Oxalate

Robin M. Sellers

Central Electricity Generating Board, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire, GL13 9PB

A simple and rapid method for the spectrophotometric determination of hydrogen peroxide using potassium titanium(IV) oxalate is described. The method can be used to measure peroxide concentrations down to about 10 μM (0.3 mg kg^{-1}) under the most favourable conditions. A variety of complexing and reducing agents, and catalysts of peroxide decomposition, known to interfere with the alternative iodide method for peroxide determination, had no effect. Fluoride was found to interfere.

Keywords: *Hydrogen peroxide determination*; *spectrophotometry*; *titanium(IV) oxalate*

Many methods have been described in the literature for the spectrophotometric determination of hydrogen peroxide.[1–10] One of the most sensitive and widely used is that based on the oxidation of I^- to I_3^-.[2] This test is not specific for hydrogen peroxide (organic peroxides and many other oxidising agents convert I^- into I_3^-), although the analysis is usually performed in the presence of molybdate, a specific catalyst of the reaction.[2] Other redox reactions, such as iron(II) to iron(III), have also been employed.[3] These too measure total peroxides, rather than hydrogen peroxide alone.

A specific spectrophotometric test based on the formation of a complex, often written as TiO_2^{2+}, between hydrogen peroxide and the titanium(IV) ion has been described.[4–9] The published procedures involve lengthy preparations of titanium(IV) sulphate, and little seems to be known about either the optimum conditions for carrying out the measurements, or interference by compounds such as complexing or reducing agents. This paper describes the development of a method for the determination of hydrogen peroxide using potassium titanium(IV) oxalate, the only analytical-reagent grade salt of titanium readily available commercially, and the influence of various additives known to interfere with the iodide method.

Experimental

Reagents

All solutions were prepared from triply distilled water. The reagents used in the determination of the hydrogen peroxide, *i.e.*, hydrogen peroxide, potassium titanium(IV) oxalate, $[K_2TiO(C_2O_4)_2.2H_2O]$ and sulphuric acid, were from BDH, AnalaR grade, and were used as received.

The organic compounds used in the tests for interference were of laboratory-reagent grade, and were obtained from BDH [ethylenediamine, iminodiacetic acid (IDA)], Aldrich [ethylenediaminediacetic acid (EDDA), *N*-(2-hydroxyethyl)ethylenediaminetriacetic acid (HEDTA)] and Eastman Organic Chemicals [ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA)]; glycine and 2,2′-bipyridyl were BDH AnalaR grade, and picolinic acid was Aldrich laboratory-reagent grade, re-crystallised once from water.

All other inorganic compounds were BDH AnalaR grade. Hydrogen peroxide solutions were standardised by the iodide method of Allen *et al.*,[2] taking ε for I_3^- at 350 nm as 25700 l mol^{-1} cm^{-1}.

Apparatus

Absorbance measurements were made with a Cecil CE505 spectrophotometer.

Results

Development of the Method

The titanium(IV) - peroxide complex is yellow - orange in colour and absorbs with a $\lambda_{max.}$ of about 400 nm. The intensity of this absorption was found to be dependent on the

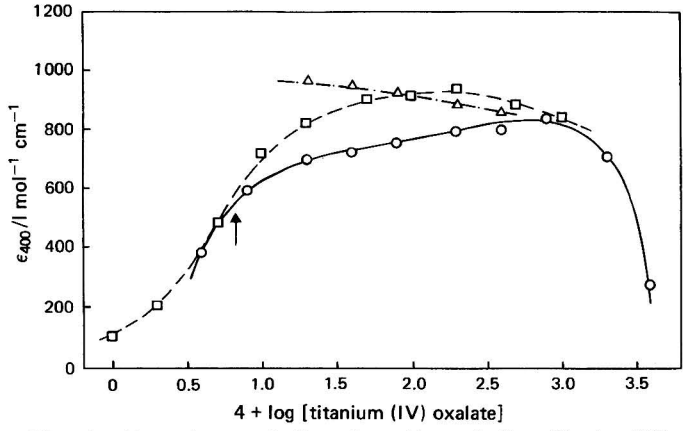


Fig. 1. Dependence of the absorption of the titanium(IV) - peroxide complex on titanium(IV) oxalate concentration. Measurements were made in solutions containing 6×10^{-4} M H_2O_2 and 0.1(△), 1.0(□) or 4.0(○) M H_2SO_4. The arrow indicates the point at which the concentrations of the titanium(IV) oxalate and hydrogen peroxide were equal.

concentrations of titanium(IV) oxalate and sulphuric acid as shown in Figs. 1 and 2. The measurements were made in solutions containing 6×10^{-4} M hydrogen peroxide; for the measurements of dependence on the concentration of titanium(IV) oxalate, the sulphuric acid concentrations were 0.1, 1.0 or 4.0 M; and for the measurements of dependence on the concentration of sulphuric acid the titanium(IV) oxalate concentrations were 0.01, 0.02 or 0.04 M. Absorption by components other than TiO_2^{2+} was allowed for by subtracting the absorbances of solutions prepared in the same way but omitting hydrogen peroxide.

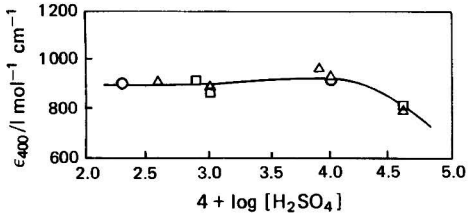


Fig. 2. Dependence of the absorption of the titanium(IV) - peroxide complex on sulphuric acid concentration. Measurements were in solutions containing 6×10^{-4} M H_2O_2 and 0.01(○), 0.02(△) or 0.04(□) M titanium(IV) oxalate.

When the titanium(IV) concentration was greater than the hydrogen peroxide concentration (*i.e.*, under conditions where all the hydrogen peroxide was complexed) only a small dependence on the concentration of titanium(IV) oxalate was found at titanium(IV) oxalate concentrations less than 0.1 M. At higher concentrations the intensity of the absorbance of the titanium(IV) - peroxide complex was much reduced. Varying the sulphuric acid concentration at a constant titanium(IV) oxalate concentration had only a small effect. The absorption was at its most intense at a titanium(IV) oxalate concentration of about 0.02 M and 0.1–1.0 M sulphuric acid. The $\lambda_{max.}$ for the absorption increased with decreasing titanium(IV) oxalate concentration or increasing sulphuric acid concentration.

Some experiments were also carried out in which the hydrogen peroxide concentration was varied at constant titanium(IV) oxalate and sulphuric acid concentrations. In all instances the absorbance at a particular wavelength varied linearly with hydrogen peroxide concentration, at peroxide concentrations up to about 2×10^{-3} M, the highest employed.

Recommended Procedure

Based on the conditions under which the absorption of the titanium(IV) - peroxide complex was at its most intense (*i.e.*, was most sensitive to hydrogen peroxide) the procedure described below appears most suitable for the determination of hydrogen peroxide using titanium(IV) oxalate.

Titanium reagent

Mix 272 ml of concentrated sulphuric acid (BDH AnalaR) with about 300 ml of distilled water (care should be taken and cooling is required). Dissolve in this mixture 35.4 g of potassium titanium(IV) oxalate, $K_2TiO(C_2O_4)_2.2H_2O$, (BDH AnalaR), and make up to 1 l with distilled water.

Procedure

Pipette 5 ml of the titanium reagent and 5 ml (or as appropriate) of the sample into a 25-ml calibrated flask and make up to the mark. Measure the absorbance of the solution at 400 nm. A blank, consisting of 5 ml of the titanium reagent and 5 ml (or as appropriate) of sample without the hydrogen peroxide present made up to 25 ml, should also be measured. (The hydrogen peroxide may be destroyed by the addition of platinum black followed by filtration, or by boiling.)

Calculation of hydrogen peroxide concentration

The concentration of hydrogen peroxide is calculated taking $\epsilon_{400} = 935$ l mol^{-1} cm^{-1} as the molar absorptivity of the titanium(IV) - peroxide complex. For x ml of sample per 25 ml of solution the concentration of hydrogen peroxide (in moles per litre) is given by:

$$[H_2O_2] = \frac{(A - A_b)}{37.4xl}$$

where A and A_b are the absorbances of the test and blank solutions, respectively, and l is the path length of the spectrophotometer cell in centimetres.

Effect of Other Solutes

The effect of other solutes on the method was investigated by measuring the apparent molar absorptivity of the titanium(IV) - peroxide complex at 400 nm in solutions made up to contain 0.02 M in titanium(IV) oxalate plus 1.0 M in sulphuric acid, 6×10^{-4} M in hydrogen peroxide and about 0.02 M in solute. The results are summarised in Table I. The solutes included a variety of complexing and reducing agents and catalysts of peroxide decomposition.

TABLE I

EFFECT OF SOME ADDITIVES ON THE TITANIUM(IV) OXALATE METHOD FOR THE DETERMINATION OF HYDROGEN PEROXIDE

All solutions also contained 0.020 M titanium(IV) oxalate, 1.0 M sulphuric acid and 6.0×10^{-4} M hydrogen peroxide.

Additive	Additive concentration/M	ϵ_{400}/l mol^{-1} cm^{-1}
None	—	935
NaF	0.020	805
$CoSO_4$	0.020	956
$CuSO_4$	0.020	935
$NiSO_4$	0.020	936
Hydrazinium sulphate	0.020	935
Ethylenediamine	0.018	949
Glycine	0.020	936
EDDA	0.002	935
EDTA	0.020	957
HEDTA	0.020	969
IDA	0.020	927
NTA	0.020	950
2,2′-Bipyridyl	0.012	949
Picolinic acid	0.024	940

Only for fluoride was any appreciable interference found. A similar series of tests was performed in which the hydrogen peroxide was determined by the iodide method of Allen *et al.*[2] The results can be summarised as follows:

(*i*) Ethylenediamine, EDTA, HEDTA, IDA and NTA, no I_3^- was formed, as these solutes reduce I_3^- back to I^-.

(*ii*) 2,2′-Bipyridyl and picolinic acid, I_3^- formed slowly, and with 2,2′-bipyridyl a purple - black precipitate was obtained. This behaviour probably results from complexation of the molybdate catalyst by these solutes.

(*iii*) Hydrazinium sulphate, no I_3^- was formed and the solution turned blue. Hydrazinium sulphate is a reducing agent and not only converts I_3^- into I^-, but also molybdate into a heteropoly blue.

(*iv*) Copper sulphate, large amounts of I_2 were formed, due to oxidation of I^- by Cu^{2+}.

(*v*) Glycine, cobalt sulphate, nickel sulphate and sodium fluoride, no interference.

Discussion

The recommended procedure gives concentrations of 0.02 M titanium(IV) oxalate and 1.0 M sulphuric acid in the analysis solution. The high acid concentration prevents precipitation of titanium(IV) hydroxide and ensures that relatively large amounts of base can be present in the peroxide containing solution without interfering unduly with the method through the consumption of protons. An upper limit of about 0.1 M of base in the peroxide sample is probably advisable.

The precision of the method is around 1% for peroxide concentrations of 0.5–2.0 $\times$ 10^{-3} M (an absorbance of 0.5–2.0 in 1-cm cells). Thus, the 13 measurements in Table I that relate to solutes that do not interfere have a relative mean deviation of 1.1%. The relative mean error of the values is also 1.1%. At lower peroxide concentrations the method becomes less precise, the size of the blank reading becoming important. This averaged about 0.001 absorbance units, as measured in 1-cm cells, in the absence of any additional solutes, and about double this in the presence of solutes, such as the complexing agents, or higher still in the presence of coloured substances such as Cu^{2+}. The upper limit for detection of peroxide is about 2 mM using 1-cm spectrophotometer cells (with an absorbance of 2). This is well below the titanium(IV) oxalate concentration in the mixture, ensuring that all peroxide is complexed. The lower limit with 1-cm cells is 10 μM under favourable circumstances.

The calculation of the hydrogen peroxide present is based on the measured molar absorptivity of the complex at 400 nm. The intensity of the absorption follows the Beer - Lambert law, so that little or no additional precision is obtained by constructing a calibration graph, although some improvement should be possible by adaptation of the method described by Neal.[9] It is clearly important to check for possible interference by other solutes that may be present. The experiments summarised in Table I suggest that the method is relatively free from interference, and appears to be particularly suitable for the determination of hydrogen peroxide in the presence of complexing and reducing agents. Interference by fluoride is not unexpected in view of the ease of formation and stability of the fluorotitanate ion, TiF_6^{2-}. It may also be noted that the method involves considerable savings in time over the earlier procedures, which required lengthy preparations of titanium(IV) by digestion of titanium(IV) oxide in sulphuric acid.

It is reported[10] that the sensitivity of the method can be improved by addition of xylenol orange, and experiments have shown[11] that potassium titanium(IV) oxalate is suitable for use as a source of titanium(IV) under these conditions. The pH of the solution seems to be critical, however, and the effect of complexing and reducing agents is unknown.

I am indebted to Mr. B. Daniel for assistance with some of the measurements. This paper is published by permission of the Central Electricity Generating Board.

References

1. Schumb, W. C., Satterfield, C. N., and Wentworth, R. L., "Hydrogen Peroxide," Reinhold, New York, 1955, p. 561.
2. Allen, A. O., Hochanadel, C. J., Ghormley, J. A., and Davies, R. W., *J. Phys. Chem.*, 1952, **56**, 575.
3. Michaels, H. B., and Hunt, J. W., *Anal. Biochem.*, 1978, **87**, 135.
4. Jackson, E., *Chem. News*, 1883, **47**, 157.
5. Richardson, A., *J. Chem. Soc.*, 1883, **63**, 1109.
6. Allsopp, C. B., *Analyst*, 1941, **66**, 371.
7. Eisenberg, G. M., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 327.
8. Humpoletz, J. E., *Aust. J. Sci.*, 1949, **12**, 111.
9. Neal, W. T. L., *Analyst*, 1954, **79**, 403.
10. Gupta, B. L., *Microchem. J.*, 1973, **18**, 363.
11. Sellers, R. M., unpublished data.

Received *April 9th*, 1980
Accepted *May 29th*, 1980

Analyst, October, 1980, Vol. 105, pp. 955–964

Improved Extraction Method for the Spectrophotometric Determination of Trace Amounts of Boron in River Water with 1,8-Dihydroxynaphthalene-4-sulphonic Acid and Removal of the Excess of Reagent

Takashi Korenaga,* Shoji Motomizu and Kyoji Tôei

Department of Chemistry, Faculty of Science, Okayama University, Tsushima-naka, Okayama-shi 700, *Japan*

A simple method for removing the excess of co-extracted reagent in the ion-association extraction of metal complex anions with quaternary ammonium salts has been applied successfully to the spectrophotometric determination of boron at the parts per 10^9 level in river water with 1,8-dihydroxynaphthalene 4-sulphonic acid (DHNS) and tetradecyldimethylbenzylammonium chloride (zephiramine). The procedure using DHNS described here greatly improves the previous method using chromotropic acid. Acetate buffer (pH 3.8), EDTA and DHNS are added to the sample solution (less than 50 ml) and the pH of the resulting solution is adjusted to 10.2. Then sodium chloride is added and the mixture is shaken with 5 ml of a 2×10^{-3} M solution of zephiramine in 1,2-dichloroethane (DCE). The organic phase is washed once with 10 ml of a back-washing solution (1.0 M in sodium chloride, pH 10.2) and the absorbance of the organic phase is measured in a quartz cell. The boron complex with DHNS is extracted quantitatively into DCE and its apparent molar absorptivity in DCE is 2.4×10^4 l mol^{-1} cm^{-1} at 341 nm. The detection limit and precision achieved with the method are 1 μg l^{-1} and 5%, respectively. EDTA allows most interferences caused by metals to be suppressed, and other sources of bias due to the effect of co-extractable anions are almost eliminated by adding relatively large amounts of sodium chloride to the extraction system. Parts per 10^9 amounts of boron present as boric acid in river water are determined spectrophotometrically, and the results obtained are successfully compared with those obtained by the methylene blue method.

Keywords: *Boron trace determination*; *river water analysis*; *spectrophotometry*; *ion-association extraction*

In a previous paper, we have proposed a spectrophotometric method for the determination of boron in natural waters involving solvent extraction with 1,8-dihydroxynaphthalene-3,6-disulphonic acid (chromotropic acid).[1] In that method, a large amount of reagent (250-fold excess of boron) was used in order to complete the complex formation and the principle presented earlier by the authors[2] was applied to the removal of a large excess of co-extracted reagent (chromotropic acid) from the organic phase. Recently, we developed 1,8-dihydroxynaphthalene-4-sulphonic acid (DHNS) as a reagent for the solvent extraction of boric acid.[3] DHNS was synthesised in order to improve the extractability of the boron complex and was found to be much superior to chromotropic acid as an analytical reagent for the solvent extraction - spectrophotometric determination of boron. In that work,[3] the method using DHNS was applied to the determination of boron at the parts per million level in sea water and hot-spring water, but not at the parts per billion (10^9) level in river water.

In this work, the previous method using chromotropic acid[1] was greatly improved by using DHNS and it could be used successfully for the concentration and determination of boron at the parts per billion level in river water, as the extractability of the boron complex with DHNS is much superior to that with chromotropic acid in the ion-association extraction using tetradecyldimethylbenzylammonium chloride (zephiramine). This paper therefore presents a greatly improved method for the spectrophotometric determination of boron present as boric acid in river water with DHNS and zephiramine and removal of the excess of DHNS reagent in the organic phase.

* Present address: School of Engineering, Okayama University, Tsushima-naka, Okayama-shi 700, Japan.

Experimental

Reagents

All of the reagents used were of analytical reagent grade.

1,8-Dihydroxynaphthalene-4-sulphonic acid (DHNS) solution, 0.01 M. Dissolve 1.311 g of DHNS, sodium salt, in 500 ml of de-ionised water and transfer the solution obtained into an amber polyethylene bottle. The solution can be kept for at least 1 month in a refrigerator.

Standard boron solution, 1.000×10^{-2} M. Prepare a stock solution by dissolving 154.6 mg of boric acid in 250 ml of de-ionised water. Store the solution in a polyethylene bottle and dilute aliquots of it with de-ionised water to give working solutions of the required concentrations.

Extraction solvent, 1,2-dichloroethane (DCE), 2×10^{-3} M in zephiramine. Dissolve 369.0 mg of dried zephiramine[4] in 500 ml of DCE (analytical-reagent grade, used as received) to give a 2×10^{-3} M solution. Store the zephiramine solution in a glass bottle; it is stable for at least 6 months.

Buffer solutions. (1) An aqueous solution 0.01 M in EDTA and 0.25 M in acetic acid - sodium acetate (pH 3.8) and (2) an aqueous solution 4 M in sodium chloride and 0.8 M in sodium hydrogen carbonate - sodium carbonate (pH 10.2) were used.

Back-washing solution. An aqueous solution 1.0 M in sodium chloride and 0.2 M in sodium hydrogen carbonate - sodium carbonate (pH 10.2) was used.

Apparatus

A Hitachi, Model 139, spectrophotometer and a Hitachi, Model EPS-3T, recording spectrophotometer were used for measuring absorbances in a quartz cell of 1-cm path length. A Hitachi-Horiba, Model F-5ss, pH meter equipped with a combined electrode (6026-05T) was used for pH measurements. An Iwaki, Model KM, shaker (frequency 250 strokes min^{-1}) was used for shaking separating funnels and stoppered test-tubes.

Recommended Procedure

Transfer by pipette the sample solution (less than 50 ml), which is not acidified and is first filtered with a membrane filter of 0.45-μm pore size, into a 100-ml polyethylene separating funnel. Dilute the solution to 50 ml with de-ionised water and add 5 ml of acetate buffer solution (1) and 5 ml of 0.01 M DHNS solution, in that order. Mix the solutions thoroughly and allow the mixture to stand for 30 min. Then add 5 ml of carbonate buffer solution (2) to adjust the pH of the resulting solution to the extraction pH, and also add 5 ml of 1.5 M sodium sulphate solution to accelerate the phase separation. Add 5 ml of 2×10^{-3} M zephiramine in DCE solution. Shake the separating funnel for 30 min and allow two phases to separate. Transfer the organic phase into a stopped 25-ml glass test-tube and add 10 ml of the back-washing solution. Shake the test-tube for 10 min and then allow it to stand for about 30 min to remove the excess of reagent from the organic phase. Measure the absorbance of the organic phase in a quartz cell of 1-cm path length at 341 nm against a reagent blank as reference.

Prepare a calibration graph by using standard boron solutions corresponding to $0–4 \times 10^{-5}$ M of boron in the organic phase.

Results and Discussion

In the extraction of the ion associate formed between the boron - DHNS complex anion and the zephiramine cation, the extraction was previously carried out with 5 ml of aqueous solution and 5 ml of DCE solution.[3] In this work, however, as the extraction was carried out with 70 ml of aqueous phase and 5 ml of organic solution, the choice of the extraction solvent (DCE or chloroform) was re-examined. The equilibrium constants for exchange of chloride (log $K_{A^{n-}}^{nCl^-}$) between DCE and water were determined for DHNS reagent and its boron complex in previous work,[3] but those between chloroform and water were not. Therefore, the constants for chloride between chloroform and water were also determined in this work, and shown in Table I for comparison with those between DCE and water. From Table I, the difference in the constants between HR^{2-} and BR_2^{3-} and the extractability of the

TABLE I

EXCHANGE EQUILIBRIUM CONSTANTS FOR CHLORIDE OBTAINED AT 25 °C

Constant*	Extraction solvent: 1,2-Dichloroethane	Extraction solvent: Chloroform
Log $K_{H_2R^-}^{Cl^-}$	2.51 ± 0.05	1.01 ± 0.02
Log $K_{HR^{2-}}^{2Cl^-}$	4.39 ± 0.07	3.55 ± 0.06
Log $K_{BR_2^{3-}}^{3Cl^-}$	9.77 ± 0.04	6.42 ± 0.11

* The exchange equilibrium constant for chloride ($K_{A^{n-}}^{nCl^-}$) refers to the following equation[2,3]:

$$A^{n-}_{(a)} + nZCl_{(o)} \rightleftharpoons nCl^-_{(a)} + Z_nA_{(o)}$$

$$K_{A^{n-}}^{nCl^-} = ([Cl^-]^n_{(a)}[Z_nA]_{(o)}) / ([A^{n-}]_{(a)}\,[ZCl]^n_{(o)})$$

where A^{n-} and Z^+ are the n-valent anion and the zephiramine cation, respectively, and subscripts a and o refer to the aqueous and organic phase, respectively.

boron - DHNS complex using DCE were found to be larger than those obtained when chloroform was used. DCE was therefore suitable for the separation of complex and reagent. As shown in the previous work,[3] the exchange constants for four univalent anions (chloride, bromide, nitrate and iodide) also indicated that chloride gave the highest concentration range for the removal of the excess of reagent and therefore caused very effective salting-out.

In this work, DCE and chloride were therefore used as the extraction solvent and the univalent anion for the removal of the excess of reagent from the organic phase, respectively. For reference, the percentage extraction of reagent and boron complex with DHNS, which was calculated by using the equilibrium constants listed in Table I, is shown in Fig. 1. It is obvious that the reagent in the form HR^{2-} is removed more easily than that in the form H_2R^-.

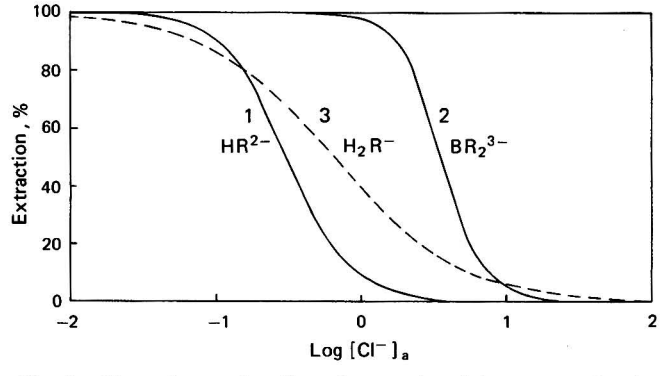


Fig. 1. Percentage extraction of reagent and boron complex into DCE against chloride concentration of aqueous solution for 2×10^{-3} M zephiramine. 1, DHNS in the form HR^{2-} at pH 9; 2, boron complex in the form BR_2^{3-} at pH 9; and 3, DHNS in the form H_2R^- at pH 3.

De-ionised water, obtained from a common de-ionisation apparatus, must be used in this work, as the absorbance of the reagent blank with de-ionised water is more constant and smaller than that with distilled water obtained from a commercially available glass distillation apparatus. The reason why the distilled water blank is higher and more variable than the de-ionised water blank is assumed to be based on the dissolution of boron present in the glass of the distillation apparatus. Hence, the former blank might decrease the accuracy, precision and detection limit of the proposed method in an actual determination.

Effect of pH on the Formation and Extraction of the Boron Complex

The effective pH range for the formation of the boron - DHNS complex in aqueous solution without EDTA was found to be 3–10.[3] The effect of pH on the formation of the complex with EDTA was also examined. The results obtained indicate that the optimum pH range is 3.5–9 in the presence of EDTA (Fig. 2) and the absorbance of the complex decreases at pH above 9. The formation of the boron complex was therefore carried out at about pH 3.8 by using an acetate buffer.

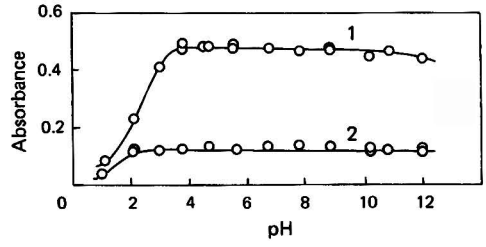


Fig. 2. Effect of pH on complex formation. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank; and 2, reagent blank as 1 but no boron present, measured against DCE.

The effect of pH on the extraction of the boron - DHNS complex with EDTA is shown in Fig. 3. Constant absorbance was obtained at pH 4–12, the optimum pH range being 7–11. Because the absorbance of the reagent blank was constant at pH above 7, but not reproducible at pH 12, the extraction of the boron complex was carried out at about pH 10.2 by using carbonate buffer. Accordingly, the complex was formed at pH 3.8 in the presence of EDTA and then extracted with zephiramine into DCE at pH 10.2 with addition of chloride.

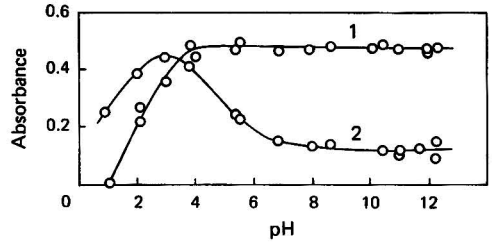


Fig. 3. Effect of pH on the extraction of the boron complex into DCE. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank; and 2, reagent blank as 1 but no boron present, measured against DCE.

The effect of pH on the back-washing of the organic phase, which was transferred into a stoppered 25-ml test-tube, was examined. When the concentration of chloride ion in the back-washing solution was 1 M, the optimum pH region was found to be 5–12. The back-washing was therefore carried out at about pH 10.2.

Effect of Chloride Concentration on the Extraction of the Boron Complex and on the Back-washing of the Organic Phase

The effect of chloride concentration on the extraction of the boron complex was examined at pH 10.2 (Fig. 4). The optimum concentration of chloride was found to be about 0.3 M. To complete and accelerate the phase separation in the ion-association extraction, 5 ml of 1.5 M sodium sulphate solution were also added as the salting-out agent in the extraction system.

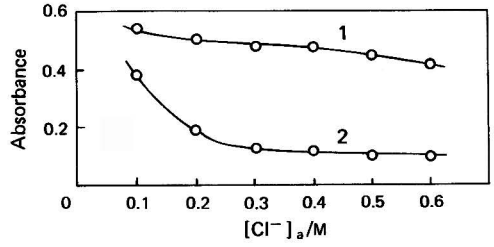


Fig. 4. Effect of chloride concentration on the extraction of the boron complex. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank; and 2, reagent blank as 1 but no boron present, measured against DCE.

Effect of pH on the Back-washing of the Organic Phase

The effect of chloride concentration on the back-washing of the organic phase, which was transferred into a stoppered 25-ml test-tube, was examined at pH 10.2, and the results obtained are shown in Fig. 5. The optimum concentration of chloride was about 1.3 M.

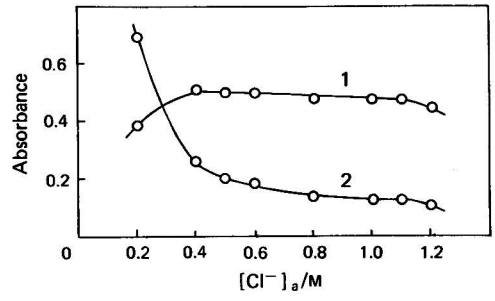


Fig. 5. Effect of chloride concentration on the back-washing of the organic phase. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank; and 2, reagent blank as 1 but no boron present, measured against DCE.

The effect of the volume of the back-washing solution on the back-washing of the organic phase was examined. The volume of the back-washing solution taken being varied from 5 to 20 ml, and 10 ml was found to be the most effective.

The efficiency of the back-washing with sodium chloride solution was examined at pH 10.2. When 13 ml of back-washing solution were used, the absorbances of the boron complex and reagent blank were 1.289 and 0.764 (no back-washing), 0.605 and 0.119 (one back-washing) and 0.564 and 0.102 (two back-washings), respectively. Accordingly, the back-washing was carried out once with 10 ml of solution.

Effect of Concentration of DHNS and Zephiramine

The effect of the DHNS concentration on the formation of the boron complex was examined. Fig. 6 shows that the amount of 0.01 M DHNS solution necessary for the complete reaction was more than 4 ml when the concentration of boron was about 1.4×10^{-6} M in 70 ml of aqueous solution and 5 ml of 2×10^{-3} M zephiramine in DCE solution was used as the extracting solvent.

The effect of the zephiramine concentration on the extraction of the boron complex was examined by using 0.01 M aqueous zephiramine solution[4] (Fig. 7). A 1-ml volume of this

solution was found to be necessary for the complete extraction of the boron complex. Therefore, 5 ml of 0.01 M DHNS solution and 5 ml of 2×10^{-3} M zephiramine in DCE solution (equivalent to 1 ml of 0.01 M aqueous zephiramine solution) were used in this work.

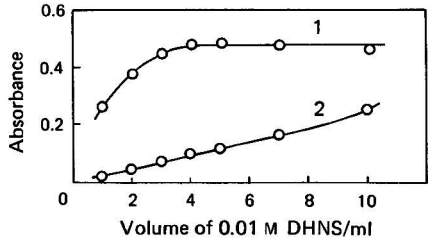


Fig. 6. Effect of the concentration of DHNS. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank: and 2, reagent blank as 1 but no boron present, measured against DCE.

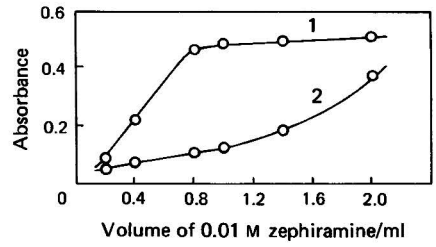


Fig. 7. Effect of the concentration of zephiramine. 1, Complex obtained with 2×10^{-5} M boron, measured against reagent blank; and 2, reagent blank as 1 but no boron present, measured against DCE.

Effect of Time

The time necessary for the complete formation of the boron complex was examined (Fig. 8). When the concentrations of boron (present as boric acid) and DHNS reagent were 1.4×10^{-6} and 7.1×10^{-4} M at pH 3.8, respectively, the complete reaction was found to be achieved within 30 min.

The time necessary for the complete extraction of the complex into DCE was examined. Fig. 9 shows that a suitable shaking time was 30 min when 5 ml of 2×10^{-3} M zephiramine in DCE solution were used.

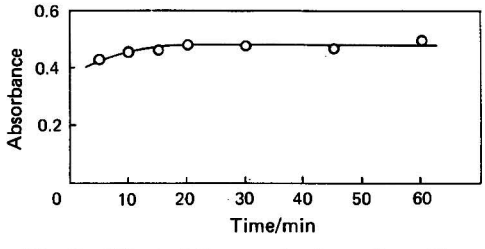


Fig. 8. Effect of time on the formation of boron complex. Absorbance measured against reagent blank.

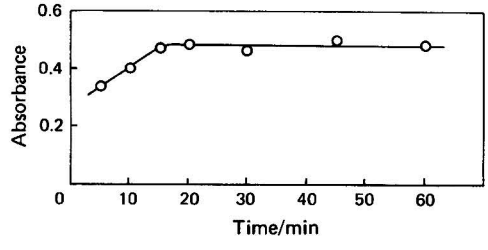


Fig. 9. Effect of shaking time. Absorbance measured against reagent blank.

The shaking time necessary for the complete back-washing of the organic phase when 10 ml of the back-washing solution were used was examined. A shaking time of 5 min was found to be sufficient, so the shaking time for the back-washing was fixed at 10 min.

The separation of the two phases was immediate on the extraction of the complex from 70 ml of aqueous solution into 5 ml of DCE solution, and standing for at most 30 min was found to be preferable to the complete separation of the two phases on the back-washing of the organic phase before measurement of the absorbance.

Absorption Spectra and Calibration Graph

The absorption spectra of the boron complex with DHNS and the reagent blank in DCE are shown in Fig. 10 (solid lines 1–3). When the excess of co-extracted reagent in the organic phase is removed, the absorption maximum is obtained at 341 nm with a minimum reagent

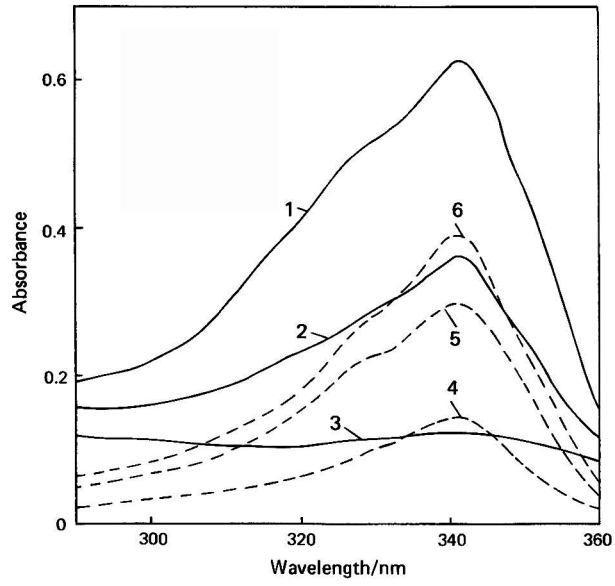


Fig. 10. Absorption spectra in DCE. 1, Complex obtained with 2×10^{-5} M boron (0.216 μg of boron), measured against DCE in reference beam; 2, complex obtained with 1×10^{-5} M boron (0.108 μg of boron), measured against DCE in reference beam; 3, reagent blank as 1 and 2 but no boron present, measured against DCE in reference beam; 4, solution obtained with 25 ml of Mino sample, measured against reagent blank in reference beam; 5, solution obtained with 50 ml of Mino sample, measured against reagent blank in reference beam; and 6, solution obtained with 25 ml of Mino sample plus 0.108 μg of boron, measured against reagent blank in reference beam.

blank. The absorbance in DCE was measured in a quartz cell of 1-cm path length at 341 nm against the reagent blank as reference.

The calibration graph at 341 nm was a straight line that passed through the origin and obeyed Beer's law over the range 0–3.6×10^{-6} M boron present as boric acid in aqueous solution (corresponding to 0–5×10^{-5} M boron in DCE). The apparent molar absorptivity in DCE calculated from the slope of the calibration graph was 2.4×10^4 l mol^{-1} cm^{-1} (converted into $\varepsilon = 3.4 \times 10^5$ l mol^{-1} cm^{-1} in aqueous solution) at 341 nm.

The reagent blank at 341 nm was 0.119 ± 0.006 (mean value of seven determinations), but reproducible when de-ionised water was used. Consequently, the detection limit and precision achieved with the method are 1 μg l^{-1} (difference in absorbance 0.03) and 5%, respectively.

Determination of Boron in River Water with DHNS

Interferences and masking agent

DHNS reacts with some metal ions, such as aluminium, copper, iron, titanium and molybdenum. The tolerance limits of these metals and other ions generally present in river water were examined without EDTA according to the recommended procedure. The tolerance limit is defined as the concentration level at which the interferent causes an error of not more than 5%. The results obtained are shown in Table II.

When EDTA was used as a masking agent for metals, the pH was adjusted to about 3.8 with acetate buffer. As shown in Table II, the tolerance limits with EDTA were also deter-

TABLE II

TOLERANCE LIMITS FOR DIVERSE IONS WITH AND WITHOUT EDTA

Ion	Tolerance limit*/M
Na^+, K^+, SO_4^{2-}	1†
HCO_3^-, $H_2PO_4^-$	0.1†
Mg^{2+}	0.01†
Ca^{2+}, Sr^{2+}, Ba^{2+}, Br^-, NO_3^-, SiO_3^{2-}	2×10^{-3}
Ag^+, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, UO_2^{2+}, Al^{3+}, Cr^{3+}, F^-, SCN^-, I^-	2×10^{-4}
Fe^{3+}, Ti^{4+}, Mo^{6+}, ClO_4^-, dodecylbenzenesulphonate	2×10^{-5}
NO_2^-	2×10^{-6}‡
Ag^+, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}	1×10^{-3}†§
Hg^{2+}, Pb^{2+}, UO_2^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Ti^{4+}, Mo^{6+}	1×10^{-4} §

* The tolerance limit is defined as the concentration level at which the interferent causes an error of not more than 5% (precision of the method).

† Maximum tested.

‡ If samples are acidified to pH below 2 by adding more than 0.5 ml of concentrated sulphuric acid per litre, nitrite ion may be almost completely removed as nitrogen monoxide gas.

§ Add 5 ml of 0.01 M EDTA solution to 70 ml of the aqueous test solution.

mined when 5 ml of 0.01 M EDTA solution were added to 70 ml of aqueous solution at pH 3.8. Hence, the interferences of these metals and other metals commonly present in river water are eliminated by adding EDTA to the extraction system at a concentration of about 10^{-3} M.

Contamination from glassware

In order to test for possible contamination from the glassware, DHNS, EDTA and acetate buffer were allowed to stand at pH 3.8 in a 100-ml glass separating funnel for 2 h. The solution was examined by the proposed procedure and little boron was found (absorbance 0.130). However, when the solution was allowed to stand at pH 10.2 (carbonate buffer) for 2 h, the absorbance was found to be higher. Polyethylene separating funnels were therefore used in order to prevent any possible contamination caused by the solution standing for a long time in glass. Polyethylene bottles were also used for storage of sample and reagents solutions.

Pre-treatment of sample solution

The effect of sample acidification was examined. When the sample was immediately treated with 0.5 ml of concentrated sulphuric acid per litre after collection, the boron content was found to be identical with that obtained without acidification.

The stability of boron in sample solutions was examined with and without sulphuric acid. The results obtained for boron content did not vary for at least a week in both instances. The sample therefore need not be acidified.

The effect of sample filtration was examined. When the membrane filter (0.45-μm pore size and 47-mm diameter circle) was used, no loss or gain of boron was found and reproducible results were obtained.

Results of determination

The results obtained by the recommended procedure for the determination of boron in water samples from the River Asahi, Okayama Prefecture, Japan, are given in Table III. An example of absorption spectra in the determination of boron in river water is shown in Fig. 10 (broken line 4).

Boron present in sample solutions as boric acid and as tetraborate can be determined by this method, but not boron present as fluoroborate. In general, river water contains boron in the form of boric acid and seldom in the form of tetraborate and fluoroborate. Accordingly, the total boron in river water can be determined by the use of the recommended procedure.

In order to check the results of the determination of boron in river water, two series of experiments were carried out. In the first experiment, the amount of sample solution taken was varied between 10 and 50 ml and de-ionised water was added to each so as to give a constant volume. For all samples, linear graphs were obtained and the lines could be

TABLE III

DETERMINATION OF BORON IN RIVER ASAHI WATER

Sample source*	Distance from estuary/km	Boron content†/μg l^{-1}	
		Proposed method	Methylene blue method[5]
Shimotokuyama	137	8.8 ± 0.3	8.4
Hatsuwa	122	8.4 ± 0.2	7.6
Katsuyama	89	8.7 ± 0.3	8.7
Ochiai	76	9.4 ± 0.4	9.7
Nishikawakami	68	11.5 ± 0.5	10.9
Eyomi	62	8.6 ± 0.4	8.1
Asahigawa-damu	54	11.4 ± 0.3	11.5
Shinada	49	10.4 ± 0.3	9.8
Kanagawa	32	11.4 ± 0.1	10.8
Ôhara	16	9.3 ± 0.4	10.0
Mino	12	11.7 ± 0.4	11.6 ± 0.9
Nanokaichi‡	4.6	360 ± 20	400
Hot-spring at Yubara	—	34 ± 2	38
Hot-spring at Misasa	—	2300 ± 100	1900
Seto Inland Sea at Shibukawa	—	4400 ± 100	4200
Seto Inland Sea at Teshima	—	4500 ± 200	4300
Pacific Ocean at Tanohama	—	4400 ± 100	4600
Japan Sea at Aoya	—	3900 ± 200	4200

* Samples from the River Asahi were sampled on May 3rd and 4th, 1978. The boron contents of hot-spring and sea waters are also given; the samples used were the same as those in previous work.[3]

† Average values of four determinations ± standard deviations. The values without standard deviations are averages of two determinations.

‡ This sample contained 0.043% of chloride, probably caused by sea water.

extrapolated to the same point, which coincided with the point obtained for 50 ml of de-ionised water. It was concluded that the determination of boron in river water was quantitative and de-ionised water could be used as the reagent blank. An example of absorption spectra in the experiment is shown in Fig. 10 (broken lines 4 and 5). In the second experiment, the recovery of boron was examined by adding various amounts of boron to the sample solutions. All of the results obtained were linear and the slopes of the graphs were identical with those of the calibration graph obtained with de-ionised water. An example of absorption spectra in this experiment is shown in Fig. 10 (broken lines 4 and 6).

Comparison with the conventional methylene blue method

The analytical values obtained by the proposed method were compared with those obtained by the methylene blue method.[5] The results obtained by the latter method, conventionally available in Japan, are shown in Table III. From Table III, two methods were found to be comparable and the sample from the River Asahi at Mino showed that the relative standard deviation obtained when using the proposed method was smaller than that with the methylene blue method. The correlation coefficient of two methods was 0.95 in the determination of boron in 11 samples from the River Asahi, except for the sample from Nanokaichi ($a = -0.05$ and $b = 0.98$ in the equation $y = a + bx$, where x is the value obtained by the proposed method and y value obtained by the methylene blue method). Consequently, the results of the proposed method using DHNS are almost identical with those of the conventional methylene blue method[5] so that the accuracy of the method is good in the practical analysis of river water samples.

Conclusion

The method proposed here could be applied to the spectrophotometric determination of parts per billion amounts of boron in river water with satisfactory results. This method of determining trace amounts of boron with DHNS is a great improvement on the previous method employing chromotropic acid,[1] and possesses the following advantages: (a) the complex formed with DHNS has a large molar absorptivity, which is about 1.7 times greater than that with chromotropic acid; (b) the extractability of the complex with DHNS is higher

than that with chromotropic acid, so that the concentration of boron by extraction from aqueous solution into DCE is complete and precise; (c) there is a large difference in the exchange equilibrium constants between the complex and reagent, *i.e.*, the removal of the excess of reagent can easily be achieved without loss of the boron complex; (d) the procedure is simple and back-washing is preferably carried out only once; and (e) the synthesis and purification of DHNS reagent are simple.

As described here, the simple method for the removal of the excess of reagent in the organic phase must lead to a greatly improved sensitivity in a given ion-association extraction system, as the measurement can be carried out at the most sensitive wavelength of the complex, which cannot be measured without removal of the excess of reagent. As a sufficient amount of the reagent and a cationic surfactant can be added, the complex can easily be formed and extracted over a wide pH range. Moreover, the addition of relatively large amounts of salts such as sodium chloride causes very effective salting-out, so that the phase separation becomes more rapid. Also, the absorbances of the complex and reagent blank become more constant and reproducible on adding salts to the ion-association extraction system, because the interferences caused by co-existing anions are effectively eliminated.

References

1. Korenaga, T., Motomizu, S., and Tôei, K., *Analyst*, 1978, **103**, 745.
2. Motomizu, S., and Tôei, K., *Anal. Chim. Acta*, 1977, **89**, 167.
3. Korenaga, T., Motomizu, S., and Tôei, K., *Anal. Chim. Acta*, in the press.
4. Tôei, K., and Kawada, K., *Bunseki Kagaku*, 1972, **21**, 1510.
5. Japan Industrial Standard (JIS), K 0102, 1974.

Received *December 10th*, 1979
Accepted *June 2nd*, 1980

Analyst, October, 1980, Vol. 105, pp. 965–973

Pyridine-2-acetaldehyde Salicyloylhydrazone as an Analytical Reagent and its Application to the Determination of Vanadium

M. Garcia-Vargas, M. Gallego* and M. de la Guardia†

Department of Analytical Chemistry, Faculty of Sciences, University of Seville, Seville, Spain

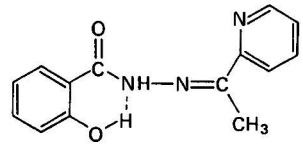
The synthesis, characteristics and analytical applications of pyridine-2-acetaldehyde salicyloylhydrazone (PASH) are described. The reagent reacts with vanadium(V) to produce a yellow 1 : 1 complex ($\lambda_{max.} = 415$ nm, $\varepsilon = 1.87 \times 10^4$ l mol^{-1} cm^{-1} in chloroform). The yellow complex, extracted into chloroform, has been used for the spectrophotometric determination of vanadium in a steel, a lead - vanadium concentrate and a phosphoric acid sample. A procedure based on the standard additions method has been applied satisfactorily to the determination of trace amounts of vanadium at the parts per billion level (parts per 10^9).

Keywords: *Pyridine-2-acetaldehyde salicyloylhydrazone reagent; vanadium determination; standard additions method; spectrophotometry*

The uses of aroylhydrazones[1–12] as analytical reagents have been described in an earlier paper.[13]

Aroylhydrazones behave as bidentate,[8,9] tridentate[10,11,13] or tetradentate[12] ligands, forming coloured chelates with transition metal ions. In moderately acidic media or alkaline solution the hydrogen atom of the –CONH– group can split off and neutral metal chelates are formed.[10,11,13]

In this paper, the synthesis, properties and analytical applications of pyridine-2-acetaldehyde salicyloylhydrazone (PASH) are reported. An extraction - spectrophotometric method for the determination of trace amounts of vanadium has been investigated and the effects of interferences have been widely studied. In order to avoid some interferences, masking and cation-exchange procedures have been used. The determination of small amounts of vanadium in different materials is described. The standard additions method has been used to determine vanadium at the sub-parts per million level.



PASH

Experimental

Apparatus

A Pye Unicam SP8000 spectrophotometer was used for recording spectra in the ultraviolet and visible regions of the spectrum. A Perkin-Elmer - Coleman 55 (digital) and a Beckmann DU spectrophotometer were used for absorbance measurements at fixed wavelengths in the visible and ultraviolet regions of the spectrum, respectively; 1-cm silica or glass cells were used. A Pye Unicam SP1000 infrared spectrophotometer was also used.

A Philips PW 9408 pH meter, with glass - calomel electrodes, was used for pH measurements.

* Present address: Faculty of Sciences, University of Cordoba, Cordoba, Spain.
† Present address: Faculty of Sciences, University of Valencia, Valencia, Spain.

Reagents

All solutions were prepared with analytical-reagent grade chemicals using distilled water.

Synthesis of PASH. A 1.05-ml volume of pyridine-2-acetaldehyde was added to 2 g of salicyloylhydrazide dissolved in 20 ml of hot absolute ethanol. Two drops of concentrated hydrochloric acid were added and the mixture was heated in a water-bath. The yellow crystals were filtered off and recrystallised twice from ethanol. The product (yield about 64%) had a melting-point of 230–234 °C, and elemental analysis gave C 65.85%, H 5.15% and N 16.70%; the calculated values for $C_{14}H_{13}O_2N_3$ are: C 65.87%, H 5.13% and N 16.46%.

Pyridine-2-acetaldehyde salicyloylhydrazone reagent solution. A 0.05% *m*/*V* solution was prepared by dissolving 0.05 g of the reagent in 3 ml of *NN*-dimethylformamide and diluting to 100 ml with chloroform. This solution was stable for several days.

Vanadium(V) standard solution, 5.295 g l^{-1} *of vanadium(V).* This solution was prepared by dissolving vanadium(V) oxide in 1 M sodium hydroxide solution, standardising it gravimetrically using cupferron.[14] Working standards were prepared from this solution as necessary.

Dowex 50-X8 resin, hydrogen form. Slurry 5.0 g of cation-exchange resin, 20–50 mesh, with distilled water and pour into a column 30 cm long and 2 cm in diameter with glass sinter bed support and a tap at the bottom.

Procedure for the Determination of Vanadium(V)

To 10–50 ml of sample solution in a separating funnel containing up to 100 μg of vanadium(V), add 2 ml of 0.5 M potassium chloride solution and 1.5 ml of 1 M hydrochloric acid and extract the mixture with one 10-ml volume of PASH reagent solution. Shake the funnel vigorously for 2 min, allow the phases to separate and transfer the lower (organic) layer into a 10-ml flask containing anhydrous sodium sulphate. Measure the absorbance of the yellow chloroform extracts after 45 min at 425 nm against distilled water or at 415 nm against a reagent blank, prepared in a similar way but without the vanadium.

The calibration graph was prepared by using standard solutions of vanadium(V) treated in the same way.

Results and Discussion

Properties of Pyridine-2-acetaldehyde Salicyloylhydrazone

The infrared spectrum of PASH in potassium bromide dics was obtained. The bands were assigned to the stretching vibrations of the N–H bond (3290 cm^{-1}), the =C–H bond (3050 cm^{-1}), the C=O bond (1640 cm^{-1}), the C=N bond (1550 cm^{-1}) and the N–N bond (920 and 875 cm^{-1}).

PASH has a solubility in *NN*-dimethylformamide of 35.7 g l^{-1}. The solubility in other solvents, such as water, methanol, ethanol, chloroform and benzene, is about 1 g l^{-1} or less. A reagent solution in ethanol or chloroform of 5×10^{-5} M concentration shows two absorption maxima at 298 and 311 or at 300 and 323 nm, with molar absorptivities of 2.6×10^4 and 2.56×10^4 or 1.94×10^4 and 1.92×10^4 l mol^{-1} cm^{-1}, respectively.

The change in pH on the spectra of aqueous solutions of the reagent (3.14×10^{-5} M) shows bathochromic shifts at pH less than 4 or greater than 6. Hydrolysis of PASH to pyridine-2-acetaldehyde and salicyloylhydrazide occurs slowly in aqueous solution. The percentage of decomposed reagent at pH 2.6, 4.4, 7.0 and 9.0 after 1 h was 33.3, 13.5, 0 and 0%, respectively.

The method used by Phillips and Merrit[15] was used for the determination of the ionisation constants. The average pK values were found to be 4.0 and 6.0. The first pK may be caused by protonation of the pyridine nitrogen atom and the second by deprotonation of the hydroxyl group.

Oxidising and reducing substances in moderate concentration do not alter the absorption spectra of PASH.

PASH appears to be a bidentate or tridentate ligand with a convenient steric arrangement of its donor groups and contains a conjugated system of π electrons connected with the donor system. The chelates of PASH are generally uncharged.

The reaction of the reagent in aqueous ethanolic solution at different pH values with 40 cations was investigated. It forms soluble, coloured complexes with lead(II), iron(III),

chromium(III), bismuth(III), cerium(IV), zirconium(IV) and vanadium(V). With uranium(VI) a soluble yellow complex that precipitates after several minutes is formed (Table I).

TABLE I

CHARACTERISTICS OF PASH REACTIONS WITH METAL IONS IN ACETATE BUFFER

Metal ion	Colour of complex in solution	$\lambda_{max.}$/nm	pD*
Fe(III)	Yellow - brown	350	4.78
Pb(II)	Yellow - green	360	4.20
Cr(III)	Yellow - green	358	4.20
Bi(III)	Yellow	378	4.78
Ce(IV)	Yellow	358	3.54
Zr(IV)	Yellow	355	3.81
V(V)	Yellow	386	4.92
U(VI)	Yellow	360	4.49

* pD = −log (minimum detectable mass of metal ion, g/volume, ml).

Study of Vanadium - PASH System

Formation of vanadium complex in aqueous ethanolic solution

The addition of a 0.05% m/V solution of PASH in ethanol to a solution of vanadium(V) ions produces a yellow complex. Absorption spectra of the complex are shown in Fig. 1(A). The complex remains stable for at least 12 h at pH 4.7 (acetate buffer).

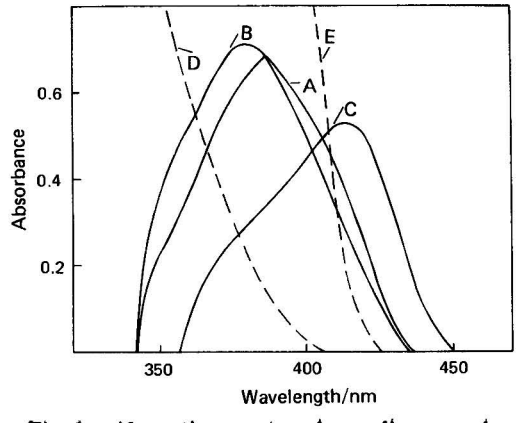


Fig. 1. Absorption spectra of vanadium complexes with PASH. A, Vanadium(V) complex in aqueous ethanolic medium at pH 4.7 (C_V = 2.3 μg ml^{-1}); B, vanadium(IV) complex in aqueous ethanolic medium at pH 4.7 (C_V = 2.3 μg ml^{-1}); and C, vanadium(V) complex extracted into chloroform at pH 1.3 (C_V = 1.4 μg ml^{-1}); D and E, reagent blanks of A and C, respectively.

The influence of pH on absorbance is shown in Fig. 2(A), at 386 nm after a 30-min reaction time. The optimum pH range is 3.5–6.0. The yellow complex is formed immediately in aqueous media (at the optimum pH range) but most of the metallic chelates are insoluble and this causes numerous interferences. It is concluded that in aqueous media the vanadium(V) - PASH complex is not of great analytical interest.

Stoicheiometry of the complex and oxidation state of vanadium

The stoicheiometry of the vanadium(V) complex was evaluated by the continuous variation method and was found to be 1:1 [Fig. 3(A)]. The metal to ligand ratio for vanadium(IV), under analogous conditions, was also 1:1 [Fig. 3(B)].

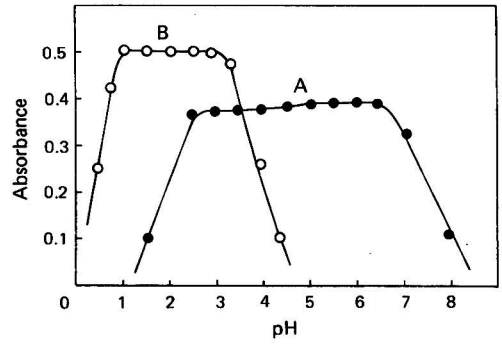


Fig. 2. Influence of pH on the formation of vanadium(V) - PASH complex: A, in aqueous ethanolic solution at 386 nm (C_V = 1.2 μg ml^{-1}); and B, extracted into chloroform at 415 nm (C_V = 1.4 μg ml^{-1}).

From the experimental evidence it was concluded that the reagent forms the yellow complex with vanadium(V). The presence of ascorbic acid in the vanadium(V) solution, before the PASH reagent was added, changed the absorption peak from 386 to 380 nm. When vanadium(IV) was used the presence of ascorbic acid did not alter the absorption peak at 380 nm [Fig. 1(A) and (B)].

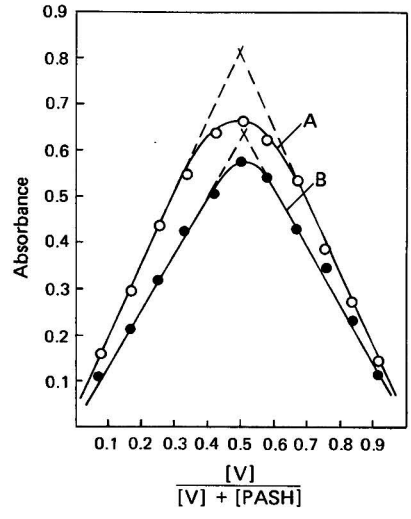


Fig. 3. Stoicheiometry of vanadium complexes, in aqueous media at pH 4.7 (continuous variation method): A, vanadium(V) - PASH complex; and B, vanadium(IV) - PASH complex.

Extraction of the complex

When a solution of PASH in an organic solvent is shaken with an aqueous acidic solution of vanadium(V), the yellow complex is formed immediately in the organic phase. When

chlorobenzene is used the resulting complex is stable; the interferences of foreign ions are high. Of the other solvents tried, chloroform proved to be the best as the complex shows a major bathochromic shift.

The absorption spectrum of the vanadium(V) - PASH complex in chloroform is shown in Fig. 1(C). It is stable for at least 10 h after a 35-min reaction time and presents one band in the visible region, at 415 nm. A bathochromic shift is produced in the absorption spectra of the complex, from 386 nm (aqueous media) to 415 nm (organic media). The reported absorbances were measured at 415 and 425 nm. The latter wavelength was used as the reagent itself does not absorb at this wavelength.

The maximum constant absorbances were obtained in the pH range 0.9–2.8 [Fig. 2(B)].

An aliquot of 10–50 ml of solution containing 14 μg of vanadium(V) was extracted with 10 ml of a solution of 0.01–0.05% *m*/*V* PASH in chloroform. The extraction was quantitative with 0.03% *m*/*V* of the reagent solution and remained constant with increasing concentration. Therefore, 10 ml of 0.05% *m*/*V* reagent solution was adopted as the concentration of solution containing the complexing ligand to be used.

The shaking time was varied from 0.25 to 5 min, while the other variables were kept constant. A shaking time of between 1 and 2.5 min did not produce any change in absorbance if the volume ratio $V_{org.}:V_{aq.}$ was between 1:1 and 1:2.5. On the other hand, shaking for 2 min was necessary for the complete extraction of vanadium(V) if the volume ratio $V_{org.}:V_{aq.}$ was 1:5.

Spectrophotometric Determination of Vanadium(V) with PASH

Based on the experimental work, a method is proposed for the determination of trace amounts of vanadium involving the formation of the yellow complex with PASH and its extraction into chloroform. Beer's law is obeyed between 0.5 and 2 μg ml^{-1} of vanadium(V) in the organic phase at 415 nm. The optimum concentration range, evaluated by Ringbom's method, is 1–1.75 μg ml^{-1} of vanadium.

The yellow complex gave a molar absorptivity of $\varepsilon = 1.87 \times 10^4$ l mol^{-1} cm^{-1} at 415 nm [in the chloroform phase (10 ml)]. The Sandell sensitivity of the method is 0.003 μg cm^{-2} of vanadium. The precision was estimated for 10–50-ml aliquots of 10 μg of vanadium(V) solution, and the relative error of the method is 0.44%. The method is more sensitive for vanadium than many existing methods, and it has been compared, advantageously, with other methods previously reported that use related ligands (Table II).

TABLE II

CHARACTERISTICS OF VANADIUM - HYDRAZONE COMPLEXES

Compound	Optimum acidity	$\lambda_{max.}$/nm	Molar absorptivity/ l mol^{-1} cm^{-1}	Metal to ligand ratio	Reference
Hydrazinium hydrazinecarbodithioate	pH 4–6	460	1100	1 : 2	16
Anthranilic acid isopropylidenehydrazide	0.2–1.0 N H_2SO_4	525	5100	1 : 2	17
Nicotinic acid hydrazide	pH 1.8–2.2	420	750	1 : 1	18
Pyridine-2-acetaldehyde salicyloylhydrazone..	pH 0.9–2.8	415	18700	1 : 1	—

Effect of Foreign Ions

For the determination of 10 μg of vanadium by this method, the foreign ions can be tolerated at the levels given in Tables III and IV. The limiting value of the concentration of foreign ions was taken as that value which caused an error of not more than 2.5% in the absorbance. Cations were added in the form of chlorides, nitrates or acetates to a maximum of 10000 μg ml^{-1}; anions were added in the form of sodium or potassium salts.

Several foreign ions, at high concentrations, produce a general decrease in the absorbance of the vanadium - PASH chelate in a wide range of concentrations. This effect is summarised in Table V. From the data in this table it may be assumed that vanadium can be determined, without great error, in the presence of these ionic concentrations, but with a lower sensitivity. In these instances, standards and samples must be matched for the foreign ion concentration.

TABLE III

TOLERANCE LIMITS OF EXTRACTIVE DETERMINATION OF VANADIUM(V)

Results obtained using a 10-μg sample of vanadium.

Ion added	Amount tolerated/ μg ml^{-1}
La(III), Cd(II), Pb(II), As(V), Ba(II), Sr(II), ammonium, alkali metals, Cl^-, Br^-, ClO_3^-, ClO_4^-, NO_3^-, BO_2^-, SO_4^{2-}, CO_3^{2-},* PO_4^{3-}, BO_3^{3-}, benzoate, acetate, citrate	10000
Mg(II), Ca(II), SiO_3^{2-}	5000
Mn(II), Be(II), tartrate	2500
Al(III), Cr(III), Se(IV), Ag(I), Hg(I), Zn(II), Tl(I), As(III), CO_3^{2-}, $B_4O_7^{2-}$, F^-, phthalate, dimethylglyoxime (DMG)	1000
Zr(IV), In(III), $S_2O_3^{2-}$	750
Th(IV), EDTA	500
U(VI), oxalate	250
H_2O_2	200
Sn(IV), Hg(II), Co(II), Fe(III), Ti(IV), CN^-, $Fe(CN)_6^{3-}$	100

* Heating gently before extracting.

TABLE IV

ELIMINATION OF INTERFERENCES BY ADDITION OF MASKING AGENTS

Foreign ion	Amount tolerated/μg ml^{-1} Without masking agent	With masking agent	Masking agent
Mo(VI)	50	250	Tartrate, 1000 μg ml^{-1}
W(VI)	10	100	Tartrate, 1000 μg ml^{-1}
Fe(II)	2	100*	H_2O_2, 200 μg ml^{-1}
Sn(II)	25	100*	HNO_3, 1 ml
Ce(IV)	2	10	PO_4^{3-}, 500 μg ml^{-1}
Pd(II)	1	25	DMG,† 10 mg
Sb(III)	1	10	Tartrate, 2500 μg ml^{-1}
I^-	50	100*	HNO_3, 1 ml

* Heating before extracting.
† DMG = dimethylglyoxime.

The tolerance limits for the extractive determination of 10 μg of vanadium for copper(II), nickel(II) and bismuth(III) are low, 1, 2 and 2 μg ml^{-1}, respectively. To remove these metal ions, when present, a cation-exchange column was used.[19,20] The following solutions were percolated through 5 g of cation-exchange resin: 10 mg of nickel(II) in 10 ml of 1 M hydrochloric acid; 5 mg of copper(II) in 10 ml of 1 M hydrochloric acid; 10 mg of nickel(II) in 10 ml of 1 M nitric acid; 5 mg of copper(II) in 10 ml of 1 M nitric acid; 10 mg of bismuth(III) in 10 ml of 1 M nitric acid. In addition each of these solutions contained 10 μg of vanadium(V) and 2 drops of a 3% *m*/*V* solution of hydrogen peroxide. The vanadium(V) was eluted using 20 ml of 1 M hydrochloric acid [when copper(II) and nickel(II) were present] or 1 M nitric acid [when copper(II), nickel(II) and bismuth(III) were present]. The flow-rate was kept at 3 ml min^{-1}. The eluate was neutralised with concentrated sodium hydroxide solution and then the extractive - spectrophotometric method was applied as described above. The recovery of vanadium was 92.6–105.7%. The precision was estimated for 10 μg of vanadium in 10 ml of 1 M hydrochloric acid or 1 M nitric acid, and the relative error of the method was less than 3%. If necessary this cation-exchange procedure may be applied to other foreign ions; for example, 10 mg of cobalt(II) can be separated from 10 μg of vanadium by applying this procedure to 10 ml of a solution in 1 M hydrochloric acid.

Applications

The method has been applied satisfactorily to the determination of vanadium in different materials.

TABLE V

EFFECT OF SOME FOREIGN IONS AT HIGH CONCENTRATIONS ON THE ABSORPTION OF VANADIUM - PASH CHELATE

Absorbance of a 10-μg sample of vanadium is 0.367.

Ion added	Concentration/ μg ml^{-1}	Absorbance	Vanadium recovery*/ μg ml^{-1}
Zn(II)	5000	0.470	1.28
	7500	0.465	1.27
Cr(III)	5000	0.210	0.57
	7500	0.216	0.59
	10000	0.212	0.58
Ag(I)	5000	0.268	0.73
	7500	0.275	0.75
	10000	0.268	0.73
Hg(I)	2500	0.250	0.68
	5000	0.253	0.69
Al(III)	2500	0.295	0.80
	5000	0.269	0.73
	7500	0.293	0.80
	10000	0.297	0.81
As(II)	2500	0.344	0.94
	5000	0.335	0.92
	7500	0.342	0.93

* Results are the means of three determinations.

Determination of vanadium in a mineral, a steel and a phosphoric acid sample

Results of the analysis of vanadium in mineral and steel samples from the Bureau of Analysed Samples Ltd. support the precision and reliability of this method. Lead - vanadium concentrates and high-speed steel were dissolved in a mixture of concentrated nitric and perchloric acids (2 + 1 *V*/*V*) and aqua regia, respectively. Triplicate results were obtained in both instances.

Lead - vanadium concentrates (BAS No. 70aG) had the following certificate composition: lead 65.4 and vanadium(V) oxide 3.20%. The vanadium content found was 3.18 ± 0.03% (for V_2O_5).

High-speed steel (BAS No. 64b) had the following certificate composition: carbon 0.9, vanadium 1.99, chromium 4.55, molybdenum 4.95 and tungsten 7.05%. The vanadium content found was 1.96 ± 0.02%.

A phosphoric acid sample, used in the detergent industry, has also been analysed for vanadium. The average composition of the phosphoric acid analysed was phosphorus(V) oxide 38, sulphuric acid 3.5, calcium oxide 0.15, silica 1, magnesium oxide 0.7, aluminium oxide 0.3, fluoride 2, iron 0.19% *m*/*V*, and vanadium and chromium 204 and 215 μg ml^{-1}, respectively. The vanadium found by the spectrophotometric determination was 207 ± 2 μg ml^{-1} (the mean result of four determinations).

Determination of vanadium by the standard additions method

The majority of the methods for the determination of parts per million and sub-parts per million amounts of vanadium require pre-concentration of vanadium by coprecipitation,[21–24] ion exchange[21,25–28] or liquid - liquid extraction.[29–34] The PASH method may also be applied to the determination of vanadium at parts per billion levels (parts per 10^9), which decreases the lower limit of the vanadium determination through the taking of a larger volume of the aqueous phase in relation to the chloroform phase and applying the standard additions method.[13]

The method consists in adding several increasing known amounts of vanadium(V) (0, 1.25, 2.5 and 3.75 μg) to four aliquots of sample solution. The extraction procedure described above is then applied and the absorbances are measured at 425 nm. The absorbances are plotted against the concentrations of the four vanadium-containing solutions of each sample. The straight line is extrapolated back to the concentration axis and the negative intercept gives the concentration of the sample solution.

All parameters in the regression equation were calculated by the principle of least squares and all regression curves were practically linear, the correlation coefficients being equal to or higher than 0.995.

This method was applied to the phosphoric acid sample mentioned above. The sample solution was first diluted 1000 times and then aliquots of 5, 10 or 20 ml were taken. The results obtained are shown in Table VI. The vanadium content was found to be 4.12 ± 0.13 μg (sample A, 20-ml aliquots of the dilute sample), 2.05 ± 0.06 μg (sample B, 10-ml aliquots of the dilute sample) and 1.07 ± 0.04 μg (sample C, 5-ml aliquots of the dilute sample).

TABLE VI

REGRESSION ANALYSIS OF CURVES BASED ON STANDARD ADDITIONS METHOD FOR DETERMINATION OF VANADIUM

Sample*	Aliquot taken/ml	Equation of regression curve	Correlation coefficient
A	20	$y = 0.0296x \pm 0.1221$	0.998
B	10	$y = 0.0304x \pm 0.0624$	0.998
C	5	$y = 0.0295x \pm 0.0316$	0.996
D	50	$y = 0.0367x \pm 0.0887$	0.999
E	50	$y = 0.0354x \pm 0.0466$	0.998

* A, B and C correspond to the phosphoric acid sample mentioned in text (dilute 1000 times, previously). D and E correspond to vanadium-containing solutions in 0.1 M potassium chloride (50 and 25 μg l^{-1}, respectively).

In order to study the accuracy of the standard additions method, two vanadium-containing solutions were made, containing 50 and 25 μg of vanadium(V) in 1000 ml of 0.1 M potassium chloride solution. The method was applied to 50-ml aliquots of both samples as described above. The results obtained are shown in Table VI, samples D and E. The vanadium recovery was 96.7% for 50 μg l^{-1} of vanadium(V) and 105.2% for 25 μg l^{-1} of vanadium(V).

Conclusion

Other methods are available for the determination of trace amounts of vanadium using hydrazones (Table II), but none of them is completely satisfactory. This paper describes a study of the optimum conditions for a selective and sensitive spectrophotometric method for the determination of vanadium. The method is relatively free from interferences because most of the metallic chelates of PASH are not extracted into chloroform and the absorption peaks of these chelates are in the ultraviolet region.

References

1. Katiyar, S. S., and Tandon, S. N., *Talanta*, 1964, **11**, 892.
2. Vasilikiotis, G. S., and Tossidis, J. A., *Microchem. J.*, 1969, **14**, 380.
3. Uno, T., and Taniguchi, H., *Bunseki Kagaku*, 1971, **20**, 997.
4. Odashima, T., and Ishii, H., *Nippon Kagaku Kaishi*, 1973, 729.
5. Vasilikiotis, G. S., *Microchem. J.*, 1968, **13**, 526.
6. Capitan, F., Salinas, F., and Gimenez Plaza, J., *Ars Pharm.*, 1975, **16**, 293.
7. Gallego, M., Garcia-Vargas, M., Pino, F., and Valcarcel, M., *Microchem. J.*, 1978, **23**, 353.
8. Aggarwal, R. C., and Rao, T. R., *Transition Met. Chem.*, 1977, **2**, 21.
9. Aggarwal, R. C., and Rao, T. R., *Transition Met. Chem.*, 1977, **2**, 59.
10. Domiano, P., Musatti, A., Nardelli, M., and Pelizzi, C., *J. Chem. Soc., Dalton Trans.*, 1975, 295.
11. Vasilikiotis, G. S., and Kouimtzis, Th. A., *Microchem. J.*, 1973, **18**, 85.
12. Rastogi, D. K., Sahni, S. K., Rana, V. B., and Dua, S. K., *Transition Met. Chem.*, 1978, **3**, 56.
13. Gallego, M., Garcia-Vargas, M., and Valcarcel, M., *Analyst*, 1979, **104**, 613.
14. Erdey, L., "Gravimetric Analysis," Part II, Pergamon Press, Oxford, 1965, p. 562.
15. Phillips, J. P., and Merrit, L. L., *J. Am. Chem. Soc.*, 1948, **70**, 410.
16. Byr'ko, V. M., Busev, A. I., Tikhonova, T. I., Baibakoba, N. V., and Shepel, L. I., *Zh. Anal. Khim.*, 1975, **30**, 1885.
17. Dolgorev, A. V., and Karpova, O. I., *Zavod. Lab.*, 1974, **40**, 771.
18. Krych, Z., and Lipiec, T., *Chem. Anal. (Warsaw)*, 1967, **12**, 535.
19. Strelow, F. W. E., Rethemeyer, R., and Bothma, C. J. C., *Anal. Chem.*, 1965, **37**, 106.
20. Strelow, F. W. E., Van Zyl, C. R., and Bothma, C. J. C., *Anal. Chim. Acta*, 1969, **45**, 81.
21. Chan, K. M., and Riley, J. P., *Anal. Chim. Acta*, 1966, **34**, 337.
22. Sugawara, K., Tanaka, M., and Naitô, H., *Bull. Chem. Soc. Jpn.*, 1953, **26**, 417.

23. Naitô, H., and Sugawara, K., *Bull. Chem. Soc. Jpn*, 1957, **30**, 799.
24. Ishiabshi, M., Fujinaga, T., Kuwamoto, T., and Sawamoto, H., *J. Chem. Soc. Jpn, Pure Chem. Sect.*, 1964, **85**, 763.
25. Kiriyama, T., and Kuroda, R., *Anal. Chim. Acta*, 1972, **62**, 464.
26. Nevoral, V., and Okáč, A., *Cesk. Farm.*, 1966, **15**, 229.
27. Linstedt, K. D., and Kruger, P., *Anal. Chem.*, 1970, **42**, 113.
28. Korkisch, J., and Krivanec, H., *Anal. Chim. Acta*, 1976, **83**, 111.
29. Chan, Y. K., and Lum-Shue-Chan, K., *Anal. Chim. Acta*, 1970, **50**, 201.
30. Crump-Wiesner, H. J., Feltz, H. R., and Purdy, W. C., *Anal. Chim. Acta*, 1971, **55**, 29.
31. Zhavoronkina, T. K., *Tr. Morsk. Gidrofiz. Inst.*, 1960, **19**, 38.
32. Morris, A. W., *Anal. Chim. Acta*, 1970, **42**, 113.
33. Nishimura, M., Matsunaga, K., Kudo, T., and Obara, F., *Anal. Chim. Acta*, 1973, **65**, 466.
34. Abbasi, S. A., *Anal. Chem.*, 1976, **48**, 714.

Received *March 27th*, 1980
Accepted *April 25th*, 1980

Analyst, October, 1980, Vol. 105, pp. 974–980

Determination of Cyanide in Animal Feeding Stuffs

J. R. Harris, G. H. J. Merson, M. J. Hardy* and D. J. Curtis

Department of Industry, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, SE1 9NQ

A method for the determination of cyanide in feeding stuffs has been developed. Naturally occurring cyano-substituted glycosides are subjected to enzymatic hydrolysis, the liberated cyanide is isolated by aeration and determined either by a spectrophotometric method or by gas chromatography. Recoveries of cyanide added to feeding stuffs at concentrations of 10 and 20 mg kg^{-1} were approximately 98%. The method is sensitive to as little as 1 mg kg^{-1} of cyanide.

Keywords: Cyanide determination; animal feeding stuffs; enzymatic hydrolysis; spectrophotometry; gas chromatography

Cyanide in trace amounts is found in a large number of plants, mainly in the form of cyano-substituted glycosides. Relatively high concentrations are found in certain grasses, pulses, roots and fruit kernels. Glycosides that have been positively identified include amygdalin in fruit kernels, especially bitter almonds; dhurrin in sorghum and other grasses; and linamarin in pulses, linseed and cassava. The structures and reactions by which these glycosides release free cyanide have been described by Montgomery[1] and Conn.[2] Of the plant products mentioned above, sorghum, linseed (after extraction of linseed oil) and cassava are important ingredients in animal feeding stuffs but their use is restricted by the presence of the cyano-substituted glycosides. The EEC Directive[3] on the control of undesirable substances in feeding stuffs prescribes maximum concentrations of hydrogen cyanide for both straight and complete feeding stuffs, ranging from 350 mg kg^{-1} (as hydrogen cyanide)for linseed cake down to 10 mg kg^{-1} for complete feeding stuffs for chicks.

The EEC method[4] prescribed for the determination of cyanide involves enzymatic hydrolysis, steam distillation and silver nitrate (Volhard) titration. The method is not specific for hydrogen cyanide and its lack of sensitivity is such that for concentrations of 10 mg kg^{-1}, only 0.2 ml of 1 N silver nitrate solution is consumed. This paper describes a procedure that is free from interferences and its sensitivity permits as little as 1 mg kg^{-1} to be determined.

As Cooke[5] pointed out, most methods for the determination of cyanide have three stages: (*i*) hydrolysis of the cyano-substituted glycoside; (*ii*) isolation of the cyanide, for example by steam distillation or aeration; (*iii*) determination of the cyanide. This author states that most of the difficulties arise in stages (*i*) and (*ii*), as there are good techniques for cyanide determination; we would agree with Cooke although in our experience stage (*ii*), the isolation of the cyanide, is the most troublesome. Although methods for the determination of cyanide in plant materials have been published by Cooke,[5] Wood,[6] Blaedel *et al.*[7] and Winkler,[8] none of these is directly applicable to compound animal feeding stuffs. In the determination stage Bark[9] considers that, for trace amounts of cyanide, the most suitable method is the spectrophotometric procedure based on the König synthesis of a pyridine dyestuff by the reaction between cyanogen bromide and a suitable aromatic amine. In a further paper, Bark and Higson[10] recommended *p*-phenylenediamine as the most suitable coupling agent; this compound is specified in the method for the determination of cyanide in water,[11] and has been adopted in the proposed method. More recently, gas-chromatographic methods[12–14] for trace amounts of cyanide have been described. In these methods the cyanide ion is reacted with bromine to produce cyanogen bromide, which, after separation on the gas chromatographic column, is determined by the use of an electron-capture detector. This procedure is included in the proposed method as an alternative to the spectrophotometric procedure.

* Present address: Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey.

Experimental

Reagents

Orthophosphoric acid, 10% *V*/*V* and 0.1 M.

Hydrochloric acid, d = 1.18 g ml^{-1} *and* 0.5 M.

Sodium hydroxide solutions, approximately 0.5 *and* 1 M.

Phosphate buffer solution, pH 7.0. Dissolve 27.22 g of potassium dihydrogen orthophosphate in water and dilute to 1 l with water. Adjust the pH of the solution to 7.0 with 5 M sodium hydroxide solution.

Bromine water. Saturated solution.

Arsenious acid solution. Dissolve 2 g of arsenic(III) oxide in 100 ml of water by boiling under reflux.

Pyridine solution. Mix 150 ml of pyridine with 100 ml of water and add 25 ml of hydrochloric acid (d = 1.18 g ml^{-1}).

p-*Phenylenediamine dihydrochloride solution*. Dissolve 0.17 g of *p*-phenylenediamine hydrochloride (or 0.1 g of *p*-phenylenediamine) in 50 ml of 0.5 M hydrochloric acid.

p-*Phenylenediamine - pyridine reagent*. For each determination mix 15 ml of pyridine solution with 5 ml of *p*-phenylenediamine dihydrochloride solution immediately before the test.

Diisopropyl ether.

Phenol, 5% m/V aqueous solution.

Potassium cyanide stock solution. Dissolve 1.563 g of potassium cyanide in water, add 25 ml of approximately 1 M sodium hydroxide solution and dilute to 250 ml. Standardise the solution against 0.1 M silver nitrate solution (1.0 ml of 0.1 M silver nitrate solution ≡ 2.5 mg of CN^-).

Potassium cyanide intermediate stock solution. To 10.0 ml of stock solution add 50 ml of approximately 1 M sodium hydroxide solution and dilute to 500 ml (1.0 ml ≡ 50 μg of CN^-).

Potassium cyanide working solution. To 10.0 ml of intermediate stock solution add 50 ml of approximately 1 M sodium hydroxide solution and dilute to 500 ml. Prepare the intermediate and working solutions freshly as required (1.0 ml ≡ 1.0 μg CN^-).

Almond suspension. Chop very finely or grind (preferably in a coffee grinder) 10 blanched sweet almonds and suspend in 100 ml of water. Shake the mixture thoroughly before use.

Apparatus

Aeration apparatus. The apparatus consists of a train of four flasks, each fitted with a Drechsel-bottle head. The reaction flask is of 500-ml capacity, round bottomed with a long neck. The flask is immersed in a water-bath at 37–40 °C.

Preceding the reaction flask is a conical flask (250 or 500 ml) containing 0.5 M sodium hydroxide solution (to remove trace amounts of sulphur dioxide from the incoming air). Following the reaction vessel are two conical flasks (250 ml), each containing 50 ml of 0.5 M sodium hydroxide solution for absorption of the liberated hydrogen cyanide.

Spectrophotometer. With 10-mm cells, operated at 515 nm. (A Cecil CE595 double-beam ultraviolet spectrophotometer was used.)

Gas chromatograph. Fitted with an electron-capture detector. (A Pye Unicam GCV fitted with a nickel-63 electron-capture detector was used.)

Column. Silanised glass, 1 m × 4 mm i.d., containing Porapak Q (80–100 mesh). Operating temperatures: column, 100 °C; injector, 125 °C; detector, 150 °C.

Procedure

Extraction

Weigh, to the nearest 0.005 g, approximately 20 g of the finely divided and mixed sample (previously ground to pass a 1-mm sieve) and transfer it into a 500-ml conical flask. Add by pipette 200 ml of 0.1 M orthophosphoric acid, stopper the flask and shake for 1 h. Allow the contents of the flask to settle and decant the supernatant liquid into a 250-ml centrifuge bottle and spin at 2500 rev min^{-1} for 5 min. Transfer the supernatant liquid into a clean dry flask.

Enzymatic hydrolysis

Place approximately 250 ml of the phosphate buffer solution into the enzymolysis flask and add 10 ml of sweet almond suspension and two drops of antifoam. Introduce by pipette 50 ml of the sample extract prepared as described in the previous section. Fit the bubbler system on to the enzymolysis flask and aerate the contents with an air flow of approximately 1.5 l min^{-1} for 18 h, maintaining the temperature of the reaction mixture at 37–40 °C by means of a water-bath. Collect the liberated hydrogen cyanide in two 250-ml conical flasks arranged in series and each containing 50 ml of approximately 0.5 M sodium hydroxide solution. Quantitatively combine the solutions in a 200-ml calibrated flask, dilute to the mark with water and mix.

Blank

Carry out a blank enzymatic hydrolysis using 50 ml of 0.1 M orthophosphoric acid in place of the sample extract and following the procedure described under *Enzymatic hydrolysis*.

Determination

Spectrophotometric method

Into a 50-ml calibrated flask transfer an aliquot portion of the sample solution obtained from the enzymatic hydrolysis, not exceeding 20 ml and expected to contain not more than 20 μg of cyanide (as CN^-). In a second 50-ml calibrated flask place an identical volume of the hydrolysis blank solution. To each flask add 0.5 ml of hydrochloric acid ($d = 1.18\,g\,ml^{-1}$), mix and then immediately add 0.5 ml of bromine water. Stopper the flasks to prevent losses of cyanogen bromide, mix and allow to stand for 2 min. Add to each flask 0.5 ml of arsenious acid, mix thoroughly to remove excess of bromine, then add 20.0 ml of *p*-phenylenediamine - pyridine reagent, dilute to the mark with water, mix and allow to stand for 40 min. Without further delay measure the absorbance of the solutions at 515 nm in 10-mm cells against water as reference. Subtract the absorbance of the hydrolysis blank from that of the sample and determine the amount of cyanide in the sample solution by reference to the calibration graph.

Calibration graph. Transfer by pipette into a series of 50-ml calibrated flasks 5, 10, 15 and 20 ml of the cyanide working solution and dilute as necessary to 20 ml with water. In addition include one flask containing 20 ml of water without cyanide as the reagent blank. Proceed as described above from "To each flask add 0.5 ml of hydrochloric acid . . ." Measure the absorbance of each solution after 40 min at 515 nm in 10-mm cells against water as reference. Subtract the absorbance of the reagent blank from those of the cyanide standards and plot the calibration graph using the net absorbance values on the ordinate and the corresponding masses of cyanide (as micrograms of CN^-) on the abscissa.

Gas-chromatographic method

Into a 100-ml separating funnel transfer an aliquot portion of the sample solution obtained from the enzymatic hydrolysis, not exceeding 20 ml and expected to contain not more than 8 μg of cyanide (as CN^-)*. Into a second separating funnel place an identical volume of the hydrolysis blank solution. Make up the volumes to 20 ml if necessary. Add 5 ml of 10% *V*/*V* orthophosphoric acid solution and 0.5 ml of bromine water. Shake each funnel and allow to stand for 15 min, then add 0.2 ml of 5% *m*/*V* aqueous phenol solution and gently shake. Add 20.0 ml of diisopropyl ether to each funnel, stopper firmly and shake vigorously for 2 min. Allow the phases to separate, then discard the lower aqueous phase and transfer the diisopropyl ether layer into a vial fitted with a septum cap. Inject 1.0 μl of each solution on to the column and measure the heights of the cyanogen bromide peaks obtained. Subtract the peak height of the cyanogen bromide in the hydrolysis blank solution (if any) from that of the sample and determine the amount of cyanide in the solution by reference to the calibration graph.

Calibration graph. Transfer by pipette into a series of 100-ml separating funnels 2, 4, 6, 8 and 10 ml of cyanide working solution, dilute as necessary to 20 ml with water. In addition

* The amount of cyanide in the solution injected into the gas chromatograph must be within the linear range of the detector. In some instances it may be necessary to reduce both the recommended maximum content in the sample solution and the amounts prescribed for the calibration graph in order to meet this requirement.

include one separating funnel containing 20 ml of water without cyanide as the reagent blank. Proceed as described above from "Add 5 ml of 10% *V*/*V* orthophosphoric acid . . ." Measure the heights of the cyanogen bromide peaks and, after correcting for the reagent blank, plot the calibration graph of corrected peak height against the corresponding mass of cyanide (as micrograms of CN^-).

Calculation

The same calculation is applicable to both procedures.

The cyanide content of the feed in milligrams per kilogram is given by

$$\frac{800 \times A}{M \times V}$$

where A = mass of cyanide (as micrograms of CN^-) present in the aliquot portion of the sample extract, M = mass of test portion (grams) and V = volume of the aliquot portion taken for the determination (millilitres).

Results and Discussion

Recovery of Cyanide from Cyano-substituted Glycosides

The efficiency of the proposed method was assessed by adding to the orthophosphoric acid used in the extraction stage known amounts of amygdalin (Aldrich Chemical Co. Ltd; cyanide content 5.69% *m*/*m* as CN^-) and linamarin (Calbiochem, Bishops Stortford, Hertfordshire; cyanide content 10.52% *m*/*m* as CN^-) and following the procedure described above. The results are given in Table I and show that near-quantitative yields of cyanide were obtained.

TABLE I

RECOVERY OF CYANIDE FROM AMYGDALIN AND LINAMARIN IN THE ABSENCE OF FEED

		Spectrophotometric method		GC method	
Glycoside	CN^- added as substrate/ μg	CN^- found/ μg	Recovery, %	CN^- found/ μg	Recovery, %
Amygdalin..	200	200	100.0	197	98.5
		197	98.5	199	99.5
		201	100.5	196	98.0
	400	405	101.3	—	—
		389	97.3	—	—
		397	99.3	—	—
Linamarin..	200	194	97.0	196	98.0
		197	98.5	201	100.5
		199	99.5	197	98.5
		191	95.5	—	—
		191	95.5	—	—
		198	99.0	—	—

Having established the efficiency and reliability of the method when applied to amygdalin and linamarin, it was then applied to a series of compound feeding stuffs fortified with these two glycosides. The results of these studies are shown in Table II, from which the recovery of cyanide and the repeatability of the method can be seen to be good. The mean recovery by the spectrophotometric method was 97.9% with a standard deviation of 1.24%. Corresponding figures for the gas-chromatographic method were 98.2% and 0.98%, respectively. For samples where both the spectrophotometric and gas-chromatographic methods were used a comparison of the results showed no significant difference between the procedures. No cyanide was detected in the unfortified feeds.

Determination of Cyanide in Straight and Compound Feeding Stuffs

Several straight feeding stuffs and a compound feeding stuff containing linseed expeller were examined by the proposed method. The results shown in Table III demonstrate the good repeatability of the method and the high level of agreement between the spectrophotometric and gas-chromatographic procedures.

As a further check on the recoveries of cyanide the method was applied to the products listed in Table III after fortification with amygdalin and linamarin at concentrations equivalent to 10 mg kg^{-1} of CN^-. Before these experiments could be conducted it was necessary to remove the cyanide arising from natural glycosides present. This was achieved by subjecting the products to the extraction and enzymatic hydrolysis procedures described above before adding known amounts of amygdalin and linamarin and then performing a second enzymolysis. Unfortified extracts treated similarly yielded no cyanide, indicating that the first enzymolysis was effective in removing all the cyanide initially present. The results of these experiments are given in Table IV and demonstrate the good recoveries given by the method.

TABLE II

RECOVERY OF CYANIDE FROM ANIMAL FEEDS FORTIFIED WITH AMYGDALIN AND LINAMARIN

			Spectrophotometric method		GC method	
Feed (20.0-g sample)	Glycoside	CN^- added/ μg	CN^- found/ μg	Recovery, %	CN^- found/ μg	Recovery, %
Chick mash A ..	Amygdalin	200	196	98.0	194	97.0
			197	98.5	199	99.5
			197	98.5	197	98.5
			191	95.5		
			194	97.0		
			197	98.5		
			194	97.0		
			191	95.5		
Chick mash B ..	Linamarin	200	194	97.0	195	97.5
			196	98.0	195	97.5
			196	98.0	198	99.0
			193	96.5		
	Linamarin	400	395	98.8		
			400	100.0		
			386	96.5		
			381	95.3		
			386	96.5		
			391	97.8		
Layers mash.. ..	Amygdalin	200	195	97.5		
			199	99.5		
			197	98.5		
	Linamarin	200	200	100.0		
			197	98.5		
			197	98.5		
Dairy ration containing approximately 18% *m/m* of rape meal	Amygadlin	200	195	97.5		
			197	98.5		
			197	98.5		
	Linamarin	200	197	98.5		
			195	97.5		
			198	99.0		
			199	99.5		

Liberation and Isolation of Cyanide

In preliminary investigations the enzymatic hydrolysis was allowed to proceed for about 16 h at 37 °C in a sealed flask. At the end of this period the liberated cyanide was steam distilled from the reaction mixture under weakly acidic conditions. Although quantitative yields of cyanide from amygdalin and potassium cyanide were recorded in the absence of a feed matrix, the inclusion of such a matrix (ground wheat) reduced the recovery to 80–90% of the theoretical yield of cyanide. When potassium cyanide was added to ground wheat and the mixture was distilled immediately complete recovery of cyanide was obtained. These observations indicated the possibility of a reaction between the liberated cyanide and the feed matrix. If the cyanide could be removed from the reaction mixture whilst the enzymatic hydrolysis was in progress then improved yields might be obtained. An aeration technique was therefore employed with an air flow-rate of 1.5 l min^{-1} and the liberated cyanide collected

in 0.5 M sodium hydroxide solution. Improved yields were obtained but these were not consistent and on re-checking the yield of cyanide from amygdalin in the absence of a feed matrix a result of about 110% was recorded using the spectrophotometric procedure. Further investigation showed that as a result of using an acetate buffer to maintain a pH of 5.0 (as recommended by the suppliers of the β-glucosidase enzyme used at this stage of the work)

TABLE III

RESULTS OF ANALYSIS: CYANIDE CONTENT OF FEEDS AND NATURAL PRODUCTS

Matrix	CN^- found/μg g^{-1}	
	Spectrophotometric method	GC method
Expeller linseed	380, 392, 395, 392	385, 380, 378, 370
Feed containing 4% *m/m* linseed	8.6, 8.5, 8.3	8.6, 8.6, 8.9
Ground cassava tubers ..	159, 157, 150	—
Sorghum (seeds)	1.5, 1.4, 1.5	—
Alfalfa	4.3, 4.0, 4.1	—

acetic acid was being carried over during the aeration stage and was enhancing the absorbance readings. This effect could not be compensated for by making blank determinations as it only became manifest in the presence of cyanide. When the acetate buffer was replaced by a phthalate buffer at pH 5.0 the carry-over problem was eliminated. In applying the method to expeller linseed using phthalate buffers at varying pH values, the maximum yield of cyanide was obtained at pH 7.0. Following the work of Cooke,[5] the phthalate buffer was subsequently replaced by one containing potassium dihydrogen phosphate at pH 7.0.

The method described by Cooke[5] employs extraction of the cyano-substituted glycosides by orthophosphoric acid, thereby obviating the need for steam-distillation or aeration. Cooke's method did not appear to be wholly applicable to animal feeding stuffs but the principle of extracting the glycosides from the feed before proceeding with the enzymatic hydrolysis aeration stage offered a further means of reducing the matrix effects discussed above. The orthophosphoric acid extraction stage was therefore combined with the aeration procedure and gave consistent, quantitative yields, as shown in the tables.

TABLE IV

RECOVERY OF CYANIDE FROM FEED MATERIALS FORTIFIED WITH AMYGDALIN AND LINAMARIN

Matrix	Glycoside	Recovery, %	
		Spectrophotometric method	GC method
Expeller linseed	Amygdalin	95.7, 98.7, 97.4	96.5, 98.3, 97.1
	Linamarin	96.4, 95.9, 97.3	97.1, 97.5, 96.9
Feed with 4% *m/m* linseed ..	Amygdalin	98.0, 98.5, 99.6	97.3, 97.3, 96.4
	Linamarin	94.2, 97.6, 97.4	95.9, 95.1, 96.7
Cassava	Amygdalin	96.3, 96.8, 99.1	—
	Linamarin	95.9, 96.3, 97.1	—
Sorghum (seeds)	Amygdalin	97.8, 96.3, 96.9	—
	Linamarin	95.6, 96.5, 95.0	—
Alfalfa	Amygdalin	92.3, 94.3, 95.1	—
	Linamarin	93.9, 95.6, 96.2	—

Enzyme Sources

The EEC method[4] for cyanide in feeding stuffs prescribes the use of blanched sweet almonds as the enzyme source in the liberation of cyanide from the glycosides. As purified enzymes are now readily available it was decided at the start of this work to use commercial β-D-glucosidase (E.C. 3.2.1.21) in place of the sweet almond suspension with a view to standardising the reagent. Although cyanide was liberated from expeller linseed in the presence of purified β-glucosidase, little or none was obtained from linamarin, the cyano-substituted glycoside of linseed. However, by using as the enzyme source an aqueous suspension of sweet almonds, good yields of cyanide were obtained from linamarin. The reasons for the

very low yields of cyanide when β-D-glucosidase was used are not known, one explanation might be that in preparing the pure enzyme (from almonds) trace amounts of other materials, including enzymes necessary for the hydrolysis of linamarin, have been removed. The fact that some cyanide was liberated from expeller linseed in the presence of purified β-D-glucosidase was undoubtedly due to the presence of endogenous enzymes, the added enzyme being superfluous to the reaction.

From these observations it was concluded that in a general method for the determination of cyanide in feeding stuffs, an enzyme system capable of acting on several glycosides must be present. The sweet almond suspension seems to supply the necessary range of enzymes and has therefore been retained in the proposed method in preference to pure enzyme sources.

We thank the Government Chemist for permission to publish this paper.

References

1. Montgomery, R. D., *in* Liener, I. E., *Editor*, "Toxic Constituents of Plant Foodstuffs," Academic Press, New York, 1969, p. 143.
2. Conn, E. E., *in* "Toxicants Occurring Naturally in Foods," National Academy of Sciences Publication 1354, Natural Research Council, Washington, D.C., 1966.
3. Council Directive 74/63 EEC, *Official Journal of the European Communities*, L38, 11th February 1974, p. 31.
4. Commission Directive 71/250 EEC, *Official Journal of the European Communities*, L155, 12th July 1971, p. 13.
5. Cooke, R. D., *J. Sci. Food Agric.*, 1978, **29**, 345.
6. Wood, T., *J. Sci. Food Agric.*, 1965, **16**, 300.
7. Blaedel, W. J., Easty, D. B., Anderson, L., and Farrell, T. R., *Anal. Chem.*, 1971, **43**, 890.
8. Winkler, W. O., *J. Assoc. Off. Agric. Chem.*, 1951, **34**, 541.
9. Bark, L. S., *Ind. Chem.*, 1962 (October), 255.
10. Bark, L. S., and Higson, H. G., *Talanta*, 1964, **11**, 471.
11. Department of the Environment, "Analysis of Raw, Potable and Waste Waters," HM Stationery Office, London, 1972, p. 227.
12. Nota, G., and Palombari, R., *J. Chromatogr.*, 1973, **84**, 37.
13. Nota, G., Palombari, R., and Improta, C., *J. Chromatogr.*, 1976, **123**, 411.
14. Bates, B. L., and Buick, D. R., *J. Assoc. Off. Anal. Chem.*, 1976, **59**, 1390.

Received *March* 31*st*, 1980
Accepted *May* 28*th*, 1980

SHORT PAPERS

A Simple Non-dispersive Atomic-fluorescence Spectrometer for Mercury Determination, Using Cold-vapour Generation

R. C. Hutton and B. Preston

Tioxide International Limited, Central Laboratories, Stockton-on-Tees, Cleveland, TS18 2NQ

Keywords: Mercury determination; vapour generation; non-dispersive atomic-fluorescence spectrometry; estuarine samples

The expansion of interest in environmental pollution has directed considerable effort towards obtaining lower limits of detection for many toxic elements. Analysis for mercury in particular has placed considerable demands on currently available instrumentation and a recent review[1] reflects the great analytical interest in this element. Both atomic-absorption and atomic-fluorescence techniques have been commonly employed to determine mercury and both have their advantages and disadvantages.[2–4]

In our laboratory, we have for many years used an atomic-fluorescence technique. However, demands for lower limits of detection coupled with the increased volume of samples presented for analysis have rendered our apparatus inadequate for present requirements.

When deciding on a replacement, it was obvious from the number of samples analysed that one instrument would have to be dedicated to mercury analysis. Rather than use an atomic-absorption procedure it was decided, after a review of the literature, that a purpose-built non-dispersive fluorescence instrument could achieve the levels required.

Non-dispersive fluorescence has not gained the popularity of dispersive techniques. The increased light-gathering power of a non-dispersive system is often offset by background scatter from the atom cell, usually a flame. However, cold-vapour generation gives minimum background scatter and considerable advantages could be obtained by using cold-vapour generation with a non-dispersive fluorescence system. Non-dispersive mercury fluorescence has been reported in the literature,[5–9] but in some instances[5] the detection limits obtained required extreme conditions, *e.g.*, solar-blind photomultipliers or sodium-lit rooms.

This work describes the performance of a simple non-dispersive fluorescence spectrometer and its application to the routine analysis of marine samples.

Experimental

Apparatus

The instrument employed in this work was a purpose-built non-dispersive fluorescence spectrometer, illustrated schematically in Fig. 1. The instrument was housed in a light-tight box with a partition in the centre separating the light source and fluorescence cell from the detection system. The fluorescence cell was a 10-mm o.d. silica tube and fluorescence was measured at a height of 3 mm above the top of the tube.

Source. Philips OZ 4-W mercury-discharge bulb, powered by a laboratory-constructed 0.35-A constant-current power supply.

Mercury cell. 10-mm o.d. silica tubing, blackened both internally and externally to reduce reflected light.

Slits. 2 mm wide × 1 cm high.

Photomultiplier. EMI 9781B (EMI Electronics Ltd., Hayes, Middlesex) operated at 600 V.

Photomultiplier power supply. Bentham 215 high-voltage supply (Bentham Instruments Ltd., Reading, Berkshire).

Amplifier. Bentham 210E current-sensitive amplifier.

Chart recorder. Chessell flat-bed recorder (Chessell Ltd., Worthing, Sussex).

Reagents

Tin(II) chloride solution, 10% m/V. Dissolve 100 g of tin(II) chloride in 500 ml of concentrated hydrochloric acid and dilute to 1 l with distilled water.

Acid solution. Add 25 ml of concentrated sulphuric acid and 10 ml of concentrated hydrochloric acid to 50 ml of distilled water. Cool and dilute to 100 ml.

Mercury standard, 1000 μg ml^{-1} of mercury. Dissolve 1.08 g of mercury(II) oxide in the minimum volume of 1 + 1 *V/V* hydrochloric acid and dilute to 1 l with distilled water.

Procedure

A procedure similar to that of Hatch and Ott[10] was employed. Tin(II) chloride solution (5 ml) and 5 ml of acid solution were placed in the reduction cell. Argon was bubbled through the solution at 1.5 l min^{-1} to remove residual mercury. A suitable aliquot of sample solution (usually 1 or 5 ml) was placed in the reactor cell using a micropipette and the fluorescence signal was read off on a chart recorder.

The tin(II) chloride solution was renewed after every 5–10 determinations, depending on mercury levels.

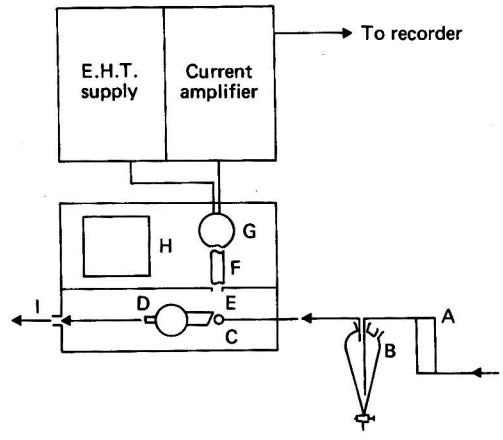


Fig. 1. Schematic layout of non-dispersive fluorescence system. A, Rotameter; B, 125-ml capacity reaction cell; C, fluorescence cell; D, mercury discharge bulb; E, slits on central partition; F, optical guide; G, photomultiplier; H, 0.35-A constant current power supply; I, exit to vacuum line.

Results and Discussion

The instrumental layout illustrated in Fig. 1 was the result of careful optical optimisation, which led to the lowest possible background light levels. It was found preferable, for example, to use an optical guide (a 15-mm diameter brass tube) rather than a lens between the slits and the photomultiplier tube. Reflections from lenses were found to degrade the signal to background ratio. The central partition, separating the source and the fluorescence cell from the photomultiplier, was necessary to reduce stray light from the source. A reduction in background from the source of approximately 300-fold was achieved with the partition in place, giving an approximate source background equivalent to 0.005 μg l^{-1}. Possible molecular background from ozone, generated by the mercury discharge bulb, was eliminated by slight suction, which also removed residual mercury vapour from the instrument.

The calibration graphs obtained are illustrated in Fig. 2. These show a linear response from the detection limit to over 100 μg l^{-1}, over three orders of magnitude. A practical detection limit of 0.04 μg l^{-1} was determined by monitoring continuous day-to-day variations on a 1-ml sample containing 1 μg l^{-1} of mercury. A typical precision run using 1 ml of a sample

containing 1 μg l^{-1} of mercury with an amplifier time constant of 1 s gave the following results (chart divisions): 43, 43, 43, 45, 43, 42, 44, 42, 44. These results give a mean of 43.2 chart divisions with $2\delta = 0.045$ μg l^{-1} and a coefficient of variation = 2.25%. Precisions of 2–3% can be achieved. Greater sensitivity could be obtained by the use of up to 5 ml of sample solution, but in general this was found not to be necessary. The detection limit obtained statistically also represents approximately twice the total blank signal obtained practically.

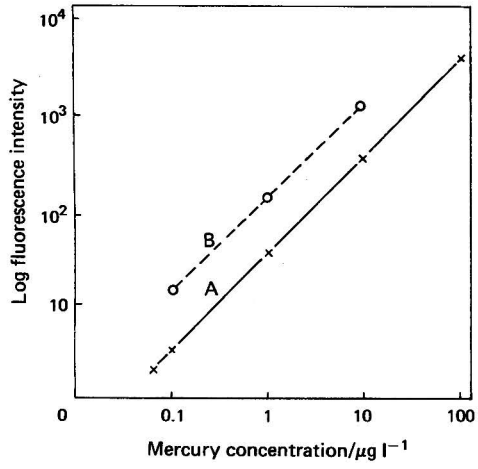


Fig. 2. Calibration graphs for mercury fluorescence on logarithmic co-ordinates. A, 1-ml sample volume; B, 5-ml sample volume.

The performance of this instrument compares favourably with that of other vapour generation systems previously reported, without requiring complex instrumentation. The instrument could possibly be improved further by the inclusion of a solar-blind response photomultiplier, but the practical problems associated with working at such low mercury levels may outweigh the expense involved in the purchase of such a tube.

The instrument has been routinely used to determine mercury levels in acid extracts of estuarine sediments and shrimps. Comparison of results obtained by dispersive fluorescence show that no disadvantages result from the use of a non-dispersive system and that lower blank levels can be quoted with confidence, owing to the increased sensitivity. This is illustrated in Table I, which gives results obtained from a typical sample batch. The instrument can typically analyse 30–40 samples per hour (including standards and blanks).

TABLE I

COMPARISON OF DISPERSIVE AND NON-DISPERSIVE DETERMINATION OF MERCURY IN EXTRACTS FROM ESTUARINE SEDIMENTS AND SEA SHRIMPS

Sample volume = 1 ml.

Mercury concentration/μg l^{-1}

	Sediments		Shrimps	
	Dispersive	Non-dispersive	Dispersive	Non-dispersive
Blank	<1	<0.05	<1	<0.05
1	14	10	3	4
2	16	7	3	3
3	12	11	3	3
4	6	6	3	3
5	9	10	8	6
6	9	12	4	3
7	11	12	3	4
8	11	9	3	4
9	12	11	3	4
10	10	13	—	—

Conclusion

It has been demonstrated that by careful optical optimisation, a simple inexpensive non-dispersive mercury fluorescence spectrometer can achieve detection limits comparable to those obtained with more complex systems. The instrument is both sensitive and precise and allows a rapid throughput of samples without the need to alter the operating conditions frequently.

The authors acknowledge the help of Mr. L. Best with the fabrication of the spectrometer. This work is published by permission of the Directors of Tioxide International Limited.

References

1. Ure, A. M., *Anal. Chim. Acta*, 1975, **76**, 1.
2. Thompson, K. C., and Reynolds, G. D., *Analyst*, 1971, **96**, 271.
3. Muscat, V. I., and Vickers, T. J., *Anal. Chim. Acta*, 1971, **57**, 23.
4. Thomerson, D. R., *Int. Lab.*, 1977 (January), 57.
5. Caupeil, J. E., Hendrikse, P. W., and Bongers, J. S., *Anal. Chim. Acta*, 1976, **81**, 53.
6. Rigin, V. I., *Zh. Anal. Khim.*, 1979, **34**, 261.
7. Shimomura, S., and Hiroto, R., *Anal. Lett.*, 1973, **6**, 613.
8. Thompson, K. C., *Lab. Pract.*, 1972, **21**, 645.
9. Nakahara, T., Tanaka, T., and Musha, S., *Bull. Chem. Soc. Jpn.*, 1978, **51**, 2020.
10. Hatch, W. R., and Ott, W. L., *Anal. Chem.*, 1968, **40**, 2085.

Received *March 25th*, 1980
Accepted *May 16th*, 1980

Determination of Sulphide in Flooded Acid - Sulphate Soils by an Indirect Atomic-absorption Spectrophotometric Method

Ramesh C. Ray, P. K. Nayar, A. K. Misra and N. Sethunathan

Division of Soil Science and Microbiology, Central Rice Research Institute, Cuttack-753006, India

Keywords: Flooded acid - sulphate soils; sulphide determination; atomic-absorption spectrophotometry

Hydrogen sulphide, evolved during the anaerobic metabolism of sulphate, is readily converted into insoluble metal sulphides, chiefly iron(II) sulphide, in flooded acid - sulphate soils that are especially rich in iron.[1] The widely used method for determining sulphide is based on the precipitation of the sulphide in hydrogen sulphide as zinc sulphide and subsequent determination by methylene blue formation[2] or titrimetry.[3] The simple, rapid and reproducible method described here essentially involves the precipitation of zinc sulphide by the action of zinc on the hydrogen sulphide liberated on acidification of metal sulphides in flooded acid - sulphate soils, and then indirect determination of sulphide by determining the zinc in the precipitate and also the zinc remaining in solution, after the precipitation, by atomic-absorption spectrophotometry.

Experimental

The assembly used for the conversion of metal sulphides into hydrogen sulphide consisted in a 250-ml Erlenmeyer flask, which was closed with a three-hole rubber bung, for holding a dropping funnel (for adding hydrochloric acid) and two glass tubes (an inlet for oxygen-free nitrogen and an outlet). The outlet was connected to two 100-ml Erlenmeyer flasks, in succession, containing an ammoniacal solution of zinc acetate to trap the hydrogen sulphide evolved. For standardisation of the method, a known amount of sodium sulphide was placed in a 250-ml flask and then treated with 100 ml of 1 N hydrochloric acid. The hydrogen sulphide evolved was swept into the zinc acetate solution with oxygen-free nitrogen for

30 min–1 h (until tests with lead acetate paper strips showed complete cessation of hydrogen sulphide evolution) in order to precipitate zinc sulphide. Zinc sulphide was accumulated in the first trap while the second trap was visibly free from any precipitate; this indicated that hydrogen sulphide was completely precipitated in the first trap.

After filtration, the precipitated zinc and soluble zinc remaining in the filtrate were assayed using a Varian Techtron atomic-absorption spectrophotometer, Model AA-1100. Sulphide equivalent to the zinc precipitated or to the decrease in the zinc content of the solution was then calculated. Care was taken to ensure that the molar concentration of sulphide was far exceeded by the molar concentration of zinc in order to provide a measurable excess of zinc in solution after complete precipitation of the sulphide. This method was compared simultaneously with the conventional iodimetric method,[3] in which the zinc sulphide precipitated in the trap was reacted with excess of iodine plus 2.5 ml of concentrated hydrochloric acid and the unreacted iodine was titrated against standard thiosulphate solution.

Sulphide formed in two acid - sulphate soils, Pokkali (pH, 5.0; organic carbon, 2.28%; sulphate-S, 0.056%; total sulphur, 0.1%) and Kari (pH, 3.9; organic carbon, 4.65%; sulphate-S, 0.039%; total sulphur, 0.13%) under flooded conditions was determined by atomic-absorption spectrophotometry and iodimetry. Soil samples (20 g) were flooded with 25 ml of distilled water in test-tubes (25 × 200 mm). After 40 d, the reduced soil samples were transferred into 250-ml flasks and treated with 100 ml of 1 N hydrochloric acid in order to liberate hydrogen sulphide from the metal sulphides, chiefly iron(II) sulphide. Hydrogen sulphide was absorbed in an ammoniacal solution of zinc acetate with precipitation of zinc sulphide. The zinc in the precipitate and the filtrate was determined by atomic-absorption spectrophotometry to give the result for the indirect determination of sulphide.

Care was taken to bubble oxygen-free nitrogen through the apparatus for 5 min prior to acidification of the soil samples to prevent the instantaneous oxidation of the hydrogen sulphide evolved in the flask. The sulphide was also determined by the conventional iodimetric method[3] by adding excess of iodine plus hydrochloric acid directly to the trap as described for the sulphide determination from sodium sulphide in the standardisation of the method. In a modification of this method, the precipitated zinc sulphide was first separated by filtration and then treated with excess of iodine plus hydrochloric acid to avoid any interferences from iodine consuming substances, if any, from the complex soil system.

Results and Discussion

The data in Table I showed that about 85% of sulphide was recovered from sodium sulphide standards by both the iodimetric and atomic-absorption spectrophotometric methods. Also, in the latter method, the sulphide values, obtained by determining the zinc either in the zinc sulphide precipitated or that remaining in solution in the filtrate, were almost identical. The atomic-absorption spectrophotometric method was simple, rapid and reproducible with variations of less than 5% within replicates, while the iodimetric method, though equally sensitive, was somewhat tedious.

TABLE I

SULPHIDE RECOVERED FROM SODIUM SULPHIDE STANDARDS

The results are for sulphide recovered.

	Atomic-absorption spectrophotometry								
	Result from determination of zinc in filtrate			Result from determination of zinc in precipitate			Iodimetry		
Sulphide added/mg	Replicate/ mg	Mean/ mg	Recovery, %	Replicate/ mg	Mean/ mg	Recovery, %	Replicate/ mg	Mean/ mg	Recovery, %
3.37	2.83 2.76 2.79	2.79	82.8	2.85 2.86 2.87	2.86	84.8	2.92 2.81 2.84	2.86	84.8
4.49	3.81 3.65 3.66	3.71	82.6	3.73 3.75 3.76	3.75	83.5	3.78 3.70 3.83	3.77	84.0

The determination of sulphide in two acid - sulphate soils after a 40-d flooding showed that the sulphide values from both soils were realistic and reproducible, ranging from 0.26 to

0.33 mg g^{-1} when determined by atomic-absorption spectrophotometry (Table II). As in the pure system when sodium sulphide was used, soil sulphide levels derived from the determination of zinc either in the precipitate or in the filtrate were identical. However, the iodimetric method in which iodine was added directly to the trap gave abnormally high sulphide values (> 1.36 mg g^{-1}) for both soils; these levels are above the theoretical values for sulphide that could be generated from Pokkali soil with 0.056% sulphate-S and 0.1% of total sulphur and from Kari soil with 0.039% sulphate-S and 0.13% of total sulphur following flooding. However, the sulphide content of the Kari soil, obtained by the modified iodimetric method, in which the zinc sulphide was treated with iodine only after filtration, was realistic, reproducible and identical with that obtained by atomic-absorption spectrophotometry. This would suggest that overestimation of soil sulphide, by the conventional method of adding iodine directly to the trap, was a result of interferences by some reduction products of the flooded soil system. For instance, reduced sulphur compounds

TABLE II

SULPHIDE FORMED IN ACID - SULPHATE SOILS ON 40-d FLOODING

The results are for sulphide formed in mg per gram of soil.

	Atomic-absorption spectrophotometry				Iodimetry			
	Result from determination of zinc in filtrate		Result from determination of zinc in precipitate		Conventional*		Precipitate†	
Soil	Replicate	Mean	Replicate	Mean	Replicate	Mean	Replicate	Mean
Pokkali ..	0.29 0.29 0.28	0.29	0.29 0.29 0.30	0.29	2.09 2.15 1.85	2.03	N.D.‡ N.D. N.D.	
Kari ..	0.29 0.30 0.26	0.28	0.29 0.29 0.29	0.29	1.70 1.68 1.56	1.65	0.31 0.31 0.33	0.32

* Iodine was added directly to the trap.
† Zinc sulphide precipitated was first separated by filtration and then treated with iodine.
‡ N.D. not determined.

such as sulphite, thiosulphate, tetrathionate and hydrosulphite may decompose during acidification leading to erratic results in the determination using iodimetry.[3] Likewise, the methylene blue method, widely used in determining sulphide, is not always reliable as not only hydrogen sulphide but other reduction products that commonly occur in anaerobic ecosystems can readily react with methylene blue to produce erroneous results. The new method of indirect determination of sulphide through the determination of zinc by atomic-absorption spectrophotometry is simple, rapid and free from interference and thus has a definite advantage over the iodimetric and methylene blue methods, especially in a complex system such as waterlogged soil.

The authors are grateful to Dr. H. K. Pande (Director) for encouragement, Dr. S. Patnaik and Dr. C. C. Biddappa for suggestions and Dr. S. B. Lodh for facilities. This project was partially funded by the Department of Science and Technology, Government of India, and the International Atomic Energy Agency, Vienna.

References

1. Ponnamperuma, F. N., *Adv. Agron.*, 1972, **24**, 29.
2. Johnson, C. M., and Nishita, H., *Anal. Chem.*, 1952, **24**, 736.
3. American Public Health Association and Water Pollution Control Federation, "Standard Methods for the Examination of Water and Waste Water," Thirteenth Edition, American Public Health Association, New York, 1971, p. 551.

Received *April 1st*, 1980
Accepted *May 8th*, 1980

Determination of Diuron Residues in Soil: Comparison of Determinations by High-performance Liquid Chromatography and Gas - Liquid Chromatography

E. G. Cotterill

Agricultural Research Council Weed Research Organization, Begbroke Hill, Yarnton, Oxford OX5 1PF

Keywords: *Diuron determination*; *soil*; *gas - liquid chromatography*; *high-performance liquid chromatography*

The herbicide diuron [*N*-(3,4-dichlorophenyl)-*NN*-dimethylurea] is used for the control of annual weeds in fruit crops and total weed control in non-crop situations. Substituted urea herbicide residues have been determined using gas - liquid chromatography (GLC) by pyrolysis to the phenyl isocyanate in the injection heater[1,2] with electron-capture detection. They have also been determined by high-performance liquid chromatography (HPLC)[3–6] and by GLC using a thermionic detector.[7] Lawrence and Laver[8] and Büchert and Lokke[9] have reported methods using GLC determination after alkylation. Bieser and Grolinmund[10] and Khan *et al.*[11] have devised methods of measuring urea herbicides by GLC without thermal decomposition. The aim of the work reported here was to determine the most reliable method of measurement for the routine determination of diuron residues in soil.

Experimental

Soils

Soils from three locations were used for the fortification studies. Table I gives some details of the soil composition at each site. Each soil was air dried and passed through a 3-mm sieve prior to fortification. Aqueous solutions of diuron were prepared from a methanolic solution containing 1 mg ml^{-1} of herbicide so that, when sufficient solution was added to the soil to achieve 75% water-holding capacity, the concentration of herbicide in soil was 1.0, 0.5 or 0.1 $\mu g\ g^{-1}$. Soils were fortified in triplicate and allowed to stand for 48 h before analysis.

In addition, an area at site 1 received a field application of diuron at 1 kg ha^{-1}. A sample from the top 10–15 cm was taken with a shovel 1 month after application and sieved. After thorough mixing the soil was split into 12 sub-samples and stored wet at −15 °C until analysis.

TABLE I

SOME PROPERTIES OF THE SOILS USED

Property	Soil 1	Soil 2	Soil 3
Organic carbon, %	1.6	4.1	25
pH	7.0	5.1	5.9
Clay, %	16	16	56
Silt, %	11	16	32
Sand, %	73	68	12
Water-holding capacity, %	16.6	27.0	38.9

Extraction

The method of McKone[1] was used. A 25-g sample of soil was extracted with 50 ml of methanol by shaking on a wrist-action shaker for 1 h. The resulting soil slurry was filtered through a Watman No. 42 filter-paper. For GLC, a 2-ml aliquot was evaporated to dryness by gently blowing dry air and the residue was re-dissolved in 2 ml of hexane. For HPLC, a

25-ml aliquot was concentrated to about 1 ml under reduced pressure whilst warming in a water-bath at 40 °C. The remaining solvent was removed with a gentle stream of dried air and the residue was then re-dissolved in 1 ml of the HPLC eluent.

Chromatography

Gas - liquid chromatography

A Pye 104 chromatograph fitted with a nickel-63 electron-capture detector and a 1.5 m × 4 mm i.d. glass column was used. The conditions employed were as follows: column packing, 5% SE-30 on Chromosorb W HP (80–100 mesh); carrier gas, oxygen-free nitrogen at a flow-rate of 50 ml min^{-1}; column temperature, 155 °C; injector temperature, 250 °C; detector temperature, 350 °C; attenuation, 10×10^2; and pulse width 150 μs.

Standard solutions with concentrations in the range 0.05–1.0 ng per 5-μl injection gave a linear response, the peak area being determined with a Perkin-Elmer Sigma 10 chromatography data station.

High-performance liquid chromatography

A constant-flow pump (HSCP, Bourne End, Buckinghamshire) was connected to a 100 mm × 5 mm i.d. stainless-steel column packed with Hypersil-ODS (5.5 μm mean diameter) (Shandon Southern). Injections were made using a Rheodyne valve and diuron was measured using a Cecil 212 variable-wavelength ultraviolet monitor set at 250 nm and 0.1 absorbance unit for full-scale deflection. Methanol - water (7 + 3) was used as the eluent at a flow-rate of about 0.5 ml min^{-1}. Standards in the range 5–50 ng per 5-μl injection gave a linear response, the peak area being determined with a Perkin-Elmer Sigma 10. The optimum absorption wavelength was determined by scanning a diuron solution between 200 and 300 nm prior to chromatography.

Results and Discussion

An initial experiment showed that the GLC conditions selected, although causing pyrolysis in the injection heater, gave more reproducible results than when conditions were arranged so as to avoid thermal degradation. As the aim of the work was to produce a routine method, derivatisation techniques were not included as they extend the analysis time.

TABLE II

RECOVERY OF DIURON FROM SOIL

Method	Soil	Diuron added/ μg g^{-1}	Recovery, %	Mean recovery, %
HPLC	1	1.0	98.8, 95.3, 102.3	98.8
		0.5	99.9, 99.9, 92.0	97.2
		0.1	101.4, 98.8, 96.2	98.8
	2	1.0	101.9, 100.0, 98.1	100.0
		0.5	101.0, 99.4, 97.9	99.4
		0.1	104.5, 100.9, 80.1	94.7
	3	1.0	87.9, 102.1, 104.9	98.3
		0.5	89.3, 92.0, 86.6	89.3
		0.1	98.8, 93.5, 87.3	93.5
GLC	1	1.0	99.6, 97.3, 94.7	97.2
		0.5	90.1, 84.8, 96.0	90.3
		0.1	84.7, 96.5, 96.1	92.4
	2	1.0	102.2, 93.2, 84.5	93.3
		0.5	96.8, 90.5, 84.6	90.7
		0.1	73.4, 97.5, 85.5	85.4
	3	1.0	Not analysable without clean-up	
		0.5		
		0.1		

Table II shows the recovery of diuron from fortified soil using HPLC and GLC determinations. There is no practical difference between the means obtained by either method but the variance within the means is greatest for GLC determination. The results obtained from the

12 sub-samples from the area treated in the field showed a mean of 0.13 μg g^{-1} and a coefficient of variation of 11.5% for HPLC determination and a mean of 0.12 μg g^{-1} and a coefficient of variation of 41.7% for GLC determination. Thus, although the means are almost the same, the variability of the GLC determination is over three times that of HPLC.

A further advantage of HPLC over GLC is shown in Figs. 1 and 2. Fig. 1 shows high-performance liquid chromatograms of (*a*) 20 ng of diuron, (*b*) extract of soil 1 treated at 0.1 μg g^{-1} and (*c*) extract of soil 3 treated at 0.1 μg g^{-1}, and Fig. 2 shows gas - liquid chromatograms of (*a*) 1.0 ng of diuron, (*b*) extract of soil 1 treated at 0.1 μg g^{-1} and (*c*) extract of soil 3 treated at 0.1 μg g^{-1}. Hence diuron can be analysed by HPLC in a soil with a high organic matter content without clean-up, so avoiding the extra manipulations and possible introduction of errors and the increased analysis time that would be required if the same soil was analysed by GLC. Fig. 2 also shows extensive peak tailing, indicative of breakdown of diuron on the column. As substituted ureas break down to the corresponding phenyl isocyanate,[2,9] diuron and its metabolites *N*-(3,4-dichlorophenyl)-*N*-methylurea (DCPMU) and *N*-(3,4-dichlorophenyl)urea (DCPU) all elute from a GLC column as 3,4-dichlorophenyl isocyanate. Hence the parent compound cannot be distinguished from its metabolites using GLC, whereas HPLC will separate all of these compounds, with retention times for diuron, DCPMU and DCPU of 4.1, 7.5 and 6.7 min, respectively, using the conditions given.

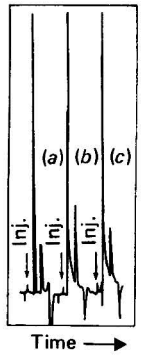


Fig. 1. High-performance liquid chromatograms of (*a*) 20 ng of diuron; (*b*) extract of soil 1 treated at 0.1 μg g^{-1}; and (*c*) extract of soil 3 treated at 0.1 μg g^{-1}.

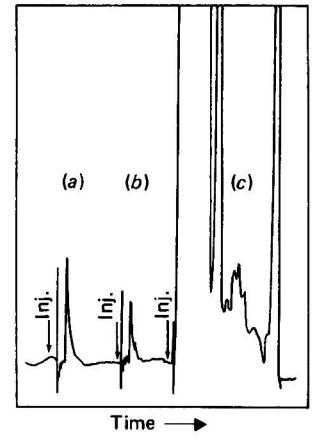


Fig. 2. Gas - liquid chromatograms of (*a*) 1.0 ng of diuron; (*b*) extract of soil 1 treated at 0.1 μg g^{-1}; and (*c*) extract of soil 3 treated at 0.1 μg g^{-1}.

Although HPLC is generally the most reproducible method, GLC has the advantage of being more sensitive. When measuring very low residues in soils with a low organic matter content, GLC could prove to be the better method but the results should be interpreted with caution because of the possible presence of unresolved metabolites. In practice, however, the limit of detection is set by the signal to background ratio, which is usually similar for both methods, but in the more organic soils HPLC is favoured. A limit of detection of 0.04 μg g^{-1} can be achieved using either method.

References

1. McKone, C. E., *J. Chromatogr.*, **1969**, **44**, 60.
2. Spengler, D., and Hamroll, B., *J. Chromatogr.*, **1970**, **49**, 205.
3. Lawrence, J. F., *J. Assoc. Off. Anal. Chem.*, **1976**, **59**, 1066.
4. Sidwell, J. A., and Ruzicka, J. H. A., *Analyst*, **1976**, **101**, 111.

5. Byast, T. H., *J. Chromatogr.*, 1977, **134**, 216.
6. Pribyl, J., and Herzel, F., *J. Chromatogr.*, 1978, **166**, 272.
7. Jarczyk, H. J., *Pflanzenschutz-Nachr.*, 1975, **28**, 334.
8. Lawrence, J. F., and Laver, G. W., *J. Agric. Food Chem.*, 1975, **23**, 1106.
9. Büchert, A., and Lokke, H., *J. Chromatogr.*, 1975, **115**, 682.
10. Bieser, H., and Grolinmund, K., *J. Assoc. Off. Anal. Chem.*, 1974, **57**, 1294.
11. Kahn, S. U., Greenhalgh, R., and Cochrane, W. P., *Bull. Environ. Contam. Toxicol.*, 1975, **13**, 602.

Received *March 31st*, 1980
Accepted *April 24th*, 1980

Rapid Extraction of Some Persistent Chlorinated Hydrocarbons from Biological Material with Low Fat Content

Gunnar Norheim and Elisabet Mo Økland
National Veterinary Institute, P.O. Box 8156 Dep., Oslo 1, Norway

Keywords: *Chlorinated hydrocarbon extraction*; *hexachlorobenzene*; *octachlorostyrene*; *biological material*; *low fat content*

Persistent chlorinated hydrocarbons of agricultural and non-agricultural interest, such as 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCBs) and hexachlorobenzene, have obtained a global distribution, and can be detected in wildlife samples in variable amounts. PCBs together with 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene (DDE) are the main types of chlorinated hydrocarbons found in Norwegian avian fauna and in fish along the Norwegian coast.[1–3]

In Frierfjorden, a fiord in South-Eastern Norway, heavy local contamination with chlorinated hydrocarbons of industrial origin has been detected. The contaminants most often found in the fish in this area are hexachlorobenzene, octachlorostyrene and decachlorobiphenyl. In addition, complex mixtures of PCBs and also of chlorinated naphthalenes have been detected.[4,5] Decachlorobiphenyl has previously been detected in arctic fox (*Alopex lagopus*) from Svalbard,[6] and octachlorostyrene was first detected in birds from The Netherlands.[7,8]

In a monitoring programme over the last 6 years, the above chlorinated hydrocarbons have been determined in samples from cod (*Gadus morhua*) obtained from the area. Flesh samples from cod have a low fat content (about 0.3%) and extraction of such samples is not especially reproducible. Some procedures are also very time consuming. A rapid and simple procedure has therefore been developed for this type of sample for the gas-chromatographic determination of some chlorinated hydrocarbons resistant to concentrated sulphuric acid.

Experimental

Apparatus

A Carlo Erba 2100 gas chromatograph equipped with a nickel-63 electron-capture detector and a 2 m × 3 mm i.d. glass column was used. The column material was 1.5% SP-2250 - 15.9% SP-2401 on 100–120-mesh Supelcon AW DMCS. The column, injector and detector temperatures were 200, 250 and 275 °C, respectively. Argon - methane (95 + 5) was used as the carrier gas, the flow-rate being 55 ml min^{-1}. The electrometer attenuation was × 128.

Reagents

Sulphuric acid, 95–97%. Pro analysi grade (Merck).
Heptane. Pro analysi grade (Merck).
Hexachlorobenzene. Pract. grade (Fluka).
Octachlorostyrene. Obtained as a gift from Norsk Hydro.

Standard Solutions

Amounts of 100 mg each of hexachlorobenzene and octachlorostyrene were dissolved in 100 ml of heptane and the mixture was diluted 1 + 50000 with heptane.

Procedure

A 0.5-g amount of sample was accurately weighed into a 10-ml Soveril glass tube fitted with a screw-cap, and 6 ml of concentrated sulphuric acid were measured into the tube. The tube was placed in a thermostatically controlled oven at 60 °C for 4 h, during which time it was shaken lightly a few times to ensure complete solubilisation of the sample. After cooling, 1.0 ml of heptane was pipetted into the tube, the screw-cap put on and the tube shaken for about 3 min. Finally, the tube was centrifuged with the screw-cap on, after which the sample was ready for gas chromatography. An injection volume of 5 μl was used.

TABLE I

RECOVERY OF HEXACHLOROBENZENE (HCB) AND OCTACHLOROSTYRENE (OCS) FROM HOMOGENISED COD FLESH AFTER ISOLATION BY THE PROPOSED METHOD AND EXTRACTION WITH DRY DIETHYL ETHER OR HEXANE - PROPAN-2-OL

Method	Recovery, %	
	HCB	OCS
Present method	95	95
Dry diethyl ether	32	58
Hexane - propan-2-ol	87	86

Results and Discussion

The proposed method is rapid and requires only small amounts of reagents and little equipment. A large number of samples can be extracted simultaneously. The recoveries of hexachlorobenzene (HCB) and octachlorostyrene (OCS) were determined after a standard addition by a method described previously.[9] The recovery of each component was 95%.

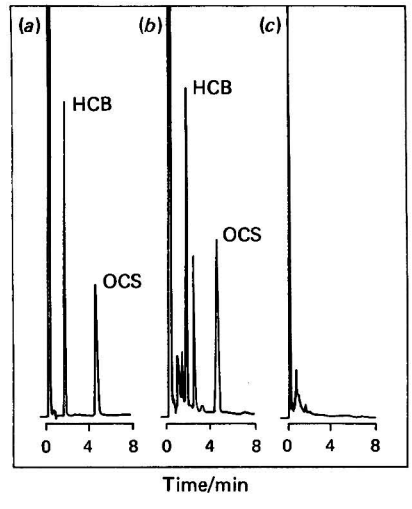


Fig. 1. Chromatograms of 5-μl injections of hexachlorobenzene (HCB) and octachlorostyrene (OCS). (*a*) Standard solution of HCB and OCS (0.1 ng of each); (*b*) extract of cod flesh from a contaminated area (in addition to HCB and OCS this chromatogram has peaks for pentachlorobenzene and heptachlorostyrene); (*c*) extract of a blank sample of cod flesh. The gas-chromatographic conditions are stated in the text.

TABLE II

RESULTS OF A COLLABORATIVE STUDY IN WHICH PENTACHLOROBENZENE (5CB), HEXACHLOROBENZENE (HCB) AND OCTACHLOROSTYRENE (OCS) WERE DETERMINED IN HOMOGENISED COD FLESH

Laboratory	Concentration/μg g^{-1} (wet mass)		
	5CB	HCB	OCS
This work	0.002	0.13	3.7
Laboratory 1	0.001	0.10	3.7
Laboratory 2	0.003	0.17	4.2
Laboratory 3	0.002	0.10	1.3
Laboratory 4	—	0.08	1.3

The recovery of HCB and OCS from homogenised cod flesh using the proposed method was compared with two other methods, namely column extraction with dry diethyl ether[10] and direct extraction for 4 h with hexane - propan-2-ol and subsequent partition with water.[4] The results are presented in Table I.

The reproducibility of the method was calculated after the determination of HCB and OCS in eight parallel samples of cod flesh. The following results were obtained after manual injection of the extracts into the gas chromatograph: HCB, 0.25 ± 0.014 μg g^{-1}; and OCS, 0.18 ± 0.017 μg g^{-1}. The coefficients of variation for HCB and OCS were 5.5% and 9.5%, respectively.

The retention time under the conditions used was 1.6 min for HCB and 4.5 min for OCS. Typical gas chromatograms are presented in Fig. 1. A blank sample from a non-contaminated area gave no interfering peaks.

The accuracy of the proposed method was tested in a collaborative study involving five laboratories.[11] The other four laboratories used other extraction procedures. The results of this study are presented in Table II. Agreement was fairly good for HCB, but less good for OCS, two of the laboratories finding lower levels. The method was also tested on homogenised herring containing about 10% of fat, and agreement with the results of ordinary extraction techniques was better than that for the cod flesh, especially for OCS.

With increasing fat content in the sample, emulsion formation after the extraction with heptane causes problems. This may be partly overcome by increasing the volume of heptane, although sensitivity is thereby decreased. Heptane was preferred to hexane as the solvent because of its lower volatility. However, if a high reproducibility is to be achieved, it is essential that centrifugation be carried out with the screw-cap on. The sensitivity of the method is dependent on the gas-chromatographic equipment used. In our laboratory we can routinely determine HCB and OCS in cod flesh at levels down to 0.001 μg g^{-1} wet mass.

An important limitation of the proposed method is that DDE is partly broken down during the digestion and cannot be determined accurately. PCBs, on the other hand, can be recovered as efficiently as HCB and OCS. In our studies on HCB, OCS and decachlorobiphenyl in fish from Frierfjorden and the surrounding fiords, the proposed method has proved useful in the gas-chromatographic determination of these chlorinated hydrocarbons.

References

1. Holt, G., Frøslie, A., and Norheim, G., *Acta Vet. Scand.*, 1979, Suppl. 70, 28 pp.
2. Brevik, E. M., Bjerk, J. E., and Kveseth, N. J., *Bull. Environ. Contam. Toxicol.*, 1978, **20**, 715.
3. Kveseth, N. J., Bjerk, J. E., Fimreite, N., and Stenersen, J., *Arch. Environ. Contam. Toxicol.*, 1979, **8**, 201.
4. Lunde, G., and Baumann Ofstad, E., *Fresenius Z. Anal. Chem.*, 1976, **282**, 395.
5. Baumann Ofstad, E., Lunde, G., Martinsen, K., and Rygg, B., *Sci. Total Environ.*, 1978, **10**, 219.
6. Norheim, G., *Acta Pharmacol. Toxicol.*, 1978, **42**, 7.
7. Koeman, J. H., ten Noever de Brauw, M. C., and de Vos, R. H., *Nature (London)*, 1969, **221**, 1126.
8. ten Noever de Brauw, M. C., and Koeman, J. H., *Sci. Total Environ.*, 1972/73, **1**, 427.
9. Norheim, G., and Økland, E., paper presented at Workshop in Gas Chromatography, Sandefjord, Norway, 1978.
10. Bjerk, J. E., and Holt, G., *Acta Vet. Scand.*, 1971, **12**, 429.
11. Martinsen, K., Baumann Ofstad, E., Lunde, G., Brevik, E. M., Kveseth, N. J., Bøe, B., Egaas, E., Crowo, J. A., Mürer, K., Frøslie, A., and Norheim, G., Report, Oslo, Norway, 1978.

Received *May 6th*, 1980
Accepted *May 29th*, 1980

Determination of the Anthelmintic Levamisole in Plasma and Gastro-intestinal Fluids by High-performance Liquid Chromatography

S. Marriner, E. A. Galbraith and J. A. Bogan

Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow, G61 1QH

Keywords: Levamisole determination; biological fluid analysis; high-performance liquid chromatography

Levamisole {*l*-tetramisole, *l*-[2,3,5,6-tetrahydro-6-phenylimidazo(2,1-*b*)thiazole]} is commonly used in many species of animal as an anthelmintic.[1–5]

Levamisole has been determined in cattle tissues and milk by differential cathode-ray polarography,[6] a technique which is not available in most chemical laboratories and which lacks sensitivity. Gas - liquid chromatography has also been used for levamisole determination in milk[7] but this method requires a lengthy clean-up procedure using organic solvent extraction and the use of a selective alkali flame-ionisation detector.

High-performance liquid chromatography (HPLC) appeared to offer a more suitable technique for the determination of levamisole and in this work a sensitive and rapid method using this technique is described.

Experimental

Reagents

All reagents were of analytical-reagent grade.

Diethyl ether.

Borate buffer, pH 9. Solution A: dissolve 0.746 g of potassium chloride and 0.618 g of boric acid in 180 ml of distilled water. Solution B: 0.2 N sodium hydroxide solution. Mix solutions A and B in the approximate proportions of 9 to 1 to give a solution of pH 9.

Hydrochloric acid, 0.1 N.

Sodium hydroxide solution, 1.0 N.

Methanol. Re-distilled before use.

Ammonium carbonate solution, 0.05 M.

HPLC Apparatus

Pump. Altex, Model 110.

Detector. Cecil, Model CE 2012, variable-wavelength spectrophotometer.

Column. 100 × 5 mm (Shandon Southern).

Packing. ODS Hypersil (Shandon Southern).

Wavelength. 220 nm.

Absorbance. 0.1 a.u.f.s.

Solvent. Methanol - ammonium carbonate solution, 0.05 M (65 + 35).

Flow-rate. 1.0 ml min^{-1}.

Under these conditions levamisole had a retention time of 2.7 min.

Procedure

To 2 ml of plasma or other body fluid contained in a 50-ml ground glass stoppered test-tube add 2 ml of pH 9 borate buffer and 15 ml of diethyl ether. Stopper firmly and shake for 10 min on a slow rotary mixer. Transfer 10.5 ml of the upper ether layer into a second 50-ml ground glass stoppered test-tube using a 5-ml adjustable pipette. (*N.B.*, The pipette tip must be pre-wetted with the ether before accurate transfer can be effected.) Add a further 15 ml of ether to the first tube and shake as before for 10 min. Transfer 15 ml of the upper, ether layer into the second test-tube, combining it with the other 10.5 ml of ether. Discard the aqueous layer. Add 3 ml of hydrochloric acid, stopper tightly and shake as above for 10 min. Remove and discard the upper ether layer using suction, taking care not to disturb the interface. Add a further 15 ml of ether to the aqueous layer followed by 0.5 ml of 1.0 N

sodium hydroxide solution and shake for 10 min as above. Transfer 12 ml of the ether layer into a 50-ml glass test-tube. Add a further 15 ml of ether to the aqueous layer and shake for 10 min as above. Remove 15 ml of the ether layer and combine it with the 12 ml of ether. Evaporate the ether to approximately 6 ml on a Dri-bath at 50–55 °C under nitrogen and then transfer it into a 10-ml glass centrifuge tube. Wash the test-tube walls three times with 1 ml of ether, each time adding the washings to the centrifuge tube. Evaporate carefully to dryness on the Dri-bath as above, wash down the walls of the tube with 0.5 ml of ether and evaporate to dryness again. Add 100 μl of methanol to the residue and sonicate for approximately 1 min whilst rotating and tilting the tube in the ultrasonic bath. Inject 5 μl of the extract on to the HPLC column.

With fresh plasma samples from sheep and cattle it was found that a shortened version of the above method could be used by evaporating the combined ether extracts from the first extraction, avoiding the need for a back-extraction. For rumen and other gastro-intestinal samples the back-extraction was found to be necessary.

The concentration of levamisole in the sample is calculated from calibration graphs prepared by adding known amounts (0.1–3.0 μg ml^{-1}) of levamisole to blank plasma or gastro-intestinal fluid. The standard samples are extracted using the procedure described and the peak heights obtained for levamisole from the sample are compared with the calibration graph prepared from the standard samples. Typical chromatograms are shown in Fig. 1.

Using this method the concentration of levamisole in the sample is calculated as follows:

By the standard method:

$$\text{Concentration in sample} = \frac{\text{Concentration in injected methanol solution}}{18.0}$$

By the short method:

$$\text{Concentration in sample} = \frac{\text{Concentration in injected methanol solution}}{18.6}$$

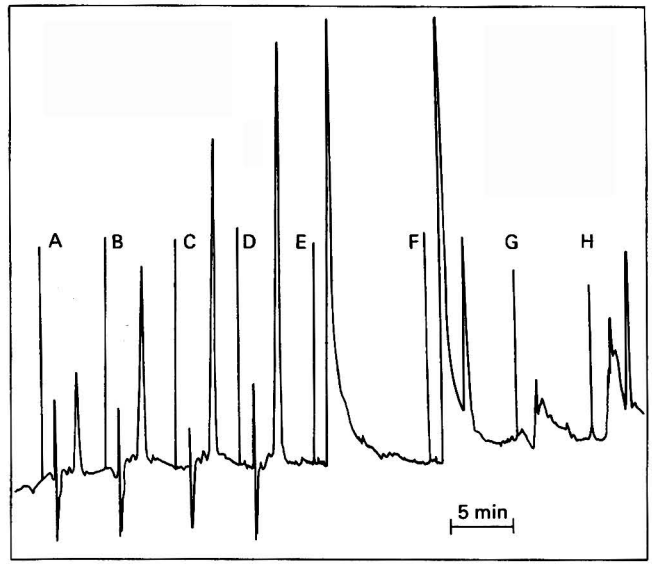


Fig. 1. High-performance liquid chromatographic responses obtained after injection of 5 μl of: A, 5; B, 10; C, 15; and D, 20 μg ml^{-1} of levamisole in methanol; and 5 μl of the extracts obtained using the standard method from: E, blank plasma; F, plasma estimated to contain 0.42 μg ml^{-1}; G, blank ruminal fluid; and H, ruminal fluid estimated to contain 0.36 μg ml^{-1}.

Results

Recoveries of Levamisole from Plasma and Gastro-intestinal Fluids

Aliquots of a solution of levamisole in methanol (20 μl) were added to samples of fresh plasma, rumen fluid and abomasal fluid to give concentrations of 1, 2, 5, 10 and 20 μg ml^{-1} of levamisole.

The plasma samples were extracted by the method outlined here and also by the short method (*i.e.*, without back-extraction). The recoveries of levamisole from these samples after extraction were calculated from a calibration graph prepared from the peak heights of known standards of levamisole in methanol. The recoveries for plasma were 83% (73–90%, $n = 12$) by the standard method and 88% (81–98%, $n = 30$) by the short method, and for rumen fluid 80% (74–84%, $n = 3$) by the standard method.

The limit of detection was between 0.02 and 0.05 μg ml^{-1}, which is adequate for the concentrations of levamisole found after normal doses (Table I).

TABLE I

DETERMINATION OF LEVAMISOLE IN PLASMA BY GC AND HPLC

Mean concentrations of two levamisole determinations in plasma, as determined by the HPLC method and a gas-chromatographic method, after administration of levamisole (7.5 mg kg^{-1} subcutaneously) to two sheep.

	Levamisole concentration/μg ml^{-1}			
	Sheep No. 1		Sheep No. 2	
Time/h	HPLC	GC	HPLC	GC
0	0	0	0	0
1	2.35	2.48	2.49	2.55
3	1.02	1.18	1.56	1.69
6	0.37	0.47	0.86	0.90

Accuracy and Precision

The accuracy and precision of the method for levamisole were determined by adding known amounts of levamisole to plasma. Samples of each known concentration were then assayed in triplicate by both the standard method and the short method (Table II). It was found that the low recoveries were due to extraction losses rather than to degradation of the levamisole.

TABLE II

LEVAMISOLE RECOVERIES

Levamisole was added to 2-ml plasma samples and assayed in triplicate by the standard procedure and by the short procedure. Allowance has been made in the levamisole measured for losses due to not taking the total amount of extraction solvent at each step.

Amount of levamisole added/μg	Method*	Levamisole measured/ μg	Mean ± standard error/μg	Standard error as percentage of the mean	Ratio of levamisole determined to that added
0.4	A	0.34, 0.34, 0.32	0.33 ± 0.01	2.02	0.83
	B	0.33, 0.30, 0.32	0.32 ± 0.01	2.79	0.80
1.0	A	0.88, 0.84, 0.90	0.87 ± 0.02	2.02	0.87
	B	0.87, 0.80, 0.82	0.82 ± 0.02	2.51	0.83
1.5	A	1.36, 1.50, 1.39	1.42 ± 0.04	3.00	0.95
	B	1.40, 1.28, 1.32	1.33 ± 0.04	2.65	0.89
2.0	A	1.72, 1.68, 1.81	1.74 ± 0.04	2.21	0.87
	B	1.79, 1.67, 1.70	1.72 ± 0.04	2.10	0.86
3.0	A	2.58, 2.76, 2.72	2.69 ± 0.05	1.86	0.90
	B	2.68, 2.56, 2.60	2.61 ± 0.04	1.34	0.87

* A = Short procedure; B = standard procedure.

Evaluation of the Method after Administration of Levamisole to Sheep and Comparison with a Gas-chromatographic Method

Levamisole* was administered subcutaneously at a dose of 7.5 mg kg^{-1} to two sheep and plasma samples were then collected at intervals. The samples were analysed in duplicate by the standard method and also by an unpublished gas-chromatographic method.† The results obtained are shown in Table I.

There was good agreement between the results obtained by the two methods.

References

1. Forsyth, B. A., *J. S. Afr. Vet. Med. Assoc.*, **1966**, **37**, 403.
2. Reid, J. F. S., Armour, J., Jennings, F. W., and Urquhart, G. M., *Vet. Rec.*, **1968**, **83**, 14.
3. Lyons, E. T., Drudge, J. H., and Tolliver, S. C., *Am. J. Vet. Res.*, **1968**, **29**, 2157.
4. Forsyth, B. A., *Aust. Vet. J.*, **1968**, **44**, 395.
5. Ciordia, H., and Baird, D. M., *Am. J. Vet. Res.*, **1969**, **30**, 1145.
6. Holbrook, A., and Scales, B., *Anal. Biochem.*, **1967**, **18**, 46.
7. Smith, J. E., Pasarela, R., and Wycroff, J. C., *J. Assoc. Off. Anal. Chem.*, **1976**, **59**, 954.

Received *March 28th*, 1980
Accepted *May 1st*, 1980

* Nilverm, Imperial Chemical Industries.

† The gas-chromatographic analyses were kindly undertaken by Dr. A. Featherstone of the Safety of Medicines Section, Imperial Chemical Industries.

Spectrophotometric Micro-determination of Silver(I) and Iodide Ions

Sudarsan Barua, B. S. Garg and R. P. Singh
Department of Chemistry, University of Delhi, Delhi-110007, India

and Ishwar Singh
Department of Chemistry, Maharishi Dayanand University, Rohtak-123001, India

Keywords: *Silver(I)*; *iodide*; *4-(2-quinolylazo)phenol*

This paper reports the analytical potential of 4-(2-quinolylazo)phenol (*p*-QAP), a new heterocyclic azo dye, as a sensitive chromogenic reagent in the spectrophotometric determination of silver(I). The silver(I) - (*p*-QAP) complex has also been used in the micro-determination of iodide ions. The principle involved is the ligand exchange reaction and the difference in absorbance between the silver(I) - (*p*-QAP) complex before and after the addition of iodide ions and the reagent blank is proportional to the concentration of iodide ions. The method is simple, rapid and the precision is high compared with some of the other published methods.[1,2]

Experimental

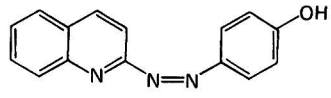
Apparatus

A Unicam SP 600 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 measurements.

Reagents

p-*QAP solution*. 2-Hydrazinoquinoline (1.5 g, 0.01 mol) dissolved in the minimum volume of dilute hydrochloric acid or acetic acid was condensed with an ethanolic solution of *p*-benzoquinone (1.08 g, 0.01 mol). The resulting solution was neutralised with ammonia solution. The orange precipitate of *p*-QAP obtained was filtered, re-crystallised from ethanol and dried over phosphorus(V) oxide in a vacuum. The purity of the compound was checked by thin-layer chromatography and by elemental analysis (calculated values for $C_{15}H_{11}N_3O$:

C 72.28, H 4.42 and N 16.86%; the values found were C 72.00, H 4.48 and N 16.75%). A 5×10^{-4} M solution of the reagent was prepared by dissolving 0.1245 g l^{-1} in ethanol. The solution is stable for several days.



4-(2-Quinolylazo)phenol (*p*-QAP)

Other reagents, standards and stock solutions. Standard solutions of silver(I) and iodide were prepared and standardised by conventional methods[3]; 0.05 M sodium tetraborate solution was prepared for adjusting the pH. All other chemicals used were of analytical-reagent grade.

Recommended Procedure

Determination of silver(I)

To a suitable aliquot of sample containing 1.5–13.0 μg of silver(I) add 1.0 ml of 5×10^{-4} M *p*-QAP solution followed by 1.0 ml of 0.05 M sodium tetraborate solution. Dilute to 10.0 ml with water and ethanol, giving a final ethanol concentration in the solution of 50%. Measure the absorbance of the solution at 530 nm against a reagent blank. The amount of silver(I) in an unknown sample can be determined from a calibration graph prepared from known silver samples as described above.

Determination of iodide ions using silver(I) - (p-QAP)

To 1.0 ml of a 1×10^{-4} M solution of silver(I) add 1.0 ml of 5×10^{-4} M *p*-QAP solution followed by suitable aliquots of iodide solution containing up to 12.7 μg of iodide ions. Add 1.0 ml of 0.05 M sodium tetraborate solution, shake well and dilute to 10.0 ml with water giving a final ethanol concentration in the solution of 50%. Record the absorbance against a reagent blank. The difference in absorbance between the complex before and after the addition of iodide ions and the reagent blank is proportional to the concentration of iodide ions.

Determination of iodide ions by adding excess of silver(I) solution

To an aliquot containing up to 15.0 μg of iodide add a known excess amount of silver(I). Allow to react for 1–2 min and then add 1.0 ml of 5×10^{-4} M *p*-QAP solution followed by 1.0 ml of 0.05 M sodium tetraborate solution. Dilute to 10.0 ml with water and ethanol, again maintaining the ethanol concentration at 50%, and record the absorbance against a reagent blank. The difference in absorbance between the complex before and after the addition of iodide ions and the reagent blank is proportional to the concentration of iodide ions.

Results and Discussion

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

An ethanolic solution of *p*-QAP forms a deep red coloured complex with silver(I), which has a maximum absorbance at 530 nm in the pH range 8.1–11.2. The complex is stable in daylight. It decomposes when the ethanolic concentration is less than 30%; subsequent studies, therefore, were carried out in 50% ethanol 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 is valid for up to 1.7 p.p.m. of silver. With the particular conditions adopted here 0.15–1.30 p.p.m. of silver can be determined accurately. The Sandell sensitivity of the colour system is 0.0013 μg cm^{-2} of silver with a molar absorptivity, ϵ, of 8.3×10^4 l mol^{-1} cm^{-1} at 530 nm.

Studies in the Presence of Diverse Ions

In the determination of 1.08 μg of silver(I) in solution, the results of the tolerance limits, in parts per million, of various ions in solution that caused a deviation smaller than ± 2% in absorbance are nitrite 800; sulphite 400; fluoride and tartrate 80; citrate and oxalate 60; chloride 20; bromide 15; lead(II) 20; lanthanides(III), uranium(VI), cobalt(II), nickel(II), zinc(II), cadmium(II) and iron(III) 10. Iodide, sulphide, thiosulphate, thiocyanate, cyanide, EDTA, palladium(II) and copper(II) interfere.

Determination of Iodide Ions

The decomposition of the silver(I) - (*p*-QAP) complex in the presence of iodide ions was studied by taking varying amounts of silver(I) (0.15–1.30 μg ml^{-1}). The amount of iodide ions determined was 0.09–1.5 μg ml^{-1} using the particular conditions adopted for the determination of silver(I). The recovery results for iodide at ten known concentration levels (each repeated four times) of between 1.27 and 12.7 μg per 10 ml [1.08 μg ml^{-1} of silver(I)] yielded coefficients of variation of less than 0.48%. In each instance the difference between the measured and known concentration was less than 1.6%. The sensitivity of the method is 0.001 5 μg cm^{-2} of iodide. Similar results were also obtained when excess of silver(I) was allowed to react with iodide ions and unreacted silver(I) was determined following the recommended procedure.

The effects of diverse ions upon the determination of 1.27 μg ml^{-1} of iodide ions were also studied. Mercury(II) interferes and other interferences were found to be of the same order as for the determination of silver(I).

One of the authors (S.B.) is grateful to the University Grants Commission, New Delhi, India, for providing him with a Teacher Fellowship and Mr. Y. S. Varma for his helpful suggestions.

References

1. Snell, F. D., "Photometric and Fluorometric Methods of Analysis, Metals, Part I," John Wiley, New York, 1978.
2. Marczenko, Z., "Spectrophotometric Determination of Elements," John Wiley, New York, 1976.
3. Vogel, A. I., "A Text Book of Quantitative Inorganic Analysis," Fourth Edition, Longmans, London, 1978.

Received *January 28th*, 1980
Accepted *May 29th*, 1980

Rapid Gas-Liquid Chromatographic Determination of Cotinine in Biological Fluids

C. Feyerabend
Poisons Unit, New Cross Hospital, London, SE14 5ER

and M. A. H. Russell
Addiction Research Unit, Institute of Psychiatry, Maudsley Hospital, London, SE5 8AF

Keywords: *Cotinine determination*; *gas chromatography*; *plasma*; *saliva*; *urine*

Radioactive tracer techniques have been described for the measurement of cotinine in biological fluids,[1] but they are cumbersome and inappropriate when large numbers of subjects are being investigated. It is preferable to use a gas - liquid chromatographic technique, especially when this can be achieved by simple modification of a routine method for nicotine measurement. We report here a rapid extraction procedure that allows the sensitive determination of cotinine in biological samples without interference or contamination.

Experimental

Apparatus

A Hewlett-Packard, Model 5730A, chromatograph fitted with an alkali flame-ionisation detector and a Model 3380A integrator was used, with an external time delay relay.

Reagents

All reagents were of analytical-reagent grade.
Acetone.
Dichloromethane.
Pheniramine maleate. Hoechst Pharmaceuticals, Hounslow, Middlesex.
Sodium hydroxide solution, 5 M.

Gas - Liquid Chromatography

The gas flow-rates were helium (carrier gas) 60, air 50 and hydrogen 3 ml min^{-1}. A glass column (2.5 m $\times$2 mm i.d.) packed with 10% (*m/m*) Apiezon L and 10% potassium hydroxide on 80–100 mesh Chromosorb W was used. The temperatures of the oven, detector and injection port were 230, 300 and 250 °C, respectively. The retention times for cotinine and pheniramine free base were 2.66 and 3.17 min, respectively. The time delay relay device was used to overcome difficulties in the integration of peak areas, as described in detail elsewhere.[2]

Procedure

To 1.0 ml of sample in a 12.5-ml centrifuge tube were added 2.0 ml of 5 M sodium hydroxide solution, 100 μl of an aqueous solution of pheniramine maleate (2.2 μg ml^{-1}) as internal standard and 3.0 ml of dichloromethane. The solution was vortex mixed for 2 min and then centrifuged for 5 min. Any emulsions were removed by discarding the aqueous layer, vortex mixing the centrifuge tube for a few seconds and centrifuging for 2 min. The organic layer was transferred into a second tube and evaporated to dryness under a stream of nitrogen at room temperature. Acetone (50 μl) was added and the tube vortexed for 1 min and centrifuged for 1 min. A 3-μl volume of the acetone solution was injected on to the chromatographic column.

Calibration

A calibration graph was constructed by adding cotinine and the internal standard to blank solutions of the sample type to be analysed to give concentrations of 25, 50, 100, 200, 400, 800 and 1000 ng ml^{-1}. These solutions were then carried through the extraction procedure.

Although the calibration graph was linear from 0 to 1000 ng ml^{-1} and passed through the origin, care was taken to ensure that all cotinine concentrates were stored away from the analytical laboratory.

Blanks

Many of the problems of positive blanks associated with nicotine analysis[3] do not occur in the cotinine method as this compound is present in cigarette smoke in much lower concentrations than nicotine. As a result, the risk of contamination is subsequently reduced.

Reproducibility

The reproducibility over a range of concentrations is shown in Table I. The average coefficient of variation over this range was 1.8% (n = 10).

TABLE I

REPRODUCIBILITY OF RESULTS OF TEN DETERMINATIONS AT VARIOUS COTININE CONCENTRATIONS

Cotinine added/ng ml^{-1} ..	25	50	100	200	400	600	800	1000
Cotinine found (mean)/ng ml^{-1}	25.0	50.1	100.1	201.6	399.2	598.8	800.4	1000.3
Standard deviation/ng ml^{-1} ..	0.4	1.0	1.4	3.9	8.6	11.6	13.1	19.8

Recovery

The absolute recovery of cotinine (90%) was determined by injecting a mixture in acetone of cotinine (2000 μg l^{-1}) and pheniramine maleate (2000 μg l^{-1}). The peak-area ratio was compared with that obtained by injecting an extract of an aqueous solution of cotinine (1 ml; 100 μg l^{-1}) taken through the extraction procedure. The internal standard was introduced by reconstituting the cotinine extract in 50 μl of acetone containing pheniramine maleate (2000 μg l^{-1}). (This procedure resulted in a final extract 20 times more concentrated than the original solution.)

Results and Discussion

A typical chromatogram of an extract from human plasma is shown in Fig. 1. The reproducible lower limit of determination of cotinine was 1 ng ml^{-1}. No interference was found from the following common drugs: atropine, amphetamine, amitriptyline, chlorpheniramine, diphenhydramine, diethylpropion, fenfluramine, imipramine, lignocaine, methylamphetamine, nortriptyline, procaine, phentermine, sodium cromoglycate, salbutamol and terbutaline.

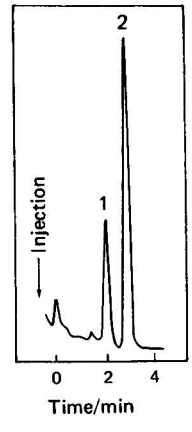


Fig. 1. Gas chromatogram of extract from human plasma: 1, cotinine and 2, pheniramine maleate.

This method for the determination of cotinine can be used in conjunction with a previously reported extraction technique for nicotine.[2,3] Nicotine is extracted into diethyl ether and the sample is then re-extracted with dichloromethane to retrieve cotinine. Unlike Hengen and Hengen,[4] who reported no loss of cotinine during an extraction with diethyl ether, we found that a loss of cotinine occurred that was inversely proportional to the ratio of the volume of sample to that of the organic phase. Thus, with sample volumes of 3, 2 and 1 ml, the cotinine losses were 10, 22 and 44%, respectively. It is therefore important to standardise the volume of sample if necessary, by the addition of tap water, and to use the equivalent volume of plasma, saliva or urine standards to construct the calibration graph.

In a preliminary experiment, a subject who had abstained from smoking for 10 days smoked a cigarette over a short period (3 min) and blood samples were withdrawn at frequent intervals via an indwelling venous cannule. Samples of saliva and urine were also collected throughout the course of the experiment (Fig. 2). An increase in plasma cotinine concentration occurred only 2 min after discarding the cigarette and the concentration rose steeply during the first hour and reached a plateau at 4 h that persisted throughout the experiment. As expected, plasma nictoine concentrations reached a maximum within 2 min after smoking had ceased, fell sharply during the next 10–15 min and then more slowly over the remaining 6.5 h to approach base-line levels. Although cotinine (pK_a 4.5[5]) is essentially unionised in blood at pH 7.4, the free base is poorly soluble in lipids and therefore its rate of distribution into

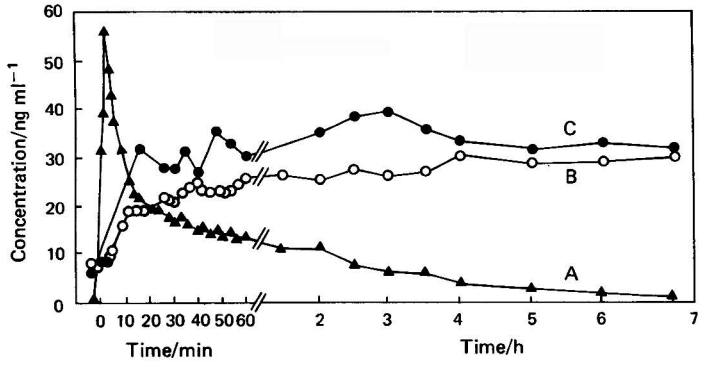


Fig. 2. Graph showing A, plasma nicotine, B, plasma cotinine and C, saliva cotinine, after smoking one 1.3-mg nicotine cigarette. Smoking period = 3 min.

tissues may be slow. This would partially explain the prolonged existence of the compound in blood. Another contributing factor to this is the low rate of renal excretion of cotinine relative to nicotine (Fig. 3). This may account for the high concentrations of this metabolite (about 800 ng ml^{-1}) often found in the plasma of habitual smokers.

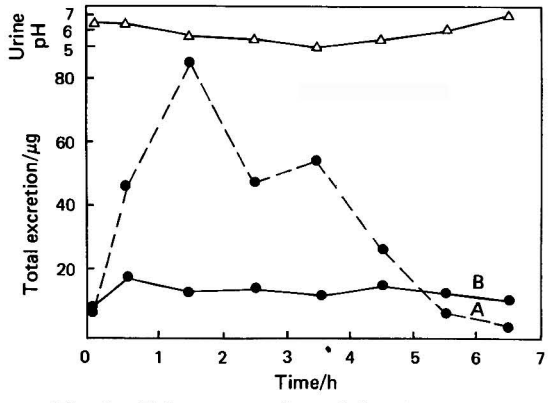


Fig. 3. Urinary excretion of A, nicotine and B, cotinine over 7 h after smoking a 1.3-mg nicotine cigarette. The pH of the urine at the different times is also shown.

Nicotine is concentrated in saliva to give levels approximately 10 times higher than those measured in plasma,[6] whereas salivary and plasma cotinine concentrations were essentially the same.

We thank A. E. Bryant for technical assistance and the Medical Research Council for financial support.

References

1. Armitage, A. K., Dollery, C. T., George, C. F., Houseman, T. H., Lewis, P. J., and Turner, D. M., *Brit. Med. J.*, 1975, **4**, 313.
2. Feyerabend, C., and Russell, M. A. H., *J. Pharm. Pharmacol.*, 1979, **31**, 73.
3. Feyerabend, C., and Russell, M. A. H., *J. Pharm. Pharmacol.*, 1980, **32**, 178.
4. Hengen, N., and Hengen, M., *Clin. Chem.*, 1978, **24**, 50.
5. Yamamoto, I., *Adv. Pest Control. Res.*, 1966, **6**, 231.
6. Russell, M. A. H., and Feyerabend, C., *Drug Metab. Rev.*, 1978, **8**, 29.

Received *March 19th*, 1980
Accepted *April 24th*, 1980

Communication

Material for publication as a Communication must be on an urgent matter and be of obvious scientific importance. Rapidity of publication is enhanced if diagrams are omitted, but tables and formulae can be included. Communications should not be simple claims for priority: this facility for rapid publication is intended for brief descriptions of work that has progressed to a stage at which it is likely to be valuable to workers faced with similar problems. A fuller paper may be offered subsequently, if justified by later work.

Manuscripts are not subjected to the usual examination by referees and inclusion of a Communication is at the Editor's discretion.

Limit of Detection in Analysis with Ion-selective Electrodes

Keywords: *Ion-selective electrodes*; *potentiometry*; *limit of detection*

In an earlier paper[1] approximations were used in deriving some of the equations [(8), (10) and (12)] expressing C_Q, the criterion of detection, and C_L, the limit of detection, and these equations were restricted to electrodes whose responses were limited by the solubility products of isovalent (1:1) salts. In this paper, exact and general solutions are derived within the same treatment as before.

Retaining the earlier notation[1] [C = analytical determinand concentration, s = concentration of determinand dissolved from electrode, b_r = reagent blank determinand, b_i = ith interference effect, K = solubility product, σ_B = standard deviation of the blank (mV), $Q = 2.33\sigma_B$, $L = 4.65\sigma_B$], the following derivations ensue for an electrode incorporating a salt A_xB_y and responding to ion A.

Response Not Limited by Solubility Product

There is no change and equation (6) in the earlier paper[1] is exact and general in application, *i.e.*, where $b = b_r + \Sigma b_i$,

$$C_Q = (10^{Q/k} - 1)b$$

Response Limited by Solubility Product Only

In this instance $b_r = \Sigma b_i = 0$ and the solubility product is given by

$$K = (C + s)^x\left(\frac{y}{x}\cdot s\right)^y \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

The e.m.f. can be expressed as follows:

$$E = E^0 + k \log\left[K\Big/\left(\frac{y}{x}\cdot s\right)^y\right]^{1/x}$$

At $C = 0$, the blank e.m.f. is given by

$$E_B = E^0 + k \log\left[K\left(\frac{x}{y}\right)^y\right]^{1/(x+y)}$$

At the criterion of detection, $C = C_Q$, $s = s_Q$ and the e.m.f. is

$$E_Q = E^0 + k \log\left[K\Big/\left(\frac{y}{x}\cdot s_Q\right)^y\right]^{1/x}$$

Hence,

$$Q = |E_Q - E_B| = k \log\left\{\left[K/\left(\frac{y}{x}\cdot s_Q\right)^y\right]^{1/x}\Big/\left[K\left(\frac{x}{y}\right)^y\right]^{1/(x+y)}\right\}$$

and

$$10^{Q/k} = \frac{\left[K^{1/x}\left(\frac{x}{y}\right)^{y/x}\right]^{y/(x+y)}}{s_Q^{y/x}}$$

Substituting in equation (1) and solving for C, we obtain

$$C^Q = \left[\frac{K}{\left(\frac{y}{x}\cdot s_Q\right)^y}\right]^{1/x} - s_Q = \left(\frac{x}{y}\right)^{y/(x+y)} K^{1/(x+y)}\, 10^{Q/k}\left(1 - 10^{-(x+y)Q/yk}\right) \quad .. \quad (2)$$

Equation (2) replaces equations (8), (14) and (16) in the earlier paper[1] and also covers other stoicheiometries. The equation for the limit of detection, C_L, is exactly analogous with L substituted for Q.

Response Limited by Solubility Product and Interference

As $b_r = 0$, $\Sigma b_i = b \neq 0$ and the solubility product is still defined as in equation (1), the e.m.f. can be expressed as follows:

$$E = E^0 + k \log\left\{b + K^{1/x}\Big/\left(\frac{y}{x}\cdot s\right)^{y/x}\right\}$$

At $C = 0$, the blank e.m.f. is given by

$$E_B = E^0 + k \log\left\{b + \left[K\left(\frac{x}{y}\right)^y\right]^{1/x+y}\right\}$$

At the criterion of detection, $C = C_Q$, $s = s_Q$ and the e.m.f. is

$$E_Q = E^0 + k \log\left\{b + K^{1/x}\Big/\left(\frac{y}{x}\cdot s_Q\right)^{y/x}\right\}$$

Now, $Q = |E_Q - E_B|$, and hence

$$10^{Q/k} = \frac{K^{1/x}\Big/\left[\frac{y}{x}\cdot s_Q\right]^{y/x} + b}{\left[K\left(\frac{x}{y}\right)^y\right]^{1/(x+y)} + b}$$

Solving for s_Q, we obtain

$$s_Q = \frac{\left(\frac{x}{y}\right)K^{1/y}}{\left\{10^{Q/k}\left[K\left(\frac{x}{y}\right)^y\right]^{1/(x+y)} + b(10^{Q/k} - 1)\right\}^{x/y}}$$

Hence, from equation (1), the criterion of detection is given by

$$C_Q = \left[\left(\frac{x}{y}\right)^y K\right]^{\frac{1}{x+y}} 10^{Q/k} + b(10^{Q/k} - 1) - \frac{\left(\frac{x}{y}\right)\cdot K^{1/y}}{\left\{\left[\left(\frac{x}{y}\right)^y K\right]^{\frac{1}{x+y}} 10^{Q/k} + b(10^{Q/k} - 1)\right\}^{x/y}} \quad .. \quad (3)$$

Equation (3) not only replaces equation (10) in the earlier paper[1] but also extends the treatment to electrodes based on non-isovalent salts. The equation for the limit of detection, C_L, is exactly analogous with L substituted for Q.

Response Limited by Solubility Product and Reagent Blank Determinand

In this case $\Sigma b_i = 0$ and $b_r \neq 0$. The solubility product equation for a salt A_xB_y in an electrode responsive to ion A is

$$K = (C + b_r + s)^x \left[\frac{y}{x} \cdot s\right]^y \quad .. \quad .. \quad .. \quad .. \quad (4)$$

At $C = 0$, $s = s_o$ and the e.m.f. of the blank is

$$E_B = E^o + k \log \left\{ K \Big/ \left[\frac{y}{x} \cdot s_o\right]^y \right\}^{1/x}$$

At the criterion of detection, $C = C_Q$, $s = s_Q$ and the e.m.f. is

$$E_Q = E^o + k \log \left\{ K \Big/ \left[\frac{y}{x} \cdot s_Q\right]^y \right\}^{1/x}$$

Now, $Q = |E_Q - E_B| = k \log \left(\frac{s_o}{s_Q}\right)^{y/x}$

Hence,

$$s_Q = s_o \, 10^{-xQ/yk}$$

From equation (4), the criterion of detection is

$$C_Q = \frac{K^{1/x}}{\left[\frac{y}{x} \cdot s_Q\right]^{y/x}} - b - s_Q$$

$$= \frac{10^{Q/k} K^{1/x}}{\left(\frac{y}{x} \cdot s_o\right)^{y/x}} - b - s_o \, 10^{-xQ/yk} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

For $x = y = 1$, equation (4) with $C = 0$ can be solved analytically to give s_o, which can be substituted in equation (5). For other stoicheiometries, s_o is best found by an iterative method, although for $x = 2$, $y = 1$ and $x = 1$, $y = 2$, s_o can be determined explicitly in terms of hyperbolic functions. The equation for the limit of detection, C_L, is exactly analogous to equation (5).

Equations (2), (3) and (5) do not involve any approximations and are, therefore, more accurate than the earlier equations,[1] as shown in Table I. The newly calculated values agree more closely

TABLE I

CRITERIA AND LIMITS OF DETECTION FOR UNIVALENT ELECTRODES WITH $\sigma_B = 1.0$ mV

	Code*			
	B	C	D	E
$10^6 b_r$/mol l^{-1}	0	0	1.5	0
$10^6 \Sigma b_i$/mol l^{-1}	0	1	0	0
$10^{12} K$	4	1	1	4×10^{-6}
Equation used	(2)	(3)	(5)	(3)
$10^7 C_Q$/mol l^{-1}—				
Graphical	3.63	3.43	2.29	5.25
This calculation	3.63	3.49	2.33	5.22
Previous calculation*	3.47	4.28	1.99	5.21
$10^7 C_L$/mol l^{-1}—				
Graphical	7.41	6.76	4.79	9.66
This calculation	7.28	6.81	4.79	10.04
Previous calculation*	7.28	8.23	4.03	10.05

* See Table III in the earlier paper.[1]

with the graphical values, especially for electrodes C and D, for which the earlier treatment involved the greatest approximations. All of the discussion in the earlier paper is applicable to the more accurate equations developed here.

It may also be noted that simple expressions exist for the limit and criterion of detection in the special cases where the e.m.f. can be measured with sufficient precision to allow limiting linear calibrations to be used.[2]

This work was carried out at the Central Electricity Research Laboratories and is published with the permission of the Central Electricity Generating Board.

References

1. Midgley, D., *Analyst*, 1979, **104**, 248.
2. Midgley, D., *Analyst*, 1980, **105**, 417.

Received *July 22nd*, 1980

Derek Midgley

Central Electricity Research Laboratories,
Kelvin Avenue, Leatherhead, Surrey, KT22 7SE

Book Reviews

INTERNATIONAL COMMISSION FOR UNIFORM METHODS OF SUGAR ANALYSIS. REPORT OF THE PROCEEDINGS OF THE SEVENTEENTH SESSION HELD IN MONTREAL, 4–9 JUNE, 1978. Pp. xxiv + 448. The International Commission for Uniform Methods of Sugar Analysis. 1979. Price £20. ISBN 0 905003 02 0.

As the title states, this book reports the Proceedings of the 17th Session of ICUMSA held in Montreal from June 4th to 9th, 1978, under its President, Dr. A. Carruthers, and attended by about 120 delegates from 27 major sugar-producing countries. The format of the book is very similar to its predecessor, which reported on the 1974 Session. The subject matter remains substantially the same, being concerned with 29 topics, each of which covers a particular field of analytical interest to the sugar industry.

The topics include specifications for laboratory apparatus and reagents, the determination of several physical properties and chemical parameters such as sucrose, reducing sugars and other saccharides, inorganic and organic non-sugars and dry substance. Additionally, microbiology, the deterioration, crystallisation and refining of sugars are discussed, and two new topics, method specification and ion-selective electrodes. For each subject the referee's presentation, summarising work carried out since the last Session, and recommendations are fully reported, together with the ensuing discussion, the final recommendations adopted and appropriate references. The proceedings are reported clearly and concisely but, as would be expected, the subject matter is highly specific.

For this reason the book is unlikely to appeal to those analysts having only a broad interest in sugar analysis. On the other hand, the book is a valuable manual for those actively engaged in this field and concerned with the latest views and developments. A. D. INCE

GEL CHROMATOGRAPHY. THEORY, METHODOLOGY, APPLICATIONS. First English Edition. By TIBOR KREMMER and LÁSZLÓ BOROSS. Pp. 299. John Wiley and Akadémiai Kiadó. 1979. Price £16.50. ISBN 0 471 99548 7.

This book is a revised version of the original Hungarian "Gélkromatográfia" (Müszaki Könyvkladó, Budapest) translated into English by Mrs. M. Gábor. The text is presented in three parts: I, Fundamentals and Theory (by T. Kremmer); II, Methods and Techniques (by T. Kremmer); and III, Applications (by L. Boross). Under headings I and II are covered the fundamental details of gels, the theory of the mechanisms of separation and the practical details covering selection and preparation of gels, selection and packing of columns and special techniques including thin-layer gel chromatography, gradient elution, zone precipitation and preparative procedures.

Applications given in the third part of the volume include the fields of proteins, nucleic acids, synthetic polymers, carbohydrates, small molecule organic compounds and inorganic ions.

From a practical viewpoint one of the most useful chapters in the book is that in which the selection, packing and use of columns are detailed, but a more detailed description of detection systems could have been included with advantage.

Much of the book comprises a review of gel chromatography rather than a practical handbook for the student or user of the technique. It thus tends to become more a work of reference and approximately 1 000 references are included. These are not numbered but are presented alphabetically by author and in date order in a section at the end of the book, and hence they may take a little longer to identify.

The reviewer feels that it would have been helpful had more discussion on the qualitative and quantitative aspects, detection limits, etc., of the technique and applications been included. Nevertheless, much useful information has been incorporated (not least the details given on bacteriostatic agents used for preserving the gels); the text is in clear English and the book seems well worth purchasing by both libraries and practical students of the technique. D. SIMPSON

CARCINOGENS AND RELATED SUBSTANCES. ANALYTICAL CHEMISTRY FOR TOXICOLOGICAL RESEARCH. By MALCOLM C. BOWMAN. Pp. xii + 316. Marcel Dekker. 1979. Price SwFr78. ISBN 0 8247 6885 X.

Recent changes in legislation concerning chemical substances have had a profound influence on the analytical aspects of toxicology. The legislation is still in a state of flux; however, this book from the National Centre for Toxicological Research (NCTR) gives a useful insight into the analytical thinking of the US Food and Drug Administration. While the book is directed specifically towards carcinogens, many of the principles described have a much wider application in analytical toxicology.

In the book, the author stresses the importance of analytical measurements in the validity and safety of todays' enormously expensive toxicology studies. "The control of a test substance begins when it enters the laboratory and ends only after its safe disposal." In pursuit of this philosophy the book covers the importance of pre-study measurements to establish the identity, purity and stability of the test substance. Specific problems that can arise in this area are illustrated from the authors' own experience at NCTR. Contaminants in animal diet, bedding material or drinking water could influence the outcome of a toxicology study. A short section of the book is devoted to methods for the detection of trace amounts of likely impurities (i.e., aflatoxins, heavy metals and pesticides).

By far the major portion of the book concentrates on the analysis of carcinogens that have been added to a variety of materials for the purpose of toxicological investigation. Here the object is to establish homogeneity, stability and approach to a nominal concentration. Aromatic amines are the subject of particular attention; however, oestrogens, specific mycotoxins, pesticides and a miscellaneous collection of other compounds are also covered in lesser detail. Methods for animal diet, drinking water, microbiological media, urine and blood are presented. There is a short section describing the experimental details of *Salmonella typhimurium* Test (Ames Test) of mutagenicity. While this is a useful description it seems a little out of place in an otherwise heavily analytical book.

When working with carcinogens the safety of laboratory personnel is obviously of paramount importance. Many judgements on safety are based on analytical measurements and consequently methods are presented for the analysis of atmospheres and work surfaces and for the measurement of test substance in urine as an index of employee exposure. Contamination of the environment also receives consideration. I feel that many readers, like myself, will find the NCTR approach to removal of carcinogens from laboratory waste water excessively expensive and largely impracticable.

In more general terms I found this a useful and clearly written book, which could be of great value to anyone engaged in the analytical aspects of toxicology. It is obvious that much of what is presented is derived from the author's own personal experience and has been carefully tailored to cover particular analytical problems. Although certain parts of the text are presented in the sort of detail one expects in a scientific paper, I feel that this does not detract from this extremely pertinent book.

G. T. STEEL

ELECTROPHORESIS. A SURVEY OF TECHNIQUES AND APPLICATIONS. PART A: TECHNIQUES. Edited by Z. DEYL, co-edited by F. M. EVERAERTS, Z. PRUSÍK and P. J. SVENDSEN. *Journal of Chromatography Library, Volume* 18. Pp. 390 + xv. Elsevier. 1979. Price $83; Dfl.170. ISBN 0 444 41721 4 (Vol. 18); ISBN 0 444 41616 1 (Series).

The Editor, aided by an eminent team of Co-editors, has collected together 17 chapters, each by a different author, to produce an impressive and worthwhile book. As the first volume of a two-part work, this deals with the principles, theory and instrumentation of modern electrophoretic methods; its companion, Part B, with a detailed survey of applications will come later.

The book may be taken in two parts, namely, an introductory part covering the first four chapters and the remainder, Chapters 5–17, being devoted to the main business of a survey of the various electrophoretic techniques taken one by one.

Taking the introductory part, Chapters 1–3, by J. Vacik, cover the essential theory, general classification of electrophoretic methods and the principles of evaluating results, and thereby form a coherent unit. Chapter 4, written by the Editor, takes a different stance and deals with the elimination of charge differences, such as by the formation of strongly negatively charged protein-detergent complexes with almost identical mobilities in free solution regardless of original charge.

This then provides a basis for relative molecular mass determination by electrophoresis on gel-type supports, where size and shape characteristics have their part to play.

The survey of techniques in Chapters 5–17 include zone electrophoresis (excepting gel-type and immunoelectrophoresis) (W. Ostrowski), gel-type techniques (Z. Hrkal), immunoelectrophoresis (P. J. Svendsen), moving boundary electrophoresis in narrow-bore tubes (F. M. Everaerts and J. L. Beckers), isoelectric focusing (N. Catsimpoolas), analytical isotachophoresis (J. Vacik and F. M. Everaerts), continuous flow-through electrophoresis (Z. Prusík), continuous flow deviation electrophoresis (the author does not regard "free-flow" as appropriate) (A. Kolin), preparative electrophoresis in gel media (Z. Hrkal) and in columns (P. J. Svendsen), preparative isoelectric focusing (P. Blanicky), and preparative isotachophoresis by wide column (P. J. Svendsen) and capillary methods (L. Arlinger).

Consideration of each technique is based on a logical division into analytical and preparative aspects and, as can be seen from the chapter titles, every effort has been made to avoid clashing. Nevertheless, discussions cannot always be complete without reference to some of the other electrophoretic methods. Thus, for example, some reference is made to SDS electrophoresis in Chapter 6 on gel-type techniques, although as mentioned above, the main principles of this will have been discussed in Chapter 4. But then, this emphasises the essential feature of this book as a practical work and it is in Chapter 6 that the more mundane details of the recipe for an SDS - gel system are to be found.

Each chapter is systematically presented. Thus, a contents list is generally followed by a concise introduction leading into the main business, frequently with practical details, and, where appropriate, some illustrative applications in order to demonstrate the scope of the variant of the technique under consideration.

The book, although very practical in its approach, gives due prominence to the underlying mathematical principles, supported by a range of clear line-diagrams. There is also a good selection of illustrative diagrams and black and white pictures of apparatus and separations, as well as copious lists of references at the end of chapters. It is a pity though that authors' names in references are not collected in a separate index as the book might also be a good reference source.

The book is enhanced by a list of the many symbols used in the text (pp. 379–384) and this is followed by an adequate subject index. For better consistency, resort might have been made to exercising some extra editorial prerogative in some quarters, such as the bringing of Chapter 12 into the ambit of SI units.

The over-all conclusion must be, "I like it," and the book is bound to be well appreciated by other readers, particularly if they read the stimulating preface by Stellan Hjerten for then they will not want to let go.

J. D. R. THOMAS

DIFFERENTIAL SCANNING CALORIMETRY. By J. L. MCNAUGHTON and C. T. MORTIMER. *Reprinted from IRS: Physical Chemistry Series* 2, 1975, *Volume* 10. Pp. ii + 44. Perkin-Elmer Corporation. 1979.

This excellent booklet describes, in detail, the many applications of differential scanning calorimetry. It is a verbatim reprint of a chapter in "IRS: Physical Chemistry Series 2, 1975, Volume 10" and is published by Perkin-Elmer Corporation. The reader may thus infer, quite correctly, that it is heavily biased towards the equipment available from this company. Indeed, competitive apparatus receives but scant attention. On pp. 2 and 3, reference is made to the IUPAC recommendations on nomenclature in thermal analysis (these were, in fact, originally made by the International Confederation for Thermal Analysis and subsequently accepted by IUPAC) and it is, therefore, a pity that throughout the booklet reference is made to "thermogram" rather than the recommended "DSC curve."

The footnote on p. 4, which states that "the theory of DSC . . . is proprietory to the Perkin-Elmer Corporation," is somewhat misleading. In fact, "DSC 1" and "DSC 2" are Trade Marks of the Perkin-Elmer Corporation and anyone is free to use the term "differential scanning calorimetry" and the abbreviation "DSC."

The text is remarkably free from printing errors and indications are that this booklet is available, free of charge, from the Perkin-Elmer Corporation.

C. J. KEATTCH

Analytical Sciences Monographs

No. 2 The Chemical Analysis of Water: General Principles and Techniques

by A. L. Wilson

The volume covers all stages of the complete analytical process including: deciding on the analytical information required; sampling, including place, time and frequency, as well as devices and techniques; the analysis proper and the reporting of results, their statistical treatment, and the factors involved in the choice of analytical methods (including on-line and automatic methods) for particular purposes; and data handling.

Hardcover 196pp 8¾″ × 6¼″ 0 85990 502 0
£7.50 ($16.50) RSC Members £5.75

No. 3 Pyrolysis-Gas Chromatography

by R. W. May, E. F. Pearson and D. Scothern

Many papers have been published, particularly over the past decade, on aspects of pyrolysis-gas chromatography. A large number of different types of apparatus have been used, on a wide range of samples. This monograph attempts to present the available knowledge in a form useful to the practising analyst, helping in the choice of an appropriate method and in the avoidance of the more common pitfalls in this, perhaps deceptively, simple technique.

Hardcover 117pp 8¼″ × 6″ 0 85186 767 7
£7.20 ($15.75) RSC Members £5.50

No. 4 Electrothermal Atomisation for Atomic Absorption Spectrometry

by C. W. Fuller

Since the introduction of atomic absorption spectrometry as an analytical technique, by Walsh, in 1953, the use of alternative atomization sources to the flame has been explored. At the present time the two most successful alternatives appear to be the electrothermal atomizer and the inductively-coupled plasma. In this book an attempt has been made to provide the author's views on the historical development, commercial design features, theory, practical considerations, analytical parameters of the elements, and areas of application of the first of these two techniques, electrothermal atomization.

Hardcover 135pp 8¾″ × 5⅝″ 0 85186 777 4
£6.75 ($14.75) RSC Members £5.00

No. 5 Dithizone

by H. M. N. H. Irving

The author of this monograph, who has been closely associated with the development of analytical techniques using this reagent for many years, and who has made extensive investigations into the properties of its complexes, has gathered together a body of historical and technical data that will be of interest to many practising analytical chemists.

Hardcover 112pp 8¾″ × 5⅝″ 0 85186 787 1
£7.25 ($16.00) RSC Members £5.50

No. 6 Isoenzyme Analysis

Edited by D. W. Moss

This monograph attempts to draw together the most important experimental techniques which have resulted from the modern recognition that enzymes frequently exist in multiple molecular forms. This monograph also indicates the advantages and limitations in isoenzyme studies of these modern experiments.

Brief Contents:
Multiple Forms of Enzymes; Separation of Multiple Forms of Enzymes; Selective Inactivation of Multiple Forms of Enzymes; Immunochemistry of Multiple Forms of Enzymes; Catalytic Differences between Multiple Forms of Enzymes, Methods of Obtaining Structural Information, Selection of Methods of Analysis.

Hardcover 171pp 8¾″ × 5⅝″ 0 85186 800 2
£9.00 ($22.00) RSC Members £6.75

Orders:

RSC Members should send their orders to:

The Membership Officer, The Royal Society of Chemistry
30 Russell Square, London WC1B 5DT

All other orders should be sent to:

The Royal Society of Chemistry, Distribution Centre,
Blackhorse Road, Letchworth, Herts. SG6 1HN

The Royal Society of Chemistry

Determination of Sulphide in Flooded Acid - Sulphate Soils by an Indirect Atomic-absorption Spectrophotometric Method

Short Paper

Keywords: *Flooded acid - sulphate soils*; *sulphide determination*; *atomic-absorption spectrophotometry*

RAMESH C. RAY, P. K. NAYAR, A. K. MISRA and N. SETHUNATHAN
Division of Soil Science and Microbiology, Central Rice Research Institute, Cuttack-753006, India

Analyst, 1980, **105**, 984–986.

Determination of Diuron Residues in Soil: Comparison of Determinations by High-performance Liquid Chromatography and Gas - Liquid Chromatography

Short Paper

Keywords: *Diuron determination*; *soil*; *gas - liquid chromatography*; *high-performance liquid chromatography*

E. G. COTTERILL
Agricultural Research Council Weed Research Organization, Begbroke Hill, Yarnton, Oxford, OX5 1PF.

Analyst, 1980, **105**, 987–990.

Rapid Extraction of Some Persistent Chlorinated Hydrocarbons from Biological Material with Low Fat Content

Short Paper

Keywords: *Chlorinated hydrocarbon extraction*; *hexachlorobenzene*; *octachlorostyrene*; *biological material*; *low fat content*

GUNNAR NORHEIM and ELISABET MO ØKLAND
National Veterinary Institute, P.O. Box 8156 Dep., Oslo 1, Norway.

Analyst, 1980, **105**, 990–992.

Determination of the Anthelmintic Levamisole in Plasma and Gastro-intestinal Fluids by High-performance Liquid Chromatography

Short Paper

Keywords: *Levamisole determination*; *biological fluid analysis*; *high-performance liquid chromatography*

S. MARRINER, E. A. GALBRAITH and J. A. BOGAN
Department of Veterinary Pharmacology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow, G61 1QH.

Analyst, 1980, **105**, 993–996.

Spectrophotometric Micro-determination of Silver(I) and Iodide Ions

Short Paper

Keywords: *Silver(I)*; *iodide*; 4-(2-*quinolylazo*)*phenol*

SUDARSAN BARUA, B. S. GARG and R. P. SINGH
Department of Chemistry, University of Delhi, Delhi-110007, India.

and ISHWAR SINGH
Department of Chemistry, Maharishi Dayanand University, Rohtak-123001, India

Analyst, 1980, **105**, 996–998.

Rapid Gas - Liquid Chromatographic Determination of Cotinine in Biological Fluids

Short Paper

Keywords: *Cotinine determination*; *gas chromatography*; *plasma*; *saliva*; *urine*

C. FEYERABEND
Poisons Unit, New Cross Hospital, London, SE14 5ER.

and M. A. H. RUSSELL
Addiction Research Unit, Institute of Psychiatry, Maudsley Hospital, London, SE5 8AF.

Analyst, 1980, **105**, 998–1001.

Limit of Detection in Analysis with Ion-selective Electrodes

Communication

Keywords: *Ion-selective electrodes*; *potentiometry*; *limit of detection*

DEREK MIDGLEY
Central Electricity Research Laboratories, Kelvin Avenue, Leatherhead, Surrey, KT22 7SE.

Analyst, 1980, **105**, 1002–1005.

TUCK IN UNDER FLAP A

THE ANALYST **October, 1980**

READER ENQUIRY SERVICE

For further information about any of the products featured in the advertisements in this issue, please write the appropriate A number in one of the boxes below.
Postage paid if posted in the British Isles but overseas readers must affix a stamp.

FIRST FOLD

(Please use BLOCK CAPITALS)

NAME .....

OCCUPATION .....

ADDRESS .....

SECOND FOLD

Postage will be Paid by Licensee

Do not affix Postage Stamps if posted in Gt. Britain, Channel Islands or N. Ireland

BUSINESS REPLY SERVICE
Licence No. W.D. 106

Reader Enquiry Service
The Analyst
The Royal Society of Chemistry
Burlington House
Piccadilly London W1E 6WF
ENGLAND

21

THIRD FOLD

FIRST FOLD

CUT ALONG THIS EDGE

FLAP A

PATENTS

British Patent No. 1 356 512.

Contractor Mixing Device.

Owner desires commercial exploitation on reasonable terms by license or sale. Inquiries, Fitzpatricks, Chartered Patent Agents, 48 St. Vincent Street, Glasgow G2 5TT, and Warwick House, Warwick Court, London WC1R 5DJ.

British Patent No. 1 490 137.

Chemical Compositions Suitable for Use as Insect Repellents.

Owner desires commercial exploitation on reasonable terms by license or sale. Inquiries, Fitzpatricks, Chartered Patent Agents, 48 St. Vincent Street, Glasgow G2 5TT, and Warwick House, Warwick Court, London WC1R 5DJ.

British Patent No. 1 346 389.

An Anti-Adhesive Composition.

Owner desires commercial exploitation on reasonable terms by license or sale. Inquiries, Fitzpatricks, Chartered Patent Agents, 48 St. Vincent Street, Glasgow G2 5TT, and Warwick House, Warwick Court, London WC1R 5DJ.

ANALAO 105 (1255) 969-1008 (1980) October 1980
ISSN 0003-2654

THE ANALYST

THE ANALYTICAL JOURNAL OF THE ROYAL SOCIETY OF CHEMISTRY

CONTENTS

Printed by Heffers Printers Ltd Cambridge England
Entered as Second Class at New York USA, Post Office